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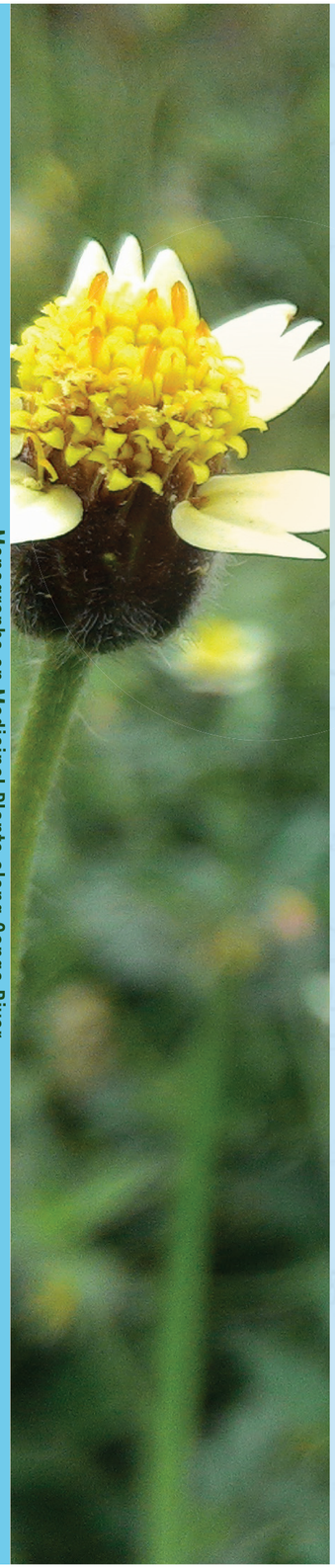
Monographs on Medicinal Plants along Ganga River
Tridax procumbens L.



Monographs on Medicinal Plants along Ganga River

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

Volume 10

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Volume 10

Sponsored by

National Mission for Clean Ganga (NMCG),
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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'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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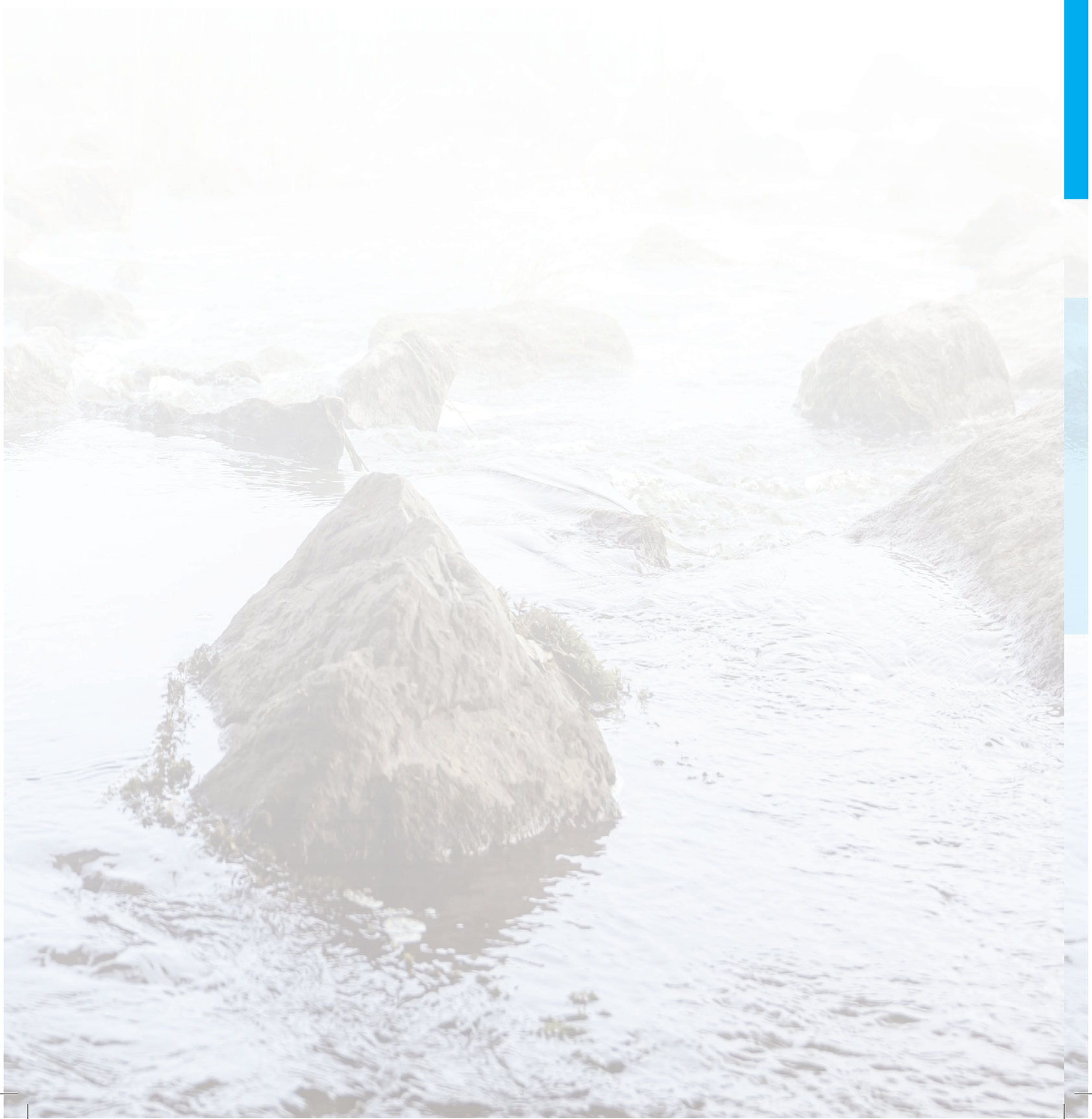

Vision of Union Minister for Jal Shakti

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/PressReleseDetail.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/
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Uttar Pradesh Forest Department & Biodiversity Board

Bihar Forest Department & Biodiversity Board

Jharkhand Forest Department & Biodiversity Board

West Bengal Forest Department & Biodiversity Board

Ganga Task Force (GTF)

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महानिदेशक
राष्ट्रीय स्वच्छ गंगा मिशन
Rajeev Kumar Mital, IAS
DIRECTOR GENERAL
NATIONAL MISSION FOR CLEAN GANGA

सत्यमेव जयते

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)

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 andbotanical 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, insilico 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

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Classical Ayurvedic Insight

Tridax procumbens L.

Vedic name : (Sadāharitkaḥ śarapatraḥ) (सदाहरित्कः शरपत्रः)

Botanical name : *Tridax procumbens* L.

Vānaspatika vivaraṇam

1. Kāyika lakṣaṇam

Taru sadāharīvaṃśasaṃjāta śākarūpadhṛk.
Vārṣikād bahuvarṣāyurdṛśyastambhitayodita.
Uccāgrabhūmiśāyī ca parvasu mūlasaṃyuta.
Kāṇḍamaroma bhūśāyi mūlabhāgeṣu śākhitam.
Śākhāstanvya stṛtāścā''ho ārohirūpata sthitāḥ.
Trimuṣṭilambitābhyaśca hastadvyaṅgulakocchritāḥ.
Dīrgharobhirāviddhāḥ sarvathā sañcakāsati.
Patramalpaṃ tathā sthūlaṃ viparītaṃ savṛntakam
Dīrghavṛttanibhākāramāho aṇḍasamākṛti.
Athavā dvitrapālībhi samāyuktakalevaram.
Upāntastu bṛhaddantasahita krakacīva ca.
Ādhāraścāpyadhovardhī tathopāntena saṃyuta.
Śīrṣaṃ tīkṣṇāgrarūpañca lambitāgraṃ tathā'thavā.
Ubhayostalayoścāpi saśūkaromabhiryutam. (Saumitreya Mahodadhi: 1-7)

Botanical Description

1. Vegetative Characters

Annual to perennial, caulescent, decumbent herb, rooting at nodes. Stem hairy, procumbent, branched at base. Branches slender, spreading or ascending, 20-50 cm, hirsute. Leaves few, opposite, thick, petiolate, elliptic or ovate or sometimes 2 or 3-lobed, margin serrate with large teeth, base with decurrent margin, apex acute or acuminate, both surface with bristly hairs. [1-8]

2. Puṣpīya lakṣaṇam

Puṣpāṇāṃ lakṣaṇe puṣpakramo muṇḍakarūpabhṛt.
Dīrgharūpastathā carjuprasūnāvalivṛntake.
Samutpannaṃ virājeta puṣpavṛntantu tatra vai.
Trimuṣṭilambitaprāyaṃ romaśaṃ pratibhāsate.
Sahapatrasthacakrantu dvayoḥ paṅktyoḥ kvacit puna.
Ekasyāmapi vai paṅktau vidyamānaṃ virājate.

Sahapatrantathā tatra saṅkucadrūpata sthitāt.
Āyāmyaṇḍanibhākāramathavā dīrghavṛttakam.
Śīrṣaṃ lambāgrarūpañca bāhyabhāgastu romaśa.
Raśmipuṣpakamapyatra tvekaliṅgitayoditam.
Ekasīmāntapaṅktau ca pītarūpaṃ kadācana.
Śvetarūpadharaṃ bhāti dviyavalambitāt khalu.
Sārdhadviyavakocchrāyacatuṣpañcaṣameva ca.
Romaguccha sadā pakṣasadṛśaśūkacakrayuk.
Dalapuñjaṃ tathā caikasaṅkucitasvarūpayā.
Pītapraṇālyā saṃyuktaṃ pratibhāti tadeva tu.
Śvete tridanture kvāpi dvidanturasuvigrahe.
Arau prasphuṭitaṃ bhāti tathā tatrā'ṇḍakāśaya.
Adhovartī tathā lagnaromabhiścāvṛta puna.
Cipaṭaścāpi bhāseta vartikā tantulā tathā.
Vartikāgraṃ dvipālīkaṃ bimbapuṣpantu pītata.
Anekaṃ romaśañcāpi romagucchastu tatra vai.
Pakṣalaśūkacakreṣu saṃsthitaṃ pratibhāsate.
Dalapuñjaṃ bhavet pītaṃ praṇālī tu viśeṣata.
Dūrasaṃsthitabhāge vai vistṛtā pañcapāliyuk.
Puṃskesarāstu pañcaivaṃ prasūnadhūlikośaka.
Antarmukhī ca tīkṣṇāgraśīrṣasthopāṅgasaṃyuta.
Bāṇābhādhārasampṛkta āṃśikarūpata khalu.
Udvardhī pratibhāseta tathā tatrā'ṇḍakāśaya.
Adhovartī tathā''lagnaromakavellanākṛti.
Vartikā tantulā tatra vartikāgraṃ dvipālikam.
Ekīnaṃ babhruvarṇābhaṃ dīrghāyatakalevaram.
Yavalambiśarīrañca sāndrakomalaromayuk.
Śūkaśca romagucchastha sapakṣa iva vartate.
Sārdhadviyavakocchrāyiśarīrācca kvacit puna.
Triyavocchrāyirūpaśca bhāsvadrūpeṇa saṃbhṛta. (Saumitreya Mahodadhi: 8-25)

2. Floral Characters

Head (capitula) borne on a long, erect peduncle. Peduncle up to 23 cm long, hairy. Involucre of 1-2 rows of bracts. Bracts narrowly to broadly ovate or elliptic, apex acuminate, hairy outside. Ray florets unisexual, in one marginal row, yellow, rarely white, 4-5 mm long, approximately 4-6. Pappus a ring of

feather-like bristles. Corolla a narrow, yellowish tube abruptly opening into a whitish tridentate or sometimes bidentate ray. Ovary inferior, flattened, covered with appressed hairs. Style filiform. Stigma bilobed. Disc flowers yellow, many, hairy. Pappus a ring of feather-like bristles. Corolla yellow, tube expanding distally, 5-lobed. Stamens 5. Anthers introrse, with acute apical appendage and sagittate base, partly exserted. Ovary inferior, cylindrical with appressed hairs. Style filiform. Stigma 2-lobed. Achenes brown, oblong, 2 mm long, densely silky pubescent. Pappus setae 5-6 mm, shiny, plumose. [1-8]

Plant Anatomy

Prayojyāṅgam

Mūlaṃ kāṇḍaṃ phalaṃ patraṃ sadāharerbhiṣagvaraiḥ.

Prayojyāṅgaṃ matantasyāntāracanopavarṇya te. (Saumitreya Mahodadhi:1)

Antaḥsaṃracanekṣaṇe

(a) Phalam

Ekabīji phalaṃ śuṣkamadhyanuprasthachitti tat.

Saṃkīrṇāyatarūpeṇa dveghātra pulabhittikā.

Bāhyamadhyasvarūpeṇa vibheditāvalokyate.

Tadbāhyaphalabhittyāṃ vai hyekapaṅktinibandhanāḥ.

Sāndraprasthūlabhittīyā mṛdutakīyakośikāḥ.

Vyavasthitāśca saṃyuktā bāhyata upacarmaṇā.

Phalabhittestu nīcairvai varṇapadārthagaḥ staraḥ.

Pādapīyaḥ sthitastatrādhimadhyaphalabhitti ca.

Sthūlabhittirhi sāndratvāt sthitā vṛttīyarūpabhāk.

Kośikāguhikāmadhye mṛdutakīyakośikāḥ.

Phalabhittyātra saṃyukto bījacolo'valokyate.

Ekapaṅktinibaddhaśca mṛdutakīyarūpabhāk.

Ūrdhvāgharasvarūpastho drumivarṇapadārthake.

Bhrūṇapoṣaḥ sthitaścaikapaṅktibaddho mṛdutakaḥ.

Sthūlabhittisvarūpo druvarṇapadārthakasya ca.

Bhāge samadhike bhrūṇapoṣaḥ supakva īkṣyate.

Bījapatradvayaṃ śārivavarṇapadārthavartinaḥ.

Akṣasya dakṣiṇe bhāge sarjarasīyanālikāḥ.

Dvādaśeha samālokyā vyavasthitasvarūyataḥ.

Sadāhareḥ phalasyāntaḥ saṃracaneti coditā. (Saumitreya Mahodadhi: 2-11)

Plant Parts

Fruit, root, stem, leaf

Microscopic Characters

Fruit

Cypsela is narrows elliptic in cross section. Pericarp is differentiated into two zones- epicarp and mesocarp. Epicarp is uni-seriate, made up of thick-walled, cubical, parenchyma cells, compactly arranged, provided with cuticle. Just below epicarpic region, phytomelanin layer is continuously arranged. Mesocarp consists of thick-walled, compactly arranged, and more or less rounded, parenchyma cell with small cell lumen.Testa is attached with pericarp, uni-seriate, parenchymatous, horizontally arranged. Endosperm persists in mature cypsela, uniseriate, thick-walled and parenchymatous. Mature embryo occupies a major part of cypsela. Cotyledons two in number, arranged at right angle to axis of cypsela, containing 12 resin ducts. [1],[9-13]

(b) Mūlam

Dvitīyakābhighāṃ vṛddhi mūlaṃ darśayate kṣatā.

Adhicarmastarā vīkṣyā laghvīhādyā kaṇāvalī.

Tanmajjakośikā atra mṛdutakīyarūpikāḥ.

Kośikāḥ sparśarekhīyā ākṛṣṭā iva santi ca.

Poṣavāho dvitīyākhyo niyatajitatāspade.

Nyagvāhakaṃ dvitīyākhyaṃ parito nālikāgataḥ.

Nyagvāhotakamadhye ca poṣavāhīyatantavaḥ.

Koṇīyā vāhikāḥ sthūlabhittīyā iha santi ca. (Saumitreya Mahodadhi: 12-15)

Root

Root exhibits secondary growth. Epidermal layer is broken and remains as small fragments. Cotex is parenchymatous and cells are tangentially stretched. Secondary phloem occurs as continuous broad zone around secondary xylem cylinder. Xylem tissue consists of libriform fibers and thick walled, angular vessels. [1],[9-13]

(c) Kāṇḍabhāgaḥ

Adhicarmaprabhāgasyaikastarīyā hi kośikāḥ.

Mṛdutakīyarūpā vai vallanākārarūpataḥ.

Āyatākārarūpiṇyastanmajjakośikātatiḥ.

Bāhyagā sthūlakoṇīyā ūtakā āntarā iha.

Mṛdutakīyasaṃjjñeṣu hyūtakeṣu vibhājitā.

Kośikāḥ sthūlakoṇīyāḥ prāyaḥ pañcastarānvitāḥ.

Bahubhujīyarūpā vā hyaṇḍākārataḥ īkṣitāḥ.

Niyatā jhillikā atra tanmajjakośikāstathā.

Mṛdutakāṇubhittīyā aṇḍākārā bhavanti tāḥ.

Bahubhujasvarūpiṇyaḥ kāṇḍāntāracanoditā. (Saumitreya Mahodadhi: 16-20)

Stem

Epidermis is single layered, parenchymatous, cells rectangular or barrel in shape. Cortex is divided in to outer collenchymatous tissue and inner parenchymatous tissue. Collenchymatous cells form a continuous sheath, cells polygonal or oval in shape and 1-2-layered. Parenchymatous cells occur in continuous cylinder, 4-7 layered. Endodermis is inconspicuous. Pericyclic sclerenchymatous forms discontinuous sheath, 1-5 layered. Pith cells are thin-walled, parenchymatous, oval or polygonal. [1],[9-13]

(d) Patram (Parṇavṛntam)

Anuprasthadyutau parṇavṛntamavatalotakāḥ.

Abhyakṣāspadake bhānti kṣudrāvatalarūpataḥ.

Saṃvahanākhyatantvantastanmajjatantukastathā.

Pārśvatantudvayī pakṣaḥ parṇavṛntasya tantuke.

Ekasmin bhāga ālokyaḥ prasūkṣmadarśikāyane.

Sadāharerhi kāṇḍāntaḥsaṃracaneti varṇitā. (Saumitreya Mahodadhi: 21-23)

Leaf

Petiole: Petiole is concavo convex in sectional view with shallow concavity on adaxial side. Vascular strands consist of a median strand, two lateral strands and one wing strand on either end of petiole. [1],[9-13]

(e) Paṭalaḥ

Pṛṣṭhāgharīyarandhrasya taladvaya upasthitaḥ.

Paṭalo hyadhicarmākhyāḥ kośikāḥ kṣudravakritāḥ.

Anityakośikaścātra randhravrajaḥ staradvayaḥ.

Parṇamadhyotako dṛśyaḥ spañjimṛdutakābhighe.

Kośikānicaye cāsau vibheditaḥ sthaladvaye.

Ekatra kośikāsthāne kevalaṃ dvārakośikāḥ.

Vikasitāḥ samālokyā ekastarasamanvitaḥ.

Stambhotakastathā spañjyūtako'tra pañcataḥ punaḥ.

Ṣaṭstarīyaḥ samālokyaḥ kāścikṣetijarūpataḥ.

Kośikāḥ karṣitābhāstā haritotakasaṃyutāḥ.] (Saumitreya Mahodadhi: 24-28)

Lamina: Lamina is dorsiventral, amphistomatous. Epidermal cells are shallowly sinuate. Stomata are anomocytic. Mesophyll is differentiated in to 1-2 layers of adaxial palisade cells and 3 or 4 layers of

spongy cells. At places instead of two guard cells only one guard cell develops. Palisade is single layered. Spongy tissue is 5-6 layered. Cells some what horizontally stretched and chlorenchymatous. [1],[9-13]

(f) Madhyaśirā

Kośikā adhicarmākhyāḥ sulaghṇyo bharaṇotakāḥ.

Mṛdutakīyarūpā vai laghurālāpraṇālikāḥ.

Saṃvahanākhyapūlānāṃ samīpasthā bhavanti tāḥ.

Pūlārūta ekale kendras'dhyadhicarmākhyakośikam.

Laghudaṇḍākṛtau naike kaṇā atra bhavanti ca.

Iti sadāharermadhyaśirāntāracanoditā. (Saumitreya Mahodadhi: 29-31)

Midrib: Epidermal cells are small. Ground tissue is parenchymatous with few small resin canals neighbouring vascular bundles. Vascular bundle is single in centre. Cells of lower epidermis show presence of small rod shaped crystals. [1],[9-13]

(g) Cūrṇam

Sagranthīnitathā granthihīnāni vātra santi vai.

Tvacāromāṇi ranghrāṇi dṛśyante sūkṣmikāyane. (Saumitreya Mahodadhi: 32)

Powder

Powder contains glandular and non-glandular and stomata. [1],[9-13]

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Abbreviation

A.Hṛ.Ci.	:	AṣṭāṇgaHṛdayaCikitsā
A.Hṛ.U.	:	AṣṭāṇgaHṛdaya Uttara Tantra
As.Hr.Ut.	:	AṣṭāṇgaHṛdaya Uttara Tantra
Bhā.Pra.Ci.	:	BhāvaprakśaCikitsā
Śā.Sa.Ut.Kha.	:	ŚāraṇgadharaSamhitā Uttara Khaṇḍa
Ca.Ci.	:	Caraka SamhitāCikitsāSthāna
Ca.Da.Ci.	:	CakradattaCikitsā
Ca.Si.	:	Caraka Samhitā Siddhi Sthāna
Ca.Sū.	:	Caraka SamhitāSūtraSthāna
Ck.Dt.Ci.	:	CakradattaCikitsā
Ga.Ni.Kā.Ci.	:	GadanigrahaKāyacikitsā
Ga.Ni.Kau.Taṃ.	:	GadanigrahaKaumaryatantra
Ga.Ni.Śā.Ta.	:	GadanigrahaŚālākya Tantra
Ga.Ni.Śa.Taṃ.	:	GadanigrahaŚalyatantra
Hā.Sa.	:	HārāitaSamhitā
Hā.Saṃ.	:	HārāitaSamhitā
Kā.Saṃ.Khilasthāna	:	KāśayapaSamhitāKhilasthāna
Rā.Mā.	:	RājaMārtaṇḍa
Ra.Ra.Sa.	:	Rasa Ratna Samuccaya
Śā.Ni.Guḍūcyādivarga	:	ŚāligrāmaNighaṇṭuGuḍūcyādivarga
Śā.Sa.U.Kha.	:	ŚāraṇgadharaSamhitā Uttara Khaṇḍa
Śā.Saṃ.Ma.Kha.	:	ŚāraṇgadharaSamhitā Madhyam Khaṇḍa
Sd.Bh.Mm. Ch. Gr.Ci.	:	Siddha BheṣajaMaṇimālāCaturthaGucchaGrahaṇāiCikitsā

Sd.Bh.Mm. Ch. Nd.Vr.Ci.	:	Siddha BheṣajaMaṇimālāCaturthaGuccha Nāḍīvraṇa Cikitsā
Sd.Bh.Mm. Ch.Nt.Rg.	:	Siddha BheṣajaMaṇimālāCaturthaGuccha Netra RogaCikitsā
Sd.Bh.Mm.Ch. Ars.Ci.	:	Siddha BheṣajaMaṇimālāCaturthaGucchaArśaCikitsā
Sd.Bh.Mm.Ch. Vrn.Ci.	:	Siddha BheṣajaMaṇimālāCaturthaGucchaVraṇaCikitsā
Sh.Gr.Ng. Gd.Vg.	:	ŚāligrāmaNighaṇṭuGuḍūcyādivarga
Su.Ci.	:	SuśrutaSamhitāCikitsāSthāna
Su.Ka.	:	SuśrutaSamhitā Kalpa Sthāna
Su.Śā.	:	SuśrutaSamhitāSārīraSthāna
Su.Ut.	:	SuśrutaSamhitā Uttara Tantra
Vai.Jī. Caturthavilāsa	:	Vaidya JāivanamCaturthavilāsa
Vai.Jī. Dvitīyavilāsaḥ	:	Vaidya JāivanamDvitīyavilāsaḥ
Vai.Ma.	:	Vaidya Manoramā
Vaṃ. Se. Āmavātarogādhikāraḥ	:	VaṃgasenaSamhitāĀmavātarogādhikāraḥ
Vaṃ.Se. Vātavyādhyidhikāraḥ	:	VaṃgasenaSamhitāVātavyādhyidhikāraḥ
Vaṃ.Se.Karṇarogaḥ	:	VaṃgasenaSamhitāKarṇarogādhikāraḥ
Vaṃ.Se.Medarogaḥ	:	VaṃgasenaSamhitāMedorogādhikāraḥ
Vaṃ.Se. Netrarog	:	VaṃgasenaSamhitāNetrarogādhikāra
Vaṃ.Se. Śothādhikāraḥ	:	VaṃgasenaSamhitāŚothādhikāraḥ
Vaṃ.Se. Strīrogaḥ	:	VaṃgasenaSamhitāStrīrogaḥ
Vaṃ.Se.Vātavyādhiḥ	:	VaṃgasenaSamhitāVātavyādhyidhikāraḥ
Vṛ.Mā.	:	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 depend 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^2 (Rahaman, 2009a). This basin is the most populated area in the world with an average density of 520 persons/km^2 (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 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

Hoogly 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^3/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 468.7×10^9 m^3 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. Since ancient times, the indigenous people have utilized the river for both water and medicinal purposes.

Among all the medicinal plants, *Tridax procumbens* L. was considered in this study. It is a perennial herb distributed throughout the tropical and subtropical world. It is commonly known as "Coat Button", "Tridax Daisy" and "Mexican Daisy". In Sanskrit "Jayanti Veda", in Hindi "Khal muriya", "Ghamra", "Vettu kaaya". Various parts of this plant, such as the seeds, roots, leaves, and stems, are used to treat a variety of illnesses due to its antioxidant, anti-hepatotoxic, analgesic, antidiabetic, anti-inflammatory, antifungal, anti-bacterial, and anti-cancer characteristics. It has diverse morphological and anatomical variations. On this basis, the current chapter is focused on the morphology, taxonomy, anatomy, and distribution assessment of *T. procumbens* around the Ganga River.

Genus *Tridax* L.

The genus *Tridax* is described by Linnaeus in 1753 in *Species Plantarum*. It belongs to the Galinsoginae subtribe of the Heliantheae tribe under family Asteraceae. The earlier workers such as Hoffmann (1894), De Candolle (1836), Bentham (1873), and Bentham and Hooker (1876) have included *Balduina*, *Bebbia,* *Blepharipappus*, *Calea*, *Dubautia*, *Galinsoga,* *Marshallia*, *Raillardia*, with *Tridax* in Galinsoginae. Later, additional taxa such as *Galinsoga trilobala* Car., 1794, *Ptilostephium coronopifolium* Kunth, 1820, and *Galinsogea balbisioides* Kunth, 1820, were described shortly which are now recognized as species of *Tridax*. From 1849 to 1895 a total of 12 taxa that were native to Mexico were described, among them, the first taxa was *T. bicolor* which was described by Gray in 1849. During the same period four South American species as *Mandonia boliviensis* Wedd., 1864; *Tridax angustifolia* Spruce ex Benth. & Hook., 1876; *T. stuebelii* Hieron., 1895; and *T. tambensis* Hieron., 1895 were described. From 1896 to 1965, nine more species were added to the genus (Powell, 1965). From 1965 to the present, a total of eleven additional taxa have been reported under this genus.

About thirty species, originally from tropical America, have been introduced into subtropical and somewhat temperate areas across the globe. *T. procumbens* is the type species of this genus. *T. accedens*, *T. angustifolia*, *T. bicolor*, *T. dubia*, *T. erecta*, *T. procumbens*, *T. rosea*, and *T. serboana* are some of the significant medicinal species in the genus (Powell, 1965; Wuhua and Pepple, 2020). *Tridax* is dispensed as "Bhringraj" and is used to treat a variety of illnesses in the Ayurvedic medical system. The plant is being used as a liver disorder remedy by several Ayurvedic practitioners (Saxena and Albert, 2005). *Tridax* grows on waste grounds, dikes, dunes, railroads, riverbanks, roadsides, and waste grounds throughout India. It is a common, significant weed with spreading stems and abundant seed production (Chauhan et al., 2008).

Etymology

The genus belongs to the family "Asteraceae" which is based on the genus "*Tridax*" derived from the Greek word *tridaknos* which means 'thrice-bitten, eaten-in-three-bites' (Gledhill, 2008). The genus name "*Tridax*" is derived from the Latin word '*thridax*' which means 'a kind of wild lettuce' (Quattrocchi, 2000); the Latin word '*tridacna*' means 'a kind of oysters' (Patil, 2007).

Habitat and Distribution

The genus *Tridax* is a herb, native to tropical America and introduced to subtropical and mild temperate regions worldwide. Currently, according to the Plants of the World Online (POWO, 2024) Database, it has 34 accepted species including both

medicinal and ornamental that are native to Argentina Northeast, Argentina Northwest, Aruba, Bahamas, Belize, Bolivia, Brazil South, Cayman Is., Colombia, Costa Rica, Cuba, Dominican Republic, Ecuador, El Salvador, French Guiana, Guatemala, Guyana, Haiti, Jamaica, Leeward Is., Mexico Central, Mexico Gulf, Mexico Northeast, Mexico Northwest, Mexico Southeast, Mexico Southwest, Netherlands Antilles, Nicaragua, Panamá, Peru, Puerto Rico, Trinidad-Tobago, Venezuela, Windward Is; and introduced in Andaman Is., Angola, Assam, Bangladesh, Benin, Botswana, Burkina, Cambodia, Cameroon, Cape Verde, Caroline Is., Chad, Chagos Archipelago, China Southeast, Christmas Is., Cocos (Keeling) Is., Comoros, Congo, East Himalaya, Eritrea, Ethiopia, Fiji, Florida, Gabon, Gilbert Is., Guinea, Gulf of Guinea Is., Hainan, Hawaii, India, Ivory Coast, Japan, Kazan-retto, KwaZulu-Natal, Laccadive Is., Laos, Line Is., Madagascar, Malaya, Maldives, Mali, Marianas, Marquesas, Marshall Is., Mauritius, Mozambique, Myanmar, Nauru, Nepal, New Caledonia, New Guinea, New South Wales, Nicobar Is., Nigeria, Northern Provinces, Northern Territory, Ogasawara-shoto, Pakistan, Philippines, Phoenix Is., Queensland, Rodrigues, Réunion, Samoa, Seychelles, Society Is., South China Sea, Sri Lanka, Sudan, Swaziland, Taiwan, Texas, Thailand, Togo, Vanuatu, Vietnam, Western Australia, Yemen, Zambia, Zaïre, Zimbabwe (Fig. 1)

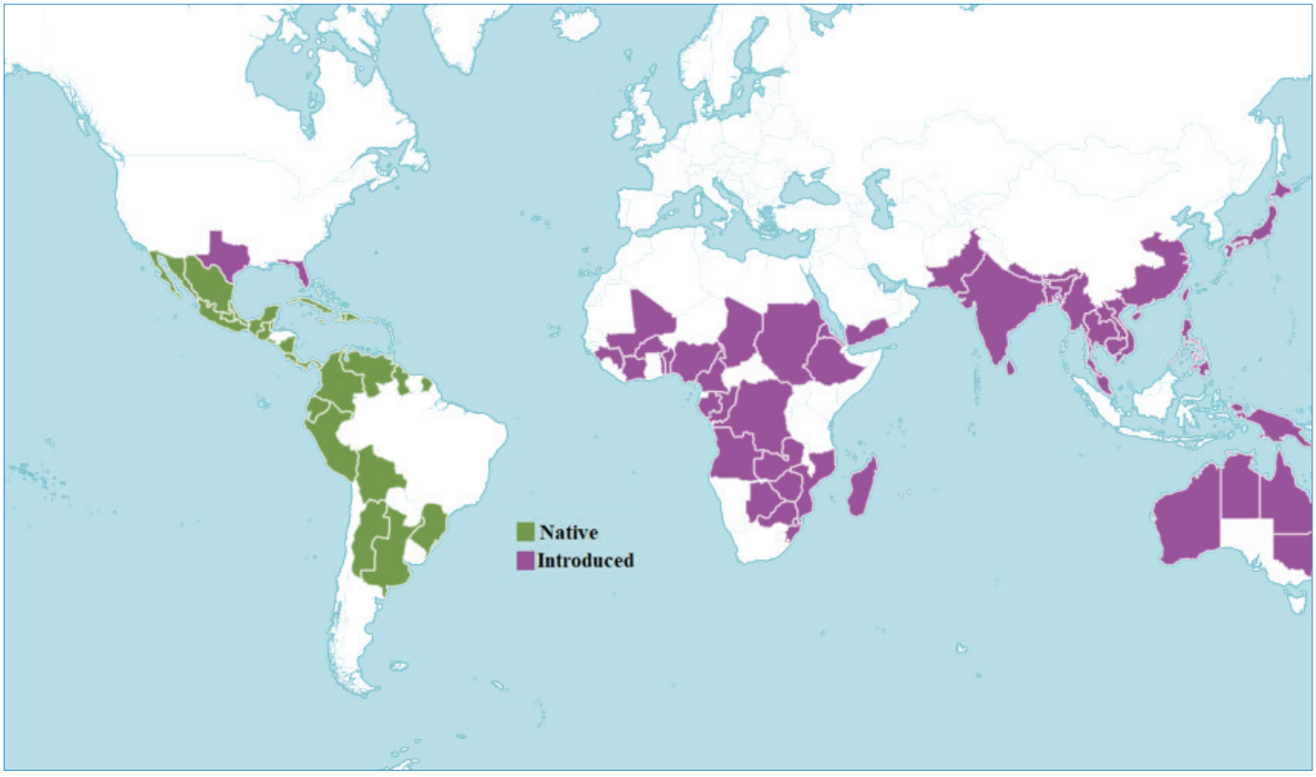

Fig. 1 Global distribution of genus *Tridax*

Botanical Characteristics

Herb, annual or perennial. Stem erect, slender, procumbent, or decumbent. Leaves opposite rarely alternate, simple or trilobed to pinnately lobed or divided; lamina ovate to linear-lanceolate, cuneate, or obtuse to subtruncated and (sometimes) decurrent at the base, margin entire, serrate, or dentate. Inflorescence solitary or a few-to many-headed cymose panicle; heads discoid or radiate, subcampanulate to broadly campanulate; peduncle on slender or stout, elongate; involucre subuniseriate or 2-3 seriate; bract 4-5, subequal, imbricated, greenish, usually purple to purple-tinged; inner bract searious-purple margin; receptacle short- or long-conical to convex or nearly flattened. Ray flower pistillate, tubular at base, with inconspicuous to conspicuous white, yellow, roseate, or purplish ligules; ligules obscurely or conspicuously bilabiate, external lip obscurely to conspicuously (2-)3(4)-lobed; pappus of the ray florets reduced. Disc florets with regular corolla yellow or less often whitish, partially cyanic, or greenish yellow; lobes equal, sublanceolate, acute or obtuse, erect to reflexed or involute at the apex. Style branches recurved to revolute, slender, and subterete or flattened on the inner surface. Fruits achene, turbinate or narrowly obconical to subcylindrical, terete to ridged, glabrous to densely pubescent.

Species

Genus *Tridax* is known to contain a total of 34 taxonomically accepted species (POWO, 2024) that are as-

1. *T. angustifolia* Spruce ex Benth. & Hook.f in Gen. Pl. 2: 392 (1873)
2. *T. balbisioides* (Kunth) A.Gray in Proc. Amer. Acad. Arts 15: 39 (1880)
3. *T. bicolor* A.Gray in Mem. Amer. Acad. Arts, n.s., 4(1): 104 (1849)
4. *T. bilabiata* A.M.Powell in S. W. Naturalist 8: 178 (1963)
5. *T. boliviensis* R.E.Fr in Ark. Bot. 5(13): 21 (1906)
6. *T. brachylepis* Hemsl in Biol. Cent. -Amer., Bot. 2: 207 (1881)
7. *T. cajamarcensis* H.Rob in Phytologia 82: 61 (1997)
8. *T. candidissima* A.Gray in Proc. Amer. Acad. Arts 15: 39 (1880)
9. *T. coronopifolia* (Kunth) Hemsl in Biol. Cent. -Amer., Bot. 2: 207 (1881)
10. *T. dubia* Rose in Contr. U.S. Natl. Herb. 1: 337 (1895)
11. *T. durangensis* García Arév in Acta Bot. Mex. 12: 9 (1990)
12. *T. erecta* A.Gray in Proc. Amer. Acad. Arts 21: 396 (1886)
13. *T. hintonii* (B.L.Turner & A.M.Powell) D.J.Keil, Luckow & Pinkava in Madroño 34: 357 (1987)
14. *T. hintoniorum* B.L.Turner in Phytologia 73: 350 (1992)

15. *T. luisana* Brandegee in Univ. Calif. Publ. Bot. 3: 392 (1909)
16. *T. mexicana* A.M.Powell in S. W. Naturalist 8: 177 (1963)
17. *T. moorei* H.Rob in Phytologia 44: 430 (1979)
18. *T. oaxacana* B.L.Turner in Phytologia 65: 139 (1988)
19. *T. obovata* Turcz in Bull. Soc. Imp. Naturalistes Moscou 24(I): 187 (1851)
20. *T. palmeri* A.Gray in Proc. Amer. Acad. Arts 15: 38 (1880)
21. *T. paneroi* B.L.Turner in Phytologia 94: 169 (2012)
22. *T. peruviensis* A.M.Powell in S. W. Naturalist 8: 178 (1963)
23. *T. petrophila* B.L.Rob. & Greenm in Proc. Amer. Acad. Arts 32: 5 (1896)
24. *T. platyphylla* B.L.Rob in Proc. Amer. Acad. Arts 43: 41 (1907)
25. *T. procumbens* L in Sp. Pl.: 900 (1753)
26. *T. purpurea* S.F.Blake in Brittonia 2: 351 (1937)
27. *T. purpusii* Brandegee in Univ. Calif. Publ. Bot. 4: 388 (1913)
28. *T. rosea* Sch.Bip in Proc. Amer. Acad. Arts 32: 6 (1896)
29. *T. serboana* B.L.Turner in Phytologia 90: 160 (2008)
30. *T. stuebelii* Hieron in Bot. Jahrb. Syst. 21: 351 (1895)
31. *T. tamaulipana* B.L.Turner in Phytologia Mem. 26a: 24 (2016)
32. *T. tenuifolia* Rose in Contr. U.S. Natl. Herb. 3: 319 (1895)
33. *T. trilobata* Hemsl in Biol. Cent. -Amer., Bot. 2: 208 (1881)
34. *T. yecorana* B.L.Turner in Phytologia 79: 286 (1996)

The recent database of World Flora Online (WFO, 2024) mentioned a total of 33 accepted species as-

1. *T. angustifolia* Spruce ex Benth. & Hook.f. in Gen. Pl. 2(1): 392 (1873)
2. *T. balbisioides* A. Gray in Proc. Amer. Acad. Arts 15: 39. 1880
3. *T. bicolor* A. Gray in Mem. Amer. Acad. Arts, ser. 2, 4(1): 104 (1849)
4. *T. bilabiata* A.M. Powell in S. W. Naturalist 8: 178 (1963)
5. *T. boliviensis* R.E. Fr in Ark. Bot. 5, n.° 13: 21 (1906)
6. *T. brachylepis* Hemsl in Biol. Cent. -Amer., Bot. 2: 207 (1881)
7. *T. cajamarcensis* H. Rob in Phytologia 82: 61 (1997)
8. *T. candidissima* A. Gray in Proc. Amer. Acad. Arts xv. (1880) 39.
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10. *T. dubia* Rose in Contr. U.S. Natl. Herb. 1: 337, pl. 33 (1895)
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Tridax procumbens L.

It is commonly known as a 'Coat Button' or 'Mexican Daisy' in English; while in Ayurveda it is known as 'Jayanti', in Siddha/Tamil as 'Vettukkaaya-thalai' and folk as 'Akala kohadi' (Christudas et al., 2012). It is native to equatorial America and adopted in equatorial India, Australia, Africa, and Asia. It is commonly found in dunes, dykes, railroads, roadsides, riverbanks, meadows, and waste grounds. It produces three-toothed ray floret with white and yellow flowers, and it has produced approximately 1500 achenes per plant, each one can catch the wind in its pappus, and carried to short distance (Choudhary and Panwar, 2018).

Different substances such as oils, teas, and skin poultices, among others, have been manufactured using this species (Beck et al., 2018). Ayurveda has been using this species since ancient times (Kethamakka and Deogade, 2014). In Ayurveda, it is used as a herbal plant to treat wounds, and liver disorders and used in hair growth (Pandey and Tripathi, 2014; Amutha et al., 2019; Pawar and Jawal, 2023). It has been used for different medicinal purposes like anemia, cold, inflammation, stomach pain, diarrhea, skin infections, wounds, bleeding, gastrointestinal and respiratory infections, high blood pressure, diabetes, malaria, leishmaniasis,

and dysentery (Beck et al., 2018). It has many pharmacological activities like antidiabetic, hepatoprotective, immunomodulatory, wound healing, antileishminicidal, anti-inflammatory, antifungal, antibacterial, hemostatic, hypotensive, anti-arthritic, defluoridation, etc. (Pawar and Jawal, 2023). It is rich in chemicals that have been isolated from this are flavonoids, terpenoids, lipids, polysaccharides, quercetin, isoquercetin, fumaric acid, centaureidin, luteolin, ß-sitosterol and puerarine (Amutha et al., 2019).

Classification

Kingdom	-	Plantae
Subkingdom	-	Tracheobionta
Super Division	-	Spermatophyta
Division	-	Magnoliophyta
Class	-	Magnoliopsida
Subclass	-	Asteridae
Order	-	Asterales
Family	-	Asteraceae
Genus	-	*Tridax*
Species	-	*procumbens*

Common Names

Coat buttons, Tridax Daisy, Mexican Daisy (English); Tridhara, Bishalya Karani (Bengali); Khal muriya, Ghamra, Akal Kohadi, Tal-muriya (Hindi); Jayanti Veda (Sanskrit); Bishalya Karani (Oriya); Gaddi Chemanthi (Marathi); Vettukaya thalai, Thatha (Tamil); and others Bhamburda, Dagdipala, Ekdandi, Tun-tuni.

Global Distribution

The plant *T. procumbens* abundantly found throughout the world mainly in tropical and subtropical regions. According to POWO (2024), it is native to Argentina Northeast, Argentina Northwest, Aruba, Bahamas, Belize, Bolivia, Brazil South, Cayman Is., Colombia, Costa Rica, Cuba, Dominican Republic, Ecuador, French Guiana, Guyana, Haiti, Jamaica, Leeward Is., Mexico Central, Mexico Gulf, Mexico Northeast, Mexico Northwest, Mexico Southeast, Mexico Southwest, Netherlands Antilles, Nicaragua, Panamá, Peru, Puerto Rico, Trinidad-Tobago, Venezuela, Windward Is; and introduced into Andaman Is., Angola, Assam, Bangladesh, Benin, Botswana, Burkina, Cambodia, Cameroon, Cape Verde, Caroline Is., Chad, Chagos Archipelago, China Southeast, Christmas Is., Cocos (Keeling) Is., Comoros, Congo, East Himalaya, Eritrea, Ethiopia, Fiji, Florida, Gabon, Gilbert Is., Guinea, Gulf of Guinea Is., Hainan, Hawaii, India, Ivory Coast, Japan, Kazan-retto, KwaZulu-Natal, Laccadive Is., Laos, Line Is., Madagascar, Malaya, Maldives, Mali, Marianas, Marquesas, Marshall Is., Mauritius, Mozambique, Myanmar, Nauru, Nepal, New Caledonia, New Guinea, New South Wales, Nicobar Is., Nigeria, Northern Provinces, Northern Territory, Ogasawara-shoto, Pakistan, Philippines, Phoenix Is., Queensland, Rodrigues, Réunion, Samoa, Seychelles, Society Is., South China Sea, Sri Lanka, Sudan, Swaziland, Taiwan, Texas, Thailand, Togo, Vanuatu, Vietnam, Western Australia, Yemen, Zambia, Zaïre, Zimbabwe (Fig. 2).

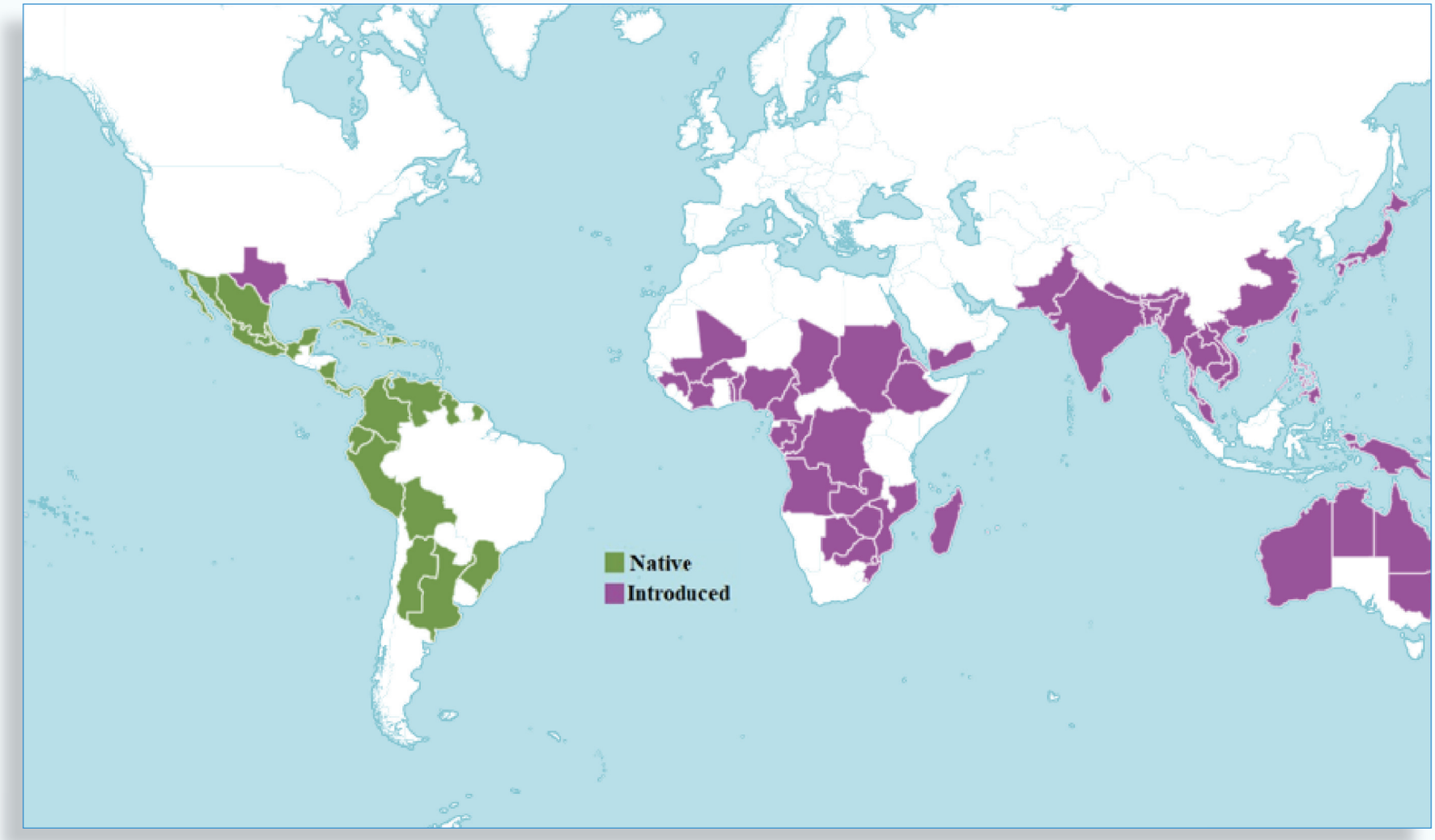

Fig. 2 Global distribution of *T. procumbens*

Distribution in India

This plant is widely distributed in India up to 2400 m above sea level. It is reported from Andhra Pradesh, Uttar Pradesh, Jharkhand, Rajasthan, Maharashtra, Madhya Pradesh, and Chhattisgarh or other Indian states (Gubbiveeranna and Nagaraju, 2016; Singh, 2022; Zade et al., 2023). It is also grown in roadsides, pastures, dykes, fallow ground, riverbanks, meadows, dunes, and waste grounds in tropical and subtropical regions (Wuhua and Pepple 2020; Mnaje et al., 2022).

Ethnopharmacology and Traditional Uses

T. procumbens is a worldwide weed and a flowering plant that contains numerous medicinal values. In India, traditionally it has been used as an anticoagulant, anti-microbial, insect repellent, and as a wound healing agent. It has been used for the treatment of bronchial catarrh, malaria, stomachache, diarrhea, epilepsy, diabetes, high blood pressure, hemorrhage, and liver problems, and as a hair tonic. A decoction of the leaves of this plant is used as an ethnomedicine to treat infectious skin diseases. It is broadly used in wound healing to stop hemorrhage from cuts, bruises, and wounds. *T. procumbens* controls high blood pressure and blood glucose levels as well as dysentery and diarrhea (Kethamakka and Deogade, 2014; Ghosh et al., 2019). Kidney and bladder stones can be removed by taking this plant juice orally (Sailaja et al., 2011).

The leaves of this plant are used by rural physicians and tribal people in West Africa to treat conjunctivitis. This herb is used to treat severe dysentery and diarrhea, as well as to regulate blood pressure and blood sugar levels (Mnaje et al. 2022). This herb was utilized orally and topically by indigenous populations across several nations for the treatment of burns, cutaneous wounds, and wound healing (Maurya and Verma, 2021). In rural parts of the world, it is one of the most widely utilized treatments for bacterial illnesses (Kakadiya and Patel, 2022). Many components of the plants exhibit anti-bleeding, anti-hypertensive, anti-dysentery, and anti-epilepsy characteristics; also used to manage metabolic syndrome (Baile and Parmar, 2023).

This plant's leaf juice is applied directly to wounds to promote their healing, and folk remedies employ leaf extracts to treat infectious skin conditions. This herb is utilized by traditional healers in some regions of India to treat wounds, boils, and blisters (Chaudhari et al., 2018). In addition to treating vaginitis, stomach discomfort, diarrhea, mucosal infections, and skin infections, it also has antibacterial, antifungal, and antiviral properties. The entire plant is used to treat typhoid, back pain, and fever. A decoction of leaves is used to treat abdominal and gastrointestinal mycosis, as well as discomfort associated with malaria (Pawar and Jawal, 2023). In addition to treating diabetes, high blood pressure, and gastrointestinal and respiratory diseases, seeds are also utilized

to manage bleeding (Chaudhari and Patil, 2022).

This plant, which possesses a variety of medicinal qualities, has been utilized for ages in the Ayurvedic system (Kaushik et al., 2020; Singh, 2022). *T. procumbens* has been widely utilized in the Ayurvedic system as a medication for heartburn, gastritis, and liver problems. Additionally, this herb is utilized as a respiratory remedy. This medicinal plant is also employed by the ethnic system to cure jaundice and liver issues (Ghosh et al., 2019). Numerous illnesses, including blood pressure, diarrhea, bronchial catarrh, malaria, dysentery, stomachache, headache, and hair loss, can be effectively treated with it. There have been reports of antiseptic, parasiticidal, and insecticidal qualities in flowers and leaves (Ahmed et al., 2019).

Selection of the Sites and Characteristics Studied

The distribution, morphological variations, and association of *T. procumbens* 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 10 km on both sides of each site. The plants were identified and deposited in the Patanjali Research Foundation Herbarium with an acronym of PRFH for future records. A bulk sample of the plant was taken from each site in a large polybag which was deposited in the analytical laboratory for phytochemical profiling.

Table 1 Different studied sites and their GPS coordinates along with *T. procumbens* 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	Present
S5	Haridwar	314	29.97	78.17	Present
S6	Bijnor	225	29.37	78.13	Present
S7	Narora	174	28.20	78.38	Absent
S8	Budaun	164	28.05	79.12	Present
S9	Farrukhabad	151	27.37	79.63	Absent

Site	Locality	GPS Coordinates			Status
S10	Bithoor	126	26.61	80.27	Present
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	Absent
S17	Patna	53	25.61	85.14	Present
S18	Barh	47	25.48	85.72	Present
S19	Bahachouki	55	25.30	86.36	Absent
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	Absent
S23	Murshidabad	18	24.18	88.27	Absent
S24	Mayapur	11	23.43	88.39	Absent
S25	Hoogli	9	22.91	88.40	Present
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 sites 4, 5, 6, 8, 10, 11, 12, 13, 14, 15, 17, 18, 20, 21, and 25 due to these sites being the source of its bulk collection. In the remaining sites 1, 2, 3, 7, 9, 16, 19, 22, 23, 24, and 26, it was either absent or invisible.

Taxonomic Treatment

Tridax procumbens L. Sp. Pl.: 900. 1753; C.B. Clarke in Hook.f., Fl. Brit. India 3: 311. 1881; Duthie, Fl. Gangetic Plain (Repr. ed.) 1: 336. 1960; Haines, Bot. Bihar Orissa (Repr. ed.) 2: 510.1961; Uniyal & al., Fl. Pl. Uttarakhand, Checkl.: 161. 2007; Sinha et. al, Fl. Uttar Pradesh 1: 433. 2016.

Synonyms

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

Homotypic Synonym

Chrysanthemum procumbens (L.) Sessé & Moc. in Fl. Mexic.: 190 (1894)

Heterotypic Synonyms

1. *Amellus pedunculatus* Ortega ex Willd. in Enum. Pl. 2: 916 (1809)
2. *Balbisia canescens* Rich. ex Pers. in Syn. Pl. 2: 470 (1807)
3. *B. divaricata* Cass. in Opusc. Phytol. 3: 91 (1834)
4. *B. elongata* Willd. in Sp. Pl., ed. 4. 3: 2214 (1803)
5. *B. pedunculata* Hoffmanns. in Verz. Pfl. -Kult. 1: 228 (1824)
6. *Tridax procumbens* var. *canescens* (Rich. ex Pers.) DC. in Prodr. 5: 679 (1836)
7. *T. procumbens* var. *ovatifolia* B.L. Rob. & Greenm. in Proc. Amer. Acad. Arts 32: 7 (1896)

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

1. *Amellus pedunculatus* Ortega ex Willd in Enum. Pl. 2: 916 (1809)
2. *Balbisia canescens* Rich in Syn. Pl. 2: 470 (1807)
3. *B. canescens* Rich. ex Pers in 1807
4. *B. divaricata* Cass in Opusc. 3: 91.; ex DC. Prod. v. 679
5. *B. elongata* Willd in Sp. Pl., ed. 4 [Willdenow] 3(3): 2214. 1803 [Apr-Dec 1803]
6. *B. pedunculata* Hoffmanns in Verz. Pfl. -Kult. 228. 1824
7. *B. pedunculata* Ortega ex O. Hoffm in Verz. Pfl. -Kult. 1: 228 (1824)
8. *Chrysanthemum procumbens* (L.) Sessé & Moc in Fl. Mexic.: 190 (1894)
9. *Tridax procumbens* var. *canescens* (Rich. ex Pers.) DC in Prodr. 5: 679 (1836)
10. *T. procumbens* var. *ovatifolia* B.L. Rob. & Greenm in Proc. Amer. Acad. Arts 32: 7 (1896)
11. *T. procumbens* var. *procumbens*

Botanical Description

Herb, 15-50 cm long, annual or perennial. Stem procumbent, prostrate, or erect. Leaves simple, opposite; petiole up to 2 cm long; lamina 2-6 x 2-4 cm, oval-lanceolate, base cuneate to attenuate, margin serrate, coarsely and often deeply dentate, undulate, apex acute or acuminate, strigose-pubescent. Inflorescence solitary capitula 1-1.5 cm in diameter; peduncle 5-25 cm long, erect, hairy; phyllaries in 2-3 series, ovate and pilose, green; paleae 6-8 mm long, linear, pilose near apex. Involucre 2-3-seriate, 5-6 x 2-4 mm, ovate, acute to shortly acuminate, roughly hirsute-hispid. Receptacle with oblong, hairy scales. Ray florets 2-6, up to 4 mm long, pale yellow. Disk floret tubular, 5-dentate, yellow, tube up to 5 mm long, limb 5-lobed, lobes reflexed, hairy. Fruits achene, narrowly

obovoid, cylindric to narrowly obconical, truncate at the apex, obtuse or rounded at the base, 2-2.5 mm long, blackish. Pappus up to 3 mm long (Fig. 3A, B).

Fig. 3A Plant habit

Fig. 3B (a) Plant twig (b) Flower head (c) Ray floret (d) Disk floret (e) Stigma (f) Fruit with pappus

Anatomical Features

Structure of the Petiole

The epidermal cells on both surfaces are parenchymatous and stretched throughout the midrib's length when seen from the surface. The epidermal cells have many multicellular uniseriate trichomes. The midrib in the transverse section is covered in an adaxial cuticle that is bent outward and an abaxial cuticle, that is wavy. The morphology of the adaxial epidermal cells is polygonal. The abaxial epidermal cells are smaller than the adaxial epidermal cells in both size and form. Thin-walled parenchymatous and collenchyma cells comprise the cortex. The circular collenchyma cells have a thickness of 1-2 layers in the abaxial direction and 3-5 layers in the adaxial direction. The adaxial and abaxial sides of the parenchyma cells are stacked four to five times. They have uneven, spherical shapes and thin walls. The vascular bundle is closed type, collateral, and has a crescent form (Fig. 4).

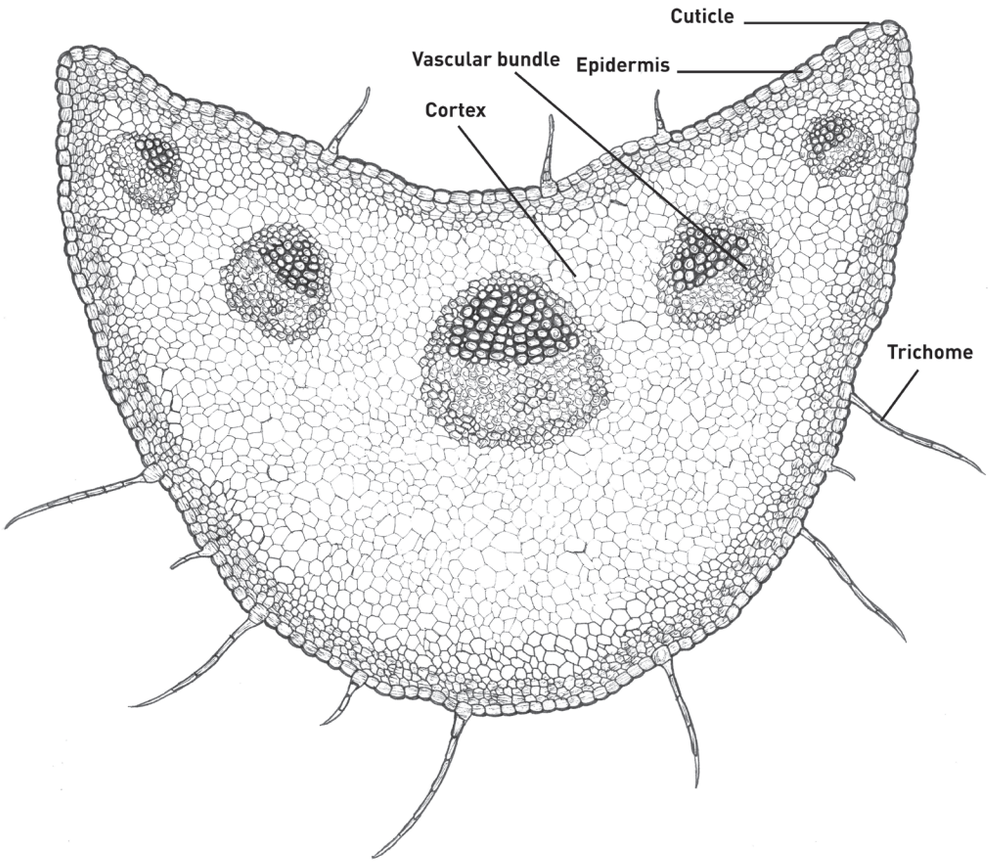

Fig. 4 TS of petiole

Structure of the Leaf

The epidermal cells on both sides have trichomes and are parenchymatous, with thin walls. Compared to the adaxial surface, the abaxial surface's cells are wavier. The lower epidermis has an abundance of anomocytic stomata. The cuticle found on the adaxial surface of the transverse section is thicker than that found on the abaxial surface. The adaxial epidermal cells had a form that ranged from barrel to rectangle. Compared to the upper epidermal cells, the abaxial epidermal cells had a similar form and size. The mesophyll underwent differentiation into spongy and palisade cells. Many chloroplasts are stacked within the palisade cells, located on the adaxial side. On the abaxial side, the spongy parenchyma cells were arranged in 5-6 layers.

The adaxial cuticle of the midrib is slightly bent outward, whereas the abaxial cuticle is wavy. The morphology of the adaxial epidermal cells is polygonal. The abaxial epidermal cells are smaller than the adaxial epidermal cells in both size and form. Thin-walled parenchymatous and collenchyma cells comprise the cortex. The collenchyma cells have a thickness of 1-2 layers in the abaxial surface and 3-5 layers in the adaxial surface. They have a rounded form. The adaxial and abaxial sides of the parenchyma cells are stacked four to five times. They have irregularly shaped shapes and thin walls. The vascular bundle is closed type, collateral, and has a crescent form.

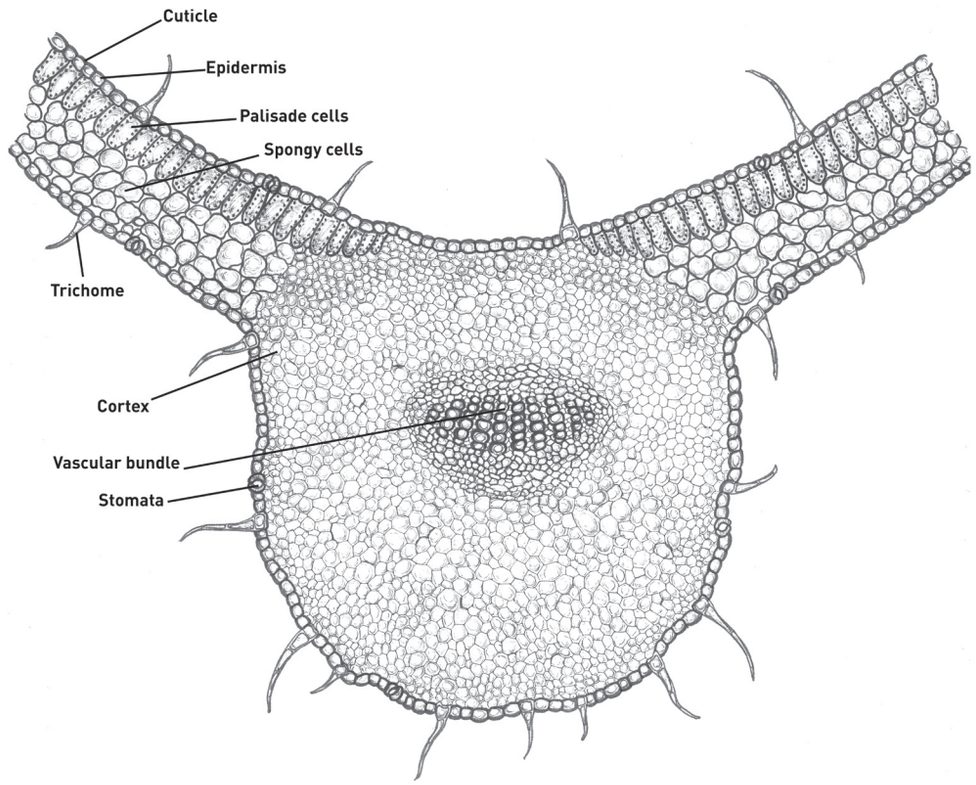

Fig. 5 TS of leaf

Structure of the Stem

The stem has a round shape in the transverse section. The epidermis is spherical, has one layer, and comprises parenchymatous cells. There are granular and multicellular trichomes. The collenchyma comprises 1-3 layers of rounded to polygonal cells and is located next to the epidermis. There are three to four layers of spherical chlorenchyma cells directly beneath the collenchyma. Patches of parenchyma and sclerenchyma with thick walls make up the pericycle. The endodermis is barely noticeable. Vascular bundles create a discontinuous ring of five to seven bundles, with parenchymatous cells interrupted in between; xylem oriented inward and phloem outward, metaxylem outward, collateral, and closed. Large parenchymatous cells comprise the middle pith (Fig. 6).

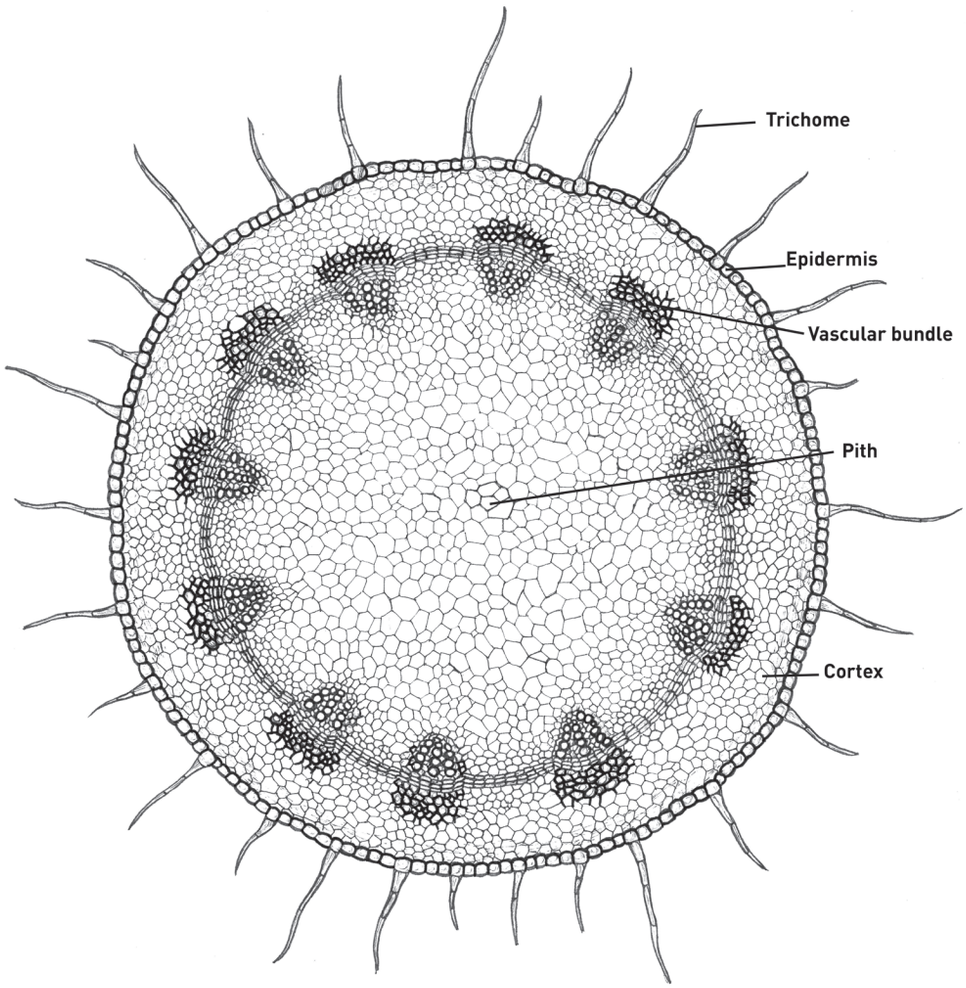

Fig. 6 TS of stem

Structure of the Root

There is a superficial layer of epiblema on the rounded transverse section of the main root. The cortex, which is made up of 4-5 layers of parenchymatous cells with thin walls, is the epiblema. The radial organization of the xylem and phloem is in distinct bundles. The metaxylem forms at the centre of the root, but the protoxylem is often exarch. Between the protoxylem groups are clusters of phloem cells. Hexarchic vascular bundles are present. The mature or secondary root has a circular shape. Phellem, Phellogen, and Phelloderm are the three regions that make up periderm. Phellem, also known as cork cells, are small, and rectangular in form. Phellogen, also known as the cork cambium, is made up of parenchymatous cells with thin walls that are polygonal in form. These cells are known as the phelloderm or secondary cortex. Encircling the xylem in a little bundle is the phloem. There are one or two layers of vascular cambium underneath the secondary phloem. The xylem tissue, which makes up the secondary root's core cylinder, is polygonal and rectangular with pits around it. Tracheids, fibres, and xylem parenchyma are all present (Fig. 7).

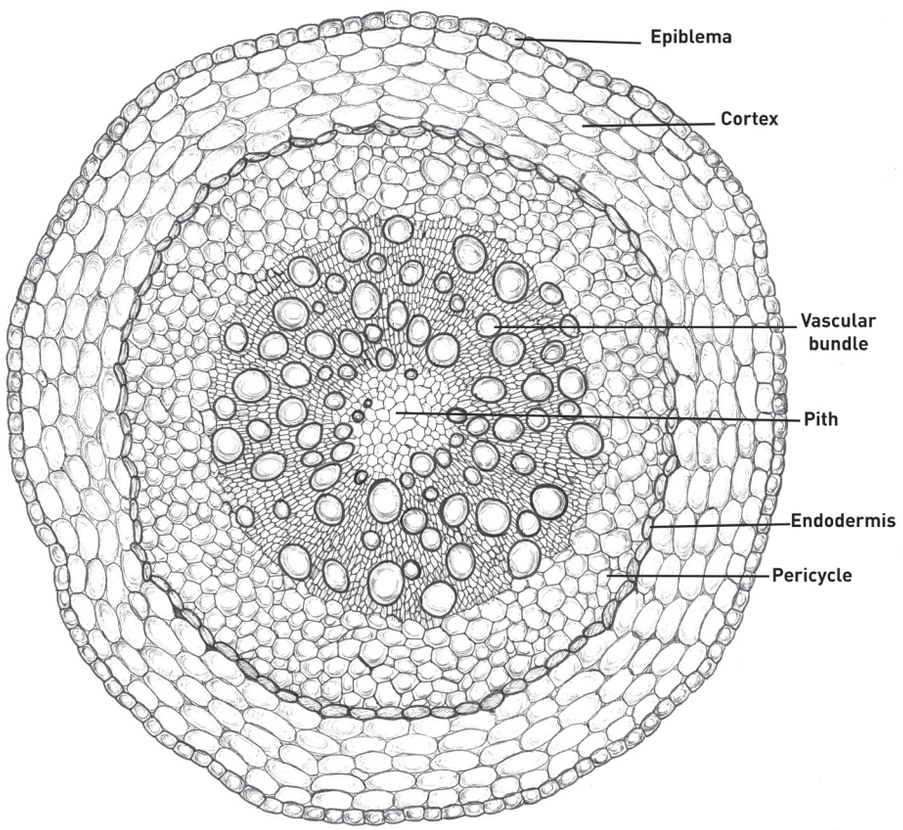

Fig. 7 TS of root

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CHAPTER

02

Bibliometric Analysis

INTRODUCTION

Tridax procumbens L., a member of the Asteraceae family, is a renowned Ayurvedic herb native to Asia, boasting a rich history of traditional utilization. *T. procumbens*, commonly known as 'coat buttons' or 'tridax daisy', is an essential plant found worldwide, valued for its medicinal properties and resilience. Commonly, it is widely distributed across regions such as Maharashtra, Madhya Pradesh, Gujarat, and Odisha in India, this species thrives in diverse habitats including open spaces, roadsides, meadows, croplands, and lawns (Mir et al., 2017). Since ancient times, it has been employed to treat various ailments. In India, it serves not only as medicine but also as a source of both food and traditional remedies (Beck et al., 2018). It is considered important since ancient times and has been employed in the treatment of liver disorders, boils, blisters, cuts, wounds, and various skin ailments, as well as for its hemostatic properties (Kaushik et al., 2020; Ingole et al., 2022). Extensive investigations into the phytochemical composition and pharmacological characteristics of *T. procumbens* have showed an accumulation of bioactive constituents, including flavonoids, essential oils, saponins, tannins, steroids, alkaloids, carbohydrates, carotenoids, and terpenoids (Kaushik et al., 2020). These chemical compounds support the various pharmacological activities exhibited by the plant, encompassing anthelmintic, antimicrobial, antiseptic, cardiovascular, insecticidal (Ingole et al., 2022), anticancer (Syed et al., 2020), antibacterial, antihyperuricemic, antioxidant (Andriana et al., 2019), anti-inflammatory (Berlin Grace et al., 2020), antileishmanial (Martín et al., 2009), larvicidal (Kamaraj et al., 2011), vasorelaxant (Salahdeen et al., 2012), and wound healing (Singh et al., 2016; Shrivastav et al., 2020). These multifunctional attributes underscore the therapeutic potential and significance of *T. procumbens* in traditional and contemporary medicine alike.

Bibliometric analysis is a powerful tool for mapping and understanding the research landscape of specific topics, and it has been increasingly applied to study the scientific literature surrounding *Tridax procumbens* (Janik et al., 2020). Through bibliometric analysis, researchers can quantitatively assess trends, identify key publications, influential researchers, prolific institutions, and the geographical distribution of

research outputs (Ali et al., 2023). This method not only highlights the evolving focus areas within the study of *T. procumbens* but also helps in finding collaborative networks and emerging research fronts. By systematically analyzing publication data, citations, and other scholarly metrics, bibliometric analysis provides a comprehensive overview of the academic contributions and the developmental trajectory of research concerning *T. procumbens*, thereby facilitating informed decision-making and strategic planning for future studies in this field.

The objective of this bibliometric analysis is to systematically evaluate the research landscape surrounding *Tridax procumbens*, a plant of significant medicinal and ecological interest. By analyzing publication patterns, citation trends, and the collaboration networks of researchers, this study aims to identify key research themes, influential studies, and prominent authors in the field. For this systematic assessment, several databases were employed to gather extensive data on peer-reviewed articles, conference papers, and reviews, enabling the mapping of research topic evolution over time, assessment of the geographical distribution of research output, and identification of high-impact journals and institutions. Ultimately, the goal is to provide a detailed overview of the scientific contributions related to *T. procumbens*, highlight gaps in the existing literature, and suggest potential directions for future research activities.

Insights with Data Source and Tools

The study utilized bibliometric analysis to explore scholarly research on *T. procumbens*, aiming to reveal its breadth, influence, and evolving trends. By systematically gathering articles from Dimensions.ai using the search term "*Tridax procumbens*" the study covered publications from 2000 to 2023. This approach ensures a comprehensive understanding of academic discourse on the plant species. The integration of analytical tools like Lens.org and VosViewer enhances the scrutiny by providing insights into thematic areas and visual representations of scholarly dynamics. Microsoft Excel was employed in managing and processing the dataset, ensuring it was organized for analysis. Lens.org and VosViewer (Version 1.6.19) were used for bibliometric insights, known for their effectiveness (Yu et al., 2020; Hajkowicz et al., 2023). Lens.org facilitated a thorough examination of *T. procumbens* research fields, revealing key thematic realms. This exploration illuminated the diverse nature of research on the plant species, enhancing its scientific understanding. VosViewer played a pivotal role in generating visual depictions

of bibliographic data, including co-authorship networks, keyword co-occurrence maps, and citation analyses, thereby providing visual elucidations of relationships and impacts within the academic community studying *T. procumbens*. The analysis revealed remarkable trends and patterns in *T. procumbens* research, furnishing invaluable insights into its evolutionary trajectory and scholarly significance, emphasizing its burgeoning importance in academic literature.

Comprehensive Data-driven Insights

The extensive analysis of scholarly publications spanning from 2000 to 2023 offered valuable insights into the global research landscape surrounding *T. procumbens*. Over the course of this period, a total of 583 scholarly articles were published, drawing upon the expertise of 2120 authors from 451 organizations representing 43 countries. Among the authors, 43 individuals stood out for their independent contributions, indicating a deep understanding of the subject matter and significant advancements in the field. The dissemination of research findings through 426 esteemed journals underscored the widespread interest and esteem for *T. procumbens* studies within the scientific community. Furthermore, the substantial average citation rate of 11.05 citations per document addressed the impact and recognition of the research's quality and relevance. The scope of *T. procumbens* research had expanded significantly, encompassing various disciplines including biology, traditional medicine, chemistry, medicine, botany, phytochemical, medicinal plants, and pharmacology. This interdisciplinary approach highlighted the plant's multifaceted applications and emphasized the broad interest in understanding its properties and benefits. Moreover, the data revealed the existence of robust collaborative networks among researchers and organizations, which facilitated knowledge exchange, resource sharing, and collective problem-solving. These collaborations were involved in advancing knowledge and fostering innovation in *T. procumbens* research.

Temporal Evolution and Growth Analysis

A total of 583 documents were discovered after extensive searches across bibliographic databases via Dimensions.ai. These demonstrated a variety of study forms, including 565 articles, 12 chapters, 4 preprints, and 2 proceedings. The bibliometric analysis of the research landscape on *T. procumbens* revealed a remarkable and sustained growth in publication output from 2000 to 2023, highlighting the increasing scientific interest in this species. In the early years, from 2000 to 2004, research activity was relatively low and stable, with the number of annual publications fluctuating between 2 and 4. This period of modest output reflected an initial phase of

exploration and interest in *T. procumbens*. A shift occurred in 2005, with the number of publications reaching 10, signaling a growing recognition of the plant's potential and importance in various research fields. Following this, the number of publications experienced some variability but generally trended upwards, with significant increases in 2008 (11 publications) and 2010 (18 publications). These years marked the beginning of more concentrated research efforts. From 2011 onwards, the publication count increased more consistently. The number of publications rose steadily each year, from 19 in 2011 to 27 in 2016. This period indicated a phase of growing research interest and expanded investigation into the applications and properties of *T. procumbens*. The years 2017 to 2022 witnessed an even sharper increase in research output, with the highest number of publications, 72, in 2022 (Fig. 1). This rise emphasized the importance and expanding scope of research topics related to it, possibly driven by its recognized medicinal properties, ecological significance, and potential applications in various industries. In 2023, there was a slight decline in the number of publications; however, the overall trend remained one of significant growth. The fall could be attributed to various factors such as changes in research funding, publication cycles, or emerging competing areas of interest. However, the long-term trajectory indicated a robust and accelerating interest in *T. procumbens*, reflecting its growing prominence and the scientific community's increasing investment in understanding and utilizing this versatile plant.

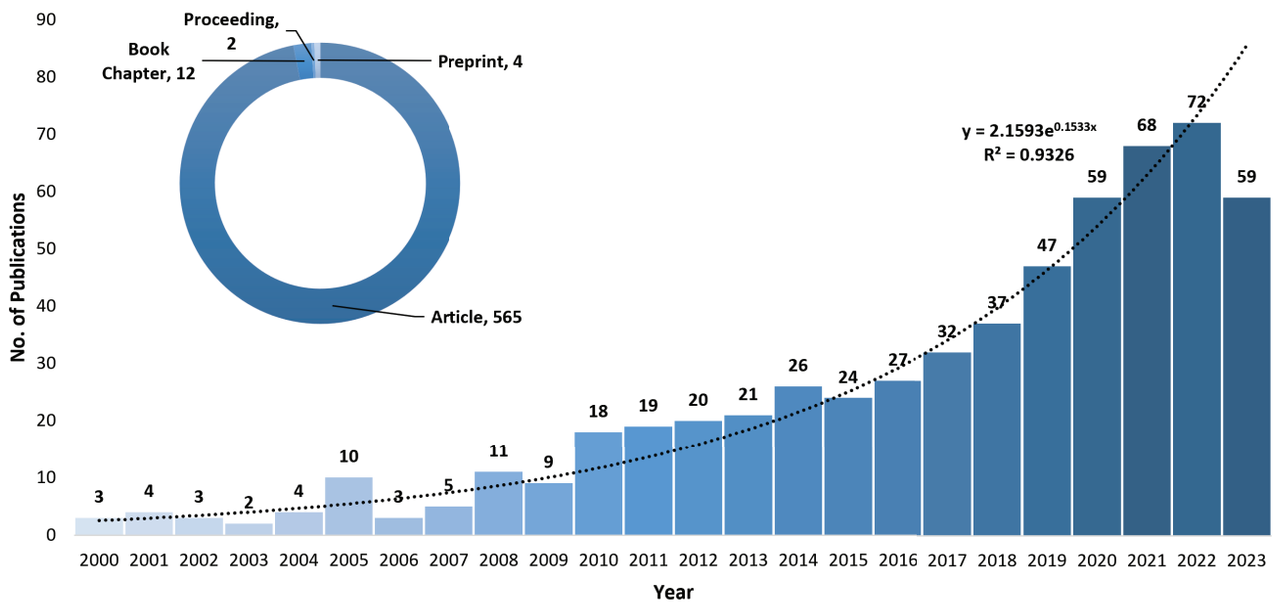

Fig. 1 Publication trends and distribution types in *T. procumbens* research

Citation Analysis

Citation analysis is an important aspect of bibliometric analysis, providing insights into the research impact and trends associated with *T. procumbens* over the years. The citation data from 2000 to 2023 revealed several key trends. In the early 2000s, citations were relatively low, with the highest being 102 in 2000, followed by a decline to 79 in 2001 and further to 43 in 2002. A remarkable decline occurred in 2003 with only 19 citations. However, a significant surge is observed in 2005, where citations rise steeply to 1323, indicating a potentially groundbreaking publication or increased research interest in *T. procumbens*. This peak is followed by a sharp decline to 28 citations in 2006, suggesting that interest or subsequent publications did not maintain the initial momentum. After 2006, the citations show a generally increasing trend with some fluctuations. For instance, in 2007, citations rose to 132 and continued to climb, reaching another peak in 2010 with 474 citations. The years following 2010 display consistent engagement, with citations fluctuating but staying relatively high, particularly in 2014 and 2018, with 355 and 677 citations, respectively (Fig. 2). This period likely reflects sustained research activity and growing acknowledgment of the plant's significance in various studies. The data from 2019 onwards suggests a gradual decline, with citations dropping from 363 in 2019 to 310 in 2020, 247 in 2021, and continuing downwards to 34 by 2023. This downward trend may indicate a shift in research focus or the saturation of studies on *T. procumbens*. Overall, the citation analysis highlights pivotal years of high research activity and citations, particularly 2005 and 2018, marking significant interest and contributions to the academic landscape of *T. procumbens*.

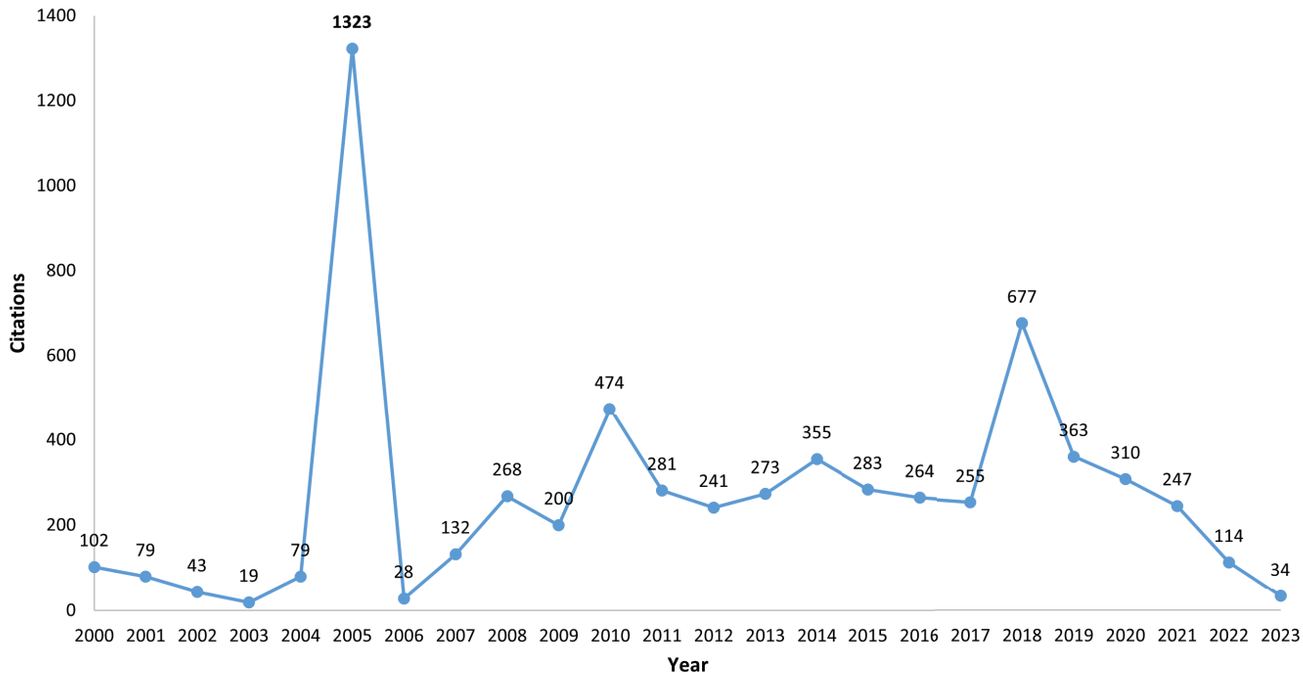

Fig. 2 Citation trends for *T. procumbens* research over time

Country-wise Publication Analysis

The bibliometric analysis of research on *T. procumbens* revealed a varied distribution of scientific contributions and citations across different countries. India emerged as the leading contributor with 119 documents, which collectively stored a substantial 2789 citations, reflecting both prolific research activity and significant impact in this field (Fig. 3). Nigeria follows with 51 publications, receiving 1547 citations, indicating strong scholarly interest and influence. Brazil, with 27 documents and 223 citations, also demonstrated prominent research output, although with comparatively fewer citations per paper. Indonesia's 15 documents received 53 citations, suggesting a more modest research presence. Saudi Arabia and Malaysia, despite having fewer publications (12 and 11 respectively), show high citation counts of 429 and 546, indicative of high-impact research. Mexico's contributions stand at 11 documents with 218 citations, showing a solid engagement in the research community. China and the United Kingdom both contributed 9 documents each, receiving 106 and 107 citations respectively, reflecting moderate research activity and impact. The United States, with 8 publications and 35 citations, showed the least engagement in terms of both output and citation count among the listed countries. This country-wise analysis represented India's leading role in *T. procumbens* research and highlighted significant contributions from Nigeria, while other countries show varied levels of research activity and influence.

Most Prominent Authors

In the bibliometric analysis of the research landscape surrounding *T. procumbens* (a medicinal plant) several authors stand out due to their contributions and the impact of their work. The 'Hussein Mofomosara Salahdeen' led the ranking with the highest number of documents (5) and has gathered 23 citations. This places him as a significant contributor to the body of knowledge on *T. procumbens*. Following closely, 'Sergio R. Peraza-Sánchez' had authored 4 documents but received 59 citations, indicated a high impact and recognition within the academic community. Similarly, 'Md Abdullah Al-Mamun', 'M Masihul Alam', and 'Amina Khatun' have each contributed 4 documents, each amassing 41 citations, which highlighted their influential research in this area. 'Mercy O. Ifeanacho', 'Catherine C. Ikewuchi', and 'Jude C. Ikewuchi', with 4 documents each and 27 citations respectively, also emerged as key researchers, demonstrating significant engagement and influence. Lastly, 'Babatunde Adekunle Murtala' and 'Shakiru Ademola Salami' each have contributed 4 documents and received 15 citations (Fig. 4). Collectively, these authors form the fundamental group driving forward the research on *T procumbens*, with varying degrees of influence as evidenced by their citation counts, thus shaping the scholarly discourse, and advancing understanding in this field.

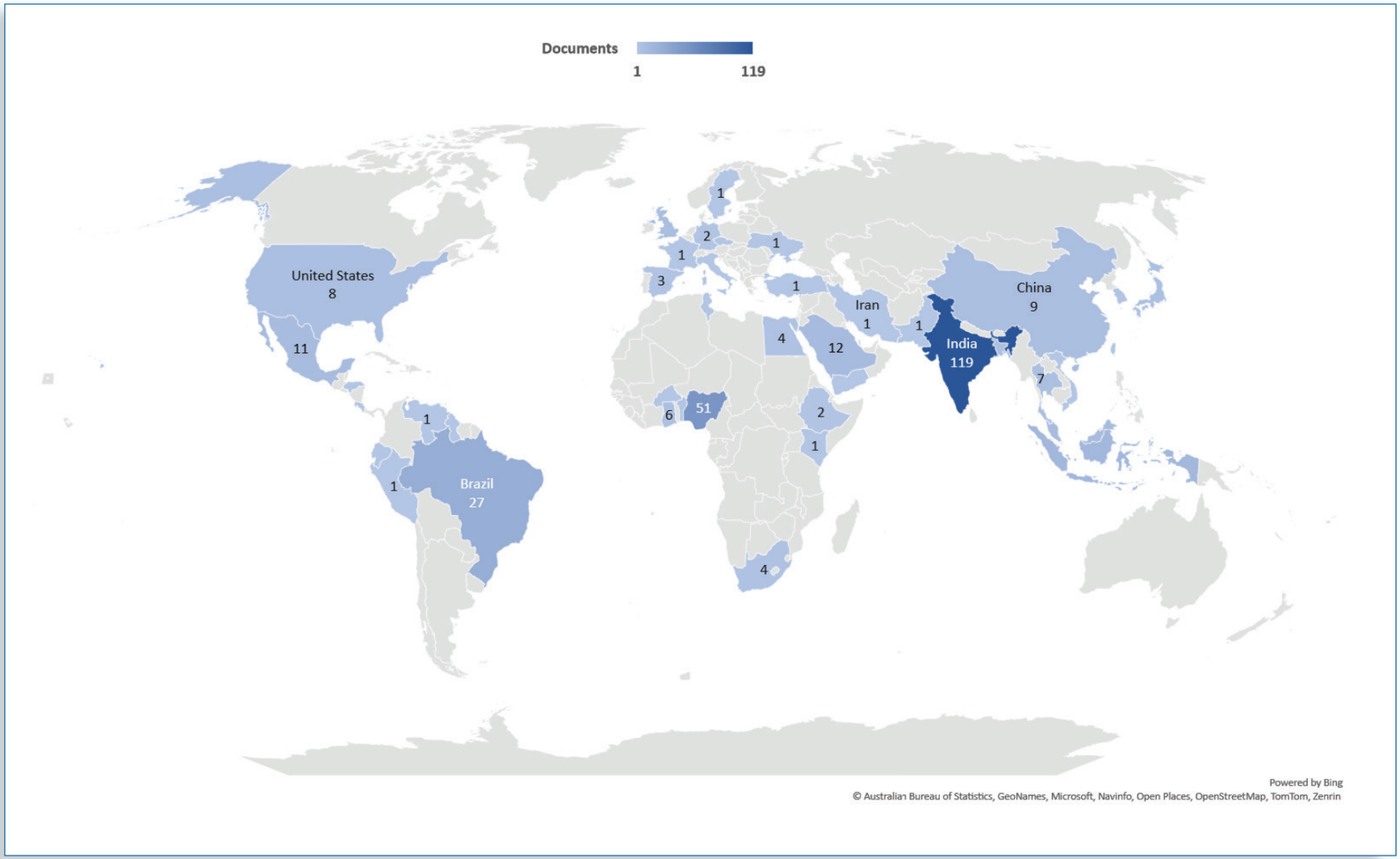

Fig. 3 Global distribution of research on *T. procumbens*

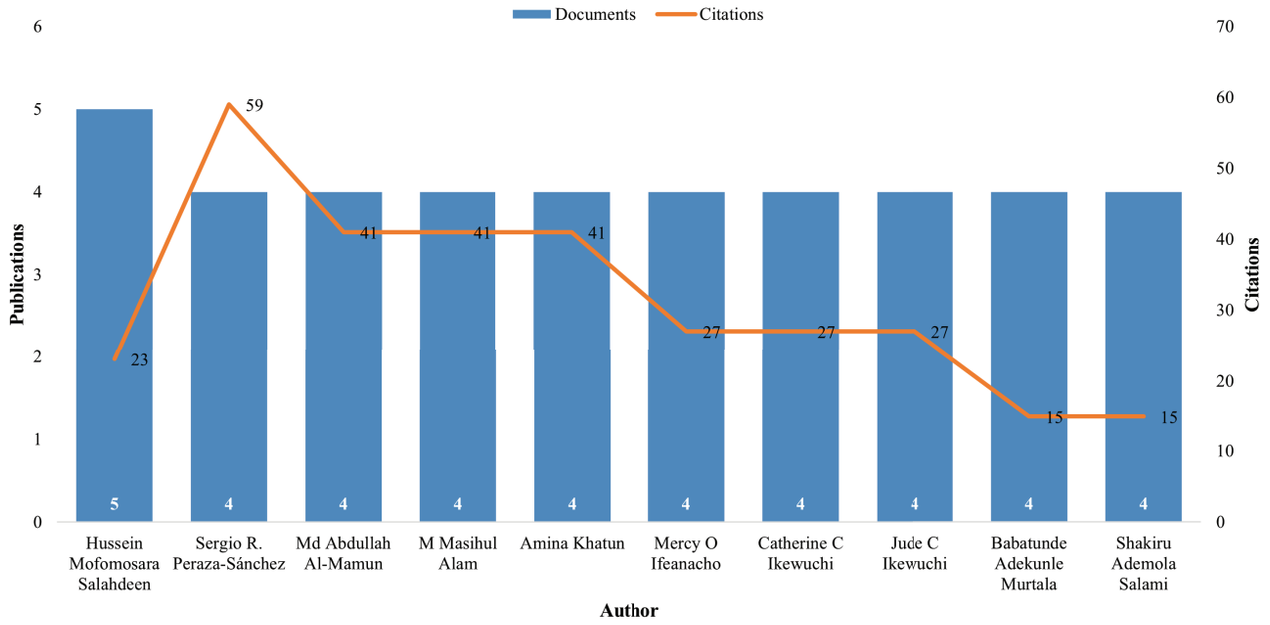

Fig. 4 Most prominent authors based on research contributions to *T. procumbens*

Highly Cited Articles

The research landscape of *T. procumbens*, a plant renowned for its diverse medicinal properties, is profoundly illustrated through a bibliometric analysis of highly cited articles (Table 1). Leading the list, with 1059 citations, is the study titled "Phytochemical constituents of some Nigerian medicinal plants" published in the African Journal of Biotechnology in 2005, highlighted the significant impact of phytochemical research within the scientific community (Edeoga et al., 2005). Another pivotal work, "Characterization of raw and alkali treated new natural cellulosic fibers from *Tridax procumbens*," published in the International Journal of Biological Macromolecules in 2018, has gained 321 citations and emphasized the material science potential of the plant (Vijay et al., 2018). The interdisciplinary reach of *Tridax procumbens* is further evidenced by the 2010 Journal of Nanoparticle Research article, "Qualitative assessment of silver and gold nanoparticle synthesis in various plants: a photobiological approach," with 129 citations, which explored its utility in nanotechnology (Rajasekharreddy et al., 2010). Bioremediation studies, as exemplified by the 2016 article in 3 Biotech, "Bioremediation of heavy metals using an endophytic bacterium *Paenibacillus* sp. RM isolated from the roots of *Tridax procumbens*," with 126 citations, highlighted its environmental applications (Govarthanan et al., 2016). Ethnopharmacological investigations also feature prominently, with two key articles in the Journal of Ethnopharmacology (2010 and 2014) focusing on medicinal uses and receiving 108 and 83 citations, respectively (Upadhyay et al., 2010; Sharma et al., 2014). The therapeutic potential of *T. procumbens* is further illustrated by studies on its immunostimulatory effects

(100 citations), hypoglycemic and anti-hyperglycemic potential (83 citations), hepatoprotective activity (82 citations), and antidiabetic properties (82 citations), published across various journals (Ravikumar et al., 2005; Punitha et al., 2008; Pareek et al., 2009; Petchi et al., 2014). This bibliometric snapshot represented the extensive and multifaceted research engagement with *T. procumbens*, reflecting its significant contributions to phytochemistry, material science, nanotechnology, bioremediation, ethnopharmacology, and therapeutic applications.

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

Rank	Title	Source	Year	Citations	Reference
1	Phytochemical constituents of some Nigerian medicinal plants	African Journal of Biotechnology	2005	1059	Edeoga et al., 2005
2	Characterization of raw and alkali treated new natural cellulosic fibers from *Tridax procumbens*	International Journal of Biological Macromolecules	2019	321	Vijay et al., 2018
3	Qualitative assessment of silver and gold nanoparticle synthesis in various plants: a photobiological approach	Journal of Nanoparticle Research	2010	129	Rajasekharreddy et al., 2010
4	Bioremediation of heavy metals using an endophytic bacterium *Paenibacillus* sp. RM isolated from the roots of *Tridax procumbens*	3 Biotech	2016	126	Govarthanan et al., 2016
5	Ethnomedicinal and ethno-pharmaco-statistical studies of Eastern Rajasthan, India	Journal of Ethnopharmacology	2010	108	Upadhyay et al., 2010
6	Immunostimulating influence of herbal biomedicines on nonspecific immunity in Grouper *Epinephelus tauvina* juvenile against *Vibrio harveyi* infection	Aquaculture International	2008	100	Punitha et al., 2008
7	Ethnomedicinal plants used to treat skin diseases by Tharu community of district Udham Singh Nagar, Uttarakhand, India	Journal of Ethnopharmacology	2014	83	Sharma et al., 2014

Rank	Title	Source	Year	Citations	Reference
8	Evaluation of hypoglycemic and anti-hyperglycemic potential of *Tridax procumbens* (Linn.)	BMC Complementary Medicine and Therapies	2009	83	Pareek et al., 2009
9	Hepatoprotective activity of *Tridax procumbens* against d-galactosamine/lipopolysaccharide-induced hepatitis in rats	Journal of Ethnopharmacology	2005	82	Ravikumar et al., 2005
10	Antidiabetic Activity of Polyherbal Formulation in Streptozotocin - Nicotinamide Induced Diabetic Wistar Rats	Journal of Traditional and Complementary Medicine	2014	82	Petchi et al., 2014

Most Active Journals

In the bibliometric analysis of the research landscape on *Tridax procumbens*, the journal "Advances in Weed Science" emerged as the most active source, publishing 11 documents, and stored 103 citations, with an impact factor of 1.882 (Table 2). This journal, published by Sociedade Brasileira da Ciência das Plantas Daninhas, led the field in terms of document output. The "Nigerian Journal of Animal Production," published by the Nigerian Society for Animal Production, followed closely with 10 documents, although it had a modest citation count of 35. The "Journal of Ethnopharmacology," an Elsevier publication, stood out for its high impact factor of 4.36 and significant citation count of 397, despite publishing only 8 documents. The "Research Journal of Pharmacy and Technology," with 6 documents and just 9 citations, is indicative of its lesser influence in the field. Other journals included the "International Journal of Biological and Chemical Sciences," which published 5 documents with an impact factor of 0.553 and received 18 citations, and "The FASEB Journal," which, despite having a high impact factor of 5.191, was less prolific with only 5 documents and 2 citations. Journals like "Biological Research," "Pharmacognosy Journal," "Aerobiologia," and "IOP Conference Series: Earth and Environmental Science" contributed 4 documents each, with varying impact factors and citation counts, reflecting a diverse but concentrated interest in the pharmacological and biological studies of *T. procumbens*.

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

Rank	Source	Publisher	Impact factor	Documents	Citations	Citations per Document
1	Advances in Weed Science	Sociedade Brasileira da Ciência das Plantas Daninhas	1.882	11	103	9.36
2	Nigerian Journal of Animal Production	Nigerian Society for Animal Production	-	10	35	3.50
3	Journal of Ethnopharmacology	Elsevier	4.36	8	397	49.63
4	Research Journal of Pharmacy and Technology	A & V Publications	-	6	9	1.50
5	International Journal of Biological and Chemical Sciences	International Formulae Group	0.553	5	18	3.60
6	The FASEB Journal	Wiley	5.191	5	2	0.40
7	Biological Research	BioMed Central	2.57	4	41	10.25
8	Pharmacognosy Journal	Pharmacognosy Network Worldwide	1.123	4	19	4.75
9	Aerobiologia	Springer	2.143	4	18	4.50
10	IOP Conference Series: Earth and Environmental Science	IOP Publishing	-	4	11	2.75

Top Productive Organizations

The bibliometric analysis focused on the research landscape of *T. procumbens,* and several organizations have emerged as key contributors. At the top list, 'the University of Ibadan', which led with 12 published documents, gathered a total of 42 citations (Table 3). This demonstrated a significant research output, although with a moderate citation impact. 'Lagos State University' followed closely with 10 publications, but outshines in terms of influence, gained

58 citations. The 'Consejo Nacional De Humanidades, Ciencias Y Tecnologías', despite having only 8 documents, stands out with an impressive 182 citations, highlighting the high impact and relevance of its research in this area. 'Anna University in Chennai' also contributed 8 documents, achieving 67 citations, indicating a strong presence in *T. procumbens* research. The 'Asian Institute of Medicine, Science and Technology', with 7 publications, is remarkable for its high citation count of 211, suggested exceptional research quality and influence. Similarly, the 'Autonomous University of Yucatán' matched this output with 7 documents and 145 citations, emphasized its significant role in advancing knowledge on *T. procumbens*. The 'University of Port Harcourt' and 'Universidade De São Paulo' each published 7 documents, with 92 and 77 citations respectively, indicating strong research activity. The 'Thiruvalluvar University', despite having only 6 publications, achieved the highest citation count in this group with 273, indicating a substantial impact of its research. Lastly, 'Shahjalal University of Science and Technology', with 6 documents and 59 citations, shows consistent research output and influence. Together, these institutions highlight the diverse and vibrant research landscape surrounding *T. procumbens*, with varying levels of productivity and impact.

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

Rank	Organization	Country	Documents	Citations	Citations per Document
1.	University of Ibadan	Nigeria	12	42	3.50
2.	Lagos State University	Nigeria	10	58	5.80
3.	Consejo Nacional De Humanidades, Ciencias Y Tecnologías	Mexico	8	182	22.75
4.	Anna University, Chennai	India	8	67	8.38
5.	Asian Institute of Medicine, Science and Technology	Malaysia	7	211	30.14
6.	Autonomous University of Yucatán	Mexico	7	145	20.71
7.	University of Port Harcourt	Nigeria	7	92	13.14
8.	Universidade De São Paulo	Brazil	7	77	11.00
9.	Thiruvalluvar University	India	6	273	45.50
10.	Shahjalal University of Science and Technology	Bangla-desh	6	59	9.83

Top Fields of Study

The current study analyzed a dataset of 805 scholarly documents from Lens.org to examine the occurrence of terms in significant academic domains, revealing a diverse spectrum of research into the plant species *T. procumbens*. The high document count for *Tridax procumbens* itself (460

documents) reflected the direct focus on this plant, indicating a broad interest in its various aspects. Biology, with 452 documents, signified a strong scientific interest in the biological aspects of *T. procumbens*. Traditional medicine, accounting for 338 documents, highlighted the plant's importance in folk medicine and ethnobotany. Chemistry, with 242 documents, pointed to extensive research into the chemical compounds present in *T. procumbens*. The focus on medicine (198 documents) highlighted interest in clinical applications and potential health benefits of *T. procumbens*, encompassing pharmacological studies, therapeutic uses, and health impact assessments. Botany, with 196 documents, indicated that botanical studies were crucial for understanding the plant's classification, morphology, growth patterns, and ecological significance. Phytochemical research (104 documents) was vital for identifying and analyzing chemical compounds derived from *T. procumbens*, crucial for discovering bioactive substances with medicinal properties. The identification of *T. procumbens* as a weed (96 documents) suggested research on its impact on agriculture, invasive potential, and management strategies to control its spread in non-native regions. The inclusion of *T. procumbens* in the broader category of medicinal plants (76 documents) emphasized its potential therapeutic applications and significance in natural product research. Pharmacology, with 74 documents, reflected investigations into the mechanisms of action, efficacy, and safety of compounds extracted from *T. procumbens*, including preclinical and clinical studies aiming to develop pharmaceutical applications (Fig. 5). Overall, the research landscape of *T. procumbens* was diverse, with significant focus areas including its biological characteristics, traditional and modern medicinal uses, chemical composition, and agricultural implications.

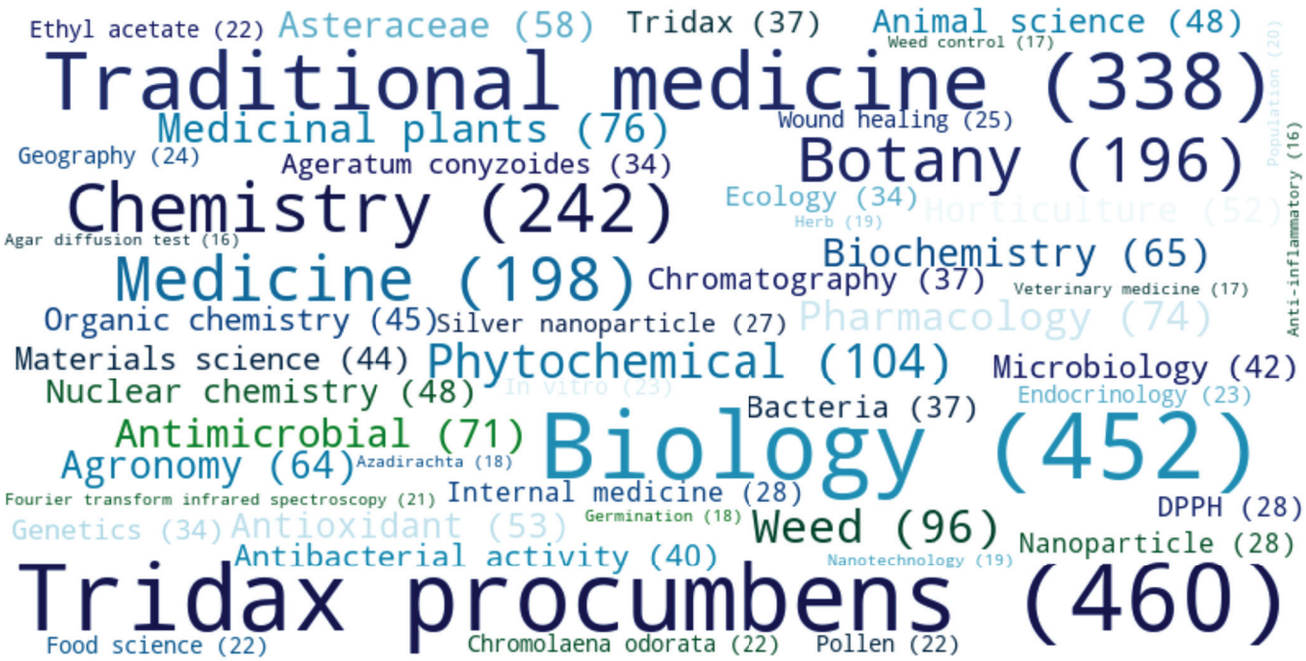

Fig. 5 Terms co-occurrence analysis within prominent terms

Research Collaboration Networks

Authors' Collaboration Network

The study on *T. procumbens* explored the collaboration network among 2,120 authors, revealing the dynamics of scholarly interaction in this research domain. Authors were included based on their contribution of at least one cited document on *T. procumbens*. The analysis identified a cohesive cluster of 190 connections, with Cluster 1 comprising 20 authors, showcasing a robust collaborative network. Gandhi Elango and Chinnaperumal Kamaraj emerged as the most influential authors, each with a high total link strength of 21 (Fig. 6). The findings highlighted 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, facilitating future collaborations and interdisciplinary exchanges within the *T. procumbens* research community.

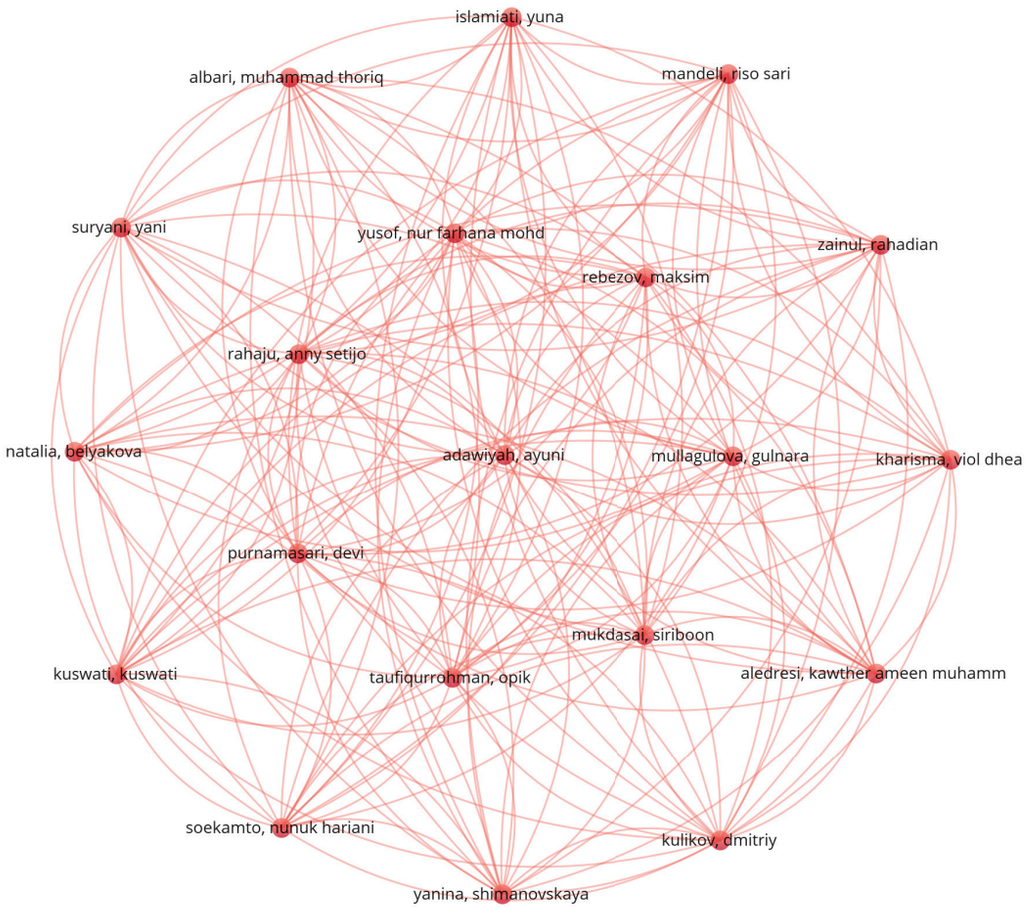

Fig. 6 Collaborative network among the authors on *T. procumbens* research

Journal-wise Collaboration Network

A comprehensive analysis of journal-wise collaboration in *T. procumbens* research was conducted using a dataset of 426 journals. This detailed examination revealed 126 interconnections among 43 journals through citation analysis. Inclusion in the collaborative network required journals to publish at least 2 documents on *T. procumbens*, each receiving a minimum of 2 citations. The analysis identified 9 distinct clusters within the network, representing groups of journals with strong interconnections. Clusters 1 and 2 were the largest, with 8 and 7 journals respectively, while clusters 3 and 4 each had 5 journals. Clusters 5, 6, and 7 included 4 journals each, and clusters 8 and 9 were smaller, containing 3 journals each (Fig. 7). The "Journal of Ethnopharmacology" emerged as the most influential journal, showing the highest connection strength at 53. "Fitoterapia" and "Phytomedicine" also demonstrated significant influence, with total link strengths of 33 and 17, respectively. This network analysis offer valuable insights into the collaborative dynamics and thematic strengths among journals focused on *T. procumbens* research. Identifying key journals and their clusters highlighted hubs of activity and influence, suggesting potential avenues for future collaborations and research within the *T. procumbens* scholarly community.

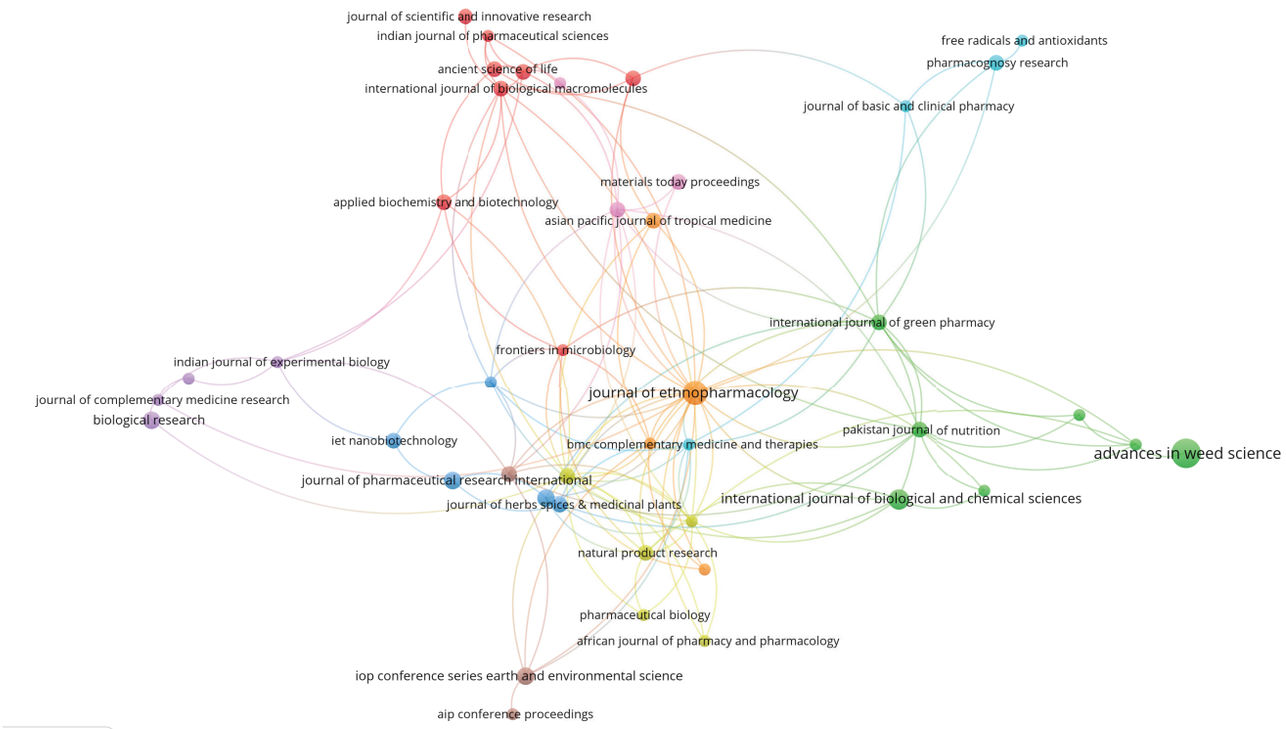

Fig. 7 Journal-wise collaboration network analysis on *T. procumbens* research

Organizational Collaborative Network

The study investigated the collaborative network among organizations involved in *T. procumbens* research through citation analysis, highlighting their interactions and connections. The analysis included 451 organizations, with 58 meeting the criteria of having published at least two documents and receiving at least two citations per document on *T. procumbens*. The resulting network revealed 223 connections, among these 58 organizations, which were grouped into 8 distinct clusters (Fig. 8). Clusters 1 and 2, the largest, each comprised 12 organizations that demonstrated significant inter-organizational cooperation, indicating a highly collaborative environment. Clusters 3 and 4 included 8 and 6 organizations respectively, showing intense internal connections and suggesting specialized research collaborations. Clusters 5, 6, 7, and 8, each consisting of 5 organizations, likely represented more specialized or emerging collaborative areas. Key findings highlighted the central role of certain organizations. "Logos State University" emerged as the most influential, with the highest connection strength and 65 links. "University of Ibadan" followed with 61 connections, and "Consejo Nacional De Humanidades" with 56 connections, indicating their substantial contributions and influence within the network. Understanding the collaborative network was crucial for encouraging effective partnerships, identifying influential players, and advancing *T. procumbens* research by creating a roadmap for improved cooperation and significant advancements.

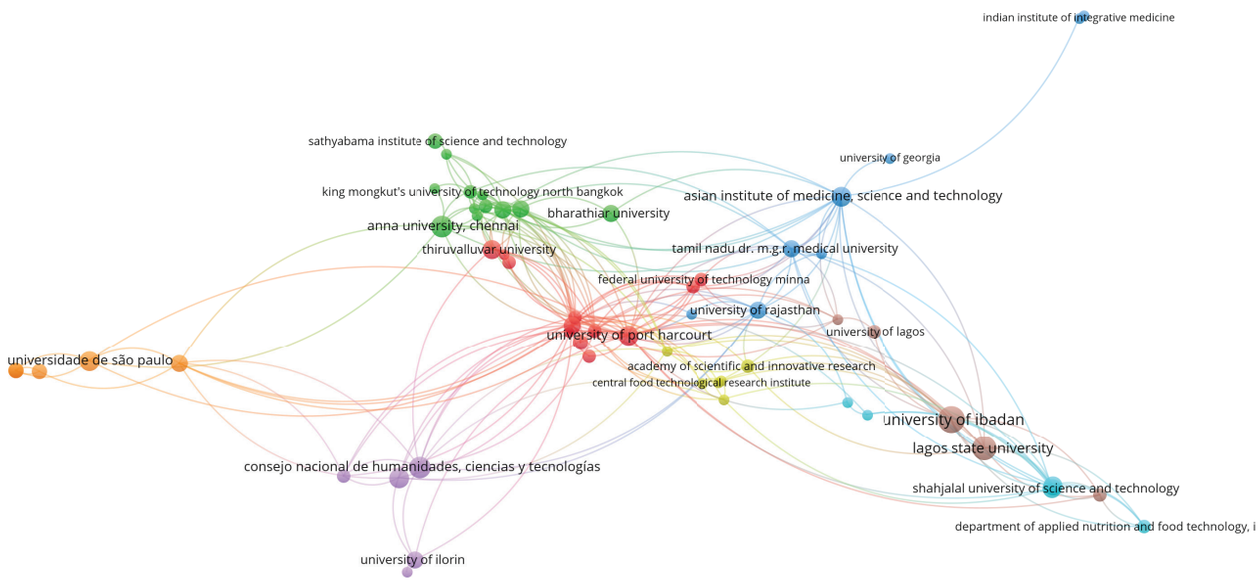

Fig. 8 Clusters of collaborative institutions for *T. procumbens* research

Country-wise Collaboration Network

The country-wise collaboration network analysis in *T. procumbens* research provides a detailed overview of global interconnectedness and cooperation among nations in this field. The study covered 33 countries, forming 134 collaborative connections. To be included, each country had to publish at least 1 document on *T. procumbens*, each with a minimum of 1 citation, ensuring that only significant contributions were considered. The analysis identified 8 distinct clusters of collaboration, reflecting varying degrees of interconnectedness and research focus. Clusters 1 and 2, with 8 countries each, showed remarkable collaborative activities. Cluster 3 included six countries, while clusters 4, 5, and 6 each comprised three countries, indicating more focused research efforts. Clusters 7 and 8 consisted of only single countries. The analysis highlighted India and Nigeria's strong connection strengths, with 283 and 88 links respectively, emphasizing their dominant roles in global *T. procumbens* research (Fig. 9). This analysis highlights the importance of international collaboration in advancing *T. procumbens* research. Global partnerships enhance knowledge sharing, techniques, and resources, driving research progress. These findings emphasize the value of promoting international research networks for innovation and deeper scientific understanding.

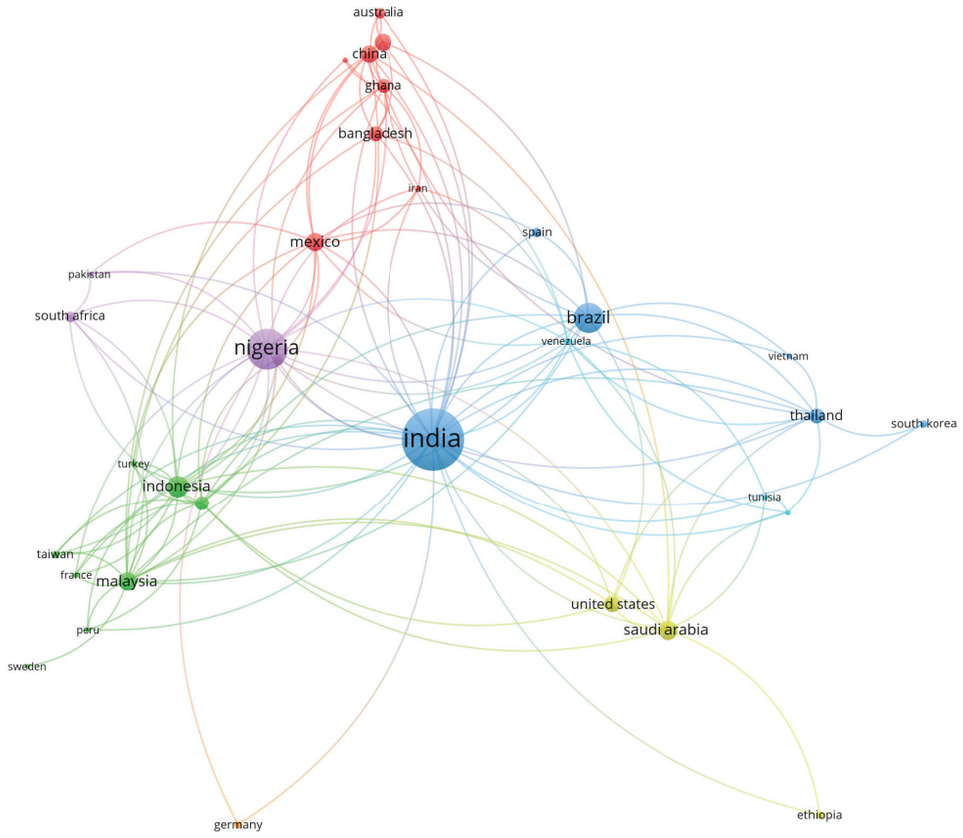

Fig. 9 Country-wise collaboration network in *T. procumbens* research

Conclusion

The bibliometric analysis of scholarly publications from 2000 to 2023 revealed significant insights into the global research landscape of *Tridax procumbens*. Over this period, 583 articles were published by 2120 authors from 451 organizations across 43 countries. The analysis showed an important surge in publications and citations in 2005, indicating growing recognition of the plant's importance. India emerged as the leading contributor with 119 documents and 2789 citations, highlighting its pivotal role in advancing research on this medicinal plant. The most prominent authors included Hussein Mofomosara Salahdeen with 5 documents and 23 citations, and Sergio R. Peraza-Sánchez with 4 documents and 59 citations. Moreover, "Advances in Weed Science" was the most active journal, publishing 11 documents with 103 citations. The University of Ibadan led among organizations with 12 documents and 42 citations, while the Asian Institute of Medicine, Science and Technology, with 7 publications and 211 citations, stood out for research quality and influence. A highly cited study, "Phytochemical constituents of some Nigerian medicinal plants," published in 2005, received 1059 citations, reflecting the impact of phytochemical research. Collaborative networks among authors, journals, organizations, and countries play a crucial role in enhancing research quality and innovation. These networks facilitate the sharing of knowledge, resources, and expertise, leading to more robust and impactful research work about *T. procumbens*. Overall, this analysis highlighted the global significance of *T. procumbens* research and the collaborative efforts driving advancements in understanding and utilizing this medicinal plant, laying a foundation for future exploration and innovation.

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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
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 ± 5°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°C to 115°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°C to 115°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 °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 = (Mw g/ Ms g) × 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, 100 ml graduated measuring cylinder, 2×50 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 ml = 1 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.
9. 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

1. Weight of 100 ml graduated cylinder = A
2. Bilk volume of the compacted soil = B
3. Weight of cylinder + compacted soil = C
4. Weight of soil sample = C-A= D
5. Bulk density (g/cm^3) = 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

1. 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.
2. In the meantime, the pH meter was switched on.

3. Initially the instrument was calibrated with buffer solution of known pH 4, 7.0, and 9.2.
4. The electrodes were washed with distilled water and wiped dry with a tissue paper.
5. Then, the electrode was dipped in the sample and the readings were taken.
6. 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 Rx, two fixed resistances R1 and R2 and a variable resistance (Rv) are linked. The variable resistance (Rv) is adjusted until no current flows through it and the reading is taken. The resistance or conductance (Rv) is measured (Richards, 1954 Chopra and Kanwar, 1976).

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

Procedure

1. 25 g of the soil samples was taken in a 100 ml beaker.
2. Added 50 ml distilled water.
3. Intermittent stirring was done with a glass rod for 30 minutes.
4. The samples were left overnight to obtain a clear supernatant.
5. The conductivity of the supernatant liquid was determined with the help of a conductivity meter.

Interpretation

EC (1:2 soil water; µS/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 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 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

1. Potassium dichromate solution (1 N $K_2Cr_2O_7$): 49.04 g of analytical grade $K_2Cr_2O_7$ was dissolved in distilled water and the volume was made up to 1 litre.
2. Concentrated sulphuric acid (H_2SO_4).
3. 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, $FeSO_4(NH_4)_2SO_4.6H_2O$]: 392 g analytical grade ferrous ammonium sulphate was dissolved in distilled water, to which 15 ml concentrated sulphuric acid was added and volume to made up to 2 litre with distilled water.

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 250ml conical flask.
2. To it, 10 ml 1 N $K_2Cr_2O_7$ 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.
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.

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 $K_2Cr_2O_7$ used for oxidation of organic carbon in soil = (X-Y)/2 ml
5. 1 ml of 1N $K_2Cr_2O_7$ =0.003 g C
6. % Organic Carbon in Soil= X-Y/2×0.003×00/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 ($KMnO_4$): 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 (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 (H_2SO_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 % 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% $KMnO_4$ and second in 2.5% 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 $KMnO_4$ and 25 ml 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 H_2SO_4 acid and note the volume of 0.02 N H_2SO_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 H_2SO_4 used to titrate ammonium borate = X ml
4. 1 ml of 0.02 N H_2SO_4 = 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^{2+} in the soil solution and the pH of the soil regulates the solubility of calcium phosphate in it. The bicarbonate (HCO_3^-) activity in the soil is increased by the 0.5 M $NaHCO_3$ 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, $NaHCO_3$ solution removes some phosphorous from Al and Fe phosphates. The precipitation of phosphate released from calcium phosphate is prevented by low Ca^{2+} 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_2PO_4 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 $NaHCO_3$: 42 g of $NaHCO_3$ 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_2SO_4 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 ($KSbO.C_4H_4O_6$) 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 0.5N $NaHCO_3$ 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 × 5 = 100 times
8. Concentration of P from spectrophotometer = X AU (absorbance units)
9. Available P in soil (ppm) = X (AU) × 100
10. Available P (Kg/ ha) = ppm × 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 (Black, 1965; Merwin and Peech, 1951; 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.

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 × total dilution-A
6. In kg/ha = A × 2.24-C

Interpretation

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

Available Sulphur

Principle: Soil was shaken with 0.15 % $CaCl_2$ 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% $CaCl_2$): 1.986 g of calcium chloride dihydrate ($CaCl_2.2H_2O$) was dissolved in distilled water and volume made up to 1 litre.
2. Barium chloride ($BaCl_2$)
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 (K_2SO_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% $CaCl_2$ solution and 1 g $BaCl_2$ 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% $CaCl_2$ 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.

Calculations

1. Weight of soil taken = 10g
2. Volume of extractant added = 50 ml
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 × 1.25 = 6.25 times
8. ppm of S from standard curve = Y
9. ppm of S in soil = Y × 6.25
10. S in kg/ha = ppm of S × 2.24

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_3 (Suprapure grade), add 1 ml of H_2O_2 (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 50ml 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 × 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 $CaCl_2.2H_2O$ buffered at pH 7.3. During this extraction, TEA gets protonated as $HTEA^+$ 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^{2+} through $CaCl_2.2H_2O$ helps avoiding dissolution of $CaCO_3$ 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 $CaCl_2$ and 0.1M TEA (Triethanolamine) and its pH was adjusted to 7.3. For preparing one litre of this solution, 13.3 ml $(HOCH_2CH_2)_3N$ i.e. TEA, 1.967 g DTPA and 1.47 g $CaCl_2.2H_2O$ 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 ± 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, 100ml 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 25ºC.
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 × 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.	*Rhizobium*	Rhizobium	HiMedia (M408-500G)
7.	*Azotobacter*	Azotobacter	HiMedia (M1944-500G)
8.	*Azospirillum*	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

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

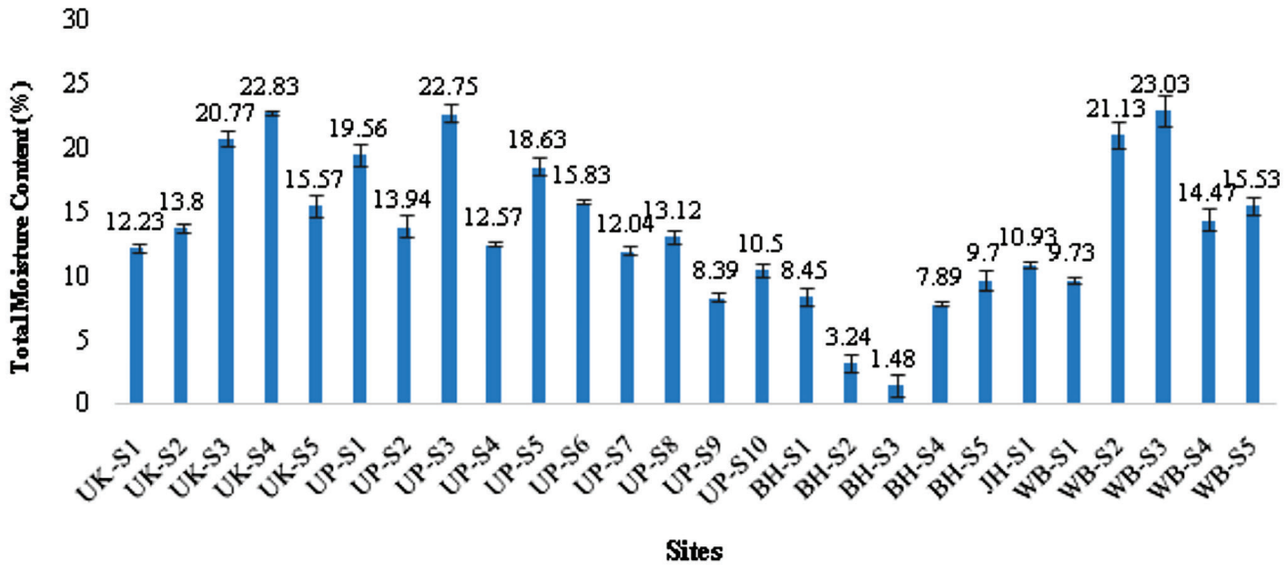


Fig. 1 Total moisture content of different sampling sites

The TMC% was found to be in the range of 1.48 ± 0.94% (lowest) to 23.03 ± 1.25% (highest). Soil sample from BH-S3 had very little moisture content i.e., 1.48% ± 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 peds, 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

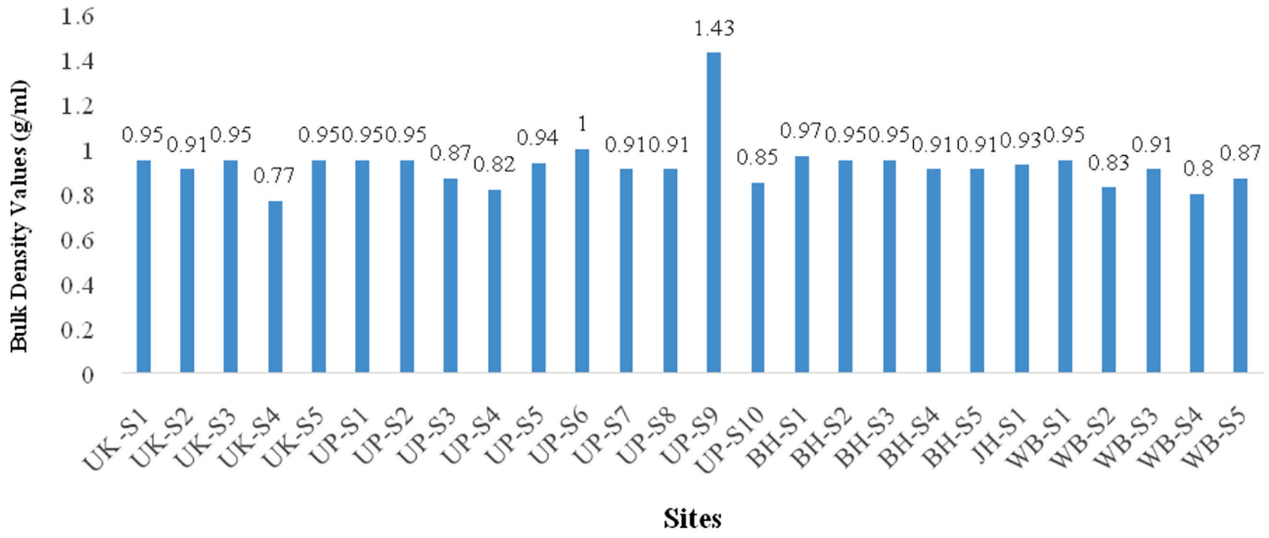


Fig. 2 Soil bulk density of different sampling sites

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

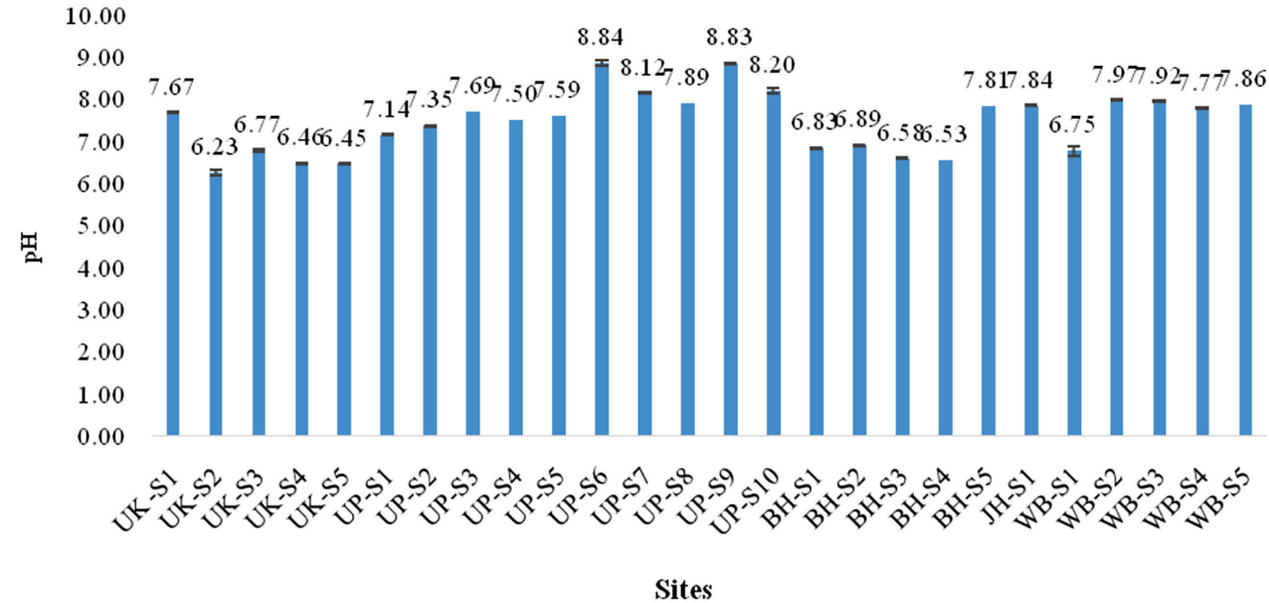


Fig. 3 Soil pH of different sampling sites

The pH of the sample sites ranged from as low as 6.23 ± 0.06 to as high as 8.84 ± 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

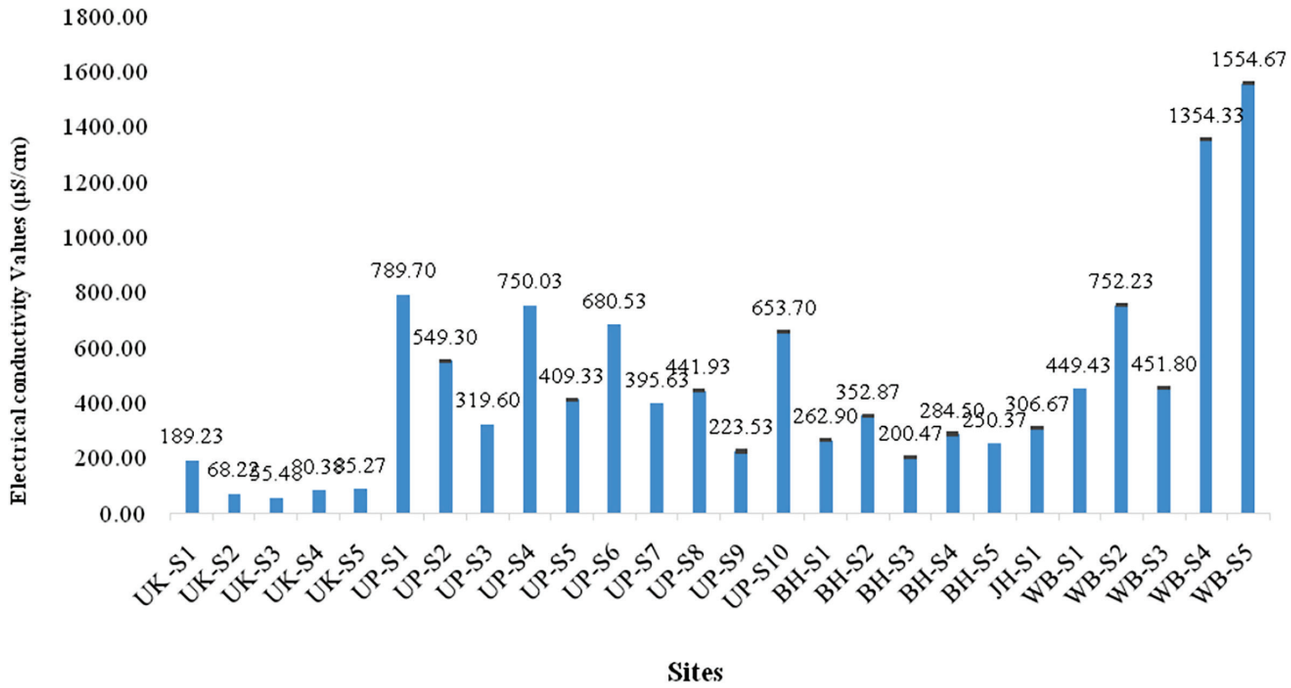


Fig. 4 Soil electrical conductivity at different sampling sites

The electrical conductivity of almost all the soil samples were within the prescribed limits of having EC below 800µS/cm. Soil samples from WB-S4 and WB-S5 showed the highest electrical conductivity value of 1354.33 ± 2.52 µS/cm and 1554.67 ± 2.52 µS/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 (Rawls et al., 2004; Allison, 1965). The organic carbon content as measured for the 26 soil samples is shown in Fig. 5

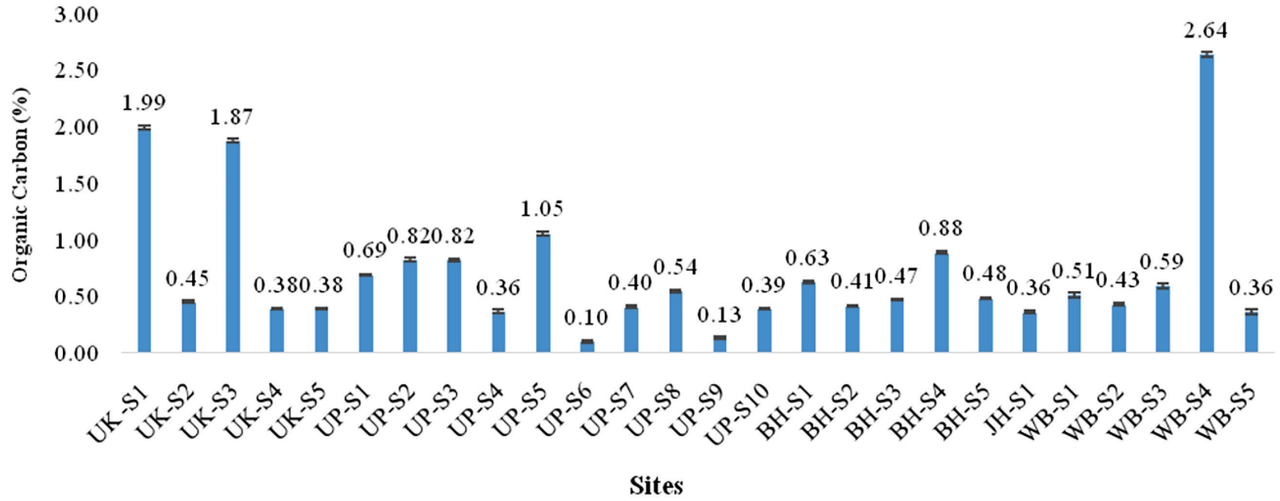


Fig. 5 Soil organic carbon content at different sites.

According to the data, the organic carbon content of soil ranged from 0.10 ± 0.01% to 2.64 ± 0.03%. UP-S6, which had the highest pH value of 8.84 ± 0.01 had the lowest organic carbon content of 0.10 ± 0.01%. The highest quantity of organic carbon content was discovered in WB-S4 (2.64 ± 0.03%), which had an alkaline pH (7.77 ± 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 (Baričevič 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

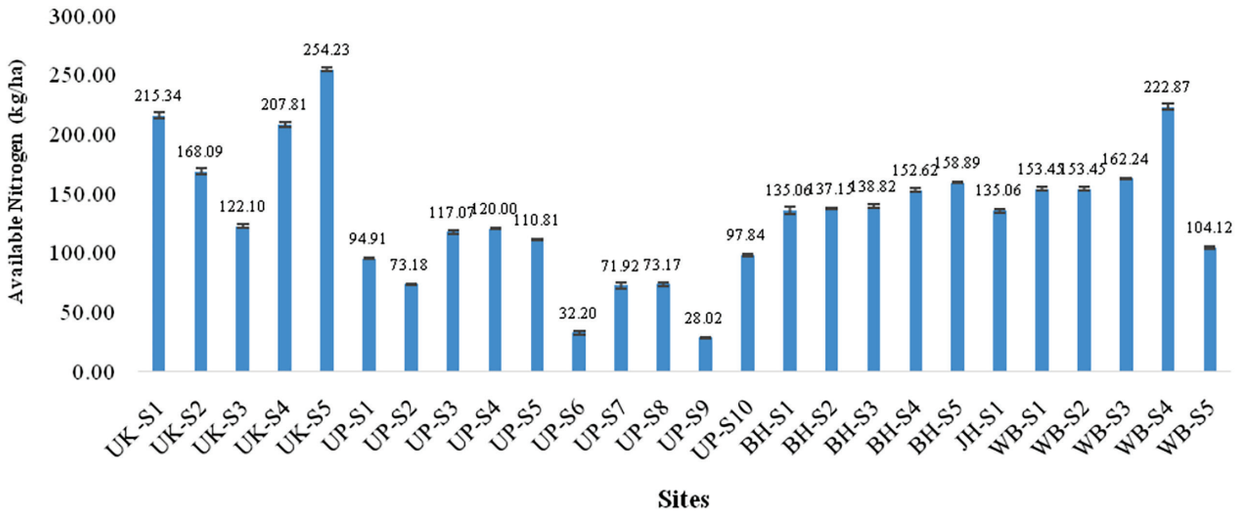


Fig. 6 Soil available nitrogen content at different sites

From the available data it was observed that site UK-S5 had the maximum available nitrogen content (254.23±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

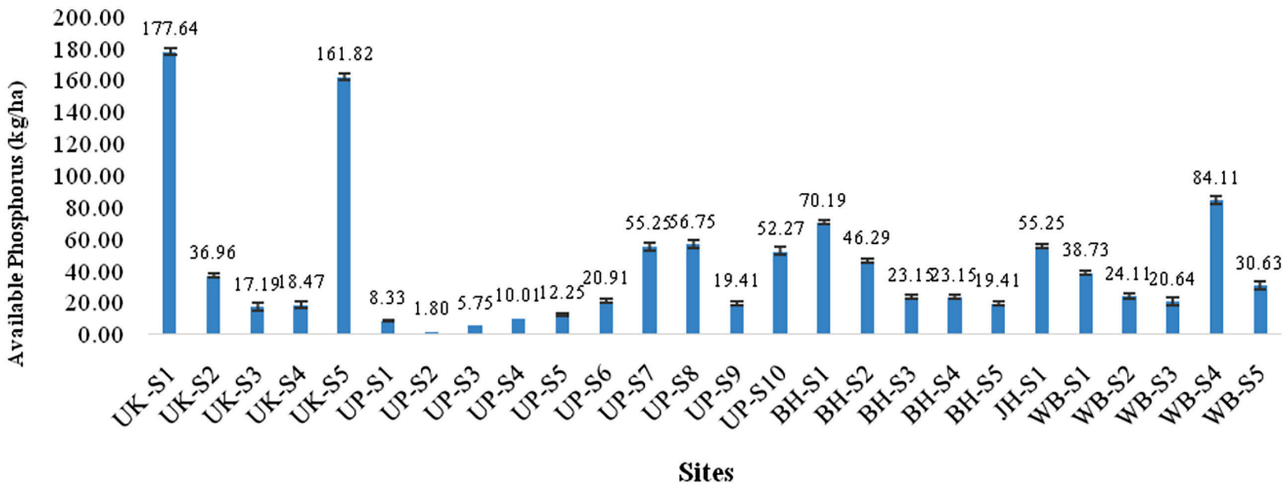


Fig. 7 Soil available phosphorus content at different sites

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

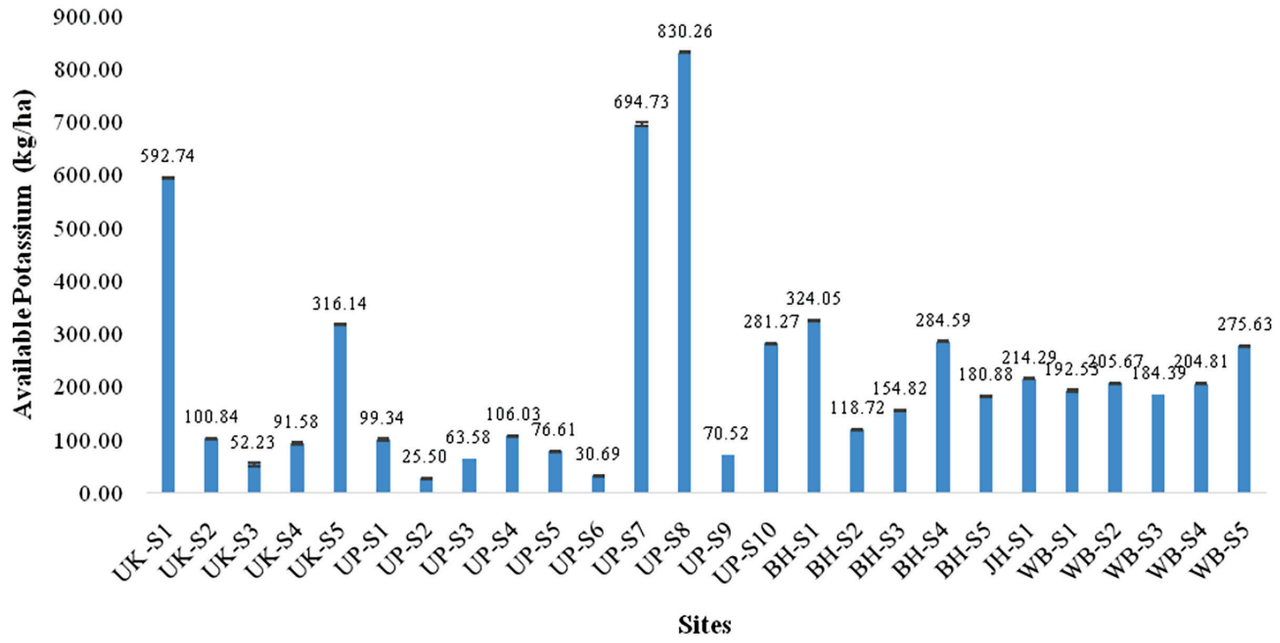


Fig. 8 Soil available potassium content at different sites

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

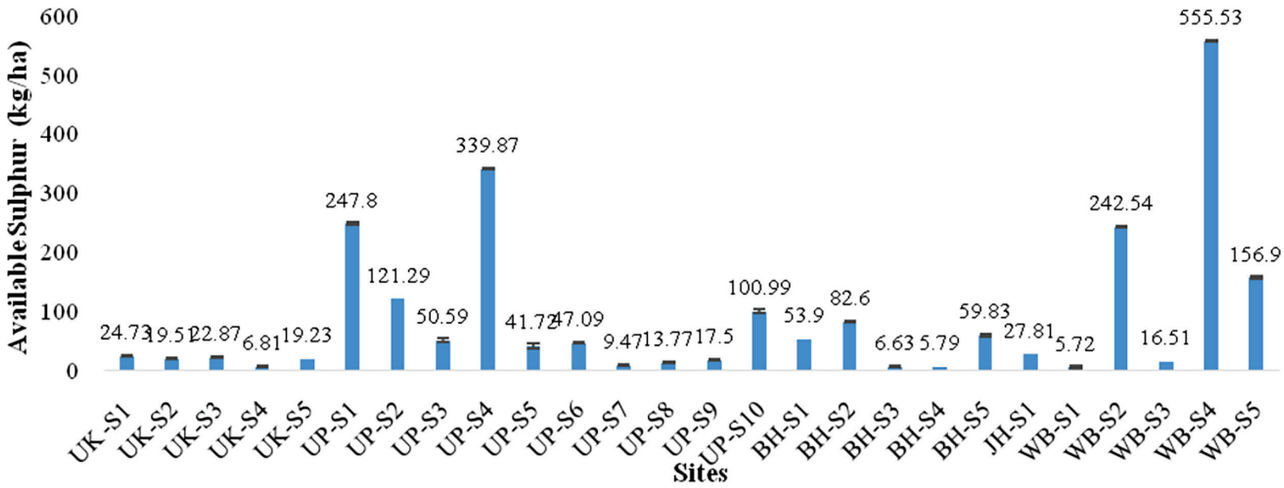


Fig. 9 Soil available sulphur content at different sites

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.

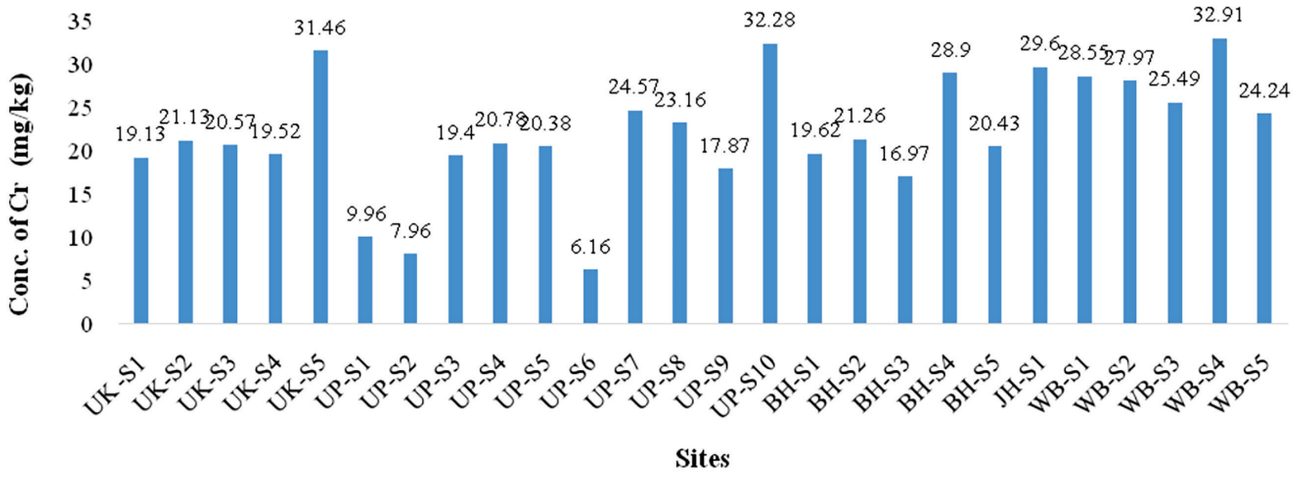


Fig. 10a Concentration of chromium at different sites

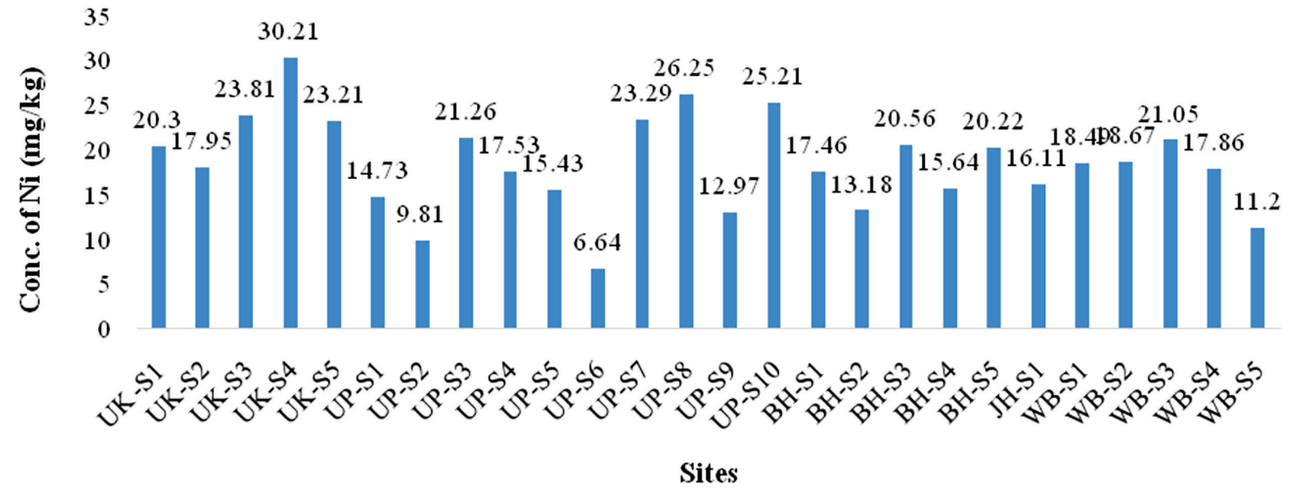


Fig. 10b Concentration of nickel at different sites

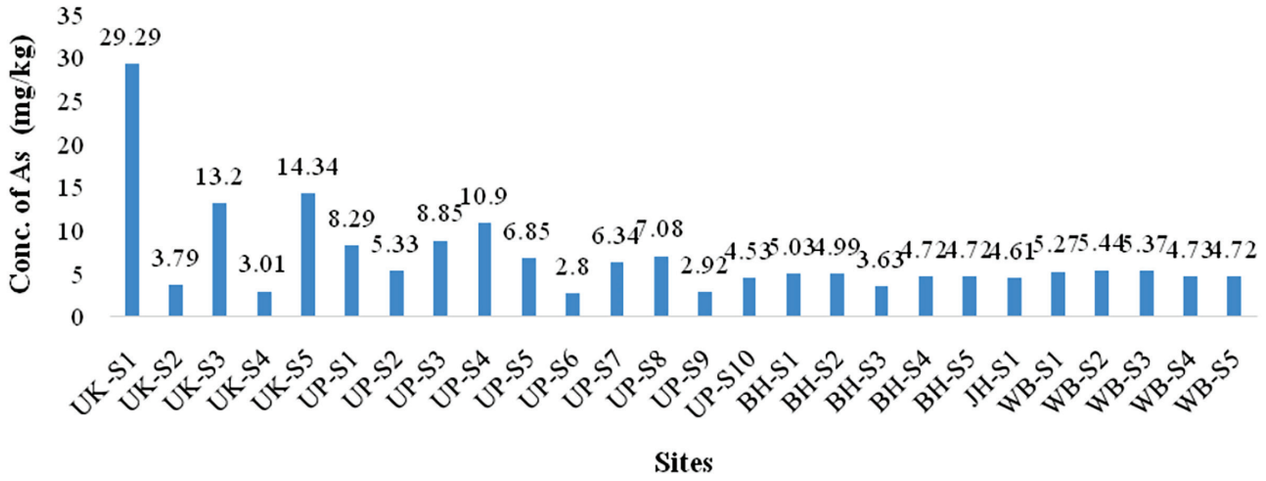


Fig. 10c Concentration of arsenic at different sites

Fig. 10d Concentration of strontium at different sites

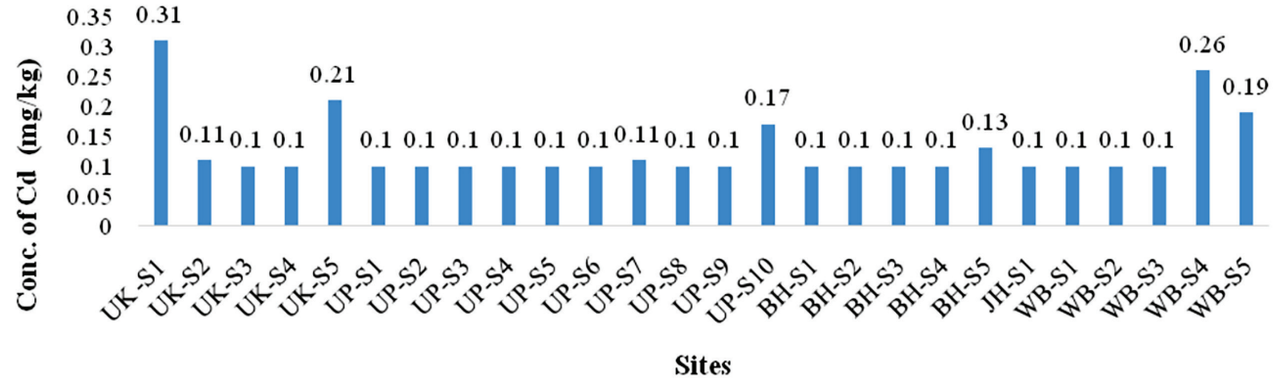


Fig. 10e Concentration of cadmium at different sites

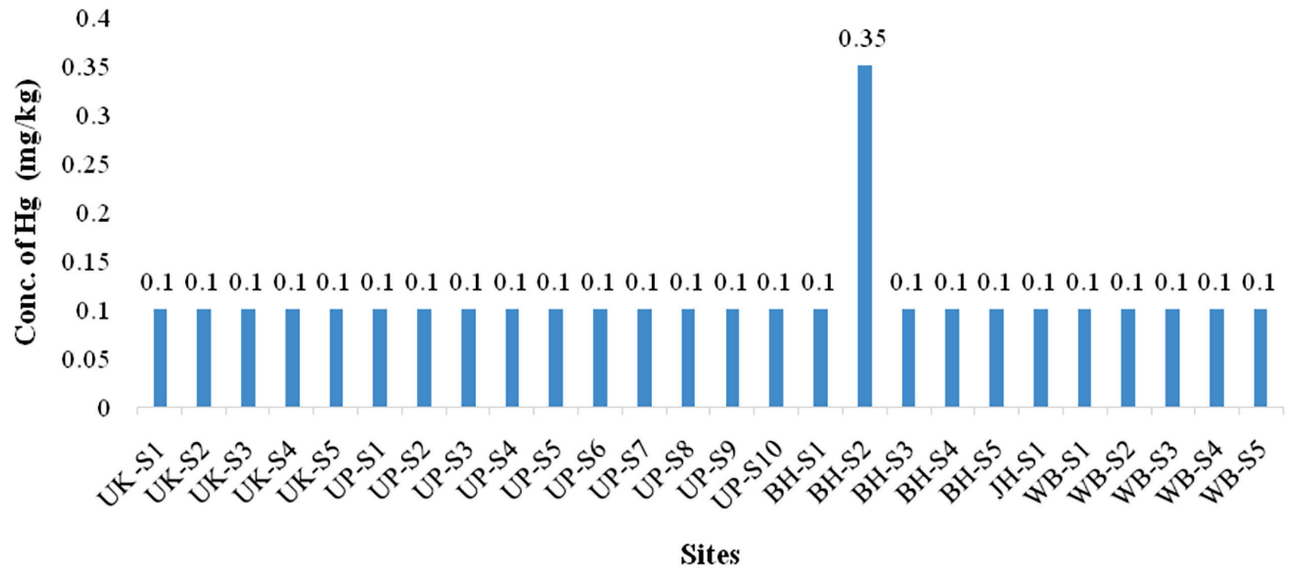


Fig. 10f Concentration of mercury at different sites

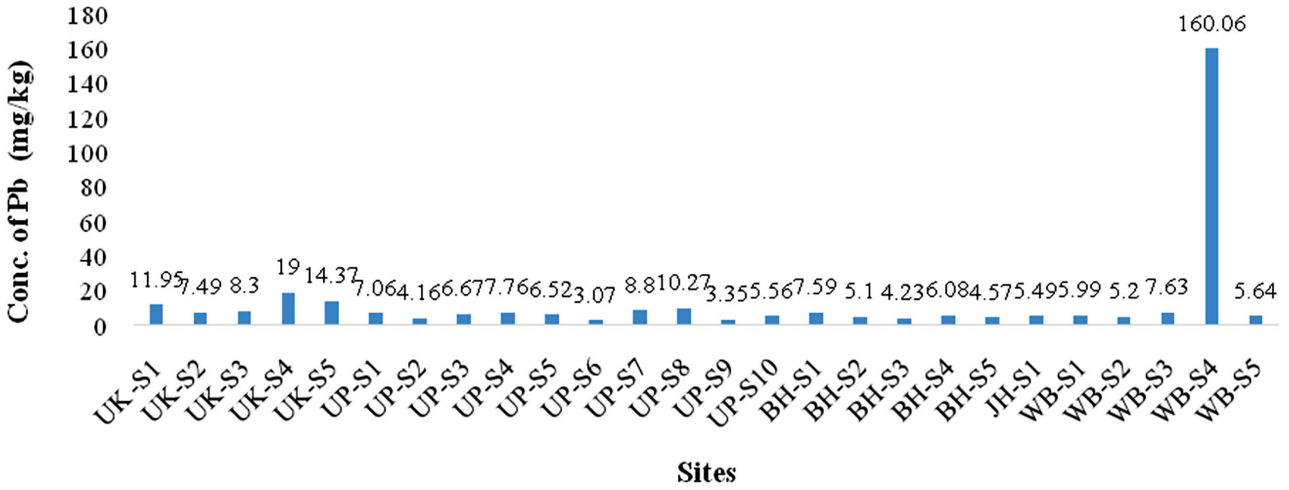


Fig. 10g Concentration of lead at different sites

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.

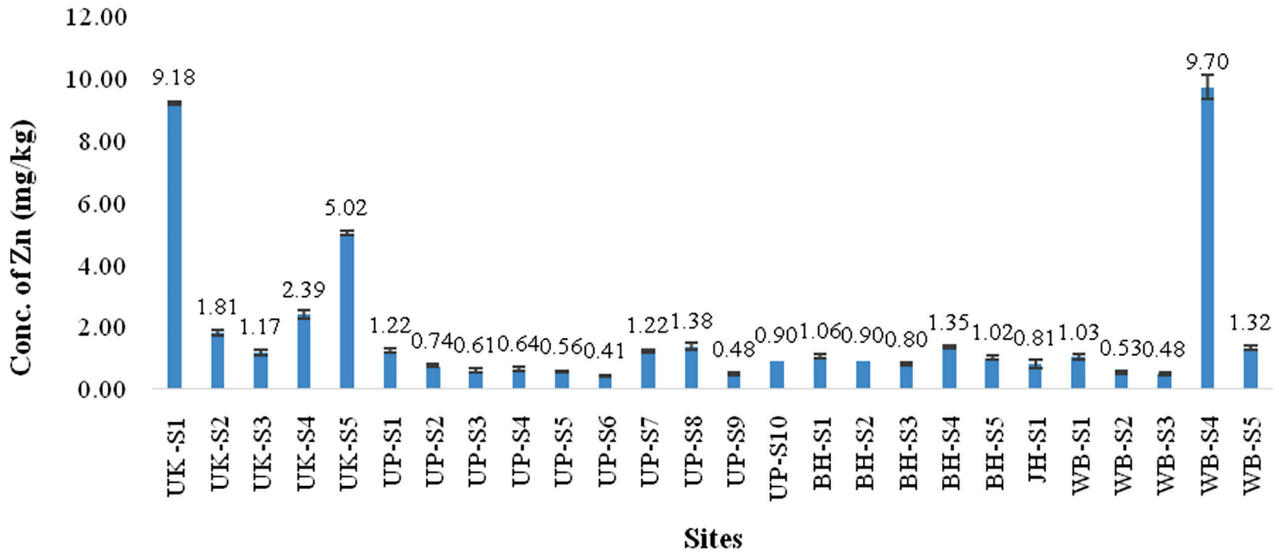


Fig. 11a Concentration of zinc at different sites

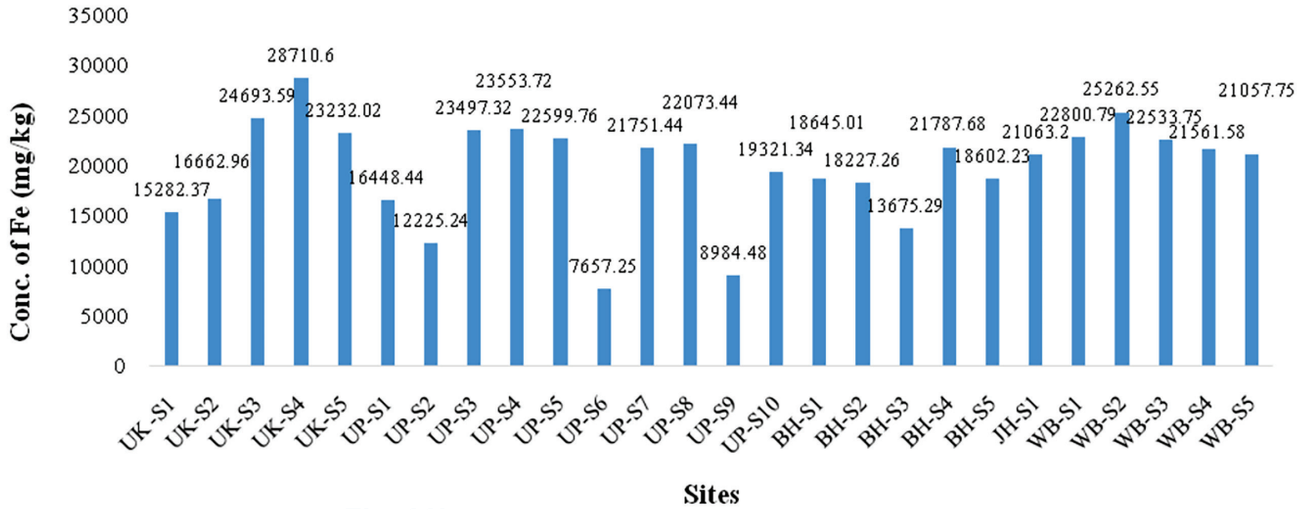


Fig. 11b Concentration of iron at different sites

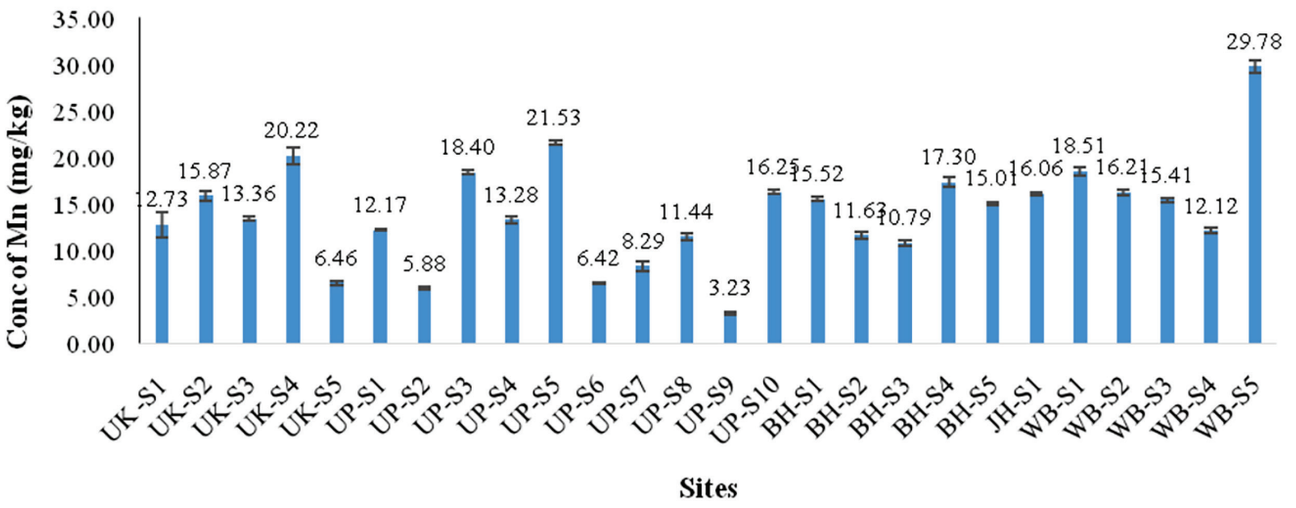


Fig. 11c Concentration of manganese at different sites

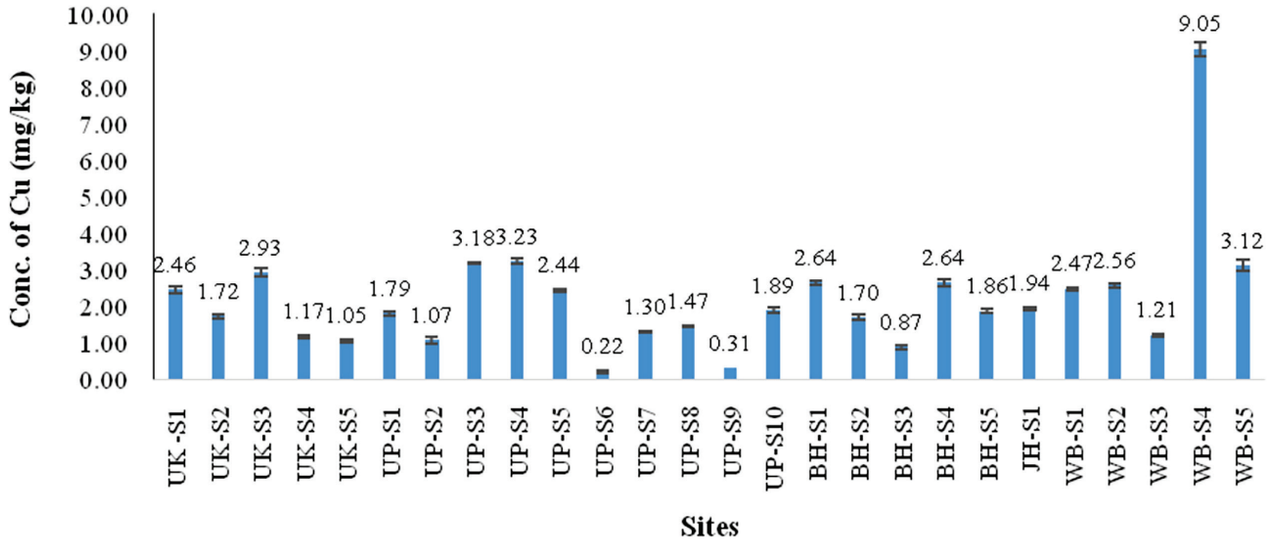


Fig. 11d Concentration of copper at different sites

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 *Tridax procumbens* L. in different locations shown in Fig. 12

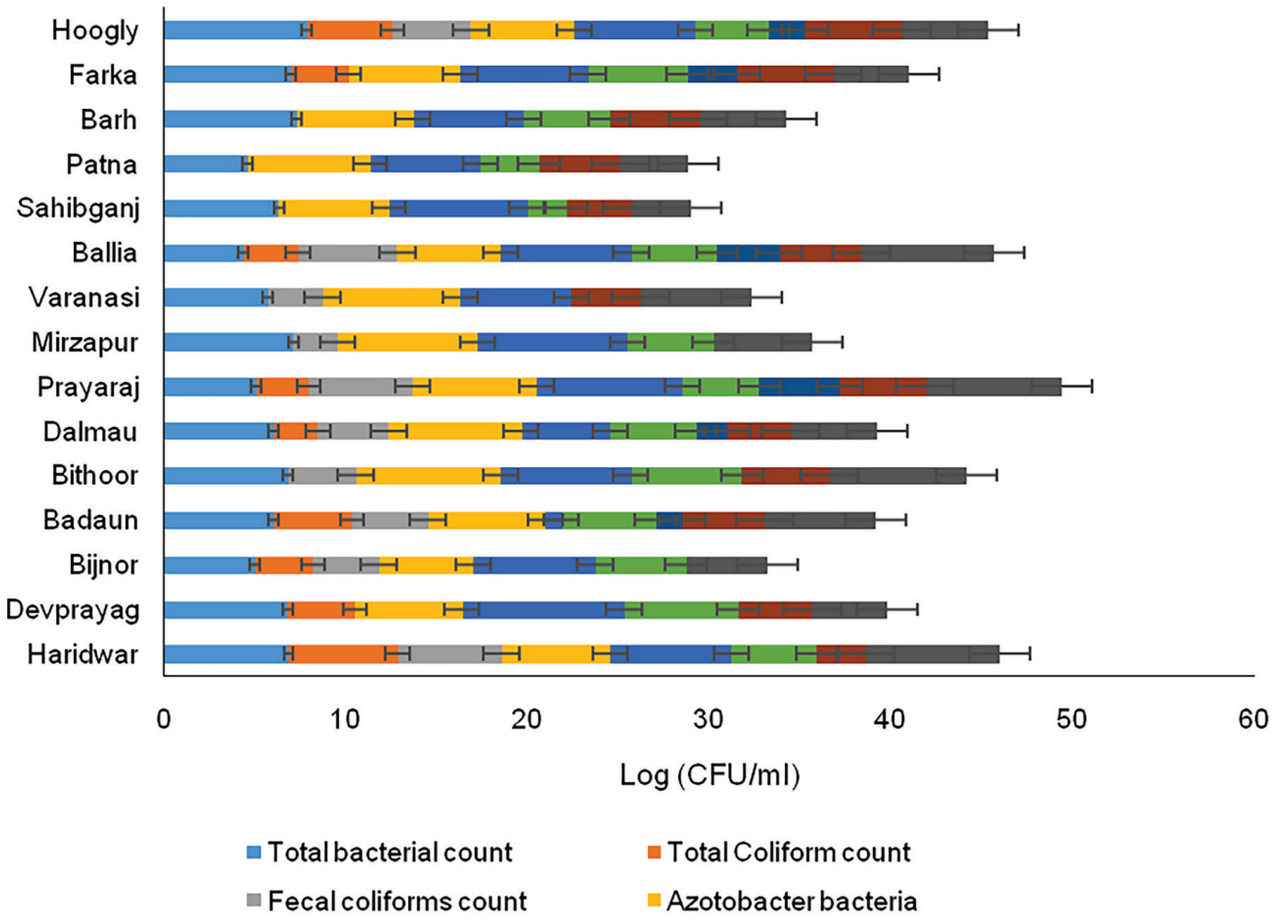
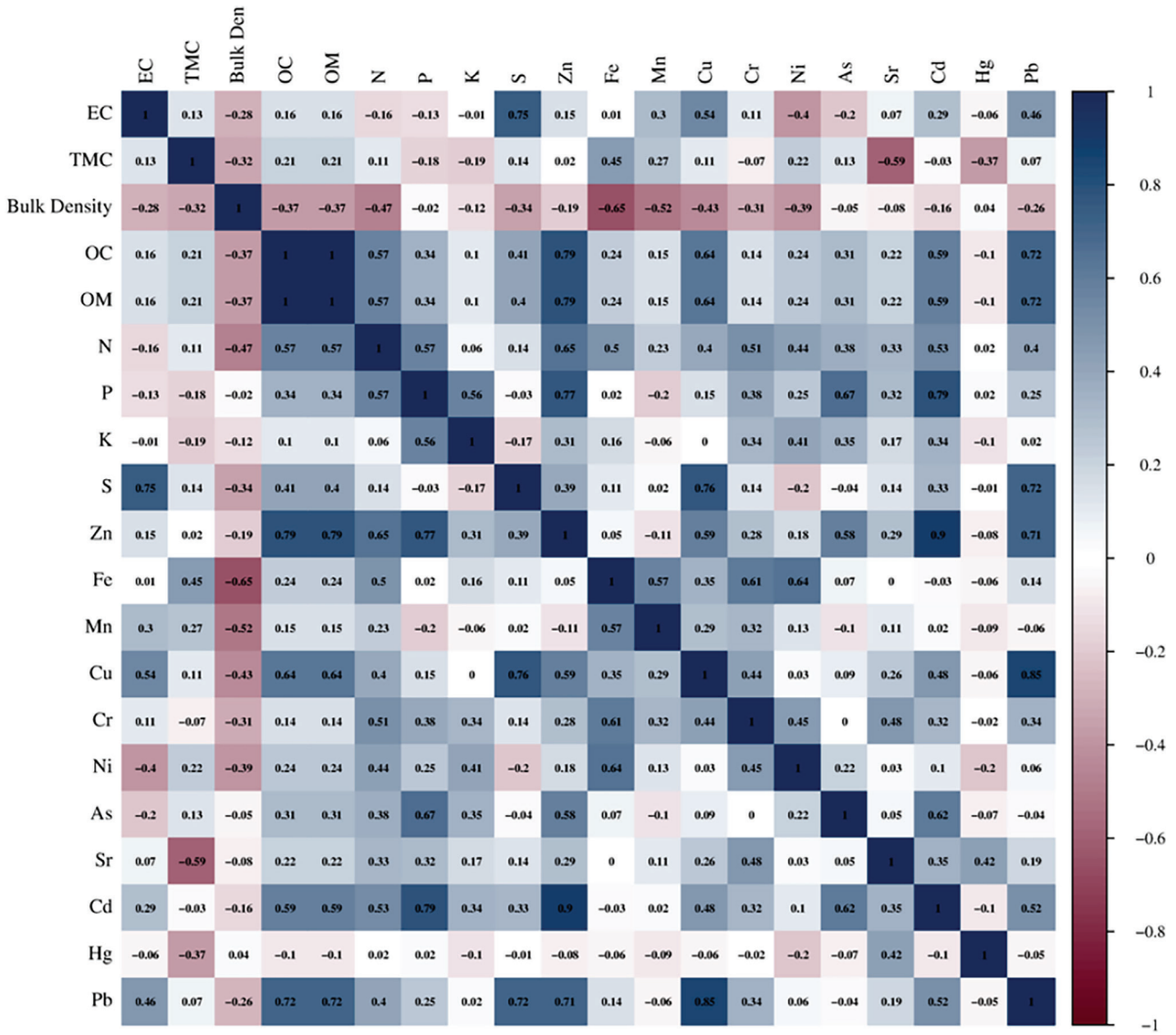


Fig. 12 Total and differential bacterial count

The total rhizosphere microbial test is performed for the identification of total bacterial count which is found highest in Hoogly 7.8636 cfu/ml and lowest in Ballia is 4.3570 cfu/ml. Whereas the total faecal and coliform count is highest in Hoogly and lowest in Dalmau 4.7075 and 2.4397 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

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

Sites	pH	EC	OC	N	P	K	S	Zn	Fe	Mn	Cu	Cr	Ni	As	Sr	Cd	Hg	Pb
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
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

Medicinal plants or plant-derived products play a valuable and significant role in the treatment of many diseases that occur in humans. There are numerous plants but, it is not easy to know the accurate number of medicinal plants available on earth till date (Shah and Baghel, 2017). Considering the ever-increasing demand for plant drugs, it is essential to maintain the quality, reproducibility, and efficacy of herbal drugs. Pharmacognostical standardization is an efficient tool to establish quality control parameters of plants. It helps to ensure the authentication of plants and the prevention of adulteration (Amponsah et al., 2014; Chanda, 2014). Standardization and quality control of plants are also essential for the worldwide approval of herbal products in the modern system of medicines. Hence, each country has adopted a set of guidelines and quality control of herbal medicine (Ahmad, 2007). *Tridax procumbens* Linn., a member of the Compositae family, is commonly known as 'Ghamra' and referred to as 'coat buttons' in English due to its flower's appearance. It has been widely utilized in Ayurvedic medicine for various ailments and is recommended as "Bhringraj," a renowned remedy for liver disorders. *T. procumbens*, native to tropical America, has naturalized in various regions, including tropical Africa, Asia, Australia, and India. This wild herb is widely distributed across India (Bhagwat et al., 2008). It grows as a low, spreading plant, often with a woody base that can root at the nodes. The plant can reach a height of up to 60 cm and has long, slender stems, about 12-24 cm in length (Jahangir, 1999). *T. procumbens* has been utilized to create various products such as oils, teas, and skin poultices. This plant species exhibits diverse pharmacological properties, including immunomodulatory, antioxidant, anti-hepatotoxic, analgesic, antidiabetic, anti-inflammatory, antifungal, and antimicrobial activities (Ravikumar et al., 2005; Bhagwat et al., 2008). The adaptability of the species is likely attributed to its defensive mechanisms, which include secondary metabolites such as flavonoids, alkaloids, tannins, carotenoids, and saponins (Beck et al., 2018).

Traditional and Ayurvedic Benefits

T. procumbens is a common plant that has a wide range of medicinal uses. It has been used as an anticoagulant, antioxidant, anticancer, immunomodulatory, and wound-healing agent in India for centuries. This medicinal plant is commonly used to treat ulcers. It is a well-known Ayurvedic medicine, used for liver diseases (Ghosh et al., 2019), respiratory problems, bronchial catarrh, dysentery, malaria, diarrhea, high blood pressure, and hemorrhage from cuts, bruises, and wounds for centuries (Pareek et al., 2009). Its leaves contain insecticidal and parasitic properties. It is also used to stop bleeding from minor injuries like cuts and bruises (Ghosh et al.,2019). The herb is also used to treat excessive blood pressure and blood sugar levels (Babu and Bairy, 2003; Gaikwadi et al., 2003). It may be used to stop hair fall and encourages hair growth. It possesses strong anti-insecticidal and immune-modulating properties (Mundada and Shivhare, 2008). Additionally, this medicinal plant was employed in the ethnic medical system to treat liver diseases and jaundice (Udupa et al., 1991). Further, the stones of the kidney were also treated using *T. procumbens* ethanol decoctions (Sailaja et al., 2011).

Therapeutic Potential

One of the major therapeutic plants found in subtropical regions, *T. procumbens* grows mostly during the rainy season. In Tamil Nadu, it is a prevalent weed that coexists alongside commercially significant crops (Suseela et al., 2002). It lives in roadside ditches, hedges, and waste areas all over India (Muthusamy et al., 2013). The foundation of many pharmaceutical industries is found in phytochemical components. Their numerous properties include antibacterial, antioxidant, anti-inflammatory, and many more (Savithramma et al., 2011). Despite being a widely accessible plant with numerous medical advantages, *T. procumbens* is nevertheless underutilized because of a lack of knowledge or awareness. In India, it has long been used as an insect-repellent, wound healer, anticoagulant, and antifungal. Leaves extracts were used in traditional medicine to treat infectious skin conditions. Other therapeutic properties of the plant include its ability to function as an antioxidant, hepatoprotective, immunomodulatory, anti-diabetic, antibacterial, promote hair growth, heal wounds, lower blood pressure, and have hematopoietic effects (Evbuomwan et al., 2023). In Table 1, a few significant medical applications along with their formulations are also discussed.

Table 1 Medicinal importance along with their preparation and mode of administration *Tridax procumbens* L.

S. No.	Parts Used	Preparations	Diseases Treated and Therapeutic Uses	References
1.	Whole plant	Juice	Wounds, boils, insect bites, diarrhea, bleeding, antiseptic, ulcer, fever, typhoid fever, cough, backache, stomachache, epilepsy	Soladoye et al., 2013; Koram and Ahorlu, 2014; Rao, 2015; Ebiloma et al., 2017; Pandey and Singh, 2017; Arunesh et al., 2018; Vishnuvardhan et al., 2018; Chinnappan, 2019; Yerramneedi et al., 2022
		Decoction along with *Phyllanthus amarus*	Anti-malarial, antibacterial, wound healing	
		Paste	Scorpion stings	
		-	Kidney stones, leprosy, ulcers, asthma, rheumatic pain, protozoal infections, chronic ulcers; anti-inflammatory, hepatoprotective, wound healing, antiseptic, hypotensive, bradycardiac.	
2.	Leaves	-	Cuts, wounds, skin diseases, dandruff, septic, scabies, dysentery, diarrhea, bleeding in bruises, boils, blisters, conjunctivitis, bronchial catarrh, dental problems, liver disorders, sores, eczema, pain, malaria, abdominal and gastrointestinal mycosis; antifungal, anticoagulant.	Cáceres et al., 1998; Ayyanar and Ignacimu-thu, 2005; Pareek et al., 2009; Upadhyay et al., 2010; Agban et al., 2013; Gairola et al., 2013; Jagtap et al., 2013; Sreeramulu et al., 2013; Bhatia et al., 2014; Singh et al., 2014; Nanadagopalan et al., 2015; Rahman and Kumar, 2015; Rao, 2015; Pardeshi and Bhiungade, 2016; Pradhan et al., 2016; Kumhar et al., 2017; Sultana and Rahman, 2017; Aadhan and Anand, 2018; Lakshmi et al., 2018; Kumar et al., 2019; Mohanty et al., 2019; Sinkar and Sa-marth, 2019; Charaya 2020; Mandal et al., 2020; Sahrawat et al., 2020; Agarwal and Rijhwani, 2021; Kumar and Singh, 2023

S. No.	Parts Used	Preparations	Diseases Treated and Therapeutic Uses	References
		Mixed with other herbs	Cough, chest complaints	
		Extract	Styptic	
		Infusion	Dysentery	
		Juice	Wounds, snake bites, bronchitis, dysentery, diarrhea, headache, skin infections, stomach ulcers, ear-ache, mouth sores, ringworm, sca-bies, inflamed skin, hemorrhage, stones in the urinary bladder, ane-mia, cold, inflammation, vaginitis, stomach pain, mucosal inflamma-tion, diabetes, insect repellent, hair loss, jaundice	
		Juice with curd	Diarrhea, dysentery, acidity, leukor-rhea, body heat, epistaxis	
		Poultice	Inflammation, gastrointestinal and respiratory infections, high blood pressure, diabetes	
		Paste	Wounds, cuts, bruises, warts, bloody stool, migraine, dysentery, bronchial disorders, eczema, sca-bies itching, swellings; hair tonic, antiseptic	

S. No.	Parts Used	Preparations	Diseases Treated and Therapeutic Uses	References
		Decoction	Eczema, wounds	
		Paste with milk	Stop bleeding during pregnancy	
3.	Aerial parts	-	Antifungal, diarrhea, blood clotting	Choudhary et al., 2023
4.	Roots	Decoction	Chronic fever	Gairola et al., 2013; Seliya and Shera, 2023
		Paste	Dysentery	
5.	Stem	-	Inflammation, gastrointestinal and respiratory infections, high blood pressure, diabetes	Poll, 2005

Ethnomedicinal and Folk treatments along the Ganga Basin of India

Uttarakhand, a natural setting of the Himalayas also known as 'Dev Bhoomi', is rich in medicinal plants and ancient medicinal expertise (Kumar et al., 2018). Several herbs are widely available in rural Uttarakhand for the treatment of various ailments on a local scale. These resources have gained a notable position in the arena of socio-culture, spirituality, and medicine (Dangwal et al., 2010). The state has five notified scheduled tribes: Tharu, Buksa, Bhotia, Jaunsari, and Raji, with a total population of 291903 (Sharma and Lata, 2022). Tharu are the largest primitive tribe in Uttarakhand who live interiorly in the forest and maintain a tight relationship with their surroundings (Rajpoot et al., 2016). They live in the Terai foothills and account for 31.3% of the state's ST population (Sharma and Lata, 2022). Several researchers (Bajpai et al., 2016; Chaudhary and Roi, 2017) have conducted ethnobotanical investigations among the Tharu tribe in Uttarakhand and its surrounding areas. People from Uttarakhand have used medicinal plants for thousands of years. *T. procumbens* leaf juice has been used to stop bleeding in cuts and wounds (Sharma and Lata, 2022). Furthermore, the inhabitants of Almora district use this plant for a variety of uses, including putting herb juice in their eyes to treat conjunctivitis and other eye problems. In glandular enlargement, leaves are boiled, ground into a paste, and applied to the affected area (Kumari et al., 2011). The inhabitants of Garhwal Himalaya use this herb to treat a variety of illnesses (Kumar et al. 2011). In addition, the Bhoksa (Buksa) community of district Dehradun knew this plant as Phulli and used the leaves infusion to treat diarrhea (Gairola et al., 2013). Further, the people in Udham Singh Nagar utilized leaf juice to heal boils and leaf paste to treat cuts and wounds (Sharma et al., 2014). Although indigenous people in the Tarai region of Kumaun Himalaya utilized the leaves to heal diarrhea, dysentery, cuts, and wounds (Chopra et al., 2019).

Uttar Pradesh has long been a thriving hub of the country's colourful culture. This area, sometimes referred to as the "rainbow land," is blessed with a wide range of unique geographical characteristics as well as cultural diversity (Singh et al, 2023). Residents of Uttar Pradesh use a variety of plants for medical, cosmetic, and other uses. The leaves of this plant, also known as phalanx, were used to treat cuts and wounds by the residents of the "Chatara" block in the District of Sonebhadra (Singh et al., 2010). Additionally, leprosy, eczema, boils, cuts, wounds, sores, stomachaches, toothaches, ulcers, and eye diseases are treated with this herb (Singh and Dubey, 2012). The leaf paste is utilized in the Ghazipur district to treat skin conditions and boils (Pandey and Sharma, 2012). In a different part of Uttar Pradesh, the Jhansi community employed the leaves of *T. procumbens* to cure skin conditions, applied an extract to wounds

to prevent sepsis, and drank the leaf decoction for seven days to manage hyperglycemia (Dassani et al., 2021). Moreover, the leaves of the plant are used to cure leukorrhea and cuts, while the paste from the plant is used to treat wounds, cuts, and scorpion stings in the Chandauli district (Srivastava and Shukla, 2018). Additionally, the inhabitants of Uttar Pradesh used this herb to treat diarrhea, dysentery, indigestion, and for wound healing (Singh et al., 2023), bronchial catarrh, dysentery, and hemorrhage (Aggarwal et al., 2012). However, *T. procumbens* leaf juice is used to alleviate earache and to treat dental issues and diarrhea in Saharanpur District (Kumar and Singh, 2023). The locals of Rampur also utilize this herb to treat wounds (Singh et al., 2020).

Additionally, many diverse types of medicinal plants have historically been found in Bihar. More than 67% of its people depend on agriculture either directly or indirectly. The biodiversity of herbal plants is abundant in the state. Nevertheless, large-scale commercial cultivation of the same has not yet begun. There has been a recent push to raise public awareness of the value and use of medicinal plants. Because of the market's rapidly increasing demand and the involvement of a few enterprising farmers, the state began cultivating therapeutic herbs (Singh, 2018). Bihar's populace and tribal people employed a variety of medicinal plants to treat a range of illnesses. They employed the juice of *T. procumbens* leaves to treat snake bites and bleeding (Kumar, 2020). This plant was considered a highly therapeutic herb in the Buxar district, where it was used to treat cuts, bruises, and toothache (Kumari et al., 2016). Moreover, its leaf paste is also applied to boils and skin conditions (Singh et al., 2013).

Further, the state Jharkhand has 26.91 million residents overall, of which 77.80% live in rural areas and 22.50% are members of schedule tribes. Tribes, forest inhabitants, and rural residents possess extensive traditional, indigenous and ethnic knowledge of surrounding flora and woods. Numerous indigenous communities, including those of Santhal, Paharia (including Saurua Paharia, Mal Paharia, and Kumar Bhag), Oraon, Munda, Kol, Kharwar, Ho, Asur, and Baiga, among others, are found in the state and possess extensive ethnobotanical knowledge. The surrounding forest areas are used by the tribes for gathering and using a variety of herbs, roots, rhizomes, tubers, flowers, fruits, leaves, and seeds of many important plants for their daily requirements and health care. One of the biggest tribes, the Santhal are of the Astro-Asian race. They and the Paharia tribes are primarily found in the Santhal Pargana region. Tribal communities residing on the Chotanagpur plateau include Munda, Oraon, Kol, Kharwer, and others (Kumar and Abbas, 2012). These Jharkhandi tribal and indigenous people employed a variety of plants for therapeutic purposes. *T. procumbens* is utilized to treat skin conditions. Additionally, the entire plant is ground up and applied as a poultice to chronic wounds by the people of the Hazaribagh district. The plant is powdered and dried before being applied to the wounds (Divakara and Prasad, 2015), its juice is helpful

in the management of diarrhea and dysentery (Lal et al., 2012). The roots of this plant, known as *muriya* in Pakur, are used as abortifacient. Eczema, skin infections, wounds, and injuries are treated using a paste made from leaves combined with the same amount of turmeric. It is used to stop hair loss, by topical application of leaf paste on the scalp. Juice from fresh flowers and leaves is administered as an antibacterial to cuts and wounds (Mukherjee and Jha, 2021). Despite being prepared as a vegetable in West Singhbhum, the plant's leaves have antidiarrheal, and antidysenteric properties. The leaf juice has insecticidal, parasiticidal, and antiseptic qualities (Horo and Topno, 2015). Tribes of Oraon in Jharkhand's Latehar district use crushed leaves and (krait and cobra) drip their juice onto the bite wound. Some juice is also applied to the area of the body where the snake bites. Similarly, to cure diarrhea and dysentery, the leaf paste is taken orally after being dissolved in a cup of water (Marandi and Britto, 2014). Tribal people in the Lohardaga district utilized this herb to cure high blood pressure, diarrhea, dysentery, and snake bites (Kumari et al., 2020).

Moreover, West Bengal has experienced notable expansion in its agriculture sector (Roy et al., 2018). Since ancient times, medicinal plants have been an essential component of human life to combat various illnesses. Around the world, more than 80,000 plants are utilized as medicines, and the majority of these are customarily used from generation to generation. It implies that the foundation of traditional or folklore remedies is made up of medicinal plants. People of Bengal employed a wide variety of medicinal plants to treat a wide range of illnesses (Hossain et al., 2014; Ghosh et al., 2019). In addition, leaves are used as an antiseptic, insecticidal, parasiticidal, and to stop bleeding from cuts, bruises, and wounds. The stem can also be soaked in water and used to cure migraines (Rao et al., 2006). Its leaves are used to treat rheumatism (Bapuji and Ratnam, 2009), diarrhea, and dysentery (Swarup et al., 2016), and cuts and wounds heal quickly when the fresh leaf juice is applied (Banerjee et al., 2016). In Puruliya District, *T. procumbens* leaf extract was also used to heal external injuries (Sur et al., 2008; Mandal and Mukherjee, 2016). Leaf juice is used as a pesticide and to halt bleeding from wounds in Jalpaiguri district (Saha et al., 2013). In Purba Medinipur, people used the leaves juice to stop the bleeding. Furthermore, stem juice is used to cure diarrhea (Patra et al., 2017). In the South of 24 Parganas District, the leaves of this plant are used to treat dysentery, diarrhea, and bronchial catarrh (Naskar et al., 2022). In the Dakshin Dinajpur district, this herb is used to treat cold and cough (Sarkar et al., 2023).

Conclusion and Future Perspectives

T. procumbens is a significant medicinal plant that has been utilized for both formal (Ayurveda, Unani) and informal (tribal, indigenous, folk) traditional medical practices from before the beginning of recorded history. Since these might be a great source of lead compounds for the treatment of a variety of medical ailments, recent technical advancements in the identification, isolation, and validation of active principles from medicinal plants have gained prominence. With its wide range of pharmacological and therapeutic uses, *T. procumbens* seems to be a very promising medicinal plant with many active compounds. Its potential for pharmacological, nutritional, phytochemical, and botanical qualities is immense. There is an enormous amount of opportunity for future studies to uncover other pharmacological properties of this plant and clarify its mode of action. In the future, the pharmaceutical industry may rely heavily on this medicinal plant as a source of herbal medications.

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CHAPTER

05

Phytochemical Analysis

INTRODUCTION

Tridax procumbens, also known as the "yellow daisy", is a traditional Ayurvedic herb native to Asia, belonging to the Asteraceae family. It has been used in natural remedies for various medicinal purposes (Beck et al., 2018). The plant has a wide range of bioactivities, including antimicrobial, antioxidant, anticancer, and anti-inflammatory effects (Ingole et al., 2022). The phytochemical composition of *T. procumbens* has been extensively studied, revealing the presence of various compounds such as tannins, saponins, steroids, alkaloids, phytosterols, essential oils, etc. These compounds have been found in different extracts of the plant, such as ethanol extracts, water extracts, and leaf extracts (Ikewuchi et al., 2015; Beck et al., 2018). The leaves of this plant have been considered a potential source of nutraceuticals and functional food due to their high content of bioactive compounds. Its traditional usage includes antimicrobial, antioxidant, anticancer, and anti-inflammatory activities (Christudas et al., 2011; Beck et al., 2018; Ingole et al., 2022).

Sampling Sites

Table 1 Sampling sites for the *Tridax procumbens* plants collected from various state-specific locations spread over the Gangetic course

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

S. No.	Sampling sites
11.	Farka, Bihar
12.	Sahibganj, Jharkhand
13.	Gomukh, Uttarakhand
14.	Gangotri, 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. Advanced methods and techniques were employed for the identification and quantification of secondary metabolites and active components within the collected samples. High-performance thin layer chromatography (HPTLC) served to detect 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 obtained and then mixed with 50 ml of high-purity milli-Q water. The mixture was carefully shaken and sonicated for 30 minutes. After that, the volume was increased to 100 ml and the mixture was filtered. From the resulting filtrate, 10 ml was taken and mixed with 750 ml of milli-Q water. Then, 25 ml of indigo sulphonic acid was added and the solution was thoroughly shaken. The titration process was started using a 0.1N potassium permanganate solution until a desired golden yellow colour was achieved. It is important to also perform a blank run without any sample to ensure accurate and precise experimental analysis.

Determination of Saponin Content

A 5 g sample was collected and mixed with 50 ml of a solvent consisting of a 1:1 ratio of methanol to water. The mixture was then heated under reflux for 1 hour, cooled, and filtered. This process was repeated three

times. The filtrate from each repetition was combined, concentrated, and evaporated until it became dry. Next, 25 ml of petroleum ether was added, and the mixture was refluxed 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 placed in a test tube and mixed with 1 ml of Folin–Ciocâlteu reagent. 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 taken in a test tube. To this, 0.4 ml of 10% aluminium chloride, 0.4 ml of sodium acetate, and 3 ml of ethanol were added. The mixture was then kept at room temperature for 30 minutes. After that, the absorbance was measured at 450 nm using a UV-visible spectrophotometer. These steps were eated with querdihydrate to create a linear plot.

HPTLC Fingerprinting

The application of high-performance thin-layer chromatography (HPTLC) in the study reflects a prevalent methodology documented in the literature for profiling secondary metabolites. The use of HPTLC not only enables the rapid separation of compounds but also aligns with the commonly reported diverse chemical fingerprints found in *Tridax* species. 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 particular herb. A colour image of typical TLC fingerprint provides a clearer guide to the users.

Sample Preparation

To analyse each batch, we took approximately 1 g of sample and dissolved it in 10 ml of methanol. After shaking and sonication for 20 minutes, the solution was centrifuged at 5000 rpm for 5 minutes. The resulting clear solution was then used for analysis. The samples were carefully spotted according to the corresponding serial numbers specified in Table 1.

Methodology and Analytical Conditions

Analysis was performed on CAMAG HPTLC (Muttenz, 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:

Stationary phase	TLC Silica gel 60 F_{254} aluminium sheet (1.0554.0007)
Mobile phase	Toluene: Formic acid: Ethyl acetate (5:1:5)
Saturation time	15 minutes
Migration distance	80 mm
Band length	8 mm
Injection volume	10 µl
Visualization	254 nm and 366 nm; under white light before derivatization

High-Performance Liquid Chromatography (HPLC)

Sample and Standard Preparation

Sample: About 1.5 gm of the sample was dissolved in 25 ml of water: methanol (50:50), sonicated for 2 hours at 60°C and filtered through 41 number Whatman filter paper. The extraction was repeated one more time before pooling all the extract together. The final volume was made up to 50 mL and filtered through a 0.45 µ filter paper before using it for further analysis.

Standard: Caffeic acid of concentration 50 ppm was prepared from 1000 ppm stock solution in water: methanol (50:50 v/v).

Analytical and Instrumentation Condition

Analysis was performed on the Prominence-i HPLC system (Shimadzu, Japan). Separation was achieved using a Shodex C18-4E (5 µm, 4.6*250 mm) column subjected to binary gradient elution. The two solvents used for the analysis were water containing 0.1% acetic acid in water (solvent A) and acetonitrile (solvent B). The column temperature was kept at 35°C and flow was set at 1.0 ml/min during the analysis. Ten microliters of standard and test solution were injected. The wavelength is set at 325 nm (for neochlorogenic acid, chlorogenic acid and cryptochlorogenic acid).

Gradient program used

Time (Min)	A%	B%
0.01	100	5
10	95	15
20	92	25
30	88	35
40	80	55
50	65	77
52	45	5
55	5	5

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

This analysis aimed to identify the major compounds in the *Tridex procumbens* test sample (PRF/CHI/1123/1085).

Sample Preparation

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 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 Acquity UPLC HSS-T3 (100 x 2.1 mm, 1.8 µm) column with the 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. One microliter of test solution was injected in UPLC/MS-QToF and chromatograms were recorded in positive and negative ionization mode.

Gradient Program

Time (min)	Flow (ml/min)	Mobile phase A %	Mobile phase B%
0	0.3	95	5
5	0.3	95	5
10	0.3	90	10
25	0.3	75	25
40	0.3	60	40
50	0.3	40	60
55	0.3	30	70
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^E	MS^E
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 Enkephalin)	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 of the plant samples exhibited the presence of tannins, saponins, total phenolics, and total flavonoids in diverse percentages as reflected accurately in the table given below (Table 2).

Table 2 Phytochemical analysis of *Tridax procumbens* collected from different locations

S. No.	Sample code	Tannin content (%w/w)	Saponin content (%w/w)	Total polyphenol content (%w/w)	Total flavonoid content (%w/w)
1.	Bijnor, Uttar Pradesh	0.099	3.189	0.391	0.006
2.	Badaun, Uttar Pradesh	0.019	10.830	0.518	0.007
3.	Bithoor, Uttar Pradesh	0.039	7.182	0.525	0.003
4.	Dalmau, Uttar Pradesh	0.039	8.360	0.303	0.006
5.	Prayagraj, Uttar Pradesh	0.080	4.811	0.288	0.004
6.	Mirzapur, Uttar Pradesh	0.040	5.614	0.288	0.001
7.	Varanasi, Uttar Pradesh	0.038	7.646	0.254	0.004
8.	Ballia, Uttar Pradesh	0.162	5.908	0.353	0.003
9.	Patna, Bihar	0.039	5.025	0.321	0.007
10.	Barh, Bihar	0.038	5.937	0.323	0.003
11.	Farka, Bihar	0.040	4.613	0.778	0.005
12.	Sahibganj, Jharkhand	0.040	6.095	0.259	0.002
13.	Gomukh, Uttarakhand	0.039	10.408	0.604	0.008
14.	Gangotri, Uttarakhand	0.060	11.760	0.448	0.007
15.	Farraka, West Bengal	0.040	3.821	0.324	0.007

The variance in the content of these phytochemicals across different sampling locations indicates environmental and geographical influence on the secondary metabolite production in *T. procumbens*. High saponin content, especially in samples from Badaun and Gangotri, aligns with reports highlighting saponins as potent bioactive compounds with antimicrobial and anticancer properties (Ikewuchi et al., 2015; Beck et al., 2018).

HPTLC Fingerprint Analysis

The HPTLC analysis provided a comprehensive fingerprint of the methanolic extracts of *T. procumbens*, revealing distinct bands at 254

nm and 366 nm. The HPTLC chromatograms (Fig. 1) under these wavelengths exhibited high-intensity bands corresponding to different phytoconstituents, reflecting the chemical diversity of the plant.

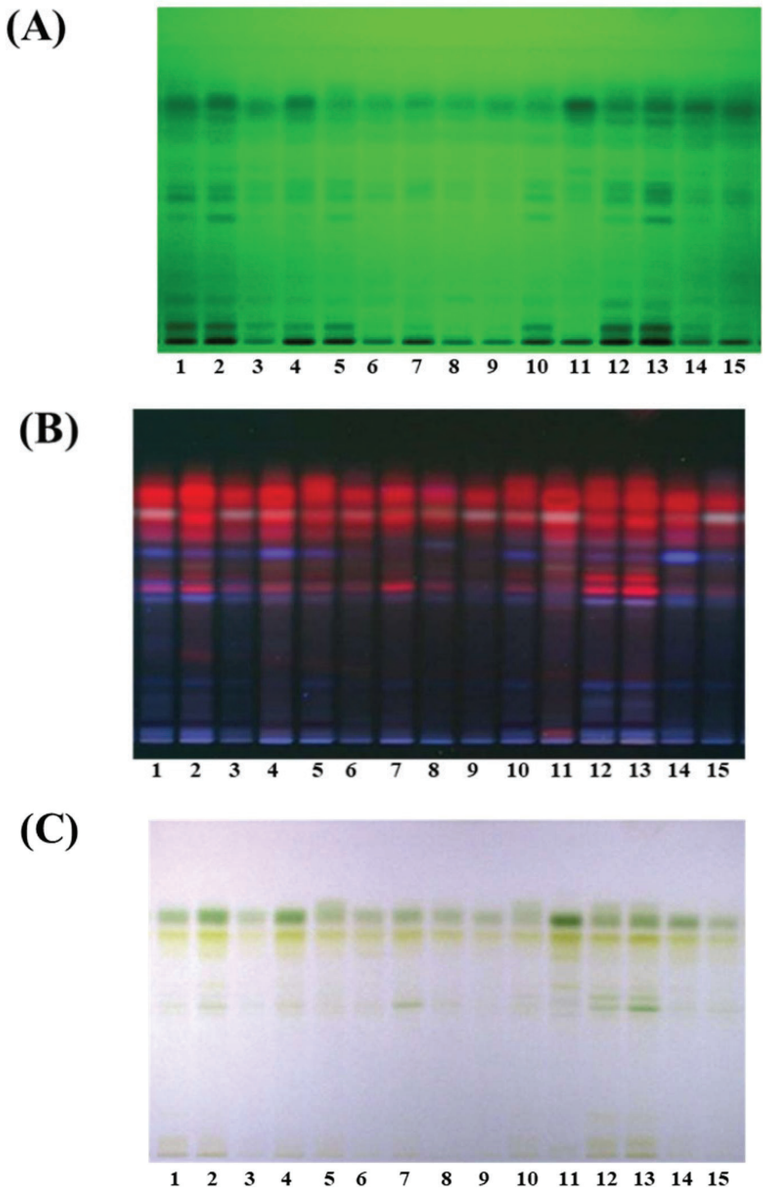

Fig. 1 HPTLC fingerprinting of methanolic extract of *Tridax procumbens*. **A.** 254 nm, **B.** 366 nm **C.** Under white light without derivatization

Previous studies have employed HPTLC to profile secondary metabolites in *Tridax* species, emphasizing its efficiency in detecting qualitative differences in chemical composition (Christudas et al., 2011; Ingole et al., 2022). The chromatographic pattern obtained aligns well with the established chemical profiles reported in the literature.

HPLC Analysis

HPLC analysis was performed using caffeic acid as a reference compound. The data exhibiting different percentages of the compound has been presented in the table below. The respective chromatograms representing the reference standard and the respective plant samples from 15 different locations against a 50 min run have been presented below for reference.

The chromatographic data (Table 3) revealed variations in caffeic acid content, with significant concentrations in samples from Gomukh and Gangotri, indicating high antioxidant potential. HPLC chromatograms (Fig. 2 and Fig. 3) depict the standard and sample profiles, highlighting the peaks corresponding to caffeic acid. The observed content is consistent with reports on the phenolic profile of *T. procumbens*, reinforcing its antioxidant properties (Beck et al., 2018).

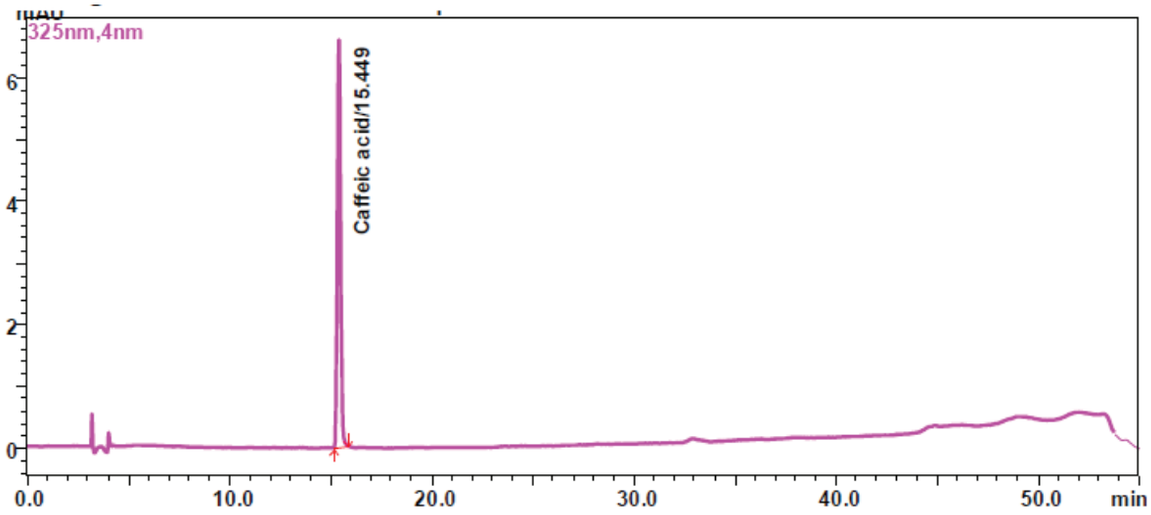

Fig. 2 HPLC profile of Caffeic acid (reference standard)

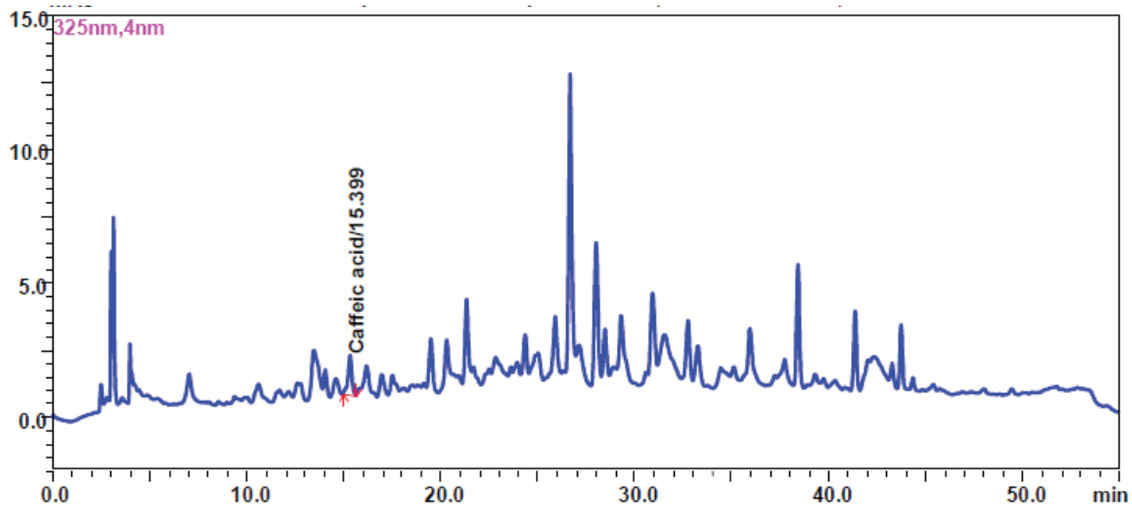

Fig. 3 HPLC profile of *Tridax procumbens* raw material

Table 3 Phytochemicals content present in *Tridax procumbens* collected from different locations

S. No	Internal code	External code	Caffeic acid (%w/w)
1	PRF/CHI/1123/1079	Bijnor, Uttar Pradesh	0.112
2	PRF/CHI/1123/1080	Badaun, Uttar Pradesh	0.122
3	PRF/CHI/1123/1081	Bithoor, Uttar Pradesh	0.107
4	PRF/CHI/1123/1082	Dalmau, Uttar Pradesh	0.043
5	PRF/CHI/1123/1083	Prayagraj, Uttar Pradesh	0.055
6	PRF/CHI/1123/1084	Mirzapur, Uttar Pradesh	0.025
7	PRF/CHI/1123/1085	Varanasi, Uttar Pradesh	0.059
8	PRF/CHI/1123/1086	Ballia, Uttar Pradesh	0.062
9	PRF/CHI/1123/1087	Patna, Bihar	0.04
10	PRF/CHI/1123/1088	Barh, Bihar	0.079
11	PRF/CHI/1123/1089	Farka, Bihar	0.032
12	PRF/CHI/1123/1090	Sahibganj, Jharkhand	0.063
13	PRF/CHI/1123/1091	Gomukh, Uttarakhand	0.338
14	PRF/CHI/1123/1092	Gangotri, Uttarakhand	0.316
15	PRF/CHI/1123/1093	Farraka, West Bengal	0.014

UPLC/MS-QToF Analysis

The UPLC/MS-QToF analysis was conducted to identify and quantify major bioactive compounds. This high-resolution technique allows for the precise separation and identification of compounds based on their mass-to-charge ratio (m/z) and retention time (RT). The analysis revealed a complex profile of phytochemicals and enabled high-resolution separation and identification of 14 major compounds in *T. procumbens*. The TIC chromatogram (Fig. 4) and compound-dependent parameters (Table 4) provide detailed insights into the phytochemical composition.

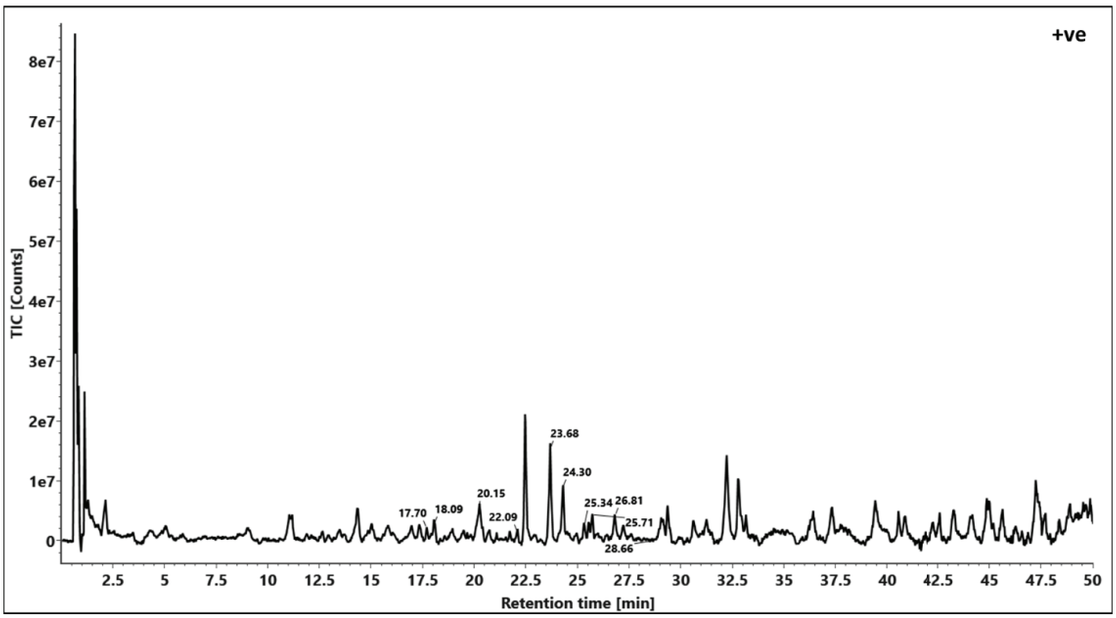

Fig. 4 TIC chromatogram of TIC chromatogram of *Tridax procumbens* (PRF/CHI/1123/1085) in positive ionization mode

Table 4 Compound-dependent parameters of analytes/identified compounds in *Tridax procumbens* (PRF/CHI/1123/1085) in positive ionization mode

S. N.	Component name	Formula	Neutral mass (Da)	Observed m/z	Mass error (mDa)	RT (min)	Response	Adducts
1	Rutin	$C_{27}H_{30}O_{16}$	610.1534	611.1613	0.6	17.70	43821	+H
2	Isoquercitin	$C_{21}H_{20}O_{12}$	464.0955	465.1015	-1.2	18.09	52814	+H
3	Luteolin-7-O-glucoside	$C_{21}H_{20}O_{11}$	448.1006	449.1075	-0.4	20.15	20500	+H
4	Isorhamnetin-3-O-glucoside	$C_{22}H_{22}O_{12}$	478.1111	479.1172	-1.2	22.09	109257	+H
5	Nevadensin 5-gentiobioside	$C_{30}H_{36}O_{17}$	668.1953	669.2044	1.8	23.68	970475	+H
6	Tridaxidone	$C_{24}H_{26}O_{13}$	522.1373	523.1452	0.6	24.30	440308	+H
7	Kaempferol	$C_{15}H_{10}O_{6}$	286.0477	287.0538	-1.2	25.34	195662	+H
8	Esulatin E	$C_{28}H_{36}O_{10}$	532.2309	555.2208	0.7	25.71	51294	+Na
9	Isorhamnetin	$C_{16}H_{12}O_{7}$	316.0583	317.0645	-1.0	26.81	231318	+H
10	Apigenin	$C_{15}H_{10}O_{5}$	270.0528	271.0583	-1.8	28.66	30586	+H

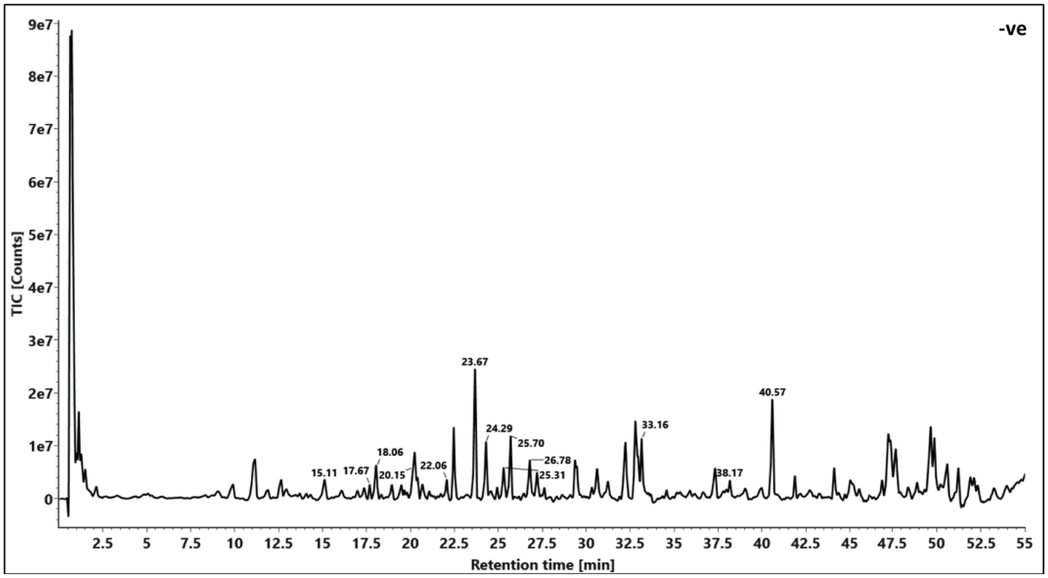

Fig. 5 TIC chromatogram of *Tridax procumbens* (PRF/CHI/1123/1085) in negative ionization mode

Table 5 Identified compounds in *Tridax procumbens* (PRF/CHI/1123/1085) in negative ionization mode

S. N.	Component name	Formula	Neutral mass (Da)	Observed m/z	Mass error (mDa)	RT (min)	Response	Adducts
1	Sinapic acid	$C_{11}H_{12}O_5$	224.0685	223.0616	0.4	15.11	586869	-H
2	Rutin	$C_{27}H_{30}O_{16}$	610.1534	609.1465	0.4	17.67	253730	-H
3	Isoquercitin	$C_{21}H_{20}O_{12}$	464.0955	463.0888	0.6	18.06	363301	-H
4	Luteolin-7-O-glucoside	$C_{21}H_{20}O_{11}$	448.1006	447.0946	1.3	20.15	283065	-H
5	Isorhamnetin-3-O-glucoside	$C_{22}H_{22}O_{12}$	478.1111	477.1033	-0.5	22.06	281333	-H
6	Nevadensin 5-gentiobioside	$C_{30}H_{36}O_{17}$	668.1953	667.1878	-0.2	23.67	3081116	-H
7	Tridaxidone	$C_{24}H_{26}O_{13}$	522.1373	567.1358	0.2	24.29	965199	+HCOO
8	Kaempferol	$C_{15}H_{10}O_6$	286.0477	285.0411	0.6	25.31	514836	-H
9	Esulatin E	$C_{28}H_{36}O_{10}$	532.2309	531.2236	0.0	25.70	935498	-H
10	Isorhamnetin	$C_{16}H_{12}O_7$	316.0583	315.0512	0.2	26.78	601997	-H
11	Apigenin	$C_{15}H_{10}O_5$	270.0528	269.0448	-0.8	28.62	63371	-H
12	Methyl 3-O-ß-laminaribiosyl polygalacate	$C_{43}H_{70}O_{16}$	842.4664	841.4591	0.0	33.16	497270	-H
13	Eclalbasaponin I	$C_{42}H_{68}O_{14}$	796.4609	841.4596	0.5	38.17	302278	+HCOO
14	Eclalbasaponin II	$C_{36}H_{58}O_9$	634.4081	633.4028	2.0	40.57	1110703	-H

The identification of rutin, isoquercetin, and other flavonoids corroborates with similar studies on *T. procumbens*, where these compounds have been highlighted for their significant pharmacological activities (Christudas et al., 2011; Ingole et al., 2022). These compounds have been extensively studied for their pharmacological properties, such as antioxidant, anti-inflammatory, and anticancer activities. For instance, rutin and quercetin are commonly found in various Asteraceae species and are known for their significant antioxidant properties.

The UPLC-QToF method provides a robust and comprehensive analysis, ensuring accurate identification and quantification of these bioactive compounds.

Conclusion

The objective of the study was to analyse the content of metabolites and their percentages in different samples of *Tridax procumbens* collected from the Gangetic course. The samples tested positive for phytochemicals such as tannins, saponins, polyphenols, and flavonoids. The content of flavonoids was similar in all samples, but the content of polyphenolics, saponins, and tannins was highest in the samples from Farka (Bihar), Gangotri (Uttarakhand), and Ballia (Uttar Pradesh) respectively.

The comprehensive analysis of *Tridax procumbens* through phytochemical, HPTLC, HPLC, and UPLC/MS-QToF techniques has revealed a rich profile of bioactive compounds, including flavonoids, phenolic acids, tannins, and saponins. The UPLC/MS-QToF analysis, in particular, provided high-resolution identification and quantification of major compounds like rutin, quercetin, isoquercitrin, and caffeic acid. These findings are consistent with previous studies and highlight the significant pharmacological potential of *T. procumbens*.

The variations in phytochemical content across different geographical locations underscore the influence of environmental factors on the secondary metabolite production in *T. procumbens*. The identified compounds are known for their antioxidant, anti-inflammatory, and anticancer properties, supporting the traditional medicinal uses of this plant.

Future research should focus on isolating these bioactive compounds and exploring their specific mechanisms of action. Additionally, the development of standardized extraction and analysis methods will be crucial for ensuring the quality and efficacy of *T. procumbens*-based herbal formulations.

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CHAPTER

06

Insilico Analysis Against Glioma

INTRODUCTION

A central nervous system (CNS) tumor, an irregular proliferation of cells arising from neural tissues within the brain or spinal cord, encompasses a diverse spectrum of over 120 distinct cancerous forms (Louis et al., 2016). Massive amounts of clinical data on patients with CNS tumors have been amassed over the past 50 years due to increasing digitalization and integration of medical records. Among all primary CNS tumors identified, 26% are identified as malignant, constituting approximately 81% of the total primary CNS tumor cases (Yearley et al., 2023). Recent data from the Surveillance, Epidemiology, and End Results (SEER) program indicate an incidence rate of 24.71 CNS tumors per 100,000 individuals in the United States annually, with 7.02 being malignant and 17.69 benign in nature. Glioma is a form of tumor that emerges from the supportive glial cells in the brain or spinal cord, varying in severity based on location, grade, and characteristics, presenting challenges in treatment due to their intricate placement within the central nervous system (Chen et al., 2017). Glioma, comprising roughly 44% of CNS tumors, stands as one of the most prevalent primary malignant brain tumors. However, the overall survival (OS) rates for glioma remain disheartening (Guo et al., 2023). Malignant glioma is a type of cancer that affects more than 22,000 Americans each year (Cuddapah et al., 2014).

In general, tumors, especially glioma, release tumor-associated contents, like circulating tumor cells (CTCs), proteins, extracellular vehicles (EVs), and cell-free nucleic acids (cfNAs) into the blood and cerebrospinal fluid (CSF) (Sumera et al., 2022). Gliomas can spread outside the brain through different pathways, such as through the lymphatic system, direct growth into bone, and invading veins. Bradykinin is an example of a chemoattractant that is produced by endothelial cells that attract glioma cells to the perivascular region around blood vessels. Additionally, perivascular invasion has been connected to glioma cells that overexpress chemokine receptors. Axonal guidance molecules, a group of proteins that function as attractive or repelling factors, control cell movement along white matter tracts, a second recognized pathway of glioma cell invasion. Genomic analysis of glioma uncovered multiple signaling pathways and gene alterations

that are essential for its growth. Epidermal growth factor receptor (EGFR), mitogen-activated protein kinase 1 (MAPK1), proto-oncogene tyrosine-protein kinase (SRC), tumor necrosis factor (TNF), tumor protein 53 (TP53) and heat shock protein 90 alpha family class A (HSP90AA1or HSP90) may be linked with the pathogenesis of the disease and aggressive tumor behavior (Khabibov et al., 2022). Despite the combination of surgery, radiotherapy, chemotherapy, targeted therapy, and tumor-treating field treatment, there have only been four drugs and one device ever approved by the FDA specifically for the treatment of glioma (Fisher and Adamson, 2021). Therefore, it is critical to understand the mechanisms of malignant glioma and develop novel therapeutic approaches. For many, ayurvedic concepts are difficult to understand, and a straightforward chemical evaluation is the better approach to take. It has numerous *in vitro* and *in vivo* tests to support its medicinal uses and is widely utilized in folkloric medicine. Consequently, traditional medicine is a crucial source for the creation of innovative chemotherapeutic drugs that are less harmful and more affordable (Datta et al., 2021).

The Asteraceae family member *Tridax procumbens* is a common tropical grass. It is an invasive weed that is also known as 'Jayanti Veda', 'Ghamra' in Hindi, and Coat Buttons in English due to the way its blossoms look. Since the dawn of time, this species has been used in Indian Ayurveda as a traditional treatment for a wide range of illnesses, such as cancer, jaundice, and liver diseases (Naqash and Nazeer, 2011). It is a very promising species that produces secondary metabolites such as alkaloids, steroids, phytosterols, tannins, carotenoids, flavonoids (catechins, centaurein, and bergenins), fatty acids, and minerals reported to have a variety of medicinal uses. Due to the presence of these secondary metabolites, *T. procumbens* shows various pharmacological activities such as anticoagulant, antimicrobial, anti-arthritic, antioxidant, anti-hyperglycemic, antifungal, insect repellent, anti-inflammatory, analgesic, liver injury, anti-cancer, etc. (Kethamakka and Deogade, 2014). The development of novel medications to treat gliomas typically employs several approaches. To reduce the time needed for identification, characterization, and structure optimization, computational drug design is a highly helpful tool for a variety of therapeutic approaches (Li et al., 2021). Computer-aided approaches make it simple to anticipate a compound's ADMET qualities, which show its efficacy and toxicity (Eissa et al., 2023). The ligand-based strategy for drug development focuses on researching molecules that interact with relevant biological targets to create pharmacologically effective drugs.

The present study focuses on two main strategies that are followed initially, blocking unwanted cell proliferation; and therefore, viral transcription and replication prevention. To do this, a thorough evaluation of the therapeutic targets and mechanisms against glioma is conducted together with an *in-silico* method to assess the pharmacological potential of phytocompounds. We also performed a molecular docking analysis to identify the most promising leads (i.e., phytochemicals). The substances were put through molecular dynamics simulations to verify the findings of the docking investigation. Our results provide new evidence for revealing the underlying mechanism of glioma.

Etiology of Glioma

Glioma, a form of tumor arising from glial cells within the brain or spinal cord, presents a complex origin with various influences contributing to their development (Louis et al., 2021). Although the precise cause remains uncertain, several factors are implicated in their onset. Genetic predisposition plays a significant role in the development of gliomas, as specific hereditary conditions are linked to an elevated risk. For instance, neurofibromatosis type 1 (NF1) and neurofibromatosis type 2 (NF2), both autosomal dominant disorders characterized by nerve tumor formation, including gliomas, are associated with increased susceptibility (Lobbous et al., 2020). Exposure to ionizing radiation, whether through prior treatments or head injuries, correlates with an augmented likelihood of glioma development. Moreover, radiotherapy, a common treatment for brain tumours, can induce secondary gliomas in irradiated regions (Ranjan et al., 2020). Furthermore, exposure to ionizing radiation from environmental sources, such as radon gas, has been associated with glioma development (Darby et al., 2001). Certain chemicals or toxins have been proposed as potential contributors to glioma development. For instance, organic solvents like benzene have been linked to an increased risk of gliomas in occupational settings (Patel et al., 2020). Similarly, pesticides and herbicides have been implicated in glioma development, although further research is necessary to establish a definitive link (Ruder et al., 2009). A small subset of glioma cases exhibits familial clustering, suggesting a genetic element. Studies have shown an elevated risk of gliomas among relatives of glioma patients, indicating the potential contribution of inherited genetic variants to glioma development (Alghuson et al., 2022). Age is also a factor influencing glioma development, with specific types of gliomas displaying predilections for distinct age groups. Low-grade gliomas like pilocytic astrocytomas are more prevalent in children and young adults. Conversely, high-grade gliomas such as glioblastoma multiforme (GBM) are more commonly found in older adults (Louis et al., 2021).

Reported Therapeutic Uses

T. procumbens has been traditionally used in various therapeutic applications, with different parts of the plant serving distinct purposes. The aerial parts have been associated with aiding liver diseases, indicating a potential hepatoprotective property. Additionally, the leaves have shown a broad spectrum of uses, ranging from addressing hair loss to acting as local anti-infective and antiparasitic agents. This plant has also been used in managing the common cold, contusions, haemorrhage, and as hemostatic, suggesting potential wound healing and bleeding control properties. Moreover, the leaf has been traditionally employed to alleviate diarrhea and dysentery symptoms. The root specifically targets diarrhea, signifying gastrointestinal benefits. The whole plant has been associated with wound healing properties (Table 1). Furthermore, the leaf has been reported to act as an insecticide, potentially useful in insect control. Although, the traditional uses highlight the broad therapeutic potential of *T. procumbens*, but there are still numerous unexplored medicinal implications that necessitate the identification of active compounds and their precise mechanisms to target a wide range of diseases.

Table 1 Various previously reported therapeutic uses of *T. procumbens*

Plant part	Therapeutic Use	Therapeutic Use identifiers	References
Aerial Parts	Liver diseases	MESH:D008107, UMLS:C0023895, DOID:409, ICD-11:SA0Z	ISBN:9788172363093
Leaves	Hair loss	MESH:D000505, UMLS:C0002170, DOID:987, ICD-11:ED70	Medicinal Plants of Nagpur and Wardha Forest Divisions (Maharashtra)
Leaves	Anti-infective agents, local	MESH:D000891, UMLS:C0003205, ICD-11:XM4VG4	ISBN:9789327275590, Medicinal Plants of Nagpur and Wardha Forest Divisions (Maharashtra)
Leaves	Antiparasitic agents	MESH:D000977, UMLS:C4708129, ICD-11:XM37L1	ISBN:9789327275590
Leaf	Common cold	MESH:D003139, UMLS:C0009443, DOID:10459, ICD-11:CA00	ISBN:9780387706375, ISBN:9789327275590, Medicinal Plants of Nagpur and Wardha Forest Divisions (Maharashtra)

Plant part	Therapeutic Use	Therapeutic Use identifiers	References
Leaves	Contusions	MESH:D003288, UMLS:C0009938, ICD-11:ND56.0	ISBN:9789327275590
Leaves	Diarrhea	MESH:D003967, UMLS:C0011991, DOID:13250, ICD-11:ME05.1	ISBN:9780387706375, ISBN:9788172361792, ISBN:9789327275590, Medicinal Plants of Nagpur and Wardha Forest Divisions (Maharashtra)
Leaves	Dysentery	MESH:D004403, UMLS:C0277526, DOID:12384, ICD-11:1A40.Z	ISBN:9780387706375, ISBN:9788172361792, ISBN:9789327275590, Medicinal Plants of Nagpur and Wardha Forest Divisions (Maharashtra)
Leaves	Hemorrhage	MESH:D006470, UMLS:C0019080, ICD-11:MG27	ISBN:9789327275590, Medicinal Plants of Nagpur and Wardha Forest Divisions (Maharashtra)
Leaves	Hemostatic	MESH:D006490, UMLS:C0019120	ISBN:9780387706375, ISBN:9789327275590
Leaves	Insecticides	MESH:D007306, UMLS:C0021576, ICD-11:XM3K66	ISBN:9789327275590, Medicinal Plants of Nagpur and Wardha Forest Divisions (Maharashtra)
Leaves	Wounds and injuries	MESH:D014947, UMLS:C0043251	ISBN:9789327275590
Root	Diarrhea	MESH:D003967, UMLS:C0011991, DOID:13250, ICD-11:ME05.1	ISBN:9788172363093
Whole plant	Wound healing	MESH:D014945, UMLS:C0043240	Contribution to the Medico-Botany of East Godavari And West Godavari Districts of Andhra Pradesh

Materials and Methods

Protein Structure Retrieval and Preparation

The proteins proto-oncogene tyrosine-protein kinase Src (SRC) and heat Shock Protein 90 (HSP90) exhibited a significant role in combating glioma, a type of brain tumour. SRC, a proto-oncogene encoding a non-receptor tyrosine kinase, is integral to cell signalling and regulates processes like proliferation and differentiation. In cancer, including gliomas, HSP90's role in stabilizing oncoproteins and promoting cell survival has been explored as a potential target for therapeutic intervention (Jafari et al., 2020; Dong et al., 2021). The protein structures SRC (PDB ID: 3g5d), and HSP90 (PDB ID: 3o0i) were downloaded from the PDB database in 'pdb' format (Burley et al., 2017). These proteins were then prepared using UCSF ChimeraX software and MODELLER (Pettersen et al., 2004). Removal of all the water molecules, co-ligands, heteroatoms, and the addition of missing residues, charge, and polar hydrogen bonds was performed to prepare all the target proteins (Madhavi Sastry et al., 2013).

Collection of Bioactive Compounds

All *Tridax procumbens* compounds were verified using the IMPPAT, Dr. Duke, and KNApSAcK databases (Afendi et al., 2012; Lans and van Asseldonk, 2020; Vivek-Ananth et al., 2023), apart from that existing literature was also reviewed (Mihigo et al., 2015; Berlin Grace et al., 2020). PubChem database was used to retrieve 3D and 2D structures of all the compounds in SDF format along with their PubChem CID, Molecular weight, and canonical SMILE (Kim et al., 2016).

Rule of Five (RO5)

For the preliminary screening of phytoconstituents, we employed the web-based platform ADMET Lab 2.0 (https://admetmesh.scbdd.com/) to assess the drug-likeness of the compounds. This online tool is instrumental in predicting the physicochemical aspects of small molecules, following an empirical rule of thumb to refine their drug ability. In our study, we scrutinized various properties, including molecular weight (MW), partition coefficient (LogP), number of hydrogen bond acceptors (nHA), and number of hydrogen bond donors (nHD). These properties are fundamental to the Lipinski Rule of Five, and any molecule deviating from the specified threshold values was excluded from further analysis (Lipinski, 2004).

Molecular Docking Study

To validate the interactions between the drugs and phytoconstituents, molecular docking was performed. The 3D conformations of the FDA-approved drug and screening phytochemicals were acquired from PubChem which is employed as ligands in molecular docking.

Energy reduction was performed using the UCSF ChimeraX software to transform the protein into a stable structure to eliminate the undesirable torsion angle, enabling stable binding of the ligand to the receptor molecule and the elimination of steric clashes (Meng et al., 2023). Molecular docking was conducted using Autodock Vina, while Open Babel was utilized to minimize energy of the protein and ligands and converting them from pdb to pdbqt format (Eberhardt et al., 2021; O'Boyle et al., 2011; Trott and Olson, 2009). The grid box size was enlarged to encompass the entire protein structure for blind docking. The ligand-protein affinities were assessed through docking score, RMSD analysis and binding energy. Discovery Studio 2021 Client was used to analyse and visualize the protein-ligand complex (Jejurikar and Rohane, 2021).

Drug-likeness and Bioavailability Analysis

The retrieved compounds were screened based on drug-likeness (DL) and oral bioavailability (OB). Drug-likeness (DL) is the degree to which a drug's chemical structure resembles that of other, well-known drugs. Oral bioavailability (OB), contrasts with the dosage of medications taken orally, which reach the bloodstream and have an impact on local tissues and organs before having a comparable pharmacological effect (Ahmed et al., 2022). To evaluate a compound's potential therapeutic value, both properties are crucial. Screening thresholds for DL and OB are ⩾ 0.18 and ⩾ 0.30 respectively. Molsoft L.L.C. was utilized for DL, and for OB analysis SwissADME tool (Daina et al., 2017) was used.

ADMET Profiling

The evolution of phytochemicals for possible therapeutic efficacy is not just limited to DL and OB; pharmacokinetic characteristics and compound toxicity are equally important. The canonical SMILE notations of the selected phytochemicals were retrieved through PubChem and fed into the online software programs ADMETlab 2.0 (Xiong et al., 2021) and SwissADME (Daina et al., 2017). The blood-brain barrier (BBB) absorption, topological polar surface area (TPSA), solubility, and Lipinski's rule of five (LoF) were among the factors that were investigated. Lipinski's rule of five includes molecular weight (⩽ 500g/mol), hydrogen bond donors (HbD ⩽ 5), hydrogen bond acceptors (HbA ⩽ 10) and octanol-water partition coefficient (log P ⩽ 5) (Chen et al., 2020). The FDA-approved drug was also evaluated with the same parameters. The compounds that meet all the aforementioned criteria were chosen for network building.

Molecular Dynamics Simulation

The Molecular dynamics (MD) simulation was conducted using GROMACS software, version 2023.1, on a Linux operating system. The results obtained were visualized through Microsoft Excel. The force fields employed for the simulation included the CHARMM36 (C36) force field for the protein structure and the CHARMM all atoms force

field for ligand parameterization. To prepare the system for simulation, the TIP 3-point solvent model was used for solvation. Post-solvation, neutralization of the system was achieved by adding appropriate quantities of Na^+ and Cl^- ions. Energy minimization of the system was performed using the steepest descent minimization algorithm, involving 50,000 steps. The convergence criterion was set at a maximum force threshold of 10.0 kJ/mol. Equilibration of the system was carried out in two phases. First, a 100 ps NVT equilibration was conducted under constant volume and temperature (300K) conditions. Subsequently, a 100 ps NPT equilibration was performed under constant pressure (1 bar) and temperature (300 K). During the simulation run, the integrator employed was the leap-frog integrator for MD simulations. The total number of steps undertaken was 150,000,000, corresponding to a simulation time of 300 ns with a time step of 2 fs. Output control parameters included saving energy data every 10.0 ps, updating log files every 10.0 ps, and saving coordinate data every 10.0 ps. To maintain bond constraints, the LINCS algorithm was used, and hydrogen bonds were constrained. Neighbour searching and van der Waals interactions were managed using a Verlet cutoff scheme and a grid-based approach, respectively. Temperature coupling was achieved using a modified Berendsen thermostat (V-rescale) with a time constant of 0.1 ps. Pressure coupling utilized the Parrinello-Rahman method with a time constant of 2.0 ps, maintaining pressure at 1.0 bar. Periodic boundary conditions (PBC) were applied in three dimensions. Long-range electrostatic interactions were accounted for using the Particle mesh Ewald (PME) algorithm. Post-simulation analysis encompassed various parameters including root mean square deviation (RMSD), root mean square fluctuation (RMSF), radius of gyration (Rg), hydrogen bonds (HB), and solvent accessible surface area (SASA). Analysis was performed using the built-in tools of GROMACS version 2023.1 (Páll et al., 2020).

Binding Free Energy Calculations

The determination of binding free energy (ΔG) in ligand-protein complexes involved employing the gmx_MMPBSA package (version 1.6.2), utilizing the MM/GBSA (Molecular Mechanics/ Generalized Born Surface Area) method (Valdés-Tresanco et al., 2021). The total binding free energy (ΔG Total) was calculated by subtracting ΔG Receptor from ΔG Complex and then further subtracting ΔG Ligand from the resultant value. Subsequently, the data underwent analysis utilizing the gmxMMBSA ana module.

Results and Discussion

Tridax procumbens, garnering attention in cancer treatment research, stands out as one of the most significant medicinal plants (Baile and Parmar, 2023). Although *T. procumbens* has been extensively studied in clinical case studies and various *in vitro* and *in vivo* research for treating different cancers. There remains a lack of in-depth research to investigate its active compounds, potential targets, and pharmacological mechanisms specifically for treating brain cancers. Glioma is a type of brain or spinal tumour that originates from glial cells, with treatment typically involving surgery, radiation therapy, and chemotherapy.

Phytochemical Library Preparation

Using various databases like IMPPAT, Dr. Duke, KNApSAcK, and literature, a total of 106 *Tridax procumbens* compounds were collected. For each phytocompound, PubChem was utilized to get its 2D and 3D structure, canonical SMILE, molecular weight, and PubChem CID. The 2D structure was necessary for the drug ability analysis in each component in addition to the 3D structure for the molecular docking investigation.

Preliminary Screening of Phytochemicals

In the quest for developing a highly effective drug with minimal adverse effects, the initial screening process, focusing on drug-likeness, assumes paramount importance. In this preliminary screening, Lipinski's Rule of Five was applied to evaluate all phytoconstituents derived from *T. procumbens*. According to Lipinski's criteria, a compound exhibits favourable oral bioavailability of its molecular weight (MW) is ⩽ 500 Da, hydrogen bond donors (HbD) are ⩽ 5, hydrogen bond acceptors (HbA) are ⩽ 10, and the octanol-water partition coefficient log P is ⩽ 5 (Lipinski, 2004). Compounds violating more than one parameter were excluded, and the remaining compounds were designated as ligands for subsequent docking studies. As depicted in Table 2, out of the total 106 phytoconstituents, 102 candidates successfully adhered to Lipinski's rule and were consequently advanced for further analysis.

Table 2 Screening of phytochemicals based on Lipinski's rule

Phytochemicals	PubChem Id	LogP	MW	nHA	nHD	nLV
1,3-Cyclohexadiene,5-(1,5-dimethyl-4-hexenyl)-2 methyl	521253	5.679	204.19	0	0	1
1H-indole-2,3-dione-5-methyl-1-(trimethylsilyl)	553349	2.857	233.09	3	0	0
2,3-Dimethylhexane	11447	4.184	114.14	0	0	0
2-Methylheptane	11594	4.53	114.14	0	0	0
9-Octadecenoic acid ethyl ester	5364430	7.746	310.29	2	0	1
(24E)-24-N-Propylidenecholesterol	5281328	7.447	412.37	1	1	1
Falcarinol	5281149	5.514	244.18	1	1	1
Beta Curcumen	91710638	4.859	220.18	1	1	0
2-Propenyl butanoate	16324	2.261	128.08	2	0	0
14-Ketostearic acid methyl ester	13991164	5.635	312.27	3	0	1
1-Decanol-2-hexyl	122715484	4.38	322.22	4	1	0
1-Dodecanol	8193	4.772	186.2	1	1	0
1-Hexadecene	12395	8.054	224.25	0	0	1
1-Octanol-2-butyl	18319148	6.87	290.22	2	0	1
1-Undecanol	8184	4.294	172.18	1	1	0
2,4-Dimethylheptane	16656	4.6	128.16	0	0	0
2a,3,4,5-Tetrahydrobenz	10895284	4.084	430.26	4	1	0
2-Hexadecanol	85779	6.498	242.26	1	1	1
2-Hexyl-1-octanol	545551	5.788	214.23	1	1	1
2-Methylhexacosane	150931	12.557	380.44	0	0	1
2-Methyltetracosane	527459	11.768	352.41	0	0	1
2-Monopalmitin	123409	5.275	330.28	4	2	1
2-Propyl-1-heptanol	24847	3.826	158.17	1	1	0
3-Octen-1-ol	5364475	2.524	128.12	1	1	0
Cholestane, 4-Epoxy-2-methyl	22213335	8.007	400.37	1	0	1
4-Octanol	11515	2.894	130.14	1	1	0
5,7,2',3',4'-Pentahydroxy-3,6-dimethoxyflavone 7-glucoside	44259890	-0.28	524.12	14	8	3
6-Hydroxyluteolin 6,3'-dimethyl eter 5-rhamnoside	44258523	1.689	476.13	11	5	1
Eicosane	8222	10.033	282.33	0	0	1

Phytochemicals	PubChem Id	LogP	MW	nHA	nHD	nLV
7-Hexadecenal-(Z)	5364438	6.171	238.23	1	0	1
9,12-Octadecadienoic acid, ethyl ester	5365672	7.217	308.27	2	0	1
9-Heptadecanone	10887	6.524	254.26	1	0	1
Arachidic acid	10467	8.389	312.3	2	1	1
Santanol acetate	5352140	4.247	262.19	2	0	0
Aspirin	2244	1.237	180.04	4	1	0
Benzofuran,2,3-Dihydro	140633	3.755	162.1	1	0	0
Beta-Amyrin	73145	7.815	426.39	1	1	1
Beta-Amyrone	12306160	7.808	424.37	1	0	1
Beta-Sitosterol	222284	7.663	414.39	1	1	1
Butyl-9- tetradecenoate	87382688	6.862	282.26	2	0	1
Butylated hydroxyanisole	24667	3.277	180.12	2	1	0
Campesterol	173183	7.308	400.37	1	1	1
Camphene	6616	3.781	136.13	0	0	0
Caryophyllene	5281515	5.906	204.19	0	0	1
Caryophyllene oxide	1742210	4.474	220.18	1	0	0
Cedrene	521207	5.161	204.19	0	0	1
Centaureidin	5315773	2.667	360.08	8	3	0
cis-Vaccenic acid	5282761	7.131	282.26	2	1	1
Cynaroside	5280637	0.317	448.1	11	7	2
Decamethyl tetrasiloxane	8852	4.947	310.13	3	0	0
Dibutyl phthalate	3026	4.337	278.15	4	0	0
Didehydrofalcarinol	6442009	4.497	240.15	1	1	0
Dihydroxyacetone	670	-1.272	90.03	3	2	0
Docosanoic acid	8215	9.198	340.33	2	1	1
Dodecane, 1-Chloro	14026611	6.478	473.99	0	0	1
Dodecanoic acid	3893	4.793	200.18	2	1	0
Dotriacontane	11008	14.749	450.52	0	0	1
Dotriacontanol	96117	13.446	466.51	1	1	1
Eicosanoic acid	10467	8.389	312.3	2	1	1
Ethanol,2-(octadecyloxy)	23688481	5.917	393.27	5	0	1
Falcarinol	5281149	5.514	244.18	1	1	1
Fosfosal	3418	0.464	218	6	2	0

Phytochemicals	PubChem Id	LogP	MW	nHA	nHD	nLV
Fumaric acid	444972	-0.175	116.01	4	2	0
Heptamethyl trisiloxane	6327366	3.836	221.08	2	0	0
Hexacosane	12407	12.394	366.42	0	0	1
Hexadecane	11006	8.415	226.27	0	0	1
Hexadecanoic acid	985	6.732	256.24	2	1	1
Hexyl eicosane	521605	12.194	380.44	0	0	1
Isopropyl linoleate	5352860	7.393	322.29	2	0	1
Isoquercetin	5280804	-0.17	464.1	12	8	2
Lauric acid	3893	4.793	200.18	2	1	0
Limonene	22311	4.368	136.13	0	0	0
Linoleic acid	5280450	6.652	280.24	2	1	1
Linolenic acid	5280934	6.156	278.22	2	1	1
Lupeol	259846	7.291	426.39	1	1	1
Luteolin	5280445	2.902	286.05	6	4	0
Myristic acid	11005	5.82	228.21	2	1	1
Neophytadiene	10446	8.007	278.3	0	0	1
Nonadecane	12401	9.64	268.31	0	0	1
Octadecanoic acid	5281	7.571	284.27	2	1	1
Octamethyl trisiloxane	24705	4.188	236.11	2	0	0
Olean-12-en-3-one	454747	7.808	424.37	1	0	1
Oxalic acid	971	-0.735	90	4	2	0
Palmitic acid	985	6.732	256.24	2	1	1
Palmitoleic acid	445638	6.293	254.22	2	1	1
2,6,10,14-Tetramethylpentadecane	15979	8.45	268.31	0	0	1
Pentadecanoic acid	13849	6.283	242.22	2	1	1
Pentaethylene glycol	62551	-1.008	238.14	6	2	0
Phthalic acid, 2-ethylhexyl neopentyl ester	6423590	6.084	348.23	4	0	1
Phytol acetate	6428538	8.311	338.32	2	0	1
Pyrazine, 2,6-dimethyl-	7938	0.593	108.07	2	0	0
Quercetin	5280343	2.155	302.04	7	5	0
Sabinene hydrate	62367	2.44	154.14	1	1	0
Salicylic acid	338	2.221	138.03	3	2	0

Phytochemicals	PubChem Id	LogP	MW	nHA	nHD	nLV
Stearic acid	5281	7.571	284.27	2	1	1
Stigmasterol	5280794	7.436	412.37	1	1	1
Vertocitral	91746677	2.068	138.1	1	0	0
trans-α-Bergamotene	6429302	5.709	204.19	0	0	1
Tridaxidone	44259891	0.725	522.14	13	6	3
Tridecane	12388	7.116	184.22	0	0	1
Tridecanoic acid	12530	5.321	214.19	2	1	1
Umbellulone	442504	2.284	150.1	1	0	0
Undecanoic acid	8180	4.304	186.16	2	1	0
Zerumbone	5470187	4.815	218.17	1	0	0
α-Humulene	5281520	6.646	204.19	0	0	1
ß-Pinene oxide	91508	2.889	152.12	1	0	0

MW: Molecular weight; nH-A: H-bond acceptor; nH-D: H-bond donor; LV: Lipinski violations

Molecular Docking Analysis

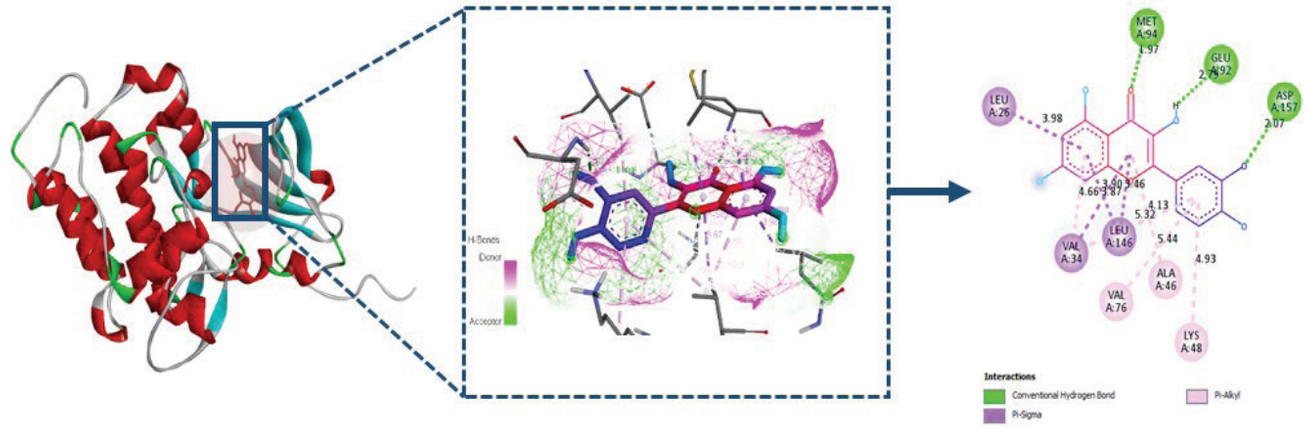
The protein structures of were downloaded from the PDB database using following SRC (PDB ID: 3g5d), and HSP90 (PDB ID: 3o0i). For each compound up to 9 conformers during docking were generated. Docking result analysis of the top 3 highly interacting phytocompounds along with FDA-approved drug is presented in Table 3. For each molecule, the pose or conformation with the lowest negative binding energy was deemed the most advantageous. The most promising lead compounds were identified as follows: Luteolin exhibited strong interaction with HSP90 protein at -10 kcal/mol, followed by quercetin interacting with SRC protein at -9.3 kcal/mol, and 6-hydroxyluteolin 6,3'-dimethyl ether 5-rhamnoside showing promising interaction with SRC protein at -9.1 kcal/mol. FDA-approved drug dabrafenib was observed to have a -6.9 kcal/mol binding affinity with protein HSP90 and trametinib (FDA-approved) drug has a -9.1 kcal/mol binding affinity with protein SRC. The Luteolin-3o0i complex was stabilized by three hydrogen bonds (3H-bond) with Pi-sigma, Pi-alkyl, and Pi- sulfur bonds. Meanwhile, the querceti-3g5d complex is stabilized by the 3H- bond along with the Pi-alkyl and Pi-sigma bond. 6-hydroxyluteolin 6,3'-dimethyl ether 5-rhamnoside-3g5d is stabilized by 3H-bond along with Pi-sigma, Pi-alkyl, and alkyl bond (Fig. 1).

Table 3 List of protein-ligand interactions between selected bioactive compounds along with FDA approved drugs and target proteins

S. No.	Ligand	PubChem ID	Target (PDB ID)	Binding affinity (kcal/mol)	Hydrogen bonds	Total no. of bonds	Type of bond	Interacting residues	Bond Length
1	Luteolin	5280445	HSP90(3o0i)	-10	3	12	Conventional Hydrogen Bond	LEU A:68 SER A:72 TYR A:159	2.62 2.21 2.28
							Pi- Sulfur	MET A:118 MET A:118	4.98 5.08
							Unfavorable Donor-Donor	THAR A:204	1.83
							Pi-Pi Stacked	PHE A:158 PHE A:158	3.66 3.12
							Pi Sigma	LEU A:127	3.78
							Pi-Alkyl	VAL A:170 VAL A A:206 LEU A:5.34	5.02 5.21 5.34
2	Querce-tin	5280343	SRC (3g5d)	-9.3	3	12	Conventional hydrogen Bond	META A:94 GLU A:92 ASP A:157	1.97 2.79 2.07
							Pi Alkyl	VAL A:76 ALA A:46 LYS A:48 VAL A:34 VAL A:34	5.44 5.32 4.93 4.13 4.66
							Pi Sigma	VAL A:34 LEU A: 26 LEU A:146 LEU A:146	3.90 3.98 3.87 3.46

S. No.	Ligand	PubChem ID	Target (PDB ID)	Binding affinity (kcal/mol)	Hydrogen bonds	Total no. of bonds	Type of bond	Interacting residues	Bond Length
3	6-Hydroxy-luteolin 6,3'-dimethyl ether 5-rhamnoside	44258523	SRC(3g5d)	-9.1	3	13	Conventional Hydrogen Bond	PHE A:31 LYS A:48	2.27 2.71
							Carbon Hydrogen Bond	THE A:91	3.48
							Alkyl	VAC A:34 VAC A:34 VAC A:34	5.03 4.71 4.50
							Pi Alkyl	ALA A:156 ALA A:46 ALA A:46	3.55 4.18 4.03
							Pi Sigma	LEU A:146 LEU A:146 LEU A:26 LEU A:26	3.83 4.58 3.95 3.91
4	Dabrafenib (FDA-approved drug)	44462760	HSP90(3o0i)	-6.9	5	9	Conventional Hydrogen Bond	GLN: A 232 PHE: A 233 ILE: A 234 UNK: N 1 UNK: N 1	2.59 2.05 2.90 2.23 2.26
							Halogen	PHE: A 233	3.61

S. No.	Ligand	PubChem ID	Target (PDB ID)	Binding affinity (kcal/mol)	Hydrogen bonds	Total no. of bonds	Type of bond	Interacting residues	Bond Length
							Alkyl	UNK: N 1 UNK: N 1	5.66 5.21
							Pi- Sulfur	UNK: N 1	5.44
5	Trame-tinib (FDA-approved drug)	11707110	SRC(3g5d)	-9.1	3	17	Conventional Hydrogen Bond	LEU: A 26	2.39
							Carbon Hydro-gen Bond	ASN: A 144 ASP: A 157	3.74 3.56
							Pi-Anion	GLU: A 63	4.80
							Pi-Sigma	LEU: A 146	3.92
							Pi-Sulfur	CYS: A 98	4.75
							Alkyl	LEU: A 26 LEU: A146 LYS: A 48 MET: A 67 ILE: A 89	4.50 4.75 4.08 5.02 3.98
							Pi-Alkyl	TYR: A 93 VAL: A 34 LEU: A 146 VAL: A 34 LYS: A 48	5.12 4.55 5.35 5.37 5.10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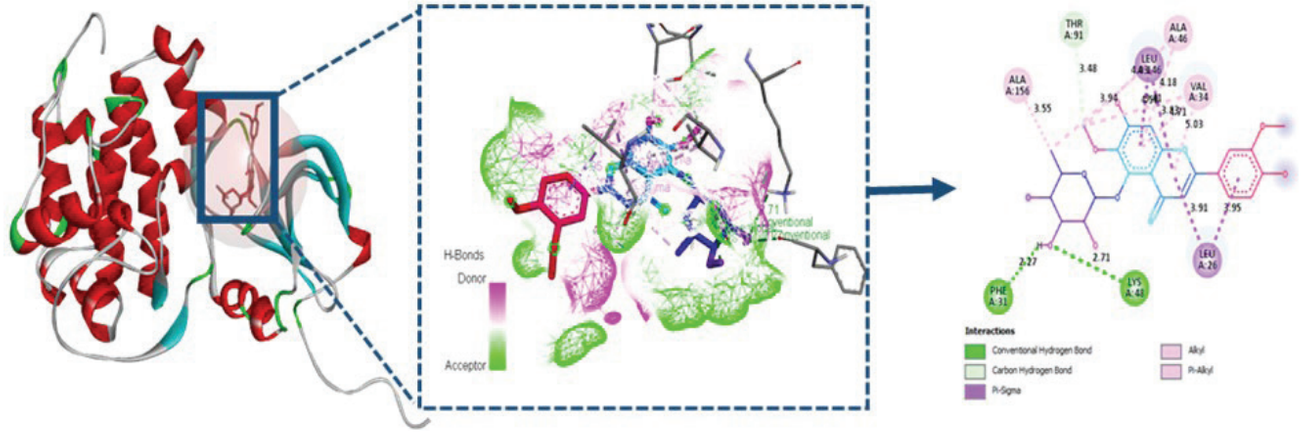
Conventional Hydrogen Bond
Pi-Alkyl
Pi-Sigma

(A)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Pi-Alkyl

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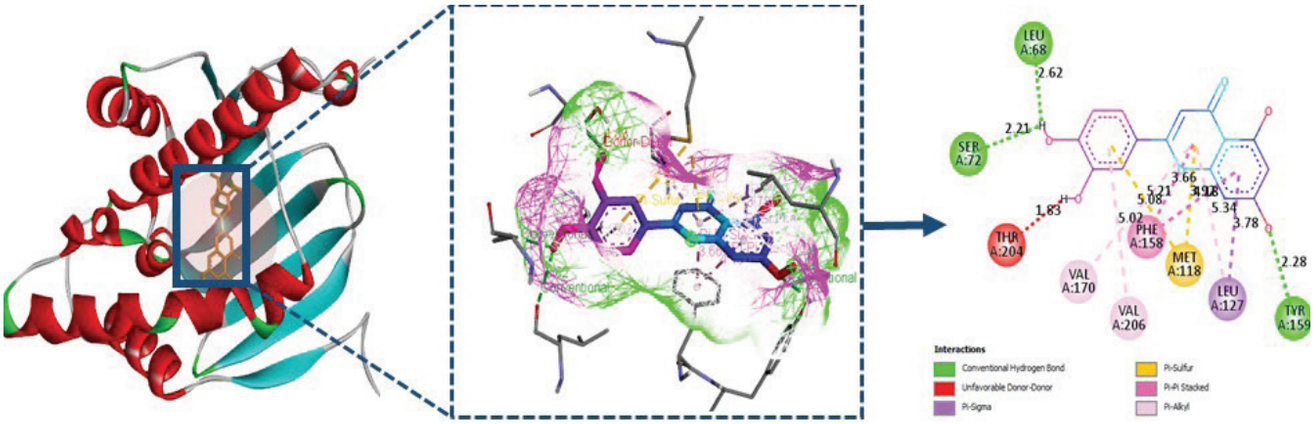
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VAL A:170
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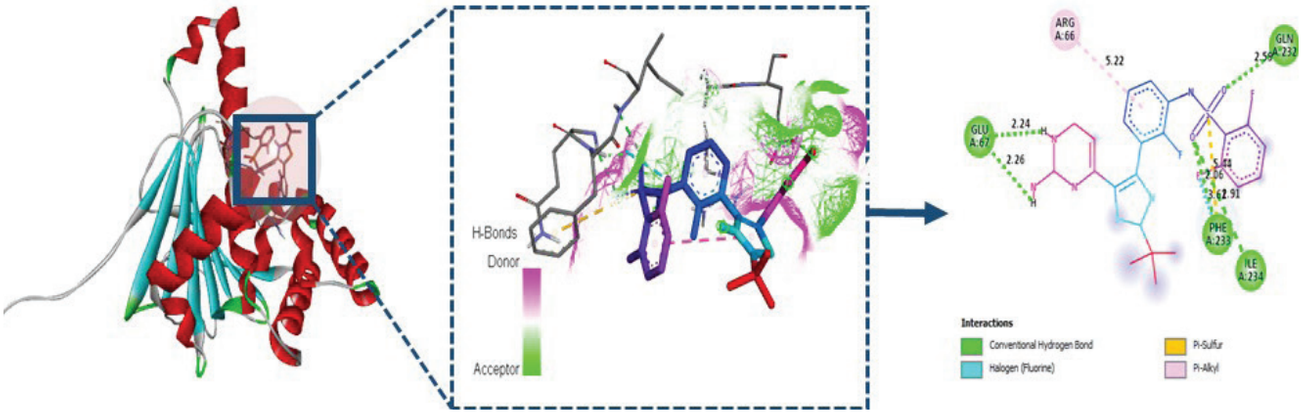
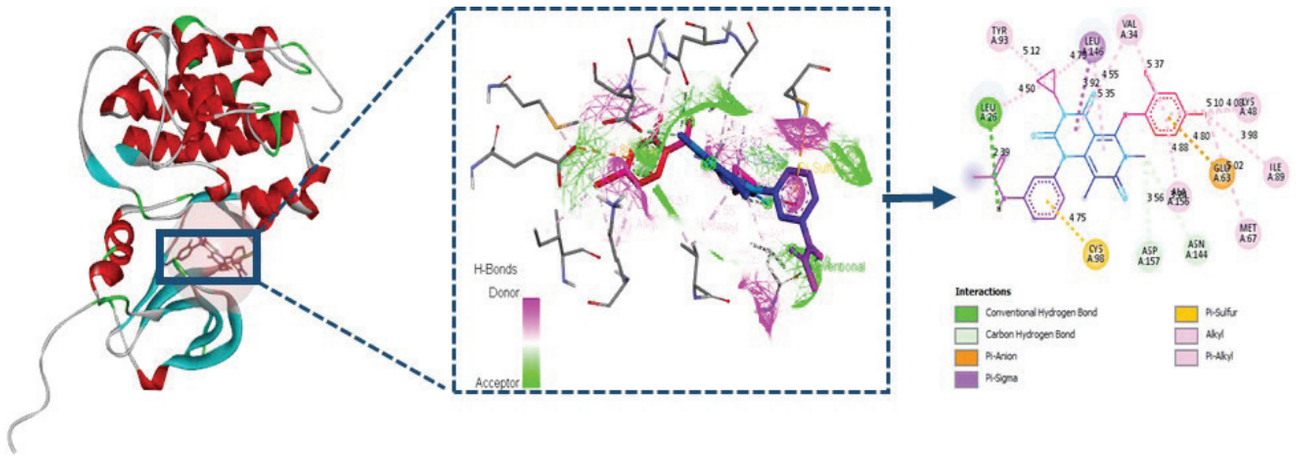
Interactions
Conventional Hydrogen Bond
Unfavorable Donor-Donor
Pi-Sigma
Pi-Sulfur
Pi-Pi Stacked
Pi-Alkyl

(C)



(D)



(E)

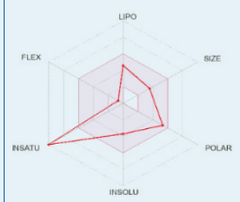
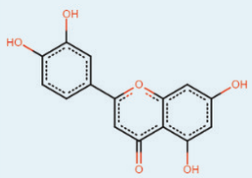
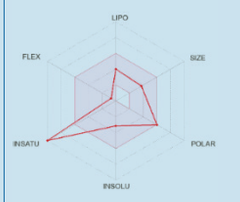
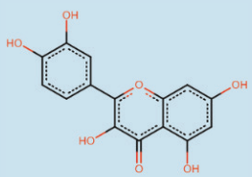
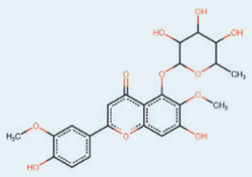
Fig. 1 (A-E) 3D and 2D structures of targeted protein docked with different phytochemicals. (A) Luteolin with the target protein HSP90 (B) Quercetin with the target protein SRC (C) 6-Hydroxyluteolin 6,3'-dimethyl ether 5-rhamnoside with the target protein SRC (D) Dabrafenib (FDA-approved drug) with the protein HSP90 (E) Trametinib (FDA-approved drug) with the protein SRC

The current study revealed that luteolin had the strongest interaction with HSP90, quercetin with SRC, and 6-hydroxyluteolin 6,3'-dimethyl ether 5-rhamnoside also with SRC, aligning with previous findings. A similar study performed on temozolomide interactions with secretory proteins in glioblastoma multiforme, identifying strong binding affinities with GDF1 and SLIT1, indicating potential targets for enhancing treatment efficacy (Sumera et al., 2022). In another study, *Taraxacum mongolicum* exhibited growth-inhibitory and apoptosis-inducing effects on hepatocellular carcinoma through molecular docking revealing strong interactions between active ingredients like austricin and quercetin with HSP90 protein, aligning with the molecular docking results of the present study (Zheng et al., 2022).

ADMET Profiling, Drug-likeness and Bioavailability Analysis

After molecular docking, the top 3 lead compounds follow all the parameters of ADMET as well as drug-likeness and bioavailability. These phytocompounds were evaluated based on DL (≥ 0.18), and OB (≥ 0.30). The 2D structure with bioavailability radar chart of all the three screened phytochemicals with their OB and DL are shown in Table 4.

Table 4 Pharmacokinetic mapping of experimental compounds with 2D Structure

S. No.	Ligand	PubChem ID	Radar Chart	2D Structure	Bioavailability	Drug-likeness
1	Luteolin	5280445			0.55	0.38
2	Quercetin	5280343			0.55	0.52
3	6-Hydroxyluteolin 6,3'-dimethyl ether 5-rhamnoside	44258523			0.55	0.77

In the comprehensive analysis of the ADMET profiles for luteolin, quercetin, and 6-hydroxyluteolin 6,3'-dimethyl ether 5-rhamnoside, various parameters with different categories were meticulously examined. For absorption parameters, P-glycoprotein inhibition (Pgp-inh), P-glycoprotein substrate potential (Pgp-sub), human intestinal absorption (HIA), absorption fractions at F (20%) and F (30%), as well as Caco-2 and MDCK cell assays were analysed. Distribution-related investigations included scrutiny of the ability to traverse the blood-brain barrier (BBB), examination of plasma

protein binding (PPB), measurement of volume of distribution at steady state (VDss), and determination of the unbound fraction (Fu). Metabolism parameters encompassed evaluations for both inhibition and substrate potential concerning various cytochrome P450 enzymes. Excretion-related considerations involved the assessment of clearance (CL) and half-life (T12). Toxicity evaluations encompassed the Ames test for mutagenicity, scrutiny of the route of administration (ROA), comparison of FDA maximum daily dose (FDAMDD), assessment of carcinogenicity, embryotoxicity (EC), endocrine disruption (EI), and analysis of genotoxic, carcinogenic, and mutagenic potential (Table 5).

Table 5 ADMET profiling of active phytochemicals

Category	Parameters	Luteolin	Quercetin	6-Hydroxyluteolin 6,3'-dimethyl eter 5-rhamnoside
Absorption	Pgp-inh	0.004	0.004	0.03
	Pgp-sub	0.274	0.005	0.949
	HIA	0.047	0.014	0.638
	F(20%)	0.998	0.93	0.009
	F(30%)	1	0.997	0.784
	Caco-2	-5.028	-5.204	-5.652
	MDCK	1.00E-05	7.69E-06	2.74E-05
Distribution	BBB	0.009	0.008	0.061
	PPB	95.44%	95.50%	80.03%
	VDss	0.533	0.579	0.811
	Fu	5.98%	7.42%	22.90%
Metabolism	CYP1A2-inh	0.981	0.943	0.044
	CYP1A2-sub	0.154	0.115	0.895
	CYP2C19-inh	0.124	0.053	0.024
	CYP2C19-sub	0.046	0.041	0.199
	CYP2C9-inh	0.576	0.598	0.018
	CYP2C9-sub	0.842	0.643	0.718
	CYP2D6-inh	0.568	0.411	0.04
	CYP2D6-sub	0.559	0.205	0.372
	CYP3A4-inh	0.549	0.348	0.09
	CYP3A4-sub	0.092	0.046	0.075

Category	Parameters	Luteolin	Quercetin	6-Hydroxyluteolin 6,3'-dimethyl eter 5-rhamnoside
Excretion	CL	8.146	8.284	5.353
	T12	0.898	0.929	0.505
Toxicity	Ames	0.536	0.657	0.623
	ROA	0.046	0.065	0.042
	FDAMDD	0.741	0.31	0.087
	Carcinogenicity	0.095	0.05	0.11
	EC	0.009	0.007	0.003
	EI	0.944	0.936	0.02
	Genotoxic_ Carcinogenic-ity_Mutagen-icity	0	0	0

Structural Stability Analysis

Molecular dynamics simulations have been conducted to investigate compound-protein interactions at a molecular level. This study focused on top-ranked interactions: quercetin binding with proto-oncogene tyrosine-protein kinase SRC (SRC), and luteolin binding with heat shock protein 90 alpha family class A member 1 (HSP90AA1 or HSP90) (Fig. 2 A-B). Due to inconsistent root mean square deviation (RMSD), radius of gyration (Rg), and solvent accessible surface area (SASA), coupled with the formation of minimal hydrogen bonds, 6-hydroxyluteolin 6,3'-dimethyl eter 5-rhamnoside was excluded from molecular dynamics (MD) simulation analysis. Through 300 nanoseconds of simulation, the stability and interaction energies of luteolin-HSP90 and quercetin-SRC complexes were assessed. The root mean square deviation (RMSD) analysis indicated complex stability. For quercetin-SRC, the average RMSD was 0.2158 nm (standard deviation 0.1474 nm), varying between 0.0005 nm and 0.6201 nm. Luteolin-HSP90 had a slightly lower average RMSD (0.1451 nm, standard deviation 0.0560 nm), ranging from 0.0005 nm to 0.4041 nm. The narrower RMSD range in luteolin-HSP90 implied greater stability compared to the quercetin-SRC interaction, which exhibited broader structural dynamics (Fig. 2C).

RMSF analysis had been performed on two molecular complexes, quercetin-SRC, and luteolin-HSP90. Quercetin-SRC had an average RMSF of 0.355 nm (±0.428 nm), fluctuating between 0.0678 nm and 3.1839 nm across 4573 residues. Luteolin-HSP90 had shown a lower average RMSF at 0.138 nm (±0.087 nm), with values ranging

from 0.0495 nm to 0.6486 nm across 3271 residues. These RMSF values indicated flexibility and dynamics, varying due to distinct molecular structures, interactions, and binding modes. Higher RMSF suggested more flexibility, while lower values indicated rigidity in specific regions (Fig. 2D). The radius of gyration was a crucial parameter in molecular biology and biophysics, revealing the size and compactness of molecules. Analyzing quercetin-SRC and luteolin-HSP90 compounds, their radius of gyration values unveiled structural insights. Quercetin-SRC had an average radius of gyration of 2.1199 nm (±0.1095 nm), indicating an extended conformation. Its range, 1.9576 nm to 2.3825 nm, had signified structural variability. In contrast, luteolin-HSP90's average radius of gyration had been 1.7357 nm (±0.0183 nm), suggesting a compact structure. Its range, 1.6844 nm to 1.8197 nm, had a narrower variation than quercetin-SRC. These values depicted the shapes and conformations of the molecules (Fig. 2 E).

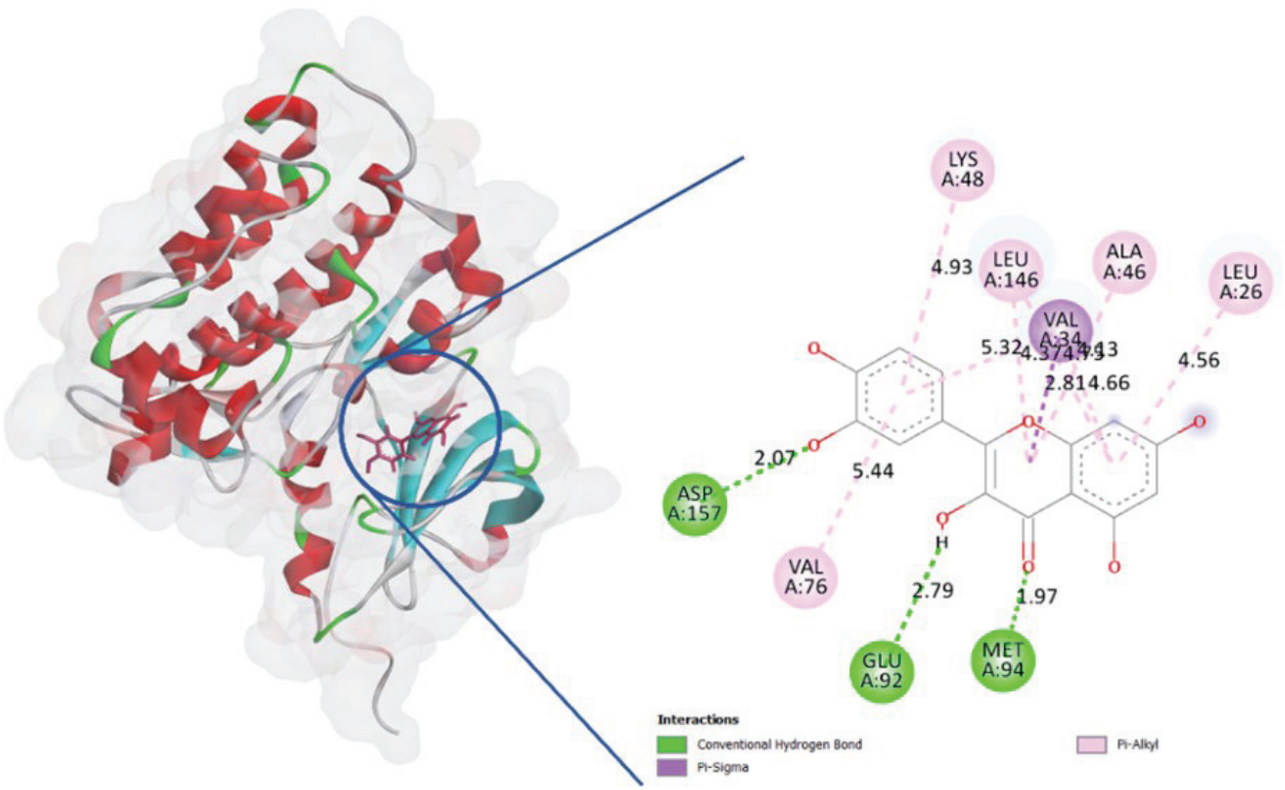
The solvent accessible surface area (SASA) analysis of quercetin-SRC and luteolin-HSP90 revealed key insights into their molecular interactions. SASA had measured solvent-exposed surface regions, reflecting structural exposure and reactivity. Quercetin-SRC's average SASA had been 161.32 nm^2, with a moderate standard deviation of 5.77 nm^2, implying structural fluctuations. The SASA range (144.88 to 186.28 nm^2) had depicted diverse solvent exposure. Similarly, luteolin-HSP90 had an average SASA of nm^2, with a smaller standard deviation (3.35 nm^2) and a narrower range (105.23 to 130.66 nm^2). The increased and fluctuating solvent-accessible surface area (SASA) of quercetin-SRC indicated a higher potential for interaction with the solvent. In contrast, luteolin-HSP90's focused, consistent SASA had implied specific solvent accessibility. These insights had elucidated their structural behaviours and roles in molecular interactions (Fig. 2 F).

MD simulations studied the stability of Luteolin-HSP90 and Quercetin-SRC complexes via hydrogen bonds. Throughout the molecular dynamics (MD) simulation process, a trajectory consisting of 30,000 frames captured over a duration of 300 nanoseconds has yielded valuable insights or revelations. Both complexes had relied on hydrogen bonds, but with varied distribution and impact. During the 300 nanosecond MD simulation reaction, luteolin-HSP90 displayed the formation of one (56.97%) and two (32.62%) hydrogen bonds (Fig. 2 G). Conversely, quercetin-SRC predominantly exhibited the formation of two (41.77%) hydrogen bonds. Luteolin-HSP90 demonstrated occasional occurrences of three (1.32%) and four (0.04%) hydrogen bonds, while quercetin-SRC intermittently showcased the formation of three (21.99%) hydrogen bonds, with the least frequent instances of four (3.59%) and five (0.27%) hydrogen bonds. Luteolin-HSP90's stability had come from numerous hydrogen bonds, while quercetin-SRC had peaked with 2 hydrogen bonds. Both complexes had intermittently formed many bonds, showing

a preference for enhanced bonding (Fig. 2 H).

In the present study, both ligands luteolin and quercetin were strongly bound to their targets, suggesting potential as drug candidates. Analysing factors like RMSD, RMSF, radius of gyration, and surface area enhanced understanding of complex stability. In a similar study by Shi et al. (2022), the RMSD values of the bosutinib-SRC complex were reported to have an average of 1.2 Å, suggesting comparable dynamics to our quercetin-SRC complex (Shi et al., 2022). Contrasting our results, another study on the interaction of quercetin with HSP90 revealed an average RMSD, indicating a more stable interaction compared to other complexes (Zheng et al., 2022). The RMSF analysis carried out in this study further emphasizes the significance of distinct binding modes. Our observation of higher RMSF values for the quercetin-SRC complex is consistent with the findings of Sundarrajan et al. (2020), where the imatinib-SRC complex exhibited increased flexibility in certain binding regions (Sundarrajan et al., 2020; Troxel and Chang, 2022). In contrast, the luteolin-HSP90 complex mirrors the tighter structural arrangement reported for the rifampicin-HSP90 complex, displaying lower RMSF values and indicating a more stable interaction (Zhou et al., 2022).

Like findings of the present study, Ndagi et al. (2018) reported a larger radius of gyration for the dasatinib-SRC complex compared to our luteolin-HSP90 complex, suggesting differences in the conformational changes induced by the ligands (Ndagi et al., 2018). Our observations regarding SASA align with the conclusions of Guo et al. (2019), who studied the interaction of naringenin with SRC kinase (Guo et al., 2023). They found a larger average SASA for their complex, indicating potential solvent exposure like our quercetin-SRC interaction (Huang et al., 2023). Contrasting our results, the investigation of sunitinib binding to HSP90 by Teranishi et al. (2023) reported narrower SASA values, indicating a more compact structure compared to our luteolin-HSP90 complex (Teranishi et al., 2023). In terms of hydrogen bond formation, our findings parallel the work of Roskoski (2016), who analysed the interaction of tofacitinib with SRC kinase (Roskoski, 2016). They identified hydrogen bonds as crucial for stability, and like our study, observed variations in bond distribution impacting complex stability (Hata et al., 2020). The study by Li et al. (2019) on the interaction of geldanamycin with HSP90 complements our results for the luteolin-HSP90 complex, emphasizing the role of hydrogen bonds in stabilizing the complex.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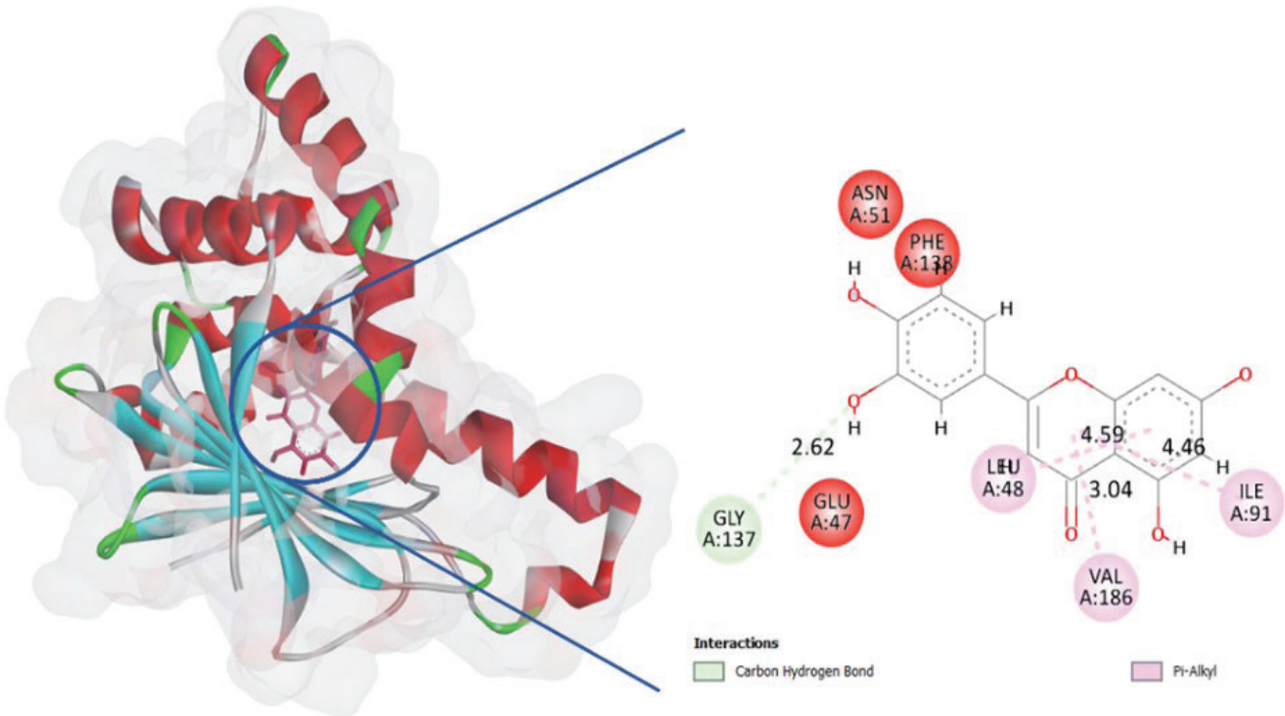
Pi-Sigma
Pi-Alkyl

(A)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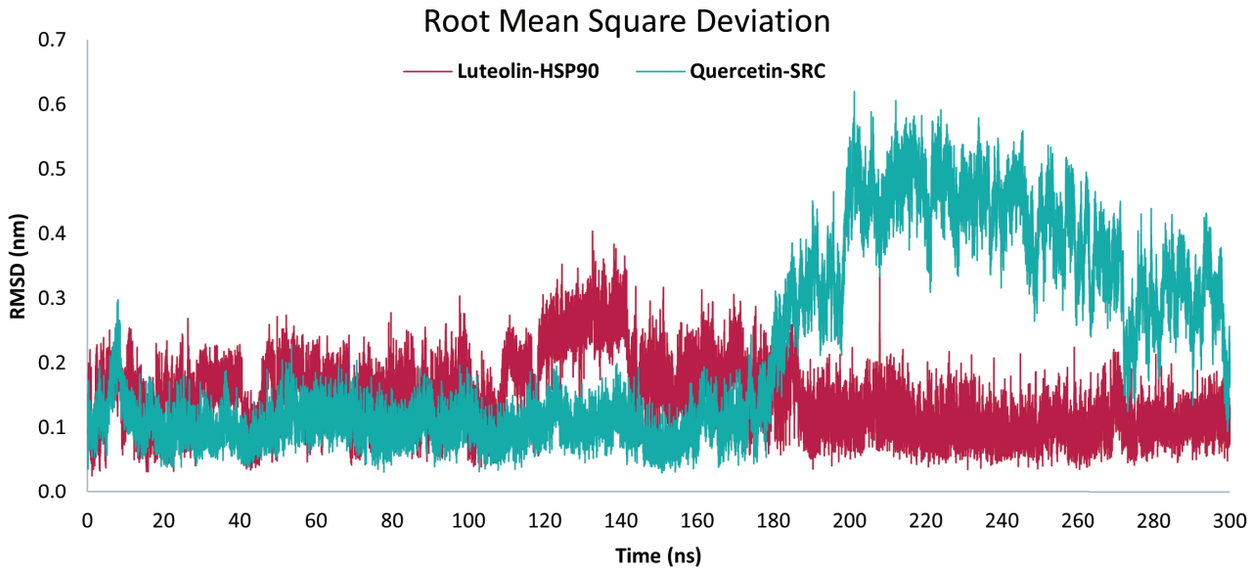
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0.4
0.3
0.2
0.1
0.0
0
20
40
60
80
100
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140
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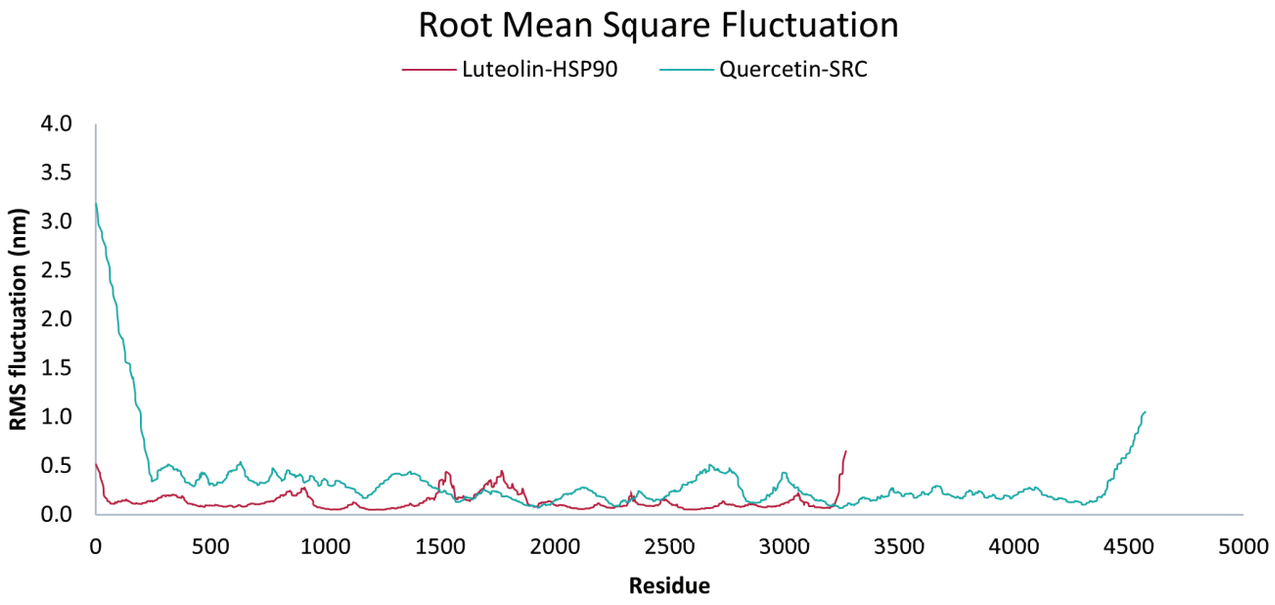
180
200
220
240
260
280
300
Time (ns)

(C)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Quercetin-SRC
RMS fluctuation (nm)
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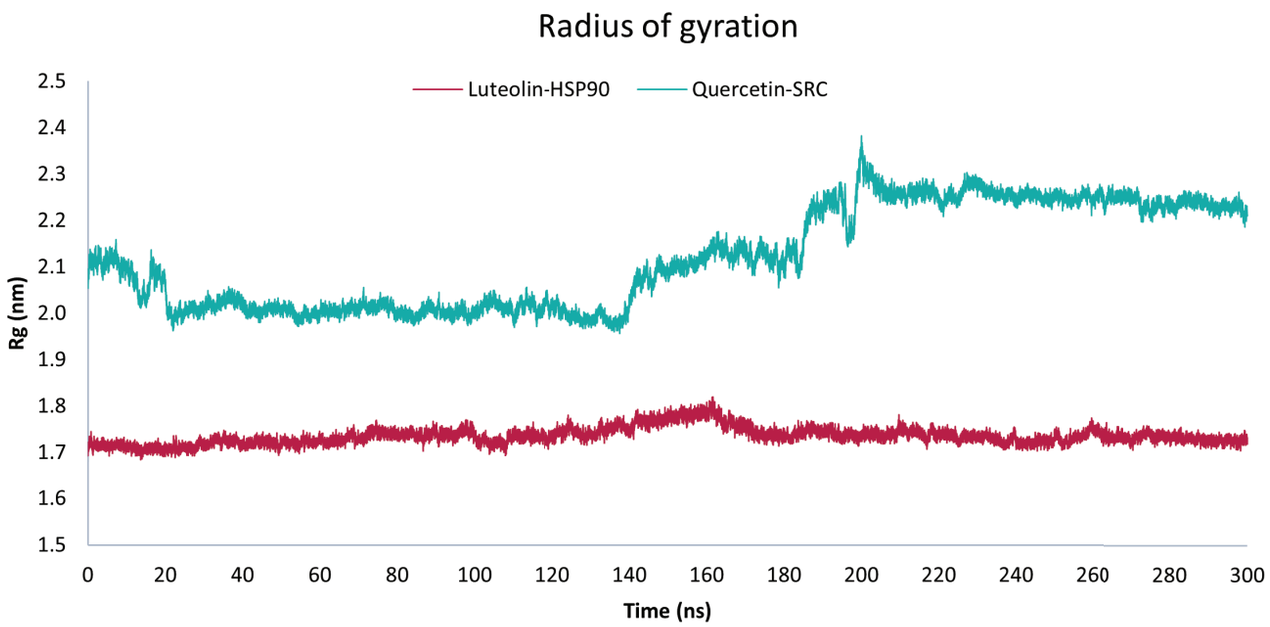
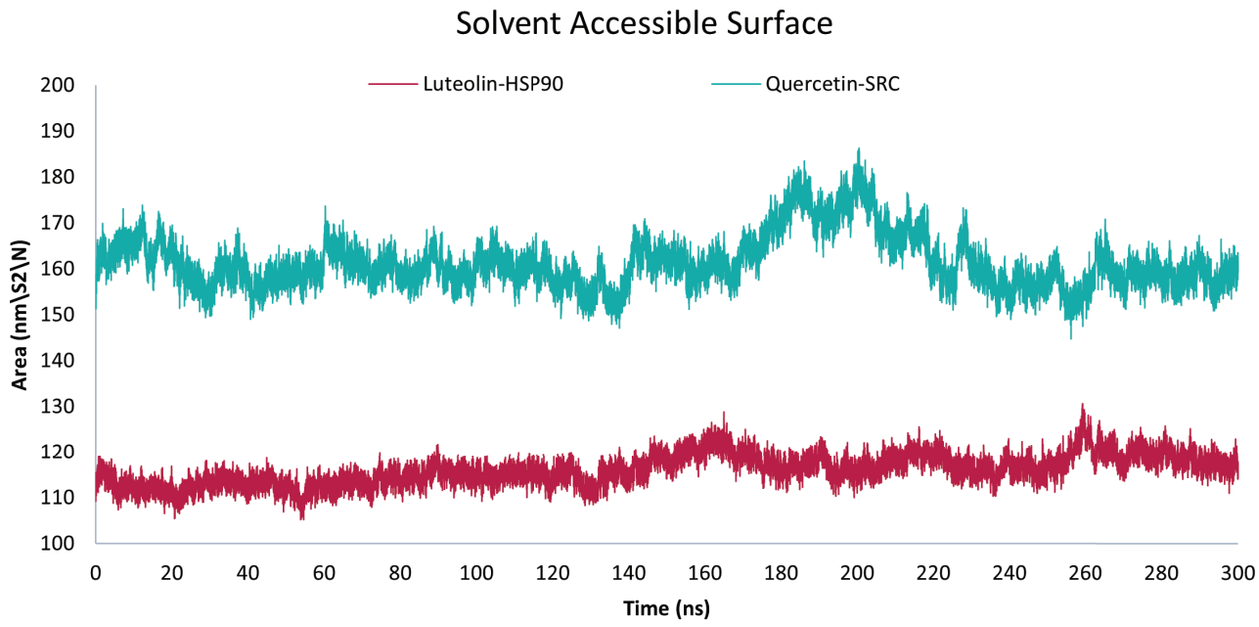
3.0
2.5
2.0
1.5
1.0
0.5
0.0
0
500
1000
1500
2000
2500
3000
3500
4000
4500
5000
Residue

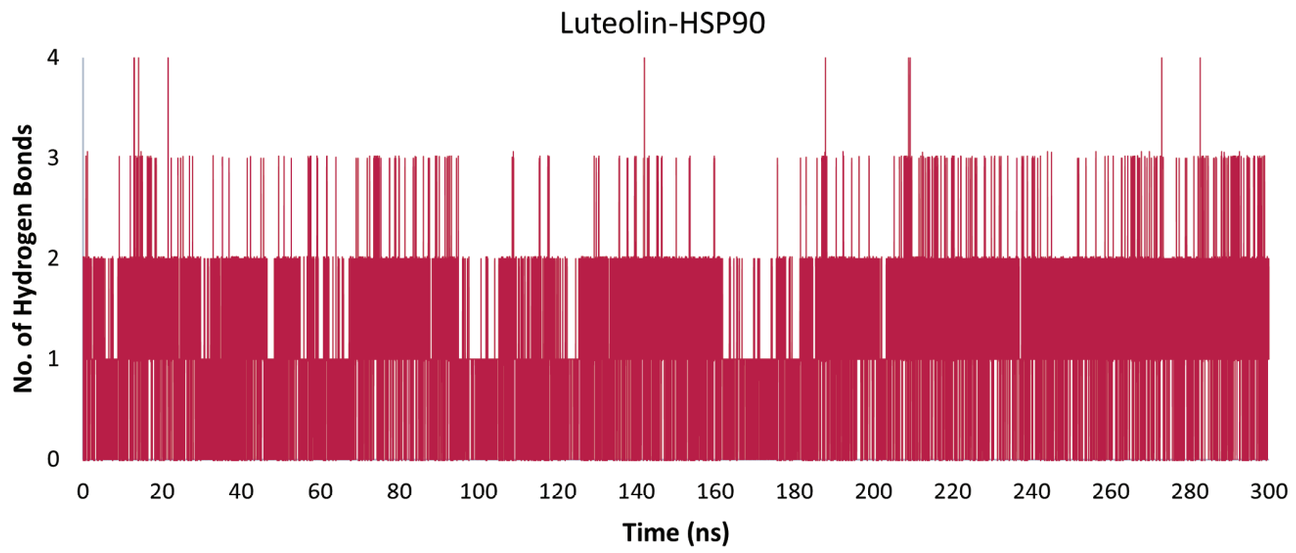
(D)


Radius of gyration
Luteolin-HSP90
Quercetin-SRC
Rg (nm)
2.5
2.4
2.3
2.2
2.1
2.0
1.9
1.8
1.7
1.6
1.5
0
20
40
60
80
100
120
140
160
180
200
220
240
260
280
300
Time (ns)

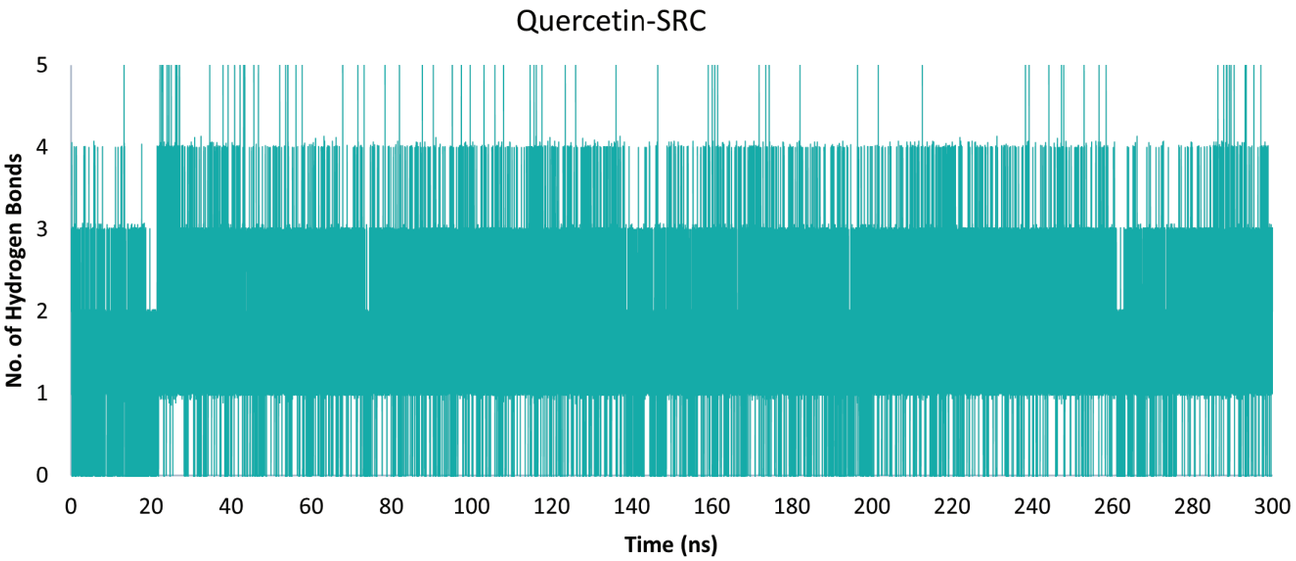
(E)


Solvent Accessible Surface
Luteolin-HSP90
Quercetin-SRC
Area (nm\S2\N)
200
190
180
170
160
150
140
130
120
110
100
0
20
40
60
80
100
120
140
160
180
200
220
240
260
280
300
Time (ns)

(F)



(G)



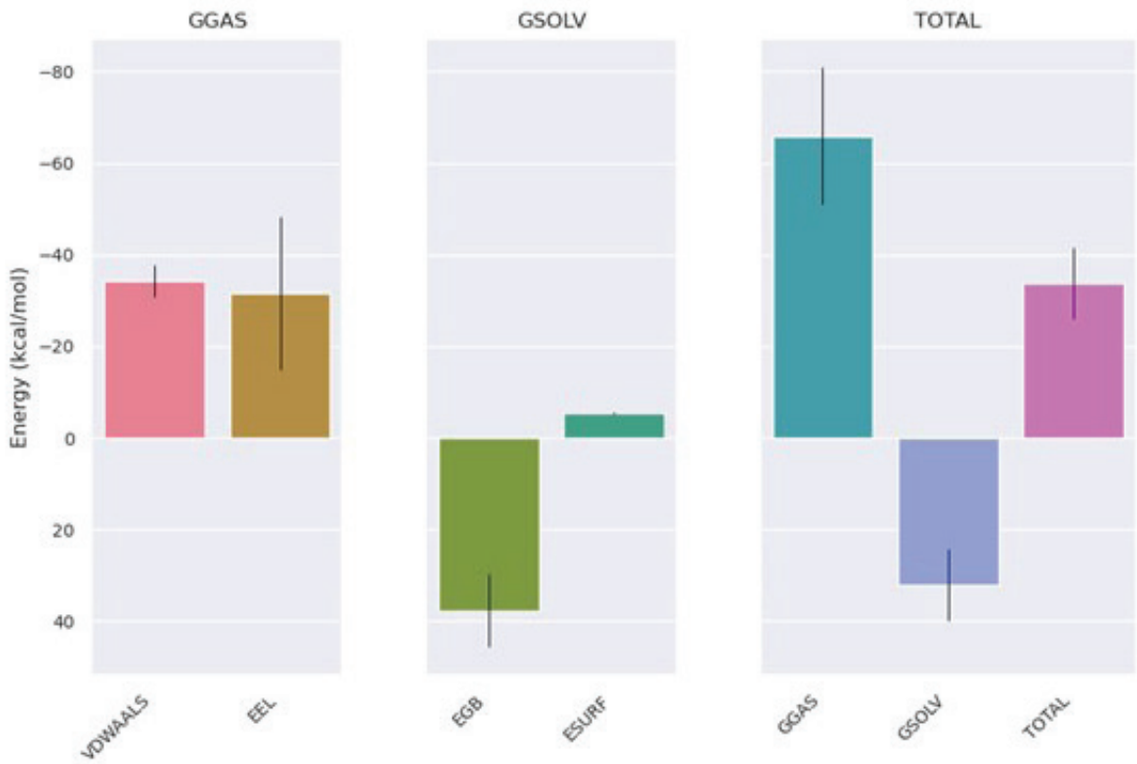
(H)

Fig. 2 (A-H) Molecular dynamics simulations endpoints are shown as (A) Quercetin-SRC (B) Luteolin-HSP90 (C) Root Mean Square Deviation (RMSD), (D) Root Mean Square Fluctuation (RMSF), (E) Radius of Gyration (total and around axes), (F) Solvent Accessible Surface, and (G) Hydrogen bond number of complex Luteolin-HSP90 (H) Hydrogen bond number of complex Quercetin-SRC (PTK2)

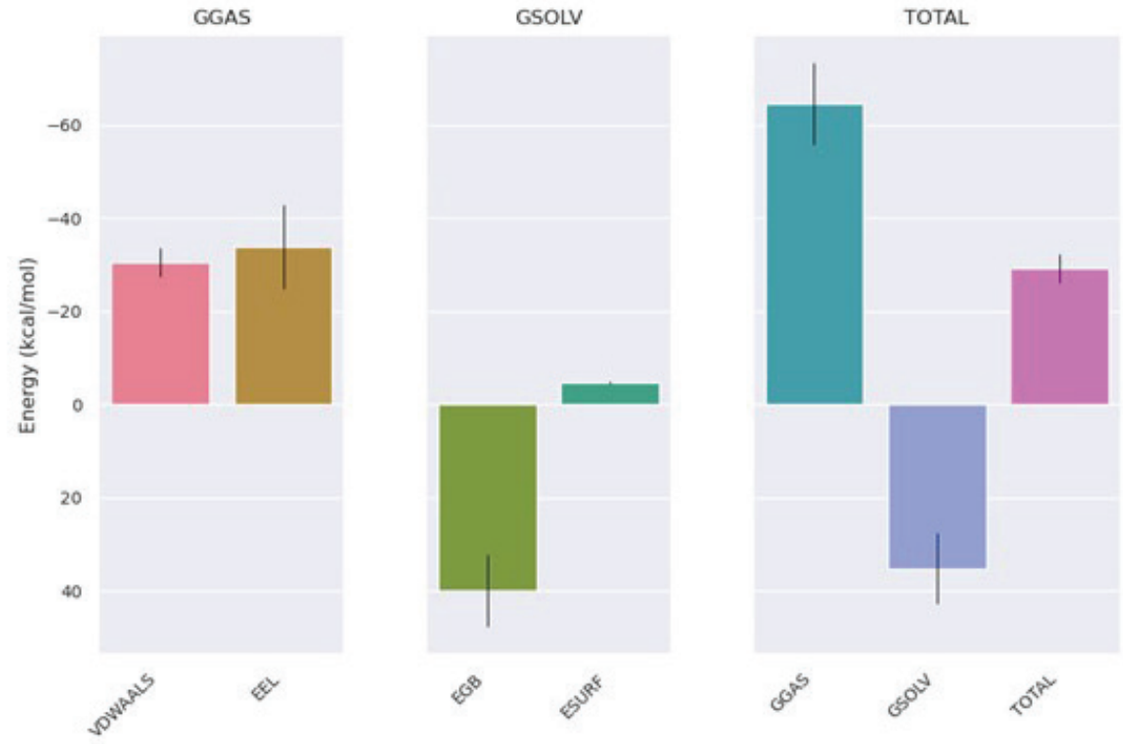
Interaction Energy Analysis

In the free binding energy analysis of the quercetin-SRC complex, the ΔTOTAL value averaged at -33.74 kcal/mol, indicating a thermodynamically favourable shift in free energy during complex formation. The change in van der Waals interactions energy (ΔVDWAALS) and change in electrostatic interactions energy (ΔEEL) components showed negative values, denoting reduced van der Waals and electrostatic interactions within the complex, while a change in solvation-free energy - generalized Born energy (ΔEGB) showed a positive contribution, signalling increased stability due to solvation effects. Despite weaker specific interactions, the overall negative ΔTOTAL value underscores the energetically favourable nature of the complex, largely driven by advantageous solvation effects indicated by ΔEGB and other solvation terms (Fig. 3 A). Similarly, in the evaluation of the luteolin-HSP90 complex, the ΔTOTAL value averaged at -29.26 kcal/mol, reflecting a favourable energy change during complex formation. ΔVDWAALS and ΔEEL components exhibited negative contributions, indicating decreased favourable interactions, whereas ΔEGB and solvation terms (ΔGGAS - change in polar solvation energy and ΔGSOLV - change in nonpolar solvation energy) contributed positively, emphasizing enhanced stability attributed to solvation effects. Despite less favourable specific interactions, the negative ΔTOTAL values in both complexes highlight their energetically favourable formations, predominantly guided by beneficial solvation effects, particularly ΔEGB, and significant contributions from solvation terms (Fig. 3 B).

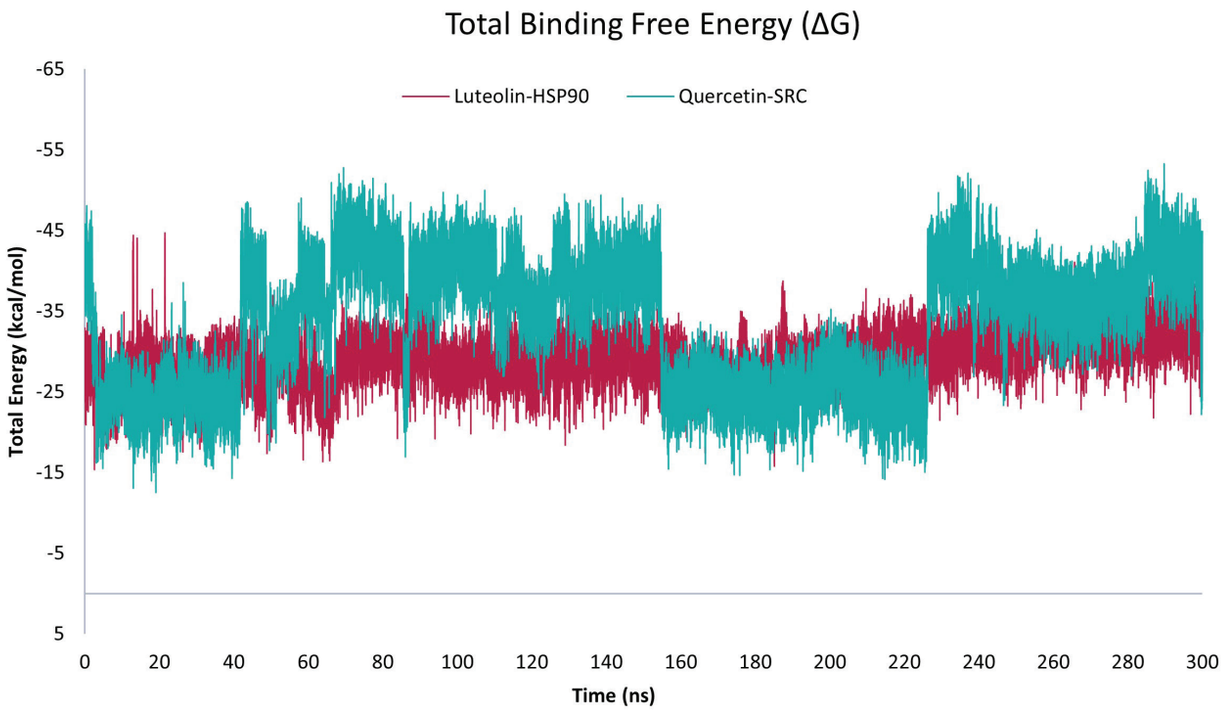
The binding free energy (BFE) trajectory analysis unveiled crucial insights into the interaction strengths between quercetin and luteolin (ligands) and their respective target proteins, SRC and HSP90. The statistical parameters revealed by the data highlighted the thermodynamic stability and binding probability of molecular interactions, with quercetin exhibiting a stronger binding affinity averaging at -33.7 kcal/mol towards the SRC protein. The minimum BFE of -53.1 kcal/mol for quercetin indicated highly favourable interactions, contrasting with the maximum value of -12.6 kcal/mol signifying weaker binding during simulation. Quercetin-SRC revealed variability in binding strengths with SD of 7.93 kcal/mol, while luteolin showed a slightly lower average binding affinity (-29.3 kcal/mol) to HSP90 compared to quercetin. However, luteolin-HSP90 showed a substantial minimum BFE (-44.7 kcal/mol) and fewer fluctuations with SD of 3.12 kcal/mol in binding strength (Fig. 3 C). Results showed that quercetin demonstrated a more robust and stable interaction with SRC, while luteolin-HSP90 exhibited slightly lower overall affinity with less variability during simulation.


GGAS
GSOLV
TOTAL
Energy (kcal/mol)
−80
−60
−40
−20
0
20
40
VDWAALS
EEL
EGB
ESURF
GGAS
GSOLV
TOTAL

(A)


GGAS
GSOLV
TOTAL
Energy (kcal/mol)
−60
−40
−20
0
20
40
VDWAALS
EEL
EGB
ESURF
GGAS
GSOLV
TOTAL

(B)



(C)

Fig. 3 (A-C) Binding Free Energy components of (A) Quercetin-SRC (B) Luteolin-HSP90 (C) BFE trajectory of Quercetin-SRC, and Luteolin-HSP90

In the quercetin-SRC complex, the ΔG Complex was found lower at -4755.76 ± 56.12 kcal/mol compared to the luteolin-HSP90 complex's ΔG complex of -3016.99 ± 44.85 kcal/mol. This difference indicated a significantly heightened binding affinity for quercetin-SRC over luteolin-HSP90. Upon dissecting the individual components, both complexes displayed more negative values for ΔG receptor than ΔG ligand, underscoring the substantial involvement and impact of the receptors in facilitating binding compared to the ligands in each complex. Additionally, the ΔG Total values, portraying the collective effect of receptor and ligand contributions, maintained negative values for both complexes, highlighting a favourable binding environment. However, the quercetin-SRC complex exhibited a notably more pronounced negative ΔG Total (-33.74 ± 7.93 kcal/mol) in contrast to luteolin-HSP90 (-29.26 ± 3.12 kcal/mol), further substantiating the superior binding affinity of quercetin-SRC (Table 6). Numerous studies employing molecular docking, and molecular dynamics simulations have examined active ingredients and molecular targets against carcinoma, all in agreement with the findings of our study (Sundarrajan et al., 2020; Zheng et al., 2022). Quercetin-SRC complex exhibited variability in binding strengths with SD of 7.93 kcal/mol, while luteolin-HSP90 demonstrated fewer fluctuations with SD of 3.12 kcal/mol

but slightly lower overall affinity. Overall, quercetin showed a more stable interaction with SRC than luteolin-HSP90 during simulations.

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

Complex	ΔG Complex	ΔG Receptor	ΔG Ligand	ΔG Total
Quercetin-SRC	-4755.76±56.12	-4749.90±55.09	27.88±4.58	-33.74±7.93
Luteolin-HSP90	-3016.99±44.85	-2969.71±44.61	-18.02±3.96	-29.26± 3.12

6. Conclusion

In this study, *T. procumbens* phytochemicals had the power to target several glioma-related target proteins. Three phytochemicals luteolin, quercetin, and 6-hydroxyluteolin 6,3'-dimethyl eter 5-rhamnoside could collectively target two proteins; HSP90 and SRC related to disease-glioma, according to research validated by numerous approaches, including molecular docking, and structural stability and interaction energy analysis via MD simulation. Furthermore, luteolin and quercetin were discovered to bind significantly with the target proteins heat shock protein 90 (HSP90) and proto-oncogene tyrosine-protein kinase (SRC) with binding affinities of 10 kcal mol^{-1} and 9.3 kcal mol^{-1}, respectively. The assessment of MD simulation results demonstrated that in a physiological context, luteolin and quercetin form highly stable complexes with their respective target proteins SRC and HSP90 with binding free energies of -33.74 kcal/mol and -29.26 kcal/mol, respectively. As a result, it can be concluded that luteolin with quercetin, taken as polyherbal medication, can be beneficial against glioma. Further *in vitro* and *in vivo* experiments are required to validate and enhance the study's outcomes.

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CHAPTER

07

Reported Pharmacological Profile

INTRODUCTION

Herbal remedies, sometimes referred to as botanical remedies or herbalism, are employed for their numerous therapeutic or medical benefits. Many drugs used in the twenty-first century came about because of research into traditional remedies that treat a wide range of illnesses using certain plants. An essential source of Ayurvedic, Unani, and traditional Chinese medicine is folk medicine. According to a WHO study, out of 119 medications made from plants, around 74% are used in contemporary medicine in a way that is closely connected to their previous usage as plant medicines by indigenous traditional societies (Ahmed et al., 2019). *Tridax procumbens* Linn. is often known as "Ghamra" in Hindi and "coat buttons" in English. It is widely used in the Ayurvedic medical system to treat various illnesses, and some Ayurvedic practitioners prescribe it as "Bhringraj," a well-known remedy for liver problems (Beck et al., 2018). This species has originated in tropical America and has spread to tropical Africa, Asia, Australia, and India. This is a wild plant found throughout India. Roadsides, waste areas, railroads, dykes, riverbanks, meadows, and dunes are all the places to find coat buttons (Bhagat et al., 2019). Its proliferating stems and copious seed production account for its broad range and significance as a weed. It is a widely used and recognized medication for liver disorders in the Ayurvedic medical system. Significant therapeutic benefits against malaria, dysentery, diarrhea, bronchial catarrh, blood pressure, hair loss, stomach discomfort, headache, and hair loss have been discovered (Andriana et al., 2019). Additionally, it is beneficial to stop bleeding from cuts and bruises and cure wounds. There have been reports of antiseptic, insecticidal, and parasiticidal qualities in flowers and leaves (Baile and Parmar, 2023). Several pharmacological activities have been associated with it, including hepatoprotective, anti-inflammatory, wound healing, anti-diabetic, hypotensive, immunomodulating, prevention of hair loss, promotion of hair growth, and anti-microbial against both Gram-positive and Gram-negative bacteria. Leaf juice has antiseptic, insecticidal, and parasiticidal qualities (Mundada and Shivhare, 2010). It is used as a treatment for conjunctivitis, insect repellent and used to stop bleeding from cuts, bruises, and wounds. A summary of *T. procumbens*' pharmacological investigations and therapeutic potential is given in this chapter.

Pre-clinical Study

Pre-clinical investigations are often undertaken in laboratories or on animals to determine a substance's pharmacological effects. This research may look at the plant's chemical makeup, impacts on biological systems, potential medicinal uses, and toxicity. *T. procumbens* is a medicinal plant that has long been used for a variety of purposes. Pre-clinical studies are critical for providing basic scientific information to support additional clinical research and the development of *T. procumbens*-based medicines or pharmaceutical products. While anecdotal evidence may support its therapeutic benefits, pre-clinical research is required to objectively analyse its effectiveness, safety, and potential mechanisms of action.

Analgesic Activity

Prabhu et al. (2011) evaluated the analgesic activity of methanolic extract (100, 200 and 400 mpk) of *T. procumbens* leaves against formalin-induced persistent pain, acetic acid-induced writhing test, and Freund's adjuvant-induced hyper analgesia in rat. In the formalin test, the extract at 200 and 400 mpk doses significantly decreased ($p<0.001$) the total paw licking and biting events only in 2nd phase. Similarly, the extract significantly ($p<0.05$) reduced the abdominal writhing in treated rats towards acetic acid-induced abdominal constriction test. In Freund's adjuvant-induced hyper analgesia method, the extract significantly ($p<0.001$) reduced mechanical hyper analgesia in rats.

Anti-arthritic Activity

Petchi et al. (2013) reported the anti-arthritic activity of ethanolic extract of *T. procumbens* (250 and 500 mg/kg, b.w.) on Freund's Complete Adjuvant (FCA)-induced arthritis in female Sprague Dawley (SD) rats. The extract at 250 mg/kg dose significantly ($p<0.01$) reduced the joint swelling in paws of rats. In addition, the extract also enhanced hemoglobin level, and red blood cell count when compared with arthritic control group.

Anti-coagulant Activity

Naqash and Nazeer (2011) reported the anti-coagulant activity of sulfated polysaccharide from the leaves of *T. procumbens* (5-100 µg/ml) on activated partial thromboplastin time (aPTT) using human blood plasma. Sulphated polysaccharides from *T. procumbens* prolonged aPTT and reached 113s at 100 µg/ml which was approximately 4.0-fold compared with the saline group (the control). Higher concentrations of sulphated polysaccharide from *T. procumbens* were required to achieve ($p<0.05$) significantly the same effect as with heparin in the aPTT assay.

Gubbiveeranna et al. (2019) demonstrated the anti-coagulant activity of aqueous extract of *T. procumbens* in mice. The extract decreased clotting time of human plasma as confirmed by recalcification time and partial thromboplastin time with 19.8 and 1.53 folds, which suggests its procoagulant nature. Also,

the extract enhanced adenosine diphosphate/ epinephrineinduced platelet aggregation by 1.35 and 1.38 folds, respectively. However, the extract did not induce hemorrhage and edema in the mice animal model study indicating its non-toxic nature.

Anti-diabetic Activity

Ikewuchi (2012) assessed the anti-diabetic activity of aqueous extract (100, 200 and 300 mg/kg, b.w.) of *T. procumbens* leaves against alloxan-induced diabetic rats. Metformin (50 mg/kg, b.w.) was used as positive control. The extract significantly lowered ($p<0.05$) plasma glucose, triglyceride, very low-density lipoprotein cholesterol, total bilirubin, urea, blood urea nitrogen; plasma alkaline phosphatase, alanine, aspartate transaminases, ocular superoxide dismutase activities and lymphocyte count when compared to test control. In addition, the extract also significantly increased ($p<0.05$) plasma calcium, ocular ascorbic acid contents, haemoglobin concentration, and neutrophil count when compared to test control.

Pareek et al. (2009) studied the acute and sub-chronic anti-hyperglycemic activity of methanol extract (250 and 500 mg/kg, b.w.) of *T. procumbens* against alloxan-induced diabetic rats using oral glucose tolerance test. The oral administration of acute and sub-chronic doses of extract showed a significant ($p<0.05$) reduction in fasting blood glucose level of diabetic rats. In acute study, the extract at tested doses showed maximum percentage of blood glucose reduction (68.26 and 71.03%) in diabetic rats at 6 h. However, the extract did not cause any significant change in blood sugar levels of normal rats.

Bhagwat et al. (2008) conducted the anti-hyperglycemic activity of aqueous, alcoholic, and petroleum ether extracts of dried leaves of *T. procumbens* (200 mg/kg, b.w.) against alloxan-induced diabetes in Wistar rats. Both aqueous and alcoholic extracts at tested dose exert decrease in blood glucose level for alloxan-induced diabetic rats, while petroleum extract exhibits very weak anti-diabetic effect.

Anti-hypertensive Activity

Salami et al. (2018) investigated the anti-hypertensive activity of aqueous extract (100 and 200 mg/kg, b.w.) of *T. procumbens* leaves on *N*-nitro-L-arginine methyl ester (L-NAME)-induced hypertensive male rats. Treatment with extract significantly ($p<0.05$) reduced the mean arterial blood pressure and body weight in L-NAME-only treated groups. The extract also exerts a significant ($p<0.05$) inhibition of contraction in response to phenylephrine, potassium chloride, and calcium chloride, while the relaxation in response to acetylcholine and sodium nitroprusside enhanced the relaxation.

Anti-inflammatory Activity

Berlin Grace et al. (2020) reported the anti-inflammatory activity of ethanolic extract of *T. procumbens* leaves (10, 50 and 100 mg/kg, b.w.) against carrageenan-induced in Albino Swiss mice. The extract showed a significant

(p≤0.001) decrease in the paw edema at 24 h, when compared to indomethacin (10 mg/kg, b.w.) used as standard drug. However, the extract at 50 mg/kg dose reduced the expressions of TNF-α and (100 mg/kg)-treated group showed very little expression of TNF-α, which was same as indomethacin. Besides, the extract also reduced COX-2 gene expression.

Devi et al. (2022) studied the anti-inflammatory activity of ethanolic extract of *T. procumbens* on alveolar type II cells (25-250 µg/ml) as well as, allergen ovalbumin (OVA) and lipopolysaccharide (LPS)-induced allergic asthma in rats (100, 200 and 400 mg/kg). The extract caused reduction in ROS production, apoptosis, and mitochondrial dysfunction in alveolar type II cells in a concentration-dependent manner. Further, the extract abrogated bronchial wall thickening, immune cell infiltration, and bronchial wall fiber deposition. In addition, the extract diminished the expression of IL-1ß and IL-6 in bronchial epithelium and vascular endothelium, and abrogated inflammation by reducing the level of inflammatory cytokines such as IL-2, IFN-ß, IL-6, and MCP-1, as well as inflammatory markers including TWEAK, TNF-α, and TNF-R1 and its downstream transcription factor NF-kB/p65 activation and nuclear translocation. Besides, the extract-treated lung tissue and alveolar type II cells reduced phosphorylation of ERK1/2.

Do Nguyen et al. (2015) assessed the anti-inflammatory activity of ethyl acetate extract (200 mg/kg, b.w.) of *T. procumbens* aerial parts against carrageenan-induced paw edema in mice. Treatment with extract significantly reduced the paw edema of mice on days 3 ($p<0.005$), 4 ($p<0.05$), and 5 ($p<0.05$) as like ibuprofen (50 mg/kg, b.w.) used as positive control.

Jachak et al. (2011) investigated the anti-inflammatory activity of ethyl acetate, methanol, 70% ethanol and dichloromethane extracts (200 mg/kg, b.w.) along with centaureidin, bergenin and centaurein isolated from *T. procumbens* aerial parts against carrageenan-induced in rat paw edema and, COX-1 and COX-2 inhibitory assay. Ibuprofen (100 mg/kg, b.w.) and indomethacin were used as positive controls. The ethyl acetate extract showed highest inhibition of paw edema (43.5 and 41.2%), followed by methanol (38.4 and 37.0%), and 70% ethanol extracts (35.8 and 35.3%) at 3 and 5 h, but less active than ibuprofen (56.4 and 52.2%). Also, the dichloromethane extract for COX-1 assays and ethyl acetate extract for COX-2 assay attained highest percentage inhibitions were 84.42 and 59.80% at 50 µg/ml when compared with indomethacin (98.23 and 50.99%). In contrast, bergenin also exerts good inhibitory effect with 70.45 and 41.59% at 100 µg/ml for both tested assays, which was higher than other tested compounds.

Anti-leishmanial Activity

Martín-Quintal et al. (2009) studied the anti-leishmanial potential of methanolic extract, its n-hexane, dichloromethane, and ethyl acetate fractions along with (3S)-16,17-didehydrofalcarinol isolated from

T. procumbens against promastigotes of *Leishmania mexicana*. The extract showed good anti-leishmanial effect with IC_{50} value of 3 µg/ml, followed by n-hexane (18 µg/ml), dichloromethane (126 µg/ml), and ethyl acetate (295 µg/ml), respectively. However, the compound showed potent effect with value of 0.478 µg/ml as comparable to amphotericin B and pentamidine (0.451 and 0.149 µg/ml) used as positive controls.

Syed et al. (2020) conducted the anti-leishmanial activity of methanolic extract of *T. procumbens* against promastigotes of *Leishmania mexicana* in serum of CD-1 mice. The extract showed a tendency to reduce the development of cutaneous lesion in mice while, four out of six mice presented minor lesions, and other two did not respond to the treatment and developed severe lesions.

Getti et al. (2009) investigated the anti-leishmanial activity of methanol, n-hexane, and dichloromethane extracts of *T. procumbens* against *Leishmania major, L. tropica, L. aethiopica*, and *L. aethiopica* axenic amastigotes by MTS assay as well as, THP-1 cells infected with *L. aethiopica*. Among tested extracts, n-hexane extract showed anti-leishmanial effect with LD_{50} values of 62.4, 7.2, and 18.5 µg/ml towards promastigotes of *L. major*, *L. tropica* and *L. aethiopica*, and 94.2, 27.1 and 95.2 µg/ml for amastigotes of *L. aethiopica*. However, the n-hexane extract significantly reduced percentage of infected monocyte-derived macrophages (THP-1), while the percentage of infected cells decreased to 14.9% when the cells were treated with n-hexane extract, without significantly decreasing the number of human cells.

Anti-microbial Activity

Syed et al. (2020) evaluated the anti-bacterial potential of methanol, ethanol, and ethyl acetate extracts of *T. procumbens* leaves against *Escherichia coli, Bacillus subtilis, Staphylococus aureus,* and *Enterobacter aerogenes* using well diffusion method. Among tested extracts, only methanol and ethyl acetate extracts showed good inhibitory effect towards all tested bacterial strains.

Cáceres et al. (1998) reported the anti-microbial effect of dichloromethane, ethanol, and water extracts from *T. procumbens* against *Pseudomonas aeruginosa, Salmonella typhi, Staphylococcus aureus, Candida albicans, Cryptococcus neoformans, Aspergillus flavus* and *Microsporum gypseum*. All tested extracts showed effectiveness with MIC values >10 mg/ml for tested strains.

Andriana et al. (2019) studied the anti-bacterial activity of n-hexane, chloroform, ethyl acetate and aqueous extracts and its chloroform: methanol fractions (F1 to F54-55) of *T. procumbens* against *Escherichia coli, Staphylococcus aureus, Bacillus subtilis,* and *Proteus mirabilis* by disc-diffusion method. Among tested extracts, the ethyl acetate extract showed highest inhibitory effect with MIC value of 25 mg/ml for *B. subtilis*, followed by *E. coli, S. aureus* and *P. mirabilis* (30 mg/ml), but less active than ampicillin (0.0195 mg/ml) and streptomycin (0.156 mg/ml) used as positive controls. Tested fractions attained

effectiveness with values ranging from 15 to 30 mg/ml for tested strains.

Taddei and Rosas-Romero (2000) reported the anti-microbial activity of n-hexane and ethyl acetate extracts of *T. procumbens* flowers and aerial parts against *Bacillus cereus*, *B. subtilis*, *Mycobacterium smegmatis*, *Staphylococcus aureus*, *S. epidermidis*, *Escherichia coli*, *Klebsiella* sp., *Proteus vulgaris*, *Pseudomonas aeruginosa*, *Salmonella* group C, *S. paratyphi*, and *Shigella sonnei* by disc-diffusion method. Among them, n-hexane flower extract showed highest inhibitory effect with zone of inhibition as 18 mm for *S. paratyphi* when compared with chloramphenicol (17 mm), erythromycin (19 mm), and tetracycline (21 mm) used as positive controls. In addition, other extracts attained effectiveness ranged from 9 to 14 mm towards other tested microorganisms. However, all extracts were found inactive for *Candida albicans*, *C. tropicalis*, *Rhodotorula rubra*, *Aspergillus flavus*, *A. niger*, *Mucor* sp., and *Trichophyton rubrum.*

Anti-osteoporotic Activity

Al Mamun et al. (2020) investigated the anti-osteoporotic activity of flavonoids from *T. procumbens* (TPF; 50 and 100 µg/ml) on lipopolysaccharides (LPS)-induced osteoclast differentiation and tartrate-resistant acid phosphatase (TRAP)-positive multinucleated cells. The flavonoids significantly (p<0.05) decreased the TRAP-positive multinucleated osteoclasts. Also, flavonoids at 100 µg/ml dose reduced multinucleated osteoclasts in culture as well as, tartrate-resistant acid phosphatase-positive multinucleated osteoclasts, inhibited TRAP, and acid phosphatase activities, and decreased the expression of osteoclast differentiating genes, including *cathepsin K*, *metalloproteinase*-2 (MMP2), MMP-9, MMP-13, and osteoclast-associated receptor (OSCAR). Moreover, the osteoclast-dependent actin ring formation and resorption pits were dramatically inhibited by TPF, which markedly decreased the expression levels of transcription factors such as c-Fos, nuclear factor of activated T cells, cytoplasmic 1, and activator protein-1.

Al Mamun et al. (2015) conducted the anti-osteoporotic activity of flavonoids (50 and 100 µg/ml) from *T. procumbens* on osteoclast differentiation and bone resorption activities using primary osteoclastic cells. Treatment with 100 µg/ml of flavonoids showed markedly fewer multinucleated osteoclasts in cultures and strongly suppressed cell-cell fusion and inhibited mature osteoclasts in terms of the formation of tartrate-resistant acid phosphatase-positive multinuclear osteoclasts. In addition, the flavonoids dose-dependently reduced the number of mature osteoclasts and inhibited RANKL-induced pit formation. In contrast, the flavonoids at 50 and 100 µg/ml inhibited mature osteoclast formation, and the resorption pit was significantly smaller in the experimental group. Further, flavonoids reduced the expression of mRNAs involved in osteoclast differentiation, such as *Trap, Cathepsin K, Mmp-9*, and *Mmp*-13, as well as the expressions of *Cathepsin* K, *Mmp*-9, and *Mmp*-13 proteins in primary osteoclastic cells.

Antioxidant Activity

Xu et al. (2010) reported the antioxidant activity of flavone derivatives from *T. procumbens* using DPPH and FRAP assays. The 8,3'-dihydroxy-3,7,4'-trimethoxy-6-O-ß-*D*-glucopyranosyl flavone showed highest antioxidant capacity in DPPH (IC_{50} of 0.03180 mg/ml) and FRAP (0.29784 mg/g), followed by 6,8,3'-trihydroxy-3,7,4'-trimethoxyflavone (0.04618 mg/ml and 0.07017 mg/g) when compared with trolox (0.00948 mg/ml and 1.00000 mg/g) used as positive control.

Brandão et al. (2021) investigated the antioxidant activity of essential oils from *T. procumbens* leaves using DPPH assay. The oils showed effectiveness with IC_{50} of 194.51 µg/ml when compared with ascorbic acid (16.71 µg/ml) used as standard.

Saxena et al. (2013) evaluated the antioxidant activity of ethyl acetate and n-butanol soluble parts of methanol extracts (100 µg/ml) of *T. procumbens* using DPPH assay. The ethyl acetate soluble part (EF-I) and n-butanol soluble part (BF-11) exhibited scavenging effect with IC_{50} of 67.26 and 80.90 µg/ml as comparable to ascorbic acid (59.62 µg/ml).

Syed et al. (2020) assessed the antioxidant activity of methanol, ethanol, and ethyl acetate extracts of *T. procumbens* leaves using DPPH and reducing power assays. Ascorbic acid is used as standard drug. Among tested extracts, the ethanol extract exhibited antioxidant effect with percentage inhibition 61.52%, whereas other extracts attained effectiveness ranging from 25.57 to 52.16%, as compared to ascorbic acid used as positive control (100%). However, the ethanol extract showed good DPPH scavenging effect with 82.5% inhibition at 200 µg/ml.

Andriana et al. (2019) evaluated the antioxidant activity of n-hexane, chloroform, ethyl acetate, aqueous extracts, and its fractions (F1 to F54-55) of *T. procumbens* using DPPH and ABTS assays. BHT is used as a positive control. The ethyl acetate extract showed the highest scavenging effects with IC_{50} values of 0.13 and 0.45 mg/ml, followed by aqueous (0.88 and 1.26 mg/ml), chloroform (1.40 and 2.11 mg/ml) and n-hexane extracts (>5 and 3.05 mg/ml) for DPPH and ABTS assays, respectively. Among tested fractions, chloroform: methanol (13.3 mg) F48-50, (11.4 mg) F51-53, and (74.3 mg) F54-55 exhibited good scavenging effect in DPPH (0.51, 0.54 and 0.82 mg/ml) and ABTS (1.04, 1.07 and 1.53 mg/ml) as comparable to BHT (0.009 and 0.045 mg/ml).

Do Nguyen et al. (2015) studied the antioxidant effect of ethyl acetate and petroleum ether extracts of *T. procumbens* aerial parts using DPPH assay. The ethyl acetate extract showed highest scavenging effect with IC_{50} value of 523.17 µg/ml when compared to petroleum ether extract (1118.7 µg/ml) for tested assay.

Anti-plasmodial Activity

Appiah-Opong et al. (2011) screened the anti-plasmodial activity of aqueous, ethanol, chloroform, and ethyl acetate extracts of *T.*

procumbens stem, flowers, and leaves against chloroquine-resistant (Dd2) *Plasmodium falciparum* parasites. Among tested extracts, the ethanol extract showed RBC protection with EC_{50} value of 121.3 µg/ml for *P. falciparum*. Further, the ethanol extract showed highest anti-plasmodial effect with IC_{50} of 143.4 µg/ml, followed by aqueous (225 µg/ml), ethyl acetate (250 µg/ml), and chloroform extracts (430.6 µg/ml) when compared with chloroquine (40 µg/ml) used as positive control.

Brandão et al. (2021) studied the larvicidal activity of essential oils (20-100 µg/ml) from *T. procumbens* leaves against *Aedes aegypti* mosquito. The oils at 100 µg/ml showed highest larvicidal effect with percentage inhibitions 60 and 65.2% after 24 and 48 h for *A. aegypti*.

Rajkumar and Jebanesan (2007) reported the anti-malarial activity of essential oils (2, 4 and 6%) from *T. procumbens* leaves against malarial vector *Anopheles stephensi*. The oils at 6% showed highest repellent activity effect of 317 min. when compared with control group (ethanol, 8.0 min).

Kaushik et al. (2015) assessed the anti-plasmodial activity of ethyl acetate and methanol extracts of *T. procumbens* leaves against *P. falciparum* (3D7 and INDO) strains. Both extracts showed anti-plasmodial effect with IC_{50} of 62 and 32 µg/ml for 3D7 strain but, less active than chloroquine (0.021 µg/ml) and artemisinin (0.0045 µg/ml) used as positive controls. However, both extracts did not show any toxicity.

Anti-trypanosomal Activity

Berger et al. (1998) studied anti-trypanosomal activity of dichloromethane, ethanol, and water extracts of *T. procumbens* against epimastigotes and trypomastigotes of *Trypanosoma cruzi* and trypomastigotes of *T. cruzi* in mice. All tested extracts showed anti-trypanosomal effect with IC_{90} values >1 mg/ml for epimastigotes of *T. cruzi*, while the ethanol extract exhibited good inhibitory effect towards trypomastigotes of *T. cruzi* (1 mg/ml). However, the ethanol extract was inactive (>500 µg/ml) against trypomastigotes of *T. cruzi* in mice.

Anti-viral Activity

Rothan et al. (2014) evaluated the anti-viral activity of ethanol and methanol extracts of *T. procumbens* leaves and stem against dengue NS2B-NS3pro virus using plaque formation assay. The ethanol extract of stem showed good anti-viral effect with IC_{50} of 25.6 µg/ml, whereas both extracts from leaves and stem attained effectiveness were ~62 and 70 µg/ml for dengue NS2B-NS3pro virus.

Naqash and Nazeer (2011) studied the anti-viral activity of sulfated polysaccharide (10 to 250 µg/ml) from *T. procumbens* leaves against herpes simplex virus (HSV-1) strain using virus plaque reduction assay. The sulfated polysaccharides at 250 µg/ml dose showed highest percentage inhibition with 80% towards HSV-1 strain.

Cardioprotective Activity

Ikewuchi et al. (2021) reported the cardioprotective activity of aqueous extract (50, 75 and 100 mg/kg, orally) of *T. procumbens* leaves against doxorubicin-induced cardiac toxicity in Wistar rats. The extract at 100 mg/kg dose significantly ($p<0.05$) increased the heart weight and heart size index. However, the extract (100 mg/kg) also significantly ($p<0.05$) decreased cardiac biomarkers of oxidative stress such as malondialdehyde, and raising cardiac levels of ascorbic acid, reduced glutathione, and superoxide dismutase (SOD) and decreases catalase (CAT) and glutathione peroxidase (GPX). In addition, the extract (100 mg/kg) increased plasma lactate dehydrogenase, cardiac cholesterol and triglyceride, and cardiac ATPase activities such as Mg^{2+}-ATPase, Na^{+}, K^{+}ATPase, and Ca^{2+}-ATPase, but significantly ($p<0.05$) decreased cardiac creatine kinase, plasma creatine kinase, and aspartate transaminase. Besides, the extract (100 mg/kg) significantly ($p<0.05$) decreased cardiac electrolytes.

Cytotoxicity

Martín-Quintal et al. (2009) studied the cytotoxicity of methanol extract, its hexane, dichloromethane, and ethyl acetate fractions along with (*3S*)-16,17-didehydrofalcarinol isolated from *T. procumbens* against blood mononuclear human cells. Amphotericin B and pentamidine were used as references drugs. The extract showed cytotoxicity with CC_{50} values of 69.18 and 64.56 µg/ml against both adherent and non-adherent blood mononuclear human cells, respectively. Moreover, the compound exhibits weak effect with values of 478.63 and 181.97 µg/ml for both cells when compared with amphotericin B (549.54 and 173.78 µg/ml) and pentamidine (575.43 and 456.51 µg/ml).

Syed et al. (2020) screened the cytotoxicity of methanol extract from *T. procumbens* leaves against human lung cancer cells (A549) and breast cancer cell lines (MCF-7) by MTT assay. The extract showed higher effectiveness against A549 cells than MCF-7 cell lines. However, the extract exerts 84% toxicity for A549 cells while, only 68% effect was observed in MCF-7 cell lines.

Naqash and Nazeer (2011) assessed the cytotoxicity of sulfated polysaccharides from *T. procumbens* leaves against Vero cells by MTT assay. The sulfated polysaccharides were found non-toxic with CC_{50} value of 200 µg/ml for tested cells. The results of cytotoxicity studies thus obtained indicate that the tested sample at different concentrations was cytotoxic only when the cultures were exposed to very high concentrations. High concentrations of any compound, under normal conditions, are cytotoxic to cell cultures.

Palshetkar et al. (2020) investigated the cytotoxicity of methanolic extract from *T. procumbens* against TZM-bl cell lines. The extract exhibit cytotoxicity with CC_{50} of 62 µg/ml for TZM-bl cell lines.

Enzyme Inhibitory Activity

Andriana et al. (2019) conducted the enzyme inhibitory activity of n-hexane, chloroform,

ethyl acetate, aqueous, hot aqueous (100°C) extracts and its fractions (F1 to F54-55) of *T. procumbens* by xanthine oxidase (XO) inhibitory assay. Among tested extracts and its fractions, the ethyl acetate extract showed highest xanthine oxidase inhibitory effect with 19.44% at 0.1 mg/ml, followed by hot aqueous extract (7.21%). Among tested fractions, chloroform: methanol (6.0:4.0) F45-47 attained highest effect (40.40%), but less active than allopurinol (90.21%) used as positive control. The other tested fractions attained effectiveness ranged from 3.99 to 37.11%.

Hepatoprotective Activity

Ravikumar et al. (2005) evaluated the hepatoprotective activity of chloroform-insoluble fraction (300 mg/kg, b.w., orally) from *T. procumbens* aerial parts against d-galactosamine/lipopolysaccharide (d-GalN/LPS)-induced hepatitis in albino Wistar rats. Pretreatment of extract showed a significant ($p<0.05$) protective effect for d-GalN/LPS-induced liver injury by maintaining the levels near normal. However, the extract did not show any significant change ($p<0.05$) in the activities of enzyme markers, bilirubin level, or lipids in the rats when compared with control group. Moreover, the extract reversed the large extent of hepatic lesions produced by d-GalN/LPS which was evident from the absence of cellular necrosis and inflammatory infiltrate around central zone and did not show any abnormal changes in the architecture of liver when compared with control group.

Ravikumar et al. (2005) studied the hepatoprotective activity of chloroform-insoluble fraction (300 mg/kg, b.w.) of *T. procumbens* against D-galactosamine/lipopolysaccharide (D-GalN/LPS)-induced hepatitis in rats. Treatment with extract increased the antioxidant enzymes (superoxide dismutase, catalase, glutathione peroxidase, glutathione transferases) and levels of non-enzymatic antioxidants (glutathione, vitamin C, vitamin E) when compared with the control rats. However, the extract-treated group did not show any significant changes in the lipid peroxide level of liver in normal and treated groups of rats.

Ikewuchi et al. (2021) investigated the hepatoprotective activity of aqueous extract (50, 75, and 100 mg/kg, b.w. orally) of *T. procumbens* leaves against doxorubicin-induced hepatic damage in Wistar rats. The extract at 100 mg/kg dose significantly decreased ($p<0.05$) the hepatic biomarkers such as malondialdehyde, and increased ascorbic acid, glutathione peroxidase, superoxide dismutase, and catalase while, decreased hepatic cholesterol and triglyceride concentrations. However, the extract (50 mg/kg) significantly increased ($p<0.05$) hepatic electrolyte profiles such as calcium, chloride, magnesium, potassium, and sodium, but decreased the levels at higher dose. In addition, the plasma biomarkers of liver function and integrity, including total protein, globulin, total bilirubin, and alkaline were significantly increased ($p<0.05$) whereas, albumin, albumin to globulin ratio, alanine transaminase, and aspartate transaminase significantly decreased ($p<0.05$) at 100 mg/

kg of extract. Besides, the extract at 100 mg/kg significantly increased ($p<0.05$) the liver weight and size indices.

Immunomodulatory Activity

Tiwari et al. (2004) evaluated immunomodulatory activity of ethanol-insoluble fraction (0.25 and 0.5 g/kg, b.w.) of *T. procumbens* aerial parts in mice. Treatment with extract at tested doses increased the phagocytic index, leukocyte count and splenic antibody secreting cells. Further, spleen weight ($p<0.05$), white blood cell ($p<0.05$), spleen leukocyte ($p<0.01$), hemagglutination antibody titer ($p<0.05$), and plaque forming cells/10^6 spleen cells ($p<0.01$) were increased with extract treatment. In addition, the extract at 0.50 g/kg attained an increase in paw volume (31%) for delayed type hypersensitivity footpad thickness of mice. In addition, the extract also reduced the number of mice presenting anaphylactic symptoms with no death and increased anti-TT IgG antibodies, implying that the extract influences both humoral and cell-mediated immune systems and, aids in the genesis of improved antibody responses against specific clinical antigens.

Nephroprotective Activity

Ikewuchi et al. (2021) demonstrated the nephroprotective activity of aqueous extract (50, 75 and 100 mg/kg, p.o.) of *T. procumbens* leaves against doxorubicin-induced renal damage in Wistar rats. The extract at 100 mg/kg dose significantly ($p<0.05$) increased kidney weight and size indices, renal ascorbic acid, glutathione peroxidase, and catalase, and decreased the renal malondialdehyde and superoxide dismutase levels. However, the urea/creatinine ratio was significantly ($p<0.05$) lowered at 100 mg/kg dose, while the plasma concentrations of creatinine, urea, and blood urea nitrogen were significantly ($p<.05$) higher at all tested doses. Further, the extract at highest dose significantly ($p<0.05$) increased renal lipid profiles (triglyceride and cholesterol) and, ATPase activities such as Mg^{2+}-ATPase, Na^+, K^+-ATPase, and Ca^{2+}-ATPase, as well as renal electrolyte profiles (calcium, chloride, magnesium, potassium, sodium).

Vasorelaxant Activity

Salahdeen et al. (2015) screened the smooth muscle relaxant activity of aqueous-ethanol extract (0.15-1.05 mg/ml) of *T. procumbens* leaves against phenylephrine (PE) and potassium chloride (KCl)-induced contractions in rat corpus cavernosum smooth muscles. The maximum relaxation responses of extract to both phenylephrine and high K^+ contracted corpus cavernosal strips were 92.2 and 52.3%, respectively. The extract showed a concentration-dependent reduction of KCl (60 mM) contraction. However, the strips incubated with the extract (0.6 mg/ml) also exert dose-dependent relaxation by acetylcholine and, attained the percentage relaxation were: 10^{-9} M: 15.8%, 10^{-8} M: 20.3%, 10^{-7} M: 44.2%, 10^{-6} M: 64.8%, 10^{-5} M: 79.4%, 10^{-4} M: 89.2% and 10^{-3} M: 88.9%, respectively. Moreover, the extract (10^{-9}, 10^{-8}, 10^{-7}, 10^{-6}, 10^{-5} and 10^{-4} M)

and attenuated dose-dependent relaxation (16.8, 40.3, 54.2, 67.8, 89.4, and 92.2%) in rat cavernosal strips. Besides, l-NAME (10^{-4} M) (15 min.) significantly ($p<0.01$) reduced the relaxation induced by extract from 1.05 mg/ml (92.2%) (in the absence) to (15.1%) (in the presence).

Salahdeen (2015) demonstrated the vasorelaxant potential of aqueous extract (0.5 to 9.0 mg/ml) of leaves of *T. procumbens* against phenylephrine (PE; 10^{-7} M) and potassium chloride (KCl; 60 mM)-induced contraction in rat's superior mesenteric arteries. The extract at tested doses significantly ($p<0.05$) reduced the contraction induced by PE (10^{-7} M) and KCL (60 mM). The extract also antagonized the calcium-induced vasoconstriction (1^{-9} to 10^{-5}) in calcium-free with high concentration of potassium as well as, in calcium- and potassium free physiological solutions. Further, the extract significantly ($p<0.05$) attenuated with preincubation of potassium channel blockers such as barium chloride, apamin, L-NAME, prostacyclin inhibitor (indomethacin), atropine, propranolol, and methylene blue. Besides, the extract was not affected by preincubation with glibenclamide, tetraethyl ammonium, 4-aminopyridine (4-AP) and oxadiazolo quinoxalin.

Wound Healing Activity

Yaduvanshi et al. (2011) screened the wound healing potential of topical ointment formulation (TP; 50 mg of either 1 or 4 mg/g) from leaves juice of *T. procumbens* using excision wound model in albino Swiss mice. TP (1 mg/g) exhibited marked dryness and, there was no visual sign of inflammation or any pathological fluid oozing out from the wounds. In contrast, TP (4 mg/g) was found wet and soft to touch. In addition, TP (1 mg/g) exhibited a significant ($p<0.05$) increase of 33.81% in collagen levels. Moreover, the collagen content of wounds treated with TP (4 mg/g) was markedly lowered by 29% when compared to control. Besides, TP (1 mg/g) on dermal wounds increased the infiltration of inflammatory cells, fibroblasts, and re-epithelization with moderate vascularity.

Toxicological Study

Pareek et al. (2009) conducted the acute toxicity study of methanol of (0.25-5 g/kg) from *T. procumbens* in rats. The extract did not show any signs of toxicity or mortality in treated rats up to a dose of 5 g/kg.

Bhagwat et al. (2008) reported the acute and chronic toxicity study of aqueous, alcoholic, and petroleum ether extracts (200 mg/kg, b.w.) of leaves of *T. procumbens* in mice. All extracts at 200 mg/kg dose showed acute toxic effect in mice after 48 and 72 h observation. In the chronic toxicity study, the extract time-dependently increased the body weight in rats.

Brandão et al. (2021) evaluated the toxicity of essential oils from the leaves of *T. procumbens* against *Artemia salina* larvae. The oils showed weak toxicity with LC_{50} value of 1238.67 µg/ml towards tested larvae as compared with

potassium dichromate (12.60 µg/ml) used as positive control.

Cáceres et al. (1998) assessed the toxicity study of dichloromethane, ethanol, and water extracts of *T. procumbens* against *A. salina* nauplii. All tested extracts showed toxicity with LD_{50} value >1000 ppm towards tested strain.

Clinical Study

Desai et al. (2015) conducted a clinical trial to evaluate the effectiveness of *T. procumbens* extract (15 ml, twice daily for 4 weeks) for anti-diabetic property including 20 individuals' (10 men and 10 women). The extract showed decrease in fasting blood glucose level by 11% in men ($p<0.01$) and, 20% in women ($p<0.05$), while post-prandial blood glucose concentrations were lowered by 26% in men ($p<0.001$) and, 29% in women ($p<0.001$) following 4 weeks. However, the extract did not show any adverse event or side effect.

Summary and Future Outlook

T. procumbens is one of the most popular herbal medicines in India and has been used for many years to treat a wide range of illnesses and paves the way for the development of novel conventional medicines. The studies on this species also desired development of novel therapeutic agents isolated from it, as isolation of oleanolic acid, a single triterpenoid, is reported from this plant. However, further research is warranted to fully understand its pharmacological properties, mechanisms of action, and clinical utility. Overall, *T. procumbens* holds significant promise as a valuable source of natural medicines. There is a great deal of potential for future studies to clarify the mechanisms of action of these plants and explore their further pharmacological effects.

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