



Monographs on Medicinal Plants  
along Ganga River

# *Terminalia arjuna* (Roxb. ex DC.) Wight & Arn.

Focusing on  
Botanical, Phytochemical, Scientific Validation and  
Insilico Analysis Including Medicinal Importance  
and Soil Properties

Volume 9



Monographs on Medicinal Plants along Ganga River  
*Terminalia arjuna* (Roxb. ex DC.) Wight & Arn.



**Patanjali Organic Research Institute Private Limited (PORI)**  
Food & Herbal Park, Village-Padartha, Laksar Road,  
Haridwar-249407, Uttarakhand  
[www.patanjalibio.com](http://www.patanjalibio.com) | [porihr@patanjolibio.com](mailto:porihr@patanjolibio.com) | +91-8755904985

# Monographs on Medicinal Plants along Ganga River

## *Terminalia arjuna*

Focusing on  
Botanical, Phytochemical, Scientific validation and Insilico  
analysis including Medicinal importance and Soil properties

Volume 09

Sponsored by



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Food & Herbal Park, Village-Padartha, Laksar Road, Haridwar-249407, Uttarakhand

[www.patanjalibio.com](http://www.patanjalibio.com) | [porihr@patanjali.bio](mailto:porihr@patanjali.bio) | +91-8755904985

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Food & Herbal Park, Village-Padartha, Laksar Road, Haridwar-249407,  
Uttarakhand (India) Tel: + 91-8755904985

**Website** : [www.patanjalibio.com](http://www.patanjalibio.com)

**Email** : [porihr@patanjalibio.com](mailto:porihr@patanjalibio.com)

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Food & Herbal Park, Village-Padartha, Laksar Road, Haridwar-249407, Uttarakhand (India)



## ABOUT THE EDITOR

*Dr.* Acharya Balkrishna, Co-founder of Patanjali Organic Research Institute, is a highly ascetic entrepreneur with a diverse personality who is a specialist in Yoga, Ayurveda, Sanskrit language, Indian sacred books, and the Vedas. Dr. Balkrishna has become a significant source of inspiration for Traditional Medicinal Practitioners and a globally recognized celebrity after dedicating his life to the resurrection of ancient healing and living practices. His maverick leadership as Co-Founder & Managing Director of Patanjali Ayurved Limited, along with overseeing Patanjali Food and Herbal Park and Divya Pharmacy, has propelled Ayurveda into a global business phenomenon with a massive following. Additionally, he has been decorated with prestigious awards such as 'Ayurveda Expert', 'Manav Ratan', 'Bharat Gaurav', 'Indian of the Year', 'Ten Versatile and Dynamic Young Men of India', 'Bheeshma Pusaka', 'Lokmanya Tilak', and 'Transformational Business Leader' for his exceptional knowledge, passion, and service to mankind. According to a study published by Stanford University in the USA and Elsevier in Europe, Dr. Balkrishna has been recognized among the top 2% of scientists worldwide for his research on Ayurveda and Yoga.

With his early age passion for plants, he has become the most renowned & respected herbal specialist for health and nutrition. For the research of novel herbal medication formulations, he explored four rare plants: 'Sanjeevani', 'Somlata', 'Swarnakshiri', 'Swarnadraka', and 'Astavavarga plants'. He has taken many initiatives for Biodiversity conservation, presently working for the establishment of Patanjali Herbal Garden & herbarium, working on the compilation of a unique multivoluminous project i.e., 'World Herbal Encyclopaedia', containing the descriptions of ~50,000 medicinal plant species with the largest collection of plant paintings and drawings. This is being done to strengthen traditional medicine systems of the world. Additionally, Dr. Balkrishna has made significant contributions to the socio-economic development of tribal communities and the upliftment of rural communities with FPOs, CLFs, and SHGs. Furthermore, his endeavors have been directed towards exploring and conserving floral diversity, phytochemical and insilico analysis, investigating soil geochemistry, understanding complex plant-microbe relationships, and monitoring water quality from Gomukh to Gangasagar.

He has published more than 400 research articles in national and international journals received around 20 patents and authored more than 200 books on Yoga, Ayurveda, Agriculture, Herbal Medicine, and Information Technology and edited more than 40 unpublished ancient Ayurveda manuscripts. With

the vision of ensuring universal health for the last two decades, more than 1.5 million patients with several persistent, chronic, and non-communicable diseases have been effectively treated. He established Patanjali Ayurved College and the University of Patanjali with the humanitarian goal of assisting youngsters in achieving their goals and serving the country. Additionally, he has been involved in various government and non-government initiatives. He is also a key proponent of agricultural transformation through organic practices, working to boost agricultural productivity, increase farmer income, and ensure equitable access to a safe, affordable, and nutritious diet year-round. Apart from this, Dr. Balkrishna is actively participating in driving agricultural transformation through the 'Patanjali Farmer Samridhi Programme' by well-trained staff and around one lakh trainers. His generous personality is also reflected through his actions like helping the nation with emergency needs like post-disaster needs assessment, providing free shelter, food, and education to orphan children, and free OPD services at Patanjali Yogpeeth. With his humanitarian attitude, Dr. Balkrishna continues his phenomenal journey of making world records, uplifting mankind through medicine & lifestyle improvement, reviving sustainable agriculture, and preserving nature's gifts in the form of literature and a sustainable living approach.





## Vision of Honourable Prime Minister

### Shri Narendra Modi on Medicinal Plants

- “
1. India's rich biodiversity of medicinal plants can provide affordable healthcare solutions.
  2. Medicinal plants are nature's gift to humanity; we must protect and utilize them wisely.
  3. Let us revive and promote the use of medicinal plants in everyday life.
  4. India's biodiversity of medicinal plants is a treasure trove waiting to be explored.
  5. Medicinal plants have been an integral part of our cultural heritage, offering natural remedies for various ailments.
  6. The knowledge embedded in our traditional systems like Ayurveda and the use of medicinal plants can address global health challenges sustainably.
  7. We must promote research and innovation in harnessing the potential of medicinal plants for healthcare and economic development.
- ”

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## **Vision of Union Minister for Jal Shakti**

**Shri Chandrakant Raghunath Patil**



1. We will understand the importance of water, conserve it and ensure rich water resources for future generations.
2. Conservation and enhancement of water resources of our country is a sacred goal. I will work with my dedication and devotion to achieve this.
3. Proper access to water is a strong step towards women's empowerment.
4. Jal Shakti is Nari Shakti, as women are the most affected by water scarcity due to their household responsibilities in rural India.



<https://pib.gov.in/PressReleaseDetail.aspx?PRID=2024324>

<https://www.newsonair.gov.in/c-r-patil-emphasizes-water-access-as-essential-for-womens-empowerment-at-catch-the-rain-2024-workshop-in-delhi/>

<https://www.indiatvnews.com/gujarat/will-transform-jal-shakti-into-rashtra-shakti-cabinet-jal-shakti-minister-c-r-patil-after-portfolio-appointment-pm-modi-2024-06-10-936272>



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- ▶ Shri Chandrakant Raghunath Patil, Hon'ble Minister
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## National Mission for Clean Ganga (NMCG)

- ▶ Rajeev Kumar Mital, Director General
- ▶ G. Asok Kumar, Director General (Former)
- ▶ Nalin Kumar Srivastava, Deputy Director General
- ▶ S. P. Vashishth, Executive Director (Admin)
- ▶ Bhaskar Dasgupta, Executive Director (Finance)
- ▶ Brijendra Swaroop, Executive Director (Projects)
- ▶ Anup Kumar Srivastava, Executive Director (Technical)
- ▶ Brijesh Sikka (Senior Consultant)
- ▶ Sandeep Behera, Consultant Biodiversity-NMCG
- ▶ Sunil Kumar, Environmental Engineer
- ▶ Harcharan Singh, Co-Lead NMCG-PMC

## Ministry of Environment, Forest and Climate Change (MoEFCC)

### Uttarakhand Forest Department & Biodiversity Board

### Uttar Pradesh Forest Department & Biodiversity Board

### Bihar Forest Department & Biodiversity Board

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राजीव कुमार मित्तल, भा.प्र.से.  
महानिदेशक  
राष्ट्रीय स्वच्छ गंगा मिशन  
Rajeev Kumar Mital, IAS  
DIRECTOR GENERAL  
NATIONAL MISSION FOR CLEAN GANGA



सत्यमेव जयते  
75  
आज़ादी का  
अमृत महोत्सव

भारत सरकार  
जल शक्ति मंत्रालय  
जल संसाधन,  
नदी विकास और गंगा संरक्षण विभाग  
GOVERNMENT OF INDIA  
MINISTRY OF JAL SHAKTI  
DEPARTMENT OF WATER RESOURCES,  
RIVER DEVELOPMENT & GANGA REJUVENATION



### MESSAGE

The Ganga, a river deeply revered in our culture, is both a source of spiritual inspiration and home to diverse ecosystems, including riparian buffers along its banks that connect terrestrial and aquatic systems. These buffers play a crucial role in supporting amphibious life, stabilizing the riverbanks, and maintaining ecosystem services. Therefore, under the "Aviral Ganga" approach for biodiversity conservation and "Arth Ganga" for enhancing livelihoods, a project on floral diversity has been initiated through Namami Gange Mission – II, in collaboration with Patanjali Organic Research Institute (PORI), Haridwar, Uttarakhand.

The project "Scientific Exploration of Floral Diversity Near the Ganga Riverbanks for Ethnobotanical Purposes along with their Conservation and Economic Development of the Region via Skill Development Programs" aims to provide valuable scientific insights into the region's ethnobotanical wealth while promoting sustainable conservation practices. The 18-month study, conducted from Gaumukh to Gangasagar along the Ganga banks, reveals the floral profile, ethno medicinal aspects, applications, livelihood perspectives, and the role of biodiversity in ecosystem resilience, highlighting the symbiotic relationship between conservation and socio-economic development. Findings presented through publications, including project reports, monographs, exploration of ethno medicinal plants, and plant wealth along river Ganga India, will benefit stakeholders, including local communities' well-being.

It gives me immense pleasure to extend my appreciation to the entire team for successfully executing the project, which advances our understanding of the unique floral diversity along the Ganga and its role in ethnobotanical purposes, conservation, and integrating science with local knowledge. The project's focus on environmental stewardship and skill enhancement for sustainable livelihoods is commendable.

This excellent work documenting the floral diversity along the banks may pave the way for the rejuvenation of the Ganga and set a precedent for biodiversity conservation and socio-economic improvement, potentially benefiting other regions of India as well.

  
(Rajeev Kumar Mital)



राष्ट्रीय स्वच्छ गंगा मिशन  
प्रथम तल, मेजर ध्यान चंद नेशनल स्टेडियम, इन्डिया गेट, नई दिल्ली-110002  
NATIONAL MISSION FOR CLEAN GANGA  
1st Floor, Major Dhyana Chand National Stadium, India Gate, New Delhi - 110002  
Ph. : 011-23049528, Fax : 23049566, E-mail : dg@nmcg.nic.in





## FOREWORD

In the timeless flow of the sacred Ganga River, lies a profound treasure trove of nature's healing bounty. As we embark on this journey through the pages of this book on Medicinal Plants along the Ganga River, we are reminded of the ancient wisdom that has sustained our civilization for millennia. The Ganga, revered not only for its spiritual significance but also for its ecological richness, nurtures a diverse array of medicinal plants. The plant, meticulously documented in this comprehensive work, are not merely botanical specimen but living reservoir of phytochemicals that hold immense therapeutic potential.

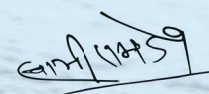
The authors have meticulously documented the plant exploration and botanical study, soil properties analysis, phytochemical analysis, and insilico analysis of the plants found in this region. Their dedication and deep respect for nature's wisdom are evident throughout the pages, making this monograph an invaluable resource for all who seek to deepen their understanding of the healing potential found in the lap of the Himalayas.

Moreover, the inclusion of phytochemical and bioinformatic aspects adds a modern scientific perspective to this ancient knowledge. By unraveling the chemical constituents and molecular mechanisms behind the therapeutic effects, this monograph bridges the gap between traditional wisdom and contemporary scientific advancements, paving the way for evidence-based herbal medicine.

I extend my heartfelt appreciation to Acharya Balkrishna Ji and his devoted team of expert scientists for their unwavering commitment for preserving and disseminating the wisdom of the Medicinal Plants along Ganga River. Their work not only contributes to the scientific community but also instills a deep reverence for nature and its intricate web of life.

May this monograph serve as a guiding light for researchers, practitioners, and enthusiasts alike, illuminating the path towards a deeper appreciation of nature's pharmacy along the revered Ganga River.

With heartfelt gratitude and blessings,



- Swami Ramdev





## PREFACE

The Ganga River, revered as the lifeblood of India, flows through the heart of the country, nourishing not only the land but also the spirit of the people. It is not just a geographical entity; it is a symbol of life, purity, and sustenance. From its origin in the pristine glaciers of Uttarakhand to its expansive delta in West Bengal, the Ganga traverses diverse landscapes, each rich in its own unique flora and fauna. For centuries, it has supported a wide variety of ecosystems, each harbouring plant species that are integral to the ecological balance, cultural heritage, and traditional practices of the region. This monograph series is dedicated to exploring and documenting the indigenous plants that thrive along this sacred river, from the high-altitude regions of Uttarakhand to the fertile plains of West Bengal. These plants are not merely biological entities; they are part of the cultural and spiritual fabric of the communities that have lived along the Ganga for generations. They are used in traditional medicine, rituals, and daily life, and they play a crucial role in the local economies.

The journey from Uttarakhand to West Bengal covers a vast array of ecosystems, from the alpine forests of the Himalayas to the mangroves of the Sundarbans. At each of the 26 sites we studied, the selected plant species reflect the unique environmental conditions and cultural practices of the region. This series of monographs is a culmination of an extensive research initiative aimed at understanding and preserving the botanical wealth of the Ganga basin. A total of 26 key sites along the river, spanning the length from its source in Uttarakhand to its mouth in the Bay of Bengal, were explored, analysed, and documented. At each of these sites, the indigenous plant species were studied which are adapted to the unique environmental conditions present. The exploration focuses on ten plant species that are not only emblematic of the region but also hold significant ecological, medicinal, and cultural value.

Each monograph in this series provides a comprehensive overview of the plant species, detailing its botanical characteristics, ecological role, traditional uses, and cultural significance. The data for distribution of these plants along the Ganga, highlighting the environmental factors that influence their growth and survival is primarily focussed. To achieve a comprehensive understanding, the selected sites were explored for their botanical diversity, phytochemical properties of the plants, and soil properties examination of each area. Additionally, *in silico* analysis was conducted to assess the plants' potential in combating several diseases. The study also investigated the ethnomedicinal and pharmacological uses and applications of these plants, complemented by a bibliometric analysis to evaluate existing research and knowledge. This exploration also presents as a fresh insight to classical literature of Ayurveda in the form of "Shlokas" and therefore extending beyond the identification of individual species; it adds a fresh perspective to the existing literature and delves into uncovering the intricate web of life that sustains these plants and the human communities that rely on them.

Patanjali Group recognized the critical need to explore the diverse botanical landscape of the Ganga River basin and to conduct scientific, evidence-based research to highlight its ecological and medicinal significance. With this vision, Patanjali Organic Research Institute embarked on an initiative to systematically document and analyse the indigenous plant species found across this region. The goal is to develop scientifically validated insights into the botanical diversity, phytochemical properties, and ethnomedicinal uses of these plants, thereby creating a comprehensive understanding of the current status and challenges associated with preserving this rich natural heritage.

We express our deepest gratitude to Param Pujya Swami Ramdev Ji for his unwavering leadership and support throughout this endeavour, as well as for his invaluable guidance in every aspect of our work. My best wishes to the dedicated and highly skilled experts, who have meticulously gathered data and conducted extensive research on the diverse botanical heritage of the Ganga River basin, utilizing cutting-edge scientific techniques and methodologies. Nature holds immense potential for sustainable practices and this project is a significant contribution to the preservation of these indigenous plant species, ultimately benefiting both ecological balance and the well-being of the communities that rely on them.

आचार्य बालकृष्ण

Dr. Acharya Balkrishna



# LIST OF CONTRIBUTORS

## OVERALL GUIDANCE AND MENTORSHIP

Dr. Acharya Balkrishna

### PRINCIPAL INVESTIGATOR

Dr. Vedpriya Arya

### CO-PRINCIPAL INVESTIGATOR

Mr. Pawan Kumar

### EDITING AND PROOF READING

Dr. Vedpriya Arya	Scientist-E
Mr. Koshal Kishor Chaturvadi	Senior Editor
Dr. Priyanka Chaudhary	Scientist-C
Er. Manisha Thapliyal	Scientist-B
Dr. Divya Joshi	Scientist-B

### FIELD SURVEY & PLANT IDENTIFICATION

Dr. Arun Kumar Kushwaha	Scientist -C
Dr. Ishwar Prakash Sharma	Scientist -C

### WRITING & SCIENTIFIC CONTRIBUTION

Dr. Pradeep Nain	DGM, Operations & Service Delivery
Dr. Rajesh Kumar Mishra	Scientist-E
Dr. Arun Kumar Kushwaha	Scientist-C
Dr. Ishwar Prakash Sharma	Scientist-C
Dr. Anurag Dabas	Scientist-C
Dr. Priyanka Chaudhary	Scientist-C
Dr. Jyotish Srivastava	HOD, Chemical Science
Dr. Divya Joshi	Scientist-B
Dr. Sumit Kumar Singh	Scientist-B
Er. Manisha Thapliyal	Scientist-B
Ms. Shalini Singh	Scientist-B
Ms. Shalini Mishra	Scientist-B
Dr. Yoganshi Sharma	Scientist-B
Ms. Himani Bhatt	Assistant Scientist
Ms. Maneesha Rana	Assistant Scientist
Mr. Mukul Kumar	Assistant Scientist
Mr. Soniya For	Assistant Scientist

### DESIGNING

Pramod Kumar Kulshreshtha	Graphic Designer
Indu Pratap Singh	Graphic Designer
Sunil Kumar	Graphic Designer
Ira Abel	Graphic Designer

### OTHER CONTRIBUTION

Mr. Pradeep Badola  
Mr. Shivam Walia  
Mr. Ashu



# Classical Ayurvedic Insight



## *Terminalia arjuna* (Roxb. ex DC.) Wight & Arn

**Vedic name** : (Vīrakaḥ arjunah) (वीरकः अर्जुनः)

**Botanical name** : *Terminalia arjuna* (Roxb. ex DC.) Wight & Arn

### Utpatti sthānam

Mūlata prāyakadvīpasambandhibhārate tathā.

Śrīlaṅkāyāṃ prajāyeta tvarjunanāmapādapa.

Brahmābhīdhānadeśe ca bhāratasya viśeṣata.

Bhūyiṣṭhabhāgajāte'pi tathā himavata khalu.

Upakaṇṭhasthabhāgeṣu choṭānāgapure'pi ca.

Madhyabhāratabhūmiṣu karṇāṭhe cotkale'pi vai.

Tamile marahaṭṭe ca sarvathā samprajāyate. (Saumitreya Mahodadhi:1-3)

### Distribution

It is native to peninsular India and Sri Lanka. In the world it is found in Myanmar, throughout the greater part of India it is found in the sub-Himalayan tract, Chota Nagpur, central India, central provinces, Odisha, Karnataka, parts of the Maharashtra, Tamilnadu. <sup>[1-12]</sup>

### Vānaspatika vivaraṇam

#### 1. Kāyika lakṣaṇam

Daśavyāmasamucchrāyāt ṣoḍaśavyāmalambita.

Vistāraśīrṣasampṛkta sarvadā haritastathā.

Bṛhadrūpo bhavedvṛkṣa kāṇḍastambhastu tatra vai.

Kṣaitijadiśi vistāriklāntinatābhireva ca.

Śākhābhīṣca samāyuktastathaiva bahudhā khalu.

Praṇālīsadrśo bhāti kāṇḍakatvattu sarvathā.

Bhūriṣṭhūlavapuṣkā ca dhūsaravarṇato'thavā.

Pāṭalaharitamchāyāsacivaścikkaṇastathā.

Śalkenāpi samāyukta pratibhāti tata param.

Navapraroḥa ābhāti cikkaṇaścā'thavā kila.

Pītābhababhrukacchāyaromarājivirājita.

Patrantu saralaṃ bhāti prāyeṇa viparītata.

Dīrghavṛttaparivyāptabhāllākārasamanvitam.

Athavā dīrghavṛttābhadīrghāyatavapurdharam.

Carmalaṃ cordhvaṃprṣṭhantu svalpaharitarṇakam.  
Prāyo'roma tathā cā'dhvaṃprṣṭhakam paṇḍuracchavi.  
Babhruvarṇamataṃ caivaṃ pañcāṅgulavilambitāt  
Dvimuṣṭilambitaṃ kvāpi sapādāṅgulakāyatāt.  
Pādonatryaṅgulāyāmaṃ śīrṣe tīkṣṇāgrato'thavā.  
Kuṇṭhāgraṃ ca tathā'dhāre golakākārato'thavā.  
Phaṅkārāṃ tathopānta svalpatakuṇṭhadantaka.  
Kraśācābha punarmukhyaśīrājātaṃ tu tatra vai.  
Daśayugmaśārīrācca pañcadaśasu yugmata.  
Jālarūpaśīrāpṛttaṃ parṇavṛntantu tatra ca.  
Sārdhadviyavakocchrāyādardhāṅgulavilambitam.  
Sādhāraṇatayā śīrṣasamīpe granthinā yutam.  
Granthidvayasamāyuktaṃ kvacid bhāti tathaiva ca. (Saumitrya Mahodadhi: 4-17)

## Botanical Description

### 1. Vegetative Characters

A large evergreen tree 18-25 m tall with a spreading crown. Stem huge, buttressed trunk with horizontally spreading, drooping branches, often fluted. Bark is very thick, grey or pinkish-green, smooth, flaking off in large, pieces. Young twigs smooth or with yellowish-brown hairs. Leaves simple, subopposite, elliptic-lanceolate or elliptic-oblong, coriaceous, pale dull green above, nearly glabrous and pale brown beneath, 10-15 cm long and 2.5-5.5 cm wide, shortly acute or obtusely acuminate at the apex, rounded or cuneate at the base, margins slightly crenate-serrate, main nerves 10-15 pairs, reticulately veined. Petiole is very short 3-10 mm long, usually with 1-2 prominent glands near the apex. <sup>[1-12]</sup>

### 2. Puṣpīya lakṣaṇam

Prasūnalakṣaṇe puṣpaṃ dalena rahitantathā.  
Vṛntahīnañca pītābhaśvetavarṇatayoditam.  
Hrasvarūpe ca kākṣīye kaṇīse cā'thavā khalu.  
Pañcāṅgulocchritāt sārdhaṣaḍaṅgulavilambini.  
Antasthagucchake bhāti sahapatraṃ tathaiva ca.  
Tatsahapatrikā bhūyohrasvākārasamanvitā.  
Rekhitatvānusambaddhabhallākārasamanvitā.  
Prasūnāddhrasvarūpaiva tathā'śūpātīnī matā.  
Bāhīkadalapuñjantu śūnyaroma mukhaṃ puna.  
Ghaṇṭākārantathā dantāḥ pratibhānti trikoṇata.  
Pālaya pañca ca hrasvāḥ prāntasparśitayoditāḥ.

Trikoṇāścāpi vidyante daśapuṃskesarāḥ puna.  
 Bāhīkadaluṅṅjasya praṇālyāṃ vai viśeṣata.  
 Baddhāḥ pañktidvaye nūnamāḥṇāḥ sañcakāṣati.  
 Aṇḍāśayastvadhovartī tathā'ḷparomaśo mata.  
 Bimbastu bhāṣate pīta āho raktābharomabhi.  
 Ācchādita phalaṃ tāvadaṣṭhilarūpata sthitam.  
 Aṇḍākārānusambaddhadīrghāyatakalevaram.  
 Sārdhāṅgulasamucchrāyāt tryaṅgulalambitantathā.  
 Yavonāṅgulakāyāmāt sārdhāṅgulāyataṃ puna.  
 Tantumayañca kāṣṭhīyaṃ prāyeṇā'romaśaṃ kila.  
 Tatra pañcatayantāvat kaṭhoram niśitāgrakam.  
 Prāya samānarūpañca tiryagūrdhvasthabhāgata.  
 Vakrapakṣayutaṃ bhāti sāndrababhrukavarṇakam.  
 Kṛṣṇavarṇam kilā''rohiśīrājātena saṃyutam.  
 Vaiśākhajyeṣṭhayoḥ puṣpaṃ mādhavaiśākhayoḥ phalam. (Saumitreyā Mahodadhī: 18-30)

## 2. Floral Characters

Flowers sessile, apetalous, yellowish white, borne in short spikes or in terminal panicles 9-13 cm long. Bract 2 mm long. Bracteoles very small, linear-lanceolate, shorter than the flowers, caducous. Calyx glabrous, mouth campanulate, teeth triangular. Lobes 5, short, valvate, triangular. Stamens 10, inserted on calyx tube in 2 series. Ovary inferior, quite glabrous. Disk clothes with yellowish or reddish hair. Fruits drupe, ovoid-oblong, 3-6 cm long and 1.8-2.8 cm wide, fibrous, woody, nearly glabrous, with 5-hard acute, subequal, oblique wing curved upwards, dark brown and blackish when dry with 5 ascending veins. Flowering April-June. Fruiting February-April. <sup>[1-12]</sup>

## Plant Anatomy

### Prayojyāṅgam

Vīrakasyārjunasyātra kāṇḍatvacā bhiṣagvaraiḥ.  
 Prayojyāṅgaṃ matañcāntaḥsamracanopavarṇyate. (Saumitreyā Mahodadhī:1)

### Antaḥsamracaneḥṣaṇe

#### (a) Kāṇḍatvak

Tvakprotakaprabhāgo'tra daśakalpastaṛīyakaḥ.  
 Sparśarekhīyalambābhiḥ kośikābhiḥ samanvitaḥ.

Bāhyastaravraje babhruvarṇapadārthasaṃhatiḥ.  
 Prapūritābhidṛśyātra tathā tvakprotakaidhikā.  
 Dvitīyakākhyikā majjakośikā na parisphuṭā.  
 Madhyāṃśaraśmisandoho bāhyatvaci pravistṛtaḥ.  
 Poṣavāhe dvitīyākhye tatakṣetreṣu nālikāḥ.  
 Cālanīsaṃjjñakā dṛśyāstatsahakośikāvrajaḥ.  
 Mṛdulāḥ poṣavāhāśca poṣavāhīyatantavaḥ.  
 Sandṛśyante tathā pāragāminaḥ poṣavāhagāḥ.  
 Raśmaya ekapaṃktisthā yadā kadā dvidhā punaḥ.  
 Tantavaḥpoṣavāhāṇāṃ daśakalpasamūhagāḥ.  
 Cūrṇātukṣāarakasyātra mṛdulapoṣavāhake.  
 Kaṇā dṛśyāstathaikatṛībhūtāśca tantusaṃcayāḥ.  
 Vicitrāḥ kośikā bhānti subṛhatkośikāśritāḥ.  
 Caturbhujīyavaiṣame samāntareṇa saṃsthitāḥ.  
 Cūrṇātukṣāarakasyātra trijmakaṇāḥ samūhagāḥ.  
 Bhānti maṇḍakaṇā bhūri saralā iha vīkṣaṇe.  
 Raśmayaḥ poṣavāhīyāḥ dṛśyanta ekapaṃktikāḥ.  
 Daśakalpata uccāśca kośikāḥ paṃktigāḥ punaḥ.  
 Dvādaśakalpataḥ proccāstathānudairghyachittitaḥ.  
 Cūrṇātukṣāarakasyātra kaṇagucchāśca tantuṣu.  
 Mṛdulapoṣavāhāṇāṃ sandṛśyante yathāyatham.  
 Tvacāyā arjunīyāyā madhyasaṃracanoditā. (Saumitreya Mahodadhi: 2-13)

## Plant Parts

Stem bark

## Microscopic Characters

### Stem bark

Cork consists of 9-10 layers of tangentially elongated cells. Few outer layers are filled with brown colouring matter. Cork cambium and secondary cortex is not distinct and medullary rays observed traversing almost up to outer bark. Secondary phloem occupies a wide zone consisting of sieve tubes, companion cells, phloem parenchyma and phloem fibres. It traversed by phloem rays, usually uniseriate but biseriate rays also occasionally seen. Phloem fibres distributed in rows and present in groups of 2-10. Crystals of calcium oxalate are present in most of the phloem parenchyma, alternating with fibres. Idioblasts consists large cells having aggregates of prismatic and rhomboidal crystals of calcium oxalate. Starch grains are mostly simple. Uniseriate phloem rays are 2-10 cells high and biseriate, 4-12 cells high.

In longitudinal section, rosette crystals of calcium oxalate were found in the form of strands in phloem parenchyma. <sup>[1],[13-16]</sup>

## (b) Cūrṇam

Cūrṇakaṃ babhruṣoṇābhamaṃ tvakprotakīyakośagāḥ.

Kaṇāścaikalapaṃktisthā raśmayaḥ poṣavāhagāḥ.

Cūrṇātukṣāarakasyātra saralaḥ kaṇasaṃcayaḥ.

Maṇḍakaṇāśca saṃyuktā dṛśyante varṇakā iha. (Saumitreya Mahodadhi: 14-15)

## Powder

Reddish-brown colour. Powder shows fragments of cork cells, uniseriate phloem rays. Crystals of calcium oxalate, starch grains are simple or compound. <sup>[1],[13-16]</sup>

## Medicinal Uses

### Thoracic disorders

Cūrṇaṃkākubhamiṣṭaṃvāsakarasaḥvāvitambahūnvārān.

Madhughṛtasitopalādilehyaṃkṣayakāsaraktaharam. (Bhā.Pra.Ci. 12: 29)

Kṣayajakāsa- Intake of *Terminalia arjuna* (Arjuna myrobalan) bark powder in a dose of about 3gm triturated with *Adhatoda vasica* (Malabar nut) juice mixed with honey, crystal sugar and *Ghrita* (Clarified butter) quickly relieves *Kṣayajakāsa*. <sup>[17] (Bh.Pr.Ci.12:29)</sup>

### Cardiac disorders

Arjunasyatvacasiddhaṃkṣīraṃyojyaṃhṛdāmāye.

Sitayāpaṃcamūlyāvābalayāmadhukēnavā. (Vṛ.Mā. 31: 10) (Ga.Ni.Kā.Ci 26: 16)

Cardiac diseases- Intake of *Terminalia arjuna* (Arjuna myrobalan) bark, crystal sugar or *Laghupañcamūla* or *Sida cordifolia* (Country mallow) or *Glycyrrhiza glabra* (Liquorice root) processed with milk is beneficial in treating cardiac diseases. <sup>[18] (Vr.Mā.31:10), [19] (Gd.Ni.Ky.Ci. 26:16)</sup>

Ghṛtenadugdhenaguḍāmbhasāvāpibanticūrṇaṃkakubhatvaco ye.

Hṛdrogajīrṇajvarapittaraktaṃhatvābhavyeṣciraḥjīvinaste.

(Vṛ.Mā. 31: 11) (Ga.Ni.Kā.Ci. 26: 17)

Intake of 3gm *Terminalia arjuna* (Arjuna myrobalan) bark powder along with *Ghrita* (Clarified butter), milk or jaggery mixed water is useful in treating cardiac diseases, chronic fever, *Raktapitta*. <sup>[18] (Vr.Mā.31:11), [19] (Gd.Ni.Ky.Ci. 26:17)</sup>

Godhūmakakubhacūrṇaṃchāgapayogavyasarpīṣāpakvam.

Madhuśarkarāsametaṃsamayatihṛdrogamuddhatapaṃpūṣām.

(Ga.Ni.Kā.Ci 26: 22) (Vṛ.Mā.31: 18)

Intake of equal quantity of *Terminalia arjuna* (Arjuna myrobalan) bark powder and *Triticum sativum* (Wheat) powder cooked in goat milk mixed with cow *Ghrita* (Clarified butter), honey and sugar is useful in treating acute cardiac diseases. <sup>[18]</sup> (Vr.Mā.31:18) , <sup>[19]</sup> (Gd.Ni.Ky.Ci. 26:22)

cūrṇaṃdugdhenapāyayet.

Hṛdrogakāsaśvāsaghnaṃkakubhasya ca valkalam.

Rasāyanamparambalyamvātajinmāsayojitam.

Samvatsaraprayogenaḥjivedvarṣaśatamdhruvam.

(Ca.Da.Ci. 31: 15-16) (Vr.Mā.31: 19-20) (Ga.Ni.Kā.Ci. 26: 23-24)

Intake of 3-5gm *Terminalia arjuna* (Arjuna myrobalan) bark powder along with milk for one month is useful in treating cardiac diseases, cough, asthma and *Vāta* diseases. It also promotes rejuvenating properties and acts as a tonic which results in longevity. <sup>[20]</sup> (Ch.Dt.Ci.31:15-16), <sup>[18]</sup> (Vr.Mā.31:19-20), <sup>[19]</sup> (Gd.Ni.Ky.Ci. 26:23-24)

Pārthasyakalkasyarasesanāsiddhamśastamghṛtaṃsarvahr̥dāmayeṣu.

Tathāgnimāndyekaṣatajapravṛttarakṛtārśasāṃcāpivadantipathyam. (Vr.Mā.31: 33)

Intake of *Arjuna Ghrita* (Clarified butter) processed with *Terminalia arjuna* (Arjuna myrobalan) paste and decoction is useful in treating cardiac diseases, dyspepsia, injury with intrinsic hemorrhage and bleeding piles. <sup>[18]</sup> (Vr.Mā.31:33)

Tailājyaguḍavipakvaṃcūrṇaṃgodhūmapārthajamvāpi.

Pibatipayo'nusabhavatijitasakalahṛdāmayaḥpuruṣaḥ. (Ga.Ni.Kā.Ci. 26:21)

Intake of equal quantity of *Triticum sativum* (Wheat) and *Terminalia arjuna* (Arjuna myrobalan) bark powder along with oil, *Ghrita* (Clarified butter), jaggery and milk is very useful in treating cardiac diseases. <sup>[19]</sup> (Gd.Ni.Ky.Ci. 26:21)

## Abdominal disorders

Śallakībadarījambupriyālāmr̥junatvacāḥ.

Pītāḥkṣīreṇamadhvāḍhyāḥpṛthakśoṇitavāraṇāḥ. (Vr.Mā. 3: 68)

Bloody diarrhea- Intake of the paste or powder prepared from *Boswellia serrata* (Indian olibanum), *Zizyphus jujuba* (Jujube), *Syzygium cumini* (Malabar plum), *Buchanania cochinchinensis* (Calumpang nut tree), *Mangifera indica* (Mango) and *Terminalia arjuna* (Arjuna myrobalan) bark alone or together or along with honey mixed milk is useful in treating bloody diarrhea. <sup>[18]</sup> (Vr.Mā.3:68)

Keśarājorjunakṣāramprātaḥpītañcamastunā.

Nihantisāmamatyarthamacirādgrahaṇīrujam. (Vaṃ.Se.Saṃgrahaṇī 189)

Sprue- Intake of *Kṣāra* prepared from *Eclipta prostrata* (Eclipta) and *Terminalia arjuna* (Arjuna myrobalan) along with whey is useful in treating *Āma* associated and chronic pain in sprue. <sup>[21]</sup> (Vg.Sn.Sangr:189)

Sauvarcalāḍhyāṃmadirāṃmūtreṭvabhīhatepibet.

.....kaṣāyamkakubhasya ca.(Vr.Mā. 28: 18)

Mūtrāvarodhajanite.....kaṣāyamkakubhasya ca. (Bhā.Pra.Ci. 31: 24)

*Udāvarta*- Intake of *Terminalia arjuna* (Arjuna myrobalan) decoction is useful in the treatment of *Udāvarta* (upward movement of *Vāta*) caused due to anuria. <sup>[18]</sup> (Vr.Mā.28:18), <sup>[17]</sup> (Bh.Pr.Ci.21:24)

## Ano-rectal disorders

Pariṣecanevidadhyādvṛṣakakubhayavāsanimbāśca. (Ca.Ci. 14: 214)

Hemorrhoids- Sprinkling done with the bark decoction of *Terminalia arjuna* (Arjuna myrobalan) over the hemorrhoids is very much beneficial in treating hemorrhoids. <sup>[22]</sup> (Ca.Ci.14:214)

## Renal and urinary bladder disorders

Kaṣāyaṃkakubhasyavā. (A.Hr.Ci. 11: 37)

Anuria- Intake of *Terminalia arjuna* (Arjuna myrobalan) bark decoction is useful in treating anuria. <sup>[23]</sup> (As.Hr.Ci. 11:37)

Dhavārjunacaṃdanaśālachallīkvāthohitaḥsyāccajalaprimehe. ....

pūyamehehitaḥkvāthodhavārjunasya.

Kadambaśālārjunadīpyakānāmviḍaṅgadārvīghavaśallakīnām.

Sarvetathaivamadhunākaṣāyāḥkaphaprimeheṣuniṣevanīyāḥ. (Hā.Saṃ. 3.28: 6-8)

Urinary diseases- Intake of the decoction prepared from *Anogeissus latifolia* (Axle wood), *Terminalia arjuna* (Arjuna myrobalan), *Santalum album* (Indian sandalwood) and *Shorea robusta* (Sal tree) bark is useful in treating *Udakameha*, decoction of *Anogeissus latifolia* (Axle wood) and *Terminalia arjuna* (Arjuna myrobalan) is useful in treating gonorrhoea and decoction of *Anthocephalus cadamba* (Wild cinchona), *Shorea robusta* (Sal tree), *Terminalia arjuna* (Arjuna myrobalan), *Apium graveolens* (Celery), *Embelia ribes* (False black pepper), *Anogeissus latifolia* (Axle wood) and *Boswellia serrata* (Indian olibanum) is useful in treating *Kapha* associated urinary diseases. <sup>[24]</sup> (Hr.Sm. 3.28:6-8)

## Reproductive disorders

Śīrīśakakubhakvāthapicūnyonauvinikṣipet.

Upadravāścaye'nyesyustānyathāsvamupācaret. (A.Hr.Śā. 2: 44)

Obstructed labour- Withholding of the vagina with a gauze piece soaked in the decoction prepared from *Albizia lebbek* (Indian siris) and *Terminalia arjuna* (Arjuna myrobalan) bark after the expulsion of the obstructed foetus and placenta is beneficial in alleviating the pain and associated complications. <sup>[23]</sup> (Aṣ. Hr. Śā. 2.44)

Śuktramehinam.....kukubhacandanakaṣāyamvā. (Su.Ci. 11: 8)

Spermatorrhea- Intake of the decoction prepared from *Terminalia arjuna* (Arjuna myrobalan) bark and *Santalum album* (Sandal wood) is useful in treating spermatorrhea. <sup>[25]</sup> (Su.Ci.11:8)

## Musculoskeletal disorders

Bhagnaḥpibettvakpayasā'rjunasyagodhūmacūrṇaṃsaghṛtenavā'tha. (Vr.Mā. 46: 16)

Fracture- Intake of *Terminalia arjuna* (Arjuna myrobalan) bark *Kṣīrapāka* along with *Ghrita* (Clarified butter) and *Triticum sativum* (Wheat) powder is useful in healing of fracture. <sup>[18]</sup> (Vr.Mā.46:16)

Cūrṇaṃpureṇasaṃyojyaghṛtenārjunalākṣayoḥ.

Bhagnaḥsandhānamāyātīlīdhaṃkṣīraghṛtāsīnā. (Bhā.Pra.Ci. 48 : 29)

Intake of equal quantity of *Terminalia arjuna* (Arjuna myrobalan) bark and *Lākṣā* powder along with *Commiphora wightii* (Gugal) and *Ghrita* (Clarified butter) followed with the diet of *Ghrita* (Clarified butter) and milk facilitates quick healing. <sup>[17]</sup> (Bh.Pr.Ci.1:754)

Saghṛtenāsthisaṃhāraṃlākṣāṃgodhūmamārjunam.

Samdhimukte'sthibhagnecapibetkṣīreṇamānaḥ. (Vṛ.Mā. 46: 13) (Bhā.Pra.Ci 48: 27)

Intake of the paste or powder prepared from equal quantity of *Cissus quadrangularis* (Treebine), *Lākṣā*, *Triticum sativum* (Wheat) and *Terminalia arjuna* (Arjuna myrobalan) along with *Ghrita* (Clarified butter) and milk is useful in the treatment of bone fracture and dislocated bone. <sup>[17]</sup> (Bh.Pr.Ci.11:44), <sup>[18]</sup> (Vṛ.Mā. 46: 13)

## Dermatological disorders

Khadirāvaghātakakubha..... Śasyantesnānapāneṣu. (Ca.Ci. 7: 129)

Leprosy- Bathing with and drinking of *Terminalia arjuna* (Arjuna myrobalan) bark decoction is beneficial in treating leprosy. <sup>[22]</sup> (Ca.Ci.7:129)

Vyaṅgeṣucārjunatvāgvāmañjiṣṭhāvāsamākṣikā. (A.Hṛ.U. 32: 16)

Facial melanosis- External application of *Terminalia arjuna* (Arjuna myrobalan) bark paste is beneficial in treating facial melanosis. <sup>[23]</sup> (As.Hr.Ut.32:16)

Kadambārjuna.....Vraṇapracchādanevidvānpatrāṇyarkasyacādiṣet.  
(Ca.Ci. 25: 95)

To cover the wound- *Terminalia arjuna* (Arjuna myrobalan) leaves are used to cover and bandage the wound. <sup>[22]</sup> (Ca.Ci. 25:95)

## Generalised body disorders

Dhanañjayodumbara.....

Pṛthakpṛthakcaṃdanayojitānitenavakalpenahitānitatra.

Nīsthītāvāsvarasīkṛtāvākalkīkṛtāvāmṛditāḥṣṛtāvā.

Etesamastāgaṇaśaḥpṛthagvāraktaṃsapittaṃśamayantiyogāḥ. (Ca.Ci. 4: 75-77)

Pibecchītakaṣāyaṃvājambvāmrārjunasambhavam. (Su.U. 45 : 23)

*Raktapitta*- Intake of *Terminalia arjuna* (Arjuna myrobalan) bark powder mixed with equal quantity of *Santalum album* (Indian sandalwood) along with the adjuvant of uncooked rice water or cold infusion, decoction, paste or juice is useful in treating *Raktapitta*. <sup>[22]</sup> (Ca.Ci.4:75-77), <sup>[25]</sup> (Su.Ut.45:23)

Kakubhatvañnāgabālāvānaribijaṃvicūrṇitaṃpayasā.

Pītaṃmadhughṛtayuktaṃsasitaṃyakṣmādikāsaharam. (Bha.Prā.Ci. 11: 44)

Tuberculosis- Intake of the powder of *Terminalia arjuna* (Arjuna myrobalan) bark, *Grewia hirsuta* (Veronicalolia) and *Mucuna pruriens* (Velvet bean) seed along with honey, *Ghrita* (Clarified butter) and crystal sugar mixed with milk is useful in alleviating cough and other diseases. <sup>[17]</sup> (Bh.Pr.Ci.11:44)

## Parts Used

Stem-bark.

## Dose

Powder 3-6gm, juice 10-20ml, decoction 50-100ml or as directed by the physician.

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## Abbreviation

<b>A.Hṛ.Ci.</b>	:	AṣṭāṅgaHṛdayaCikitsā
<b>A.Hṛ.U.</b>	:	AṣṭāṅgaHṛdaya Uttara Tantra
<b>As.Hr.Ut.</b>	:	AṣṭāṅgaHṛdaya Uttara Tantra
<b>Bhā.Pra.Ci.</b>	:	BhāvaprakśaCikitsā
<b>Śā.Sa.Ut.Kha.</b>	:	ŚāraṅgadharaSamhitā Uttara Khaṇḍa
<b>Ca.Ci.</b>	:	Caraka SamhitāCikitsāSthāna
<b>Ca.Da.Ci.</b>	:	CakradattaCikitsā
<b>Ca.Si.</b>	:	Caraka Samhitā Siddhi Sthāna
<b>Ca.Sū.</b>	:	Caraka SamhitāSūtraSthāna
<b>Ck.Dt.Ci.</b>	:	CakradattaCikitsā
<b>Ga.Ni.Kā.Ci.</b>	:	GadanigrahaKāyacikitsā
<b>Ga.Ni.Kau.Taṃ.</b>	:	GadanigrahaKaumaryatantra
<b>Ga.Ni.Śā.Ta.</b>	:	GadanigrahaŚālākya Tantra
<b>Ga.Ni.Śa.Taṃ.</b>	:	GadanigrahaŚalyatantra
<b>Hā.Sa.</b>	:	HārāitaSamhitā
<b>Hā.Saṃ.</b>	:	HārāitaSamhitā
<b>Kā.Saṃ.Khilasthāna</b>	:	KāśayapaSamhitāKhilasthāna
<b>Rā.Mā.</b>	:	RājaMārtaṇḍa
<b>Ra.Ra.Sa.</b>	:	Rasa Ratna Samuccaya
<b>Śā.Ni.Guḍūcyādivarga</b>	:	ŚāligrāmaNighaṇṭuGuḍūcyādivarga
<b>Śā.Sa.U.Kha.</b>	:	ŚāraṅgadharaSamhitā Uttara Khaṇḍa
<b>Śā.Saṃ.Ma.Kha.</b>	:	ŚāraṅgadharaSamhitā Madhyam Khaṇḍa
<b>Sd.Bh.Mm. Ch. Gr.Ci.</b>	:	Siddha BheṣajaMaṇimālāCaturthaGucchaGrahaṇāiCikitsā
<b>Sd.Bh.Mm. Ch. Nd.Vr.Ci.</b>	:	Siddha BheṣajaMaṇimālāCaturthaGuccha Nāḍāivraṇa Cikitsā
<b>Sd.Bh.Mm. Ch.Nt.Rg.</b>	:	Siddha BheṣajaMaṇimālāCaturthaGuccha Netra RogaCikitsā
<b>Sd.Bh.Mm.Ch. Ars.Ci.</b>	:	Siddha BheṣajaMaṇimālāCaturthaGucchaArśaCikitsā
<b>Sd.Bh.Mm.Ch. Vrn.Ci.</b>	:	Siddha BheṣajaMaṇimālāCaturthaGucchaVraṇaCikitsā
<b>Sh.Gr.Ng. Gd.Vg.</b>	:	ŚāligrāmaNighaṇṭuGuḍūcyādivarga
<b>Su.Ci.</b>	:	SuśrutaSamhitāCikitsāSthāna

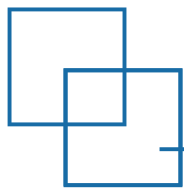
<b>Su.Ka.</b>	:	SuśrutaSamhitā Kalpa Sthāna
<b>Su.Śā.</b>	:	SuśrutaSamhitāSārīraSthāna
<b>Su.Ut.</b>	:	SuśrutaSamhitā Uttara Tantra
<b>Vai.Jī. Caturthavilāsa</b>	:	Vaidya JāivanamCaturthavilāsa
<b>Vai.Jī. Dvitīyavilāsaḥ</b>	:	Vaidya JāivanamDvitīyavilāsaḥ
<b>Vai.Ma.</b>	:	Vaidya Manoramā
<b>Vaṃ. Se. Āmavātarogādhikāraḥ</b>	:	VaṃgasenaSamhitāĀmavātarogādhikāraḥ
<b>Vaṃ.Se. Vātavyādhidhikāraḥ</b>	:	VaṃgasenaSamhitāVātavyādhidhikāraḥ
<b>Vaṃ.Se.Karṇarogaḥ</b>	:	VaṃgasenaSamhitāKarṇarogādhikāraḥ
<b>Vaṃ.Se.Medarogaḥ</b>	:	VaṃgasenaSamhitāMedorogādhikāraḥ
<b>Vaṃ.Se. Netrarog</b>	:	VaṃgasenaSamhitāNetrarogādhikāra
<b>Vaṃ.Se. Śothādhikāraḥ</b>	:	VaṃgasenaSamhitāŚothādhikāraḥ
<b>Vaṃ.Se. Strīrogaḥ</b>	:	VaṃgasenaSamhitāStrīrogaḥ
<b>Vaṃ.Se.Vātavyādhiḥ</b>	:	VaṃgasenaSamhitāVātavyādhidhikāraḥ
<b>Vṛ.Mā.</b>	:	Vṛndamādhava

CHAPTER

01

# Plant Exploration and Botanical Study





## INTRODUCTION

Water is the key resource required to sustain life on this planet. It is found in most of the earth as in the ocean, rivers, ponds, streams, ice, etc. The river Ganga is an important source of water in India that makes an important river system from the Himalaya to the Bay of Bengal. The Indian civilization and economy depended on this river system due to its water availability throughout the year (Paul and Sinha, 2013). The river Ganga alone accounts for 25% of India's total water resources (Paul, 2017). Globally more than 300 million people from India, Nepal, and Bangladesh depend on the river Ganga (Gopal, 2000). This is the thirtieth longest river in the world and covers a basin area of 861,404 km<sup>2</sup> (Rahaman, 2009a). This basin is the most populated area in the world with an average density of 520 persons/km<sup>2</sup> (Das and Tamminga, 2012). The basin is very rich in heritage, cultural, and religious values. India's about one-fourth area drains by the river Ganga.

The river originates from the Gangotri glacier at Gomukh (30° 36' N; 79° 40' E; 3800 m) in the Uttar Kashi district of Uttarakhand under the name of Bhagirathi; and another tributary *i.e.*, Alaknanda which is originated from the Bhagirath-Kharak (30° 49' N; 79° 17' E) and Satopanth (30° 45' N; 79° 21' E) glaciers. Both the tributaries join at Devprayag from where the name Ganga started. The river has a total length of 2600 km from its main source Gomukh.

Up to Haridwar it flows in the hills after here it enters the plain area. From here, it flows southwards, passing through the plains of Uttar Pradesh starting from Bijnor district. Up to Ballia, it continuously flows in Uttar Pradesh through different districts after here it enters Bihar near Chausa of Buxar district. It flows up to Katihar district in Bihar and from here it enters the Sahebganj district of Jharkhand. Then it turns southwards and enters the West Bengal from the Murshidabad district. About 40 km from the Farakka, it divides into two streams; the left stream flows eastwards toward Bangladesh while the right stream, known as Bhagirathi, continues to flow south through West Bengal. Its name changed to Hooghly when it flows in the west and south-west of Kolkata. The Hooghly at Diamond Harbor flows southward and is split into two streams before reaching the Bay of Bengal (Rahaman, 2009b).



The annual water discharge from the river Ganga is about 18700 m<sup>3</sup>/s which is the fifth highest in the globe (Paul, 2017). The maximum flow of the Ganga exists within the catchment area with a mean maximum flow is  $468.7 \times 10^9$  m<sup>3</sup> which is a total of 25.2 % of the total water resources of India (Sarkar et al., 2012). The main source of the river Ganga is the melting of snow in the Himalayas and monsoon rains. The water of the Ganga is regularly used by the living being for drinking. Outdoor bathing has a spiritual significance for Hindus in which millions of people take a holy dip at least once a year throughout the course of the river, from Gangotri to Ganga Sagar.

The Ganga basin supports biodiversity, species richness, and uniqueness, attracting people from different parts of the earth. Plant communities have been a major magnetism for investigation since time immemorial. Vegetation composition and assemblages of an area form a significant habitat that contributes to the structure and function of such ecosystems. The vegetation pattern along the Ganga varies according to the seasonal changes, flood level, and species composition differs by the function of water supply and different soil types, which has a sharp influence on plant species distribution. Macrophytes such as submerged, emergent, and free-floating aquatic plants are known to accumulate and bioconcentrate heavy metals producing an internal concentration several folds greater than their surroundings (Chen et al., 2008; Allen-Diaz et al., 2008). The river basin has large numbers of medicinal plant species. The natives depend on the river for water and medicinal importance to fulfill their requirements from the beginning.

Among all the medicinal plants, *Terminalia arjuna* was considered in this study. It is commonly known as "Arjuna". In Sanskrit, "Kakubha", "Pārtha", "Indrādru", "Dhavala", "Devasāla"; in Hindi "Anjan", "Anjani", "Arjun". It is a tree that can grow up to the height of 18-24 m. The Yunani and Ayurvedic medicinal systems consider this tree to be important, and it is commonly used to make Ayurvedic formulations such as Kashaya, Ksheerpaka, Arjunaishtam, Cintamanirasam, and Laksagugula (Choudhary and Arya, 2022). Most people use all the parts of this plant for therapeutic purposes. This plant is used to cure obesity, it is anti-dysentric, cardiogenic, lithotriptic, antipyretic, astringent, treats cirrhosis of the liver, and relieve symptomatic hypertension (Hoq, 2018). On this basis, the current chapter is focused on the morphology, taxonomy, anatomy, and distribution assessment of *T. arjuna* around the Ganga River.

## Genus *Terminalia* L.

*Terminalia* is a genus of 279 accepted species that are widely distributed in tropical areas of Africa, Asia, America, and subtropical areas of Australia and the Pacific Islands. The genus belongs to the family Combretaceae (Waly et al., 2020). Around 250 species of *Terminalia* are found in the tropical region of the world and approximately 12 species are native to India (Choudhary and Arya, 2022) and 54 species are distributed in western, eastern, and southern Africa (Lebrun and Stork, 1991; Smith, et al., 2004). The two most important species found in west and central Africa are *T. ivorensis* and *T. superba* (Norgrove and Hauser, 2002a). However, they have also established in plantations both inside and outside of their natural habitat, for example, in South and Central America, East Africa, Hawaii, Fiji, and the Solomon Islands (Jones, 1969). The most common species in eastern and southern Africa are *T. prunioides*, *T. brachystemma*, *T. sericea*, *T. gazensis*, *T. mollis*, and *T. sambesiaca* (Coates-Palgrave, 1988; Masoko et al., 2005).

In many countries in tropical areas, *Terminalia* trees are planted as a source of high-quality solid wood for fine woodwork, joinery, construction, flooring, and plywood production (Schmidt et al., 2002; Smith et al., 2004). *Terminalia* spp. is also often planted in mixed crop systems to form a "taungya" agri-sylvicultural system in which they give shade and contribute significantly to soil fertility (Nichols et al., 2001; Norgrove and Hauser 2002b). Members of the *Terminalia* genus are also among the plants that are most

often used for medicinal purposes (Masoko et al., 2005; Kamtchouing et al., 2006). Relatively little research has been done on the fungal diseases that infect *Terminalia* spp., despite their significance. Die-back, leaf spot, and canker have all been linked to *Terminalia* spp. (Lamb and Ntima, 1971; Ofosu Siedu and Cannon, 1976; Hodges and Ferreira, 1981).

### Etymology

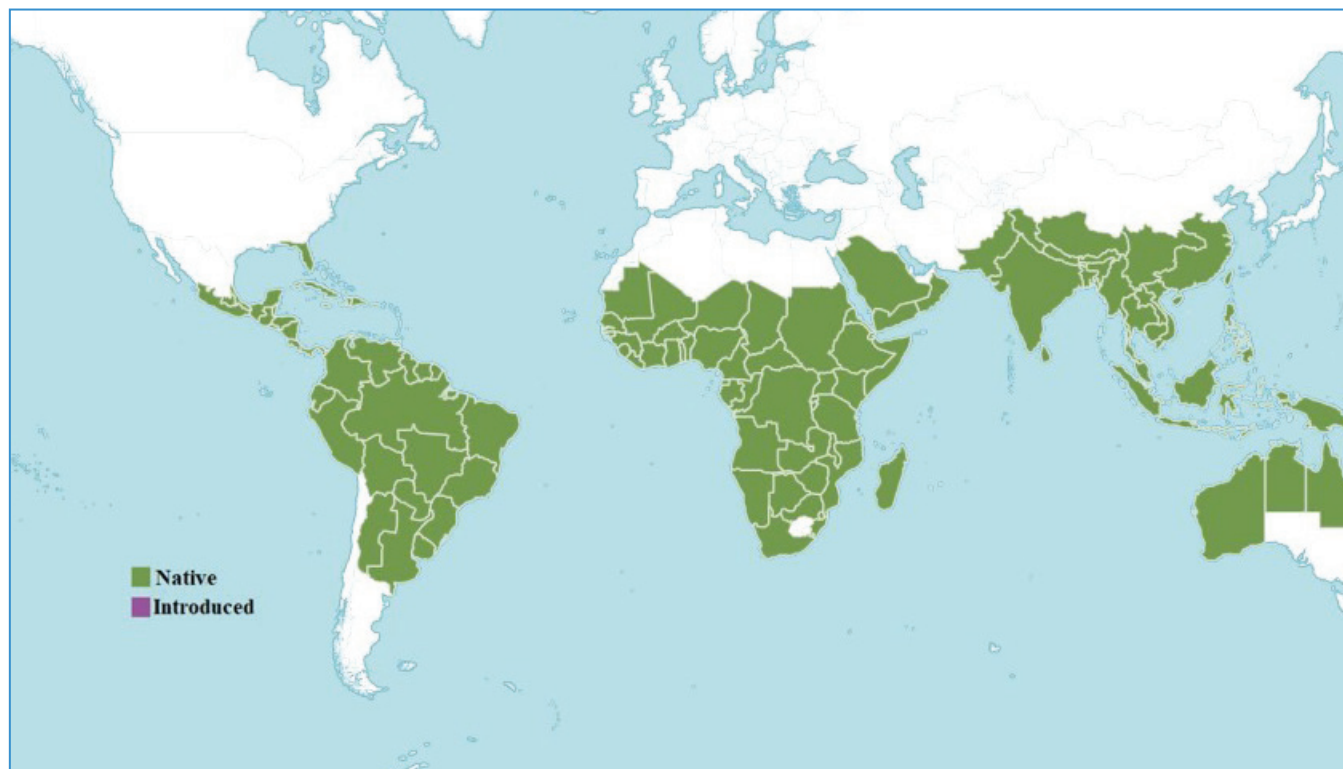
The genus *Terminalia* name is derived from the Latin word *terminus* which means 'end', referring to the fact that the leaves are located at the very tips of the shoots; branchlets, and branches. *Terminus* was also the name of the Roman God of boundaries and frontiers, and *Terminalia* was the festival celebrated (23rd February) in honor of God *Terminus* at the end of the Roman year (Gledhill, 2008; Cock, 2015).

### Habitat and Distribution

The genus *Terminalia* is a tree distributed throughout the tropical and subtropical regions of the world; abundantly found in Africa and Asia. Currently, according to the Plants of the World Online database (POWO, 2024), it is native to Aldabra, Andaman Is., Angola, Argentina Northeast, Argentina Northwest, Assam, Bahamas, Bangladesh, Belize, Benin, Bismarck Archipelago, Bolivia, Borneo, Botswana, Brazil North, Brazil Northeast, Brazil South, Brazil Southeast, Brazil West-Central, Burkina, Burundi, Cabinda, Cambodia, Cameroon, Cape

Provinces, Caprivi Strip, Caroline Is., Central African Repu, Chad, China South-Central, China Southeast, Christmas Is., Cocos (Keeling) Is., Colombia, Comoros, Congo, Cook Is., Costa Rica, Cuba, Djibouti, Dominican Republic, East Himalaya, Ecuador, El Salvador, Equatorial Guinea, Eritrea, Ethiopia, Fiji, Florida, French Guiana, Gabon, Gambia, Ghana, Gilbert Is., Guatemala, Guinea, Guinea-Bissau, Guyana, Hainan, Haiti, Honduras, India, Ivory Coast, Jamaica, Jawa, Kazan-retto, Kenya, KwaZulu-Natal, Laos, Leeward Is., Lesser Sunda Is., Liberia, Madagascar, Malawi, Malaya, Maldives, Mali, Maluku, Marianas, Marquesas, Marshall Is., Mauritania, Mauritius, Mexico Central, Mexico Gulf, Mexico Southeast, Mexico Southwest, Mozambique, Myanmar, Namibia, Nanseishoto, Nauru, Nepal, New Caledonia, New Guinea, Nicaragua, Nicobar Is., Niger, Nigeria, Niue, Northern Provinces, Northern Territory,

Ogasawara-shoto, Oman, Pakistan, Panamá, Paraguay, Peru, Philippines, Phoenix Is., Puerto Rico, Queensland, Rodrigues, Rwanda, Réunion, Samoa, Saudi Arabia, Senegal, Sierra Leone, Society Is., Solomon Is., Somalia, South China Sea, Southwest Caribbean, Sri Lanka, Sudan, Sulawesi, Sumatera, Suriname, Swaziland, Taiwan, Tanzania, Thailand, Tibet, Togo, Tokelau-Manihiki, Tonga, Trinidad-Tobago, Tuamotu, Tubuai Is., Turks-Caicos Is., Tuvalu, Uganda, Uruguay, Vanuatu, Venezuela, Venezuelan Antilles, Vietnam, Wallis-Futuna Is., West Himalaya, Western Australia, Windward Is., Yemen, Zambia, Zaire, Zimbabwe and introduced into Cape Verde, Cayman Is., Central American Pac, Chagos Archipelago, Gulf of Guinea Is., Hawaii, Laccadive Is., Line Is., Mozambique Channel I, Netherlands Antilles, Pitcairn Is., Seychelles, Wake Is. (Fig. 1)



**Fig. 1** Global distribution of genus *Terminalia*

## Botanical Characteristics

Tree or rarely shrub. Stem branched, branches often in tiers. Leaves alternate, spiral, or rarely opposite; lamina oblong, elliptic, obovate, or orbicular, hairy, or glabrous, often minutely verruculose. Inflorescence axillary or terminal spikes or racemes, sometimes panicles, more rarely in terminal or terminal and axillary panicles. Flowers bisexual. Calyx tube proximally broadly cylindrical to ellipsoid or ovoid, distally cupular or sometimes scarcely developed; lobes 4 or 5, deltoid or ovate, triangular. Corolla absent. Stamen 8-10; anther dorsifixed, versatile. Ovary inferior; style simple, free, exserted. Fruit drupelike, 2-5-winged, or -ridged.

## Ethnomedicinal Importance

The *Terminalia* genus has been recorded as having cultural values, medicinal values, and social significance. Plant parts such as roots, bark, leaves, and fruit extract may be extracted to provide a variety of dye hues that are employed in several industrial processes and products (Waly et al., 2020). *Terminalia* members are widely utilized in traditional medicine on different continents throughout the world to cure a variety of diseases such as abdominal disorders, bacterial infections, backache, colds, sore throats, conjunctivitis, diarrhea, dysentery, fever, gastric ulcers, headache, heart diseases, hookworm, hypertension, jaundice, leprosy, nosebleed, edema, pneumonia, and skin disease (Eloff et al., 2008; Fahmy et al., 2015). The fruits

of *T. bellerica* and *T. chebula* are essential components of 'triphala', a well-known Ayurvedic remedy that has several uses in Indian traditional medicine. In Tibet, *T. chebula* is referred to as the "King of medicine" because its fruits have a remarkable capacity for healing and are used to cure a wide range of illnesses (Fahmy et al., 2015).

Traditional uses of *T. arjuna* include the treatment of bile infections, ulcers, is cardiotonic, poison antidote, hepatoprotective and cardioprotective (Zhang et al., 2019; Das et al., 2020). *T. arjuna* bark, which has a significant number of antioxidants such as glycosides, flavonoids, tannins, and inorganic minerals, is used to cure and relieve angina (Cock, 2015). The *T. argentea* leaves are traditionally used to cure digestion and respiratory-related diseases. *T. bellirica* fruits are used to treat diarrhea, cough, scorpion-sting, etc. and have antibacterial and antioxidant qualities. *T. catappa* leaves have antipyretic, hemostatic, and can treat hepatitis, and liver-related illness. Because of its ability to lower oxidative stress, inflammation, angiogenesis, lipid profile correction, and direct hypoglycemic activities, leaves are also used to treat diabetes. Traditionally, *T. sericea* has been used to treat infections, diabetes mellitus, hypertension, and stomach problems (Das, et al., 2020).

*T. spinosa* is used to treat abdominal disorders, pain, bilharzia, cancer, cough and cold, dysentery, diarrhea, fever, venereal diseases, heart disorders, hypertension, jaundice, diabetes, and antiseptics. *T.*

*paniculata* is used in the treatment of cholera, inflammation, menstrual disorders, cough, bronchitis, cardiac disorders, diabetes, wounds, and skin diseases (Cock, 2015). The fruits of *T. citrina* are used in the treatment of chronic fever and appetizer, act as a sexual stimulant, asthma, constipation, boils, migraine, dental disorders, dizziness, hemorrhoids, anemia, eye diseases, infections, wound cuts, cardiac disorders, hepatomegaly and urolithiasis, inflammation, pain, diarrhea and other digestive disorders and helminthic contaminations. Seeds cure stomachache and intestinal diseases such as colitis, possess antioxidant properties and the bark act as diuretic and cardio tonic (Das et al., 2015; Akhtar et al., 2016; Beigi et al., 2018).

The genus *Terminalia* is widely used in various traditional medicines such as traditional Chinese medicine, Tibetan medicine, and Indian Ayurvedic medicine practices (Zhang et al., 2019). The native Asian plants *T. arjuna* and *T. chebula* are particularly well documented due to their numerous applications in Ayurvedic medicine. A few species of *Terminalia* have been reported for their high antioxidant contents in the African and Asian countries. Asian species are *T. chebula*, *T. bellerica*, *T. paniculata*, *T. arjuna*, *T. catappa*, and African species are *T. prunioides*, *T. brachystemma*, *T. gazensis*, *T. mollis* and *T. sambesiaca*. In Ayurveda *Terminalia* species is used for many medicinal purposes, including abdominal and back pain, cough and cold, conjunctivitis, diarrhea and dysentery, fever, headache, heart disorders, inflammation, leprosy, pneumonia, sexually transmitted

diseases, worms, wounds, hemorrhages, ulcers, and as a general tonic. Of the ayurvedic plants *T. arjuna*, *T. bellerica*, *T. chebula* and *T. catappa* are arguably the most useful, being utilized for multiple ailments (Cock, 2015).

## Chemical Constituents

Many phytochemicals have been studied in *Terminalia* species, and these substances are classified as active ingredients in numerous groups. These include flavonoids, other phenolic compounds, pentacyclic triterpenes and their glycoside derivatives, and tannins. A literature survey has recorded that *Terminalia* is a rich source of tannins and pseudotannins, including gallic acid and its simple gallate esters, chebulic and non-chebulic ellagitannins, ellagic acid derivatives and ellagic acid glycosides, phenolic acids, flavonoid, triterpenes, and triterpenoid glycosides are also present in high amounts in various *Terminalia* species, few lignan and lignan derivatives have been isolated from genus *Terminalia* (Fahmy et al., 2015; Zhang et al., 2019). The important phytochemicals are-

**Triterpenes-** 2 $\alpha$ ,3 $\beta$ ,19 $\alpha$ -trihydroxyolean-12-en-20-oic acid 3-O- $\beta$ -D-galactosyl-(1 $\rightarrow$ 3)- $\beta$ -D-glucoside, 2 $\alpha$ ,3 $\beta$ ,19 $\alpha$ -trihydroxyolean-12-en-28-oic acid methylester 3 $\beta$ -orutinoside, 2 $\alpha$ ,3 $\beta$ ,19 $\beta$ ,23-tetrahydroxyolean-12-en-28-oic acid 3 $\beta$ -O- $\beta$ -D-galactosyl-(1 $\rightarrow$ 3)- $\beta$ -D-glucoside-28-O- $\beta$ -D-glucoside, 3-acetylmassic acid, arjunic acid, arjunoside I, oleanolic acid, ursolic acid, massilic acid, 2 $\alpha$ ,3 $\alpha$ ,24-trihydroxyolean-11,13(18)-dien-28-oic acid, terminoside A,

arjungenin, arjunglucoside I, crataegioside, 23-O-galloylarjunic acid, quercotriterpenoside I, sericic acid, 24-deoxy-sericoside, arjunglucoside I, quercotriterpenoside I, crataegioside, 23-O-galloylarjunolic acid, ursolic acid, betulinic acid, terminalin A,  $\beta$ -caryophyllene,  $\alpha$ -humulene, punicalin, 1- $\alpha$ -O-galloylpunicalagin, 1,3-di-O-galloyl-2,4-chebuloyl- $\beta$ -d-glucose, 1( $\alpha$ )-O-galloylpedunculagin, procyanidin B-1, dimethyl neochebulinate.

**Tannins-** 1,2,3,6-tetra-O-galloyl- $\beta$ -d-glucose, gallotannin (1,2,3,4,6 penta galloyl glucose), 1,3,4,6-tetra-O-galloyl- $\beta$ -d-glucose, sanguin H-4, corilagin, tercatanin, 1,3-di-O-galloyl- $\beta$ -d-glucose, tellimagrandin I, punicalin,  $\alpha/\beta$ -punicalagin, 1- $\alpha$ -O-galloylpunicalagin, dimethyl neochebulaglate, dimethyl 4'-epi-neochebulaglate, chebulinic acid, 2-O-galloylpunicalin, 1-desgalloyleugeniin, acutissimin A, eugenigrandin A, neochebulinic acid, 4,6-O-isoterchebuloyl-d-glucose, 1'-O-methyl neochebulanin, 3'-O-galloyl procyanidin B-2.

**Flavonoids-** Arjunone, 8-Methyl-5,7,2',4'-tetramethoxy-favanone, naringin, hesperitin, arjunolone (6,4-dihydroxy-7-methoxy favone), bicalein (5,6,7-trihydroxy favone), isovitexin, apigenin-6-C-(2'-O-galloyl)- $\beta$ -d-glucoside, amentofavone, catechin-epigallocatechin, gallocatechin, quercetin, rutin, kaempferol.

**Phenols and glycosides-** Ellagic acid, methyl ellagic acid, 3-O-methylellagic acid, 3,4,3'-tri-O-methylfavellagic acid, flavogallonic acid, vanillic acid 4-O- $\beta$ -d-(6'-O-galloyl) glucoside, brevifolincarboxylic acid, phyllembin (ethyl

gallate isomers1 progallin A), 5-O-galloyl(-)-shikimic acid, 6'-O-methyl chebulate, chebulic acid trimethyl ester, p-coumaric acid, ferulic acid.

**Steroids-**  $\beta$ -sitosterol,  $\beta$ -sitosterol-3-acetate,  $\beta$ -sitosteryl palmitate, stigmasterol 3-O- $\beta$ -d-glucoside, stigmasterol, stigma-4-ene-3-one

**Polyol-** 2-hexanol, octanol, methoxycarbonyloxymethyl methylcarbonate, ribonolactone, apionic acid, ascorbic acid, gluconolactone, glucohepatonic acid-1,4-lactone, galacturonic acid

**Esters-** Geranyl formate, citronellyl acetate, geranyl acetate, geranyl tiglate, laxiforin, (1S,5R)-4-oxo-6,8-dioxabicyclo [3.2.1] oct-2-ene-2-carboxylic acid.

**Others-** Glucuronic acid, eujavonic acid, 5-(4-hydroxy-2,5-dimethylphenoxy)-2,2-dimethylpentanoic acid, p-hydroxytiaprofenic acid, arjunaphthanoloxide, resveratrol- $\beta$ -d-glucoside, resveratrol-3- $\beta$ -rutinoside glycoside, terminalianone, termicalcicolanone A, benzoyl- $\beta$ -d-(4' $\rightarrow$ 10'geranilanoxy)-pyranoside.

## Species

Genus *Terminalia* is known to contain a total of 281 taxonomically accepted species (POWO, 2024) that are as-

1. *T. actinophylla* Mart in Flora 24(2 Beibl.): 22 (1841)
2. *T. acuminata* (Allemão) Eichler in C.F.P.von Martius & auct. suc. (eds.), Fl. Bras. 14(2): 92 (1867)

3. *T. adamantium* Cambess in A.F.C.P.de Saint-Hilaire & al., Fl. Bras. Merid. 2: 241 (1830)
4. *T. adenopoda* Miq in Fl. Ned. Ind., Eerste Bijv.: 327 (1861)
5. *T. albida* Scott Elliot in J. Linn. Soc., Bot. 30: 79 (1894)
6. *T. amazonia* (J.F.Gmel.) Exell in A.A. Pulle, Fl. Suriname 3: 173 (1935)
7. *T. anisoptera* (Welw. ex M.A. Lawson) Gere & Boatwr in Bot. J. Linn. Soc. 184: 319 (2017)
8. *T. ankaranensis* Capuron in Bull. Mus. Natl. Hist. Nat., Sér. 3, Bot. 11: 104 (1973)
9. *T. anogeissiana* Gere & Boatwr in Bot. J. Linn. Soc. 184: 319 (2017)
10. *T. apetala* (Vollesen) Gere & Boatwr in Bot. J. Linn. Soc. 184: 319 (2017)
11. *T. arbuscula* Sw in Prodr. Veg. Ind. Occ.: 68 (1788)
12. *T. archboldiana* Exell in Brittonia 2: 137 (1936)
13. *T. archipelagi* Coode in Kew Bull. 23: 299 (1969)
14. *T. arenicola* Byrnes in Contr. Queensland Herb. 20: 35 (1977)
15. *T. argentea* Mart in Nov. Gen. Sp. Pl. Bras. 1: 43 (1824)
16. *T. aridicola* Domin in Biblioth. Bot. 22(89): 446 (1928)
17. *T. arjuna* (Roxb. ex DC.) Wight & Arn in Prodr. Fl. Ind. Orient. 1: 314 (1834)
18. *T. aroidoi* Bisse in Feddes Repert. 85: 608 (1974)
19. *T. arostrata* Ewart & O.B.Davies in Fl. N. Territory: 212 (1917)
20. *T. aubletii* Gere & Boatwr in Bot. J. Linn. Soc. 184: 319 (2017)
21. *T. australis* Cambess in A.F.C.P.de Saint-Hilaire & al., Fl. Bras. Merid. 2: 240 (1830)
22. *T. avicapitis* Coode in Contr. Herb. Austral. 2: 3 (1973)
23. *T. avicennioides* Guill. & Perr in Fl. Seneg. Tent.: 277 (1832)
24. *T. barbosae* (Exell) Gere & Boatwr in Bot. J. Linn. Soc. 184: 319 (2017)
25. *T. basilei* Chiov in Nuovo Giorn. Bot. Ital., n.s., 36: 364 (1929)
26. *T. beccarii* Exell in Blumea 7: 325 (1953)
27. *T. belini* Capuron in Bull. Mus. Natl. Hist. Nat., Sér. 3, Bot. 11: 111 (1973)
28. *T. bellirica* (Gaertn.) Roxb in Pl. Coromandel 2: t. 198 (1805)
29. *T. bentii* (Baker) Gere & Boatwr in Bot. J. Linn. Soc. 184: 319 (2017)
30. *T. bentzoe* (L.) L.f in Suppl. Pl.: 434 (1782)
31. *T. bialata* (Roxb.) Steud in Nomencl. Bot., ed. 2, 2: 668 (1841)
32. *T. bipileura* Borhidi & O.Muñiz in Acta Bot. Acad. Sci. Hung. 26: 262 (1980 publ. 1981)
33. *T. boivinii* Tul in Ann. Sci. Nat., Bot., sér. 4, 6: 95 (1856)
34. *T. brachystemma* Welw. ex Hiern in Cat. Afr. Pl. 1: 340 (1898)
35. *T. brassii* Exell in J. Bot. 73: 134 (1935)
36. *T. brevipes* Pamp in Bull. Soc. Bot. Ital.

- 1915: 16 (1915)
37. *T. brownii* Fresen in Mus. Senckenberg. 2: 152 (1837)
38. *T. buceras* (L.) C.Wright in Anales Acad. Ci. Méd. Habana 5: 409 (1869)
39. *T. bucidoides* Standl. & L.O. Williams in Ceiba 3: 214 (1953)
40. *T. bursarina* F.Muell in Fragm. 2: 149 (1861)
41. *T. calamansanai* (Blanco) Rolfe in J. Linn. Soc., Bot. 21: 310 (1884)
42. *T. calcicola* H.Perrier in Ann. Mus. Colon. Marseille, sér. 7, 1(1): 24 (1953)
43. *T. calogemma* Coode in Contr. Herb. Austral. 2: 7 (1973)
44. *T. calophylla* Tul in Ann. Sci. Nat., Bot., sér. 4, 6: 102 (1856)
45. *T. cambodiana* Gagnep in Notul. Syst. (Paris) 3: 284 (1916)
46. *T. camuxa* Pickel in Arq. Bot. Estado São Paulo 3: 199 (1958)
47. *T. canaliculata* Exell in Blumea 7: 327 (1953)
48. *T. canescens* (DC.) Radlk in T.A. Durand, Index Gen. Phan.: 500 (1888)
49. *T. capitanea* A.C.Sm in Brittonia 23: 401 (1971)
50. *T. capitulata* Exell in Blumea 7: 322 (1953)
51. *T. carinata* Sabatier & J.Engel in Adansonia, sér. 3, 42: 262 (2020)
52. *T. carolinensis* Kaneh in Bot. Mag. (Tokyo) 46: 672 (1932)
53. *T. catappa* L in Mant. Pl.: 128 (1767)
54. *T. celebica* Exell in Blumea 7: 325 (1953)
55. *T. cephalota* McPherson in Bull. Mus. Natl. Hist. Nat., B, Adansonia 13: 21 (1991)
56. *T. chebula* Retz in Observ. Bot. 5: 31 (1788)
57. *T. cherrieri* MacKee in Bull. Mus. Natl. Hist. Nat., B, Adansonia 6: 116 (1984)
58. *T. citrina* (Gaertn.) Roxb in Asiat. Res. 11: 183 (1810)
59. *T. clemensae* Exell in Blumea 7: 324 (1953)
60. *T. complanata* K.Schum in K.M.Schumann & U.M.Hollrung, Fl. Kais. Wilh. Land: 83 (1889)
61. *T. congesta* (Ducke) Gere & Boatwr in Bot. J. Linn. Soc. 184: 319 (2017)
62. *T. coronata* (Stapf) Gere & Boatwr in Bot. J. Linn. Soc. 184: 319 (2017)
63. *T. corrugata* (Ducke) Gere & Boatwr in Bot. J. Linn. Soc. 184: 320 (2017)
64. *T. corticosa* Pierre ex Craib & Hutch in Bull. Misc. Inform. Kew 1909: 358 (1909)
65. *T. costaricensis* (Stace) Gere & Boatwr in Bot. J. Linn. Soc. 184: 320 (2017)
66. *T. crassipes* Kaneh. & Hatus in Bot. Mag. (Tokyo) 53: 156 (1939)
67. *T. creaghii* Ridl in Bull. Misc. Inform. Kew 1933: 493 (1933)
68. *T. crebrifolia* A.C.Sm in Brittonia 23: 402 (1971)
69. *T. crenata* Tul in Ann. Sci. Nat., Bot., sér. 4, 6: 91 (1856)
70. *T. crispialata* (Ducke) Alwan & Stace in Ann. Missouri Bot. Gard. 76: 1126 (1989)

71. *T. cunninghamii* C.A.Gardner in Bull. Woods Forests Dept. Western Australis 32: 73 (1923)
72. *T. cyanocarpa* Capuron in Bull. Mus. Natl. Hist. Nat., Sér. 3, Bot. 11: 126 (1973)
73. *T. darfeuillana* Pierre ex Laness in Pl. Util. Col. Franç.: 314 (1886), nom. subnud.
74. *T. darlingii* Merr in Philipp. J. Sci., C 5: 202 (1910)
75. *T. densiflora* Craib in Bull. Misc. Inform. Kew 1930: 162 (1930)
76. *T. dhofarica* (A.J.Scott) Gere & Boatwr in Bot. J. Linn. Soc. 184: 320 (2017)
77. *T. dichotoma* G.Mey in Prim. Fl. Esseq.: 177 (1818)
78. *T. diptera* (Sagra) Greuter & R.Rankin in Espermat. Cuba Invent. Prelim.: XII (2016)
79. *T. disjuncta* H.Perrier in Ann. Mus. Colon. Marseille, sér. 7, 1(1): 31 (1953)
80. *T. divaricata* H.Perrier in Ann. Mus. Colon. Marseille, sér. 7, 1(1): 35 (1953)
81. *T. diversipilosa* H.Perrier in Ann. Mus. Colon. Marseille, sér. 7, 1(1): 25 (1953)
82. *T. domingensis* Urb in Symb. Antill. 7: 524 (1913)
83. *T. duckei* Gere & Boatwr in Bot. J. Linn. Soc. 184: 320 (2017)
84. *T. eddowesii* Coode in Contr. Herb. Austral. 2: 13 (1973)
85. *T. eichleriana* Alwan & Stace in Ann. Missouri Bot. Gard. 76: 1127 (1989)
86. *T. elliptica* Willd in Sp. Pl., ed. 4. 4: 969 (1806)
87. *T. engleri* Gere & Boatwr in Bot. J. Linn. Soc. 184: 320 (2017)
88. *T. erici-rosenii* R.E.Fr in Wiss. Erg. Schwed. Rhodesia-Kongo-Exp. 1911-1912, 1: 173 (1914)
89. *T. eriostachya* A.Rich in R.de la Sagra, Hist. Phys. Cuba, Pl. Vasc.: 524 (1846)
90. *T. erythrocarpa* F.Muell in Fragm. 2: 150 (1861)
91. *T. exelliana* Capuron in Bull. Mus. Natl. Hist. Nat., Sér. 3, Bot. 11: 130 (1973)
92. *T. exsculpta* Tul in Ann. Sci. Nat., Bot., sér. 4, 6: 93 (1856)
93. *T. fagifolia* Mart in Nov. Gen. Sp. Pl. Bras. 1: 42 (1824)
94. *T. fanshawei* (Exell & Maguire) Gere & Boatwr in Bot. J. Linn. Soc. 184: 320 (2017)
95. *T. fatraea* (Poir.) DC in Prodr. 3: 12 (1828)
96. *T. ferdinandiana* Exell in J. Bot. 73: 263 (1935)
97. *T. fitzgeraldii* C.A.Gardner in W. Austral. Forests Dept. Bull., Bot. Notes 32: 73 (1923)
98. *T. flavicans* Tul in Ann. Sci. Nat., Bot., sér. 4, 6: 99 (1856)
99. *T. foetidissima* Griff in Not. Pl. Asiat. 4: 685 (1854)
100. *T. franchetii* Gagnep in Notul. Syst. (Paris) 3: 287 (1916)
101. *T. gatopensis* Guillaumin in Bull. Mus. Natl. Hist. Nat., sér. 2, 14: 455 (1943)
102. *T. gazensis* Baker f. in J. Linn. Soc., Bot. 40: 69 (1911)

103. *T. glabrata* G.Forst in Fl. Ins. Austr.: 74 (1786)
104. *T. glabrescens* Mart in Flora 20(2 Beibl.): 124 (1837)
105. *T. glaucifolia* Craib in Bull. Misc. Inform. Kew 1928: 68 (1928)
106. *T. gossweileri* Exell & J.G.García in Bol. Soc. Brot., sér. 2, 36: 95 (1962)
107. *T. gracilipes* Capuron in Bull. Mus. Natl. Hist. Nat., Sér. 3, Bot. 11: 137 (1973)
108. *T. gracilis* Tul in Ann. Sci. Nat., Bot., sér. 4, 6: 96 (1856)
109. *T. grandiflora* Benth in Fl. Austral. 2: 503 (1864)
110. *T. grandis* (Ducke) Gere & Boatwr in Bot. J. Linn. Soc. 184: 320 (2017)
111. *T. griffithsiana* Liben in Bull. Jard. Bot. État Bruxelles 35: 181 (1965)
112. *T. guaiquinimae* Maguire & Exell in Mem. New York Bot. Gard. 10(1): 93 (1958)
113. *T. guyanensis* Eichler in C.F.P.von Martius & auct. suc. (eds.), Fl. Bras. 14(2): 88 (1867)
114. *T. habeensis* (Aubrév. ex Keay) Gere & Boatwr in Bot. J. Linn. Soc. 184: 320 (2017)
115. *T. hadleyana* W.Fitzg in J. Proc. Roy. Soc. Western Australia 3: 183 (1918)
116. *T. harmandii* Gagnep in Notul. Syst. (Paris) 3: 285 (1916)
117. *T. hoehneana* (N.F.Mattos) Gere & Boatwr in Bot. J. Linn. Soc. 184: 320 (2017)
118. *T. hylobates* Eichler in Vidensk. Meddel. Naturhist. Foren. Kjøbenhavn 1870: 195 (1870)
119. *T. hylodendron* (Mildbr.) Gere & Boatwr in Bot. J. Linn. Soc. 184: 320 (2017)
120. *T. hypargyrea* K.Schum. & Lauterb in Fl. Schutzgeb. Südsee: 467 (1900)
121. *T. impediens* Coode in Kew Bull. 23: 308 (1969)
122. *T. ivorensis* A.Chev in Vég. Ut. Afr. Trop. Franç. 5: 152 (1909)
123. *T. januarensis* DC in Prodr. 3: 11 (1828)
124. *T. kaernbachii* Warb in Bot. Jahrb. Syst. 18: 201 (1893)
125. *T. kaiseriana* F.Hoffm in Beitr. Fl. Centr.-Ost-Afr.: 26 (1889)
126. *T. kajewskii* Exell in J. Bot. 73: 133 (1935)
127. *T. kangeanensis* Slooten in Bull. Jard. Bot. Buitenzorg, sér. 3, 6: 35 (1924)
128. *T. katikii* Coode in Contr. Herb. Austral. 2: 17 (1973)
129. *T. kilimandscharica* Engl in Pflanzenw. Ost-Afrikas, C: 294 (1895)
130. *T. kjellbergii* Exell in Blumea 7: 322 (1953)
131. *T. kleinii* (Exell) Gere & Boatwr in Bot. J. Linn. Soc. 184: 320 (2017)
132. *T. kuhlmannii* Alwan & Stace in Ann. Missouri Bot. Gard. 76: 1126 (1989)
133. *T. kumpaja* R.L.Barrett in Austral. Syst. Bot. 28: 36 (2015)
134. *T. laeteviridis* Gilg & Ledermann ex Engl in H.G.A.Engler & O.Drude, Veg. Erde 9(III 2): 720 (1921)
135. *T. latifolia* Sw in Prodr. Veg. Ind. Occ.: 68 (1788)
136. *T. latipes* Benth in Fl. Austral. 2: 501 (1864)

137. *T. laxiflora* Engl in Monogr. Afrik. Pflanzen-Fam. 4: 12 (1900)
138. *T. leandriana* H.Perrier in Ann. Mus. Colon. Marseille, sér. 7, 1(1): 28 (1953)
139. *T. leiocarpa* (DC.) Baill in Hist. Pl. 6: 265 (1876)
140. *T. litoralis* Seem in Fl. Vit.: 94 (1866)
141. *T. longespicata* Slooten in Bijdr. Combret. Flacourt. Ned. -Ind.: 19 (1919)
142. *T. lucida* Hoffmanns. ex Mart in Nov. Gen. Sp. Pl. Bras. 1: 43 (1824)
143. *T. lundquistii* Exell in Blumea 7: 326 (1953)
144. *T. luteola* A.C.Sm in J. Arnold Arbor. 33: 100 (1952)
145. *T. macadamii* Exell in Blumea 7: 324 (1953)
146. *T. macrantha* Rojo in Blumea 17: 93 (1969)
147. *T. macrophylla* (Spruce ex Eichler) Gere & Boatwr in Bot. J. Linn. Soc. 184: 320 (2017)
148. *T. macroptera* Guill. & Perr in Fl. Seneg. Tent.: 276 (1832)
149. *T. macrostachya* (Standl.) Alwan & Stace in Fl. Neotrop. Monogr. 107: 250 (2010)
150. *T. maestrensis* Bisse in Feddes Repert. 85: 606 (1974)
151. *T. mameluco* Pickel in Arq. Bot. Estado São Paulo 3: 200 (1958)
152. *T. mantaliopsis* Capuron in Bull. Mus. Natl. Hist. Nat., Sér. 3, Bot. 11: 110 (1973)
153. *T. mantaly* H.Perrier in Ann. Mus. Colon. Marseille, sér. 7, 1(2): 24 (1953)
154. *T. megalocarpa* Exell in J. Bot. 73: 132 (1935)
155. *T. megalophylla* (Van Heurck & Müll.Arg.) Gere & Boatwr in Bot. J. Linn. Soc. 184: 320 (2017)
156. *T. melanocarpa* F.Muell in Fragm. 3: 92 (1862)
157. *T. menezesii* Mendes & Exell in Garcia de Orta, Sér. Bot. 1: 22 (1973)
158. *T. microcarpa* Decne in Nouv. Ann. Mus. Hist. Nat. 3: 457 (1834)
159. *T. modesta* Tul in Ann. Sci. Nat., Bot., sér. 4, 6: 102 (1856)
160. *T. molii* Exell in Blumea 7: 324 (1953)
161. *T. molinetii* M.Gómez in Bol. Secr. Agric. Comerc. Trab., Cuba 22: 76 (1914)
162. *T. mollis* M.A.Lawson in D.Oliver & auct. suc. (eds.), Fl. Trop. Afr. 2: 417 (1871)
163. *T. morobensis* Coode in Contr. Herb. Austral. 2: 23 (1973)
164. *T. muelleri* Benth in Fl. Austral. 2: 500 (1864)
165. *T. myanmarensis* W.J.Kress & DeFilipps in Contr. U.S. Natl. Herb. 45: 483 (2003)
166. *T. myriocarpa* Van Heurck & Müll.Arg in Observ. Bot. Descript. Pl. Nov. Herb. Van Heurckiani 2: 215 (1871)
167. *T. myrtifolia* (M.A.Lawson) Gere & Boatwr in Bot. J. Linn. Soc. 184: 320 (2017)
168. *T. narnorokensis* H.Perrier in Ann. Mus. Colon. Marseille, sér. 7, 1(1): 31 (1953)
169. *T. neglecta* Bisse in Feddes Repert. 85: 607 (1974)
170. *T. neotaliala* Capuron in Bull. Mus. Natl.

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171. *T. nildae* R.T.M.Ribeiro, Loiola & M.F.Sales in Syst. Bot. 45: 268 (2020)
172. *T. nipensis* Alain in Contr. Ocas. Mus. Hist. Nat. Colegio "De La Salle" 12: 8 (1953)
173. *T. nitens* C.Presl in Abh. Königl. Böhm. Ges. Wiss., ser. 5, 6: 214 (1851)
174. *T. nitidissima* Rich in Actes Soc. Hist. Nat. Paris 1: 109 (1797)
175. *T. novocaledonica* Däniker in Vierteljahrsschr. Naturf. Ges. Zürich 79(Beibl. 19): 289 (1933)
176. *T. oblonga* (Ruiz & Pav.) Steud in Nomencl. Bot., ed. 2, 2: 668 (1841)
177. *T. oblongata* F.Muell in Fragm. 2: 152 (1861)
178. *T. ochroprumna* (Eichler) Gere & Boatwr in Bot. J. Linn. Soc. 184: 321 (2017)
179. *T. oliveri* Brandis in Hooker's Icon. Pl. 23: t. 2202 (1892)
180. *T. ombrophila* H.Perrier in Ann. Mus. Colon. Marseille, sér. 7, 1(1): 23 (1953)
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182. *T. oreadum* Diels in Bot. Jahrb. Syst. 57: 429 (1922)
183. *T. orientensis* Monach in Caribbean Forester 8: 79 (1947)
184. *T. oxycarpa* Mart in Flora 24(2 Beibl.): 22 (1841)
185. *T. oxyphylla* Miq in Fl. Ned. Ind., Eerste Bijv.: 326 (1861)
186. *T. pachystyla* Borhidi in Acta Bot. Acad. Sci. Hung. 21: 224 (1975 publ. 1976)
187. *T. pallida* Brandis in Indian Trees: 308 (1906)
188. *T. pallidovirens* (Cuatrec.) Gere & Boatwr in Bot. J. Linn. Soc. 184: 321 (2017)
189. *T. paniculata* B.Heyne ex Roth in Nov. Pl. Sp.: 383 (1821)
190. *T. papuana* Exell in Brittonia 2: 246 (1936)
191. *T. parvifolia* (Ducke) Gere & Boatwr in Bot. J. Linn. Soc. 184: 321 (2017)
192. *T. parvula* Pamp in Bull. Soc. Bot. Ital. 1915: 17 (1915)
193. *T. pedicellata* Nanakorn in Nordic J. Bot. 4: 195 (1984)
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195. *T. pendula* (Edgew.) Gere & Boatwr in Bot. J. Linn. Soc. 184: 321 (2017)
196. *T. pennyana* Anozie in Edinburgh J. Bot. 52: 347 (1995)
197. *T. perrieri* Capuron in Bull. Mus. Natl. Hist. Nat., Sér. 3, Bot. 11: 135 (1973)
198. *T. petiolaris* A.Cunn. ex Benth in Fl. Austral. 2: 502 (1864)
199. *T. phaeocarpa* Eichler in C.F.P.von Martius & auct. suc. (eds.), Fl. Bras. 14(2): 89 (1867)
200. *T. phanerophlebia* Engl. & Diels in H.G.A. Engler, Monogr. Afrik. Pflanzen-Fam. 4: 19 (1900)
201. *T. phellocarpa* King in J. Asiat. Soc. Bengal, Pt. 2, Nat. Hist. 66: 330 (1897)
202. *T. phillyreifolia* (Van Heurck & Müll.Arg.)

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## ***Terminalia arjuna* (Roxb. ex DC.) Wight & Arn.**

*T. arjuna* is also known as 'Arjun' or 'Arjuna' is legendary after the greater hero of 'Mahabharata'. It is a member of the Combretaceae family and found in the Sri Lanka and Mauritius forest and grow almost in all types of soils, but prefers humid, fertile loam and red lateritic soils (Dwivedi and Chopra, 2014). It has been observed that *T. arjuna* can cure a variety of illnesses (Zafar et al., 2015). A decoction of arjuna bark has been used for generations by traditional Indian subcontinental physicians to treat pain, hypertension, congestive heart failure, and dyslipidemia (Dwivedi and Chopra, 2014). Major phytoconstituents of *T. arjuna* are arjunic acid, arjunolic acid, arjunone,

arjungenin, arjunolone, gallic acid, ellagic acid and luteolin (Thakur et al., 2021).

In India, arjuna is one of the holy trees. It has acquired the social and religious sanctity with the passage of time. It is said that Arjuna was born of Kubair's two sons following his curse by Saint Narada. On many religious festivals, the leaves and blooms of this tree are presented to Lord Ganpati and Lord Vishnu (Choudhary and Arya, 2022). It is an Ayurvedic treatment that has been described in several ancient Indian medicinal literatures since the Vedic period, including 'Charaka Samhita', 'Sushruta Samhita', and 'Astang Hridayam'. Vagabhatta was the first to recommend the use of stem bark powder for

cardiac diseases (Dwivedi and Chopra, 2014). In the Ayurvedic, Siddha, and Unani systems of medicine, *T. arjuna* is widely utilized for a various medicinal purpose (Khare, 2007). *T. arjuna* is one of the medicinal plants recognized to be good for many cardiac problems. "Atharva Veda" "Vagbhattacharya" was the first to describe the usage of this plant in the treatment of heart diseases in his book 'Astang Hridayam', and this was confirmed by Chakradattam and Bhavamisram (Gupta et al., 2018).

Arjuna has been used as an ingredient of 103 internal and 22 external formulations, in external formulations, indicated for *Striroga* (diseases of women), *Charmaroga* (diseases of skin), *Upadansha* (poisoning), *Balaroga* (diseases of child) etc. Formulations indicated for internal administration disease condition like *Hridayaroga* (heart disease), *Raktapitta* (bleeding disorder), *Mutrakruchcha* (dysuria), *Prameha* (increased frequency of urine), *Shula* (pain), *Striroga* (gynecological disorder), *Ashmari* (kidney stone), etc. The highest number of formulations was found in *Hridayaroga*, *Prameha* and *Upadansha* followed by *Agnimandhya-Ajirna*, *Striroga*, *Kasa*, *Raktapitta*, *Amlapitta* etc (Mehta, 2016).

## Classification

Kingdom	-	Plantae
Subkingdom	-	Tracheobinota
Super Division	-	Spermatophyta

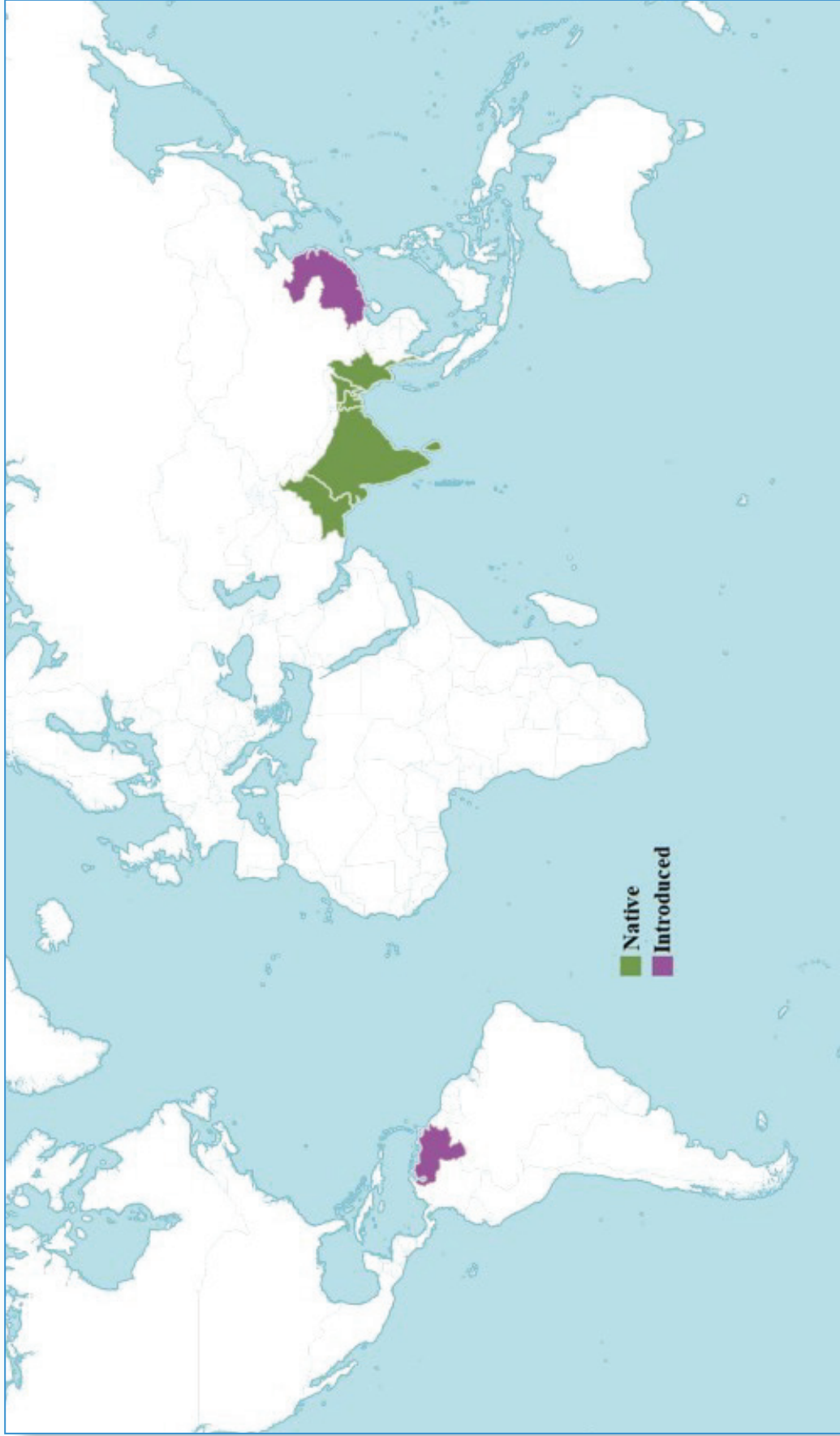
Division	-	Mangoliophyta
Class	-	Magnoliopsida
Subclass	-	Rosidae
Order	-	Myrtales
Family	-	Combretaceae
Genus	-	<i>Terminalia</i>
Species	-	<i>arjuna</i>

## Common Names

Arjuna, Kakubha Kireetu (Sanskrit); Arjun, Arjuna, Arjan, Anjani, Jamla, Koha, Kava, Kahuva (Hindi); Arjun (Bengali); Dhaula Sadar, Arjuna, Sadada (Gujarati); Arjuno, Panda, Sahajo, Hanjal, Kowha (Oriya); Vella-marda, Attumaruthe, Vella-matti, Tanikai, Marudai, Vellabili-mathi, Nir-mathi, Bolu-mathi (Tamil); Yermaddi, Tella Madu (Telugu); Vellu-marnthu (Malayalam).

## Global Distribution

The species *T. arjuna* is found in Indian Subcontinent to Myanmar. According to POWO (2024), it is native to Bangladesh, India (Assam), Myanmar, Pakistan, Sri Lanka; and introduced into China Southeast, Mauritius, Rodrigues, Trinidad-Tobago, Venezuela, Venezuelan Antilles (Fig. 2)



**Fig. 2** Global distribution of *T. arjuna*

## Distribution in India

It is distributed throughout Indo-sub-Himalayan region of Uttar Pradesh, Assam, Gujarat, Chota Nagpur, Burma, South Bihar, Gujarat, Jharkhand, Madhya Pradesh, Maharashtra, Punjab, South Bihar, Orissa, Tamil Nadu, West Bengal, Delhi, and Deccan region mainly along riverside, rivulets, and ponds. This plant is grown commonly in all types of soils, but prefers humid, fertile loam and red lateritic soils (Dwivedi and Chopra, 2014; Amalraj and Gopi, 2017; Thakur et al., 2021; Choudhary and Arya, 2022).

## Ethnopharmacology and Traditional Uses

*T. arjuna* is an Ayurvedic plant with important medicinal value. The whole plant is used as cardiogenic in heart failure, ischemic, cardiomyopathy, atherosclerosis, myocardium necrosis and has been used for the treatment of different human diseases like blood diseases, anemia, venereal and viral disease, fractures, ulcers, hepatic and hypercholesterolemia, and possess antibacterial, antimicrobial, antitumorogenic, antioxidant, antiallergic and antifertility (in high doses) and anti-HIV activities (Amalraj and Gopi, 2017).

The bark, leaves, and fruits of the *T. arjuna* plant have been used in traditional medicine to treat a variety of illnesses. Bark is used as an anti-dysentery, cardiogenic, lithotriptic, antipyretic, astringent, and tonic. The bark powder has been found to possess cardioprotective properties, anti-ischemic,

antioxidant action, hypocholesterolemic effect, fungicidal, antimicrobial, antibacterial, anti-fertility, treatment of ulcers, skin disorders and as antidote to poison. Arjuna leaves are mainly used in curing earache and treatment of cracked heels. The fruit is useful as tonic and deobstruent, helps in wound healing. Root paste of *T. arjuna* is used to treat headache (Hafiz et al., 2014; Soni and Singh, 2019; Susikumar et al., 2022). The paste of arjuna bark, turmeric, water is mixed and applied over fracture to help promote early healing and the twig of arjuna is used for teeth cleansing (Dhruti, 2020).

Arjuna is used to control high blood pressure and regulate the heartbeat (Babasaheb and Arif, 2018). Bangladeshi indigenous people used the bark to cure diabetes without using any modern diagnostic procedures. Bark is used to cure heart, dysentery, asthma, hypertension, menstrual problems, anemia, wounds and pains and other disease (Hafiz, et al., 2014). In the Kancheepuram area of Tamil Nadu, traditional healers use *T. arjuna* fruit paste topically to wounds, boil the bark powder in water, and inhale the resulting mixture to relieve headache and destroy tooth worms (Muthu, et al., 2006). Malamalasar tribes in Kerala used its fresh leaf juice in the treatment of earache; bark powder is used to treat heart troubles (Yesodharan and Sujana, 2007).

## Ayurvedic Uses

Arjuna is a well-known Ayurvedic plant that is utilized for a variety of medicinal purposes.

In Ayurveda, the stem and bark are utilized for medicinal purposes in various forms such as *Kshirpaka*, *Siddha Gruta*, *Arishta* etc. It is indicated in *Medoroga* (Obesity), *Vrana* (Wounds), *Hridroga* (heart diseases), *Kshatashaya* (Debility), *Prameha* (Diabetes), *Vyanga* (Chloasma), etc. (Dongre and Shishir, 2022). It is recommended by the renowned ancient physician Chakradatta to be taken as a *ghrita* or as a bark decoction with milk. Bark decoction used as an ulcer wash, while bark ashes have been prescribed for snakebite and scorpion sting (Dwivedi and Chopra, 2014). In Ayurveda, the two main formulations are known, *Kashaya* (water decoction) and *Ksheerapaka* (milk extract). *Kashaya* is mostly made by boiling the plant material in a specified quantity amount of water until the active components are extracted. This liquid is then filtered using a muslin cloth and utilized fresh. Decoction can be used both externally and internally. In ancient time, *Ksheerapaka* was considered highly nutritive and medicinal. It is used as food and base of medicament. Protein, lipids, fatty acids, vitamins, enzymes, and minerals are the primary ingredients of *ksheerapaka* (Gupta et al., 2018).

The primary component of the Arjuna plant, the bark, is utilized in both Ayurveda and Allopathy to treat a wide range of illnesses. Bark has glucosides, calcium salts, and magnesium salts. According to Vagbhata, glucosides have been employed in traditional ayurvedic herbalism. The bark of the Arjuna tree is used as a cooling, calming, *kaphapitta*, and cardiac restoration. It also aids in the treatment of wounds, tuberculosis, and

poisoning. Bark is utilized as a cardioprotective and a tonic for heart illness (Babasaheb and Arif, 2018).

*T. arjuna* stem bark is used as an ingredient in several Ayurvedic formulations, including *Parthadyarista*, *Nagarjunabhra Rasa*, *Arjuna Ghrta*, *Arjuntwakchurna*, *Arujunaksheerpaka*, *Arjunarista*, *Godhumarjunavlehya*, *Godhumarjunapaka*, *Kakubhadichurna*, and *Lakshmi Guggulu*. The powdered bark is used as a tonic for heart diseases and normalizes high blood pressure. The bark is beneficial for myocardial infarction, angina, coronary artery diseases, heart failure, hypercholesterolemia, ischemia, and hypertension (Vijaya et al., 2015; Susikumar et al., 2022). The bark powder is useful for the treatment of *Kshata* (injury or wound), *Kshaya* (emaciated condition), *Visha* (poison), *Raktavikara* (as a styptic), *Medaroga* (diabetic issues), *Prameha* (urinary disorders), *Vrana* (ulcer/wound), *Rasa panchak* (Thakur et al., 2021).

## Selection of the Sites and Characteristics Studied

The distribution, morphological variations, and association of *T. arjuna* were investigated across the Ganga River. The Ganga began from the Gomukh in Gangotri glacier and ended at Gangasagar in the Bay of Bengal. A total of twenty-six sites were selected with two sites ranging around 100 km in distance while the whole distance is about 2600 km (Table 1). The plant diversity was assessed up to the 10 km on both sides of each site. The plants were identified and deposited in the

Patanjali Research Foundation Herbarium site in a large polybag which was deposited with an acronym of PRFH for future records. A bulk sample of the plant was taken from each site in the analytical laboratory for phytochemical profiling.

**Table 1** Different studied sites and their GPS coordinates along with *T. arjuna* Status

Site	Locality	GPS Coordinates			Status
		Altitude (m)	Latitude (N)	Longitude (E)	
S1	Gomukh	4023	30.80	79.15	Absent
S2	Gangotri	3415	30.98	78.93	Absent
S3	Uttarkashi	1158	30.73	78.44	Absent
S4	Devprayag	830	30.15	78.60	Absent
S5	Haridwar	314	29.97	78.17	Present
S6	Bijnor	225	29.37	78.13	Present
S7	Narora	174	28.20	78.38	Present
S8	Budaun	164	28.05	79.12	Present
S9	Farrukhabad	151	27.37	79.63	Absent
S10	Bithoor	126	26.61	80.27	Absent
S11	Dalmau	115	26.07	81.03	Present
S12	Prayagraj	98	25.45	81.85	Present
S13	Mirzapur	80	25.15	82.58	Present
S14	Varanasi	81	25.32	83.01	Present
S15	Ballia	67	25.76	84.15	Present
S16	Revelganj	52	25.78	84.67	Present
S17	Patna	53	25.61	85.14	Absent
S18	Barh	47	25.48	85.72	Present
S19	Bahachouki	55	25.30	86.36	Present
S20	Farka	42	25.23	87.09	Present
S21	Sahebganj	16	25.25	87.65	Present
S22	Farakka Bar- rage	30	24.82	87.90	Present
S23	Murshidabad	18	24.18	88.27	Absent
S24	Mayapur	11	23.43	88.39	Absent
S25	Hoogli	9	22.91	88.40	Absent
S26	Gangasagar	4	22.19	88.19	Absent

## Local Occurrence

The plant was recorded in most of the sites but it was collected for the analytical studies only from the sites 5, 6, 7, 8, 11, 12, 13, 14, 15, 16, 18, 19, 20, 21, and 22, due to these sites being the source of its bulk collection. In the remaining sites 1, 2, 3, 4, 9, 10, 17, 23, 24, 25, and 26, it was either absent or difficult to collect.

## Taxonomic Treatment

*Terminalia arjuna* (Roxb. ex DC.) Wight & Arn., Prodr. Fl. Ind. Orient. 1: 314. 1834; C.B. Clarke in Hook.f., Fl. Brit. India 2: 447. 1878; Duthie, Fl. Gangetic Plain 1: 336. 1903; Haines, Bot. Bihar Orissa (Repr. ed.) 2: 368.1961; Uniyal & al., Fl. Pl. Uttarakhand, Checkl.: 131. 2007; Sinha et. al, Fl. Uttar Pradesh 1: 551.2016.

## Synonyms

The Plants of the World Online database mentioned it with its one homotypic synonym and ten heterotypic synonyms (POWO, 2024) as-

### Homotypic Synonyms

*Pentaptera arjuna* Roxb. ex DC. in Prodr. 3: 15 (1828), nom. cons.

### Heterotypic Synonyms

1. *Myrobalanus cuneata* (B. Heyne ex Roth) Kuntze in Revis. Gen. Pl. 1: 236 (1891)
2. *Pentaptera angustifolia* Roxb. in Fl. Ind., ed. 1832. 2: 437 (1832)
3. *P. glabra* Roxb. in Fl. Ind., ed. 1832. 2: 440 (1832)

4. *P. obovata* DC. in Prodr. 3: 14 (1828)
5. *Terminalia berryi* Wight & Arn. in Prodr. Fl. Ind. Orient. 1: 314 (1834)
6. *T. cuneata* B. Heyne ex Roth in Nov. Pl. Sp.: 380 (1821)
7. *T. glabra* Wight & Arn. in Prodr. Fl. Ind. Orient. 1: 314 (1834)
8. *T. ovalifolia* Rottler ex C.B. Clarke in J.D. Hooker, Fl. Brit. India 2: 447 (1878)
9. *T. psidiifolia* Delile in F. Cailliaud, Voy. Méroé 4(prepr.): 92 (1826)
10. *T. urjan* Royle in Ill. Bot. Himal. Mts.: 209 (1835)

While another database i.e., World Flora Online (WFO, 2024) mentioned it with a total of following eleven synonyms-

1. *Myrobalanus cuneata* Kuntze in Revis. Gen. Pl. 1: 236 (1891)
2. *Pentaptera angustifolia* Roxb in Fl. Ind. ed. 1832, 2: 437 (1832)
3. *P. arjuna* Roxb. ex DC in Prodr. 3: 15 (1828)
4. *P. glabra* Roxb in Fl. Ind. (Roxburgh) 2: 440. 1832
5. *P. obovata* DC in Prodr. 3: 14 (1828)
6. *Terminalia berryi* Wight & Arn in Prodr. Fl. Ind. Orient.: 314 (1834)
7. *T. cuneata* Roth in Nov. Pl. Sp.: 380 (1821)
8. *T. glabra* Wight & Arn in Prodr. Fl. Ind. Orient.: 314 (1834)
9. *T. ovalifolia* Rottler ex C.B. Clarke in Fl. Brit. India 2: 447 (1878)
10. *T. psidiifolia* Delile in Voy. Méroé 4(prepr.): 92 (1826)

11. *T. urjan* Royle in Ill. Bot. Himal. Mts. [Royle] 209. 1835 [Apr 1835]

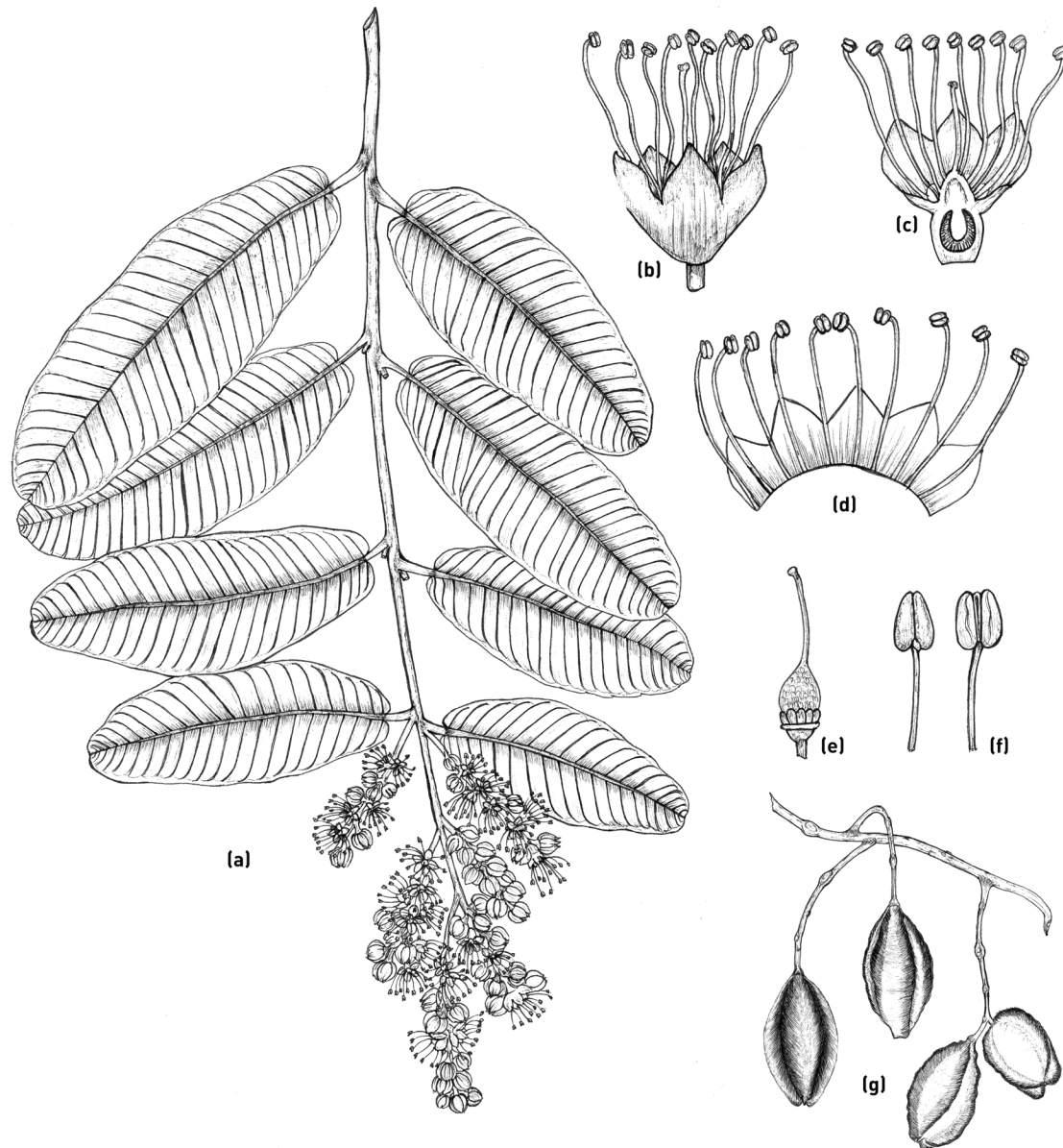
### Botanical Description

Large and deciduous tree, up to 30 m tall. Bark grey or pinkish-grey, smooth, thick. Leaves simple, alternate to sub-opposite; petiole up to 1.5 cm long; lamina 7-12 x 3.5-5 cm, oblong or obovate-oblong, base rarely subacute with rounded or cordate, margin crenate-serrate, apex obtuse or sub-

acute, glabrous to subglabrous. Inflorescence spikes axillary, in panicles, up to 10 cm long; bracteole small, linear-lanceolate; peduncle up to 5 cm long. Flowers yellowish white, sessile. Hypanthium 4-5 mm long, broadly campanulate; teeth up to 15 mm long, triangular, glabrous. Stamen 10 or more; filament 3-4 mm long. Ovary up to 1.5 mm long, ovoid, glabrous. Fruits drupe, obovoid-oblong, 5-angled, 2.5-6 x 1.8-2.8 cm long, 5-winged, dark brown to reddish-brown fibrous, glabrous (Fig. 3A, B).



Fig. 3A Plant habit



**Fig. 3B** (a) Plant twig (b) Flower (c) L.S of Flower (d) Stamens arrangement (e) Stigma (f) Stamen (g) Fruit

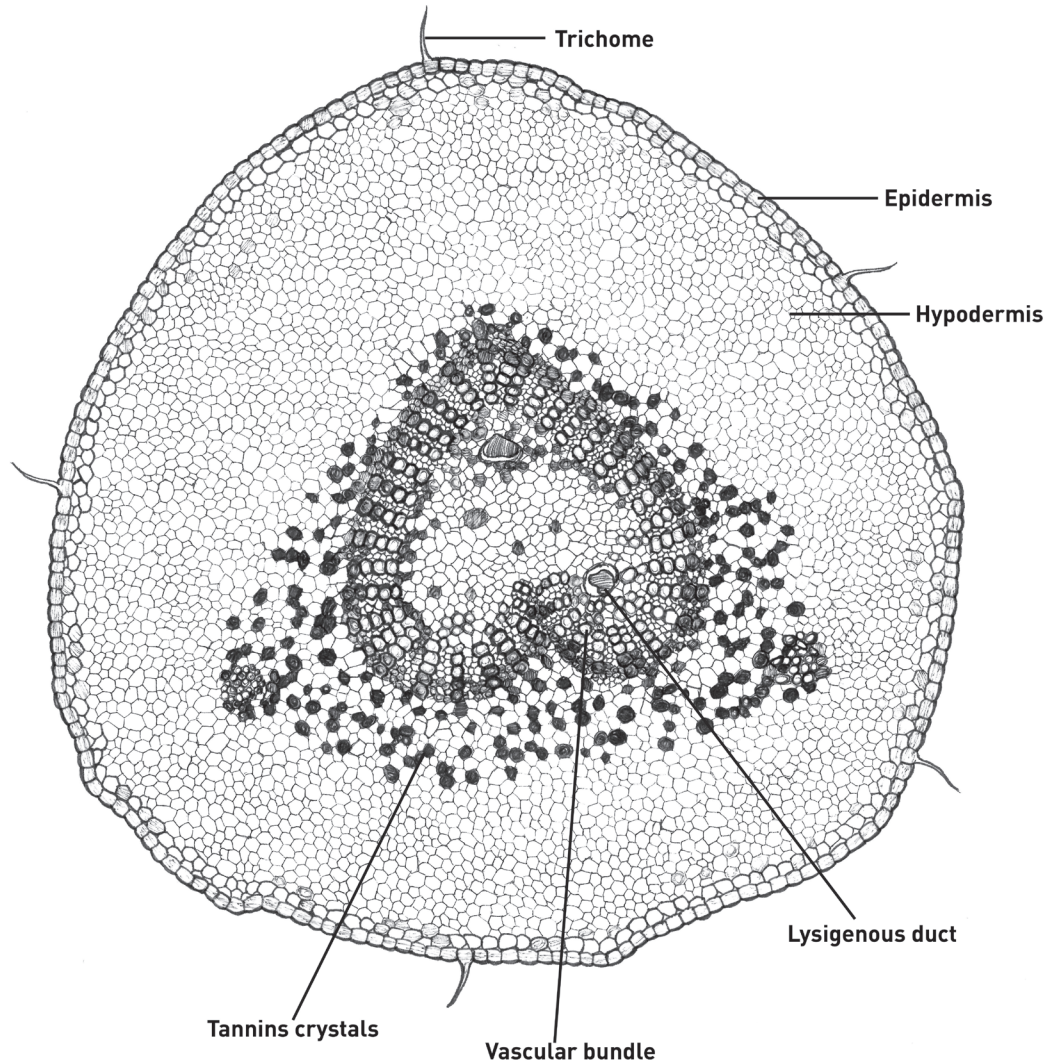
## Anatomical Features

### Structure of the Petiole

The outline of the petiole is ovate. The epidermis with their many unicellular trichomes and lenticels make up the outermost layer. There is a large hypodermis located

beneath the epidermis. The cells that make up the ground tissues are parenchymatous and collenchymatous. The vascular bundle has a heart-shaped form, with sclerenchymatous sheath patches on the outside. There are two accessory bundles—one on each side of the main vascular bundle. The kind of

vascular bundle is primitive. There are several lysigenous ducts inside and outside of the bundles. A few crystals and a few of tannins are found with these cells (Fig. 4).

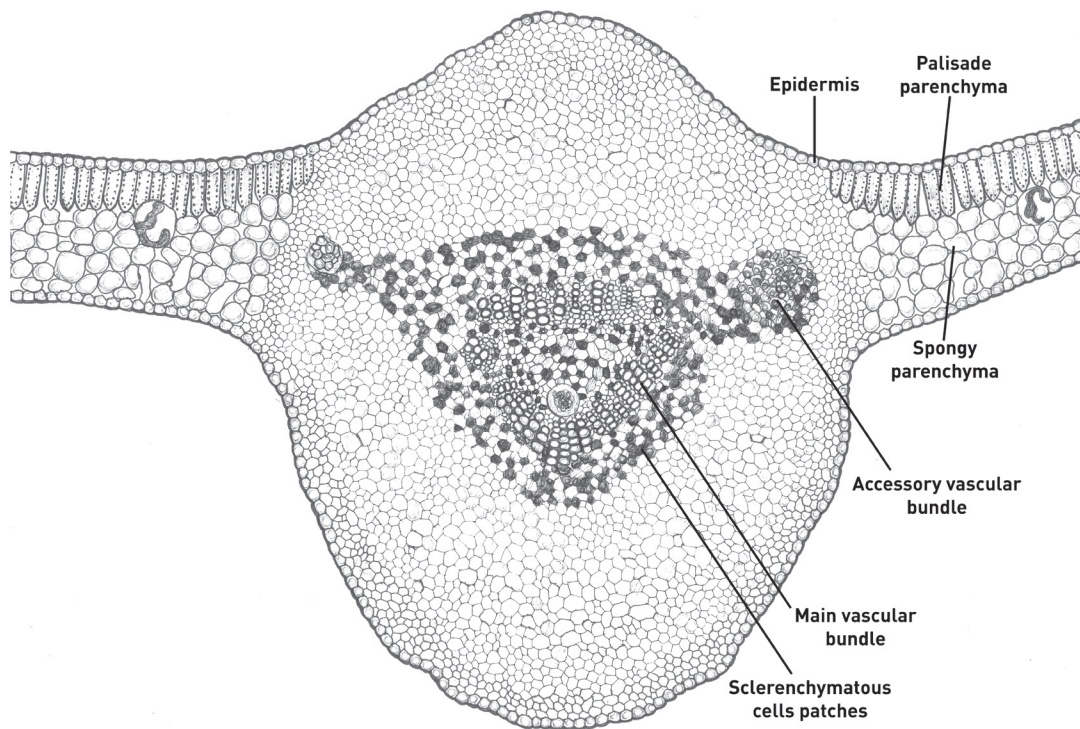


**Fig. 4** TS of petiole

### Structure of the Leaf

The shape of the upper epidermis is rectangular. Palisade and spongy parenchymatous cells make up the leaf wing. The spongy cells are thick in numerous layers, but the palisade cells are found in a single bare layer on the upper side of the leaf. In the midrib area, there are two different kinds of cells. There are two

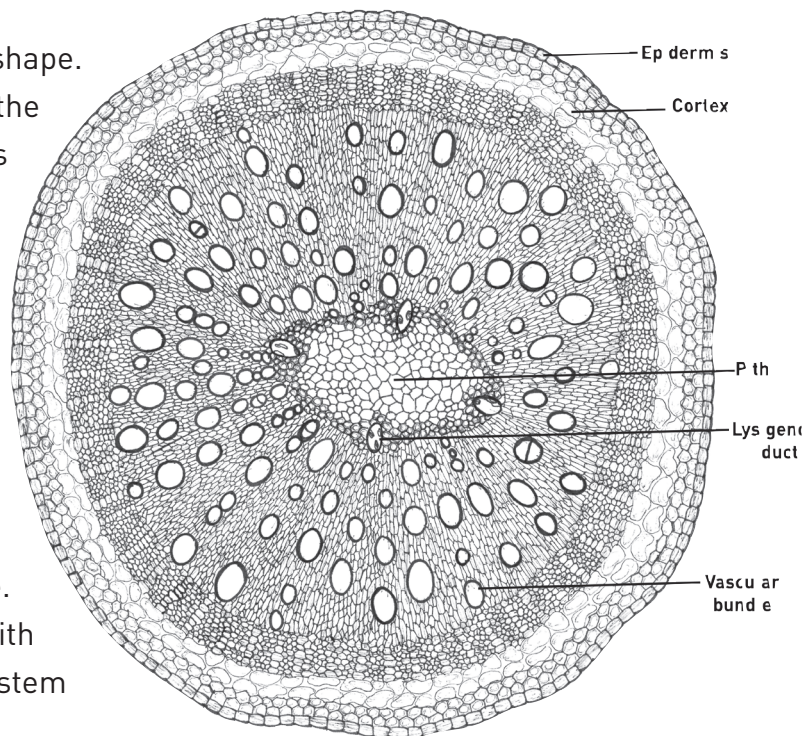
different kinds of vascular bundles: main and accessory vascular bundles. There are two accessory bundles—one on either side of the main vascular bundle—while the main bundle is solitary and medium in size. The sclerenchymatous cell patches envelop the bundles. In and around the bundles, different crystals have been seen (Fig. 5).



**Fig. 5** TS of leaf

### Structure of the Stem

The stem has a rounded shape. Trichomes are absent from the epidermis. The parenchymatous and sclerenchymatous cells that comprise the cortex are located underneath the epidermis. The vascular bundles are arranged in a ring. Phloem cells are found on the outside of the endodermis. The secondary xylem is located on the inner side of the endodermis. The quadrangular pith is in the centre. There is a lysigenous duct at every pith angle. The central portion of the stem contains several crystals (Fig. 6).

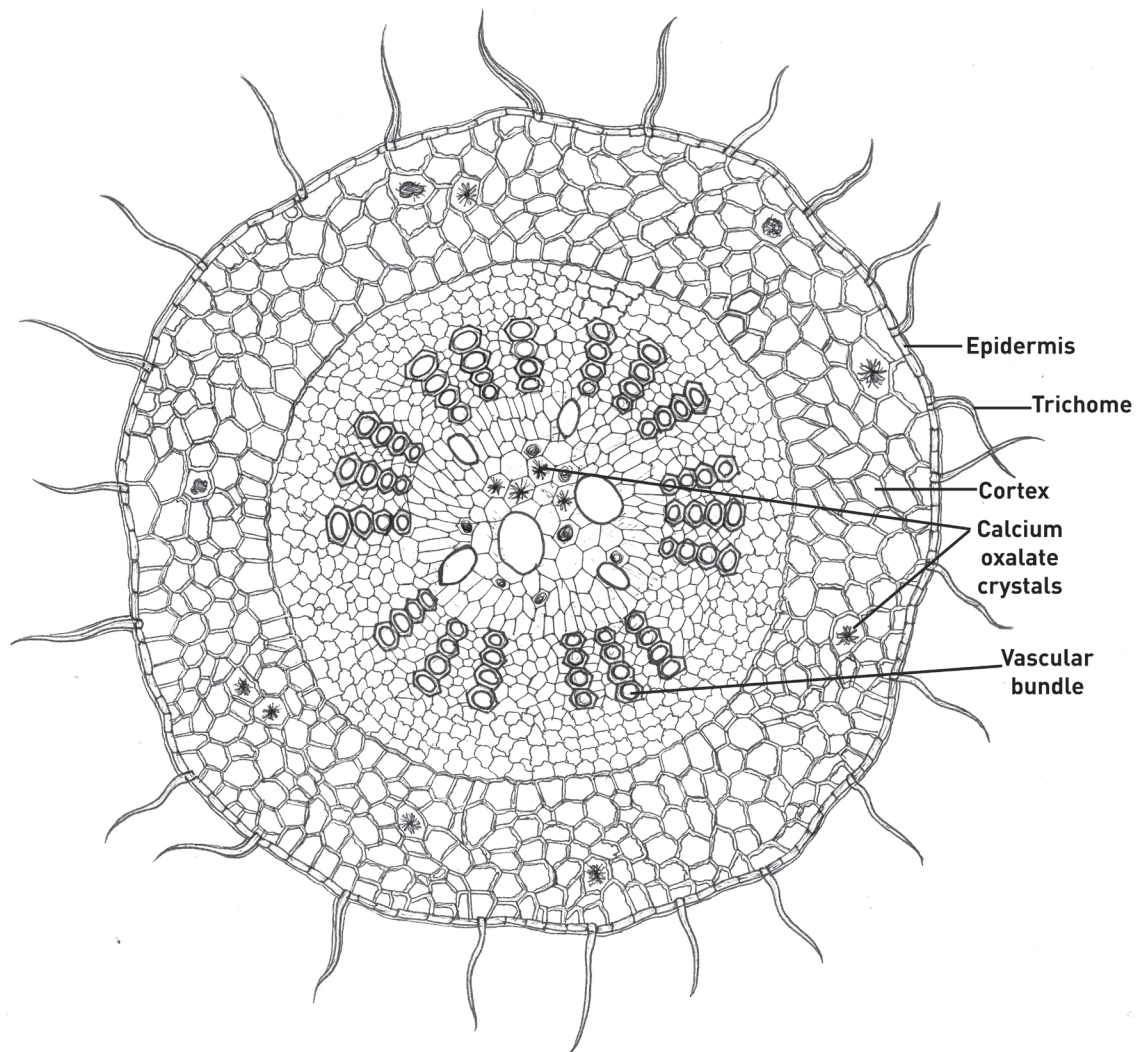


**Fig. 6** TS of stem

## Structure of the Peduncle

The peduncle's transverse section has a single-layered outer epidermis that is heavily coated with tiny to slender trichomes. It trailed the several layers of parenchymatous cortex, which has thin walls. The rosette and clusters of calcium oxalate crystals are visible

in some cortical cells. The xylem and phloem were divided by the endodermis. The vascular bundles are conjoint, open, and collateral. The calcium oxalate crystals are packed in several layers of parenchymatous cells with thin walls in the centre. The resin ducts are recorded above the vascular bundles (Fig. 7).



**Fig. 7** TS of peduncle

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CHAPTER  
**02**

# Bibliometric Analysis



## INTRODUCTION

The Combretaceae family comprises about 200 species distributed throughout the humid, semi-humid, tropical, and subtropical regions of the world. About 24 species of *Terminalia* were reported in India including some prominent species such as *T. arjuna*, *T. bellerica*, *T. chebula*, *T. tomentosa*, *T. catappa*, *T. elliptica*, *T. porphyrocarpa*, and *T. mantaly* (Kumar et al., 2023). The bark of *T. arjuna* (Roxb.) Wight and Arnot is known in India as “Arjuna”. *Terminalia arjuna*, is a large tree widely distributed in South Asian region. It is well-known for its medicinal properties; it exhibits a wide range of biological activities. In Ayurveda, it is highly recognized for its diverse therapeutic benefits. The plant has long been recognized for its potent cardiogenic properties (Jaiswal et al., 2021). In Ayurvedic medicine, it is known for its effectiveness in treating ecchymosis, spermatorrhea, and sexually transmitted diseases such as gonorrhoea. *T. arjuna* possesses a variety of medicinal properties such as astringency, cooling effects, aphrodisiac properties, and is a cardiogenic. This plant is also used in treating cough, leukorrhoea, excessive perspiration, ulcers, diabetes, tumors, asthma, inflammation, and various skin disorders (Soni and Singh, 2019). It is reported that powdered bark from this plant holds significant potential for treating coronary heart disease. Several chemical constituents have also been reported from the stem bark portion of *T. arjuna* such as hydrolyzable tannins triterpenoids acid and their glycosides, flavonoids, phenolics, phytosterol (Amalraj and Gopi, 2017). In addition, arjunic acid, arjunolic acid, arjunglucoside, arjunetin, and terminolic acid belong to the group of important constituents of the bark. *T. arjuna* also has the potential to cure hepatic, congenital, venereal, and viral diseases, bark powder is reported to exhibit hypocholesterolemic and antioxidant effects (Jaiswal et al., 2021).

Bibliometric analysis is pivotal in modern scientific research, providing a comprehensive assessment of the scholarly landscape surrounding a particular topic (Ellegaard and Wallin, 2015). This study explored the research environment of *T. arjuna* through a bibliometric examination, analyzing a wide range of literature,



research trends, and knowledge dissemination related to this highly versatile and extensively studied plant (Karakose et al., 2022). By employing bibliometric analysis, researchers can investigate both the quantitative and qualitative aspects of academic publications, enabling them to identify patterns, trajectories, and the development of scientific discourse on *T. arjuna*. This methodological approach involves a detailed investigation of publication metrics, citation dynamics, collaborative networks among authors, geographical distribution of research activities, and thematic areas across the body of scientific literature (Mejia et al., 2021). Through systematic analysis of bibliographic data related to *T. arjuna*, scholars gain a comprehensive understanding of the scope and depth of research activities concerning this botanical species.

The primary objective of this research is to conduct a comprehensive bibliometric analysis of the research landscape surrounding *T. arjuna*. This involves systematically evaluating and mapping the existing literature to identify trends, patterns, and gaps in the research. Specifically, the study aims to quantify the volume and growth trajectory of publications related to *T. arjuna* over time, highlighting key periods of increased research. Moreover, it seeks to categorize the research themes and topics most frequently addressed in the literature, thereby identifying dominant areas of focus and underexplored aspects of *T. arjuna* research. The analysis also includes examining the geographical distribution of research outputs to identify leading countries and institutions contributing to *T. arjuna* research, and to assess international collaboration patterns. Furthermore, the study aims to determine the impact and influence of *T. arjuna* research through citation metrics, identifying highly cited works and influential authors within the field. Lastly, the research seeks to map the collaboration networks among researchers, journals, institutions, and countries revealing the structure and dynamics of research partnerships in the field.

## Insights with Data Source and Tools

The study investigated the research landscape of *Terminalia arjuna* through a comprehensive bibliometric analysis, aiming to provide an in-depth understanding of the scope, impact, and evolving trends in academic publications related to this plant species. The methodology involved a systematic collection and analysis of scholarly articles sourced from Dimensions.ai, utilizing the search phrase "*Terminalia arjuna*" to capture relevant publications from 2000 to 2023. Microsoft Excel was employed for managing and pre-processing the large dataset, offering robust data management capabilities that ensured the data was structured appropriately for detailed analysis. For advanced bibliometric analysis, the study utilized Lens.org and VosViewer (Version 1.6.19). These tools were chosen for their established efficiency in similar studies (Yu et al., 2020; Hajkowicz et al., 2023). Lens.org

facilitated an in-depth exploration of top fields of study, enabling the identification of key thematic areas within the research on *T. arjuna*. Its integrated features were particularly valuable in mapping out various domains and subfields, providing a comprehensive overview of the research landscape. VosViewer was essential for creating visual representations of bibliographic data, such as co-authorship networks, keyword co-occurrence maps, and citation analysis, which offered visual insights into the relationships and impact within the academic community studying *T. arjuna*. The analysis revealed significant trends and patterns in the research on *T. arjuna*. This comprehensive approach provided valuable insights into the development and impact of research on *T. arjuna*, underscoring its scientific significance and growing importance in academic literature.

## Comprehensive Data-driven Insights

From 2000 to 2023, scholarly publications on *T. arjuna* provided comprehensive data-driven insights into the global research landscape, highlighting the species' extensive academic and practical significance. During this period, a total of 872 scholarly articles were published, involving 2954 researchers from 712 organizations across 38 countries. This broad and diverse international engagement illustrated the universal relevance of *T. arjuna* and fostered a range of perspectives and methodologies. A

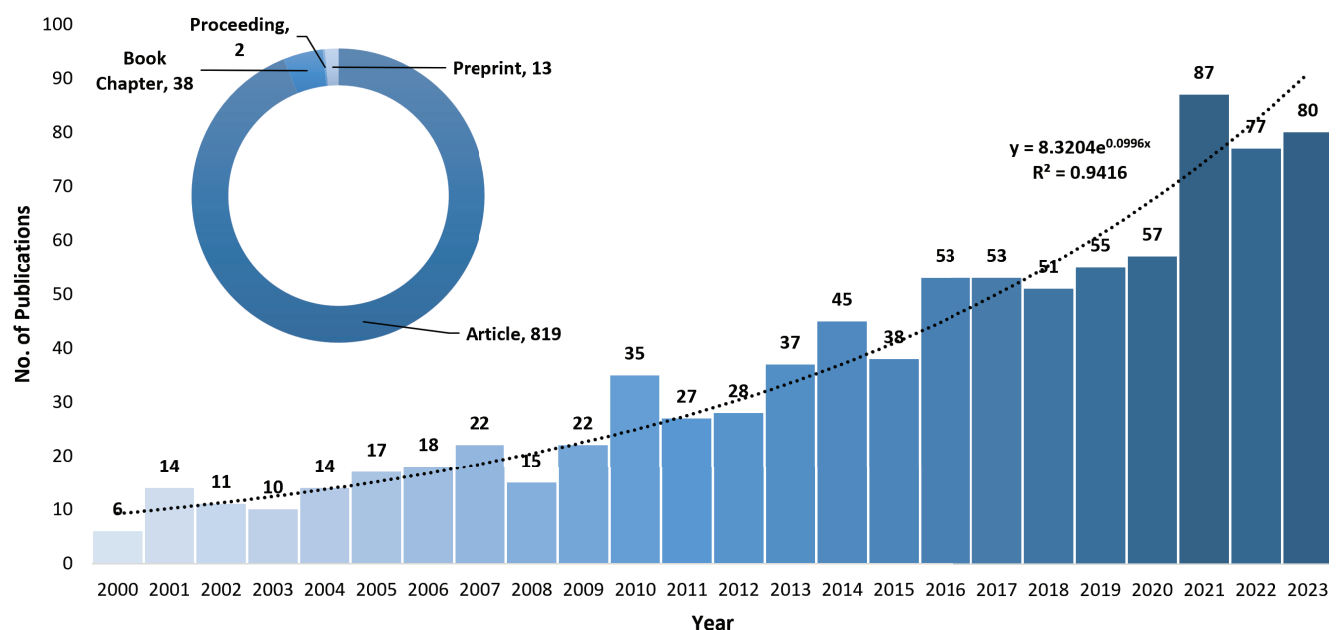
total of 55 authors distinguished themselves by publishing independently, showcasing deep expertise and significant individual contributions to the field. The research findings were disseminated through 573 esteemed journals, reflecting the high regard and wide interest in *T. arjuna* studies within the scientific community. An average citation rate of 17.50 citations per document underscored the strong impact and recognition of the research's relevance and quality. The scope of *T. arjuna* research broadened

significantly, encompassing various fields such as biology, traditional medicine, chemistry, botany, pharmacology, biochemistry, and medicinal plants. This interdisciplinary approach highlighted the plant's multifaceted applications and the broad interest in understanding its properties and benefits. Furthermore, the data revealed robust collaborative networks among researchers and organizations, which were essential for advancing knowledge and fostering innovation. These data-driven insights underlined the potential for continued innovation and advancement in understanding and utilizing *T. arjuna*, driven by a diverse and interconnected scientific community.

## Temporal Evolution and Growth Analysis

A total of 872 documents were discovered after extensive searches across bibliographic databases via Dimensions.ai. These demonstrated a variety of study forms,

including 819 articles, 38 chapters, 13 preprints, and 2 proceedings. The bibliometric analysis of research publications on arjuna over a span of 24 years from 2000 to 2023 revealed a significant upward trend in scholarly interest and output. The number of publications has increased markedly from 6 in the year 2000 to 80 in 2023. This growth trajectory highlights several phases of accelerated research activity: from a modest rise in the early 2000s, with occasional fluctuations such as a dip in 2003 (10 publications) and a subsequent peak in 2010 (35 publications), to a more substantial surge starting around 2014. The highest number of publications i.e., 87 were found in 2021 (Fig. 1). This exponential growth indicates a burgeoning interest and expanding research landscape concerning *T. arjuna* have recognized medicinal properties and potential health benefits. Overall, the data indicated a strong and growing academic engagement with *T. arjuna*, reflecting its emerging significance in scientific research.



**Fig. 1** Publication trends and distribution types in *T. arjuna* research

## Citation Analysis

The citation analysis for the bibliometric study on the research landscape of *T. arjuna* represented a dynamic and fluctuating pattern of scholarly attention over the years. Starting with a modest 79 citations in 2000, there was a significant surge in interest peaking in 2009 with 1,628 citations. This peak is part of an overall increasing trend from 2001 to 2009, marked by highs in 2005 (1,065 citations) and 2007 (1,185 citations). After 2009, citation

counts show considerable variability, with another substantial rise in 2013 (975 citations) and 2014 (1,143 citations), followed by a gradual decline in recent years, dropping to 122 citations by 2023 (Fig. 2). This pattern indicated that while the research on *T. arjuna* has experienced periods of intense focus and high scholarly output, recent years have seen a tapering in citation activity, suggesting either a maturation of the research field or shifting academic interests.

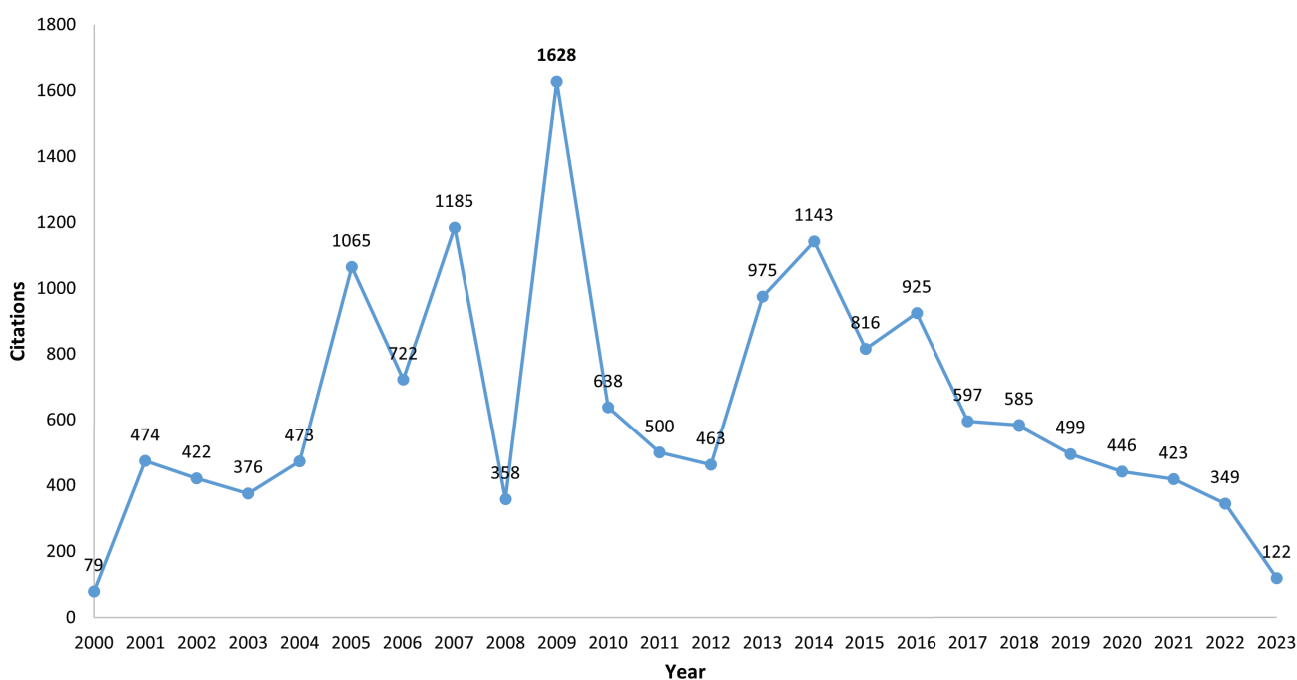


Fig. 2 Citation trends for *T. arjuna* research over time

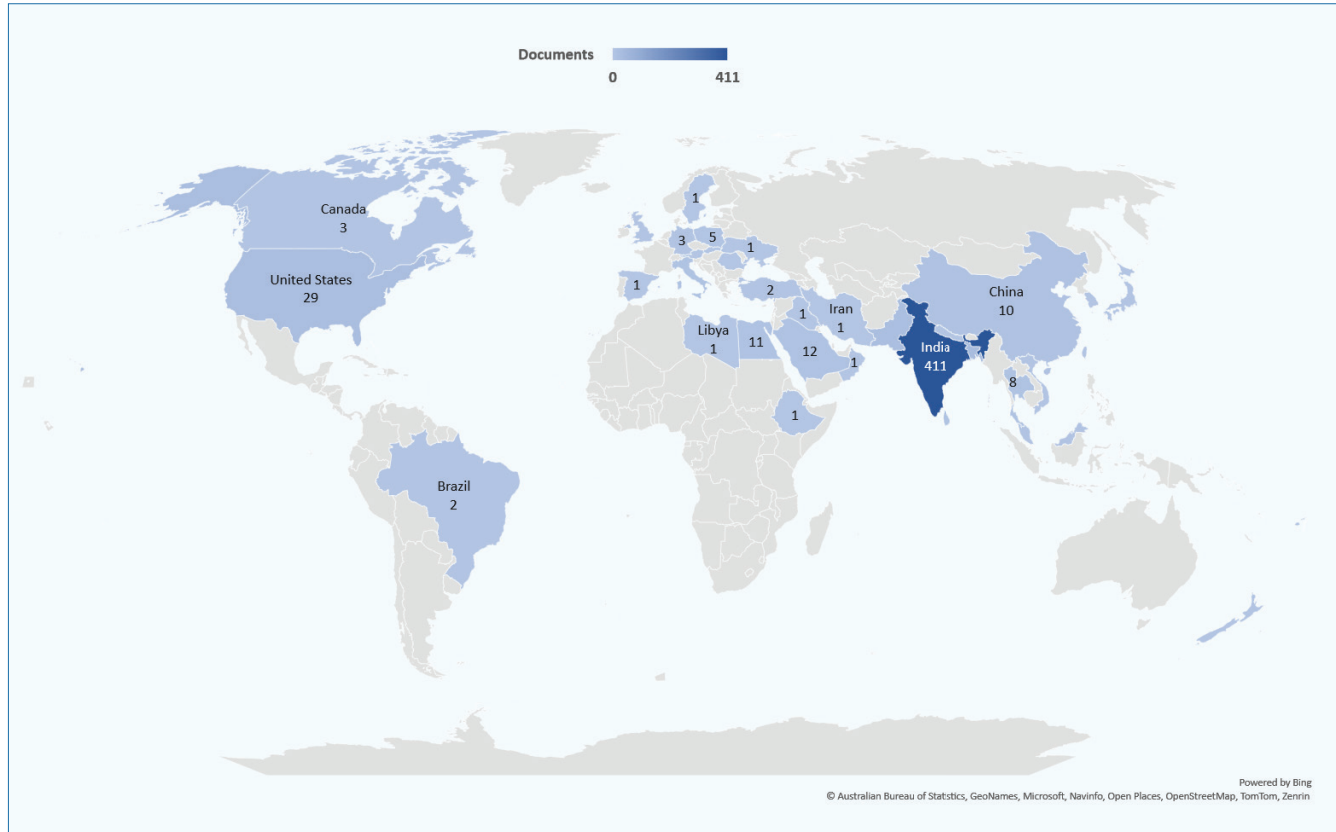
## Country-wise Publication Analysis

The bibliometric analysis of the research landscape on *T. arjuna* revealed a significant difference in publication outputs across different countries. India leads the list with 411 publications and 10,829 citations, highlighting

its dominant role in research on this medicinal plant. Bangladesh and the United States follow distantly, contributing 33 and 29 publications with 266 and 399 citations respectively, indicating moderate engagement with the topic. Pakistan represented 25 publications collected a relatively high citations of 1,364, reflecting substantial impact per publication.

Other countries like Saudi Arabia, Egypt, and China show limited research activity with 12, 11, and 10 publications respectively, each contributing modest citation counts (Fig. 3). Similarly, Sri Lanka and Italy also exhibited lower publication numbers, 9

and 8 respectively, with correspondingly minor citation impacts. This country-wise analysis highlighted important role of India's contribution and variation in global interest in *T. arjuna* research.



**Fig. 3** Global distribution of research on *T. arjuna*

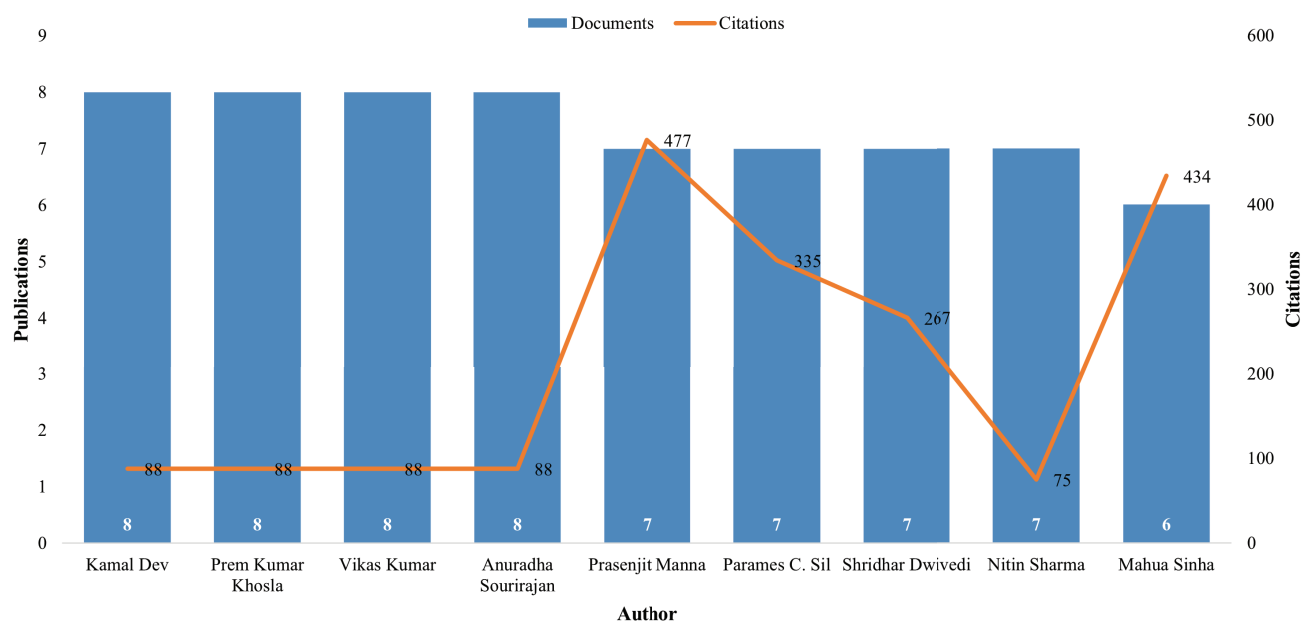
## Most Prominent Authors

In the bibliometric analysis of research on *T. arjuna*, several authors stand out for their significant contributions. Among all authors, Kamal Dev, Prem Kumar Khosla, Vikas Kumar, and Anuradha Sourirajan, each have authored eight documents, each with 88 citations, highlighting their collaborative impact in this field. Prasenjit Manna and Parames C. Sil also have substantial influence, with 7

documents each but with much higher citation counts of 477 and 335 respectively, indicating high recognition and influence of their work (Fig. 4). Shridhar Dwivedi and Mahua Sinha, with 7 and 6 documents respectively, have prominent citation counts of 267 and 434, reflecting the high relevance and quality of their research. Other contributors like Nitin Sharma, Veena Dhawan, and Mohammad Zashim Uddin also play a role, though with

lower citation impacts, emphasize a broader but varied research engagement on *T. arjuna*. These authors collectively shape the research

landscape through their prolific output and impactful work, paving the way for further advancements in the field.



**Fig. 4** Most prominent authors based on research contributions to *T. arjuna*

### Highly Cited Articles

The top 10 highly cited research articles on *T. arjuna* provide a comprehensive overview of its diverse applications and properties (Table 1). The most highly cited article, published in *Molecules* in 2009, “Effect of Extraction Solvent/Technique on the Antioxidant Activity of Selected Medicinal Plant Extracts”, with 701 citations (Sultana et al., 2009). Another significant study in *Food Chemistry* (2007) “Antioxidant activity of phenolic components present in barks of *Azadirachta indica*, *Terminalia arjuna*, *Acacia nilotica*, and *Eugenia jambolana* Lam. Trees” gained 359 citations

(Sultana et al., 2007). The article “Removal of chromium (VI) from dilute aqueous solutions by activated carbon developed from *Terminalia arjuna* nuts activated with zinc chloride” published in *Chemical Engineering Science* (2005), has 308 citations (Mohanty et al., 2005). A study “Antimicrobial Activity of Five Herbal Extracts Against Multi Drug Resistant (MDR) Strains of Bacteria and Fungus of Clinical Origin” collected 264 citations in 2009 (Khan et al., 2009). These articles collectively represented the diverse applications and ecological significance of *T. arjuna* in phytoremediation, pharmacology, and environmental safety.

**Table 1** Top 10 highly cited research articles published on *T. arjuna*

Rank	Title	Source	Year	Citations	Reference
1	Effect of Extraction Solvent/Technique on the Antioxidant Activity of Selected Medicinal Plant Extracts	Molecules	2009	701	Sultana et al., 2009
2	Antioxidant activity of phenolic components present in barks of <i>Azadirachta indica</i> , <i>Terminalia arjuna</i> , <i>Acacia nilotica</i> , and <i>Eugenia jambolana</i> Lam. trees	Food Chemistry	2007	359	Sultana et al., 2007
3	Removal of chromium (VI) from dilute aqueous solutions by activated carbon developed from <i>Terminalia arjuna</i> nuts activated with zinc chloride	Chemical Engineering Science	2005	308	Mohanty et al., 2005
4	Antimicrobial Activity of Five Herbal Extracts Against Multi Drug Resistant (MDR) Strains of Bacteria and Fungus of Clinical Origin	Molecules	2009	264	Khan et al., 2009
5	Phenolic contents and antioxidant activity of some food and medicinal plants	International Journal of Food Sciences and Nutrition	2005	254	Bajpai et al., 2005
6	Antimicrobial evaluation of some medicinal plants for their anti-enteric potential against multi-drug resistant <i>Salmonella typhi</i>	Phytotherapy Research	2004	213	Rani and Khullar, 2004
7	Microwave assisted rapid synthesis and biological evaluation of stable copper nanoparticles using <i>T. arjuna</i> bark extract	Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy	2013	212	Yallappa et al., 2013
8	Enzymatic natural dyeing of cotton and silk fabrics without metal mordants	Journal of Cleaner Production	2007	188	Vankar et al., 2007
9	Green synthesis of gold nanoparticles from fruit extract of <i>Terminalia arjuna</i> , for the enhanced seed germination activity of <i>Gloriosa superba</i>	Journal of Nanostructure in Chemistry	2014	184	Gopinath et al., 2014
10	Biogenic Synthesis of Selenium Nanoparticles and Their Effect on As(III)-Induced Toxicity on Human Lymphocytes	Biological Trace Element Research	2014	157	Prasad et al., 2014

## Most Active Journals

The present study showed the top journals of *T. arjuna* research based on their impact factor, publications, and citations (Table 2). In the research landscape of *T. arjuna*, the 'Journal of Ethnopharmacology' stands out as the most active journal for bibliometric analysis, with the highest number of documents (11), significant impact factor of 5.4 and highlighted pharmacological, and therapeutic aspects of this plant. 'Phytomedicine' and 'Phytotherapy Research' represented clinical application

of medicinal plants and natural products of this plant, also contributed efficiently, each publishing 8 documents, with impact factors of 4.99 and 5.92, respectively. Other important journals include 'Planta Medica' and 'Natural Product Research', each with 8 documents and moderate impact factors of 2.42 and 2.74, respectively. The diversity of publishers and impact factors, ranging from the highly influential to more specialized regional journals, highlighted a broad interest and multidisciplinary approach in the study of *T. arjuna*.

**Table 2** Top journals in *T. arjuna* research have the highest published document and citations

Rank	Source	Publisher	Impact factor	Documents	Citations	Citations per Document
1	Journal of Ethnopharmacology	Elsevier	5.4	11	499	45.36
2	Phytomedicine	Elsevier	4.99	8	460	57.50
3	Phytotherapy Research	John Wiley & Sons Ltd.	5.92	8	454	56.75
4	Planta Medica	Georg Thieme Verlag KG	2.42	8	129	16.13
5	Natural Product Research	Taylor & Francis Ltd.	2.74	8	88	11.00
6	International Journal of Current Microbiology and Applied Sciences	Excellent Publishers	1.59	8	8	1.00
7	Indian Journal of Experimental Biology	National Institute of Science Communication and Information Resources (NISCAIR)	0.79	7	167	23.86

Rank	Source	Publisher	Impact factor	Documents	Citations	Citations per Document
8	Asian Journal of Pharmaceutical and Clinical Research	Innovare Academic Sciences Pvt. Ltd.	0.51	7	27	3.86
9	Indian Journal of Forestry	Indian Council of Forestry Research and Education	-	7	7	1.00
10	Bangladesh Journal of Scientific and Industrial Research	Bangladesh Council of Scientific and Industrial Research (BCSIR)	-	6	22	3.67

## Top Productive Organizations

In the research landscape of *Terminalia arjuna*, “Jamia Hamdard” tops as the most productive organization, contributing 22 documents with 319 citations, all originating from India. The “University of Madras” follows with 16 documents, but with a significantly higher citation count of 619, indicating impactful research. The “Banaras Hindu University” also contributed 16 documents, with 474 citations, while the “All India Institute of Medical Sciences” produced 14 documents

and 343 citations. The “University of Delhi” and “Amity University” have 12 and 11 documents respectively, with citations indicating varied research influence (Table 3). The “University of Dhaka” from Bangladesh was found as the only non-Indian institution in the top ranks, with 11 documents but a lower citation counts of 20. Other prominent Indian institutions included the “Post Graduate Institute of Medical Education and Research”, the “National Botanical Research Institute”, and “Jadavpur University”, highlighting the regional concentration of research on *T. arjuna*.

**Table 3** Top organizations' contributions to *T. arjuna* research

Rank	Organization	Country	Documents	Citations	Citations per Document
1.	Jamia Hamdard	India	22	319	14.50
2.	University of Madras	India	16	619	38.69
3.	Banaras Hindu University	India	16	474	29.63
4.	All India Institute of Medical Sciences	India	14	343	24.50
5.	University of Delhi	India	12	230	19.17
6.	Amity University	India	11	91	8.27
7.	University of Dhaka	Bangladesh	11	20	1.82
8.	Post Graduate Institute of Medical Education and Research	India	10	141	14.10
9.	National Botanical Research Institute	India	9	721	80.11
10.	Jadavpur University	India	9	150	16.67

### Top Fields of Study

The present study examined the occurrence of terms in significant academic domains by analyzing a dataset of 1,188 scholarly documents from Lens.org, revealing a diverse spectrum of scholarly investigations into the plant species *T. arjuna*. With 774 documents dedicated specifically to *Terminalia arjuna*, it was evident that researchers had shown considerable interest in exploring its various facets. Biology emerged as a dominant field with 528 documents, indicating a thorough examination of the plant's biological characteristics, including its anatomy, physiology, and ecological roles. Subsequently, traditional medicine with 497 documents, reflecting the enduring allure of traditional healing practices. Chemistry research, represented by 392 documents, explored the intricate chemical composition of the plant, aiming to identify bioactive compounds.

Medicine, with 339 documents, highlighted the plant's potential therapeutic applications in modern healthcare. Botany (254 documents) and *Terminalia* (236 documents) signified broader botanical inquiries, encompassing taxonomy, morphology, and distribution studies. Pharmacology (166 documents) explored the pharmacological properties and mechanisms of action of *Terminalia arjuna*, while research conducted on the bark (149 documents) and its biochemistry (149 documents) explored specific aspects of its medicinal and biochemical attributes. Antioxidant research (148 documents) emphasized *Terminalia arjuna*'s reputation for its antioxidant properties, offering insights into its potential health benefits. Studies on medicinal plants (125 documents) compared *Terminalia arjuna* with other botanical remedies, while horticulture (103 documents) addressed cultivation and management practices. Ecology (86 documents)

investigated the plant's ecological role, and phytochemical analysis (84 documents) and highlighted its chemical constituents (Fig. 5). Overall, this analysis showcased the interdisciplinary nature of *Terminalia arjuna* research, spanning fields such as

biology, medicine, chemistry, and traditional knowledge systems. It accentuated the plant's significance in scientific assessment and its potential applications across various sectors, from healthcare to agriculture.

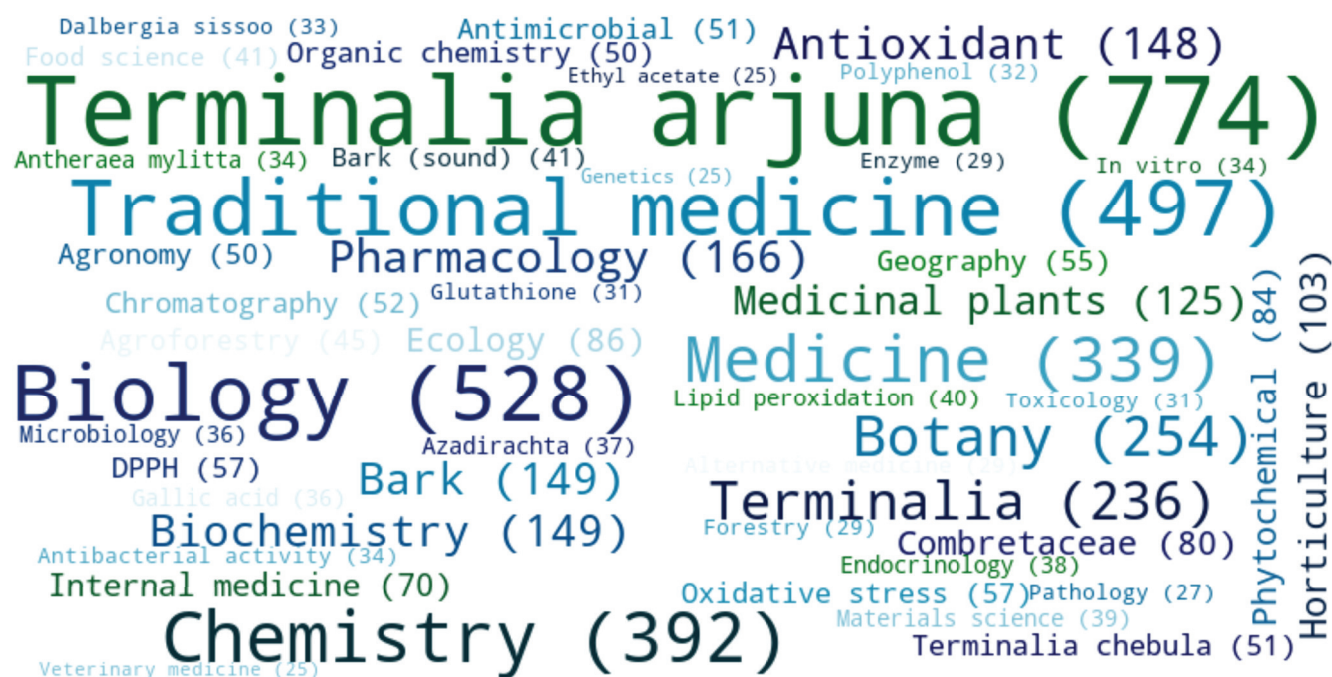


Fig. 5 Terms co-occurrence analysis within prominent terms

## Research Collaboration Networks

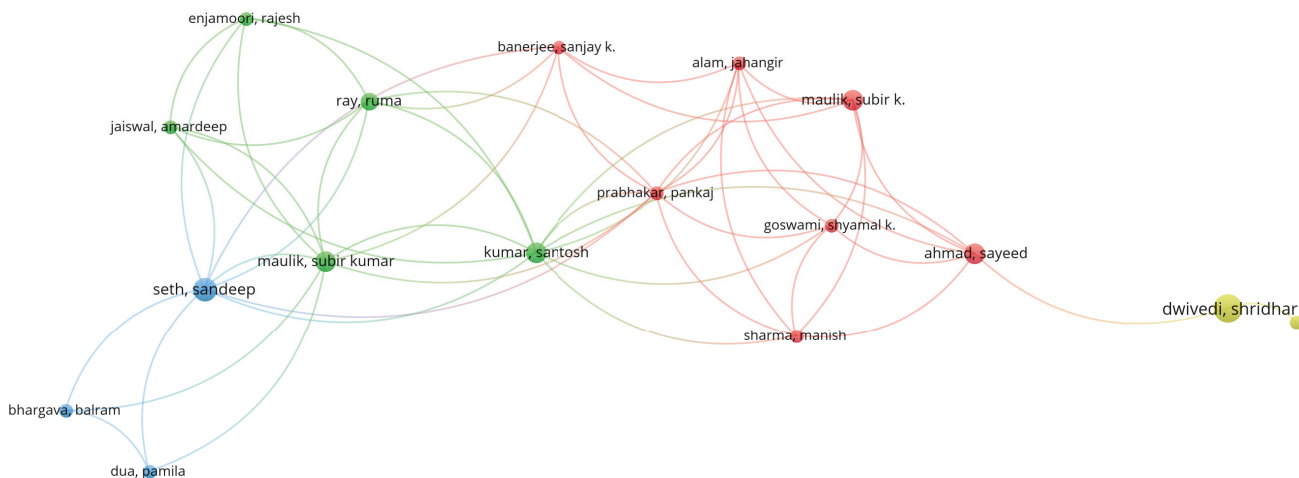
### Authors' Collaboration Network

The collaboration network among the authors in the study on *T. arjuna* provides a fascinating glimpse into the dynamics of scholarly interaction within a specific subject domain. Through an exhaustive examination of 2954 authors and their publications, the study explored the interconnectedness and collaborative efforts within the research community. The criteria for inclusion in the

network were stringent, requiring authors to have contributed at least 2 documents on *T. arjuna*, each with a minimum citation count of 2 citations per document. The analysis results in the identification of 4 distinct clusters within the network, indicating cohesive and collaborative author groups. Cluster 1 emerged as the largest, comprising 7 authors, suggesting a robust collaborative network within this group. Clusters 2, 3, and 4 were found to be smaller but still significant, with 5, 3, and 2 authors respectively, indicating a

diversity of collaborative efforts within the field. Among the authors, Kamal Dev, Prem Kumar Khosla, Vikas Kumar, and Anuradha Sourirajan stand out as the most influential, each boasting a high total link strength of 35. Additionally, Nitin Sharma and Santosh Kumar also emerge as prominent authors, with total link strengths of 32 and 20 respectively, further highlighting the diversity of expertise and collaborative networks

within the field (Fig. 6). Overall, the findings illustrate a dynamic and diverse research community characterized by varying degrees of specialization and collaboration. This network analysis provides valuable insights into the collaborative dynamics and knowledge dissemination processes within this research community, facilitating future collaborations and interdisciplinary exchanges.



**Fig. 6** Collaborative network among the authors on *T. arjuna* research

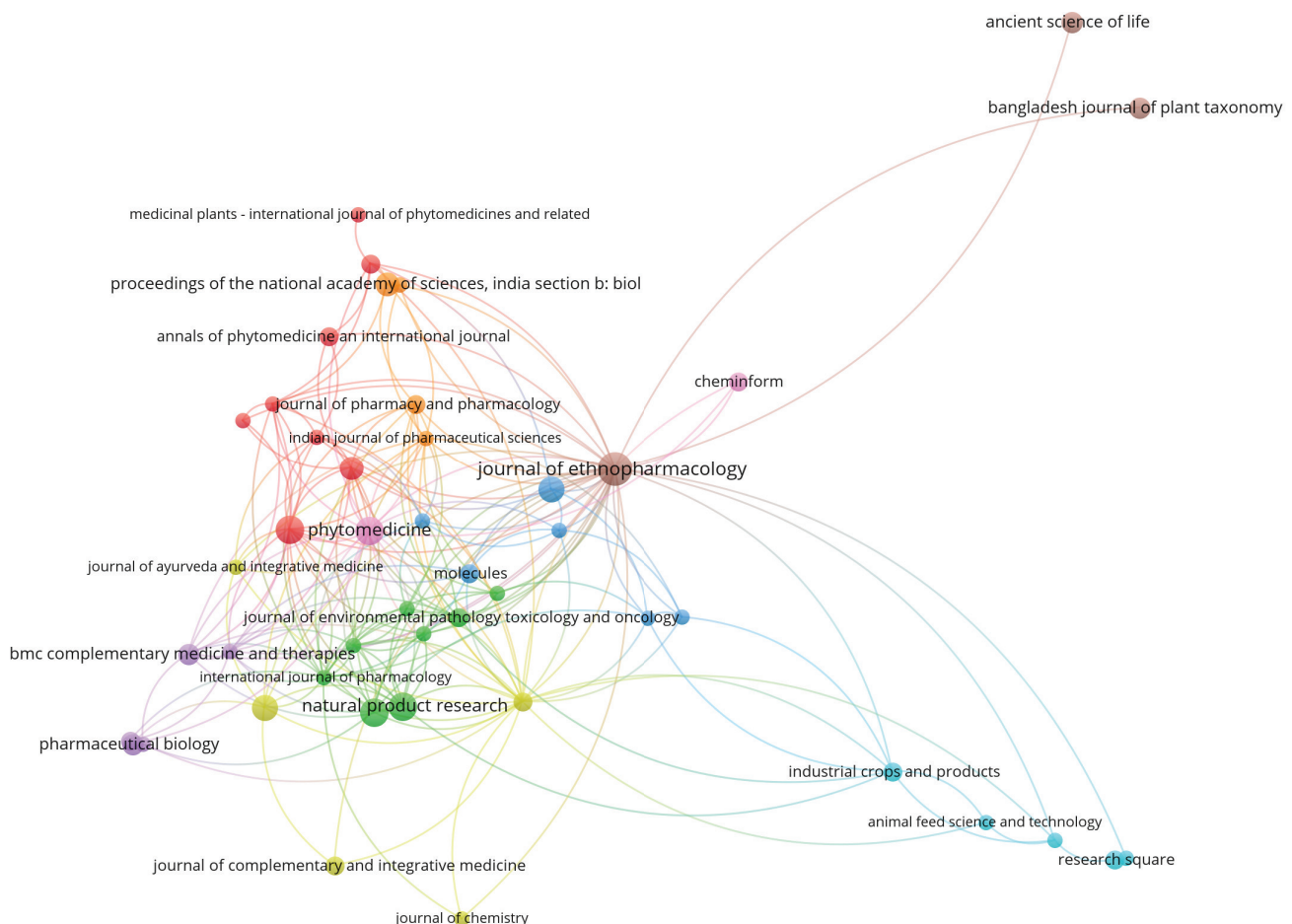
## Journal-wise Collaboration Network

The analysis of journal-wise collaboration within the field of *T. arjuna* research was conducted using a comprehensive dataset comprising 573 journals. This dataset was meticulously examined to reveal 194 interconnections among 46 journals through

citation analysis. For a journal to be included in this collaborative network, it had to meet stringent criteria: publishing at least 3 documents on *T. arjuna*, each receiving a minimum of 3 citations. The analysis identified 9 distinct clusters within the network, each representing a group of journals with strong interconnections. Clusters 1 and 2 emerged as the largest, each containing 8 journals, while

cluster 3 consisted of 6 journals. Clusters 4, 5, and 6 each comprised 5 journals, and clusters 7, 8, and 9 were smaller, containing 4, 3, and 2 journals, respectively (Fig. 7). The "Journal of Ethnopharmacology" was identified as the most influential journal within this network, showing the highest connection strength at 100. Additionally, "Journal of Traditional and Complementary Medicine" and "Phytomedicine" were also remarkable for their significant influence, with total

link strengths of 54 and 35, respectively. This network analysis provides valuable insights into the collaborative dynamics and thematic strengths among journals focused on *T. arjuna* research. The identification of key journals and their respective clusters not only highlights the hubs of activity and influence but also suggests potential avenues for future collaborative efforts and research focus within the *T. arjuna* scholarly community.

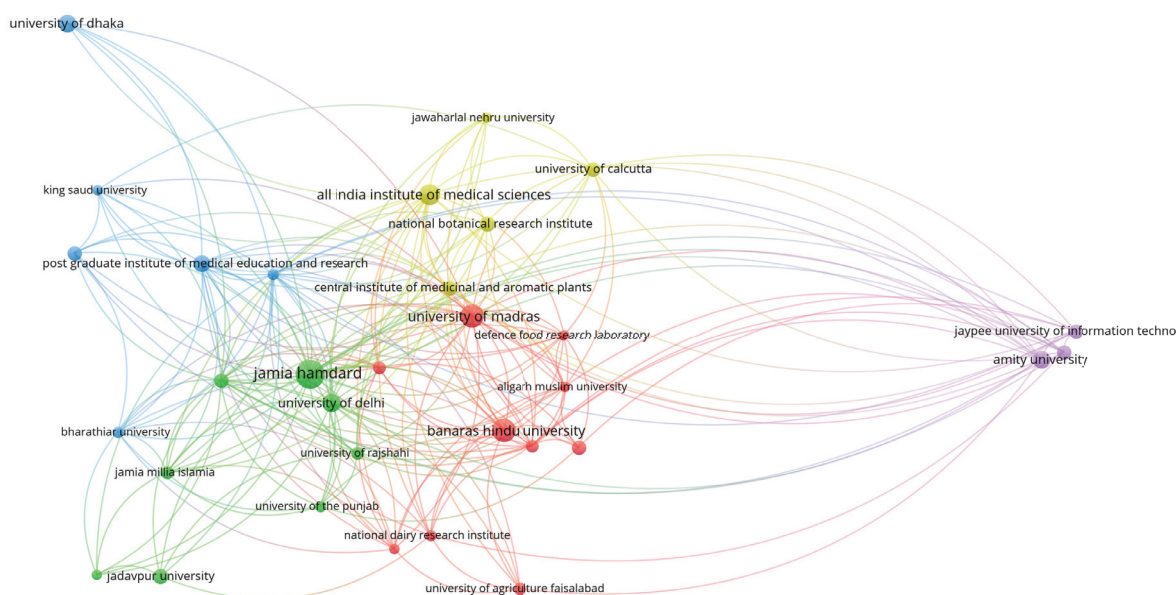


**Fig. 7** Journal-wise collaboration network analysis on *T. arjuna* research

## Organizational Collaborative Network

The study investigated the collaborative network among organizations involved in *T. arjuna* research through citation analysis, focusing on how these entities interact and connect. The analysis covered 712 organizations, out of which 32 met the criteria for inclusion: each had to publish at least 5 documents and receive at least 5 citations per document on *T. arjuna* research. The resulting network identified 225 connections among these 32 organizations, which were grouped into 5 distinct clusters (Fig. 8). Cluster 1, as largest cluster comprised of 10 organizations and demonstrated significant inter-organizational cooperation, indicating a highly collaborative environment. Clusters 2 and 3 included 8 and 6 organizations respectively, each showing intense connections within their groups, suggesting focused and possibly specialized research collaborations. Clusters 4 and 5 were smaller, consisting of 5 and 3 organizations respectively,

potentially indicating more specialized or emerging areas of collaboration. A key finding was the central role of certain organizations within this network. "Jamia Hamdard" emerged as the most influential, with the highest overall connection strength and 143 links. Subsequently, "All India Institute of Medical Sciences" with 113 connections and the "University of Madras" with 93 connections, showed their substantial contributions and influence within the research network. Understanding this collaborative network is crucial for fostering effective partnerships and advancing *T. arjuna* research. By recognizing the central and influential players within this network, researchers and institutions can better navigate the collaborative landscape, identify potential partners, and create more synergetic and productive research environments. This study provides a roadmap for improving cooperation, driving research forward, and ultimately making significant advancements in *T. arjuna* research.

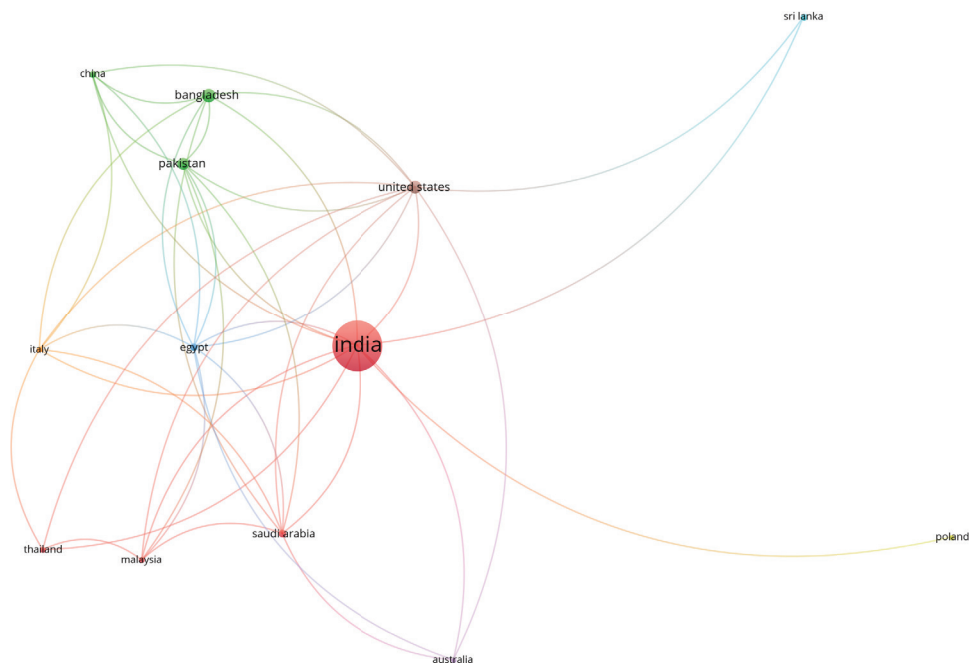


**Fig. 8** Clusters of collaborative institutions for *T. arjuna* research

## Country-wise Collaboration Network

The country-wise collaboration network analysis in *T. arjuna* research offers a comprehensive look at the global interconnectedness and cooperative efforts among nations engaged in this specific field of study. The study encompassed 13 countries, which together formed 42 collaborative connections. To be included in this network, each country was required to have published at least 5 documents on *T. arjuna*, each document accruing a minimum of 5 citations, ensuring that only impactful and relevant research contributions were considered. The meticulous citation analysis identified 8 distinct clusters of collaboration, indicating varying degrees of interconnectedness and research focus. The clusters 1 and 2, containing 4 and 3 countries respectively, showcased significant

collaborative activities. Subsequently, clusters 3 through 8, each consisting of a single country, indicated more insular but potentially focused research efforts. The high point of the analysis is the connection strengths of India and the United States, which exhibited 612 and 249 links respectively. These figures highlight the dominant roles these countries play in the global *T. arjuna* research landscape (Fig. 9). Overall, this analysis emphasizes the importance of international collaboration in enhancing our understanding of *T. arjuna*. It illuminates how global partnerships can facilitate the sharing of knowledge, techniques, and resources, thereby advancing research efforts. For researchers, institutions, and policymakers, these findings offer valuable insights into the dynamics of scientific collaboration, emphasizing the need to foster and support international research networks to drive innovation and deepen scientific understanding in *T. arjuna* research.



**Fig. 9** Country-wise collaboration network in *T. arjuna* research

## Conclusion

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The bibliometric analysis conducted on the research landscape of *T. arjuna* from 2000 to 2023 provides a comprehensive overview of the global scholarly engagement with this significant medicinal plant. Over the examined period, 872 scholarly articles were published involving 2954 researchers from 712 organizations across 38 countries, highlighting the widespread academic and practical importance of *T. arjuna*. Temporal evolution and growth analysis represented an outstanding surge in publications, with the highest number recorded in 2021, indicating a growing interest and investment in *T. arjuna* research. Similarly, citation analysis demonstrated peaks in interest in 2009, emphasizing the impact and influence of research output during certain periods. Country-wise publication analysis identifies India as a leader in *T. arjuna* research, both in terms of publication volume and citation count. The dominance of Indian research emphasized the country's significant role in advancing knowledge about this medicinal plant. The contributions of individual authors are also noteworthy, with Kamal Dev, Prem Kumar Khosla, Vikas Kumar, and Anuradha Sourirajan emerging as prominent figures in

the field, each having authored 8 documents with considerable citation counts. The research findings have been disseminated widely through esteemed journals, with the 'Journal of Ethnopharmacology' emerging as a hub for *T. arjuna* research. Among the organizations, Jamia Hamdard from India stands out as the most productive, contributing the highest number of scholarly articles and significant citations. Researchers have shown considerable interest in *Terminalia arjuna*, as evidenced by the 774 documents dedicated to this topic. Furthermore, collaborative networks among authors, journals, organizations, and countries explored the research quality, increase impact, facilitate resource sharing, and promote innovation by combining diverse expertise and perspectives. Overall, this bibliometric analysis offers valuable insights into the global research landscape of *T. arjuna*, highlighting its significance and the collaborative efforts driving advancements in understanding and utilizing this medicinal plant. As research continues to evolve, this analysis serves as a foundation for further exploration and innovation in the field of *T. arjuna* research.

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CHAPTER  
**03**

# Soil Properties





## INTRODUCTION



Soil is a complex matrix generated by the weathering of rocks that compose the Earth's crust's outer layer. It is generated through the dynamic interplay of minerals, organic matter, air, water, and other living species. It promotes plant growth due to its nature and the availability of nutrients. Plant development and yield are enhanced when the soil is nutrient rich (The State of Queensland, 2013). Soils, in addition to serving as a medium for plant growth, play an important role in gaseous exchange by absorbing and releasing gases such as carbon dioxide, oxygen, and methane. They can also hold water, which is an important resource for plant growth. Soils are host to various living organisms such as animals, fungi, bacteria, and other organisms because of their qualities. As a result of the presence of numerous living creatures, organic matter is constantly recycled through breakdown and accumulation. Soil is also a valuable basis for construction material from a commercial perspective (Soil Science Society of America, 2023).

Soil is made up of biotic and abiotic constituents, which comprise live creatures as biotic factors and minerals, organic matter, gas, and water as abiotic ones. All the components are responsible for plant growth and serve a significant role in preserving soil quality (Needelman, 2013). The percentages of each component vary amongst soil types, such as sandy, clayey, loamy, silty, and so on. The quantities of distinct components in soil are determined by the sites where the soil is available and the parent material from which it forms. The area where the soil is available and the parent material from which it forms determines the respective proportions of individual components in the soil. For instance, there are many different types of soils available in India. The first type is the alluvial soil which covers 46% of the Indian soil area. Rocks of the Himalayas are the parent material, and this kind of soil formation occurs by silt deposition by Indo-Gangetic-Brahmaputra Rivers. The next kind is the black soil which is formed from volcanic rocks in the Deccan Plateau. These soils are rich in clay and cover 16.6% of the total soil cover in India. The other types of soil include red and yellow soil, desert soil, laterite soil, peaty and marshy soil, alkaline soil, and mountain soil (Bhattacharyya et al., 2013).



The percentage composition, quantity, and quality of the components or nutrients in the soil all affect how fertile it is. The fertility of the soil in turn determines the type of crop that can grow in it and its yield and nutritional parameters. Ogundola et al., (2021) found different concentrations of essential oil from the shoots of *Solanum nigrum* L. that were grown in different types of soil. Due to the change in the oil content of the shoot, the free radical scavenging activity varied. It was reported in the study that plants grown of clay loam soil showed the highest radical scavenging activity. Similar results were obtained from extracts of plant shoots grown on clay loam soil for ABTS radical scavenging. Andrade et al., (2011) also reported a similar phenomenon in the case of *Piper dilatatum* Rich. They showed that the essential oil composition of plants collected from different regions of Amazon, Brazil during rainy seasons varied due to environmental variations including variations in soil type. Jimoh et al., (2019) reported that *Amaranthus caudatus* L. exhibited the highest phytochemical yield when grown in clayey loam soil. A similar thing was replicated for the antioxidant activity of the plant extract. Zargoosh et al., (2019) showed that the interaction of site and elevation played a significant effect on the antioxidant potential and total phenol content in *Scrophularia striata* Boiss. The antioxidative capacity was indirectly correlated to elevation, phosphorous, potassium, organic carbon, organic matter, and nitrogen levels for the first site. Similarly, the acidity content had the highest negative correlation with antioxidant capacity. The lime and sand percentage of soil was also negatively related to the phytochemical content.

It has previously been demonstrated that a plant's environment has an impact on the number of secondary metabolites it produces and, ultimately, on the phytochemical composition of that plant. (Walker et al., 2001; Srivastava and Shym, 2002; Dorri et al., 2009). The temperature and the humidity of the area in which a plant grows affect the phytochemical profile of the plant (Fox et al., 1999). How the environment regulates phytochemicals composition and quantity of plants is yet to be completely understood. Yet theories state that environmental factors may influence metabolic processes in plants which thereby affects the phytochemical content. At different altitudes, even the same plant shows different growth characteristics due to differences in light intensity, exposure to radiation, wind speed, humidity, water content in soil, and nutrient content in soils. The microbial composition of the soil may also affect the plant phytochemicals. Makgato et al., (2020) showed that inoculation of beneficial soil microbes like *Rhizobium* into soil can also stimulate plant phytochemical content. They showed that total phenolic

and flavonoid content increased because of *Rhizobium* inoculation in soil as compared to control. This occurred even though plant biomass and nitrogen fixation did not show any increase. Similarly, Egamberdieva et al., (2015) have reviewed in their book that plant growth promoting rhizobacteria helps plants by improving their salt and heavy metal tolerance, preventing plant diseases, and boosting soil fertility. In the case of medicinal plants specifically, they can enhance the phytochemical levels by inducing secondary metabolite production. It is thus beneficial to enrich soils with beneficial microbes which can further be utilized for commercial purposes. Thus, it is evident that environmental variations including humidity, temperature, radiations as well and soil microbiomes influence the secondary metabolite production of medicinal plants and hence their phytochemical production. These attributes are beneficial to understanding plant physiology and the best possible conditions for the cultivation of medicinal plants. In the current study, emphasize on studying the physicochemical variations in soil quality from different altitudes of sampling sites is given in Table 2. Soil physicochemical parameters like pH, electrical conductivity, organic carbon content, available nitrogen, phosphorous, potassium, sulphur and micronutrients, total moisture content, heavy metal, and bulk density have been studied to understand the quality at different altitudes and how they may influence vegetation in the different sites.

## Sampling Sites

The bulk soil and plants (along with the rhizospheric soil) were collected from a total of 26 sites. The numbering of the sites, name of sites along coordinates are given in Table 1.

**Table 1** Study sites and coordinates

S.No.	States	Site	Site Code	Latitude	Longitude
1.	Uttarakhand	Gomukh	UK -S1	30.56790	79.03081
2.		Gangotri	UK-S2	31.03920	78.74655
3.		Uttarkashi	UK-S3	30.74619	78.48377
4.		Devprayag	UK-S4	30.1358	81.1218
5.		Haridwar	UK-S5	29.9169	81.5315

S.No.	States	Site	Site Code	Latitude	Longitude
6.	Uttar Pradesh	Bijnor	UP-S1	29.2857	82.3243
7.		Narora	UP-S2	28.1465	83.1315
8.		Badaun	UP-S3	27.9394	83.5612
9.		Farrukhabad	UP-S4	27.4104	84.496
10.		Bithoor	UP-S5	26.6159	85.1242
11.		Dalmau	UP-S6	26.3485	85.5979
12.		Prayagraj	UP-S7	25.2537	86.2076
13.		Mirzapur	UP-S8	25.8588	87.0908
14.		Varanasi	UP-S9	25.1518	87.396
15.		Ballia	UP-S10	25.3546	87.9107
16.	Bihar	Revelganj	BH-S1	25.4388	88.2213
17.		Patna	BH-S2	25.3808	88.3809
18.		Barh	BH-S3	25.2311	88.3895
19.		Bahachoki	BH-S4	25.1782	88.1959
20.		Farka	BH-S5	25.2323	78.8535
21.	Jharkhand	Sahibganj	JH-S1	25.1461	79.6282
22.	West Bengal	Farraka	WB-S1	24.8227	80.277
23.		Hazarduari	WB-S2	23.9969	81.1218
24.		Mayapur	WB-S3	23.4129	81.5315
25.		Hoogly	WB-S4	22.8491	82.3243
26.		Gangasagar	WB-S5	22.1774	83.1315

The temperature of a particular sites is dependent on its altitude as well as climate conditions. At the time of sample collection, the temperature of the sites as well as altitude of the sites as shown in live GPS map via GPS map camera app was noted down. The altitude and temperature of different sites for sample collection are shown in the Table 2.

**Table 2** Temperature and altitude of different sampling sites

Site	Temperature (°C)	Altitude (m)
UK -S1	09	3794
UK -S2	17	2506
UK -S3	18	1232
UK -S4	23	1014

Site	Temperature (°C)	Altitude (m)
UK -S5	34	265
UP-S1	29	217
UP-S2	31	179
UP-S3	25	162
UP-S4	27	136
UP-S5	28	113
UP-S6	31	91
UP-S7	33	91
UP-S8	32	91
UP-S9	38	65
UP-S10	34	67
BH-S1	25	69
BH-S2	25	36
BH-S3	28	30
BH-S4	30	18
BH-S5	25	23
JH-S1	25	17
WB-S1	28	32
WB-S2	30	14
WB-S3	27	8
WB-S4	30	9
WB-S5	31	3

## Soil Analysis

### Physicochemical Analysis of Soil

#### Total Moisture Content (%)

Soil moisture content, often known as water content, is an indication of the quantity of water in the soil. Moisture content is stated as a proportion of the mass of water contained in the pore spaces of soil to the solid mass of particles in that substance. The mass of the sample is determined using a reference temperature of  $110 \pm 5^\circ\text{C}$ . Almost, all soil tests

detect the natural moisture content of the soil, which is critical knowledge for all soil mechanics. The natural moisture content indicates the condition of the soil in the field (Hossain et al., 2022).

**Apparatus and equipment required:** non-corrodible vented container, thermostatically controlled drying oven that maintains temperatures between  $105^\circ\text{C}$  to  $115^\circ\text{C}$ , Balance of sufficient sensitivity (sensitive to 0.01 g) and container handling apparatus.

### Procedure

1. Clean, dry and weigh  $W_1$  the container. The balance needs to be tared before it is used to measure the weight.
2. Weigh  $W_2$  a sample of the specimen in the container.
3. Keep the container in the oven for 24 hours. Dry the specimen to a constant weight, maintaining the temperature between  $105^{\circ}\text{C}$  to  $115^{\circ}\text{C}$ . (The time will vary with the type of soil, but 16 to 24 hours is usually sufficient.)
4. Record the final constant weight  $W_3$  of the container with the dried soil sample. Peat and other organic soils should be dried at a lower temperature (approximately  $60^{\circ}\text{C}$ ) for a longer period.

### Calculations

1. Weight of the container =  $W_1$  g
2. Weight of the container + Weight of the wet sample =  $W_2$  g
3. Weight of the container + Weight of the dried sample =  $W_3$  g
4. Weight of water in the soil sample =  $W_2 - W_3 = M_w$  g
5. Weight of the dry soil =  $W_3 - W_1 = M_s$  g
6. Moisture content in the given soil sample =  $(M_w \text{ g} / M_s \text{ g}) \times 100\%$

### Bulk Density

Bulk density is a commonly measured soil property by agriculturalists and engineers. High bulk density soils are soils with little pore space, so water infiltration is reduced, root penetration is inhibited, and aeration is restricted – reducing agricultural productivity.

Low bulk density soils are easily compacted and may settle considerably to the detriment of roads, sidewalks, and building foundations (Bowen, 2016).

**Apparatus and equipment required:** Top load balance, soil spatula, 100ml graduated measuring cylinder, 2x50 ml beaker, paper towels and mud bucket.

### Procedure

1. Add slightly more than 50 ml of the soil sample to 50 ml beaker.
2. Clean and thoroughly dry a 100 ml graduated cylinder. Weigh and record weight (A).
3. Slowly add soil sample to pre-weighed graduated cylinder to the 10 ml line. Compact the soil by dropping onto a padded surface like a book, notebook, etc. at least ten times from a height of about 2-3 inches.
4. Repeat this process in 10 ml intervals until you reach the 50 ml mark.
5. Use a soil spatula to level the top of the sample in the graduated cylinder and add soil with the spatula until the top of the soil sample is exactly even with the 50 ml line – this is the bulk volume of compacted soil (B) ( $1 \text{ ml} = 1 \text{ cm}^3$ ).
6. Weigh and record graduated cylinder plus compact soil weight (C).
7. After drying the beaker, place any soil sample that is still in it back into the sample storage container.
8. Return 50 ml sample in graduated cylinder to 50 ml beaker. Remove all of sample within graduated cylinder.

- Slowly pour approximately 25 ml of soil sample from beaker into water in the graduated cylinder. Gently stir soil/water mixture to remove any air bubbles. Add the second 25 ml of soil sample and stir again to remove air bubbles.

### Calculations

- Weight of 100 ml graduated cylinder = A
- Wt. volume of the compacted soil = B
- Weight of cylinder + compacted soil = C
- Weight of soil sample = C-A= D
- Bulk density (g/cm<sup>3</sup>) = D/B

### pH

**Principle:** The pH of sample is measured with a pH meter, in which the potential of a hydrogen ion indicating electrode (glass electrode) is potentiometrically measured against a calomel saturated reference electrode, which also functions as a salt bridge. Most pH meters now contain a single integrated electrode. The equipment must be calibrated with a standard buffer solution of known pH before measuring the pH of the soil. As temperature affects pH, the pH meter is set to according to the

temperature of the solution (Varley, 1972; Jackson, 1973).

**Reagents:** Buffer solutions (pH 4.0, 7.0, and 9.2)

**Equipment required:** A balance, 100 ml beaker, measuring cylinder, glass rod, pH meter and ordinary tissue paper.

### Procedure

- 25 g of the soil was weighed in a 100 ml beaker and make it to 50 ml final volume by adding of distilled water. The mixture was stirred well for at least four times within a 30-minute period to allow the soil and water to reach equilibrium.
- In the meantime, the pH meter was switched on.
- Initially the instrument was calibrated with buffer solution of known pH 4, 7.0, and 9.2.
- The electrodes were washed with distilled water and wiped dry with a tissue paper.
- Then, the electrode was dipped in the sample and the readings were taken.
- Finally, the electrodes were washed with distilled water and placed back into a beaker containing 4.00-7.00 pH buffer or 3-4M KCl.

### Interpretation

pH	Category	Soil Rating/Recommendation
<6.5	Acidic	Requires liming for reclamation
6.5-8.7	Normal	Optimum for most crops
8.8-9.3	Alkaline	Requires application of organic manures
>9.3	Alkali (Sodic)	Requires gypsum for amelioration

## Electrical Conductivity

**Principle:** A conductivity meter known as “Solu Bridge” is used to measure the electrical conductivity of a soil solution. It is based on the Wheat Stone Bridge principle, in which alternating current is utilized instead of direct current to prevent electrode polarization and electrolysis of the solution. In a branched circuit with the conductance cell having resistance  $R_x$ , two fixed resistances  $R_1$  and  $R_2$  and a variable resistance ( $R_v$ ) are linked. The variable resistance ( $R_v$ ) is adjusted until no current flows through it and the reading is taken. The resistance or conductance ( $R_v$ ) is measured (Chopra and Kanwar, 1976; Richards, 1954).

### Interpretation

EC (1:2 soil water; $\mu\text{S}/\text{m}$ )	Soil Rating
Below 800	Normal
800 - 1600	Critical for salt sensitive crops
1600 - 2500	Critical for salt tolerant crops
Above 2500	Injurious to all crops

## Organic Carbon

**Principle:** In the presence of concentrated sulphuric acid, a known weight of soil was treated with an excess of standard potassium dichromate solution. The heat of the sulphuric acid gently digests the soil at a low temperature, oxidizing the organic carbon in the soil to  $\text{CO}_2$ . The excess potassium dichromate was titrated against a standard solution of ferrous ammonium sulphate in the presence of a diphenylamine indicator and sodium fluoride or phosphoric acid, which distinguishes the

**Apparatus and equipment required:** Weighing balance, 100 ml beaker, measuring cylinder, glass rod and conductivity meter.

### Procedure

- 25 g of the soil samples was taken in a 100 ml beaker.
- Added 50 ml distilled water.
- Intermittent stirring was done with a glass rod for 30 minutes.
- The samples were left overnight to obtain a clear supernatant.
- The conductivity of the supernatant liquid was determined with the help of a conductivity meter.

colour due to their flocculating effect. The hue of the suspension varies from violet to blue to vivid green at the terminal point. (Walkley and Black, 1934; Jackson, 1973).

### Chemicals and reagents used

- Potassium dichromate solution (1 N  $\text{K}_2\text{Cr}_2\text{O}_7$ ): 49.04 g of analytical grade  $\text{K}_2\text{Cr}_2\text{O}_7$  was dissolved in distilled water and the volume was made up to 1 litre.
- Concentrated sulphuric acid ( $\text{H}_2\text{SO}_4$ ).
- Orthophosphoric acid (85%) or sodium fluoride (NaF).

4. Diphenylamine indicator: 0.5 g diphenylamine indicator was dissolved in a mixture of 100 ml concentrated sulphuric acid and 20 ml distilled water. This was stored in an amber colour bottle.
5. Ferrous ammonium sulphate solution [0.5 N,  $\text{FeSO}_4(\text{NH}_4)_2\text{SO}_4 \cdot 6\text{H}_2\text{O}$ ]: 392 g analytical grade ferrous ammonium sulphate was dissolved in distilled water, to which 15 ml concentrated sulphuric acid was added and volume made up to 2 litre with distilled water.
5. After cooling, about 0.5 g of NaF or 5 ml orthophosphoric acid, 100 ml of distilled water and 10 drops of diphenylamine indicator solution were added. These were shaken vigorously for complete mixing.
6. This was titrated against N/2 ferrous ammonium sulphate solution till the colour changes from violet to bright green through blue.
7. The volume of ferrous ammonium sulphate solution used for titration was noted down every time.
8. A blank titration was carried out without any soil.

**Apparatus required:** 250 ml Erlenmeyer (Conical) flask, pipette, burette and measuring cylinder.

### Procedure

This process is also known as Walkley and Black's rapid titration method (1934).

1. 2 gm of dried, ground, and sieved soil was taken in a 250 ml conical flask.
2. To it, 10 ml 1 N  $\text{K}_2\text{Cr}_2\text{O}_7$  solution was added and shaken gently to mix the contents.
3. Next, 20 ml of concentrated sulphuric acid was added while swirling the flask slowly as the reaction is exothermic and a lot of heat is produced.
4. The flask was kept on a dry tile or asbestos sheet for 30 minutes and left to attain room temperature.

### Calculations

1. Weight of soil taken (W) = 2 g
2. Vol. of N/2 ferrous ammonium sulphate used for blank titration = X ml
3. Vol. of N/2 ferrous ammonium sulphate used to titrate excess nascent oxygen = Y ml
4. Vol. of 1N  $\text{K}_2\text{Cr}_2\text{O}_7$  used for oxidation of organic carbon in soil =  $(X-Y)/2$  ml
5. 1 ml of 1N  $\text{K}_2\text{Cr}_2\text{O}_7$  = 0.003 g C
6. % Organic Carbon in Soil =  $(X-Y)/2 \times 0.003 \times 100/W$

### Interpretation

Organic Carbon (%)	Soil Rating
<0.4	Low
0.4-0.75	Medium
>0.75	High

## Available Nitrogen

**Principle:** An excess of alkaline potassium permanganate is applied to a known weight of soil, extracting a relatively easily oxidizable fraction of organic nitrogen. Ammonia is absorbed in excess of boric acid, forming ammonium borate, which is titrated against standard acid to determine the amount of ammonium absorbed (Subbaiah, 1956).

### Chemicals and reagents required

1. Potassium permanganate solution ( $\text{KMnO}_4$ ): 3.2 g/l of potassium permanganate was dissolved in distilled water and the volume was made up to 1 litre.
2. Sodium hydroxide solution ( $\text{NaOH}$ ): 25 g/l of sodium hydroxide pellets were dissolved in distilled water and the volume was made up to 1 litre.
3. 0.02 N sulphuric acid ( $\text{H}_2\text{SO}_4$ ).
4. Mixed indicator: 0.066 g of methyl red and 0.099 g of bromocresol green were mixed in 100 ml of ethanol.
5. Boric acid: 25 g/l of boric acid was dissolved in one litre of distilled water and to it 40 ml mixed indicator was added. Then, the pH was adjusted to 4.5-5.0 by adding 2.5 %  $\text{NaOH}$ .

**Apparatus and equipment required:** Kjeldahl distillation assembly, measuring cylinder, burette, balance, pipettes, and 100 ml conical flask.

### Procedure

1. The Kjeldahl assembly was prepared by dipping the respective inlet tubes, one in

0.32%  $\text{KMnO}_4$  and second in 2.5%  $\text{NaOH}$  reagent tanks.

2. 5 g soil sample was weighed and put into distillation tube carefully so that the soil sample does not stick to the sides of the tubes. Soil particles attached to the sides of the tube were washed down with distilled water.
3. The distillation tube was fixed in distillation unit and the sample was moistened with distilled water by pressing the dilution key.
4. 25 ml of 2.5% boric acid was taken in a conical flask and the receiving end of the distillation tube was dipped in it.
5. Next, 25 ml of  $\text{KMnO}_4$  and 25 ml  $\text{NaOH}$  were added in the sample by pressing the respective keys.
6. The heating unit was switched on, and the process ran for 6 minutes.
7. The ammonia gas released from the sample was distilled and collected into the receiver containing acid. After complete digestion, the receiver flasks were removed.
8. The ammonium borate formed in the receiver flask was titrated against 0.02 N  $\text{H}_2\text{SO}_4$  acid and note the volume of 0.02 N  $\text{H}_2\text{SO}_4$  utilized. The colour changes from bluish green to wine red.

### Calculations

1. Weight of soil taken = 5 g
2. Vol. of 2.5% boric acid taken = 25 ml
3. Vol. of 0.02 N  $\text{H}_2\text{SO}_4$  used to titrate ammonium borate = X ml

4. 1 ml of 0.02 N H<sub>2</sub>SO<sub>4</sub> = 0.00028 g of N
5. Available N (%) = 0.00028 g of N
6. Available N (ppm) = percentage N × 10,000
7. Available N (Kg/ha) = ppm × 2.24

### Interpretation

Available Nitrogen (kg/ha)	Soil Rating
<272	Low
272-544	Medium
>544	High

### Available Phosphorous

**Principle:** The activity of Ca<sup>2+</sup> in the soil solution and the pH of the soil regulates the solubility of calcium phosphate in it. The bicarbonate (HCO<sub>3</sub><sup>-</sup>) activity in the soil is increased by the 0.5 M NaHCO<sub>3</sub> solution buffered to pH 8.5, which reduces calcium activity. As a result, some phosphate from the surface of calcium phosphate gets dissolved in the soil. Similarly, due to the inactivation of Al and Fe, NaHCO<sub>3</sub> solution removes some phosphorous from Al and Fe phosphates. The precipitation of phosphate released from calcium phosphate is prevented by low Ca<sup>2+</sup> activity. The soluble phosphate forms heteropoly complexes with molybdate ion freed from ammonium molybdate solution when added to the soil extract. (Bray and Kurtz, 1945; Black, 1965).

### Chemicals and reagents required

1. Standard phosphorous solution
  - i. Standard solution of P (100 ppm): 0.4387 g of KH<sub>2</sub>PO<sub>4</sub> was dissolved in distilled water and the volume was made up to 1 litre.
  - ii. Standard solution of P (5 ppm): 5 ml of 100 ppm P solution was diluted to 100 ml with distilled water.

2. 0.5 M NaHCO<sub>3</sub>: 42 g of NaHCO<sub>3</sub> was dissolved in distilled water and the volume was made up to one litre after adjusting the pH to 8.5 with sodium hydroxide using a pH meter.
3. Sulphuric acid (5N): 139 ml of concentrated H<sub>2</sub>SO<sub>4</sub> was diluted to 1 litre.
4. Reagent A: Dissolve 12 g of ammonium paramolybdate in 250 ml distilled water. Separately, 0.2908g of potassium antimony tartrate (KSbO.C<sub>4</sub>H<sub>4</sub>O<sub>6</sub>) was dissolved in 100 ml of distilled water. Both these dissolved reagents were added to 1 litre of 5 N sulphuric acid. Both were mixed thoroughly and diluted with distilled water to 2 litres.
5. Reagent B: 1.056 g of L-ascorbic acid was dissolved in 200 ml of reagent A and mixed. It was prepared freshly before use.

**Apparatus and equipment required:** 100 ml conical flasks, funnels, pipette, (1 and 5 ml), 25 ml volumetric flasks, 100 ml measuring cylinder, electric shaker, Whatman No. 1 filter paper and spectrophotometer.

## Procedure

### a) Preparation of a standard curve

The relationship between the intensity of the coloured solution of a substance and the percent transmittance or absorbance of the light rays flowing through the solution was depicted by a standard curve. It was used to figure out how much of a certain element is present in an unknown sample. 0, 0.5, 1, 2, 3, 4, 5 ml of P solution was taken in seven different 25 ml volumetric flasks to make the standard curve. It will produce a solution with a final concentration of 0, 0.1, 0.2, 0.4, 0.6, 0.8 and 0.1 ppm, respectively. To it, 5 ml of extracting solution and 0.5 ml 5N  $H_2SO_4$  were mixed and shaken well until the evolution of  $CO_2$  stopped. 10ml of distilled water was added while washing the neck of the flask and 4 ml of reagent B and the volume was made up to the mark with distilled water and mixed. All flasks developed a blue colour of variable intensity. The intensity of blue colour was read on spectrophotometer at 880 nm. A standard curve was plotted using P concentration against absorbance value to establish their relationship.

### b) Analysis of the test sample

- 1) 1 g of soil sample was taken in a 100 ml flask.

- 2) To it, a pinch of Darco-G 60 and 20 ml of 0.5 N  $NaHCO_3$  solution were added.
- 3) The flask was placed on an electric shaker and mixed at a constant speed for half an hour. Then the contents were filtered through Whatman No. 1.
- 4) A blank was prepared by following all the steps without addition of soil.
- 5) In case, the filtrate was not clear, a pinch of Darco – G was added.
- 6) 5 ml of the filtrate was taken in a 25 ml volumetric flask and proceed further for colour development as described under preparation of standard curve.

## Calculations

1. Weight of soil sample taken = 1 g
2. Vol. of 0.5 N  $NaHCO_3$  solution added = 20 ml
3. First dilution = 20 times
4. Vol. of the filter taken for colour development = 5 ml
5. First volume made = 25 ml
6. Second volume = 5 times
7. Total dilution =  $20 \times 5 = 100$  times
8. Concentration of P from spectrophotometer = X AU (absorbance units)
9. Available P in soil (ppm) = X (AU)  $\times$  100
10. Available P (Kg/ ha) = ppm  $\times$  2.24

## Interpretation

Available Phosphorus (kg/ha)	Soil Rating
>12.4	Low
12.4-22.4	Medium
22.4-50	High
>50	Very High

## Available Potassium

**Principle:** In a neutral normal ammonium acetate solution, a known weight of soil was shaken. Potassium (K) ions absorbed on soil colloids exchange ammonium ions. The amount of exchangeable and water-soluble potassium in the extract was measured using a flame photometer. The transfer of non-exchangeable K to exchangeable form was hampered during ammonium acetate extraction because ammonium ions, like  $K^+$ , retain strongly charged layers together (Merwin and Peech, 1951; Black, 1965; Jackson, 1973).

### Chemicals and reagents required

1. Neutral normal ammonium acetate solution ( $CH_3COONH_4$ ): 77.09 g/l of ammonium acetate was dissolved in distilled water and the volume was made up to 1 litre. The pH of the solution was adjusted to 7 with ammonium solution or acetic acid.
2. Standard solution of K (1000 ppm K): 1.91 g of potassium chloride (KCl) was dissolved in distilled water and the volume was made to 1 litre.
3. Working standard solution of K: The stock solution was diluted 100 times to get 10 ppm K solution.

### Interpretation

Available Potassium (kg/ha)	Soil Rating
<137	Low
137– 337	Medium
>337	High

**Apparatus and equipment required:** A weighing balance, 150 ml conical flasks, a shaker, funnels, beaker, Whatman filter paper No 1, pipettes and a flame photometer.

### Procedure

1. 5 g of soil was weighed in a 150 ml conical flask.
2. To this, 25 ml of neutral normal ammonium acetate solution was added.
3. The mixture was shaken for 5 minutes on an electric shaker and then filtered through Whatman No. 1 filter paper.
4. 5 ml of the filtered extract was taken in a 25 ml volumetric flask and the volume was made up with distilled water. This solution was fed into the atomizer of the flame photometer and readings were noted down.

The amount of K in the test sample was calculated by using the dilution factor.

### Calculations

1. Weight of soil sample taken = 5 g
2. Volume of the neutral normal  $CH_3COONH_4$  solution added = 25 ml
3. Dilution = 5 times
4. Reading of K (ppm) in flame photometer = Y
5. In ppm K =  $Y \times \text{total dilution} - A$
6. In kg/ha =  $A \times 2.24 - C$

## Available Sulphur

**Principle:** Soil was shaken with 0.15 %  $\text{CaCl}_2$  solution. During extraction, chloride ions displace adsorbed sulphate while calcium ions decrease soil organic matter extraction and hence eliminate contamination caused by extractable organic sulphur. The turbidity produced by the precipitation of sulphate as barium sulphate is measured on a spectrophotometer at a wavelength of 420 nm. The turbidity is stabilized using gum acacia solution, which prevents the barium sulphate formed from settling (Lisle et al., 1994).

### Chemicals and reagents required

1. Extracting solution (0.15%  $\text{CaCl}_2$ ): 1.986 g of calcium chloride dihydrate ( $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ ) was dissolved in distilled water and volume made up to 1 litre.
2. Barium chloride ( $\text{BaCl}_2$ )
3. Gum acacia solution (0.25%): 0.25 g of gum acacia was dissolved in distilled water and diluted to 100 ml.
4. Standard sulphur solution (100 ppm): 0.5434 g of the reagent grade potassium sulphate ( $\text{K}_2\text{SO}_4$ ) was dissolved in distilled water and diluted to 1 litre.

**Apparatus and equipment required:** 150 ml conical flask, funnel, pipettes, 25 ml volumetric flasks, electric shaker, and spectrophotometer and Whatman No. 42 filter paper.

### Procedure

#### a) Preparation of standard curve--:

1. 0.25, 0.5, 1.0, 2.5 and 5.0 ml of 100 ppm S solution were taken in different 25 ml volumetric flasks. It was theoretically

calculated to give 0, 1, 2, 4, 7, 10, 20 ppm concentration of sulphur in the final volume.

2. To every flask 10 ml 0.15%  $\text{CaCl}_2$  solution and 1 g  $\text{BaCl}_2$  were added.
3. Mixed for 1 minute to dissolve all the crystals.
4. Next, 1ml 0.25% solution of gum acacia was added.
5. The volume was made up to the mark for all the flasks and shaken properly for thorough mixing. Within 5-30 minutes after the development of turbidity, the standards were read on a spectrophotometer at 420 nm.
6. 0 absorbance at 0 ppm solution (blank) was adjusted.
7. A standard curve was plotted showing relationship between concentration of S (turbidity) and transmittance/absorbance readings.

#### b) Analysis of test samples

1. 10g air dried soil was weighed and transferred to a 150 ml conical flask.
2. 50 ml of 0.15%  $\text{CaCl}_2$  solution was added, and the mixture was shaken for 30 minutes on an electric shaker.
3. Filter the suspension through Whatman No. 42 filter paper.
4. 20 ml of the filtrate was taken in a 25 ml volumetric flask and the same steps as in case of standard curve were followed.

5. A blank was run with all the chemicals except the soil.
6. The sulphate concentration of unknown samples was determined from the standard curve.
3. First dilution= 5 times
4. Volume of aliquot taken= 20 ml
5. Final volume= 25 ml
6. Second dilution= 1.25 times
7. Total dilution=  $5 \times 1.25 = 6.25$  times
8. ppm of S from standard curve= Y
9. ppm of S in soil=  $Y \times 6.25$
10. S in kg/ha= ppm of S  $\times 2.24$

### Calculations

1. Weight of soil taken= 10g
2. Volume of extractant added= 50 ml

### Interpretation

Available Sulphur (ppm)	Soil Rating
<10 ppm	Deficient
>10 ppm	Sufficient

### Heavy Metal

All the heavy metal i.e. Cr, Ni, As, Sr, Cd, Hg and Pb, are extracted with the help of ICP-MS (Make-Thermo Scientific instrument) (Retka et al., 2010).

#### Procedure

1. Take the sample and homogenize properly.
2. Take approximately 0.1 to 0.2 g sample in microwave vessels and add 4 ml of HNO<sub>3</sub> (Suprapure grade) add 1 ml of H<sub>2</sub>O<sub>2</sub> (Suprapure grade).
3. Kept at room temperature 20-30 minutes for open digestion.
4. Afterwards samples were put in microwave for close digestion.
5. After digestion, samples were transferred into the 50 ml volumetric flasks and volume make up to the mark.
6. Samples were vortexed properly and run of ICP-MS against the Linearity.

### Calculations

Calculate the concentration of the elements as follows.

Sample Conc.= (Sample reading-reagent blank reading  $\times$  dilution factor)/ Sample Wt.

### Micronutrient

**Principle:** All the four micronutrient cations i.e. Zn, Mn, Fe and Cu, are extracted by shaking the soil with DTPA extracting solution containing 0.005M DTPA, 0.1 M TEA (Triethanol amine) and 0.01 M CaCl<sub>2</sub>.2H<sub>2</sub>O buffered at pH 7.3. During this extraction, TEA gets protonated as HTEA<sup>+</sup> because of which micronutrient cations from the solid phase comes into solution and are chelated by the DTPA. Buffering of the extractant in the slightly alkaline pH range and inclusion of soluble Ca<sup>2+</sup> through CaCl<sub>2</sub>.2H<sub>2</sub>O helps avoiding dissolution of CaCO<sub>3</sub> and thus excludes from the estimation of the occluded micronutrients,

which do not form a part of the pool that is available for absorption by plant roots. After that the contents of the micronutrients cations in the soil extract are estimated on atomic absorption spectrophotometer (Katyal and Sharma, 1991).

### Chemicals and reagents required

1. DTPA extraction solution: This solution was prepared to contain 0.005M DTPA, 0.01M  $\text{CaCl}_2$  and 0.1M TEA (Triethanolamine) and its pH was adjusted to 7.3. For preparing one litre of this solution, 13.3 ml  $(\text{HOCH}_2\text{CH}_2)_3\text{N}$  i.e. TEA, 1.967 g DTPA and 1.47 g  $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$  were dissolved in about 500 ml of deionized water. Sufficient time was given for DTPA to dissolve, and the contents are diluted to approximately 900 ml. The pH of the solution was then adjusted to  $7.30 \pm 0.05$  by adding 6N HCL while stirring and finally the contents were diluted to 1 litre. This solution was stable for several months.

**Apparatus and equipment required:** 1 litre volumetric flask, 100 ml narrow mouth polyethylene bottles, pipettes, electric shaker,

Whatman No. 1 filter paper and atomic absorption spectrophotometer.

### Procedure

1. 12.5 g of air-dried soil sample was taken and transferred to a 100 ml narrow mouth polyethylene bottle.
2. 50 ml DTPA solution was added, and the bottle was closed with the stopper.
3. The bottle was shaken on an electric shaker for 2 hours at  $25^\circ\text{C}$ .
4. The contents were then filtered through Whatman No. 42 filter paper to obtain a clear solution.
5. Also, a blank was run with only DTPA solution without the soil.

### Calculations

1. Weight of soil used for extraction = 12.5 g
2. Volume of the extractant used = 50 ml
3. Dilution = 2 times
4. Concentration of the given micronutrient in extract = A mg/kg
5. Available micronutrient in given soil sample =  $A \times 2$  mg/kg

### Interpretation

Content below which the soil is deficient	
Metal	mg/kg soil
Zn	0.6
Cu	0.2
Fe	4.5
Mn	3.5

## Rhizosphere Soil Microbiology

**Principle:** The principle for the analysis of microbe's different media is used such as nutrient agar, Eosin Methylene blue, MacConkey Agar, Azotobacter, Azospirillum, Rhizobium, zinc solubilising, phosphate solubilising and potash mobilizer. The microbial count is usually expressed in CFU (colony-forming units) per gram or millilitre. The direct count method for enumerating bacteria in natural environments is widely used (Kirchman, 1982).

### Procedure

- Different growth medium is used to grow different types of microorganisms is given in Table 3.
- The medium is sterilized in an autoclave.
- Petri dishes are used to hold the growth media.
- A small number of bacteria is needed to inoculate the growth media.
- A 100 µl culture of sample is picked up with the help of pipette and transferred to the growth media and spread with the help of L-shaped spreaders.
- The inoculated growth media is incubated at the optimal temperature and conditions for the bacteria to grow.
- After incubation, the bacterial growth can be observed by looking closely at the colonial growth on the surface of a solid medium.

**Table 3** Lists the various growth media that are used to cultivate the various kinds of microorganisms

Serial No.	Target Organism	Synthetic Media Used	Make
1.	Total Bacterial Count	Nutrient Broth	HiMedia (M002-500G)
2.	Total Fecal Count	MacConkey Agar	HiMedia (M008S-500G)
3.	Total Coliform Count	Eosin methylene Agar	HiMedia (M317-500G)
4.	Potash Mobilizer	Aleksandrow	HiMedia (M1997-500G)
5.	Zinc Solubilizer	Zinc sulphate	HiMedia (M2023-500G)
6.	<i>Rhizobium</i>	Rhizobium	HiMedia (M408-500G)
7.	<i>Azotobacter</i>	Azotobacter	HiMedia (M1944-500G)
8.	<i>Azospirillum</i>	Azospirillum + KOH	HiMedia (M1720-500G + M1720-500G)
9.	Phosphate Solubilizes	Pikovskaya	HiMedia (GM1719-500G)

**Apparatus and equipment required:** Weighing balance, spatula, distilled water, measuring cylinder, and a 1000 ml conical flask.

### Calculation

1. Colony forming Units (CFU/ml) = Number of colonies × Dilution factor/Volume of culture

## Correlation Studies and Statistical Analysis

The tests have been performed in triplicates and the mean of values along with the standard deviation has been represented graphically. A total of 8 parameters have been studied for 26 sites and each parameter has shown considerable variation. The effect of one parameter on the other may be studied through correlation. A correlation coefficient is an indicator of the relationship between two variables. The correlation coefficient is a statistical measure that indicates the strength and direction of the relationship between

two variables. Its value ranges from -1 to +1, representing different degrees and types of correlation. When the correlation coefficient is closer to +1, it signifies a strong positive relationship between the variables. This means that as one variable increases, the other tends to increase as well. A correlation coefficient around 0 implies no linear relationship between the variables. Changes in one variable do not predict or affect changes in the other. As the correlation coefficient approaches -1, it denotes a strong negative relationship. This suggests that as one variable increases, the other tends to decrease.

## Results And Discussion

### Physicochemical Characterization of Soil

Moisture content is one of the most essential index qualities for determining the relationship between soil behaviour and index values. Soil moisture content expresses the phase

relationships of water, air, and solids in each volume or weight of material. The consistency of a specific soil, combined with its liquid and plastic limitations, is used to represent its relative consistency in cohesive soil (Hossain et al., 2022). The moisture content of different sampling sites is shown in Fig. 1

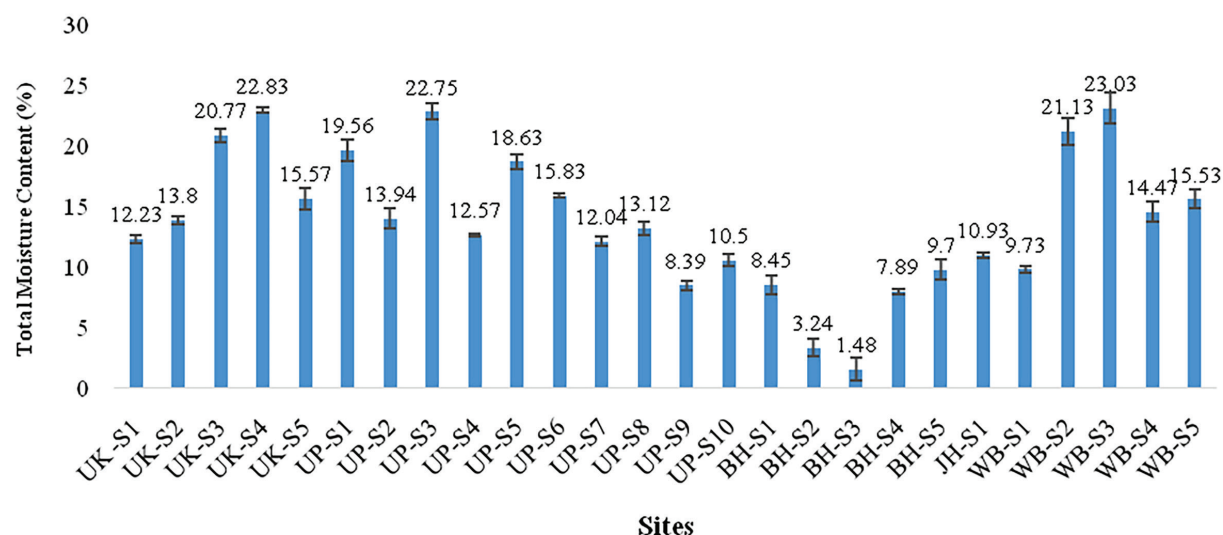
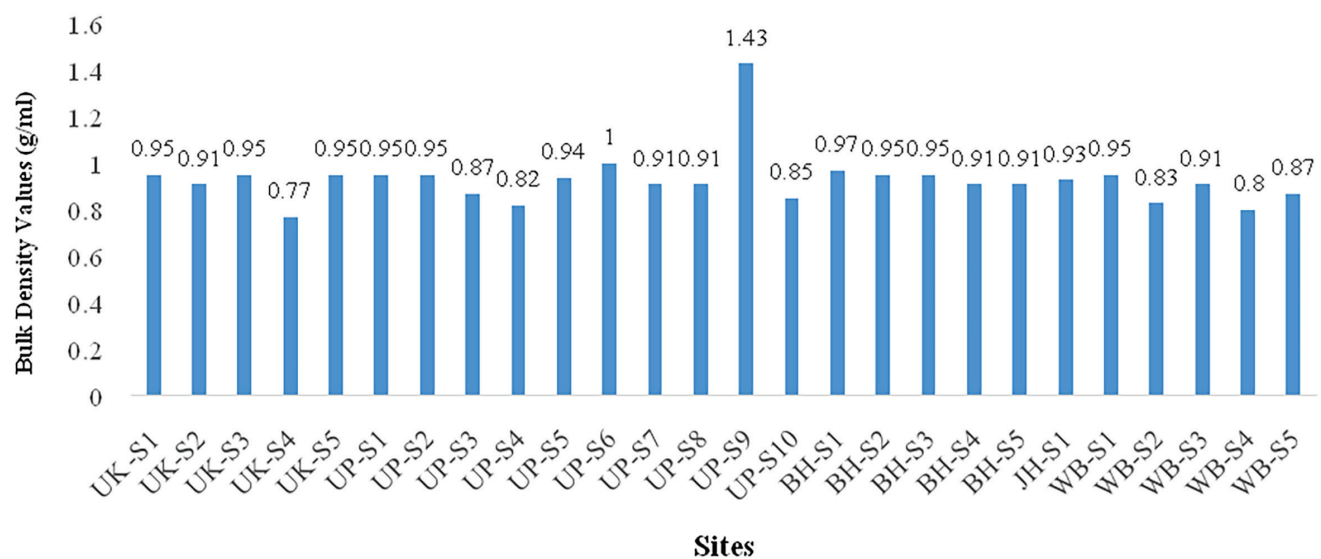


Fig. 1 Total moisture content of different sampling sites

The TMC% was found to be in the range of  $1.48 \pm 0.94\%$  (lowest) to  $23.03 \pm 1.25\%$  (highest). Soil sample from BH-S3 had very little moisture content i.e.,  $1.48\% \pm 0.94$ . Seven of the soil samples had moisture content below 10%, while in most samples, moisture content was found to be within 20%. Sample from five sites had their TMC% over 20%.

Soil bulk density was another characteristic investigated. It is a measure of how thick or

firmly packed the soil is. The composition of the soil, the structure of the soil ped, the distribution of sand, silt, and clay particles, the volume of pore space, and how densely the particles are packed all influence soil bulk density. Bulk density indicates how easily roots can develop and water can filter through a profile's multiple soil strata (The Globe Program). The soil bulk density of different sampling sites is illustrated in Fig. 2

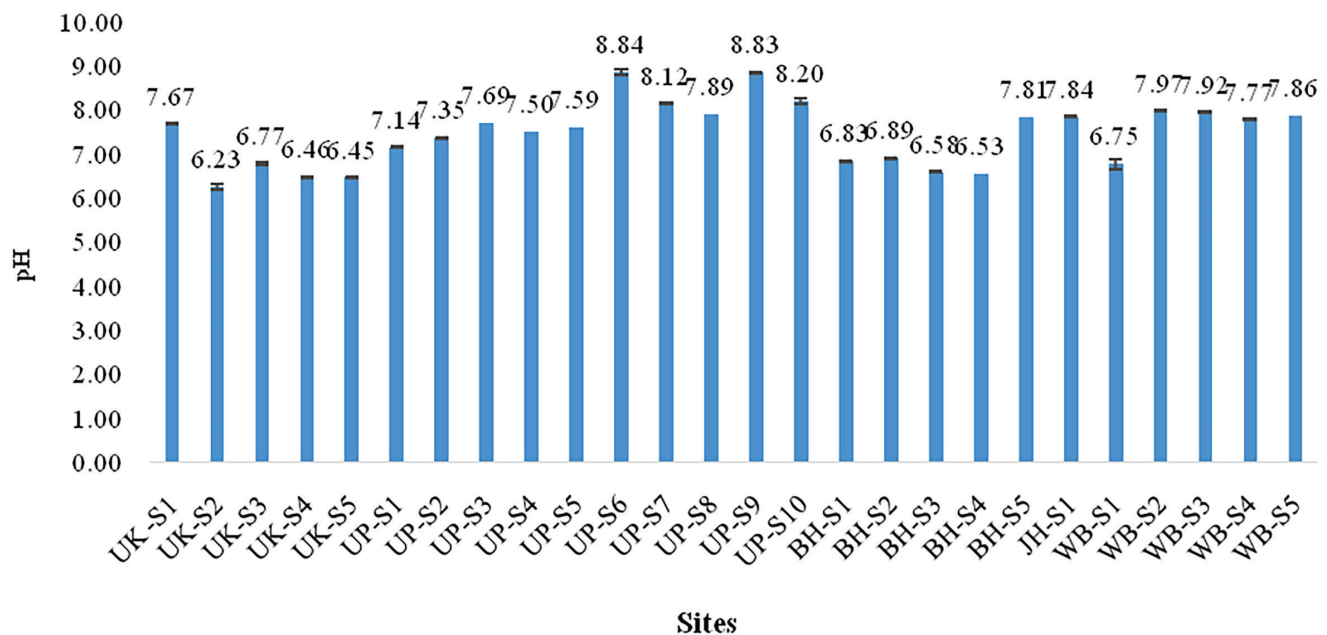


**Fig. 2** Soil bulk density of different sampling sites

From the above data it was deduced that soil bulk density ranged from 0.77 g/ml to 1.43 g/ml. Soil bulk density of UP-S9 was highest i.e. 1.43 g/ml but that of UK-S4 was lowest i.e. 0.77 g/ml. Six of the sites had their bulk density below 0.90 g/ml while the remaining sites had their bulk density values above 0.90 g/ml.

The pH of soil is an important physical attribute that determines species richness

and density (Gough et al., 2000). This happens as soil pH determines the amount of nutrients that are soluble in soil water and their availability to plants. Some nutrients are more available under acidic conditions while some are available under alkaline condition. However extreme condition of acidity or alkalinity are harmful for the soil as well as plant growth. The pH of the different sampling sites is shown in Fig. 3.

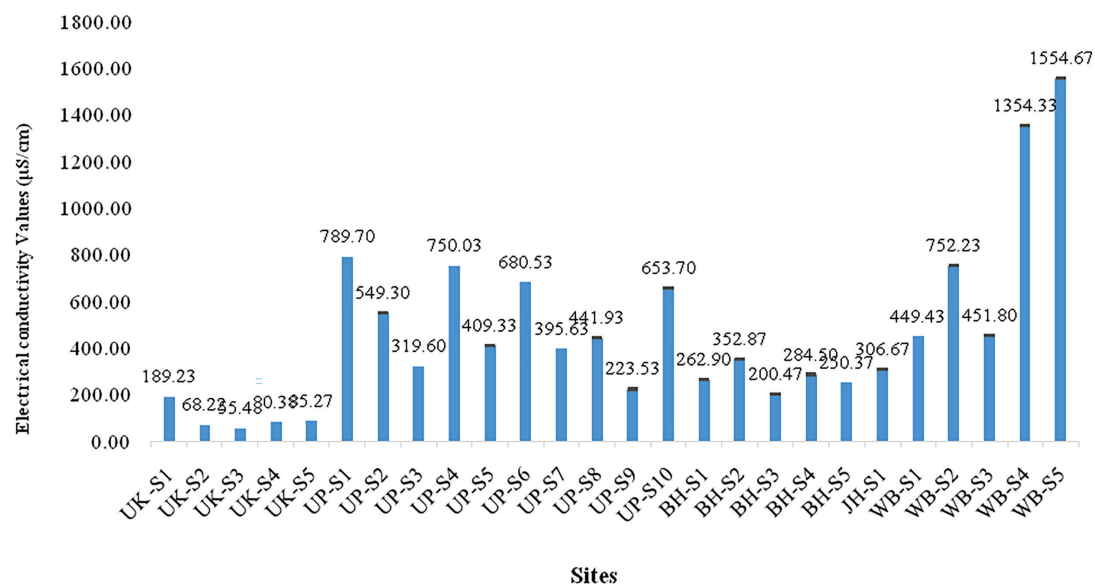


**Fig. 3** Soil pH of different sampling sites

The pH of the sample sites ranged from as low as  $6.23 \pm 0.06$  to as high as  $8.84 \pm 0.01$ . Soil samples from UK-S2, UK-S4 and UK-S5 were found to be acidic, with pH levels lower than 6.5. The pH of 9 sites was neutral, whereas the pH of the remaining sites was alkaline.

The next parameter studied, was electrical conductivity of the soil. Soil electrical conductivity is an indicator of soil salinity, clay

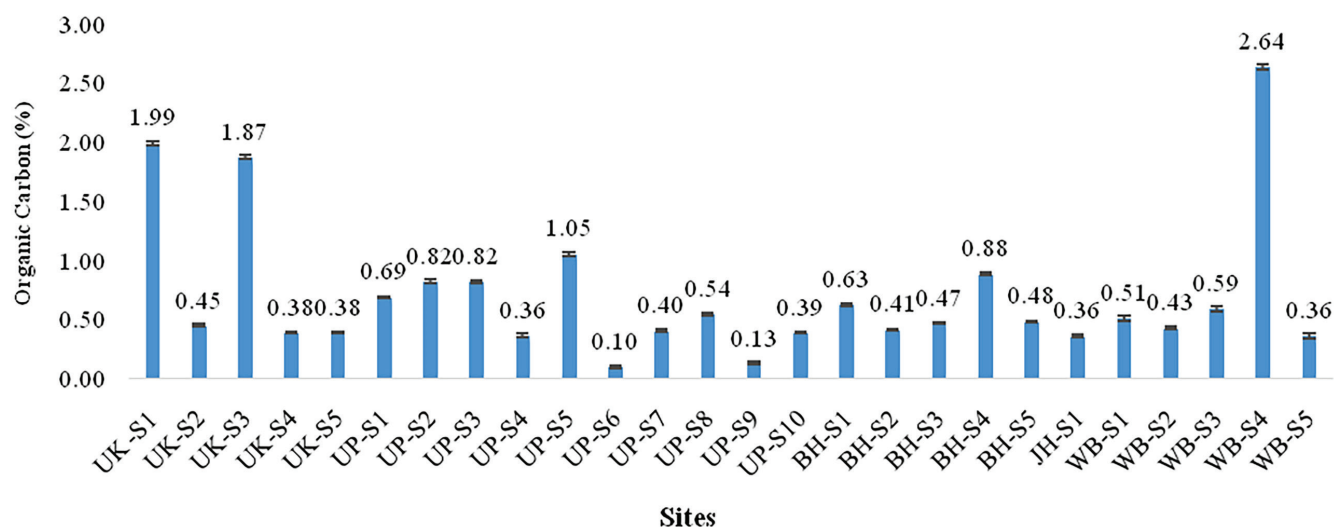
content and the nutrient concentration in the field (Heiniger et al., 2003). As shown above, electrical conductivity may be categorized in different divisions and that may be used to determine the salinity of the soil so that which crop could be sown may be decided. The electrical conductivity of soils from different sampling sites was determined and is represented in Fig. 4



**Fig. 4** Soil electrical conductivity at different sampling sites

The electrical conductivity of almost all the soil samples were within the prescribed limits of having EC below 800 $\mu$ S/cm. Soil samples from WB-S4 and WB-S5 showed the highest electrical conductivity value of 1354.33 $\pm$ 2.52  $\mu$ S/cm and 1554.67 $\pm$ 2.52  $\mu$ S/cm respectively. Thus, it may be said that the soil electrical conductivity was within the range at remaining all sampling sites and hence favourable for plant growth.

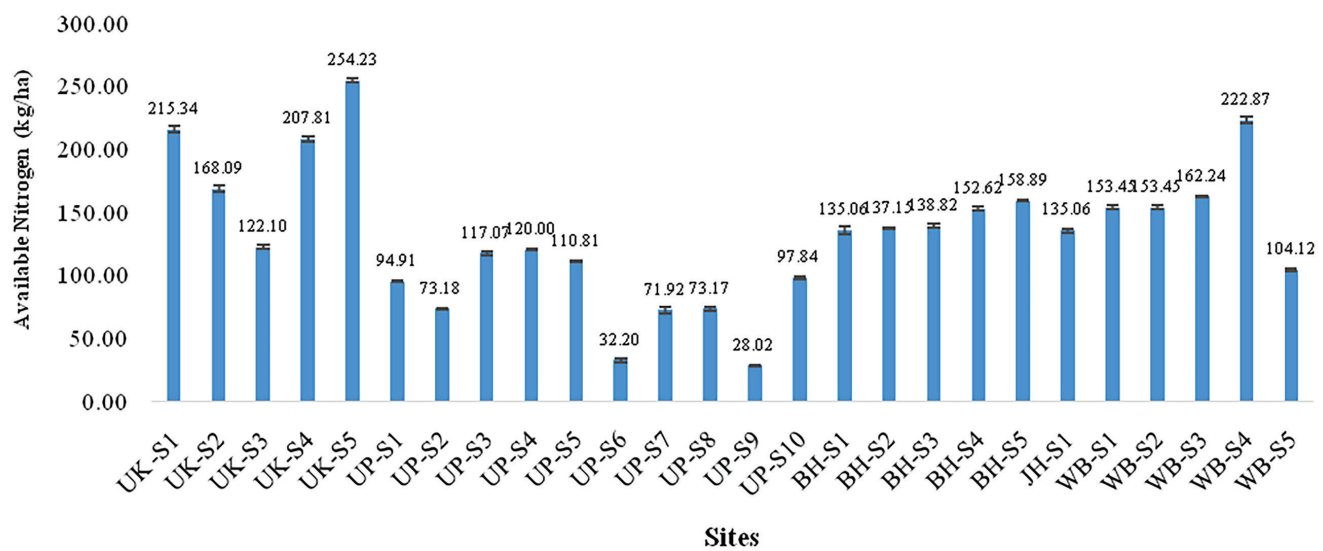
Soil organic carbon is a measure of the organic content present in soils. It is an important determinant of the hydraulic properties of soil like porosity, water retention and hydraulic conductivity. Thus overall, it is an important parameter determining food content in soil for uptake by plants (Allison, 1965; Rawls et al., 2004). The organic carbon content as measured for the 26 soil samples is shown in Fig. 5.



**Fig. 5** Soil organic carbon content at different sites

According to the data, the organic carbon content of soil ranged from 0.10 $\pm$ 0.01% to 2.64 $\pm$ 0.03%. UP-S6, which had the highest pH value of 8.84 $\pm$ 0.01 had the lowest organic carbon content of 0.10 $\pm$ 0.01%. The highest quantity of organic carbon content was discovered in WB-S4 (2.64 $\pm$ 0.03%), which had an alkaline pH (7.77 $\pm$ 0.02). Soil samples from 8 different sites showed low organic carbon concentration, whereas soil samples from 11 different sites had medium organic carbon content. A total of 7 sites had high levels of organic carbon.

The next soil parameter studied was available nitrogen content. Nitrogen is the most important plant macronutrient, and its proper availability is a crucial determinant for plant growth and its optimal health. Researchers (Baričević and Zupančič, 2002) showed that increasing concentration of N added to soil resulted in increased concentration of alkaloids which is a critical component in extracts of medicinal plants. The available nitrogen content in soils from the different sites is shown in Fig. 6

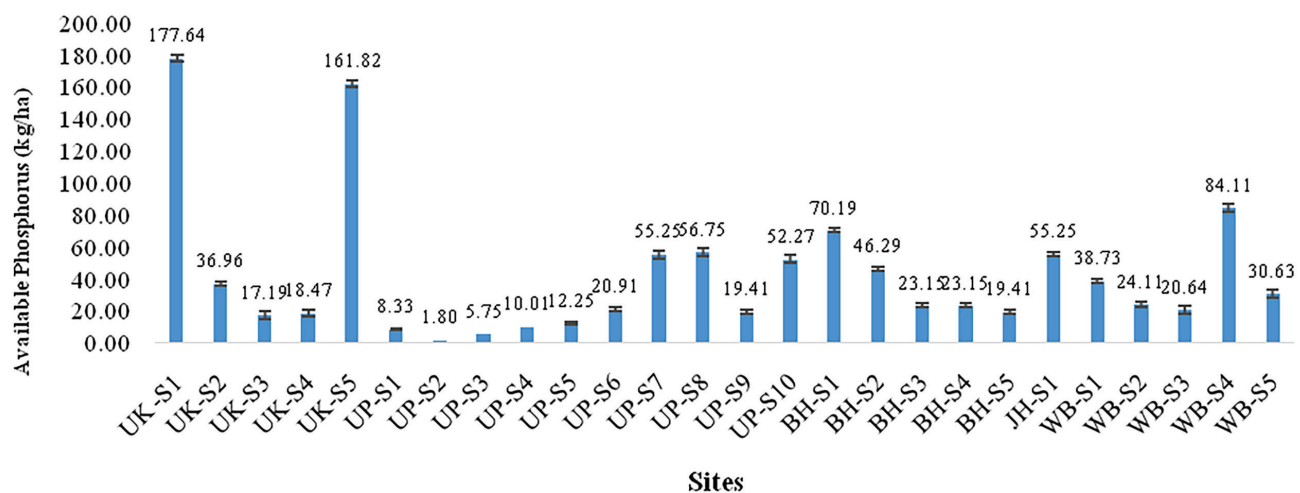


**Fig. 6** Soil available nitrogen content at different sites

From the available data it was observed that site UK-S5 had the maximum available nitrogen content ( $254.23 \pm 1.45$  kg/ha) and as per soil categories the content was low. For all the other types of soil, the available nitrogen content was low. This could be because all these samples were randomly picked from different sites, which necessarily did not receive any fertilization.

The next parameter tested was that of phosphorous which is another important

plant macronutrient. Phosphorous is a macronutrient that controls the water use efficiency, winter hardiness and promotes root formation (Chakraborty and Prasad, 2021). In case of medicinal plants enough phosphorous in soils promotes development of leaf biomass which is the main part of the medicinal plant in most cases (Stewart, 2003). The concentration of available phosphorous in soils from the different sites is shown in Fig. 7



**Fig. 7** Soil available phosphorus content at different sites

As per the data obtained and divisions demarcating phosphorous content in soil, the maximum P was recorded in site UK-S1 (177.64±1.83 kg/ha) while a very less amount was recorded in site UP-S2 (1.80±0.06 kg/ha). The remaining sites have enough amount of the P in soil. Four of the sites had available phosphorus content in low range, while 6 sites were having available phosphorus in medium range. Similarly, 4 sites had their range in high category and the remaining sites had available phosphorus in very high category.

The next parameter is the third major macronutrient for plants, potassium. In general, potassium plays a critical role in the closing and opening of stomata and thus regulates the uptake of water by plants. It also regulates plant growth and yield (Perrenoud, 1977). Literature also suggests that proper potassium content in soil results in higher concentration of essential oils in medicinal plants. Thus, such plants when grown in properly fertilized soil will result in good quality of medicinal plants for maximum benefit of consumers. The potassium content of the different soil samples is shown in Fig. 8

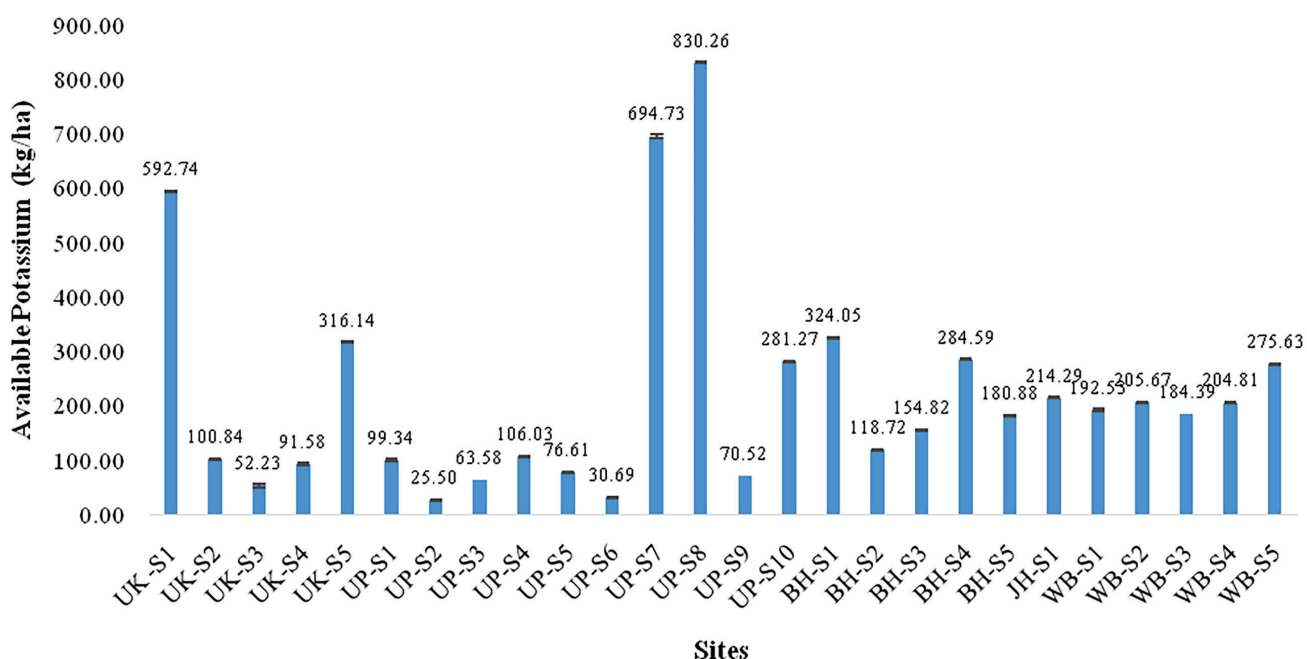
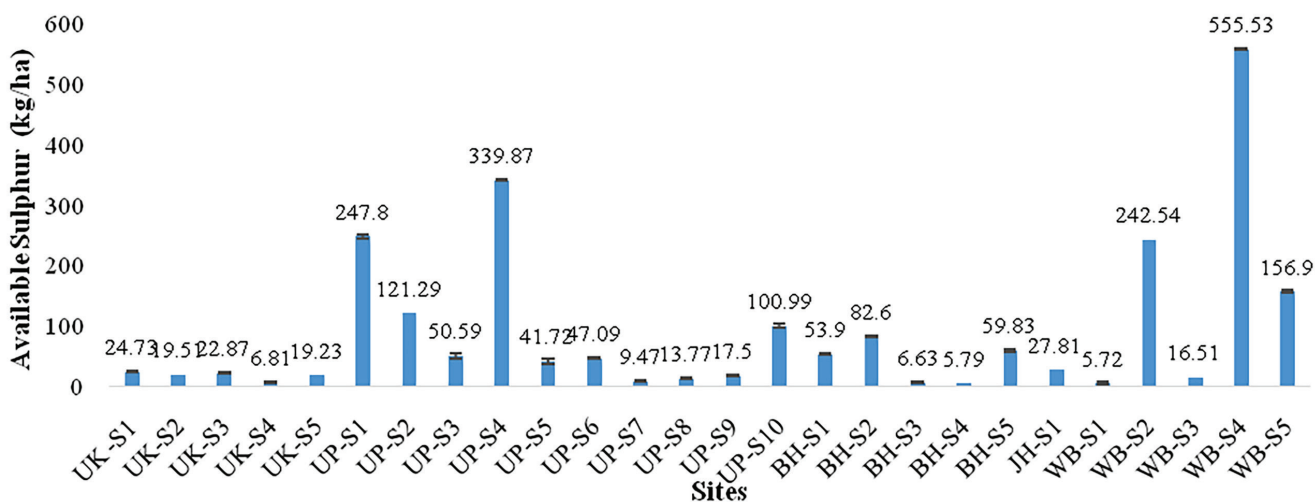


Fig. 8 Soil available potassium content at different sites

From the obtained results, it was seen that site UK-S1, UP-S7 and UP-S8 had high potassium content. As per the divisions of potassium content in soil, the potassium content was medium at twelve sites. The remaining sites had low potassium content.

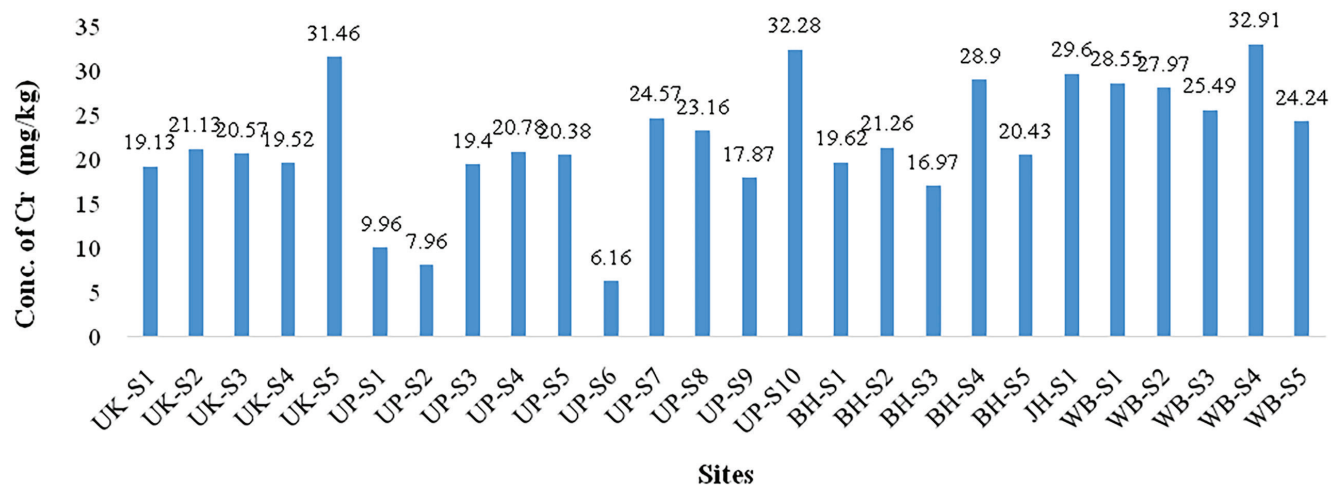
Sulphur content in soil plays a role as it is up taken by plants for the formation of sulphur containing amino acids and hence the buildup of proteins, chlorophyll, and oils (Tabatabai, 1984). The sulphur content in the soil is shown in Fig. 9



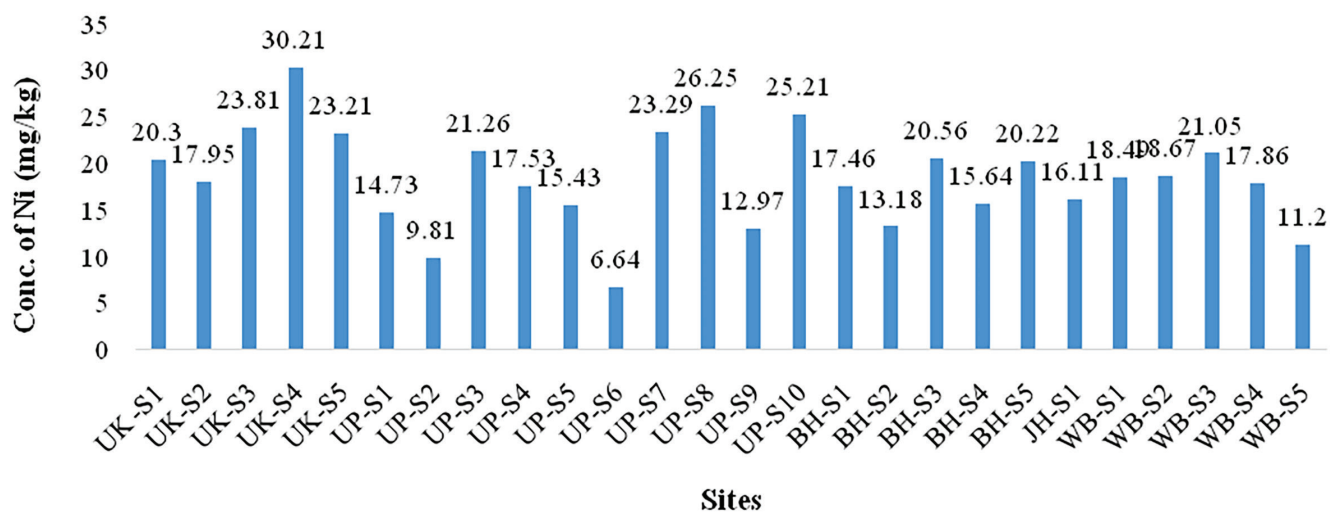
**Fig. 9** Soil available sulphur content at different sites

For the results obtained for the sulphur content, it was found that 10 sites were sulphur deficient. The remaining sites had enough sulphur in their soil. Further, correlation studies will help us to understand the effect of pH, EC, or even microbial parameters on sulphur content and vice versa. Additionally, the impact of both high and low sulphur on the composition of phytochemicals can be examined.

Fig. 10 a-g shows the different heavy metals content in all the sampling sites. The concentration of 7 heavy metals Cr, Ni, As, Sr, Cd, Hg and Pb were determined using ICP-MS. Their concentration further talks about the level of pollution and contamination in the soil sampling sites.



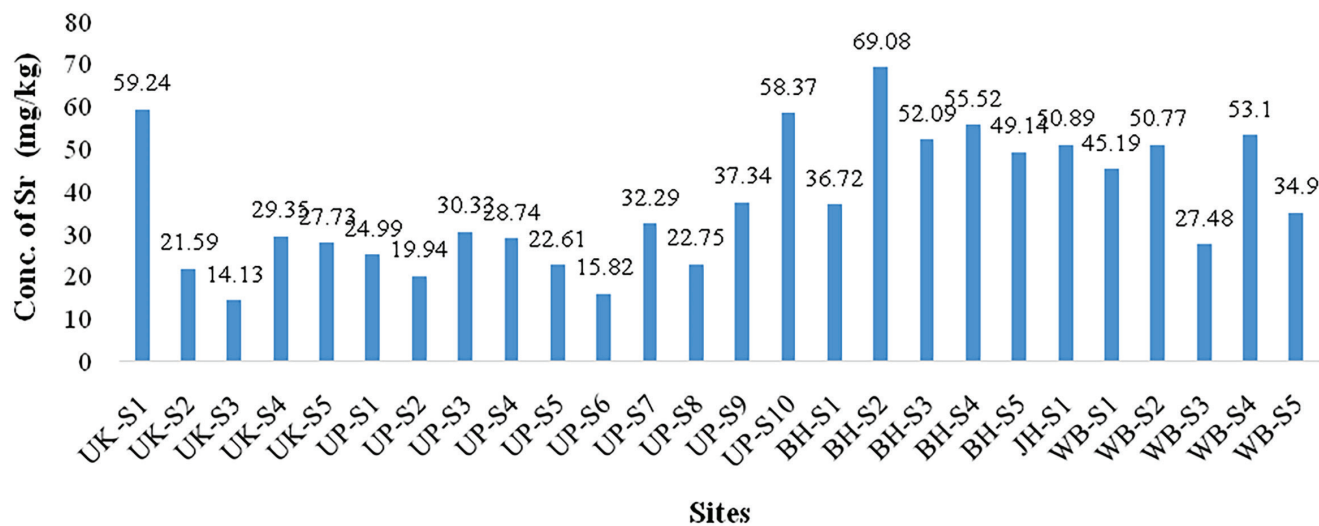
**Fig. 10a** Concentration of chromium at different sites



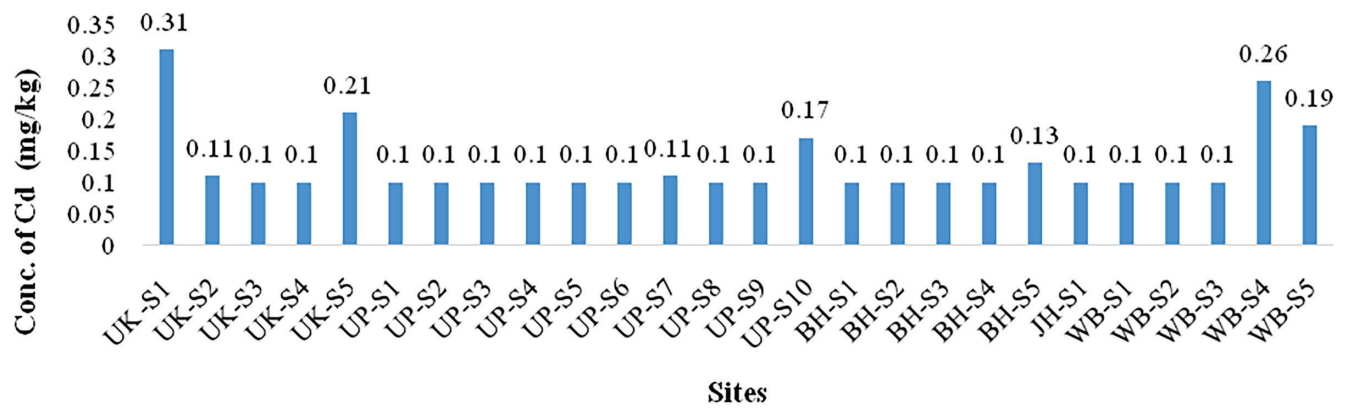
**Fig. 10b** Concentration of nickel at different sites



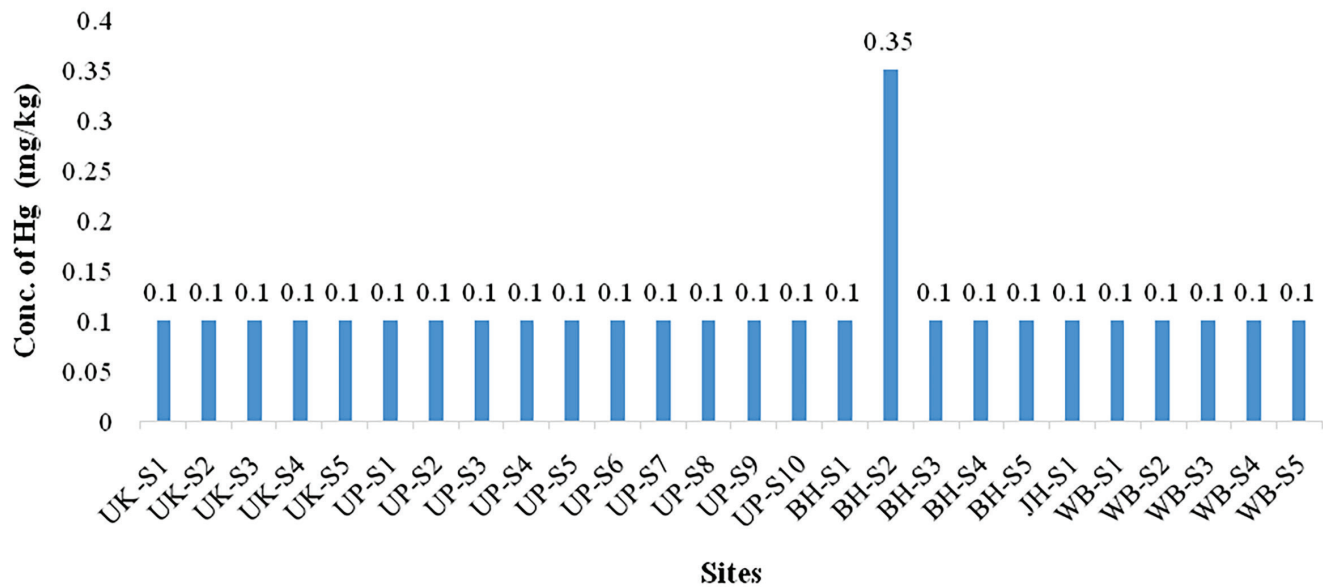
**Fig. 10c** Concentration of arsenic at different sites



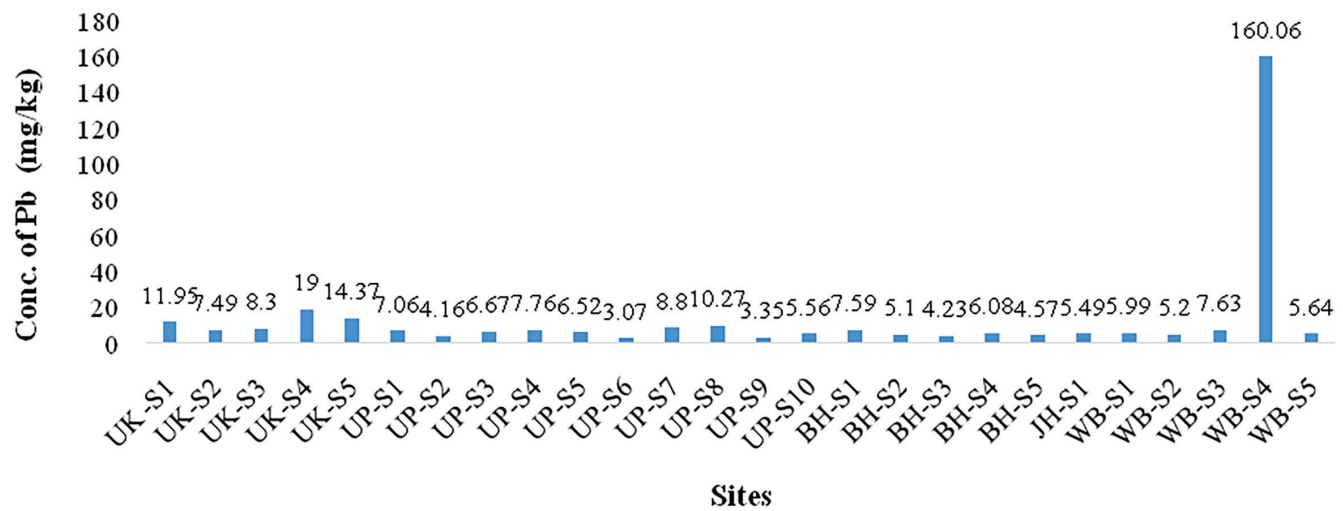
**Fig. 10d** Concentration of strontium at different sites



**Fig. 10e** Concentration of cadmium at different sites



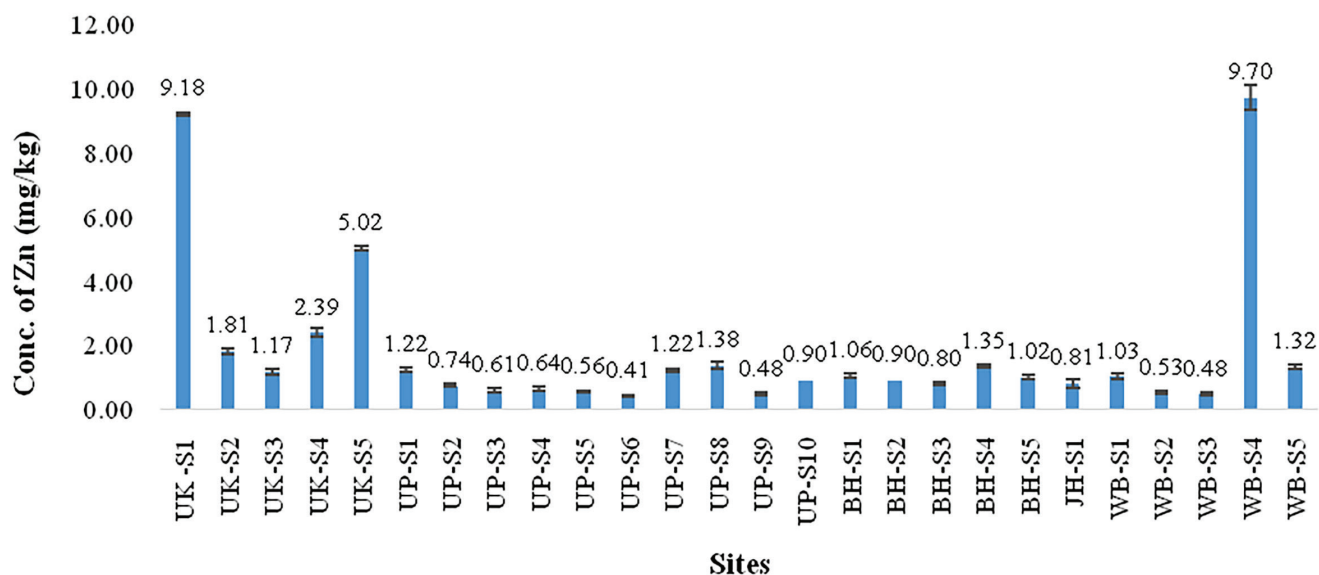
**Fig. 10f** Concentration of mercury at different sites



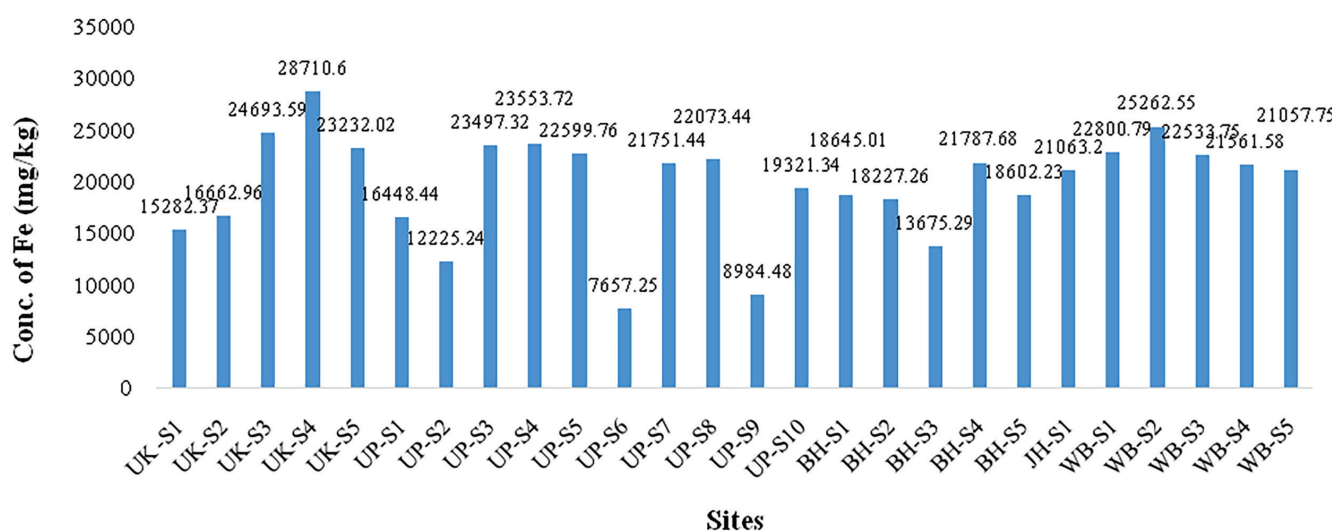
**Fig. 10g** Concentration of lead at different sites

Micronutrients are elements which are required by plants in very small quantities. They mainly act as co-factor of enzymes. When available to plants in the right quantity, they help in proper metabolism and bring about optimal plant growth and yield. The

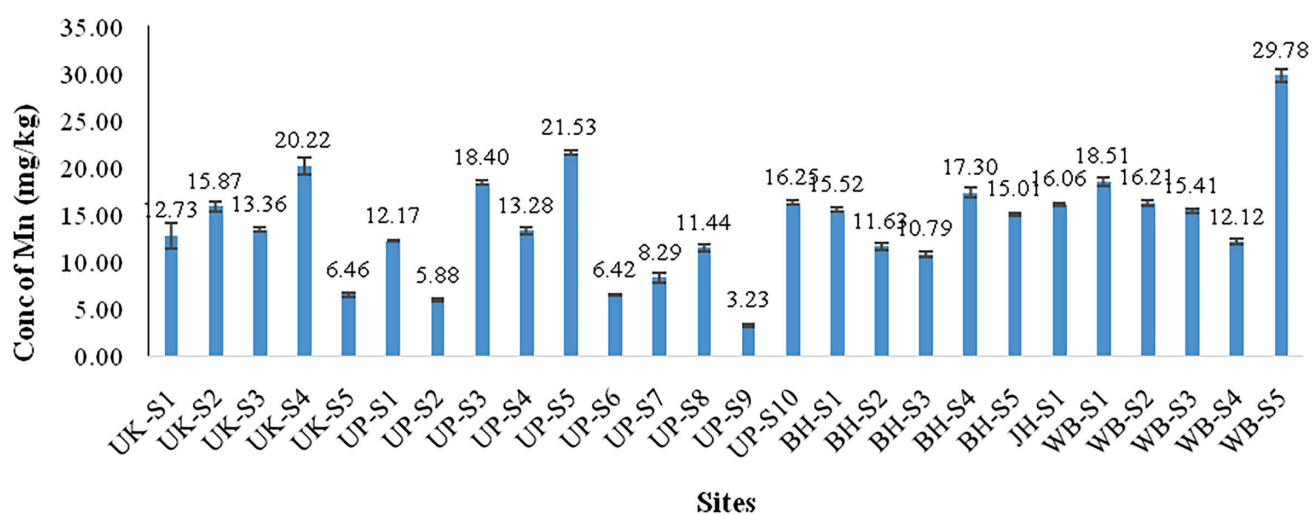
concentration of the four micronutrients Zn, Fe, Mn and Cu were determined using Atomic Absorption Spectrophotometer. The values obtained for all the sites are represented in Fig. 11 a, b, c and d.



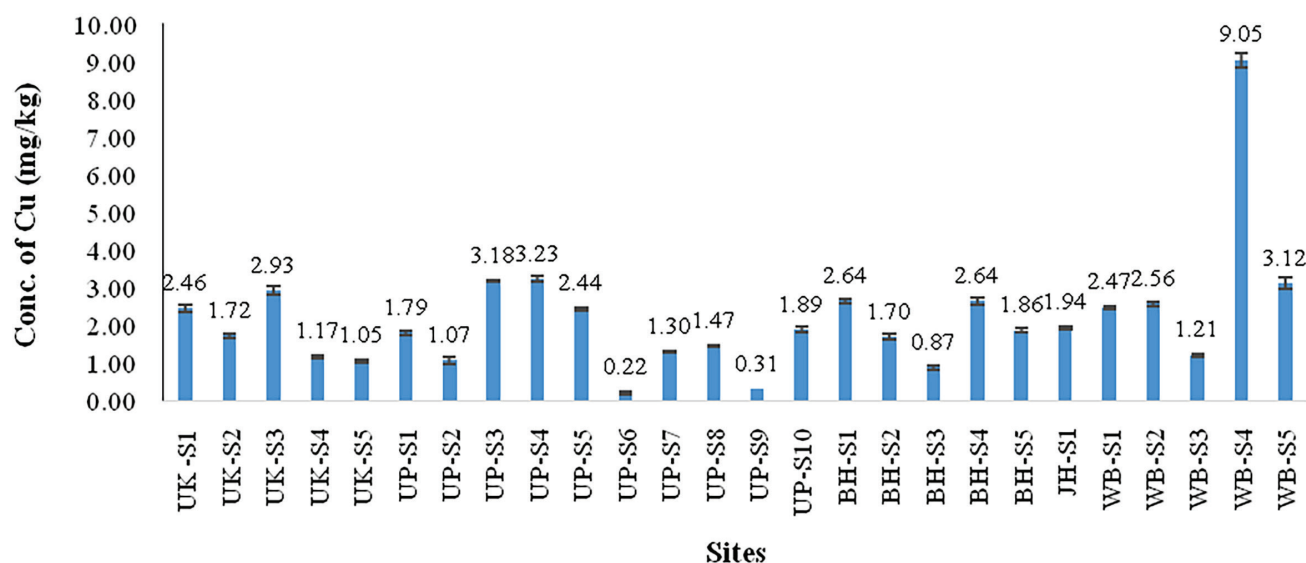
**Fig. 11a** Concentration of zinc at different sites



**Fig. 11b** Concentration of iron at different sites



**Fig. 11c** Concentration of manganese at different sites



**Fig. 11d** Concentration of copper at different sites

As per the requirement of micronutrients by plants, there are different prescribed limits. The minimum amount of a particular micronutrient to be present in the soil is considered as the threshold. Deficient soils are those with concentrations below them, whereas sufficient soils have concentrations above them.

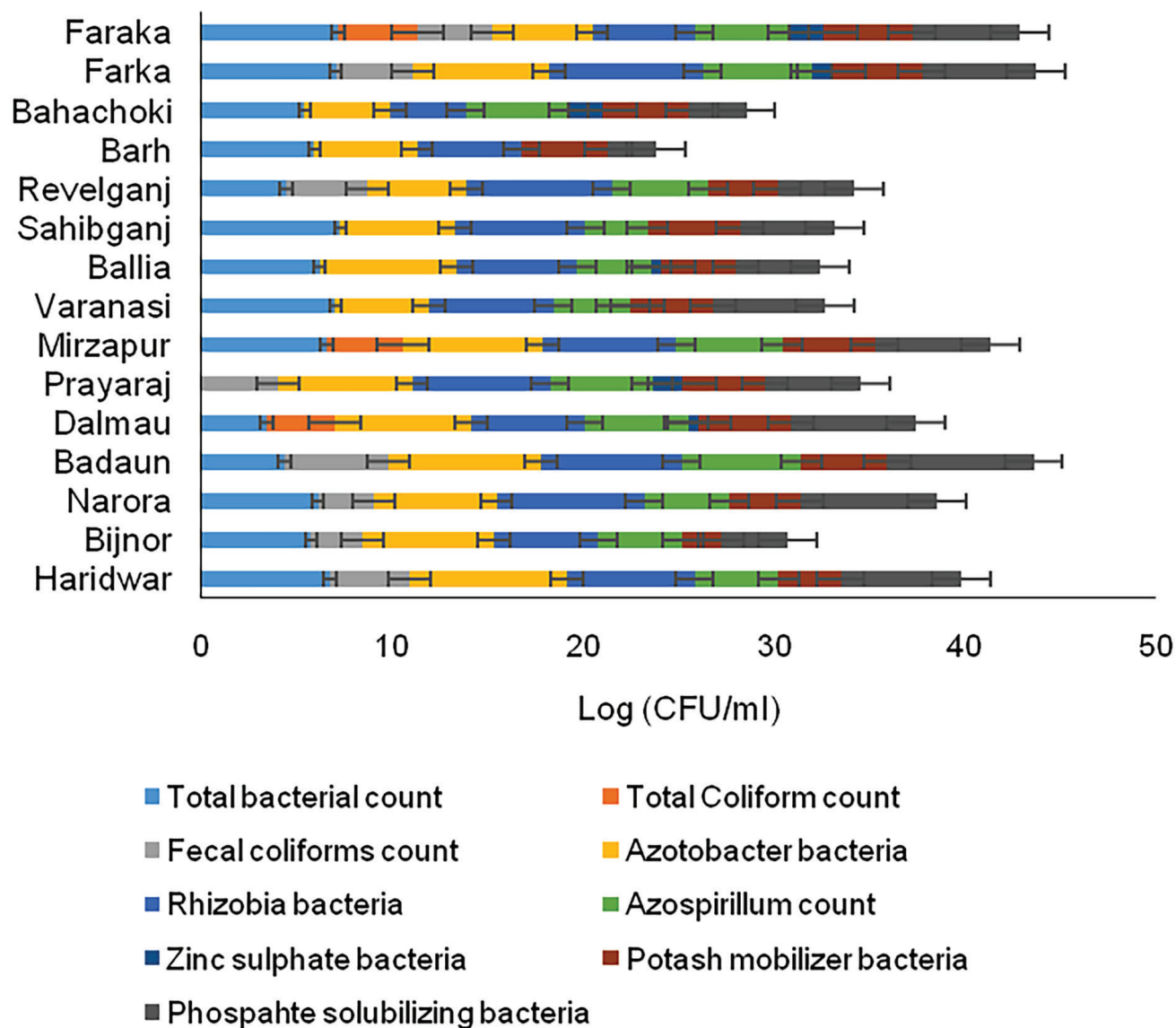
Though micronutrients are required by plants in very small quantities, they are

important for proper metabolic functioning. Soils where micronutrients are present in insufficient quantities will result in plant having inadequate metabolism and hence, less of phytochemical content too. Thus, cultivation of medicinal plants must be done in soils with proper nutritional content (Katyal et al., 1991).

## Total and Differential Bacterial Count from Rhizosphere

Rhizosphere microbial testing involves analysing the microbial communities in the

soil, rhizosphere, and roots of plants. The rhizosphere microbial test is performed in different media to calculate the bacterial count for the *Terminalia arjuna* (Roxb. ex DC.) Wight & Arn in different locations shown in Fig. 12



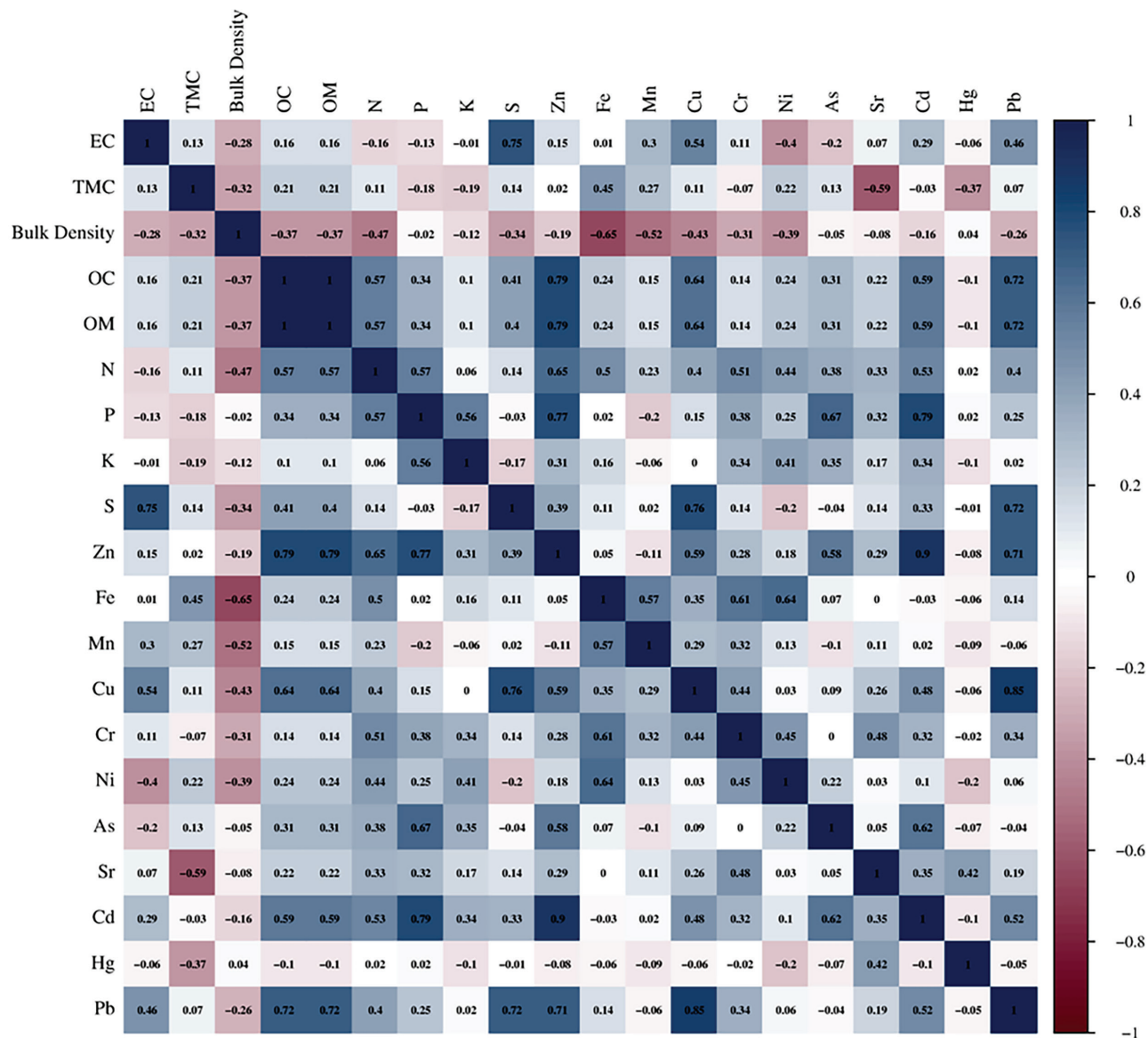
**Fig. 12** Total and differential bacterial count

The total rhizosphere microbial test is performed for the identification of total bacterial count which is found highest in Varanasi 7.25042 cfu/ml while lowest in Dalmau

3.402695 cfu/ml. Whereas the total faecal and coliform count is highest in in Farakka 4.18525 cfu/ml. The results of correlation studies have been represented as a matrix in Table 4.

## Correlation Coefficient Matrix

**Table 4** Correlation coefficient matrix



The correlation matrix in Table 6 clearly shows the micronutrient status at different sites and according to the correlation coefficient values of one parameter against the other, the values have been colour coded. The colour coding key also shown above shows the colour codes

as per the range of the correlation coefficient value. According to correlation coefficient values of each parameter against the other, few observations can be made. These are relevant for every parameter from every site because the values were determined using

information gathered for every site for every parameter.

- pH showed weak negative correlation with total moisture content, potassium, sulphur, and lead while it showed moderate positive correlation with electrical conductivity.
- A high degree of strong positive correlation between electrical conductivity and sulphur was observed, while moderate positive correlation was seen between the former and copper and lead.
- Total moisture content showed moderate positive correlation with iron; furthermore, bulk density showed weak negative correlation with mercury.
- Organic carbon showed negative correlation with mercury, moderate positive correlation with nitrogen, phosphorus, sulphur and cadmium and strong positive correlation with zinc, copper, and lead.
- Nitrogen showed a very strong positive correlation with zinc. Phosphorus showed the same with zinc, arsenic, and cadmium. Potassium had moderate positive correlation with zinc, chromium, nickel, arsenic, and cadmium.
- A strong positive correlation was seen between sulphur, copper, and cadmium. The same was also observed between zinc, cadmium, and lead. Iron, chromium, and nickel. Manganese and chromium shared moderate positive correlation between each other.
- Strong positive correlation was seen between copper and lead. Chromium, arsenic, and mercury had negative correlation amongst themselves. The same was also observed between nickel and mercury. A strong positive correlation was seen between arsenic and cadmium.
- Strontium showed moderate positive correlation between cadmium and mercury. The same was also seen between cadmium and lead. Mercury on the other hand showed negative correlation with lead.

## Conclusion

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From the physicochemical and microbial analysis of soil it was found that the parameters were different at each site. If the soil of each site is characterized as per the availability of nutrients and bacteria

according to the recommendations, then the best soil as per the area can be determined. The Table 5 shows the conclusive results of all the results obtained for different parameters from all the sites.

**Table 5** Conclusive results for different parameters from all sites

Sites	pH	EC	OC	N	P	K	S	Zn	Fe	Mn	Cu	Cr	Ni	As	Sr	Cd	Hg	Pb
UK -S1	AB	WR	AR	BR	Very AR	AR	WR	WR	WR	WR	WR	WR	WR	AB	WR	WR	WR	WR
UK-S2	BR	WR	WR	BR	AR	BR	BR	WR	WR	WR	WR	WR	WR	WR	WR	WR	WR	WR
UK-S3	WR	WR	BR	BR	WR	BR	WR	WR	WR	WR	WR	WR	WR	WR	WR	WR	WR	WR
UK-S4	BR	WR	AR	BR	WR	BR	BR	WR	WR	WR	WR	WR	WR	WR	WR	WR	WR	WR
UK-S5	BR	WR	BR	BR	Very AR	WR	BR	WR	WR	WR	WR	WR	WR	WR	WR	WR	WR	WR
UP-S1	WR	WR	WR	BR	BR	BR	WR	WR	WR	WR	WR	WR	WR	WR	WR	WR	WR	WR
UP-S2	WR	WR	AR	BR	BR	BR	WR	WR	WR	WR	WR	WR	WR	WR	WR	WR	WR	WR
UP-S3	AB	WR	AR	BR	BR	BR	WR	WR	WR	WR	WR	WR	WR	WR	WR	WR	WR	WR
UP-S4	WR	WR	BR	BR	BR	BR	WR	WR	WR	WR	WR	WR	WR	WR	WR	WR	WR	WR
UP-S5	AB	WR	AR	BR	BR	BR	WR	BR	WR	WR	WR	WR	WR	WR	WR	WR	WR	WR
UP-S6	AB	WR	BR	BR	WR	BR	WR	BR	WR	WR	WR	WR	WR	WR	WR	WR	WR	WR
UP-S7	AB	WR	WR	BR	Very AR	AR	BR	WR	WR	WR	WR	WR	WR	WR	WR	WR	WR	WR
UP-S8	AB	WR	WR	BR	Very AR	AR	BR	WR	WR	WR	WR	WR	WR	WR	WR	WR	WR	WR
UP-S9	AB	WR	BR	BR	WR	BR	BR	BR	WR	BR	WR	WR	WR	WR	WR	WR	WR	WR
UP-S10	AB	WR	BR	BR	Very AR	WR	WR	WR	WR	WR	WR	WR	WR	WR	WR	WR	WR	WR
BH-S1	WR	WR	WR	BR	Very AR	WR	WR	WR	WR	WR	WR	WR	WR	WR	WR	WR	WR	WR
BH-S2	WR	WR	WR	BR	AR	WR	WR	WR	WR	WR	WR	WR	WR	WR	WR	WR	AB	WR
BH-S3	WR	WR	WR	BR	AR	WR	BR	WR	WR	WR	WR	WR	WR	WR	WR	WR	WR	WR



Sites	pH	EC	OC	N	P	K	S	Zn	Fe	Mn	Cu	Cr	Ni	As	Sr	Cd	Hg	Pb
BH-S4	WR	WR	AR	BR	AR	WR	BR	WR	WR	WR	WR	WR	WR	WR	WR	WR	WR	WR
BH-S5	AB	WR	WR	BR	WR	WR	WR	WR	WR	WR	WR	WR	WR	WR	WR	WR	WR	WR
JH-S1	AB	WR	BR	BR	Very AR	WR	WR	WR	WR	WR	WR	WR	WR	WR	WR	WR	WR	WR
WB-S1	WR	WR	WR	BR	AR	WR	BR	WR	WR	WR	WR	WR	WR	WR	WR	WR	WR	WR
WB-S2	AB	WR	WR	BR	AR	WR	WR	BR	WR	WR	WR	WR	WR	WR	WR	WR	WR	WR
WB-S3	AB	WR	WR	BR	WR	WR	BR	BR	WR	WR	WR	WR	WR	WR	WR	WR	WR	WR
WB-S4	AB	CSSC	AR	BR	Very AR	WR	WR	WR	WR	WR	WR	WR	WR	WR	WR	WR	WR	WR
WB-S5	AB	CSSC	BR	BR	AR	WR	WR	WR	WR	WR	WR	WR	WR	WR	WR	WR	WR	WR

The given parameter range is expressed in CSSC (Critical for Salt-Sensitive Crops): This range is vital for salt-sensitive crops. WR (Within Range): The parameter falls within the acceptable range. AR (Above Range): The parameter exceeds the recommended range. BR (Below Range): The parameter is below the recommended range. These parameter ranges are associated with the studies conducted by the Katyal et al., 1991, Hossain et al., 2022, Bowen, 2016, Varley, 1972; Jackson, 1973, Chopra and Kanwar, 1976; Richards, 1954, Jackson, 1973; Walkley and Black, 1934, Subbaiah, 1956, Bray and Kurtz, 1945; Black, 1965, Black, 1965; Merwin and Peech, 1951; Jackson, 1973, Lisle et al., 1994 and Retka et al., 2010.

The comprehensive investigation can also provide a better understanding of how soil quality factors, environmental fluctuations, and

altitudinal variations affect the phytochemical composition of medicinal plants.

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CHAPTER  
**04**

**Traditional and  
Ethnomedicinal  
Applications**



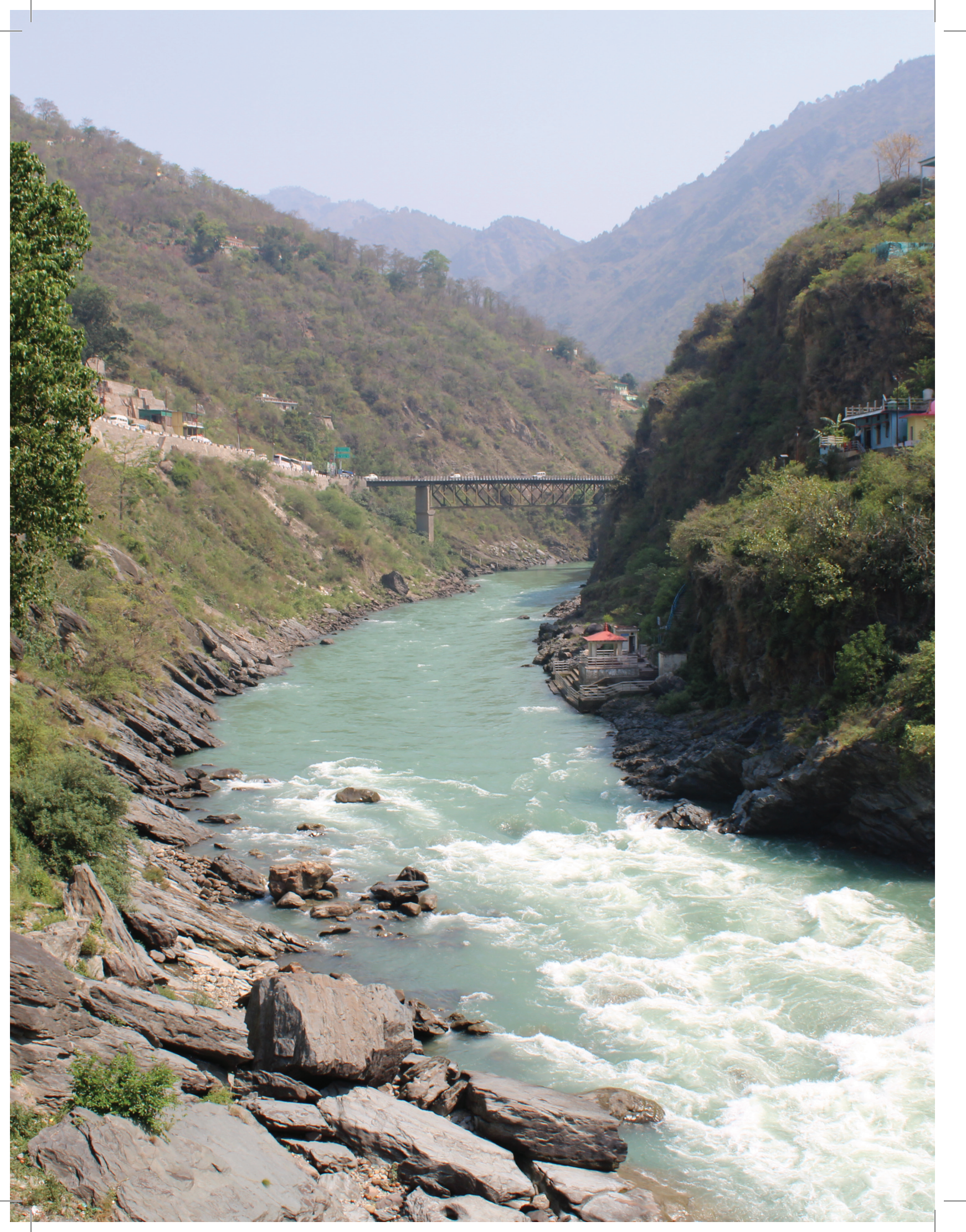


## INTRODUCTION

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Even though medicinal plants are vital to health care and serve as a key source of raw materials for both conventional and traditional medicine preparations, most people still prefer herbal remedies to conventional ones (WHO, 2002). Their effectiveness, the lack of available medical alternatives, the rising expense of contemporary medications, and cultural preferences all contributed to their increased attention (Heinrich, 2003; Tabuti et al., 2003). Globally, traditional knowledge has gained prominence in preservation, sustainable development, and the exploration of novel approaches to the use of plant resources. The knowledge, abilities, and practices of traditional medicine are derived from the experiences, beliefs, and presumptions of folk communities, to safeguard their health issues. Nearly 80% of people worldwide rely on traditional medicine, and 60% of rural Indians utilize herbal remedies, according to the WHO (WHO, 2002). With over 200 species, the genus *Terminalia* L. is the second biggest in the family Combretaceae after *Combretum* (Lima et al., 2012). The species of *Terminalia* include shrubs and huge deciduous forest trees. Most of them are enormous trees that can grow to a height of 75 meters (Stace, 2007). Standing 20-30 metres above the ground, *Terminalia arjuna* (Roxb. ex DC.) Wight & Arn. is a deciduous and evergreen tree that is a member of the Combretaceae family. There are roughly 24 *Terminalia* species known from different regions of India. *T. arjuna* is found in India, Burma, Mauritius, and Sri Lanka. It is roughly 60-80 feet tall with a buttressed trunk, a horizontally spreading crown, and drooping branches (Kapoor et al., 2014). It is an important medicinal plant, used in Ayurveda and goes by the names 'Arjuna', 'Dhavala', 'Kaubha', 'Nadisaraja Partha', 'Indradru', and 'Veeravriksha'. It is found in most of the Indian subcontinent, including the Himalayan face of Uttar Pradesh, West Bengal, Deccan, Bihar, Madhya Pradesh, Orissa, Punjab, and Konkan. It usually grows on the banks of streams and rivers (Sharma et al., 2000; Ali et al., 2003; Kapoor et al., 2014). Every component of the Arjuna plant has been utilized for its medicinal benefits, beneficial for heart health, and for easing the symptoms of anxiety and discomfort (Emran et al., 2011). It has anti-bacterial (Samy et al., 1998), anti-mutagenic, hypolipidemic, antioxidant, hypocholesterolemic, and anti-





inflammatory effects. By enhancing anti-oxidative defence mechanisms, *T. arjuna* can shield the liver and renal tissues from oxidative stress caused by  $\text{CCl}_4$  (Manna et

al., 2006). Magnesium, glycosides, tannins, flavonoids, triterpenoids, and  $\beta$ -sitosterol are among its many active ingredients (Paarakh, 2010).

## Traditional and Ayurvedic Benefits

People have begun to consider traditional healing systems like Ayurveda, Siddha, and Unani after decades of intense fixation on the modern medical system. This is because these systems have fewer side effects and are less expensive than synthetic drugs (Sarker et al., 2011). Since the Vedic era, people have used this therapeutic plant. Within Ayurvedic literature, the plant is synonymous with Arjun, the heroic figure from the renowned epic, Mahabharata. Several ancient Indian medical texts, such as the 'Charaka Samhita', 'Sushruta Samhita', and 'Astang Hridayam', have referred to the herb Arjuna. The first person to employ Arjuna stem bark powder for heart conditions was Vegabhatta. It is referred to as *Hridya* (cardiac tonic) in Ayurveda. Arjuna bark powder is used to cure a variety of conditions, including *kshata* (wound or injury), *kshaya* (emaciated condition), *visha* (poison), *raktavikara* (as a styptic), *medaroga* (diabetes difficulties), *prameha* (urinary diseases), *vrana* (wound/ulcer), etc. (Premila, 2006). The bark extract

of *T. arjuna* can prevent myocardial alterations brought on by long-term beta-adrenoceptor stimulation, as well as significantly reduce endogenous antioxidant levels, avoid fibrosis without raising the heart-to-body weight ratio, and prevent isoprenaline-induced increase through oxidative stress (Paarakh, 2010). The powdered bark has diuretic properties in cases of liver cirrhosis and relieves symptoms of hypertension as well as has anti-dysenteric, cardiogenic, lithotriptic, antipyretic, astringent, and tonic properties (Kumar et al., 2009). It has been discovered that the bark powder contains antioxidant, anti-ischaemic, cardioprotective qualities (Miller, 1998), hypocholesterolemic effect, fungicidal (Row, 1970), antimicrobial, antifertility, used as an antidote for poisons and to heal ulcers and skin conditions. It can also be used to treat diabetes, hypertension, and obesity (Hoq, 2018). Some of the medicinal values listed in ayurvedic literature are shown in Table 1.



**Table 1** Some important preparations used against various diseases in ancient Ayurveda

S. No.	Diseases/ Conditions	Mode of Administration/Uses	References
1.	Thoracic disorders	<b>Cūrṇaṅkākubhamiṅṅaṅvāsakarāsabhāvitaṅbahūnvārān. Madhughṅtasitopalādilehyaṅkṅayakāsarakṅtāharam-</b> <i>Terminalia arjuna</i> (Arjuna myrobalan) bark powder, in a dose of about 3 g, triturated with <i>Adhatoda vasica</i> (Malabar nut) juice, mixed with honey, crystal sugar and <i>Ghrita</i> (Clarified butter), used to relieve <i>Kṣayajakāsa</i>	Misra and Vaisya, 2012a
2.	Cardiac disorders	<b>Arjunasyatvacasiddhaṅkṅīraṅyojyaṅḥḍāmāye. Sitayāpaṅcamūlyāvābalayāmadhukēnavā-</b> <i>T. arjuna</i> bark, crystal sugar or <i>Laghupaṅcamūla</i> or <i>Sida cordifolia</i> (Country mallow) or <i>Glycyrrhiza glabra</i> (Liquorice root) processed with milk, beneficial in cardiac diseases; <b>Gḅṅtenadugḅhenaguṅām-bhasāvāpibanticūrṅaṅkakubhatvaco ye. Hḅdrogajīrṅaj-varapittarakṅtaṅhatvābhavye uścira jīvinaste-</b> 3 g <i>T. arjuna</i> bark powder along with <i>Ghrita</i> (Clarified butter), milk or jaggery, mixed water, useful in cardiac diseases; <b>Godhūmakakubhacūrṅaṅchāgapayogavyasarpiṅāpakvam. Madhuśarkarāsametaṅśamayatiḅdrogamuddhataṅpuṅsām-</b> equal quantity of <i>T. arjuna</i> bark powder and <i>Triticum sativum</i> (Wheat) powder, cooked in goat milk, mixed with cow <i>Ghrita</i> (Clarified butter), honey and sugar, useful in acute cardiac diseases; ..... <b>cūrṅaṅdugḅhenapāyayet. Hḅdrogakāśāśvāsaghṅaṅkakubhasya ca valkalam. Rasāyanaṅparaṅbalyaṅvātajinmāsajitam. Saṅvatsara-prayogeṅajīvedvarṅśataṅdhruvam-</b> 3-5 g <i>T. arjuna</i> bark powder along with milk, for one month, used in cardiac diseases; <b>Pārthasyakalkasyarāsenasiddhaṅśastāṅghṅtaṅsarvahḅḍāmāyeṅu. Tathāḅgnimāṅdyekṅatajapraṅṅttarak-tārśasāṅcāpivadantiṅpathyam-</b> <i>Arjuna Ghrita</i> (Clarified butter) processed with <i>T. arjuna</i> paste and decoction, used in cardiac diseases; <b>Tailājyaguṅavipakvaṅcūrṅaṅ-godhūmapārthajaṅvāpi. Pibatipayo'nusabhavatiṅjitasakalahḅḍāmāyaṅpuruṅaṅ-</b> equal quantity of <i>T. sativum</i> and <i>T. arjuna</i> bark powder along with oil, <i>Ghrita</i> (Clarified butter), jaggery and milk, very effective for cardiac diseases	Tiwari, 2007a; Tiwari, 2007b; Tiwari, 2007c; Tiwari, 2007d; Tiwari, 2007e; Tripathi, 2010; Tripathi, 2012a; Tripathi, 2012b; Tripathi, 2012c; Tripathi, 2012d; Tripathi, 2012e

S. No.	Diseases/ Conditions	Mode of Administration/Uses	References
3.	Abdominal disorders	<b>Śallakībadarījambupriyālāmrārjunatvacāṇ.</b> <b>Pītāṅkīreṇamadhvāṇhyāṅṇṭhakśoṇitavāraṇāṇ-</b> a paste or powder prepared from <i>Boswellia serrata</i> (Indian olibanum), <i>Zizyphus jujuba</i> (Jujube), <i>Syzygium cumini</i> (Malabar plum), <i>Buchanania cochinchinensis</i> (Calumpang nut tree), <i>Mangifera indica</i> (Mango) and <i>T. arjuna</i> (Arjuna myrobalan) bark, alone or together or along with honey, mixed with milk and used in bloody diarrhea; <b>Keśarājorjunakṇāraṇprātaṇpītaṇcam-astunā.</b> <b>Nihantisāmamatyarthamacirādgrahaṇīrujam-</b> <i>Kṣāra</i> prepared from <i>Eclipta prostrata</i> (Eclipta) and <i>T. arjuna</i> (Arjuna myrobalan) along with whey, useful in treating <i>Āma</i> associated and chronic pain in sprue	Tiwari, 2007f; Tripathi, 2009
4.	Anorectal disorders	<b>Parīṇecanevidadhyādṇṇakakubhayavāsanimbāśca-</b> sprinkling with the bark decoction of <i>T. arjuna</i> over the affected area, beneficial for hemorrhoids	Śāstrī and Chaturvedi, 2011a
5.	Renal and Urinary bladder disorders	<b>Kaṇāyaṅkakubhasyavā-</b> <i>T. arjuna</i> bark decoction, useful against anuria; <b>Dhavārjunacaṇdanaśālachallīkvātho-hitaṇsyāccajalaprāmehe.....pūyamehehitāṅkvāthodhavārjunasya.</b> <b>Kadambaśālārjunadīpyakānāṇviṇaṇgadārvīghavaśallakīnām.</b> <b>Sarvetathaivamadhunākaṇāyāṅkaphaprāmeheṇuniṇevanīyāṇ-</b> decoction prepared from <i>Anogeissus latifolia</i> (Axle wood), <i>T. arjuna</i> , <i>Santalum album</i> (Indian sandalwood) and <i>Shorea robusta</i> (Sal tree) bark, useful in <i>Udakameha</i> ; decoction of <i>Anthocephalus cadamba</i> (Wild cinchona), <i>Shorea robusta</i> (Sal tree), <i>T. arjuna</i> , <i>Apium graveolens</i> (Celery), <i>Embelia ribes</i> (False black pepper), <i>Anogeissus latifolia</i> (Axle wood) and <i>Boswellia serrata</i> (Indian olibanum), useful in <i>Kapha</i> associated urinary diseases	Pandey, 2010; Tripathi, 2011a

S. No.	Diseases/ Conditions	Mode of Administration/Uses	References
6.	Reproductive disorders	<b>Śirīṇakakubhakvāthapicūnyonauvinikṇipet.Upadravāś-caye'nyesyustānyathāsvamupācaret-</b> withholding of the vagina with a gauze piece soaked in the decoction prepared from <i>Albizia lebbek</i> (Indian siris) and <i>T. arjuna</i> bark after the expulsion of the obstructed fetus and placenta, beneficial in the pain and associated complications; <b>Śuktramehinaṇṇ.....kukubhacandanakaṇāyaṇṇvā-</b> decoction prepared from <i>T. arjuna</i> bark and <i>Santalum album</i> (Sandalwood), useful in spermatorrhea	Tripathi, 2011b; Sharma, 2012
7.	Musculo-skeletal disorders	<b>Bhagnaṇṇipibettvakpayasā'rjunasya-godhūmacūrṇaṇṇasaghṇtenavā'tha-</b> <i>T. arjuna</i> bark <i>Kṣīrapāka</i> along with <i>Ghrita</i> (Clarified butter) and <i>Triticum sativum</i> (Wheat) powder, useful against fractures; <b>Cūrṇaṇṇapureṇasaṇṇyojyaghṇtenārjunalākṇayoṇ.</b> <b>Bhagnaṇṇsandhānamāyātīlīṇṇhaṇṇkṇīraghṇtāsīnā-</b> an equal quantity of <i>T. arjuna</i> bark and <i>Lākṣā</i> powder along with <i>Commiphora wightii</i> (Gugal) and <i>Ghrita</i> (Clarified butter) followed with the diet of <i>Ghrita</i> (Clarified butter) and milk facilitates quick healing; <b>Saghṇtenāsthisaṇṇhāraṇṇlākṇāṇṇgodhūmamārjunam.Saṇṇdhimukte'sthibhagnecapibetkṇīreṇamānavaṇṇ-</b> a paste or powder prepared from an equal quantity of <i>Cissus quadrangularis</i> (Treebine), <i>Lākṣā</i> , <i>Triticum sativum</i> (Wheat) and <i>T. arjuna</i> along with <i>Ghrita</i> (Clarified butter) and milk, useful in bone fractures and dislocated bone	Tiwari, 2007g; Misra and Vaisya, 2012b; Misra and Vaisya, 2012c
7.	Dermatological disorders	<b>Khadirāvaghātakakubha.....Śasyantesnānapāneṇu-</b> bathing with and drinking with <i>T. arjuna</i> bark decoction, beneficial for leprosy; <b>Vyaṇṇgeṇucārjunatvagrāmāṇṇjiṇṇhāvāsamākṇikā-</b> external application of <i>T. arjuna</i> bark paste, beneficial for facial melanosis; <b>Kadambārjuna.....Vraṇṇapracchādanevidvānpatrāṇṇyarkasyacādiśet-</b> <i>T. arjuna</i> leaves, used to cover and bandage the wound	Śāstrī and Chaturvedi, 2011b; Śāstrī and Chaturvedi, 2011c; Tripathi, 2011c

S. No.	Diseases/ Conditions	Mode of Administration/Uses	References
8.	Generalized body disorders	<b>Dhanañjayodumbara.....Pñthakpñthakcañdanayojitānitenavakalpenahitānitatra.Niśisthitāvāsvarasīkñtāvākalkīkñtāvāmñditāñśñtāvā.Etesamastāgañśaññthagvāraktañsapittañśamayantiyogāñ;Pibecchī-takañāyañvājambvāmñrñjunasambhavam-</b> <i>T. arjuna</i> bark powder, mixed with an equal quantity of <i>Santalum album</i> (Indian sandalwood) along with the adjuvant of uncooked rice water or cold infusion, decoction, paste or juice, useful in <i>Raktapitta</i> ; <b>Kakubhatvaññāgabalāvāñaribijañvicūrñitañpayasā.Pītañmadhughñtayuktañsasitañyakñmādikāsahara-</b> powder of <i>T. arjuna</i> bark, <i>Grewia hirsuta</i> (Veronicalolia) and <i>Mucuna pruriens</i> (Velvet bean) seed along with honey, <i>Ghrita</i> (Clarified butter) and crystal sugar, mixed with milk, useful in cough and other diseases	Śāstrī and Chaturvedi, 2011d; Misra and Vaisya, 2012d

## Therapeutic Potential

Medicinal plants play a significant role in health care and are the main natural materials used for both conventional and traditional medicine preparations as most people choose herbal medicines over conventional medicines. Herbal medicinal usage continues to play an important role in the treatment of various diseases. People expanded their attention to herbal medicines due to their effectiveness, lack of current medical alternatives, cultural preferences, and increasing cost of modern medicines (Heinrich, 2000). Since ancient times, medicinal plants have been a major source of substances used to treat illnesses. *T. arjuna* is one of the most popular and effective medicinal herbs in the traditional medical system for treating a wide range of critical ailments. Based on the observations

of ancient physicians dating back centuries, its bark decoction is used on the Indian subcontinent for angular discomfort, hypertension, congestive heart failure, and dyslipidemia. Since 500 BC, *T. arjuna* has been associated with cardiovascular benefits, and its bark has been utilized as a cardi tonic in Indian traditional medicine for more than three centuries (Chopra and Chopra, 1994; Miller, 1998). The use of bark in traditional medicine may lead it to become endangered. Therefore, the antimicrobial and antioxidant potential of leaves and bark are promoting the utilization of leaves (non-destructive method) in therapeutics (Kumar et al., 2018). Table 2 shows some of the significant medical properties associated with their formulations.

**Table 2** Medicinal importance along with their preparation and mode of administration *Terminalia arjuna* (Roxb. ex DC.) Wight & Arn.

S. No.	Parts Used	Preparations	Diseases Treated and Therapeutic Uses	References
1.	Bark	-	Dysentery, jaundice, diabetes, cardiotonic, cardioprotective, expectorant, burn, asthma, bronchitis, hypertension, anti-dysenteric, diuretic, heart palpitation, angina, poor coronary circulation, chest pain, liver disorder, low blood pressure, leukorrhea, blood dysentery, ulcers, fracture, anemia, cirrhosis, cardiopathy, sprain, fever, cancer, heart diseases, stones in kidney, scorpion stings, poisoning	Chopra and Chopra, 1958; Warriar, 1993; Nayak et al., 2004; Muthu et al., 2006; Kumar et al., 2007; Prusti and Behera, 2007; Dhanapal et al., 2009; Jain et al., 2009; Jain et al., 2010; Das et al., 2012; Gairola et al., 2013; Shukla et al., 2013; Sinhababu and Banerjee, 2013; Doley et al., 2014; Modak and Basu, 2014; Singh et al., 2014; Chowdhury, 2015; Hada, 2015; Kaur, 2015; Saha et al., 2016; Sharma et al., 2016; Prakash et al., 2017; Upasani et al., 2017; Kumari et al., 2018; Panda, 2018; Kumar and Duggal, 2019; Padhy et al., 2020; Patel et al., 2020; Rout and Panda, 2020; Kumar et al., 2021; Mandal et al., 2021; Sen and Behera, 2021; Naskar et al., 2022; Paul and Dey, 2022; Singh et al., 2022
		Decoction	Lowers blood pressure, reduces blood cholesterol levels, dysentery, jaundice, cholera, cardiac tonic, ulcer, astringent, cleaning of urinary tract, support uterus and regulate the hormonal cycle, antioxidant, fractures, fever, wounds, heart attack, urinary diseases	
		Decoction with milk	Cardioprotective	
		Infusion	Anti-diabetic	

S. No.	Parts Used	Preparations	Diseases Treated and Therapeutic Uses	References
		Powder	Cardiac disorders, blood pressure, headache, to kill worms in teeth, weakness, mouth blister, bone fracture, throat problems	
		Juice along with <i>Lannea coromandelica</i> , <i>Mangifera indica</i>	Chronic dysentery	
		Soaked in water	Atherosclerosis, cardiomyopathy	
		Ash	Snakebites or scorpion stings	
		Extract	Heart diseases, toothache	
		Powder with milk and water	Strengthen the stomach	
		Decoction, tea	Heart trouble, stomachic	
		Paste with pepper	Swollen muscle	
		Along with bark of <i>Mycanthes arbortristis</i>	Internal injuries	
		Powder with rice	Blood in urine	
		Paste	Skin diseases, herpes, leukoderma, dysentery, snake bites, bone fracture, maggots from the wounds to facilitate fast healing	



S. No.	Parts Used	Preparations	Diseases Treated and Therapeutic Uses	References
2.	Leaf, Bark	- Juice	Heart disease, tonic, wounds Heart diseases, heart pain	Das et al., 2012; Singh and Dubey, 2012
3.	Whole plant	- Powder	Oral cancer, spermatorrhoea, acne, heart disease, diabetes, wounds, headache, malaria Wounds, haemorrhages, ulcers, tumours, skin and gynaecological problems, earaches, dysentery, sexual diseases, urinary tract infections	Amin et al., 2009; Senthilkumar et al., 2014; Kaur, 2015; Panigrahy et al., 2016; Tiwari et al., 2022
4.	Stem-bark	Decoction with cow milk Decoction	Chest pain Fever, dysentery, cholera, malaria fever, cold, cough, reduces sugar level, blood filtering, and removing of clots from blood vessel	Behera, 2006; Khan and Singh, 2010; Sahu et al., 2013; Saini and Sood, 2017; Rao and Reddi, 2018; Pandey, 2021; Singh et al., 2022
		- Powder with milk Paste	Malaria, heart disease Cardiotonic, helps in reducing blood cholesterol level To improve sexual desire in men and women	
5.	Leaves	Juice Extract -	Earache Skin diseases, urinary infection, expectorant, acne Ulcer, earache	Yesodharan and Sujana, 2007; Gautam and Batra, 2014; Rath and Padhy, 2012; Singh et al., 2022

S. No.	Parts Used	Preparations	Diseases Treated and Therapeutic Uses	References
6.	Fruits	- Paste	Cardiac problems Wounds, astringent, purgative	Muthu et al., 2006; Kujur and Ahirwar, 2015; Naskar et al., 2022
7.	Bark, Young twigs	-	Internal injuries, as a toothbrush and tongue cleaner	Behera et al., 2021
8.	Stem	Decoction with goat milk	Debility	Dhal et al., 2014
9.	Stem bark, Root bark, Fruits, Leaves	-	Stress-induced heart problems, chronic respiratory disorders, urinary tract infections, high cholesterol (LDL), fractured bones, hormonal imbalance, obesity, tonic for rejuvenation, deobstruent, earache	Maheshwari and Sharma, 2019
10.	Latex	-	Menorrhagia, dysentery, vomiting	Gautam and Batra, 2014

\*[-] Not determine



## Ethnomedicinal and Folk treatments along the Ganga Basin of India

The Himalayan region has a long history of developing plant-based therapeutics. Worldwide, medicinal plants are essential to people's livelihoods. India's Himalayan state of Uttarakhand also relies on traditional medicine and medicinal herbs. This state's population relies primarily on traditional knowledge of medical practices and medicinal herbs as their major source of healthcare. Numerous prior research investigations on traditional medicine as scientific products have conventional claims of efficacy that are beneficial in treating a range of illnesses (Dwivedi et al., 2019). The Himalaya is a hotspot for biodiversity in the world, supporting 18,440 different species of plants, of which 25.3% are indigenous. Its many diverse geographical, ecological, and evolutionary characteristics contribute to species richness (Singh and Hajra, 1996; Samant et al., 1998). With a vast amount of natural diversity, the Himalayan hotspot state of Uttarakhand in India makes up 17.3% of the country's total land area, with 92.57% of that area being covered in hills and 7.43% in plains (Samant et al., 1998). In the state of Uttarakhand, 78% of the population lives in rural areas. The state has a relatively small number of primary health centres. Every basic health centre serves about 31,000 individuals, even though the hilly region of Uttarakhand is projected to have 20,000 people (Samal et

al., 2004). Because of their remote location and limited access to contemporary medical services, the people of Uttarakhand still rely on traditional herbal healers, or *Vaidhyas*, to treat their illnesses (Maikhuri et al., 1998; Kala, 2002; Kala, 2005). For their traditional system of therapy, the people community-primarily the Bokshas, Tharus, Bhotias, Van-Gujjars, Marchchas, Tolchas, Jaunsaris, Koltas, Gangwal, Banw-rauat, etc. generally depends on the wild flora (Gaur, 2008; Joshi and Pant, 2012). The people and tribes of Uttarakhand made extensive use of both edible and medicinal plants. Uttarakhand is home to numerous significant medicinal plants. People in Uttarakhand employed the bark of this plant to treat pneumonia, therefore they used *T. arjuna* to treat a variety of ailments (Joshi and Pant, 2012) such as fractures, ulcers, hypocholesterolemia, and hepatic function, antioxidant, antitumor, antibacterial, antimicrobial, antifeedant, and antifertility properties, as well as anti-HIV activity (Bachaya et al., 2009; Gopinath et al., 2013). The scarcity of paramedical personnel and doctors in rural Uttarakhand causes people living in the hilly regions of the state to be more severely affected and to have less access to high-quality medical care. Additionally, the medical facilities in this area are not functional. The ethnic population of Uttarakhand depends on native plant species for use in the traditional

healthcare system, both financially and logistically (Bhardwaj, et al., 2019). The bark of this shrub was utilized by the natives to cure diarrhea (Gairola et al., 2013). Additionally, the bark of this plant is utilized by the people of Dehradun as a treatment for hypertension, cancer, dermatological conditions, cardiac problems, and urinary ailments (Kanta et al., 2018) and for menstrual, mental, and cardiac conditions as well (Kumar, 2017).

Further, the state Uttar Pradesh has a tropical monsoonal climate with 600-2000 mm of yearly rainfall (Shukla et al., 2013). People and indigenous tribes of Uttar Pradesh employed *T. arjuna* to treat a variety of illnesses such as cholera, jaundice, and dysentery can be cured with the decoction of bark (Shukla et al., 2013). The people of Saharanpur employed this herb as a powerful medicine. The leaves of this plant are beneficial for diabetes, and it was once used as a heart tonic. Fruit can help lower high blood pressure. While treating oral issues, twigs are used as toothbrushes (Kumar and Singh, 2022). People in the Jalun district treat diarrhea with a decoction made from the bark (Saxena et al., 2014). Additionally, in Kannauj district, it is utilized to treat cardiac ailments (Verma and Yadav, 2021), by the locals and communities in the Sonbhadra district to treat high blood pressure and diarrhea (Anand et al., 2013). People in the Bundelkhand region employed its bark extract, fruits, and leaf extract to alleviate earaches, as well as the fruit as tonic, astringent, and deobstruent (Unial et

al., 2011). Fruits and bark are also used to treat jaundice and as a diuretic in Chandauli district (Kumar et al., 2015).

One of the most well-known Indian states, Bihar is significant to the country's economy (Roy et al., 2016). It is largely service based, with a significant share of agricultural and industrial sectors. The state has been working to enhance field-produced goods. To meet the expanding relevance of herbs as a source of therapeutic agents, essential oils, and raw materials for creating a variety of health-promoting goods, emphasis is being placed on the systematic cultivation of high-value medicinal and aromatic plants under current agro-ecological conditions (Ambast et al., 2016). Many medicinal plants were employed both ethnomedicinally and ethnobotanically by the people of Bihar. *T. arjuna* was utilized by the tribal people and the indigenous people in Bihar to treat a variety of illnesses (Kumari and Prabhat, 2020). The bark of this plant was used by the people of the Gopalganj district to treat heart disease and symptomatic hypertension (Kumar and Singh, 2014). The stem bark of this plant was used in the Rohtas area of Bihar to treat cardiac problems. A decoction of the bark was also used to treat digestive issues (Singh et al., 2018). Moreover, in Buxar, the bark is boiled in milk and used for heart disease (Singh et al., 2013), cough, chronic bronchitis, and fractures (Kumari et al., 2016). Additionally, residents in Bihar's Saran district used the bark to treat pain, diarrhea, cold, and asthma (Singh, 2018). The leaves and bark of this plant were also

utilized in Siwan to treat liver and heart conditions (Morya et al., 2018).

Moreover, the 28th state in India, Jharkhand has an area of 79,714 square kilometres and has 26,907,428 residents, or 2.62% of India's total population. 32 tribes and ethnic groups make up 11.85% of the state's total population. Asur, Birhor, Birjia, Korwa, Malpaharia, Paharia, Saurya Pahariya, and Savar are among its eight primitive tribal groups (PGT). The state is blessed with the greatest wealth of minerals and forests, as well as a beautiful natural environment and an abundance of plants (Kumari and Kerketta, 2019). The state of Jharkhand dominated by tribals is very rich in terms of cultural heritage and natural resources: minerals and biodiversity (Mairh et al., 2010). Many plants were employed by the Jharkhandi population for culinary and medicinal uses. They employed *T. arjuna* as a highly therapeutic herb; for example, they used the bark of this plant to treat heart conditions and enhance the function of the heart muscle. Juice from the leaves reduces diarrhea and ear pain (Kumari and Kerketta, 2019). Its bark was used as a plaster on pimples and other small skin lesions in the Sahibganj district of Jharkhand. The ground bark is a diuretic for liver cirrhosis and relieves symptoms of symptomatic hypertension. It is also a cardiac tonic. Fever and diarrhea are also treated with roasted seeds (Hebrom and Kumar, 2017). Bark powder is taken orally to cure bone fractures (Gupta and Kumar, 2018), anxiety, angina discomfort, irregular heartbeat (Gupta et al.,

2015), and heartburn (Tomar et al., 2012). This plant was also employed by the rural communities of district Chatra to balance the *vata*, *pitta*, and *kapha*. Additionally, it has been used to treat poisonings, bile duct problems, asthma, and scorpion stings. (Kumari et al., 2019).

Likewise, West Bengal is the only state in India where the plains and plateaus cover the remaining area, with the Himalayas to the North, bordering Sikkim and Bhutan, and the Sea to the South, bordering Assam and Bangladesh to the East (Chatterjee et al., 2006). People of West Bengal employs a wide variety of plants to treat a wide range of illnesses and *T. arjuna* is a highly medicinal plant that is used for a variety of uses. For example, the Santal people took a bath made from the bark of this plant to cure physical pain (Mandal et al., 2020). To treat gastric problems, residents in the Hooghly district used this plant (10-15 g crushed) together with 21 leaves of *Centella asiatica* and one leaf each of *T. chebula* and *T. belerica*, to produce a paste that was consumed for 15 days on an empty stomach (Chatterjee and Mukherjee, 2015). Powder of the bark is used for treatment of diarrhea, and roasted fruits are used for rheumatism, skin burns, and skin diseases (Chaudhury et al., 2018). People in the Cooch Behar District utilize this plant's powdered dried bark to treat a condition known as "Dhatudosa," which affects men (Mandal et al., 2020). The bark is also utilized in the Nadia district to treat leukorrhea, diabetes, anemia, hypertension, and cirrhosis of the liver (Banerjee, 2014).

Bark decoction is also administered on an empty stomach to cure heart problems and is practiced in the Jalpaiguri district (Bose et al., 2015). Its extract is used to treat heart problems in the Alipurduar district (Mandal et al., 2021). Further, the bark is soaked in water (for one night) and prepared pills

are utilized in the Jhargram district to treat cardiomyopathy and atherosclerosis (Paul and Day, 2022). The dried bark powder can be had with rice for lunch to lower blood pressure and to treat toothaches (Saha et al., 2016).

## Conclusion and Future Perspectives

Virtually every part of the plant has a great ethnopharmacological value with a wide array of traditional as well as pharmaceutical applications due to the presence of many bioactive chemical constituents. The plant was found to be very useful in antibacterial, antiviral, antimutagenic, anti-inflammatory, and wound-healing activities. The most exciting aspects of the plant were the treatment of diabetes, cancer, and heart diseases. However, the continuous research progress of using *T. arjuna* is very

much needed regarding exact molecular mechanism, drug administration, drug-drug interactions, and toxicological studies. Studies on molecular mechanisms in different cells, immunological markers, and synthesis of phytochemical and evaluation of its toxicity must be furnished. Increasing the awareness regarding its medicinal usage can give a direction to the physicians to respond to the challenges in treating cardiovascular diseases.

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CHAPTER  
**05**

# Phytochemical Analysis



## INTRODUCTION

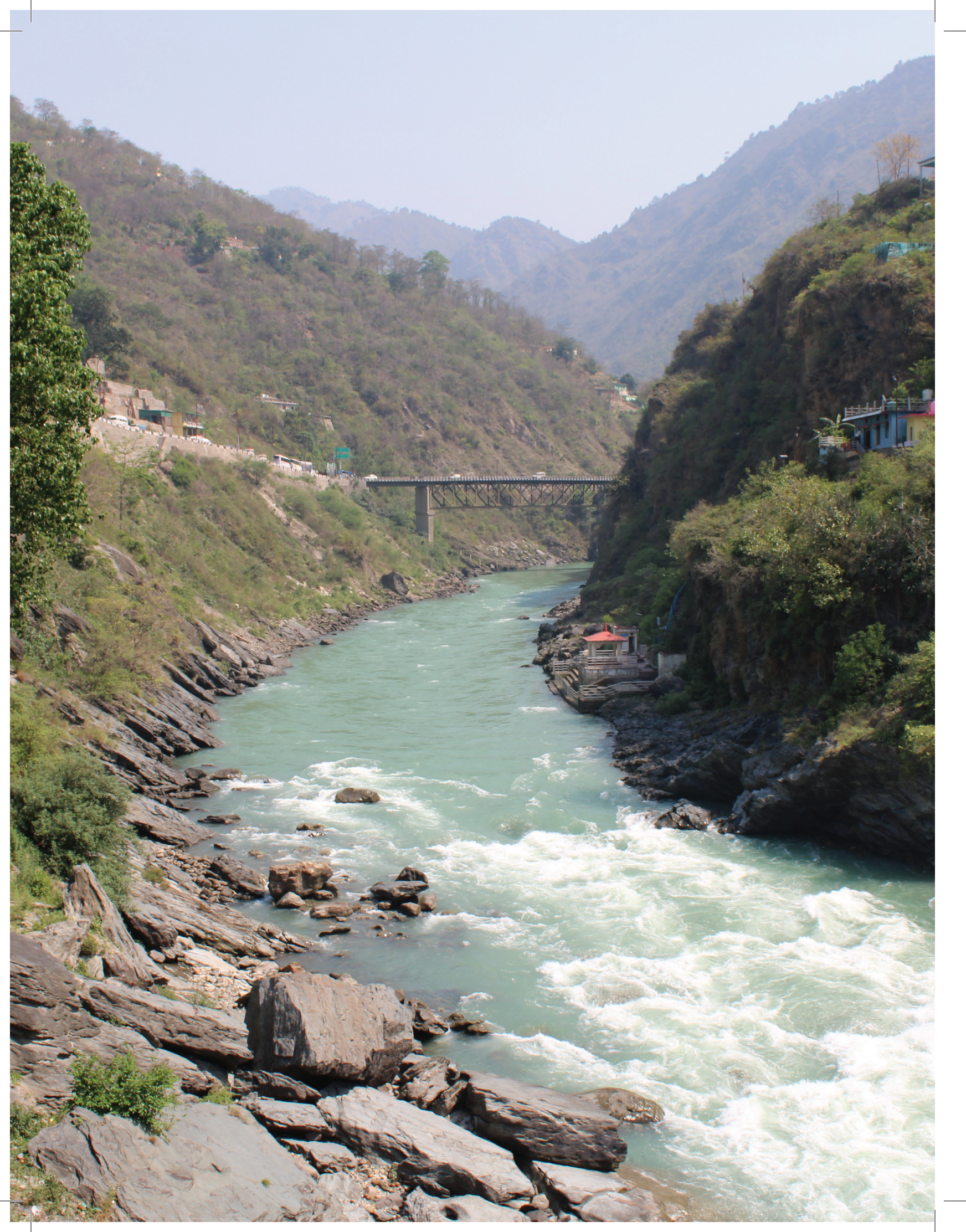
*Terminalia arjuna*, commonly known as 'Arjuna', is a deciduous large-sized fluted tree found throughout the South Asian region (Mandal et al., 2013). It has been used in traditional medicine for various purposes, including cardiogenic in heart failure, ischemic, cardiomyopathy, atherosclerosis, myocardium necrosis, and the treatment of blood anemia, venereal, and viral diseases (Amalraj and Gopi, 2016). The plant is known for its medicinal properties, which can be attributed to its phytochemical composition and bioactivities. The phytochemical composition of *T. arjuna* includes flavonoids, tannins, lactones, phenolic compounds, phytosterols, glycosides, etc. (Ramesh and Palaniappan, 2022). Further, it has been shown to have various bioactivities, including antioxidant, analgesic and anti-inflammatory, cardioprotective, antibacterial, and hypocholesterolemic activities (Mandal et al., 2013; Amalraj and Gopi, 2016).

Its usage in traditional medicine, particularly in cardiovascular diseases, is supported by scientific evidence. Hence, further research is needed to fully understand the potential therapeutic applications of *T. arjuna*.

## Sampling Sites

**Table 1** Sampling sites for the *Terminalia arjuna* collected from various state-specific locations spread over the Gangetic course

S. No.	Sampling sites
1.	Bijnor, Uttar Pradesh
2.	Narora, Uttar Pradesh
3.	Badaun, Uttar Pradesh
4.	Dalmau, Uttar Pradesh
5.	Prayagraj, Uttar Pradesh
6.	Mirzapur, Uttar Pradesh
7.	Varanasi, Uttar Pradesh
8.	Ballia, Uttar Pradesh
9.	Revelganj, Bihar



S. No.	Sampling sites
10.	Barh, Bihar
11.	Bahachoki, Bihar
12.	Farka, Bihar
13.	Sahibganj, Jharkhand
14.	Gomukh, Uttarakhand
15.	Farraka, West Bengal

## Phytochemical Analysis

Phytochemical components like tannin (by titration), total saponins (by gravimetry), total polyphenols, and total flavonoids (by UV-visible spectrophotometer), were determined for their respective contents via in-house protocols using API standards and literature, developed in Chemical Science Division, Drug Discovery and Development Department, Patanjali Research Foundation, Haridwar. For the identification and quantification of secondary metabolites and active components of the samples collected, advanced methods and techniques were used. High performance thin layer chromatography (HPTLC) was used to detect the presence of marker compounds and provide a chromatographic fingerprint of the plant sample. The hyphenated techniques have been proven as a powerful tool for the identification and structural characterization of known compounds. Hence, for further identification and quantification of compounds, high-performance liquid chromatography (HPLC) and ultra-performance liquid chromatography quadrupole time-of-flight mass spectrometry (UPLC/MS-QToF) were employed.

### Determination of Tannin Content

A sample weighing 1-10 g was taken and mixed with 50 ml of milli-Q water. The mixture was shaken and sonicated for 30 minutes. Then, the volume was adjusted to 100 ml and filtered. From this, 10 ml was taken and mixed with 750 ml of milli-Q water. Next, 25 ml of indigo sulphonic acid was added, and the mixture was shaken. The solution was titrated with 0.1N potassium permanganate solution until a golden yellow color appeared, indicating the endpoint. A blank test without a sample was also conducted to serve as a control.

### Determination of Saponin Content

A 5 g sample was taken and mixed with 50 ml of a solvent consisting of a 1:1 ratio of methanol and water. The mixture was then heated and allowed to boil for 1 hour. After cooling, the mixture was filtered. This process was repeated three times. The filtrate from each

round was combined and then concentrated and evaporated until it became dry. Next, 25 ml of petroleum ether was added to the dry mixture and heated for 10 minutes. After cooling, the layer of ether was separated. Then, 10 ml of methanol and 100 ml of acetone were added to the remaining mixture and filtered. The filter paper with the residue was dried at 80°C for 1 hour and weighed.

### Determination of Total Polyphenol Content

A 1 ml sample was taken in a test tube and 1 ml of Folin–Ciocâlțeu reagent was added. The mixture was then incubated for 5 minutes. After that, 1 ml of a 10% sodium carbonate solution was added. The test tube was kept in the dark

for 1 hour. The absorbance was measured at 760 nm using a UV-visible spectrophotometer. The same steps were repeated for gallic acid to create a linear plot.

### Determination of Total Flavonoid Content

A 1 ml sample was first placed in a test tube. Then, 0.4 ml of 10% aluminum chloride, 0.4 ml of sodium acetate, and 3 ml of ethanol were added to the sample. The mixture was kept at room temperature for 30 minutes. After that, the absorbance at 450 nm was measured using a UV-visible spectrophotometer. These steps were repeated for quercetin dihydrate to create a linear plot.

## HPTLC Fingerprinting

This technique has been recognized for its effectiveness in exploring the qualitative aspects of secondary metabolites in various plant species. As per WHO Technical Report Series, No. 1010, (2018), HPTLC chromatographic pattern, generally refer as “fingerprints”, are used for identification of phytochemicals. The band or spots obtained during test are characteristic of herb. A color image of typical TLC fingerprint provides a clearer guide to the users.

### Sample Preparation

To analyze each batch, approximately 1 g of sample was dissolved in 10 ml of methanol.

The samples were then shaken and sonicated for 20 minutes. Afterward, the solution was centrifuged at 5000 rpm for 5 minutes. The resulting clear solution was used for analysis.

### Methodology and Analytical Conditions

Analysis was performed on CAMAG HPTLC (Muttensz, Switzerland), equipped with an Automatic TLC Sampler (ATS 4), TLC scanner 4 and TLC visualize. Data processing acquisition and visualization were achieved using win-CATS software (version 1.4.10). The chromatographic conditions for the HPTLC analysis were as follows:

<b>Stationary phase</b>	TLC Silica gel 60 F <sub>254</sub> aluminum sheet (1.0554.0007)
<b>Mobile phase</b>	Ethyl acetate: Toluene: Formic acid: Acetic acid (5:3:1:0.5 v/v/v/v)
<b>Saturation time</b>	15 minutes
<b>Migration distance</b>	80 mm
<b>Band length</b>	8 mm
<b>Injection volume</b>	10 µl
<b>Visualization</b>	366 nm and under white light after derivatized with anisaldehyde-sulphuric acid

## High-Performance Liquid Chromatography (HPLC)

### Sample and Standard Preparation

Sample: Approximately 5 g of a sample was heated in 100 ml of diethyl ether for 15 minutes in a water bath. The solution was then cooled and filtered. The residue was refluxed two more times with diethyl ether, cooled, and filtered again. All the filtrates were combined, and the solvent was evaporated under reduced pressure until dry. The resulting residue was dissolved in 10 ml of methanol and filtered using a 0.45 µm nylon filter. The filtered solution was used for analysis.

Standard: Arjungenin reference standard was dissolved in methanol to create a solution with a concentration of 0.1 mg/ml. Arjunetin, arjungenin, arjunolic acid, and arjunic acid were determined based on the relative retention time (RRT) of arjungenin. The RRT values were as follows: arjunetin 0.67 min,

arjungenin 1.00 min, arjunolic acid 1.5 min, and arjunic acid 1.7 min.

### Analytical and Instrumentation Condition

Analysis was performed by Prominence-XR UHPLC system (Shimadzu, Japan) equipped with a quaternary pump (NexeraXR LC-20AD XR), DAD detector (SPD-M20 A), auto-sampler (Nexera XR SIL-20 AC XR), degassing unit (DGU-20A5R) and column oven (CTO-10ASVP). Separation was achieved using a Shodex C18-4E (5 µm, 4.6 x 250 mm) column, subjected to binary gradient elution. The two solvents used for the analysis consisted of water containing 0.140 mg potassium hydrogen phosphate in 1000 ml water and 0.5 ml orthophosphoric acid (solvent A) and acetonitrile (solvent B). The flow rate was set at 1.5 ml/min during the analysis. Twenty microliters of standard and test solution were injected. The wavelength was set at 205 nm.

**Gradient program used**

Time (Min)	A %	B%
0	70	30
18	40	60
20	15	85
22	15	85
25	70	30
30	70	30

## Identification of compounds by Ultra Performance Liquid Chromatography Quadrupole Time-of-Flight Mass Spectrometry (UPLC/MS-QToF)

Test sample: *Terminalia arjuna* (PRF/CHI/1123/1065): Narora, Uttar Pradesh

### Sample Preparation

A 500 mg powdered sample was dissolved in 10 ml methanol and sonicated for 30 min. The solution was centrifuged at 10000 rpm for 5 minutes and filtered through a 0.22 µm nylon filter.

### Analytical and Instrumentation Conditions

Analysis was performed on a Xevo G2-XS QToF (Waters Corporation, USA) with Acquity UPLC-I Class and Unifi software. Separation was

carried out using ChromCore 120 C18 (100 x 2.1 mm, 1.8 µm) column (China) with a flow rate of 0.3 ml/min using gradient elution of 0.1 % v/v formic acid in water (mobile phase A) and 0.1 % v/v formic acid in acetonitrile (mobile phase B). The column was maintained at 40°C throughout the analysis and sample temperature was kept at 20°C. Detection was carried out by Xevo G2-XS QToF. Two microliters of test solution was injected in UPLC/MS-QToF and chromatograms were recorded in positive and negative ionization mode.

**Gradient program used**

Time (min)	Flow rate (ml/min)	Mobile phase A %	Mobile phase B%
0	0.3	95	5
2	0.3	95	5
10	0.3	85	15

Time (min)	Flow rate (ml/min)	Mobile phase A %	Mobile phase B%
25	0.3	70	30
40	0.3	50	50
50	0.3	20	80
55	0.3	20	80
56	0.3	95	5
60	0.3	95	5

### Xevo G2-XS QToF Parameters

Parameter	Polarity (+ve)	Polarity (-ve)
Ionisation type	ESI	ESI
Mode	MS <sup>E</sup>	MS <sup>E</sup>
Mass range (m/z)	50-1200 m/z	50-1200 m/z
Scan time	0.5 s	0.5 s
Cone Voltage	40 V	40 V
Capillary	1.0 kV	2.0 kV
Low CE	6.0 eV	6.0 eV
High CE	15-60 eV(ramp)	15-60 eV(ramp)
Source temperature	120°C	120°C
Desolvation Temperature	500°C	500°C
Cone gas flow	50 L/h	50 L/h
Desolvation gas flow	900 L/h	900 L/h
Lock Spray (Leucine Enkaphalin)	556.2766 m/z	554.2620 m/z
Lock mass scan time	0.5 s	0.5 s
Lock mass interval	30 s	30 s

## Results and Discussion

### Phytochemical Analysis

The quantitative phytochemical analysis revealed the presence of tannins, saponins, total polyphenols, and total flavonoids in the plant samples from various locations. The content of these phytochemicals varied across different samples, indicating the diversity in chemical composition based on geographical location. For example, the samples from Varanasi, Uttar Pradesh, exhibited the highest content of tannins, while Gomukh, Uttarakhand, showed the highest saponin content (Table 2).

These findings are consistent with previous studies that have reported the diverse phytochemical composition of *T. arjuna*, including flavonoids, tannins, and phenolic compounds. Mandal et al. (2013) also highlighted the antioxidant and antimicrobial properties of *T. arjuna* bark extract, further supporting the bioactivity of the phytochemicals identified in this study.

- ◆ **Tannins:** Tannins are known for their antioxidant properties and have been reported to exhibit anti-inflammatory, antimicrobial, and cardioprotective effects. Studies have shown that tannins present in *T. arjuna* contribute to its cardioprotective activity by helping to maintain cardiovascular health and reduce oxidative stress. Additionally, tannins have been linked to anti-diabetic and anti-cancer properties, highlighting the diverse bioactivity of these compounds.
- ◆ **Saponins:** Saponins are bioactive compounds with various pharmacological activities, including anti-inflammatory, anti-cancer, and immunomodulatory effects. Research suggests that saponins found in *T. arjuna* may contribute to its anti-inflammatory properties and potential therapeutic benefits for conditions such as cardiovascular diseases and diabetes. Furthermore, saponins have been studied for their hepatoprotective and neuroprotective effects, indicating the multifaceted bioactivity of these compounds.
- ◆ **Flavonoids:** Flavonoids are well-known for their antioxidant and anti-inflammatory properties, making them valuable in the prevention and treatment of various diseases. *T. arjuna* contains flavonoids such as arjunetin and arjungenin, which have been associated with cardioprotective effects and the modulation of lipid metabolism. Flavonoids in *T. arjuna* may also contribute to their anti-hypertensive and anti-atherosclerotic activities, highlighting their potential therapeutic significance.
- ◆ **Phenolic Compounds:** Phenolic compounds, including arjunolic acid and arjunic acid, have been studied for their antioxidant, anti-inflammatory, and anti-cancer properties. These compounds present in *T. arjuna* may play a role in protecting against oxidative stress,

reducing inflammation, and potentially inhibiting the growth of cancer cells. The bioactivity of phenolic compounds in *T.*

*arjuna* aligns with its traditional use in Ayurvedic medicine for cardiovascular health and overall well-being.

**Table 2** Phytochemical analysis of *Terminalia arjuna* collected from different locations

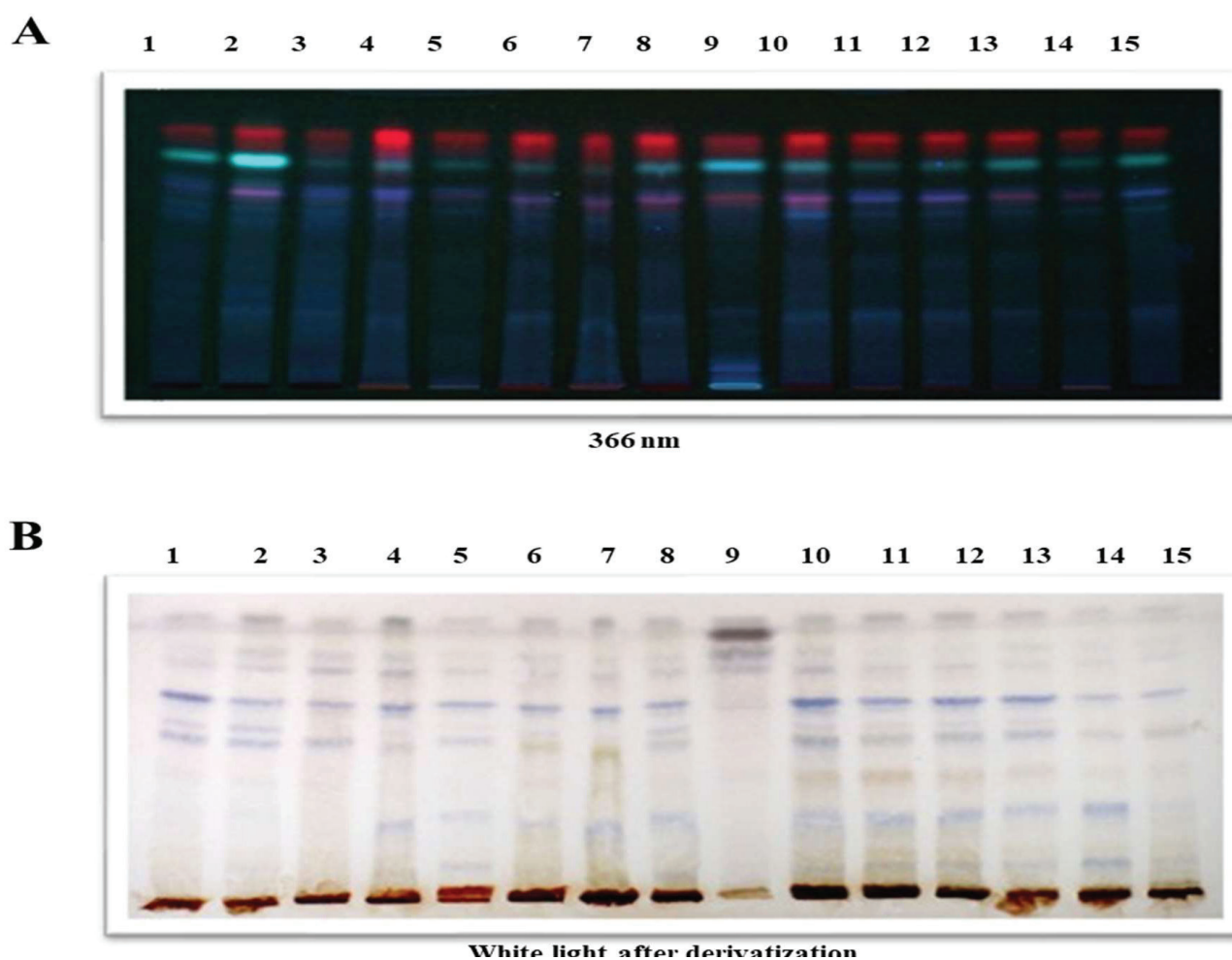
S. No.	Samples	Tannin (%w/w)	Saponin (%w/w)	Total polyphenol (%w/w)	Total flavonoid (%w/w)
1.	Bijnor, Uttar Pradesh	0.133	7.76	0.649	0.455
2.	Narora, Uttar Pradesh	0.071	8.88	0.717	0.247
3.	Badaun, Uttar Pradesh	0.071	7.44	0.416	0.358
4.	Dalmau, Uttar Pradesh	0.133	7.21	0.624	0.394
5.	Prayagraj, Uttar Pradesh	0.049	13.08	0.571	0.164
6.	Mirzapur, Uttar Pradesh	0.152	16.90	0.588	0.269
7.	Varanasi, Uttar Pradesh	0.272	13.64	0.401	0.439
8.	Ballia, Uttar Pradesh	0.104	8.400	0.770	0.503
9.	Revelganj, Bihar	0.050	2.05	0.129	0.321
10.	Barh, Bihar	0.174	7.53	0.628	0.329
11.	Bahachoki, Bihar	0.110	8.94	0.672	0.334
12.	Farka, Bihar	0.174	14.14	0.661	0.095
13.	Sahibganj, Jharkhand	0.090	8.83	0.604	0.274
14.	Gomukh, Uttarakhand	0.216	19.41	0.449	0.390
15.	Farraka, West Bengal	0.150	8.84	0.108	0.408

### HPTLC Fingerprint Analysis

HPTLC analysis provided a visual representation of the chemical diversity within the samples, offering insights into the unique phytochemical profile of the plant samples. The results of the HPTLC analysis highlighted the presence of a diverse range of phytochemicals, further emphasizing the plant's chemical complexity. HPTLC chromatograms obtained under multiwavelength detection using short wave

(254 nm) and long wave (366 nm) -UV light revealed a different pattern in the chemical composition of the analyzed samples (Fig. 1).

Similar studies have utilized HPTLC to characterize the phytochemical composition of medicinal plants, demonstrating its efficacy in profiling bioactive compounds. The results of this analysis align with the broader literature on the chemical complexity of *T. arjuna* and its potential therapeutic applications.



**Fig. 1** HPTLC fingerprinting of methanolic extract of *Terminalia arjuna*. **A.** 366 nm, **B.** Under white light after derivatized with anisaldehyde-sulphuric acid

## HPLC Analysis

HPLC analysis was conducted using reference compounds such as arjunetin, arjungenin, arjunolic acid, and arjunic acid to determine their content in the plant samples. The chromatograms obtained from the HPLC analysis showcased the different percentages

of these compounds in the respective samples, providing valuable information on the chemical composition of *T. arjuna* (Table 3). The respective chromatograms representing the reference standards and the respective plant samples from 15 different locations against a 30-minute run have been presented below for reference (Fig 2, 3).

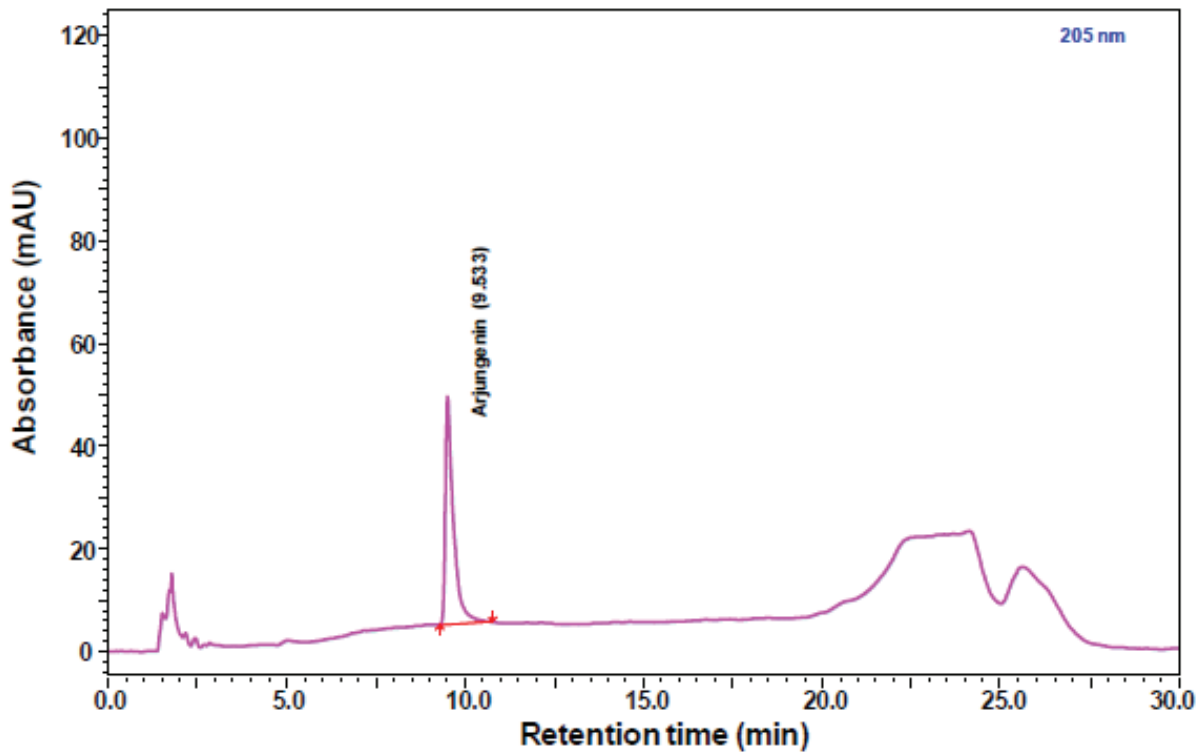


Fig. 2 HPLC chromatogram of standard Arjungenin RS at 205 nm

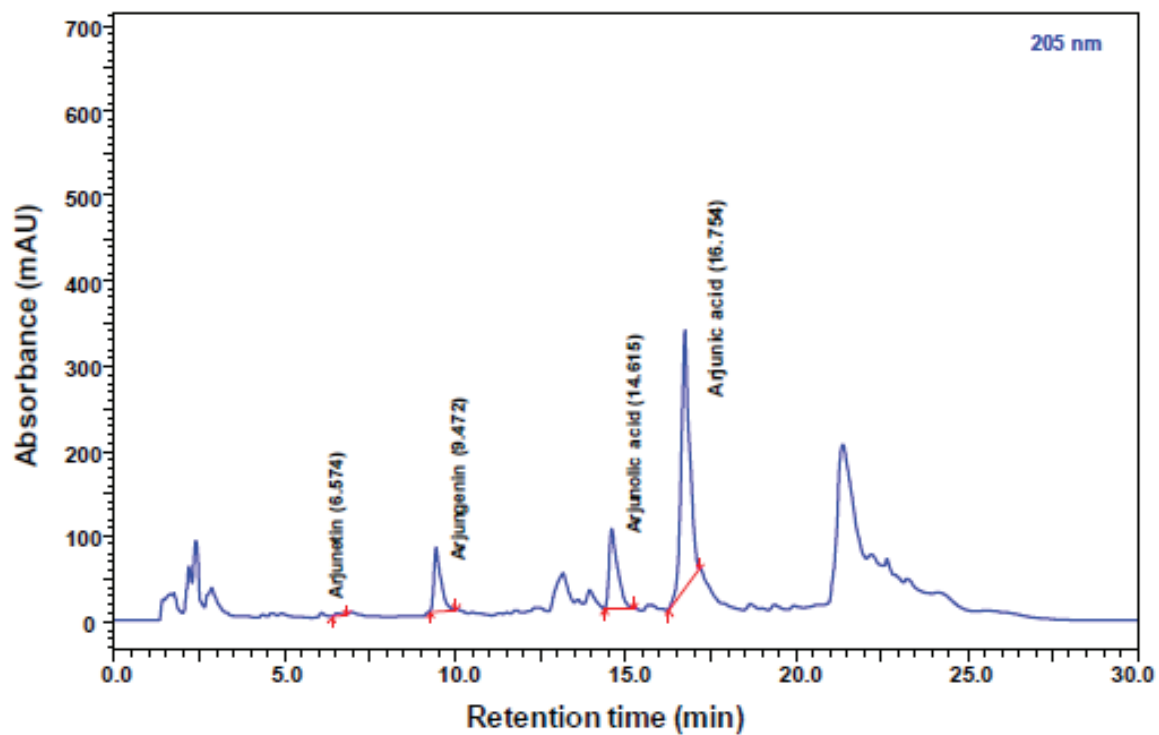


Fig. 3 HPLC chromatogram of *Terminalia arjuna*(PRF/CHI/1123/1075) at 205 nm

**Table 3** Phytochemicals content present in *Terminalia arjuna* collected from different locations

S. No	Internal code	Samples	Arjunetin (µg/mg)	Arjungenin (µg/mg)	Arjunolic acid (µg/mg)	Arjunic acid (µg/mg)
1	PRF/CHI/1123/1064	Bijnor, Uttar Pradesh	ND	0.210	0.529	1.325
2	PRF/CHI/1123/1065	Narora, Uttar Pradesh	ND	0.298	0.177	0.455
3	PRF/CHI/1123/1066	Badaun, Uttar Pradesh	ND	0.077	0.083	0.094
4	PRF/CHI/1123/1067	Dalmou, Uttar Pradesh	ND	0.119	0.367	0.862
5	PRF/CHI/1123/1068	Prayagraj, Uttar Pradesh	ND	0.182	0.499	1.028
6	PRF/CHI/1123/1069	Mirzapur, Uttar Pradesh	ND	0.019	0.053	0.174
7	PRF/CHI/1123/1070	Varanasi, Uttar Pradesh	ND	0.145	0.417	1.108
8	PRF/CHI/1123/1071	Ballia, Uttar Pradesh	ND	0.140	0.302	0.860
9	PRF/CHI/1123/1072	Revelganj, Bihar	ND	ND	ND	ND
10	PRF/CHI/1123/1073	Barh, Bihar	ND	0.309	0.296	1.474
11	PRF/CHI/1123/1074	Bahachoki, Bihar	0.011	0.209	0.472	1.005
12	PRF/CHI/1123/1075	Farka, Bihar	0.010	0.325	0.494	1.596
13	PRF/CHI/1123/1076	Sahibganj, Jharkhand	ND	0.411	0.341	2.321
14	PRF/CHI/1123/1077	Gomukh, Uttarakhand	ND	0.078	0.126	0.333
15	PRF/CHI/1123/1078	Farraka, West Bengal	ND	0.098	0.259	0.258

The results indicated variations in the levels of these compounds across different geographical locations, reflecting the influence of environmental factors on phytochemical production. These compounds are known for their pharmacological activities, including

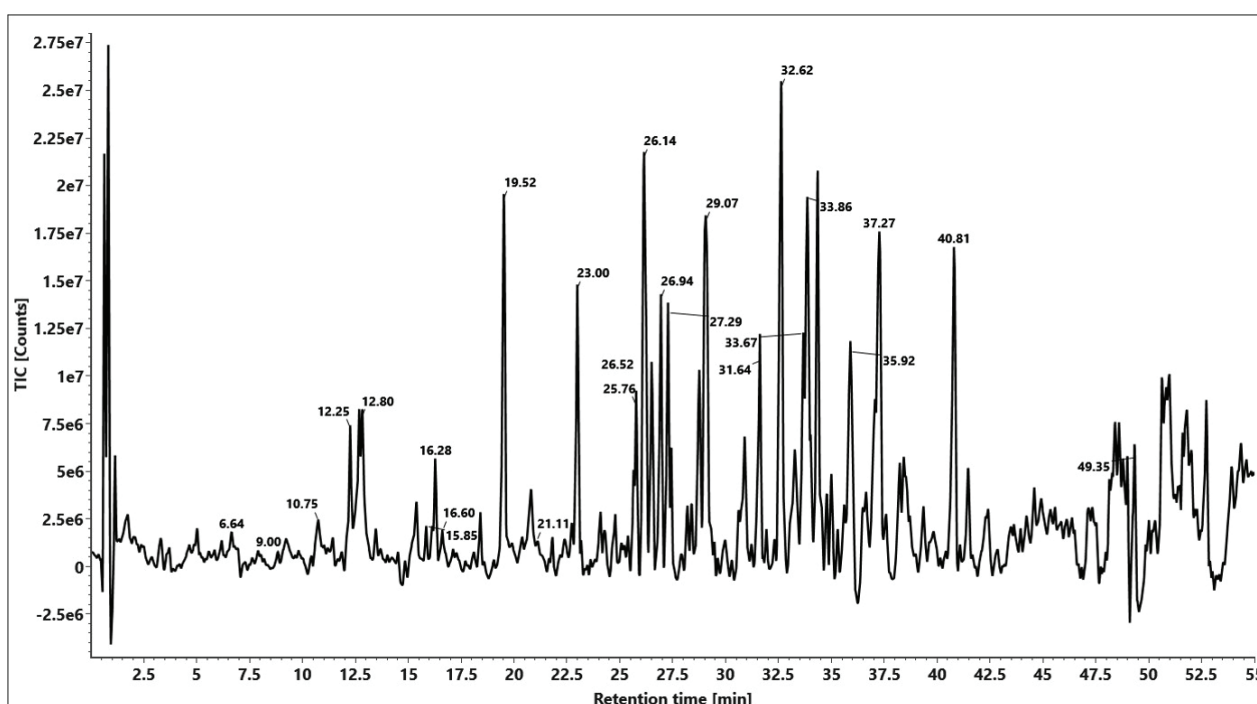
cardioprotective and anti-inflammatory effects. Previous research has also identified these compounds in *T. arjuna* and highlighted their significance in traditional medicine and modern pharmaceuticals.

## UPLC/MS-QToF Analysis

The analysis resulted in a fast, high-resolution separation with high sensitivity. The qualitative (identification of compounds), and relative quantification (response curve) were obtained for a variety of substances within the complex mixture. The TIC chromatogram as well as the list of identified compounds and their associated parameters in both positive and negative modes have been presented below. A total of 30 compounds were consequently identified.

This methodology allowed for a detailed

characterization of the chemical constituents present in the plant species, enhancing our understanding of its phytochemical complexity. Similar studies utilizing UPLC/MS-QToF have demonstrated its efficacy in identifying bioactive compounds in medicinal plants and providing comprehensive chemical profiles. The results from UPLC/MS-QToF analysis further support the rich phytochemical diversity of *T. arjuna* and its potential therapeutic applications.

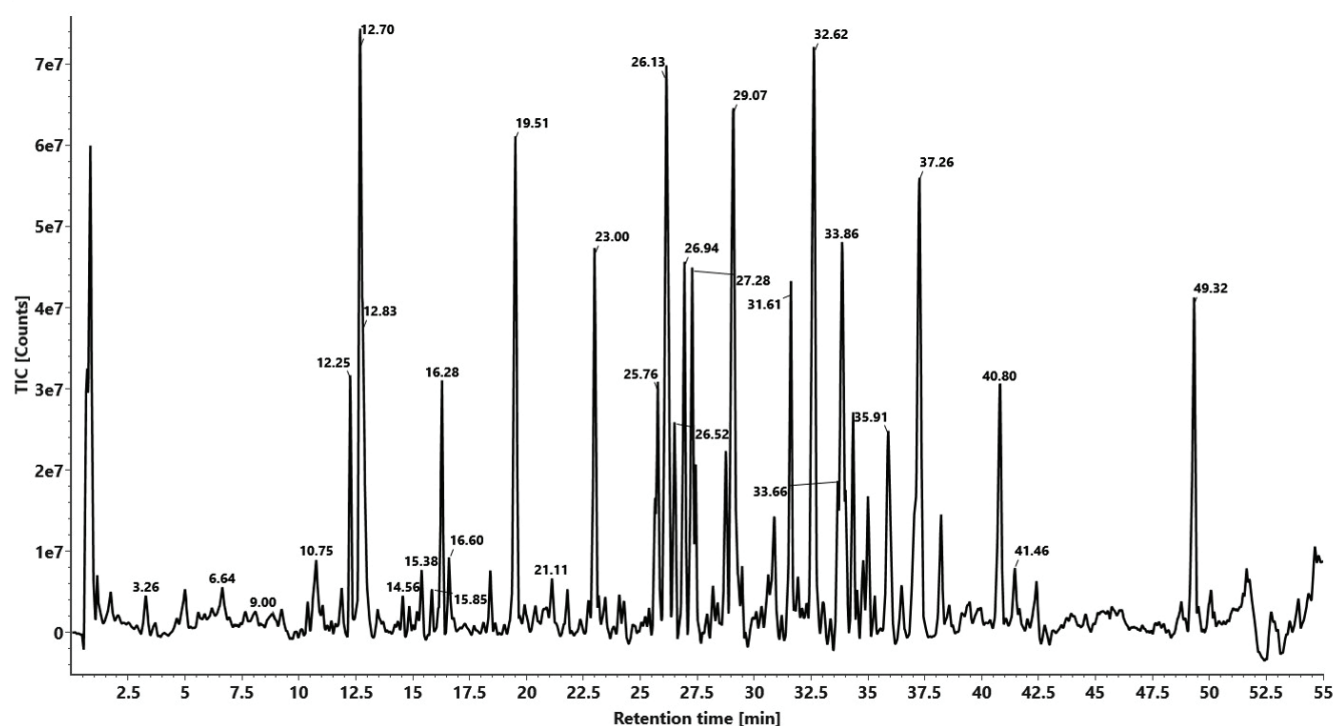


**Fig. 4** TIC chromatogram of *Terminalia arjuna* (PRF/CHI/1123/1065) in positive ionization mode

**Table 4** Compound-dependent parameters of analytes/identified compounds in *Terminalia arjuna*(PRF/CHI/1123/1065) in positive ionizationmode

S. N.	Component name	Formula	Neutral mass(Da)	Observed m/z	Masserror (mDa)	RT (min)	Response	Adducts
1	Catechin	C <sub>15</sub> H <sub>14</sub> O <sub>6</sub>	290.0790	291.0850	-1.3	6.64	104447	+H
2	(-)-Epicatechin	C <sub>15</sub> H <sub>14</sub> O <sub>6</sub>	290.0790	291.0853	-1.0	9.00	23082	+H
3	3,3'-Di-ellagicacid	C <sub>28</sub> H <sub>100</sub> O <sub>16</sub>	601.9969	603.0048	0.7	10.75	293737	+H
4	Ellagicacid4-xylloside	C <sub>19</sub> H <sub>140</sub> O <sub>12</sub>	434.0485	435.0543	-1.5	12.25	270244	+H
5	Ellagicacid	C <sub>14</sub> H <sub>60</sub> 8	302.0063	303.0117	-1.8	12.80	243665	+H
6	3,4'-Di-O-Methylellagicacid-4-O- $\alpha$ -L-arabinofuranoside	C <sub>21</sub> H <sub>180</sub> O <sub>12</sub>	462.0798	463.0861	-1.0	15.85	37520	+H
7	3-O-Methylellagicacid-3'-O- $\alpha$ -L-rhamnopyranoside	C <sub>21</sub> H <sub>180</sub> O <sub>12</sub>	462.0798	463.0855	-1.6	16.28	191525	+H
8	3-O-Methylellagicacid	C <sub>15</sub> H <sub>80</sub> 8	316.0219	317.0272	-2.0	16.60	48058	+H
9	Arjunglucosidel	C <sub>36</sub> H <sub>580</sub> O <sub>11</sub>	666.3979	689.3901	2.9	19.52	57594	+Na
10	3,3'-Di-O-methylellagicacid	C <sub>16</sub> H <sub>100</sub> 8	330.0376	331.0427	-2.1	21.11	47512	+H
11	Termiarjunosidel	C <sub>36</sub> H <sub>580</sub> O <sub>11</sub>	666.3979	689.3891	2.0	23.00	32220	+Na
12	ArjunasideA	C <sub>36</sub> H <sub>560</sub> O <sub>10</sub>	648.3874	671.3789	2.4	25.76	30773	+Na
13	Arjunglucosidel/Arjunetin/its isomer	C <sub>36</sub> H <sub>580</sub> O <sub>10</sub>	650.4030	673.3960	3.8	26.14	85941	+Na
14	BellericageninB	C <sub>30</sub> H <sub>46</sub> O <sub>7</sub>	520.3400	521.3478	0.5	26.52	38231	+H
15	Arjunglucosidel/Arjunetin/its isomer	C <sub>36</sub> H <sub>58</sub> O <sub>10</sub>	650.4030	673.3973	5.1	26.94	64118	+Na

S. N.	Component name	Formula	Neutral mass(Da)	Observed m/z	Masserror (mDa)	RT (min)	Response	Adducts
16	Arjunglucosidel/Arjunetin/its isomer	C <sub>36</sub> H <sub>58</sub> O <sub>10</sub>	650.4030	673.3951	2.9	27.29	46123	+Na
17	Arjungenin/Terminolicacid/its isomer	C <sub>30</sub> H <sub>48</sub> O <sub>6</sub>	504.3451	527.3341	-0.2	29.07	92316	+Na
18	Arjungenin/Terminolicacid/its isomer	C <sub>36</sub> H <sub>58</sub> O <sub>10</sub>	650.4030	673.3960	3.8	31.64	28271	+Na
19	Arjungenin/Terminolicacid/its isomer	C <sub>30</sub> H <sub>48</sub> O <sub>6</sub>	504.3451	527.3347	0.4	32.62	58174	+Na
20	19,23-Dihydroxy-3-oxours-12-en-28-oicacid	C <sub>30</sub> H <sub>46</sub> O <sub>5</sub>	486.3345	509.3249	1.1	33.67	11989	+Na
21	Arjungenin/Terminolicacid/its isomer	C <sub>30</sub> H <sub>48</sub> O <sub>6</sub>	504.3451	505.3526	0.3	33.86	67297	+H
22	Arjunolicacid	C <sub>30</sub> H <sub>48</sub> O <sub>5</sub>	488.3502	511.3397	0.3	35.92	35750	+Na
23	Arjunicacid	C <sub>30</sub> H <sub>48</sub> O <sub>5</sub>	488.3502	489.3567	-0.8	37.27	38270	+H
24	Terminoicacid	C <sub>30</sub> H <sub>48</sub> O <sub>5</sub>	488.3502	511.3392	-0.2	40.81	21113	+Na
25	Ursolicacid	C <sub>30</sub> H <sub>48</sub> O <sub>3</sub>	456.3604	457.3684	0.8	49.35	7769	+H



**Fig. 5** TIC chromatogram of *Terminalia arjuna* (PRF/CHI/1123/1065) in negative ionization mode

**Table 5** Compound-dependent parameters of analytes/identified compounds in *Terminalia arjuna* (PRF/CHI/1123/1065) in negative ionization mode

S. N.	Component name	Formula	Neutral mass(Da)	Observed m/z	Mass error (mDa)	RT (min)	Response	Adducts
1	Gallocatechin	C <sub>15</sub> H <sub>14</sub> O <sub>7</sub>	306.0740	305.0670	0.3	3.26	291773	-H
2	Catechin	C <sub>15</sub> H <sub>14</sub> O <sub>6</sub>	290.0790	289.0726	0.8	6.64	428156	-H
3	(-)-Epicatechin	C <sub>15</sub> H <sub>14</sub> O <sub>6</sub>	290.0790	289.0714	-0.4	9.00	101601	-H
4	3,3'-Diellagicacid	C <sub>28</sub> H <sub>100</sub> O <sub>16</sub>	601.9969	600.9873	-2.3	10.75	2376860	-H
5	Ellagicacid4-xyloside	C <sub>19</sub> H <sub>14</sub> O <sub>12</sub>	434.0485	433.0432	1.9	12.25	2523630	-H
6	Ellagicacid2-rhamnoside	C <sub>20</sub> H <sub>16</sub> O <sub>12</sub>	448.0642	447.0568	-0.1	12.70	4228197	-H
7	Ellagicacid	C <sub>14</sub> H <sub>6</sub> O <sub>8</sub>	302.0063	300.9989	0.0	12.83	3107241	-H
8	3-Methylellagicacid8-rhamnoside	C <sub>21</sub> H <sub>18</sub> O <sub>12</sub>	462.0798	461.0733	0.8	14.56	450501	-H

S. N.	Component name	Formula	Neutral mass(Da)	Observed m/z	Mass error (mDa)	RT (min)	Response	Adducts
9	3-O-Methylelagic acid 4-O-beta-D-Xylopyranoside	C <sub>20</sub> H <sub>16</sub> O <sub>12</sub>	448.0642	447.0632	6.3	15.38	1038832	-H
10	3,4'-Di-O-Methylelagic acid-4-O-α-L-Arabinofuranoside	C <sub>21</sub> H <sub>18</sub> O <sub>12</sub>	462.0798	461.0729	0.4	15.85	517661	-H
11	3-O-Methylelagic acid-3'-O-α-L-rhamnopyranoside	C <sub>21</sub> H <sub>18</sub> O <sub>12</sub>	462.0798	461.0726	0.0	16.28	2003651	-H
12	3-O-Methylelagic acid	C <sub>15</sub> H <sub>8</sub> O <sub>8</sub>	316.0219	315.0152	0.6	16.60	826167	-H
13	Arjunglucosidel	C <sub>36</sub> H <sub>58</sub> O <sub>11</sub>	666.3979	711.3942	-1.9	19.51	6592347	+HCOO
14	3,3'-Di-O-Methylelagic acid	C <sub>16</sub> H <sub>10</sub> O <sub>8</sub>	330.0376	329.0292	-1.1	21.11	750793	-H
15	Termiarjunosidel	C <sub>36</sub> H <sub>58</sub> O <sub>11</sub>	666.3979	711.4010	4.8	23.00	4812834	+HCOO
16	ArjunasideA	C <sub>36</sub> H <sub>56</sub> O <sub>10</sub>	648.3874	693.3857	0.1	25.76	3208575	+HCOO
17	Arjunglucosidel/Arjunetin/its isomer	C <sub>36</sub> H <sub>58</sub> O <sub>10</sub>	650.4030	695.3994	-1.8	26.13	6868757	+HCOO
18	BellericageninB	C <sub>30</sub> H <sub>48</sub> O <sub>7</sub>	520.3400	565.3373	-0.9	26.52	2219798	+HCOO
19	Arjunglucosidel/Arjunetin/its isomer	C <sub>36</sub> H <sub>58</sub> O <sub>10</sub>	650.4030	695.4005	-0.7	26.94	4917918	+HCOO
20	Arjunglucosidel/Arjunetin/its isomer	C <sub>36</sub> H <sub>58</sub> O <sub>10</sub>	650.4030	695.4021	0.9	27.28	4362639	+HCOO
21	Arjungenin	C <sub>30</sub> H <sub>48</sub> O <sub>6</sub>	504.3451	549.3494	6.1	29.07	5986244	+HCOO
22	Arjungenin/Terminolic acid/its isomer	C <sub>36</sub> H <sub>58</sub> O <sub>10</sub>	650.4030	695.4005	-0.7	31.61	3433447	+HCOO
23	Arjungenin/Terminolic acid/its isomer	C <sub>30</sub> H <sub>48</sub> O <sub>6</sub>	504.3451	503.3378	0.0	32.62	3035297	-H

S. N.	Component name	Formula	Neutral mass(Da)	Observed m/z	Mass error (mDa)	RT (min)	Response	Adducts
24	19,23-Dihydroxy-3-oxo-12-en-28-oic acid	C <sub>30</sub> H <sub>46</sub> O <sub>5</sub>	486.3345	531.3328	0.1	33.66	1353153	+HCOO
25	Arjungenin/Terminolic acid/its isomer	C <sub>30</sub> H <sub>48</sub> O <sub>6</sub>	504.3451	549.3427	-0.5	33.86	5023322	+HCOO
26	Arjunolic acid	C <sub>30</sub> H <sub>48</sub> O <sub>5</sub>	488.3502	533.3482	-0.2	35.91	3399804	+HCOO
27	Arjunic acid	C <sub>30</sub> H <sub>48</sub> O <sub>5</sub>	488.3502	533.3497	1.3	37.26	4767679	+HCOO
28	Terminoic acid	C <sub>30</sub> H <sub>48</sub> O <sub>5</sub>	488.3502	533.3476	-0.7	40.80	2410232	+HCOO
29	19,24-Dihydroxy-3-oxo-12-en-28-oic acid	C <sub>30</sub> H <sub>46</sub> O <sub>5</sub>	486.3345	485.3273	0.1	41.46	686810	-H
30	Ursolic acid	C <sub>30</sub> H <sub>48</sub> O <sub>3</sub>	456.3604	455.3541	1.0	49.32	3581410	-H

## Conclusion

A comprehensive analysis was conducted on leaves obtained from 15 distinct *Terminalia arjuna* samples, sourced from various locations along the Gangetic belt. The purpose of this research was to explore the phytochemical composition of *T. arjuna*, shedding light on its diverse secondary metabolites. The samples were tested for phytochemicals such as tannins, saponins, polyphenols, and flavonoids. The content of total polyphenolics and flavonoids were found to be similar in the samples, but the content of tannin and saponin was highest in the samples from Varanasi (Uttar Pradesh) and Gomukh (Uttarakhand), respectively. The HPTLC also showed a diverse range of phytochemicals in the samples.

HPTLC was employed to fingerprint the samples, allowing for the identification of major compounds or secondary metabolites. The results of this technique provided a visual

representation of the chemical diversity within the samples, enhancing our understanding of the plant's chemical profile. Using HPLC, we determined the exact content of the reference compounds, arjunetin, arjungenin, arjunolic acid, and arjunic acid in the samples. This step allowed for a more targeted examination of key bioactive compounds, shedding light on their abundance in different samples along the Gangetic belt.

Finally, the study employed advanced hyphenated techniques, namely ultra-performance liquid chromatography/mass spectrometry quadrupole time-of-flight (UPLC/MS-QToF), for the identification and quantification of a total of 30 compounds. This advanced methodology allowed for a highly detailed and accurate characterization of the chemical constituents present in the species, further enhancing our understanding of its phytochemical complexity.

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CHAPTER

06

# Insilico Analysis Against Endometrial Cancer





## INTRODUCTION



Endometrial cancer (EC) is the fourth most common cancer among women worldwide. It is the most common gynecological malignancy in developed countries, with increasing incidence rates. Endometrial cancer typically arises from abnormal growth of cells in the endometrial lining, leading to the formation of tumours that can invade nearby tissues and spread to other parts of the body (Kataki et al., 2023). The incidence of EC correlates closely with hormonal factors, particularly estrogen exposure. Postmenopausal women, who experience prolonged exposure to estrogen unopposed by progesterone, are at higher risk. Conversely, conditions associated with estrogen excess, such as obesity, polycystic ovary syndrome, and estrogen replacement therapy without progesterone, also elevate risk. These hormonal imbalances disrupt the normal cellular turnover in the endometrium, increasing the likelihood of malignant transformation (Wong et al., 2024). Diagnosis typically involves a combination of medical history assessment, pelvic examination, imaging studies (e.g., transvaginal ultrasound), and tissue sampling (biopsy) for pathological examination. Endometrial biopsy remains the gold standard for confirming the diagnosis and determining the histologic subtype of endometrial cancer, which influences treatment decisions and prognosis (Dellino et al., 2023). Treatment strategies for endometrial cancer depend on several factors, including the stage and grade of the cancer, the presence of hormone receptors, and the patient's overall health and preferences (Kuhn et al., 2023). Advances in molecular profiling and targeted therapies promise personalized treatment options for endometrial cancer. Targeted therapies directed at specific molecular alterations, such as mutations in the PI3K/AKT/mTOR pathway or hormone receptor status, are being investigated in clinical trials to improve outcomes and reduce treatment-related toxicity (Soberanis and Lheureux, 2024). Approximately 10-15% of cases are diagnosed at an advanced stage, and for those who have advanced EC or experience recurrence, their survival outcomes are poor, with an estimated five-year overall survival of 17%. In 2020, there were 417,367 new diagnoses and 97,370 new deaths in the world (Mullaguri et al., 2024). The incidence and associated mortality rates of EC ubiquitously increase worldwide and are projected to rise during the next 10 years. The early-stage EC exhibits favourable prognostic indicators. Patients diagnosed at stage I can achieve a 5-year survival rate ranging from 80% to 90%.



In contrast, those at stage III typically experience a 5-year survival rate of 50% to 65%, with stage IV presenting significantly lower rates at 15% to 17% (Van et al., 2020).

Recent improvements in molecularly targeted therapy have shown progress, yet effective treatments for endometrial carcinoma remain inadequate. The development of potent new therapies is urgently required to address this gap. Moreover, natural compounds from plants have emerged as promising candidates for novel anti-cancer drugs, complementing existing adjuvant therapies like radiotherapy, chemotherapy, and hormonal therapy in treating endometrial carcinoma (Deep et al., 2023). Additionally, with the advancement in scientific research, natural compounds from plants, including bioactive compounds used traditionally among plants and herbs, have emerged as promising candidates for novel anti-cancer drugs. The Combretaceae family comprises about 200 species distributed throughout the humid, semi-humid, tropical, and subtropical regions of the world. Nearly 24 species of *Terminalia* were reported in India, including some prominent species such as *T. arjuna*, *T. bellerica*, *T. chebula*, *T. tomentosa*, *T. catappa*, *T. elliptica*, *T. porphyrocarpa*, and *T. mantaly* (Kumar et al., 2023). The bark of *T. arjuna* (Roxb) Wight and Arnot is known in India as “Arjuna”. *Terminalia arjuna*, is a large tree widely distributed in South Asian region. It is well-known for its medicinal properties; it exhibits a wide range of biological activities. In Ayurveda, it is highly recognized for its diverse therapeutic benefits. The plant has long been recognized for its potent cardiotoxic properties (Jaiswal et al., 2021). In Ayurvedic medicine, it is known for its effectiveness in treating ecchymosis, spermatorrhea, and sexually transmitted diseases such as gonorrhoea. *T. arjuna* possesses a variety of medicinal properties such as astringency, cooling effects, aphrodisiac properties, and cardiotoxic effects. This plant is also used in treating cough, leukorrhoea, excessive perspiration, ulcers, diabetes, tumours, asthma, inflammation, and various skin disorders (Soni and Singh, 2019). It is reported that powdered bark from this plant holds significant potential for treating coronary heart disease. Several chemical constituents have also been reported from the stem bark portion of *T. arjuna* such as hydrolysable tannins, triterpenoid acids and their glycosides, flavonoids, phenolics and phytosterols (Amalraj and Gopi, 2017). In addition, arjunic acid, arjunolic acid, arjunglucoside, arjunetin, and terminoic acid belong to the group of important constituents of the bark. *T. arjuna* has also the potential to treat hepatic, congenital, venereal, and viral diseases. Bark powder is reported to exhibit hypocholesterolemic and antioxidant effects (Jaiswal et al., 2021).

## Etiology of Disease

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Endometrial cancer, originating in the uterine lining, is influenced by key factors like prolonged estrogen exposure without progesterone opposition, associated with obesity, nulliparity, early menarche, or late menopause (Passarello et al., 2019). These conditions disrupt hormone balance, promoting unrestricted endometrial cell growth. Genetic predispositions, such as Lynch syndrome and mutations in DNA repair genes, also increase susceptibility by impairing cell growth regulation (Carbone et al., 2020). Certain medical conditions,

like diabetes and hypertension, exacerbate risk through hormonal disturbances and inflammation. Postmenopausal estrogen-only hormone replacement therapy increases endometrial cancer risk by stimulating tissue growth without progesterone's protective effects, potentially leading to malignancy over time (Gompel, 2020). Understanding these factors is critical for prevention and early detection, with healthcare providers focusing on mitigating risks through lifestyle changes and regular screenings, crucial for improving outcomes in affected individuals.

## Therapeutic Uses

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*Terminalia arjuna*, commonly known as 'arjuna', exhibits a remarkable collection of therapeutic potentials across its various plant parts. The bark of *T. arjuna* is particularly valued for its diverse medicinal uses, including its role as a solution for alcoholic intoxication and its effectiveness against anemia (Alam et al., 2022). *T. arjuna* plays an important role in managing various disease conditions such as angina pectoris and asthma, acting as both an anti-arrhythmic

and antihypertensive agent. Moreover, the bark of *T. arjuna* exhibits anti-inflammatory properties, aids in wound healing, and acts as a diuretic. The bark and fruits are utilized in treating cardiovascular diseases, including ischemia and coronary artery disease, and are known for their cardioprotective effects. *T. arjuna* is considered as a valuable plant in medicine systems due to its wide array of therapeutic applications (Table 1).

**Table 1** List of previously reported therapeutic uses of *T. arjuna*

Plant part	Therapeutic use	Therapeutic use identifiers	References
Bark	Alcoholic intoxication	MESH:D000435, UMLS:C0001969, ICD-11:6C40.3	Pharmacognosy of Indigenous Drugs Volume I
Bark	Anemia	MESH:D000740, UMLS:C0002871, DOID:2355, ICD-11:3A9Z	ISBN:9788171360536, ISBN:9788173717062
Bark, fruit	Angina pectoris, variant	MESH:D000787, UMLS:C0002962, ICD-11:BA40, MESH:D000788, UMLS:C0002963, ICD-11:BA85.Z	ISBN:9788172363093, ISBN:9789327275590, ISBN:9780387706375
Bark	Anti-arrhythmia agents	MESH:D000889, UMLS:C0003195, ICD-11:B-C9Z	ISBN:9788172363093
Bark, fruit	Antidiuretic agents	MESH:D050034, UMLS:C1563717	ISBN:9789327275590, ISBN:9788172360818
Bark	Antidotes	MESH:D000931, UMLS:C0003295, ICD-11:XM1S43	ISBN:9788172361266
Leaves	Antifungal agent	MESH:D000935, UMLS:C0003308, ICD-11:X-M83G4	ISBN:9788172363093
Bark	Antihypertensive agents	MESH:D000959, UMLS:C0003364, ICD-11:X-M2PT6	ISBN:9788172363093
Bark	Anti-inflammatory agent	MESH:D000893, UMLS:C0003209, ICD-11:X-M7XD1	ISBN:9788172363093
Bark	Antipyretics	MESH:D058633, UMLS:C0003419, ICD-11:X-M1RS7	ISBN:9788172360818, ISBN:9788172361266, Pharmacognosy of Indigenous Drugs Volume I

Plant part	Therapeutic use	Therapeutic use identifiers	References
Whole plant	Anxiety	MESH:D001007, UMLS:C0003467, ICD-11:MB24.3	ISBN:9788172363093
Bark	Aphrodisiacs	MESH:D001046, UMLS:C0003567	Contribution to the Medico-Botany of East Godavari and West Godavari Districts of Andhra Pradesh, ISBN:9788173717062
Bark	Asthma	MESH:D001249, UMLS:C0004096, DOID:2841, ICD-11:CA23	ISBN:9788171360536, ISBN:9788173717062
Bark	Astringents	MESH:D001252, UMLS:C0004110, ICD-11:X-M0VK6	ISBN:9788172360818, ISBN:9788172361266, ISBN:9788173717062, Pharmacognosy of Indigenous Drugs Volume I
Stem	Blister	MESH:D001768, UMLS:C1579830, ICD-11:ME63.3	Pharmacognosy of Indigenous Drugs Volume I
Bark	Bronchitis	MESH:D001991, UMLS:C0006277, DOID:6132, ICD-11:CA20	ISBN:9788171360536, ISBN:9788173717062
Bark, fruit	Cardiotonic agents	MESH:D002316, UMLS:C0007209, ICD-11:XM91S1	ISBN:9789327275590, ISBN:9788173717062, ISBN:9788172360818, ISBN:9788172361266, ISBN:9788172363093, ISBN:9789327275590, Medicinal Plants of Nagpur and Wardha Forest Divisions (Maharashtra), Pharmacognosy of Indigenous Drugs Volume I
Bark	Celiac disease	MESH:D002446, UMLS:C0007570, DOID:10608, ICD-11:DA95	Pharmacognosy of Indigenous Drugs Volume I

Plant part	Therapeutic use	Therapeutic use identifiers	References
Bark	Contusions	MESH:D003288, UMLS:C0009938, ICD-11:ND56.0	Pharmacognosy of Indigenous Drugs Volume I
Bark	Cooling effect on body	UMLS:C0678568	Pharmacognosy of Indigenous Drugs Volume I
Bark	Coronary artery disease	MESH:D003324, UMLS:C0010054, DOID:3393, ICD-11:BA8Z	ISBN:9788172363093
Bark, fruit	Coronary circulation	MESH:D003326, UMLS:C0010067	ISBN:9789327275590
Bark	Diabetes mellitus	MESH:D003920, UMLS:C0011849, DOID:9351, ICD-11:5A14	ISBN:9788171360536, ISBN:9788172363093, ISBN:9788173717062
Bark	Diarrhea	MESH:D003967, UMLS:C0011991, DOID:13250, ICD-11:ME05.1	ISBN:9788171360536, Pharmacognosy of Indigenous Drugs Volume I
Bark	Diuretics	MESH:D004232, UMLS:C0012798, ICD-11:X-M4D06	ISBN:9788172363093, Medicinal Plants of Nagpur and Wardha Forest Divisions (Maharashtra), Pharmacognosy of Indigenous Drugs Volume I, Medicinal Plants of Nagpur and Wardha Forest Divisions (Maharashtra)
Bark	Dysentery	MESH:D004403, UMLS:C0277526, DOID:12384, ICD-11:1A40.Z	ISBN:9788171360536, ISBN:9788172360818, ISBN:9788173717062, Pharmacognosy of Indigenous Drugs Volume I
Bark, leaves	Earache	MESH:D004433, UMLS:C0013456, ICD-11:AB70.2	ISBN:9788173717062, ISBN:9788171360536, ISBN:9788172360818, ISBN:9788172361266, Pharmacognosy of Indigenous Drugs Volume I

Plant part	Therapeutic use	Therapeutic use identifiers	References
Bark	Emaciation	MESH:D004614, UMLS:C0013911, ICD-11:5B51	ISBN:9788190648943
Bark	Encephalitis	MESH:D004660, UMLS:C0014038, DOI:9588, ICD-11:1D00.Z	ISBN:9788190648943
Whole plant	Expectorants	MESH:D005100, UMLS:C0015314	ISBN:9788173717062
Whole plant	Fatigue	MESH:D005221, UMLS:C0015672, ICD-11:MG22	ISBN:9788173717062
Bark	Fibrosis	MESH:D005355, UMLS:C0016059, ICD-11:DB93.Z	ISBN:9780387706375
Bark	Fractures, bone	MESH:D050723, UMLS:C0016658, ICD-11:ND56.2	ISBN:9788173717062, ISBN:9788172363093, Pharmacognosy of Indigenous Drugs Volume I
Bark	Freckles	MESH:D008548, UMLS:C0025209, ICD-11:ED61.0	ISBN:9788171360536
Bark, fruit	General tonic for rejuvenation	MESH:D012060, UMLS:C0035016	ISBN:9788172360818, ISBN:9788172361266, Pharmacognosy of Indigenous Drugs Volume I
Bark	Heart diseases	MESH:D006331, UMLS:C0018799, DOI:114, ICD-11:BC4Z	ISBN:9788172363093, ISBN:9788173717062, ISBN:9788172361266, Pharmacognosy of Indigenous Drugs Volume I
Bark	Hemorrhage	MESH:D006470, UMLS:C0019080, ICD-11:MG27	ISBN:9788173717062, Pharmacognosy of Indigenous Drugs Volume I

Plant part	Therapeutic use	Therapeutic use identifiers	References
Bark	Hemostatics	MESH:D006490, UMLS:C0019120	ISBN:9788173717062, ISBN:9788172360818, Pharmacognosy of Indigenous Drugs Volume I
Bark, fruit	Herpes simplex	MESH:D006561, UMLS:C0019348, DOID:8566, ICD-11:1F00.Z	ISBN:9780387706375, ISBN:9789327275590
Whole plant	Hyperhidrosis	MESH:D006945, UMLS:C0020458, ICD-11:EE00.Z	ISBN:9788173717062
Bark	Hyperpigmentation	MESH:D017495, UMLS:C0162834	ISBN:9788190648943
Bark, fruit	Hypertension	MESH:D006973, UMLS:C0020538, DOID:10763, ICD-11:BA00.Z	ISBN:9788172360818, ISBN:9788173717062ISBN:9789327275590, Medicinal Plants of Nagpur and Wardha Forest Divisions (Maharashtra)
Whole plant	Infection	MESH:D007239, UMLS:C3714514, ICD-11:1H0Z	ISBN:9788173717062
Whole plant	Inflammation	MESH:D007249, UMLS:C0021368	ISBN:9788173717062
Bark	Ischemia	MESH:D007511, UMLS:C0022116, DOID:326	ISBN:9788172363093
Bark	Jaundice	MESH:D007565, UMLS:C0022346, ICD-11:ME10.1	ISBN:9788172363093
Bark	Leukorrhea	MESH:D007973, UMLS:C0023533, DOID:3766, ICD-11:MF3A	ISBN:9788171360536

Plant part	Therapeutic use	Therapeutic use identifiers	References
Bark	Liver diseases	MESH:D008103, UMLS:C0023890, DOID:5082, ICD-11:DB93.1, MESH:D008107, UMLS:C0023895, DOID:409, ICD-11:SA0Z	ISBN:9780387706375, ISBN:9788172361266, ISBN:9788173717062, Medicinal Plants of Nagpur and Wardha Forest Divisions (Maharashtra), Pharmacognosy of Indigenous Drugs Volume I
Whole plant	Meniere disease	MESH:D008575, UMLS:C0025281, DOID:9849, ICD-11:AB31.0	ISBN:9788172363093
Bark	Myocardial depressant factor	MESH:D009201	ISBN:9788172363093
Bark	Myocardial infarction	MESH:D009203, UMLS:C0027051, DOID:5844, ICD-11:BA41	ISBN:9788172363093
Whole plant	Neoplasms	MESH:D009369, UMLS:C0027651, DOID:162, ICD-11:2E2Z	ISBN:9788173717062
Bark	Obesity	MESH:D009765, UMLS:C0028754, DOID:9970, ICD-11:5B81	ISBN:9788190648943
Stem	Oral ulcer	MESH:D019226, UMLS:C0149745, ICD-11:DA01.15	Pharmacognosy of Indigenous Drugs Volume I
Bark	Scorpion stings	MESH:D065008, UMLS:C0238417, ICD-11:NE61	ISBN:9788172361266
Bark, fruit	Skin diseases	MESH:D012871, UMLS:C0037274, DOID:37, ICD-11:EM0Z	ISBN:9780387706375, ISBN:9788171360536, ISBN:9789327275590
Bark	Stress, physiological	MESH:D013312, UMLS:C0449430, ICD-11:6E40.4	ISBN:9788190648943
Bark	Thirst	MESH:D013894	ISBN:9788190648943

Plant part	Therapeutic use	Therapeutic use identifiers	References
Bark	Ulcer	MESH:D014456, UMLS:C0041582	ISBN:9788172360818, ISBN:9788173717062, ISBN:9788190648943, Pharmacognosy of Indigenous Drugs Volume I
Whole plant	Urethral discharge	UMLS:C0152447	ISBN:9788173717062
Bark	Urination disorders	MESH:D001744, UMLS:C0005683, ICD-11:GB71.0, MESH:D014555, UMLS:C0042035, ICD-11:GBOY	ISBN:9788172360818, ISBN:9788173717062, ISBN:9788190648943
Bark	Vasodilator agents	MESH:D014665, UMLS:C0042402	ISBN:9788172363093
Bark, fruit	Vitiligo	MESH:D014820, UMLS:C0042900, DOID:12306, ICD-11:ED63.0	ISBN:9789327275590
Bark	Wound healing	MESH:D014945, UMLS:C0043240	ISBN:9788172360818, ISBN:9788172361266, Pharmacognosy of Indigenous Drugs Volume I

Several studies have reported that phytochemicals from *T. arjuna* show significant cytotoxic effects against various type of cancer (Bishop and Liu, 2017; Liu et al., 2019). Despite these efforts, as of now, the therapeutic efficacy of *T. arjuna* against endometrial cancer has not been explored. This study aimed to investigate the potential of *T. arjuna* in treating endometrial cancer by examining the genes

associated with its phytoconstituents against endometrial cancer. The present study utilized ADMET analysis, molecular docking, and molecular dynamics simulations to provide a detailed understanding of how these phytochemicals might alleviate pathogenesis of endometrial cancer. The multidimensional computational methods used in this chapter are represented in Fig.1.

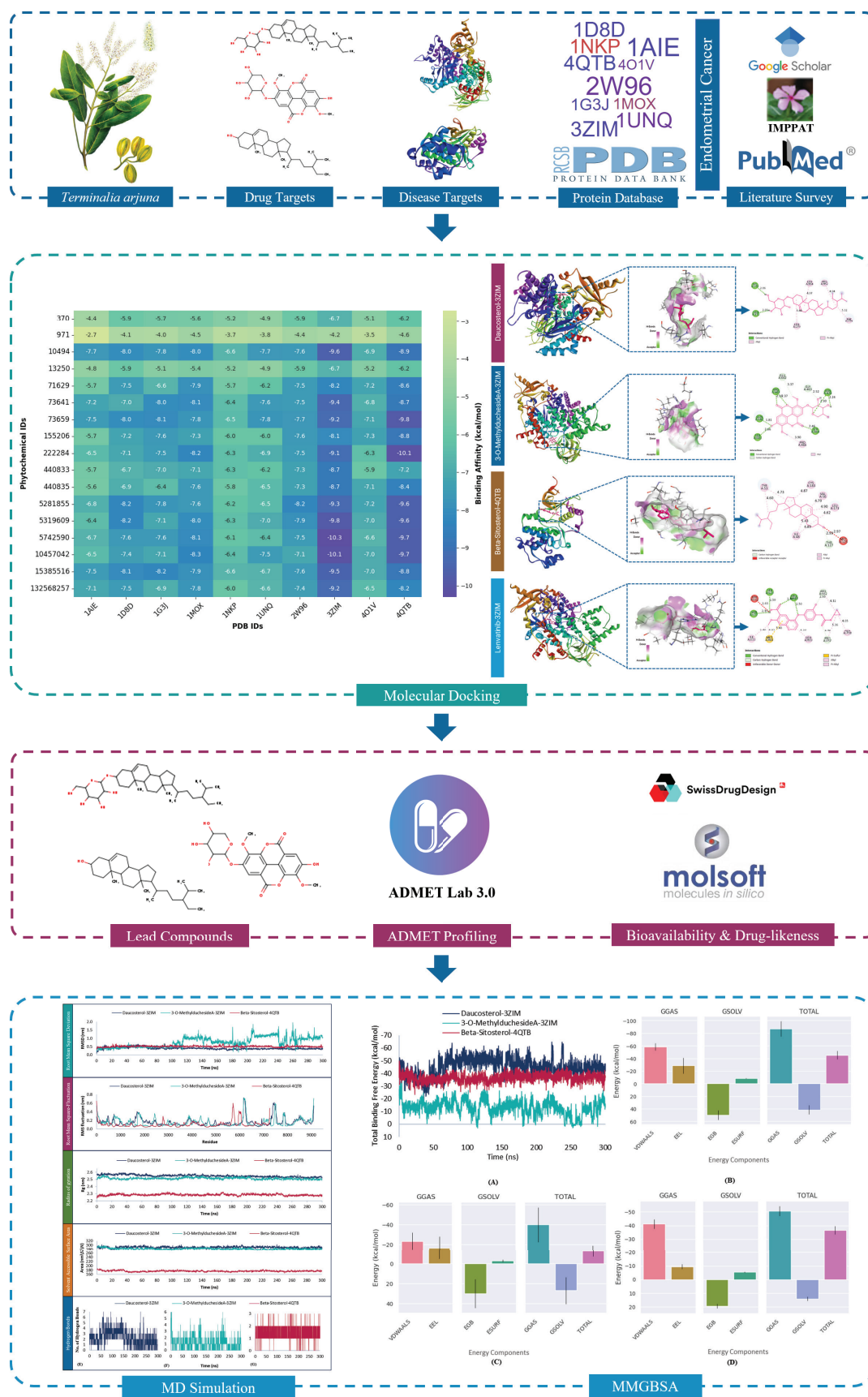


Fig. 1 General procedure of computational approaches

## Materials and Methods

### Assembling of Phytochemicals Library

All phytochemicals from *T.arjuna* were collected from IMPPAT and Dr. Duke databases, and a thorough literature review was conducted to compile a comprehensive library (Lans and van Asseldonk, 2020; Asghar et al., 2023; Kumar et al., 2023; Vivek-Ananth et al., 2023). The PubChem database was employed to obtain canonical SMILES, molecular weights, 3-D structures, and PubChem compound identifiers (CIDs) for an extensive analysis (Kim et al., 2016). Canonical SMILES were utilized to evaluate the ADMET (absorption, distribution, metabolism, excretion, and toxicity) profiles of all phytochemicals and to screen potential lead compounds for drug-likeness and bioavailability. Moreover, the 3D structures are used in molecular docking studies, allowing for a comprehensive evaluation of compound properties, and supporting their potential as lead compounds in drug development.

### Lipinski's Rule of Five

Canonical SMILES of phytochemicals were analysed based on Lipinski's rule of five parameters, which include molecular weight (MW), hydrogen bond donors (nHD), hydrogen bond acceptors (nHA), and the octanol-water partition coefficient (LogP). Phytochemicals that did not meet Lipinski's rule of five criteria were classified as Lipinski violations (LV). Those that met the criteria were subjected to molecular docking analysis (Lipinski, 2004).

### Protein Data Extraction and Preparation

A total of 10 disease targets were selected for endometrial cancer, according to comprehensive review of the literature (Zhao et al., 2022; Jin et al., 2023; Yang et al., 2023). The 3-D protein structure of genes was obtained from the Protein Data Bank (Burley et al., 2017). MODELLER (version 10.5) was used for structure modelling and structure was prepared using UCSF ChimeraX software (version 1.7). This process involved addition of any missing residues in the protein structure and eliminating water molecules, co-ligands, and heteroatoms. Moreover, polar hydrogen atoms and charges were added to confirm the ideal preparation of all target proteins for molecular docking (Meng et al., 2023).

### Molecular Docking

Molecular docking was conducted to assess the interactions between phytochemicals and target proteins associated with a specific disease. The 3-D structures of both FDA-approved drugs and screened phytochemicals were obtained from the PubChem database and utilized as ligands. The optimization of protein structures was conducted using UCSF ChimeraX. This process involved modifying unfavourable torsional angles and nonstandard residues, resulting in reduced energy and enhanced structural stability (Meng et al., 2023; Balkrishna et al., 2024). Molecular docking was performed using



Autodock Vina, a widely recognized software for predicting ligand-protein interactions. The energy minimization of ligands and the conversion of receptor-ligand complexes from PDB to PDBQT format were accomplished using Open Babel, a comprehensive toolkit for molecular modelling (Butt et al., 2020; Chaurasia et al., 2023). Blind docking was performed by expanding the grid box to encompass the entire protein structure. The evaluation of ligand-protein binding affinities was based on docking scores (kcal/mol) and root mean square deviation (RMSD). Subsequently, the protein-ligand complexes were analyzed and visualized using Discovery Studio 2021 Client software.

## ADMET Profiling of Lead Compounds

In the absorption, distribution, metabolism, excretion, and toxicity (ADMET) profiling, parameters such as P-glycoprotein inhibitor (p<sub>gp\_inh</sub>) and substrate (p<sub>gp\_sub</sub>), human intestinal absorption (HIA), fraction unbound in plasma (f<sub>u</sub>), BCRP, PPB, and drug metabolism-related parameters such as inhibitor (inh) and substrate (sub) for CYP1A2, CYP2C9, and CYP2D6, as well as plasma clearance (cl<sub>plasma</sub>), elimination half-life (t<sub>0.5</sub>), Ames test, route of administration (ROA), and assessments of carcinogenicity, hematotoxicity, nephrotoxicity, and neurotoxicity were considered (Fu et al., 2024). Compounds that met all criteria within the medium range of empirical decisions according to ADMETlab 3.0 were selected for further analysis.

## Lead Compounds Drug Profiling

All compounds were systematically assessed for their drug-likeness (DL) and oral bioavailability (OB) to identify potential lead candidates. Drug-likeness is a measure of the extent to which a compound's chemical structure resembles those of established pharmaceuticals. Oral bioavailability refers to the proportion of a medication taken by mouth that enters the bloodstream and affects local tissues and organs, ultimately producing the desired pharmacological effect (Ahmed et al., 2022). The therapeutic potential of phytochemicals is assessed by evaluating their DL and oral OB. Screening thresholds are set at DL  $\geq$  0.18 and OB  $\geq$  0.30. These parameters are analysed using Molsoft L.L.C. and the SwissADME tool, respectively (Daina et al., 2017).

## Molecular Dynamics Simulation

The molecular dynamics (MD) simulation was performed using GROMACS software, version 2023.3, within a Linux environment. For the simulation, the force fields used were the CHARMM36 force field for the protein structure and the CHARMM all-atom force field for ligand parameterization (Valdés-Tresanco et al., 2021). The system was prepared for simulation using the TIP 3-point solvent model for solvation, followed by neutralization with the addition of (0.1M) of Na<sup>+</sup> and Cl<sup>-</sup> ions. Energy minimization was performed using the steepest descent algorithm for 50,000 steps with a convergence criterion of 10.0 kJ/

mol. The system was equilibrated in two phases: a 1000 ps NVT equilibration at 300K, followed by a 1000 ps NPT equilibration at 300K and 1 bar. The simulation used the leap-frog integrator with 150,000,000 steps, corresponding to 300 ns with a 2-fs time step. Energy data, log files, and coordinate data were saved every 100 ps. The post-simulation analysis covered several parameters, such as root mean square deviation (RMSD), root mean square fluctuation (RMSF), radius of gyration (Rg), hydrogen bonds (HB), and solvent accessible surface area (SASA). All MD simulation analyses utilized the integrated tools of GROMACS version 2023.3 (Balkrishna et al., 2023).

### Binding Free Energy Analysis

The gmx\_MMPBSA package (version 1.6.2) was employed to calculate the binding free energy

( $\Delta G$ ) of ligand-protein complexes. The analysis of binding free energy utilized the molecular mechanics generalized born surface area (MM/GBSA) method (Balkrishna et al., 2023). The GROMACS trajectory, topology, and index files of the target complexes were used to assess the total change in free energy ( $\Delta \text{TOTAL}$ ), along with its standard deviation. The energy components such as electrostatic (Coulombic) energy ( $\Delta \text{EEL}$ ), changes in van der Waals energy ( $\Delta \text{VDWAALS}$ ), generalized born (GB) solvation energy ( $\Delta \text{EGB}$ ), solvent-accessible surface area (SASA) term ( $\Delta \text{ESURF}$ ), solvation free energy ( $\Delta \text{GSOLV}$ ), gas-phase free energy ( $\Delta \text{GGAS}$ ), and overall change in total free energy ( $\Delta \text{TOTAL}$ ) were analyzed in the term of average value with standard deviation to assess the binding free energy (Joshi et al., 2023; Balkrishna et al., 2024).

## Results and Discussion

### Potential Phytochemical Profiling

A total of 46 phytochemicals were collected from various databases, along with an extensive literature survey. The obtained phytochemicals were screened based on Lipinski's rule of five and ADMET parameters (Table 2). After screening, only 17 phytochemicals were found, and these phytochemicals were used for molecular docking analysis. The results of this study aligned with those of a previous study which

identified beta-sitosterol as the main active substance with prominent drug-likeness and oral bioavailability, demonstrating excellent molecular docking results. *In vivo* and *in vitro* experiments showed that it could inhibit the PI3K/Akt/HIF-1 $\alpha$  signalling pathway in lung cancer (Cao et al., 2024). In another study, *in silico* methods predict ADME parameters, pharmacokinetics, and drug-likeness of phytosterols, affirming their suitability as potential drugs for breast cancer treatment (Raju et al., 2021).

**Table 2** Screening of phytochemicals based on Lipinski's rule and ADMET

Phytochemical	Pubchem ID	MW	nHA	nHD	logP	LV	ADMET
(+)-Leucocyanidin	155206	306.07	7	6	0.35	0	A
(1R,3aS,5aS,5bR,7aR,11aR,11bR,13aS,13bS)-9,13a-Dihydroxy-5a,5b,8,8,11a-pentamethyl-1-prop-1-en-2-yl-2,3,4,5,6,7,7a,9,10,11,11b,12,13,13b-tetradecahydro-1H-cyclopenta[a]chrysene-3a-carboxylic acid	132568257	472.36	4	3	3.36	0	A
(3R,4R,4aS,6aR,6bS,8aS,12aS,14aS,14bR)-4a-Hydroxy-14b-(hydroxymethyl)-4,6a,6b,11,11-pentamethyl-3-[(2R,3R,4S,5S,6R)-3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl]oxy-2,3,5,6,7,8,9,10,12,12a,14,14a-dodecahydro-1H-picene-4,8a-dicarboxylic acid	102146986	680.38	12	8	0.96	1	NA
(4S,4aS,6aR,6aR,6bS,8aS,10S,12S,12aR,14bS)-4,6a,12-Trihydroxy-2,2,6a,6b,9,9,12a-heptamethyl-10-[(2R,3R,4S,5S,6R)-3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl]oxy-3,4,5,6,7,8,8a,10,11,12,13,14b-dodecahydro-1H-picene-4a-carboxylic acid	102146985	666.4	11	8	1.36	1	NA
[(2S,3R,4S,5S,6R)-3,4,5-Trihydroxy-6-(hydroxymethyl)oxan-2-yl] (1S,4aR,6aR,6aS,6bR,8aR,10R,11R,12aS,14bS)-1,10,11-trihydroxy-2,2,6a,6b,9,9,12a-heptamethyl-13-oxo-3,4,5,6,6a,7,8,8a,10,11,12,14b-dodecahydro-1H-picene-4a-carboxylate	102117122	664.38	11	7	1.92	1	NA
2-(2,4-Dimethoxyphenyl)-5,7-dimethoxy-4H-1-benzopyran-4-one	153387	342.11	6	0	2.44	0	NA
2,3-(S)-Hexahydroxydiphenoyl-D-glucose	492390	482.07	14	9	-2.08	1	NA
3-O-Methyl Ducheside A	10457042	462.08	12	4	-0.39	0	A

Phytochemical	Pubchem ID	MW	nHA	nHD	logP	LV	ADMET
3-O-Methylellagic acid 3'-O-alpha-rhamnopyranoside	5319609	462.08	12	5	-0.55	0	A
8-Hydroxyhexadecanoic acid	15569773	272.24	3	2	4.98	0	NA
Afrormosin	5281704	298.08	5	1	2.29	0	NA
Arjugenin	12444386	504.35	6	5	2.55	0	NA
Arjunetin	21152828	650.4	10	7	2.82	1	NA
Arjunglucoside I	14658050	666.4	11	8	1.56	1	NA
Arjunglucoside II	52951052	650.4	10	7	2.30	1	NA
Arjunic acid	15385516	488.35	5	4	3.07	0	A
Arjunolic acid	73641	488.35	5	4	3.00	0	A
Arjunone	14034821	344.13	6	0	3.02	0	NA
Baicalein	5281605	270.05	5	3	2.63	0	NA
beta-Amyrin	73145	426.39	1	1	6.20	0	NA
beta-Sitosterol	222284	414.39	1	1	8.11	0	A
Catechol	289	110.04	2	2	1.03	0	NA
Cerasidin	14034812	344.13	6	1	3.29	0	NA
Ellagic Acid	5281855	302.01	8	4	0.95	0	A
Ethyl gallate	13250	198.05	5	3	1.32	0	A
Friedelin	91472	426.39	1	0	6.66	0	NA
Gallic Acid	370	170.02	5	4	0.69	0	A
Kaempferol	5280863	286.05	6	4	1.97	0	NA
Leucocianidol	440833	306.07	7	6	0.35	0	A
Leucocyanidin	71629	306.07	7	6	0.35	0	A
Leucodelphidin	440835	322.07	8	7	-0.06	0	A
Lisinopril	5362119	405.23	8	5	-1.10	0	NA
Losartan	3961	422.16	7	2	3.23	0	NA
Losmapimod	11552706	383.2	5	2	3.83	0	NA
Luteolin	5280445	286.05	6	4	2.25	0	NA

Phytochemical	Pubchem ID	MW	nHA	nHD	logP	LV	ADMET
Maslinic Acid	73659	472.36	4	3	3.79	0	A
Methyl oleanolate	92900	470.38	3	1	5.58	0	NA
Norartocarpetin	5481970	286.05	6	4	1.92	0	NA
Oleanolic Acid	10494	456.36	3	2	4.22	0	A
Oxalic Acid	971	90	4	2	-0.42	0	A
Phosphocholine	1014	184.07	5	2	-1.44	0	NA
Quercetin	5280343	302.04	7	5	1.45	0	NA
Rutin	5280805	610.15	16	10	-0.03	1	NA
Simvastatin	54454	418.27	5	1	4.36	0	NA
Sitogluside	5742590	576.44	6	4	4.65	0	A
Vincetoxicoid B	5748601	448.1	11	7	0.40	1	NA

LogP: Partition coefficient; MW: Molecular weight; nH-A: H-bond acceptor; nH-D: H-bond donor; LV: Lipinski violations; A: Accepted; NA: Not Accepted

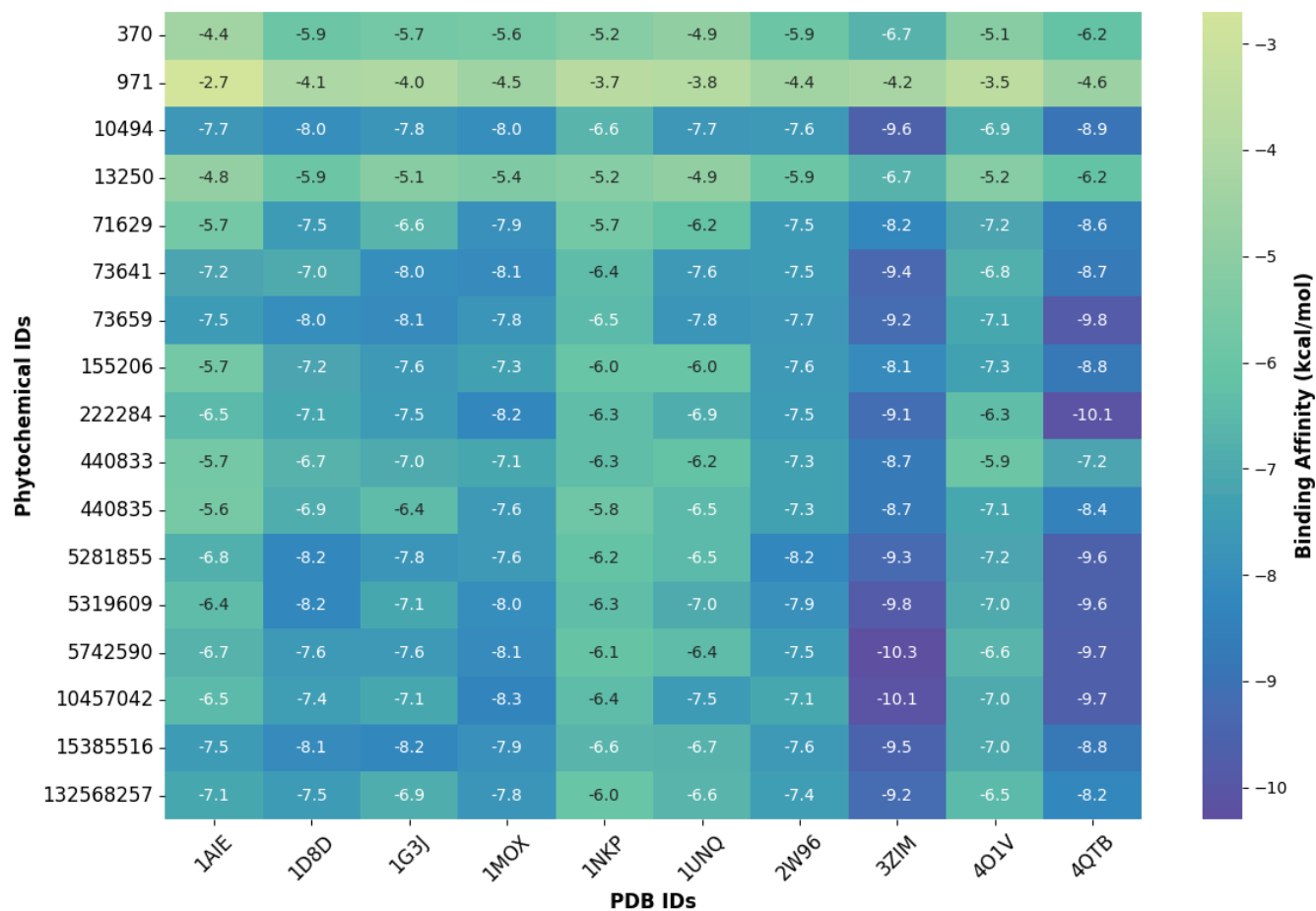
## Molecular Docking Analysis

The molecular docking process utilized drug targets with disease-related targets corresponding to the 10 genes and 17 phytochemicals were docked with protein structures of key hub genes, including TP53, AKT1, MYC, EGFR, PTEN, CTNNB1, CCND1, KRAS, PIK3CA, and MAPK3 identified by PDB IDs including 1AIE, 1UNQ, 1NKP, 1MOX, 4O1V, 1G3J, 2W96, 1D8D, 3ZIM, and 4QTB, respectively. The resulting interactions were visualized in a heat map, depicting affinity between phytochemicals and targeted proteins associated with the 10 genes, with darker colours indicating lower receptor-ligand affinity in kcal/mol (Fig. 2). The molecular docking analysis of

3 phytochemicals and FDA-approved drug, lenvatinib, with the phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha (PIK3CA) and mitogen-activated protein kinase 3 (MAPK3) receptor revealed insightful differences in binding affinity and interaction characteristics. The daucosterol (PubChem ID: 5742590), targets 3ZIM protein with a high binding affinity of -10.3 kcal/mol, forming 2 hydrogen bonds and a total of 6 bonds. Similarly, 3-O-methyliduchesindeA (PubChem ID: 10457042) also targets 3ZIM protein with a binding affinity of -10.1 kcal/mol although, forms a higher number of hydrogen bonds (9) and total bonds (10). On the other hand, Beta-Sitosterol (PubChem ID: 222284) binds to the 4QTB protein with a binding affinity of -10.1 kcal/mol, establishing 1 hydrogen bond

and 9 total bonds. The FDA-approved drug lenvatinib (PubChem ID: 9823820), with 3ZIM protein, showed a lower binding affinity of -9.2 kcal/mol while forming a 7 hydrogen bonds and 15 total number of bonds. The findings suggested that lenvatinib's interaction with

3ZIM protein is less robust compared to the tested phytochemicals (Table 3). The 2D and 3D structures of the targeted protein, along with the corresponding bonds formed during the interaction with neighbouring amino acids, is shown in Fig. 3 (A-D).



**Fig. 2** Heat map of molecular docking illustrates interactions between phytochemicals and proteins linked to the top 10 hub genes

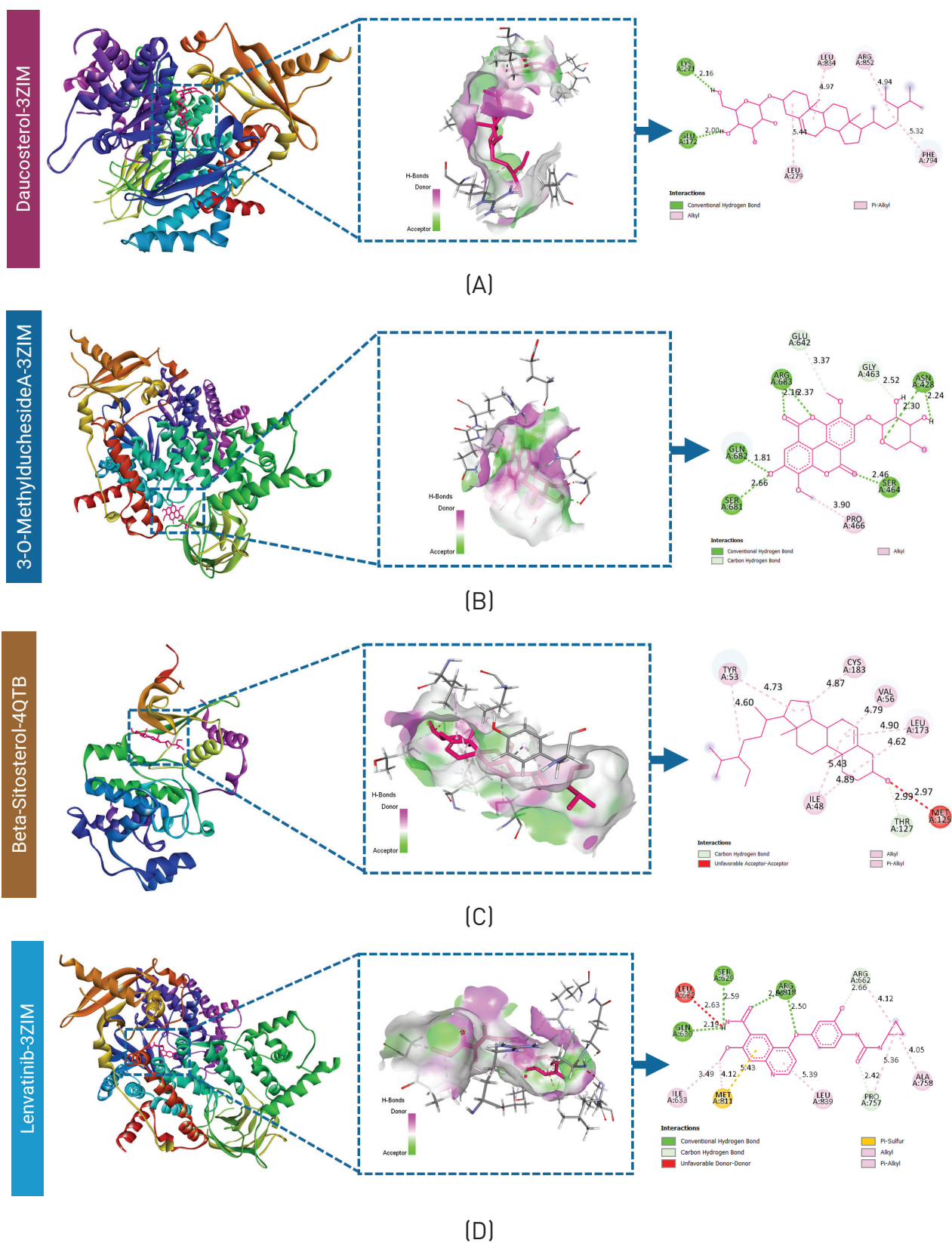
Table 3 Interactions between selected bioactive compounds and target proteins

S. N.	Ligand	PubChem ID	Target (PDB ID)	Binding affinity (kcal/mol)	Hydrogen bonds	Total bonds	Type of Bond	Interacting residues	Bond Length (Å)
1.	Daucosterol	5742590	PIK3CA (3ZIM)	10.3	2	6	Conventional Hydrogen Bond	N:UNK1:H - A:LYS271:O	2.16
							Conventional Hydrogen Bond	N:UNK1:H - A:-GLU172:OE2	2.00
							Alkyl	A:LEU279 - N:UNK1	5.44
							Alkyl	A:LEU834 - N:UNK1	4.97
							Alkyl	A:ARG852 - N:UNK1	4.94
							Pi-Alkyl	A:PHE794 - N:UNK1	5.32
2.	3-O-Methyl- ylduch- esideA	10457042	PIK3CA (3ZIM)	10.1	9	10	Conventional Hydrogen Bond	A:ASN428:HD21 - N:UNK1:O	2.30
							Conventional Hydrogen Bond	A:SER464:HN - N:UNK1:O	2.46
							Conventional Hydrogen Bond	A:SER681:HN - N:UNK1:O	2.66
							Conventional Hydrogen Bond	A:GLN682:HN - N:UNK1:O	1.81
							Conventional Hydrogen Bond	A:ARG683:HE - N:UNK1:O	2.16

S. N.	Ligand	PubChem ID	Target (PDB ID)	Binding affinity (kcal/mol)	Hydrogen bonds	Total bonds	Type of Bond	Interacting residues	Bond Length (Å)
							Conventional Hydrogen Bond	A:ARG683:HH22 - N:UNK1:O	2.37
							Conventional Hydrogen Bond	N:UNK1:H - A:ASN428:O	2.24
							Carbon Hydrogen Bond	A:GLY463:HA2 - N:UNK1:O	2.52
							Carbon Hydrogen Bond	N:UNK1:C - A:- GLU642:O	3.37
							Alkyl	N:UNK1:C - A:- PRO466	3.90
3.	Beta-Sitos-terol	222284	MAPK3 (4QTB)	10.1	1	9	Carbon Hydrogen Bond	A:THR127:HA - N:UNK1:O	2.99
							Alkyl	A:ILE48 - N:UNK1	5.43
							Alkyl	A:VAL56 - N:UNK1	4.79
							Alkyl	A:LEU173 - N:UNK1	4.90
							Alkyl	A:CYS183 - N:UNK1	4.87
							Alkyl	N:UNK1 - A:ILE48	4.89
							Alkyl	N:UNK1 - A:LEU173	4.62

S. N.	Ligand	PubChem ID	Target (PDB ID)	Binding affinity (kcal/mol)	Hydrogen bonds	Total bonds	Type of Bond	Interacting residues	Bond Length (Å)
							Pi-Alkyl	A:TYR53 - N:UNK1	4.73
							Pi-Alkyl	A:TYR53 - N:UNK1	4.60
4.	Lenvatinib (FDA- approved Drug)	9823820	PIK3CA (3ZIM)	9.2	7	15	Conventional Hydrogen Bond	A:ARG818:HH12 - N:UNK1:O	2.50
							Conventional Hydrogen Bond	A:ARG818:HH21 - N:UNK1:O	2.56
							Conventional Hydrogen Bond	A:ARG818:HH22 - N:UNK1:O	2.56
							Conventional Hydrogen Bond	N:UNK1:H - A:SER629:O	2.59
							Conventional Hydrogen Bond	N:UNK1:H - A:GLN630:O	2.19
							Carbon Hydrogen Bond	A:ARG662:HD1 - N:UNK1:Cl	2.66
							Carbon Hydrogen Bond	A:PRO757:HD1 - N:UNK1:O	2.42
							Pi-Sulfur	A:MET811:SD - N:UNK1	5.43
							Alkyl	A:ARG662 - N:UNK1	4.12

S. N.	Ligand	PubChem ID	Target (PDB ID)	Binding affinity (kcal/mol)	Hydrogen bonds	Total bonds	Type of Bond	Interacting residues	Bond Length (Å)
							Alkyl	A:PRO757 - N:UNK1	5.36
							Alkyl	A:ALA758 - N:UNK1	4.05
							Alkyl	N:UNK1:CL - A:ARG662	4.69
							Alkyl	N:UNK1:C - A:ILE633	3.49
							Alkyl	N:UNK1:C - A:MET811	4.12
							Pi-Alkyl	N:UNK1 - A:LEU839	5.39



**Fig. 3 (A-D)** 3D and 2D structures of targeted protein docked with different phytochemicals. (A) Daucosterol with the target protein 3ZIM (B) 3-O-MethylduchesideA with the target protein 3ZIM (C) Beta-Sitosterol with the target protein 4QTB (D) Lenvatinib (FDA-approved drug) with the protein 3ZIM

These findings are aligned with a previous study indicating that phytosterol inhibits the proliferation and migration of EC cells by inducing G1 cell cycle arrest and suppressing cancer stemness through the IGF1R/AKT/mTOR pathway (Wang et al., 2022). Another studies demonstrated that phytosterol induces ovarian cancer cell death through apoptosis, ROS production, and dysfunction of the endoplasmic reticulum and mitochondria, while also acting as a potential Nrf2 inhibitor, enhancing the therapeutic effect of cisplatin in EC cells (Bae et al., 2020; Liao et al., 2020). In a previous study, the phytosterols of *Cynodon dactylon* showed remarkable efficacy to combat epithelial ovarian cancer, with beta-sitosterol showing a strong affinity for PARP-1 and MAPK3 in molecular docking, resulting to target the EGFR/MAPK signalling pathway (Balkrishna et al., 2024). In a similar study, molecular docking studies demonstrated strong binding affinities between phytosterol and MAPK3, highlighting their potential as effective agents in combating breast cancer (Arif et al., 2024). Beta-sitosterol also emerges as a promising therapeutic agent against glioma by inhibiting proliferation, inducing apoptosis and cell cycle arrest, suppressing

migration, and targeting the EGFR/MAPK signalling pathway, highlighting its potential in glioma treatment (Xie et al., 2024).

## Drug Profiling of Lead Compounds

Drug profiling of lead compounds involves evaluating various pharmacokinetic and pharmacodynamic properties to determine their potential as viable drug candidates. All three ligands exhibited the same bioavailability score of 0.55, indicating similar absorption and utilization in the body (Table 4), however, their drug-likeness are different. Beta-sitosterol has the highest drug-likeness score at 0.78, suggesting it has more favourable properties for drug development compared to daucosterol (0.50) and 3-O-methylduchesideA (0.32). These leading compounds also exhibited values below the moderate threshold for various ADMET parameters, including Pgp-inh, Pgp-sub, HIA, PPB, Fu, BCRP, CYP1A2-inh, CYP1A2-sub, CYP2C19-inh, CYP2C19-sub, CYP2D6-inh, CYP2D6-sub, CL,  $T_{1/2}$ , Ames, ROA, hematotoxicity, nephrotoxicity, neurotoxicity, and carcinogenicity (Table 5).

**Table 4** Profiling of lead compounds for drug development

S. N.	Ligand	PubChem ID	Radar Chart	2D Structure	Bioavailability	Drug-likeness
1	Daucosterol	5742590			0.55	0.50
2	3-O-Methylduch- esideA	10457042			0.55	0.32
3	Beta-Sitosterol	222284			0.55	0.78

**Table 5** Characterization of lead compounds through ADMET profiling

Category	Parameters	Daucosterol	3-O-MethylduchesideA	Beta-Sitosterol
Absorption	pgp_inh	3E-05	3E-05	2E-03
	pgp_sub	2E-02	6E-01	1E-02
	hia	2E-02	6E-01	3E-04
Distribution	BCRP	8E-03	1E-03	2E-02
	PPB	8E+01	6E+01	9E+01
	Fu	2E+01	4E+01	2E+01
Metabolism	CYP1A2-inh	4E-17	4E-05	2E-10
	CYP1A2-sub	7E-03	3E-04	1E-02
	CYP2C19-inh	5E-12	6E-07	1E-07
	CYP2C19-sub	7E-01	2E-04	8E-01
	CYP2D6-inh	4E-09	2E-06	6E-04
	CYP2D6-sub	2E-01	9E-02	8E-01
Excretion	cl-plasma	5E+00	2E+00	1E+01
	t0.5	1E+00	3E+00	4E-01
Toxicity	Ames	8E-02	6E-01	4E-02
	ROA	2E-01	5E-01	9E-02
	Carcinogenicity	2E-01	5E-01	4E-01
	Neurotoxicity-DI	3E-01	6E-02	3E-01
	Hematotoxicity	5E-02	2E-01	1E-01
	Nephrotoxicity-DI	4E-02	2E-01	1E-01

## Structural Stability Analysis

Molecular dynamics simulations were conducted to study the interactions of daucosterol and 3-O-methylduchesideA with phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha (PDB ID: 3ZIM) and beta-sitosterol with mitogen-activated protein kinase 3 (PDB ID: 4QTB) over 300 nanoseconds, capturing 3000 frames. Key parameters, including root mean square

deviation (RMSD), root mean square fluctuation (RMSF), radius of gyration (Rg), solvent accessible surface area (SASA), and hydrogen bonds (HB), were assessed to evaluate the structural stability of the complexes, providing insights into their molecular interactions and potential biological activities.

### Root Mean Square Deviation (RMSD)

The backbone RMSD values for the complexes of daucosterol, 3-O-methylducheside A, and

beta-sitosterol with their respective targets 3ZIM, and 4QTB provided insights into their stability. The average RMSD values were found to be 0.40 nm, 0.76 nm, and 0.51 nm, respectively, indicating that daucosterol formed the most stable complex on average, while 3-O-methyl DuchesideA exhibited the highest average RMSD, suggesting less stability. Standard deviations (SD) of 0.07 nm, 0.30 nm, and 0.04 nm revealed that 3-O-methyl DuchesideA experienced the greatest conformational changes, whereas daucosterol and beta-sitosterol showed more limited changes. During the first 100 ns of the MD simulation, 3-O-methyl DuchesideA with 3ZIM remained structurally stable, but subsequently showed the highest conformational variability. Overall, the data suggested that while all complexes demonstrated reasonable stability, daucosterol was the most stable on average, whereas 3-O-methyl DuchesideA was the least stable and most flexible (Fig. 4 A).

### Root Mean Square Fluctuation (RMSF)

The RMSF values were analyzed for three compounds to ensure the flexibility of their molecular structures during MD simulation. For daucosterol-3ZIM, the average RMSF was 0.161 nm, with a minimum of 0.1 nm, maximum of 0.7 nm, and SD of 0.10 nm. Similarly, 3-O-methyl DuchesideA-3ZIM exhibited an average RMSF of 0.2 nm, with minimum, maximum, and SD values similar to daucosterol-3ZIM. Beta-sitosterol-4QTB showed slightly lower average RMSF at 0.1

nm, with maximum value of 0.6 nm, and SD of 0.07 nm. These findings indicated that all three compounds possessed varying degrees of backbone flexibility. Daucosterol and 3-O-methyl DuchesideA-3ZIM, differing in their target receptor proteins, exhibited slightly higher average RMSF values compared to beta-sitosterol. The variability in molecular structures resulted in a significant degree of flexibility observed across various regions of each compound's backbone structure (Fig. 4 B).

### Radius of Gyration (Rg)

The radius of gyration is used to assess the compactness of each molecule or group of atoms within the molecule, calculated as the root mean square distance from the center of mass. The radius of gyration of daucosterol-3ZIM exhibited the highest average value at 2.50 nm, indicating a broader mass distribution compared to 3-O-methyl DuchesideA-3ZIM (2.46 nm) and beta-sitosterol-4QTB (2.23 nm). The gyration radii varied across datasets including daucosterol-3ZIM ranged from 2.46 to 2.54 nm, 3-O-methyl DuchesideA-3ZIM from 2.43 to 2.52 nm, and beta-sitosterol-4QTB from 2.19 to 2.28 nm, indicating differing distributions. Moreover, daucosterol-3ZIM had standard deviation of 0.016 nm, slightly higher than 3-O-methyl DuchesideA-3ZIM (0.014 nm) and beta-sitosterol-4QTB (0.01 nm). Overall, daucosterol with 3ZIM exhibited the widest distribution of mass compared to 3-O-methyl DuchesideA with 3ZIM, and beta-sitosterol with 4QTB (Fig. 4 C).

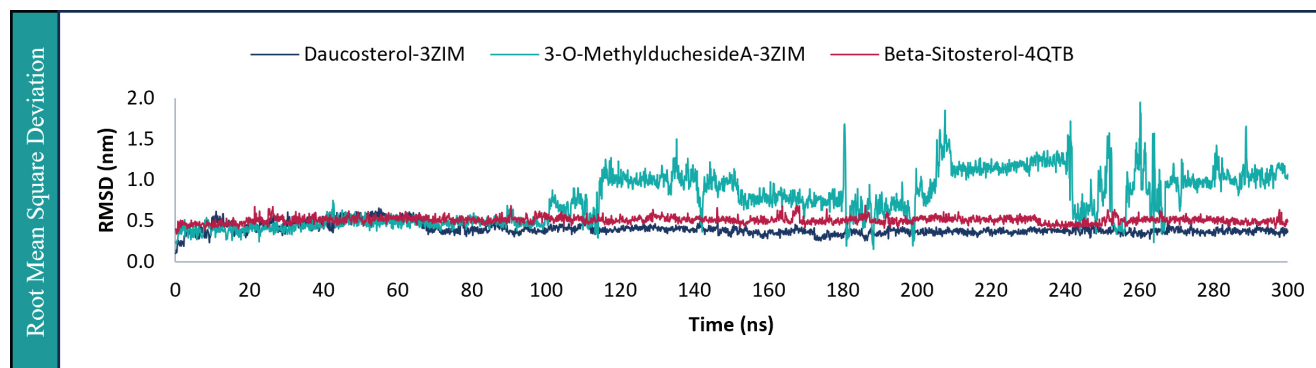
## Solvent Accessible Surface Area (SASA)

The SASA data for daucosterol-3ZIM, 3-O-methyl DuchesideA-3ZIM, and beta-sitosterol-4QTB revealed insightful information about their molecular characteristics. Daucosterol-3ZIM exhibited the highest average SASA at 290.0 nm<sup>2</sup>, with a narrow range from 275.7 to 303.6 nm<sup>2</sup>, suggesting consistent surface accessibility across its structure. Similarly, 3-O-methyl DuchesideA-3ZIM exhibited a slightly lower average SASA of 282.8 nm<sup>2</sup>, suggesting a comparable yet marginally less accessible surface area compared to daucosterol-3ZIM, although these variances were considered statistically insignificant. Being relatively smaller size of receptor protein, beta-sitosterol with 4QTB showed the lowest average SASA among the compounds at 195.9 nm<sup>2</sup>, with a broader range from 165.2 to 254.6 nm<sup>2</sup>. This variability suggested potential structural flexibility or differing degrees of exposure to solvent molecules. Overall, daucosterol-3ZIM and 3-O-methyl DuchesideA-3ZIM demonstrated relatively stable and comparable SASA values, although beta-sitosterol with 4QTB exhibited significantly lower and more variable surface accessibility due to its smaller size of target receptor (Fig. 4 D).

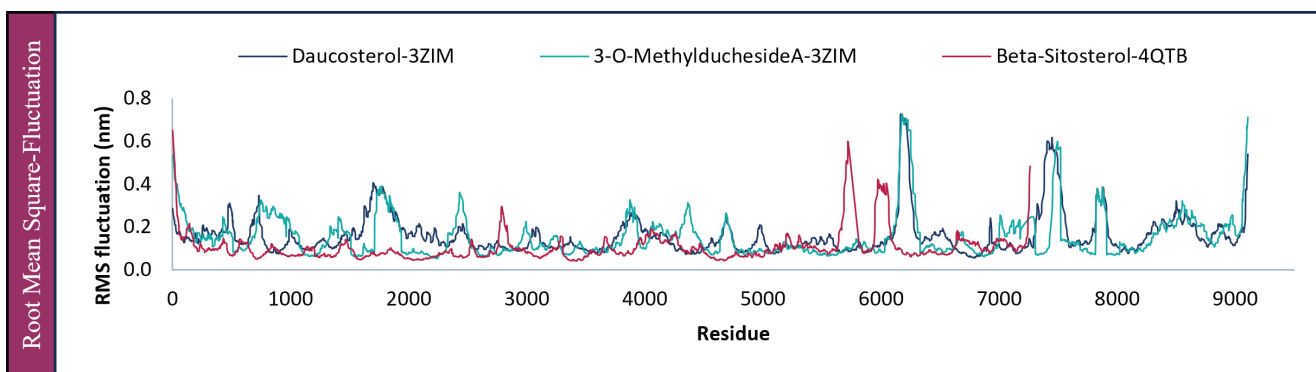
## Hydrogen Bonds (HB)

Hydrogen bonds are essential in molecular interactions, influencing the structure, stability, and function of biological molecules.

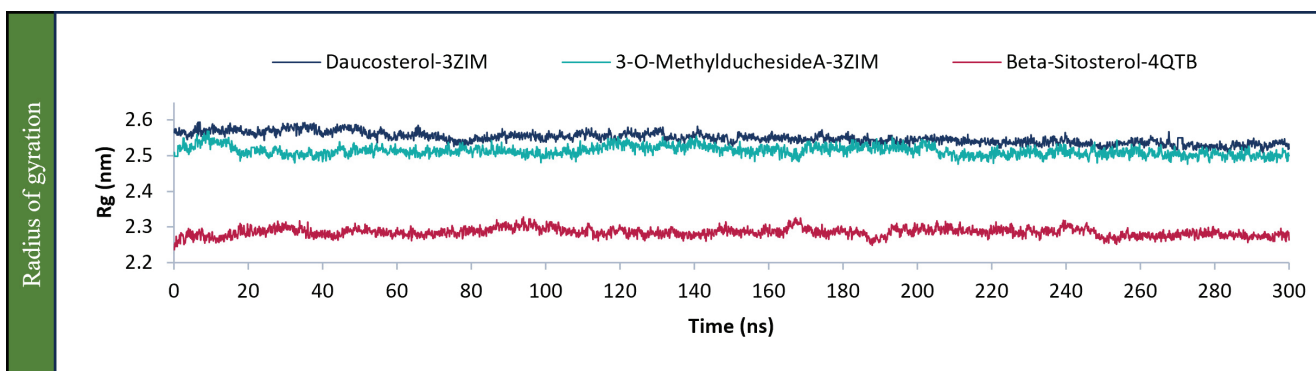
Daucosterol-3ZIM formed a total of 2823 hydrogen bonds. It peaked at 842 instances for one-HB interactions, indicating substantial HB formations. The high number of instances for hydrogen bonds suggests increased stability in its molecular interactions. The data, with 716 instances for two HBs, 767 for three HBs, and 356 for four HBs, contributes to the increased stability and robustness of the daucosterol-3ZIM complex. Moreover, a total of 106 instances for five HBs, 33 for six HBs, and 3 for seven HBs were observed during the 300-nanosecond MD simulation reaction (Fig. 4 E). As the number of HBs increases, the frequency of interactions decreases, indicating a preference for fewer but significant HB formations. Similarly, 3-O-methyl DuchesideA-3ZIM exhibited a total of 2068 hydrogen bonds, peaking at 1239 instances for two-HB interactions. The higher number of instances for hydrogen bonds, particularly in the case of two-HB interactions, suggests a less robust and stable molecular network compared to daucosterol-3ZIM complex. Substantial instances were also observed for one HB (577), three HBs (189), four HBs (41), five HBs (13), and 9 instances for six HBs, demonstrating its capacity to form multiple stable interactions (Fig. 4 F). Furthermore, beta-sitosterol-4QTB formed a total of 2959 hydrogen bonds, with a peak at 728 bonds for two-HB interactions. The substantial number of instances for two-HB interactions indicates a stable molecular complex. It also formed 2187 bonds involving a single hydrogen bond (Fig. 4G).



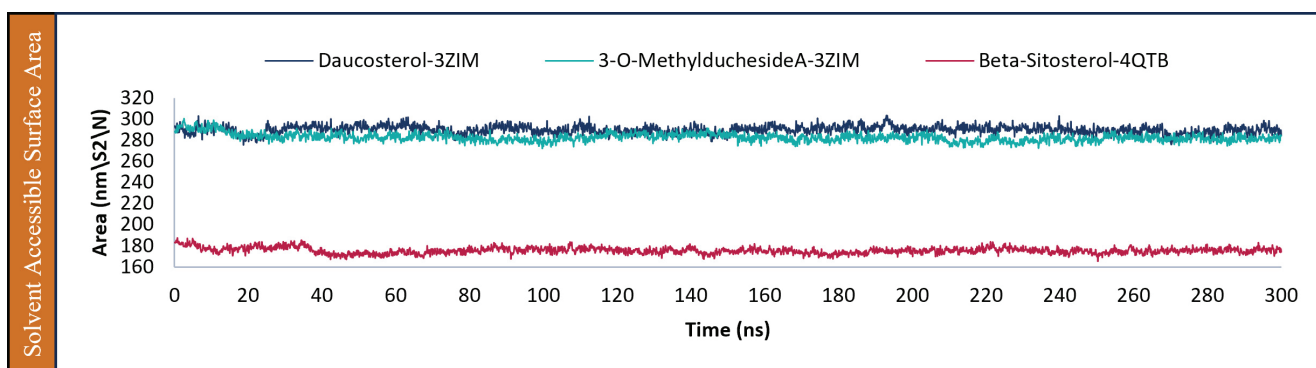
(A)



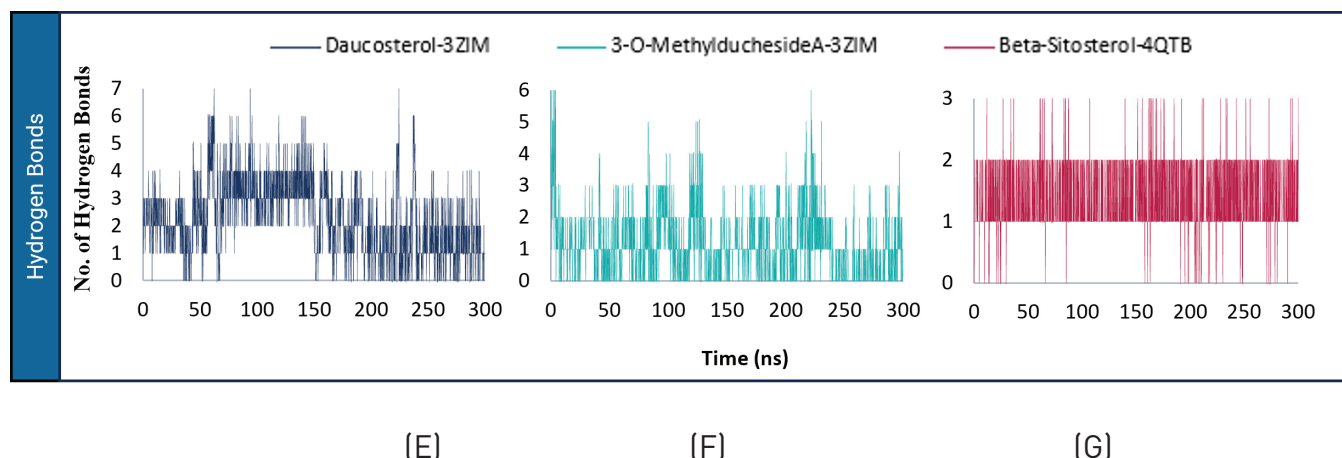
(B)



(C)



(D)



**Fig. 4 (A-G)** Molecular dynamics simulations endpoints as (A) Root Mean Square Deviation, (B) Root Mean Square Fluctuation, (C) Radius of Gyration (total and around axes), (D) Solvent Accessible Surface, (E) Hydrogen bond formed in Daucosterol-3ZIM (F) Hydrogen bond formed in 3-O-MethylduchesideA-3ZIM, and (G) Hydrogen bond formed in Beta-Sitosterol-4QTB complex

Overall, daucosterol and beta-sitosterol formed more hydrogen bonds with their receptor protein than 3-O-methylduchesideA, indicating the daucosterol-3ZIM and beta-sitosterol-4QTB can be considered potentially more stable and robust complex. These findings highlight the potential of daucosterol and beta-sitosterol complexes for stable molecular interactions in biological systems. These findings align with a previous study where docking and simulation studies demonstrated beta-sitosterol's strong binding affinity with human DNA topoisomerase I, highlighting its potential as a promising candidate for cancer therapy (Pareek et al., 2023). Similarly, another study integrated molecular docking, and molecular dynamics simulations to elucidate the effectiveness of phytosterols in breast cancer treatment, highlighting their potential therapeutic role and offering insights into the involved molecular

mechanisms (Jiao et al., 2023). A similar decline in SASA values of beta-sitosterol was recorded against gastric cancer (Chen et al. 2022).

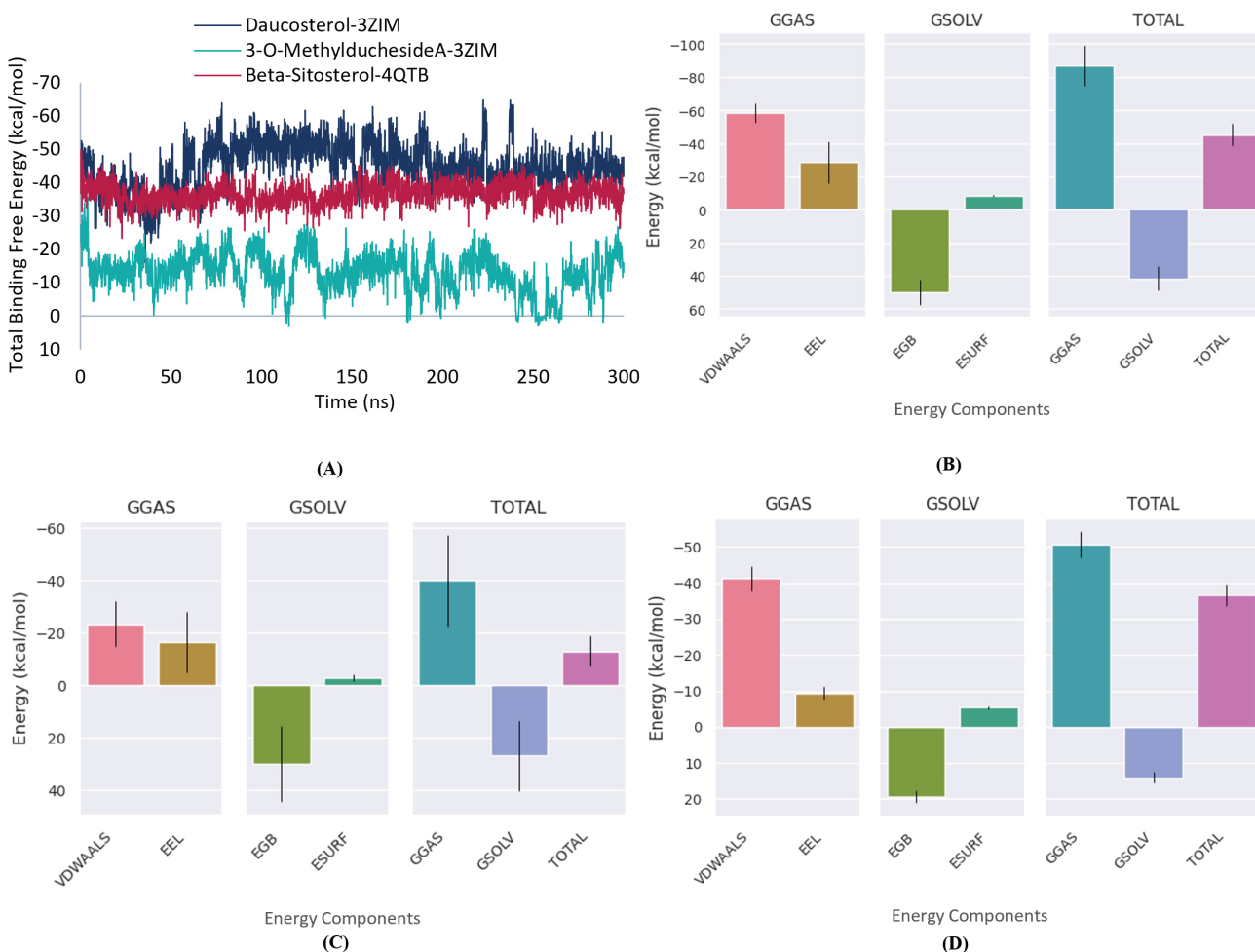
### Interaction Energy Analysis

The binding free energy (BFE) trajectory analysis utilizing MMGBSA was conducted for ligands (daucosterol, 3-O-methylduchesideA, beta-sitosterol) and their receptor proteins to provide insights into their stability and strength of interactions. The average BFE values indicated that daucosterol-3ZIM (-45.16 kcal/mol) formed the strongest interaction, followed by beta-sitosterol-4QTB (-36.61 kcal/mol), while 3-O-methylduchesideA-3ZIM (-13.16 kcal/mol) showed the weakest binding affinity. The minimum and maximum BFE values emphasized the range of interaction strengths observed, with daucosterol-3ZIM showing the

highest variability. Overall, these BFE values suggested that daucosterol-3ZIM formed the most stable and favourable complex with its receptor protein, while 3-O-methylduchesideA-3ZIM formed the least stable complex among the studied ligands (Fig. 5A).

The components of binding free energy (BFE) provide a detailed breakdown of the energetic contributions influencing the stability of ligand-receptor complexes. Daucosterol with 3ZIM showed the strongest van der Waals interactions (-58.42 kcal/mol) and electrostatic interactions (-28.4 kcal/mol), indicating robust bonding with its receptor protein. It also exhibited favourable polar solvation energy (49.75 kcal/mol), suggesting effective interaction of polar groups with the surrounding solvent. Non-polar solvation energy for daucosterol-3ZIM was relatively small (-8.1 kcal/mol), indicating minimal interference from non-polar groups with the solvent. In the absence of solvent, the gas phase energy ( $\Delta$ GGAS) for daucosterol-3ZIM was highly negative (-86.82 kcal/mol), reflecting its strong overall interaction energy. Upon considering solvation effects, daucosterol-3ZIM maintained a high solvation free energy

(41.65 kcal/mol), indicating favourable energetics upon solvation of the complex. Consequently, daucosterol-3ZIM exhibited the most negative total binding free energy (-45.16 kcal/mol), suggesting it formed the most stable and favourable complex with its receptor protein compared to beta-sitosterol-4QTB and 3-O-methylduchesideA-3ZIM (Fig. 5 B). In contrast, 3-O-methylduchesideA with 3ZIM exhibited weaker interaction energies including  $\Delta$ VDWAALS,  $\Delta$ EEL,  $\Delta$ EGB,  $\Delta$ ESURF,  $\Delta$ GGAS, and  $\Delta$ GSOLV of -23.42, -16.55, 29.88, -3.06, -39.98, and 26.82 kcal/mol, resulting a less favourable solvation free energy (-13.16 kcal/mol), as a resultant it formed the least stable complex (Fig. 5 C). On the other hand, with a different receptor protein, beta-sitosterol with 4QTB showed substantial  $\Delta$ VDWAALS,  $\Delta$ EEL,  $\Delta$ EGB,  $\Delta$ ESURF,  $\Delta$ GGAS, and  $\Delta$ GSOLV of -41.2, -9.4, 19.36, -5.37, -50.6, and 13.99 kcal/mol, respectively, resulting in a highly stable complex with binding energy of -36.61 kcal/mol (Fig. 5 D). These findings emphasized that both the phytosterols (daucosterol and beta-sitosterol) showed strong affinity and stability in interaction with their receptor protein.



**Fig. 5** (A) BFE trajectory of Daucosterol-3ZIM, 3-O-MethylduchesideA-3ZIM, and Beta-Sitosterol-4QTB complexes. Binding Free Energy components of (B) Daucosterol -3ZIM (C) 3-O-MethylduchesideA-3ZIM, (D) and Beta-Sitosterol-4QTB

The binding free energy was further divided into three distinct components alike  $\Delta G$  Complex,  $\Delta G$  Receptor, and  $\Delta G$  Ligand. Daucosterol-3ZIM showing the most negative value ( $-9012.18 \pm 86.24$  kcal/mol), indicating the strongest interaction, followed by beta-sitosterol-4QTB ( $-8448.52 \pm 210.12$  kcal/mol) and 3-O-methylduchesideA-3ZIM ( $-8275.78 \pm 74.73$  kcal/mol). The  $\Delta G$  Receptor values represented the energy of the receptor alone, with daucosterol-3ZIM again showing the most negative value ( $-9220.95 \pm 85.84$

kcal/mol), followed by beta-sitosterol-4QTB ( $-8519.42 \pm 210.03$  kcal/mol) and 3-O-methylduchesideA-3ZIM ( $-8506.86 \pm 74.67$  kcal/mol). The  $\Delta G$  Ligand values, representing the energy of the ligand alone, were lowest for beta-sitosterol-4QTB ( $107.51 \pm 7.16$  kcal/mol), followed by 3-O-methylduchesideA-3ZIM ( $244.24 \pm 5.29$  kcal/mol) and daucosterol-3ZIM ( $253.93 \pm 6.83$  kcal/mol). The  $\Delta G$  Total, representing the overall binding free energy, indicated that daucosterol-3ZIM ( $-45.16 \pm 6.78$  kcal/mol) formed the most stable complex,

followed by beta-sitosterol-4QTB (-36.61±2.41 kcal/mol) and 3-O-methylduchesideA-3ZIM (-13.16±5.95 kcal/mol). These findings suggested that daucosterol formed the most stable and favourable complex with its receptor

protein (3ZIM), exhibiting the strongest binding interactions, while beta-sitosterol also formed a relatively stable complex with 4QTB, and 3-O-methylduchesideA formed the least stable complex with 3ZIM (Table 6).

**Table 6** Net binding free energies of both the complexes (kcal/mol)

Complex	$\Delta G$ Complex	$\Delta G$ Receptor	$\Delta G$ Ligand	$\Delta G$ Total
Daucosterol-3ZIM	-9012.18±86.24	-9220.95±85.84	253.93±6.83	-45.16±6.78
3-O-MethylduchesideA-3ZIM	-8275.78±74.73	-8506.86±74.67	244.24±5.29	-13.16±5.95
Beta-Sitosterol-4QTB	-8448.52±210.12	-8519.42±210.03	107.51±7.16	-36.61±2.41

The findings of current investigation, supported by MM-PBSA and MM-GBSA calculations, demonstrated the significant antineoplastic potential of beta-sitosterol (B-SITO) against invasive breast carcinoma by preferentially binding to BII and BIII  $\beta$ -tubulin isotypes, which were often associated with drug resistance, thereby disrupting microtubule structure and function and suggesting its promising therapeutic value in overcoming chemoresistance (Pradhan et al., 2018). These findings are also demonstrated strong potential of *Ocimum gratissimum*'s phytochemicals like isovitexin, vitexin, rosmarinic acid, nepetoidin A, and luteolin, identified through MMGBSA and molecular docking, for combating breast cancer with stable binding affinities and safe oral administration profiles (Ajiboye et

al., 2024). Overall, daucosterol was found to inhibit the PI3K/Akt signalling pathway, crucial in promoting cell growth and survival, thereby potentially suppressing endometrial cancer progression. On the other hand, Beta-Sitosterol disrupted the MAPK signalling pathway, which is vital for cell proliferation and survival regulation. Aberrant MAPK activation is common in cancer and contributes to tumour progression. By targeting these pathways, daucosterol and beta-sitosterol showed promise in preventing endometrial cancer by inhibiting oncogenic signalling, promoting apoptosis, and hindering tumour cell proliferation. Further preclinical and clinical investigations are necessary to fully understand their therapeutic potential and optimize their use in clinical contexts.

## Conclusion

The present investigation emphasized the potential therapeutic efficacy of *T. arjuna* in treating endometrial cancer, employing advanced computational approaches such as molecular docking, ADMET analysis, molecular dynamics simulations, and MMGBSA calculations. In this study, 17 compounds of *T. arjuna* underwent comprehensive ADMET profiling and Lipinski's rule of five assessments. These compounds were further subjected to molecular docking analysis targeting pivotal cancer-related proteins such as TP53, AKT1, MAPK3, PIK3CA, and EGFR, among others. Especially, daucosterol and beta-sitosterol exhibited strong binding affinities and stable interactions with their respective protein targets, indicating their potential as lead compounds for further investigations. Structural stability and interaction energy analyses through molecular dynamics simulations and MMGBSA calculations revealed that daucosterol formed the most stable complex with its 3ZIM receptor protein,

exhibiting consistent structural integrity and favourable binding free energy. Similarly, beta-sitosterol formed a relatively stable complex with its receptor protein 4QTB, indicating robust binding interactions in MD simulations. Conversely, 3-O-methyl-daucosterol demonstrated greater flexibility and lower binding affinity, suggesting potential challenges in its application as a therapeutic agent. Furthermore, drug profiling assessments indicated favourable pharmacokinetic properties for daucosterol and beta-sitosterol, with a high drug-likeness score and acceptable ADMET parameters, highlighting its potential as a promising candidate for future therapeutic strategies against endometrial cancer. Overall, this research provides valuable insights into the pharmacological potential of *Terminalia arjuna* and its phytochemical constituents, emphasizing the importance of further exploration and clinical validation of daucosterol and beta-sitosterol for their efficacy and safety in combating endometrial cancer.

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CHAPTER  
**07**

**Reported  
Pharmacological  
Profile**



## INTRODUCTION

Medicinal plants are vital to health care and are the primary source of raw materials for both conventional and traditional medicine formulations; nonetheless, the majority of people still prefer herbal remedies to conventional ones (WHO, 2002). *Terminalia arjuna* (Roxb.) Wight & Arn., commonly referred to as *arjuna*, is a member of the Combretaceae family. In the indigenous medical system, it is one of the most well-recognized and beneficial medicinal herbs for treating a variety of serious illnesses. It is a large tree that grows throughout South Asian region (Chander et al., 2004). It is one of the most resourceful medicinal plants with a wide range of biological actions. Several ancient Indian medicinal texts, such as the 'Charaka Samhita', 'Sushruta Samhita', and 'Astang Hridayam', have mentioned this species in ayurvedic medicine since the Vedic era. Vagabhatta was the one who initially recommended using stem bark powder to treat cardiac conditions (Premila, 2006). It is beneficial for ecchymosis, spermatorrhoea, and sexually transmitted illnesses including gonorrhoea, according to Ayurvedic scriptures (the Indian medicinal system). This species is used to cure cough, leukorrhoea, excessive sweating, ulcers, diabetes, tumours, asthma, inflammation, and numerous skin ailments. It also possesses astringent, cooling, aphrodisiac, and cardiotonic characteristics. Various pharmacological characteristics, such as inotropic, anti-ischemic, antioxidant, blood pressure-lowering, antiplatelet, hypolipidemic, antiatherogenic, and antihypertrophic effects, have been demonstrated by several extracts from the stem bark of *arjuna* (Paarakh, 2010). This chapter provides a brief overview of the pharmacological studies and therapeutic potential of *T. arjuna*.

### Pre-clinical Study

*T. arjuna* is a tree native to India, Sri Lanka, and Southeast Asia. The bark is traditionally used in Ayurvedic medicine for a variety of conditions, including heart disease, high blood

pressure, and diarrhea. Several pre-clinical studies have suggested that *T. arjuna* have a number of potential health benefits.



## Analgesic Activity

Tahsin et al. (2021) investigated the analgesic activity of ethanolic extract of *T. arjuna* bark (500, 750 and 1000 mg/kg, b.w.) against acetic-acid-induced writhing response and tail flick methods in rats. The extract (1000 mg/kg) inhibited the number of writhes by 60.75% when compared with aspirin (64.13%) at 200 mg/kg dose used as positive control. Moreover, the extract (750 and 1000 mg/kg) significantly ( $p < 0.05$ ) enhanced the reaction time of rats after 30, 45 and 60 min when compared with control group.

Biswas et al. (2011) reported the analgesic activity of methanol extract of *T. arjuna* leaves against acetic acid-induced writhing, hot plate, and formalin tests in albino Swiss mice. The extract significantly reduced ( $p < 0.001$ ) the acetic acid induced writhing in mice when compared to aspirin (100 mg/kg). Further, the extract exhibited percentage inhibitions as 66.16 and 69.16% in hot plate and formalin tests, respectively.

## Anti-atherogenic Activity

Bhansali et al. (2019) evaluated the anti-atherogenic activity of aqueous bark extract (50 to 200  $\mu\text{g/ml}$ ) of *T. arjuna* on oxidized low-density lipoprotein (ox-LDL)-induced human leukemia monocytic cell line (THP-1)-derived foam cell formation. Treatment with extract significantly ( $p < 0.05$ ,  $< 0.01$  and  $< 0.001$ ) decreased the Oil Red O-positive cells while, enhanced the apoptosis and protein expression levels of X-box binding protein

1 (XBP1) and C/EBP homologous protein in ox-LDL-stimulated macrophages when compared with ox-LDL alone group.

## Anti-arthritis Activity

Tyagi et al. (2018) reported the anti-arthritis activity of bark extract of *T. arjuna* (100 and 200 mg/kg) against collagen-induced arthritis (CIA) in female Wistar rats. The extract at 100 and 200 mg/kg significantly ( $p < 0.05$ ) decreased paw thickness in CIA rats after 15, 18 and 21 days of immunization when compared with CIA control group. Moreover, the extract significantly ( $p < 0.05$ ) reduced tissue articular elastase (ELA) activity and nitrite and TBARS levels while, enhanced the tissue superoxide dismutase (SOD) and catalase (CAT) activity, glutathione (GSH) levels in CIA rats when compared with CIA control group. In histopathological analysis, the extract also attenuated neutrophil infiltration in CIA rats.

## Anti-cancer Activity

Moulisha et al. (2010) evaluated the anti-cancer activity of ursolic acid isolated from *T. arjuna* leaves against human chronic myelogenous leukemia cell line K562. The compound exhibited anti-cancer effect with  $\text{IC}_{50}$  value of 7.40  $\mu\text{g/ml}$  for tested cell line.

Kuo et al. (2005) investigated the anti-cancer activity of casuarinin (0.5 to 10  $\mu\text{M}$ ) isolated from *T. arjuna* bark against human breast adenocarcinoma (MCF-7) cells. The compound showed anti-cancer effect with  $\text{IC}_{50}$  of 6.04  $\mu\text{M}$  as compared to 5-FU (fluorouracil)

used as positive control (8.27  $\mu\text{M}$ ) for MCF-7 cell line. The compound (0.5 to 10  $\mu\text{M}$ ) increased the population of G0/G1 phase from 44.7 to 67.65 on cell cycle of progression of MCF-7. The compound also increased the percentage of apoptosis and did not affect the protein expression of p53. In contrast, the amount of p21/WAF1 concomitantly and caspase-8 increased in MCF-7 cells by compound.

### Anti-colitis Activity

Cota et al. (2019) studied the anti-colitis activity of hydroalcoholic extract of *T. arjuna* (125, 250 and 500 mg/kg) against trinitrobenzenesulphonic acid (TNBS)-induced colitis in female Wistar rats. The extract reduced the disease activity index score in rats when compared with TNBS group. Moreover, the extract (500 mg/kg) significantly ( $p < 0.01$ ) reduced the macroscopic and histological scores, levels of myeloperoxidase (MPO), malondialdehyde (MDA), nitric oxide (NO), interleukin-1 $\beta$  (IL-1 $\beta$ ), MCP-1 (monocyte chemoattractant protein-1) and tumor necrosis factor alpha (TNF- $\alpha$ ) while, enhanced CAT, SOD and plasma zinc levels of rats when compared with TNBS treated group. Further, IL-6, GSH and *Bifidobacteria* levels were increased, and *Clostridium* level was decreased by extract (500 mg/kg) in rats when compared with TNBS treated group.

### Anti-depressant Activity

Tahsin et al. (2021) assessed the anti-depressant activity of ethanolic extract of *T. arjuna* (500, 750 and 1000 mg/kg) against

reserpine-induced depression in rats using tail suspension (TST) and sucrose preference tests (SPT). Citalopram (5, 10 and 15 mg/kg) was used as a positive control. The extract significantly ( $p < 0.05$ ) increased the consumption of sucrose water from total intake of water in SPT but, decreased the immobility time of rats in TST when compared with reserpine treated group.

### Anti-dermatophytic Activity

Bhattacharyya and Jha (2011) demonstrated the anti-dermatophytic activity of crude extract of *T. arjuna* bark against *Trichophyton mentagrophytes*, *T. rubrum*, *T. tonsurans*, *Microsporum gypseum* and *M. fulvum*. The extract exhibited inhibitory effect with MIC values of 75  $\mu\text{g/ml}$  (*T. tonsurans*), 125  $\mu\text{g/ml}$  (*T. mentagrophytes*), 250  $\mu\text{g/ml}$  (*T. rubrum*, *M. fulvum*) and 2000  $\mu\text{g/ml}$  (*M. gypseum*).

### Anti-diabetic Activity

Tahsin et al. (2021) conducted the anti-diabetic activity of ethanolic extract of *T. arjuna* (250, 500 and 1000 mg/kg) against alloxan-induced diabetes in rats. The extract (500 and 1000 mg/kg) significantly ( $p < 0.05$ ) reduced the levels of blood glucose, serum glutamic pyruvic transaminase (SGPT), serum glutamic oxaloacetic transaminase (SGOT), creatinine, urea, total cholesterol, low-density lipoprotein (LDL), high-density lipoprotein (HDL) and triglyceride in rats when compared with alloxan group. Moreover, the extract significantly increased the insulin and hepatic glucose levels of rats when compared with alloxan group.

Parveen et al. (2011) evaluated the anti-diabetic activity (500 mg/kg) of *T. arjuna* on high-fat diet (HFD; 40%) and streptozotocin (STZ; 40 mg/kg) induced in type 2 diabetes mellitus (T2DM) in rats. Treatment with extract reductions in fasting blood glucose (FBG) and glycated hemoglobin (HbA1c) levels. Extract led to decreased levels of total cholesterol, triglycerides, LDL-C, and VLDL-C, while increasing HDL-C. The extract significantly ( $p < 0.05$ ) decreased renal injury markers in serum, BUN, serum creatinine (Scr) and ALP). Moreover, extract significantly ( $p < 0.05$ ) ameliorated thiobarbituric reactive substances (TBARS), MDA and protein carbonyl (PC), and GSH, glutathione-s-transferase (GST) and CAT in liver and pancreas in HFD/STZ rats.

### Anti-hypercholesterolemic Activity

Rather et al. (2016) demonstrated the anti-hypercholesterolemic activity of aqueous extract of *T. arjuna* against high-fat diet (HFD)-induced hypercholesterolemic rabbits. The extract significantly ( $p < 0.05$ ) decreased the serum lipids levels in rabbits at 3 and 6 months when compared with HFD group. Moreover, extract significantly suppressed the protein expression of TNF- $\alpha$ , cyclooxygenase-2, matrix metalloproteinase-9 (MMP-9), HSP60, intercellular adhesion molecule 5 (ICAM-5), endothelin-3, vimentin, protein S100-A9 in rabbits when compared with HFD group.

### Anti-hypertensive Activity

Meghwani et al. (2017) evaluated the anti-hypertensive activity of aqueous extract of *T.*

*arjuna* stem bark (125 and 250 mg/kg) against monocrotaline (MCT)-induced pulmonary hypertension (PH) in male Wistar rats. The extract significantly ( $p < 0.05$ ) reduced the right and left ventricular systolic pressure, intraventricular septum, and lung weight of rats. Moreover, extract at 250 mg/kg significantly ( $p < 0.01$ ) upregulated the RVoTD/AoD while downregulated PAAT/ET in rats. The extract (250 mg/kg) significantly ( $p < 0.05$ ,  $< 0.01$  and  $< 0.001$ ) reduced the medial wall thickness of pulmonary artery at  $< 100 \mu\text{m}$  and lung TBARS levels whereas lung SOD and catalase levels were enhanced in rats. Additionally, extract (250 mg/kg) significantly ( $p < 0.01$ ) suppressed the right ventricular hypertrophy index where protein expression of NOX1 was significantly increased in lung and gene expression of Bcl2/Bax ratio was significantly decreased in right ventricle in MCT-induced PH rats.

### Anti-inflammatory Activity

Tahsin et al. (2021) studied the anti-inflammatory activity of ethanolic extract (500, 750 and 1000 mg/kg) from *T. arjuna* against carrageenan-induced acute inflammation in rats. The extract dose-dependently inhibited paw edema by 33.36, 37.05 and 39.26%, respectively after 4 h when compared with ibuprofen (35.88, 36.59 and 37.99% at 10, 20 and 25 mg/kg, respectively) used as positive control.

Vijayalakshmi et al. (2023) reported the anti-inflammatory activity of aqueous and ethanolic extracts (10, 20, 30, 40 and

50 µl) of *T. arjuna* bark using egg albumin denaturation assay. Both the extracts exhibited good anti-inflammatory effect with percentage of inhibition 82 and 85% as compared to diclofenac sodium was used as standard (~81%) for using egg albumin denaturation assay.

Biswas et al. (2011) reported the anti-inflammatory activity of methanol extract (100 and 200 mg/kg) of *T. arjuna* leaves against carrageenan, histamine, and dextran-induced paw edema in albino Wistar rats. The extract at 200 mg/kg exhibited anti-inflammatory effect with percentage of inhibition 80.70, 74.25 and 72.47% in carrageenan, histamine, and dextran-induced paw edema, respectively, in rats as compared to indomethacin (86.12, 77.25 and 73.60%, respectively) used as positive control.

### Anti-leishmanial Activity

Moulisha et al. (2010) evaluated the anti-leishmanial activity of ursolic acid isolated from *T. arjuna* leaves against promastigotes of *Leishmania donovani* (strain AG 83). The compound exhibited anti-leishmanial effect with IC<sub>50</sub> value of 3.51 µg/ml for the tested strain.

### Anti-microbial Activity

Gupta and Kumar (2017) reported the anti-microbial activity of the extract of *T. arjuna* against *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Candida albicans* using agar well diffusion method. The

extract showed anti-microbial activity with zones of inhibition as 14, 12, 12 and 18 mm, respectively, towards tested microorganisms.

Bano et al. (2020) investigated the anti-bacterial activity of volatile oil from *T. arjuna* leaves and fruits against *Klebsiella pneumoniae*, *Escherichia coli*, methicillin resistant *Staphylococcus aureus* (MRSA), total drug-resistant *Pseudomonas aeruginosa* (TDRPA) and *P. aeruginosa*. Both oils showed anti-bacterial effect with MIC values as 0.32, 0.32, 0.64, 0.64, 2.56 mg/ml and, 0.16, 0.16, 0.32, 0.64 and 1.28 mg/ml, respectively towards the tested strains.

Vijayalakshmi et al. (2023) reported the anti-bacterial activity of aqueous and ethanolic extracts (25, 50 and 100 µL) of *T. arjuna* bark against *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas* spp. using agar well diffusion method. Both extracts exhibited anti-bacterial effect with zones of inhibition ranging from ~20 to 25 mm for tested strains as compared to standard (amoxicillin; ~35 to 40 mm).

### Anti-mutagenic Activity

Shastri Viswanatha et al. (2010) evaluated the anti-mutagenic activity of alcoholic extract of *T. arjuna* stem bark against cyclophosphamide-induced mutagenesis in albino Swiss mice using micronucleus test. In micronucleus test, the extract (100 and 200 mg/kg) showed a significant ( $p < 0.001$ ) decrease in percentage of micronucleus for both polychromatic erythrocytes (PCE) and normochromatic erythrocytes (NCE) and, also a significant reduction in P/N ratio.

## Anti-osteoporotic Activity

Kakadia and Kanaki (2021) investigated the anti-osteoporotic activity of aqueous and methanol extracts (250 and 500 mg/kg) of *T. arjuna* stem bark against bilateral ovariectomized induced post-menopausal osteoporosis in rats. Both extracts suppressed atrophy of uterus and descent of bone mineral density (BMD) in treated rats. Moreover, the methanol extract (500 mg/kg) decreased the serum concentration of calcium, phosphorus, alkaline phosphate, and TRAP in rats. Both extracts exert an increase in biomechanical strength. Further, the methanol extract increased the uterine and femoral bone weight, while histological analysis also revealed its protective action through elevation of bone formation.

Trivedi et al. (2015) investigated the anti-osteoporotic activity of ethanol extract (100, 300 and 500 mg/kg) of *T. arjuna* on ovariectomized female Sprague Dawley model rats. Treatment with extract showed an increase in uterine weight and femoral bone length, weight, density and significant increase of ash weight, ash percentage, ash calcium and hardness in lumbar vertebrae as compared to control group. The extract attained significant decrease in the levels of creatinine, phosphorus, and calcium ( $p < 0.01$  to  $p < 0.001$ ) as compared to control group.

## Anti-oxidant Activity

Mittal et al. (2015) studied the anti-oxidant activity of aqueous extract of *T. arjuna* dried

bark using DPPH assay. The extract exhibited moderate scavenging effect with  $IC_{50}$  value of 13.11  $\mu\text{g/ml}$  when compared with ascorbic acid (5.84  $\mu\text{g/ml}$ ) used as positive control.

Ramya et al. (2017) reported the anti-oxidant activity of hydro-alcoholic extract of *T. arjuna* using FRAP, DPPH, hydrogen peroxide ( $\text{H}_2\text{O}_2$ ), ABTS and nitric oxide (NO) assays. The extract exhibited scavenging effect with  $IC_{50}$  values of 85, 180, 13.3 and 109  $\mu\text{g/ml}$  for DPPH,  $\text{H}_2\text{O}_2$ , ABTS and NO assays, respectively. In addition, the extract showed FRAP value of 683 mmol Fe (II)/g.

Mittal et al. (2016) assessed the anti-oxidant activity of Tris-Cl extract of *T. arjuna* bark using DPPH assay. The extract exhibited scavenging effect with  $IC_{50}$  values as 51.72  $\mu\text{g/ml}$  when compared with ascorbic acid (5.84  $\mu\text{g/ml}$ ) used as positive control.

Bhattacharyya and Jha (2011) demonstrated the anti-oxidant activity of crude extract (12.5 to 200  $\mu\text{g/ml}$ ) of *T. arjuna* bark using DPPH assay. The extract exhibited scavenging effect with percentage inhibition ranging from 31.20 to 87.1% as compared to gallic acid used as positive control (92.03 to 95.12%).

## Anti-spermatogenic Activity

Banerjee et al. (2021) investigated the anti-spermatogenic activity of bark extract (60, 80 and 100 mg/l) of *T. arjuna* on rat Leydig cells culture from rat testis. The extract at all tested doses significantly ( $p < 0.05$  and  $< 0.01$ ) enhanced the cytotoxic percentage, viability of Leydig cells and interleukin IL-6 while,

reduced the tumor necrosis factor (TNF)- $\alpha$ , IL-10, steroid 5- $\alpha$ -reductase 1 (SRD5A1) and androgen receptor (AR) levels when compared to control group.

### Anti-tussive Activity

Sivová et al. (2016) assessed the anti-tussive activity of water extract and its fractions: acetone-soluble (TA-S) and acetone precipitated (TA-P) of *T. arjuna* stem bark against citric acid-induced cough efforts (NE) and specific airway resistance in male Guinea pigs (*in vivo*). Codeine phosphate (10 mg/kg) was used as a positive control. The extract and TA-S (50 mg/kg) significantly ( $p < 0.05$  and  $< 0.01$ ) decreased the number of cough efforts induced by citric acid in guinea pigs after 30 to 300 min when compared with vehicle treated group. However, no effect was observed on specific airway resistance in pigs.

### Anti-ulcer Activity

Devi et al. (2007) reported the anti-ulcer activity of methanol extract (100, 400 and 200 mg/kg) of *T. arjuna* bark against ethanol (ETH), diclofenac sodium (DIC) and dexamethasone (DEX)-induced ulcer in rats. The pre-, post and co-administration of extract showed 100% protection to the gastric mucosa against ETH, DIC and DEX induced ulcer in rats. The extract significantly decreased the levels of lipid peroxides and activities of SOD and CAT, whereas the levels of GSH increased in gastric mucosa compared with DEX rats. No significant changes observed in GPx level of treated rats. Further, the extract exerts an increase in total hexoses, hexosamine, sialic acid,

fucose, total carbohydrate and TC: P contents as compared with DEX rats.

### Anti-urolithic Activity

Mittal et al. (2015) demonstrated the anti-urolithic activity of aqueous extract (100, 200, 500 and 1000  $\mu\text{g/ml}$ ) of *T. arjuna* dried bark against crystallization of calcium oxalate (CaOx) *in vitro*. The extract (100 and 1000  $\mu\text{g/ml}$ ) inhibited CaOx crystal nucleation by 42.63 and 61.35%, when compared with cysteine (57.53%), used as positive control. Moreover, the extract dose-dependently inhibited the CaOx crystal aggregation by 34.47, 39.9, 44.6 and 49.8% when compared with cysteine (69.7%). The extract (500 and 1000  $\mu\text{g/ml}$ ) reduced the growth of CaOx crystals by 96.4 and 86.89% when compared with cysteine (90.67%). In addition, the extract reduced the area, perimeter, length, and width of crystal when compared with control group.

Mittal et al. (2017) reported the anti-urolithic potential of aqueous extract (20, 30 and 40  $\mu\text{g/ml}$ ) of *T. arjuna* bark against calcium oxalate (CaOx)-induced injury to renal tubular epithelial cells (MDCK cells). The extract significantly ( $p < 0.01$ ) enhanced the viability of MDCK cells when compared with CaOx alone group. Moreover, the extract (40  $\mu\text{g/ml}$ ) upregulated the number of viable cells with intact cellular membrane and, no remarkable nuclear changes in MDCK cells. Further, the extract (40  $\mu\text{g/ml}$ ) decreased the number of apoptotic cells by 33.8% while 57.5% number of apoptotic cells were reduced using anti-Active Caspase-3 antibody staining.

Mittal et al. (2016) conducted the anti-urolithic activity of Tris-Cl extract (10, 20 and 40 µg/ml) of *T. arjuna* bark on oxalate-induced cell injury of NRK-52E renal epithelial cells. The extract (100 µg/ml) inhibited CaOx crystal nucleation, aggregation, and growth by 39.9, 46.9 and 31.06% when compared with cysteine (24.38, 42.88 and 27.78%) used as positive control. Moreover, the extract significantly ( $p < 0.05$  and  $< 0.005$ ) increased the viability of NRK-52E cells when compared with CaOx alone group. Further, the extract showed healthy cellular morphology showing no adverse effect NRK-52E on cells when compared with CaOx alone group.

### Anti-viral Activity

Cheng et al. (2002) reported the anti-viral activity of casuarinin isolated from bark of *T. arjuna* on herpes simplex type 2 (HSV-2) *in vitro*. The compound showed anti-viral effect with  $IC_{50}$  values of 3.69 and 1.59 µM using XTT and plaque reduction assays, respectively.

### Cardioprotective Activity

Manu et al. (2019) screened the cardioprotective activity of hydroalcoholic extract and arjunic acid (AA) from *T. arjuna* bark against cobalt chloride ( $CoCl_2$ )-induced hypoxia damage and apoptosis in rat cardiomyocytes (H9c2). The extract (25-100 µg/ml) and AA (6-10 µg/ml) significantly ( $p < 0.05$ ,  $< 0.01$  and  $< 0.001$ ) enhanced the cell viability. Moreover, extract (50 µg/ml) and AA (8 µg/ml) significantly ( $p < 0.001$ ) decreased the LDH leakage while enhanced the expression of HIF-1α in H9c2

cells. The extract and AA significantly ( $p < 0.05$  and  $< 0.01$ ) decreased the reactive oxygen species (ROS),  $H_2O_2$ , nitrite and MDA levels while mitochondrial membrane potential was increased in H9c2 cells. Both extract and AA significantly ( $p < 0.05$ ) suppressed the levels of SOD, CAT, glutathione peroxidase (GPx), and glutathione reductase (GR) and GSH, expressions of HSP-70, iNOS, Bax, JNK, pJNK, c-jun, and caspase 3 whereas elevated Bcl-2 expression in H9c2 cells.

Mohanty et al. (2019) studied the cardioprotective activity of hydro-alcoholic dried extract of *T. arjuna* on DPP-IV inhibition (*in vitro*) and streptozotocin (45 mg/kg) and isoproterenol (ISP; 85 mg/kg b.w.)-induced myocardial infarction in male Wistar rats (*in vivo*). The extract inhibited the DPP-IV activity by 86.39% when compared with vildagliptin (90.42%) used as positive control. Moreover, extract (500 mg/kg) significantly ( $p < 0.001$ ) suppressed the serum levels of creatinine phosphokinase (CPK-MB), blood glucose and DPP-IV in rats when compared with ISP control group. In histopathological analysis, extract reduced the degree of edema, inflammation and necrosis in rats when compared with ISP control group.

Bhat et al. (2018) evaluated the cardioprotective activity of aqueous stem bark extract of *T. arjuna* against interleukin-18 (IL-18)-induced atherosclerosis in Apo E2/2 mice. The extract significantly ( $p < 0.05$ ) reduced the expression levels of IL-18, IL-18 Ra, TC and LDL-C levels while, enhanced the HDL-C in IL-18-treated Apo E2/2 mice.

The extract significantly ( $p < 0.05$ ) increased the mRNA expressions of PPAR- $\gamma$ , LXR- $\alpha$  whereas, decreased mRNA expression of CD36, MMP-9, NF- $\kappa$ B, ICAM-1 and VCAM-1 in IL-18-treated Apo E2/2 mice. Further, the extract significantly ( $p < 0.05$ ,  $< 0.01$  and  $< 0.001$ ) upregulated the protein expression levels of PPAR- $\gamma$ , LXR- $\alpha$  but, protein expression of CD36, MMP-9, ICAM-1 and VCAM-1 was downregulated in IL-18-treated Apo E2/2 mice. In histopathological analysis, the extract decreased atherogenic lipids, and atherosclerotic lesion area in IL-18-treated Apo E2/2 mice.

Karunakaran (2015) reported the cardioprotective activity of methanol extract of *T. arjuna* bark (6.75 and 9.75 mg/kg) against myocardial ischemic-reperfusion injury in male albino Wistar rats. The extract at both doses showed recovery of myocardial function and, significantly decreased the myocardial thiobarbituric acid reactive substance (TBARS) level while, SOD, GSH and catalase activities were increased in rats when compared with vehicle treated group.

Kumar et al. (2017) evaluated the cardioprotective activity of aqueous extract of *T. arjuna* bark against isoproterenol (ISO)-induced cardiac hypertrophy in male Wistar rats. The extract significantly ( $p < 0.05$ ) reduced the heart to body weight ratio in rats. Also, the extract significantly ( $p < 0.001$ ) decreased the left ventricular wall thickness (LVPW and IVS), levels of  $\beta$ -MHC, skeletal  $\alpha$ -actin, brain natriuretic peptide (BNP) and TGF- $\beta$ 2, protein expression of AKT in rats. Furthermore,

extract downregulated the expressions of Grp78, HDAC5, pERK and MEF2D while upregulated the expressions of NF $\kappa$ B and AP-1 in rats when compared with ISO alone group. In addition, the extract improved the subset of proteins up- and down-regulated the hypertrophied hearts of rats.

## Cytoprotective Activity

Hebbani et al. (2021) screened the cytoprotective activity of aqueous extract of *T. arjuna* bark against alcohol-induced oxidative damage of albino Wistar rats' erythrocyte membranes. The extract reduced lipid peroxidation, protein carbonyl contents, phosphatidyl choline, phosphatidyl ethanolamine, phosphatidyl serine, phosphatidyl inositol and sphingomyelin, erythrocyte membrane anisotropic value in alcohol treated rats. Moreover, the extract significantly ( $p < 0.05$ ) increased the GSH, GPx, SOD and catalase levels while, decreased total cholesterol and total phospholipids in alcoholic rats' erythrocyte. Further, the extract elevated the resistance to hypotonic shock induced by NaCl and hemolysis in alcoholic rat's erythrocyte.

Bodiga et al. (2022) investigated the cytoprotective activity of ellagic acid (25, 50 and 100  $\mu$ M) from *T. arjuna* fruits against chromium and cobalt-induced toxicity in primary human lymphocytes. The compound effectively ameliorated the viability and proliferative responses of resting and activated lymphocytes whereas, suppressed the apoptotic index when compared with

Cr<sup>6+</sup> and CO<sub>2</sub> group. Moreover, ellagic acid significantly ( $p < 0.05$ ) increased the levels of IL-2 and IFN- $\gamma$  anti-CD3 and, anti-CD3 $\pm$ anti-CD28 activated lymphocytes exposed for 48 h when compared with Cr<sup>6+</sup> and CO<sub>2</sub> treated group. Further, ellagic acid exert a significant ( $p < 0.05$ ) decrease in number of apoptotic cells at resting and anti-CD3-activated lymphocytes for 24 hr when compared with Cr<sup>6+</sup> and CO<sub>2</sub> treated group.

Ramya et al. (2017) evaluated the cytoprotective activity of hydroalcoholic extract (10, 20 and 30  $\mu\text{g/ml}$ ) along with ursolic acid, kaempferol, ellagic acid, arjunolic acid, arjungenin isolated from *T. arjuna* bark against domoic acid (DA)-induced toxicity in human epithelial colorectal adenocarcinoma cells (Caco-2). The extract and tested compounds significantly ( $p < 0.05$ ) increased the cell viability of Caco-2 cells. Different doses of TA pre-treatment attenuated domoic acid-induced loss of mitochondrial membrane potential and improved the fluorescence intensity by  $49.5 \pm 4.3\%$  (TA-10  $\mu\text{g/ml}$ ),  $64.8 \pm 3.38\%$  (TA-20  $\mu\text{g/ml}$ ) and  $86.2 \pm 6.1\%$  (TA-30  $\mu\text{g/ml}$ ). Moreover, extract at all doses reduced the ROS and NO levels. However, the extract did not show any significant effect on CAT and GR levels.

### Effect on Cerebral Vascular Leakage

Kumar et al. (2016) studied the effectiveness of hydroalcoholic extract (150, 200 and 250 mg/kg) of *T. arjuna* bark on acute hypobaric hypoxia-induced cerebral vascular leakage in male Sprague Dawley rats. The extract significantly

( $p < 0.05$ ) enhanced the glomerular filtration rate (GFR), atrial natriuretic peptide (ANP) concentration and urine volume in normoxic rats. Moreover, the extract significantly ( $p < 0.05$ ) increased cerebral vascular leakage (CVL) and aldosterone levels but, decreased the GFR and ANP concentration in hypoxic rats. Further, the extract (150 mg/kg) significantly ( $p < 0.05$ ) downregulated the CVL and serum sodium levels whereas upregulated the urine volume and serum potassium levels in hypoxic and normoxic rats. In addition, the extract (150 mg/kg) significantly ( $p < 0.05$ ) elevated the GFR, ANP, CAT and SOD activities but, decreased serum renin, angiotensin-II concentration, aldosterone, and MDA levels in hypoxic rats.

### Effect on Central Nervous System

Sekhar et al. (2017) assessed the effectiveness of alcoholic extract (25, 50 and 100 mg/kg) of *T. arjuna* bark against picrotoxin (PTX)-induced anxiety in BALB/c mice using open field, elevated plus maze, light-dark paradigm, and Vogel's conflict tests. Diazepam (DIZ; 1.5 mg/kg) was used as positive control. The extract significantly ( $p < 0.05$ ) increased open arm entries, time spent in open arm and central position while, decreased time spent in closed arm in mice using elevated plus maze test. Moreover, the extract significantly ( $p < 0.05$ ) enhanced total number of crossings, time spent in light box in seconds and total rearing frequency, locomotion, time spent in central position and total rearing frequency using light/dark box and open filled conflicts

in mice. In Vogel's conflict test, the extract also significantly ( $p < 0.05$ ) upregulated total number of licks and shocks in mice. Further, the extract elevated the expressions of BDNF,  $IP_3$ , CREB,  $GABA_A$ , SOD, GPx, and GR whereas, suppressed  $5HT_{1A}$  expression in mice.

### Enzyme Inhibitory Activity

Varghese et al. (2018) evaluated the enzyme inhibitory effect of methanol extract and its fractions (petroleum ether, chloroform, ethyl acetate, n-butanol) and sub-fractions from n-butanol fraction (sub-fractions 1-6) of *T. arjuna* bark against CYP3A and CYP2D inhibition in rat liver microsomes. The extract and all fractions showed CYP3A and CYP2D inhibition with  $IC_{50}$  ranging from 7.46 to 29.84 and, 13.03 to 35.91  $\mu\text{g/ml}$ , respectively, when compared with ketoconazole and quinidine (0.051 and 0.151  $\mu\text{g/ml}$ ) used as a positive control. All sub-fractions inhibited CYP3A and CYP2D isoenzyme potential with values ranging from 5.64 to 19.30 and, 16.11 to 31.14  $\mu\text{g/ml}$ , respectively, as compared to positive control (0.057 and 0.142  $\mu\text{g/ml}$ ).

Varghese et al. (2015) investigated the inhibitory activity of alcoholic and aqueous extracts along with arjunic acid, arjunetin and arjungenin isolated from *T. arjuna* against CYP3A4, CYP2D6 and CYP2C9 enzymes in human liver microsomes (HLM). The alcoholic extract inhibited CYP3A4, CYP2D6 and CYP2C9 enzymes with  $IC_{50}$  values of 16.6, 15.28 and 34.52  $\mu\text{g/ml}$  whereas, aqueous extract showed effectiveness of 17.4, 11.97 and 27.78  $\mu\text{g/ml}$ , respectively. However, arjunic acid, arjunetin

and arjungenin, did not show any significant inhibition of CYP3A4, CYP2D6 and CYP2C9 enzymes in human liver microsomes up to final concentrations of 50  $\mu\text{M}$ .

### Gastroprotective Activity

Devi et al. (2007) evaluated the gastroprotective activity of methanolic extract (100 to 500 mg/kg, b.w.) of *T. arjuna* on diclofenac sodium-induced gastric ulcer in rats. The extract showed significant reduction ( $p < 0.001$ ) in lesion index in ulcer induced animals. Moreover, the extract exerts an increase in pH, NP-SH, GSH, enzyme antioxidants, protein bound carbohydrate complexes, adherent mucus content, nucleic acids with decrease in volume of gastric juice, free and total acidity, pepsin concentration, acid output, LPO levels and MPO activities in treated rats.

### Hepatoprotective Activity

Toppo et al. (2018) demonstrated the hepatoprotective activity of arjunolic acid isolated from *T. arjuna* heartwood against palmitate, oleate, BSA complex (PO-BSA)-induced steatosis in HepG2 cell lines as well as, high-fat diet (HFD)-induced non-alcoholic fatty liver disease (NAFLD) in male Wistar rats. The compound (12.5, 25 and 50  $\mu\text{M}$ ) significantly ( $p \leq 0.05$ ) decreased the oil red O (ORO) concentration, TG levels, LDH release, ALT, and AST in spent media of HepG2 cells. Moreover, the compound attained  $GI_{50}$  value of 746.34  $\mu\text{M}$  for HepG2 cells. The compound (25 and 50 mg/kg) also significantly ( $p \leq 0.05$ ) suppressed the levels of TC, TG, LDL cholesterol and total

cholesterol/H L cholesterol and serum levels of AST, ALT, ALP, LDH and GGT levels in rats. Further, the compound significantly ( $p \leq 0.05$ ) upregulated the expressions of PPAR $\alpha$  and FXR $\alpha$  whereas, PPAR $\gamma$  expression level was downregulated in rats. In histopathological analysis, the compound also reduced multifocal fatty hepatocytes having non-eosinophilic cytoplasm and MNC infiltration in rats.

### Immunomodulatory Activity

Saxena et al. (2008) reported the immunomodulatory activity of arjunic acid (0.1, 1 and 10  $\mu\text{g/ml}$ ) isolated from *T. arjuna* bark in murine splenocytes using MTT assay. The compound at tested doses showed percentage stimulation and modulation with 42.4, 34.3 and 37.6% and, 1.3, -18.1, -10.3% inhibition of splenic lymphocytes when compared with levamisole HCl (53 and 26.4%) used as positive control.

### Organoprotective Activity

Bhattacharjee et al. (2019) conducted the organoprotective activity of aqueous extract of *T. arjuna* bark against cadmium (Cd)-induced hepatic and cardiac injuries in male Wistar rats. The extract significantly ( $p < 0.001$ ) reduced the liver and heart weight to body weight ratio, levels of SGPT, SGOT, TLDH, LDH5, LDH1, ALP and CK-MB in rats. The extract significantly ( $p < 0.001$ ) downregulated the levels of LPO, PCO, GPx, XO and XDH while GSH, TSH and GR levels were upregulated in heart and liver of rats. The extract significantly ( $p < 0.001$ ) suppressed the Cu-ZnSOD and

MnSOD and, increased in heart tissues whereas, elevated the CAT activity in liver tissues but decreased in heart tissues of rats. The extract significantly ( $p < 0.001$ ) increased the activities of pyruvate dehydrogenase, isocitrate dehydrogenase,  $\alpha$ -ketoglutarate dehydrogenase, succinate dehydrogenase, cytochrome c oxidase and cytochrome c-oxidoreductase but, attenuated DNA band density, fluorescence intensity and hepatic and cardiac mitochondria in both tissues of rats. In addition, the extract significantly ( $p < 0.001$ ) upregulated the collagen volume in heart tissues and, downregulated the liver tissues of rats.

### Nephroprotective Activity

Kanthe et al. (2021) reported the nephroprotective activity of ethanolic extract from *T. arjuna* bark against high fat diet-induced nephrotoxicity and oxidative stress in albino Wistar rats. The extract significantly ( $p \leq 0.05$ ) reduced the final body weight, blood urea, serum creatinine, MDA and SOD levels while GSH level was enhanced in rats when compared with high fat diet group. Moreover, the extract significantly ( $p \leq 0.05$ ) suppressed the MDA, SOD and GSH levels in renal tissues of rats. In histopathological analysis, the extract improved architecture of kidney of rats when compared with high fat diet group.

Sherif (2015) screened the nephroprotective activity of arjunolic acid isolated from *T. arjuna* against cisplatin-induced nephrotoxicity in male Sprague-Dawley rats. The compound significantly ( $p < 0.05$ ) decreased the levels

of creatinine, blood urea nitrogen, MDA and NO while, GSH level was increased in rats. Moreover, the compound significantly ( $p < 0.05$ ) downregulated the mRNA expression of TGF- $\beta$ , NF- $\kappa$ B and Kim-1 whereas, upregulated Bcl-2 mRNA expression of rats when compared with cisplatin treated group. In histopathological analysis, the compound also reduced tubular cell degeneration and necrosis in rats when compared with cisplatin administered group.

### Neuroprotective Activity

Yaidikar and Thakur (2015) evaluated the neuroprotective activity of arjunolic acid (10 and 20 mg/kg) isolated from *T. arjuna* bark against middle cerebral artery occlusion (MCAO)-induced focal cerebral ischemia reperfusion (I/R) injury in male Wistar rats. The compound at both doses significantly ( $p < 0.001$ ) reduced the neurologic deficit score, brain water content, ROS levels, carbonyl, MDA and NO contents while, enhanced Na(+)/K(+)ATPase activity, SOD, catalase, GR, GSH and GPx activities in rats when compared with MCAO group.

### Wound Healing Activity

Rane et al. (2003) evaluated the wound healing activity of oral administration and

topical application of alcoholic extract (150 and 300 mg/kg or 1.5 and 0.1%, w/w) along with tannins isolated from *T. arjuna* bark on incision and excision wounds in Wistar rats. The oral administration and topical application of extract showed a significant ( $p < 0.01$ ) increase in the tensile strength of wounds as well as, increase in the percentage reduction of wound size for excision wounds in rats as compared to control. Tannins at 0.1% dose showed highest tensile strength of 462 g for wounds. The oral administration of extract and tannin exhibited a significant ( $p < 0.01$ ) increase in hydroxyproline content of the granulation tissue of excision wounds as compared to control.

Chaudhari and Mengi (2006) investigated the wound healing activity of fractions I, II and III (1%, w/w) fractionated from hydroalcohol extract from *T. arjuna* bark against healing of dermal wounds in albino Wistar rats. The fraction I showed maximum increase in the tensile strength and fastest rate in epithelialization of incision wounds. Also, fraction I at 1% dose showed maximum increase in hexosamine content from granulation tissue of excision wounds as compared to control.

## Clinical Study

Dwivedi et al. (2019) conducted a clinical study to evaluate the effectiveness of arjuna (*T. arjuna*) bark extract in 35 patients of chronic coronary artery disease (CAD). The extract was found to be safe and effective in patients with chronic CAD and did not show any adverse effects on patients.

Another double-blind, randomized controlled clinical trial was established by Maulik et al. (2019) to examine the effects of aqueous stem bark extract of *T. arjuna* including 100 patients of chronic heart failure compared to placebo (carvedilol). The extract enhanced the levels of 6 MWT, RBC-SOD, RBC

catalase and RBC glutathione when compared with placebo treated group.

Kapoor et al. (2015) also reported a placebo-controlled, randomized, multi-centric clinical trial to investigate the effect of *T. arjuna* extract in 116 patients on classical and immunoinflammatory markers for CAD as an adjuvant therapy. The extract significantly ( $p < 0.05$ ,  $< 0.01$  and  $< 0.001$ ) decreased the levels of TC, TG, VLDL-C, IL-6, IL-18 and hsCRP while, increased HDL-C, Ox-LDL and IL-10 levels, expressions of JUN, IL-8, IL-1 $\beta$  and NF- $\kappa$ BIZ/MAIL after 6 months when compared with placebo treated group.

## Toxicological Study

Kumar et al. (2016) investigated the acute and sub-chronic toxicity of hydroalcohol extract from *T. arjuna* bark in male Sprague Dawley rats. The extract did not show mortality at 2000 mg/kg b.w. Moreover, the extract did not show any adverse effect on food and water intake, body weight and behavior as well as on vital organs of rats. Further, the extract enhanced the urine output during the initial 6 h.

The methanol extract along with 7-methyl gallic acid (7MG) isolated from bark of *T. arjuna* (TAB) were evaluated for cytotoxicity in human peripheral blood mononuclear cells (PBMCs) using MTT assay, hemolytic activity in human erythrocytes, mutagenicity using Ames test and genotoxicity by comet assay

*in vitro* as well as assessed for *in vivo* toxicity in albino Swiss mice. TAB and 7MG did not show cytotoxic and genotoxic effect in PBMC, no hemolytic effect in RBC and, no mutagenic effect in TA 98 and TA 100 up to 2000 mg/ml dose. Moreover, TAB and 7MG did not exhibit any changes in body weight, behavior, hematology, biochemical parameters, organ weight and histopathological analysis, as reported by Suganthy et al. (2018).

Toppo et al. (2018) also reported the toxicity study of arjunolic acid isolated from ethyl acetate extract of *T. arjuna* heartwood in Wistar rats. The compound did not show any noticeable adverse effect up to 2 g/kg in rats.

## Summary and Future Outlook

In conclusion, *T. arjuna* demonstrates significant pharmacological activities relevant to cardiovascular health, including cardioprotection, antihypertensive effects, antiarrhythmic properties, lipid-lowering activity, antioxidant, and anti-inflammatory effects. Pharmacological studies have revealed several beneficial effects of this species, particularly in relation to heart health. More clinical trials are needed to confirm its efficacy and safety for specific conditions. Efforts to standardize the

composition and quality of *T. arjuna* products are crucial for consistent results. A deeper understanding of its mechanisms of action can guide further development and clinical application. Exploring *T. arjuna* in combination with conventional medications may offer new treatment strategies. Overall, the future of *T. arjuna* pharmacology appears promising, but rigorous research and standardization are crucial to unlock its full potential and ensure safe and effective use.

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