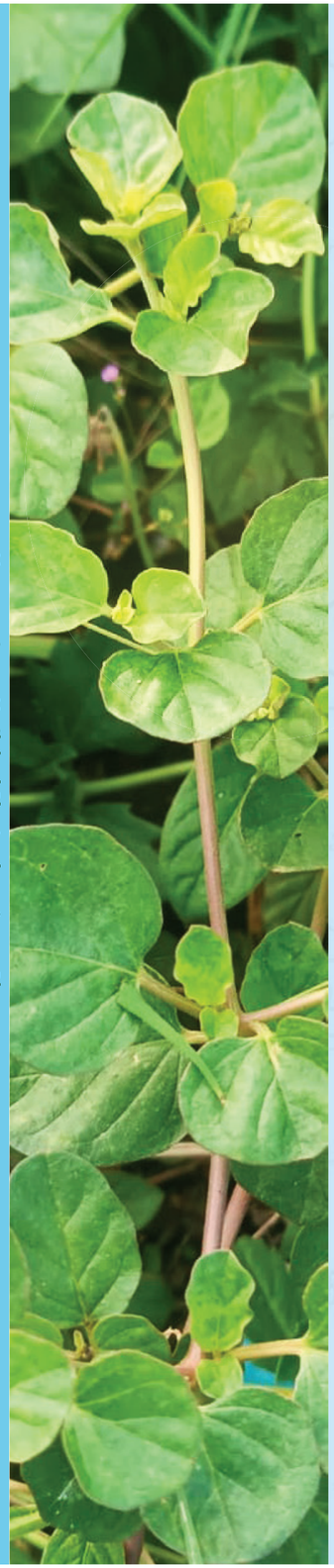
Patanjali Organic Research Institute Private Limited (PORI)
Food & Herbal Park, Village-Padartha, Laksar Road,
Haridwar-249407, Uttarakhand
www.patanjalibio.com | porihr@patanjalibio.com | +91-8755904985

Monographs on Medicinal Plants along Ganga River
Boerhavia diffusa L.

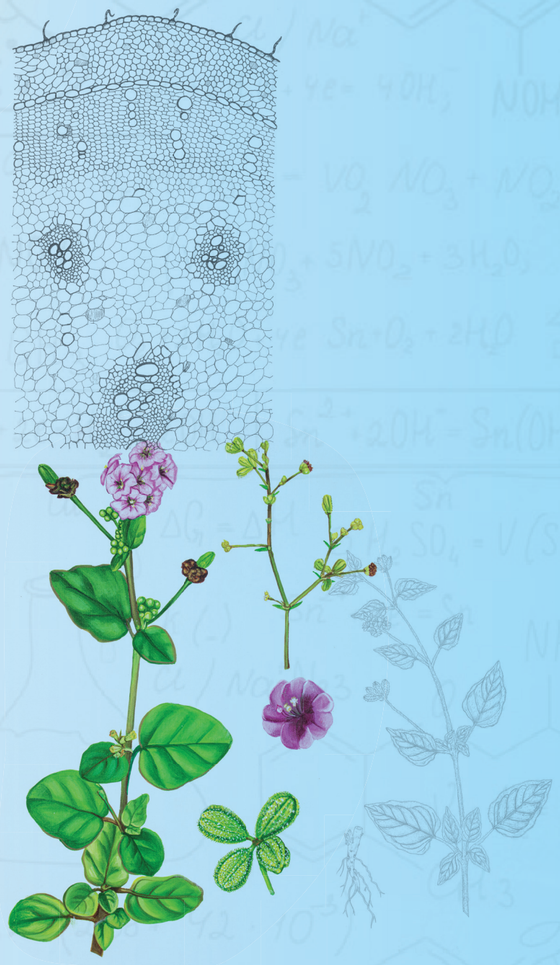


Monographs on Medicinal Plants along Ganga River

Boerhavia diffusa L.

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

Volume 1



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Volume 01

Sponsored by

National Mission for Clean Ganga (NMCG),
Department of Water Resources, River Development & Ganga Rejuvenation,
Ministry of Jal Shakti, New Delhi

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Implemented by Patanjali Organic Research Institute Pvt. Ltd. (PORI)

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

ABOUT THE EDITOR

Dr. Acharya Balkrishna, Co-founder of Patanjali Organic Research Institute, is a highly ascetic entrepreneur with a diverse personality who is a specialist in Yoga, Ayurveda, Sanskrit language, Indian sacred books, and the Vedas. Dr. Balkrishna has become a significant source of inspiration for Traditional Medicinal Practitioners and a globally recognized celebrity after dedicating his life to the resurrection of ancient healing and living practices. His maverick leadership as Co-Founder & Managing Director of Patanjali Ayurved Limited, along with overseeing Patanjali Food and Herbal Park and Divya Pharmacy, has propelled Ayurveda into a global business phenomenon with a massive following. Additionally, he has been decorated with prestigious awards such'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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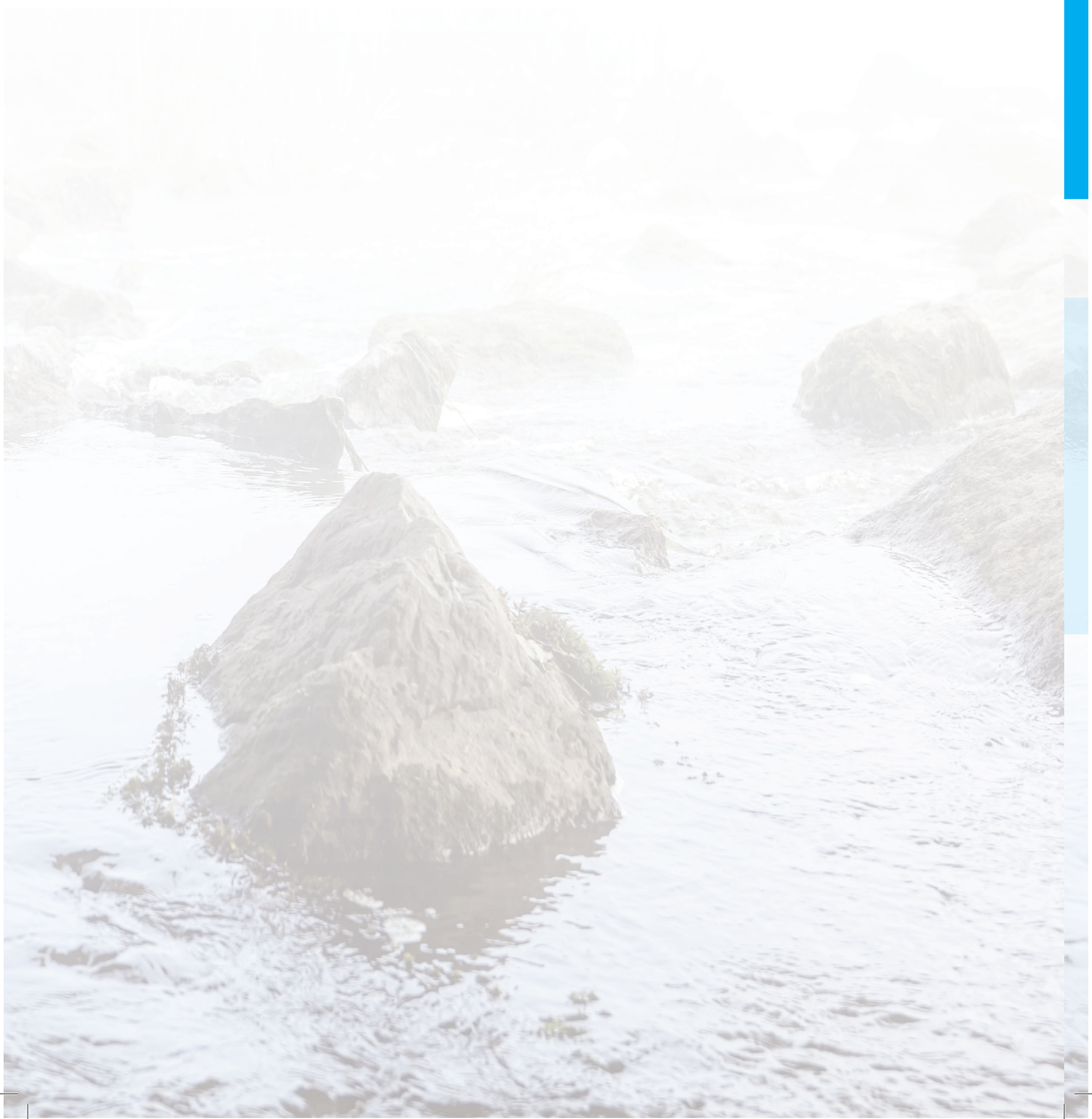

Vision of Union Minister for Jal Shakti

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.

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

Ministry of Environment, Forest and Climate Change (MoEFCC)

Uttarakhand Forest Department & Biodiversity Board

Uttar Pradesh Forest Department & Biodiversity Board

Bihar Forest Department & Biodiversity Board

Jharkhand Forest Department & Biodiversity Board

West Bengal Forest Department & Biodiversity Board

Ganga Task Force (GTF)

Table of CONTENTS

03

Soil Properties

04

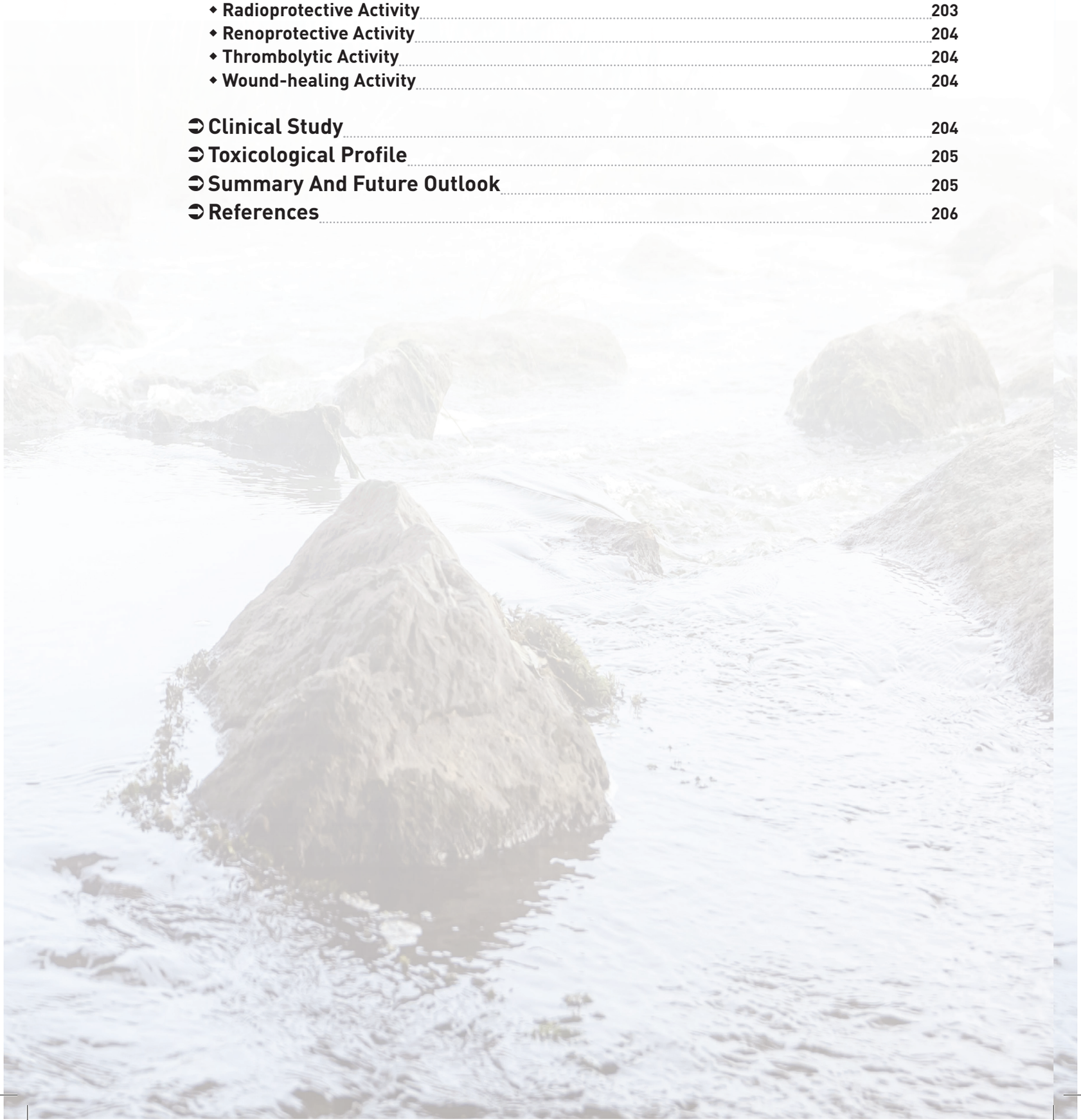
Traditional and Ethnomedicinal Applications

05

Phytochemical Analysis

06

Insilico Analysis Against Lung Cancer



राजीव कुमार मित्तल, भा.प्र.से.
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

LIST OF CONTRIBUTORS

OVERALL GUIDANCE AND MENTORSHIP

Dr. Acharya Balkrishna

PRINCIPAL INVESTIGATOR

Dr. Vedpriya Arya

CO-PRINCIPAL INVESTIGATOR

Mr. Pawan Kumar

EDITING AND PROOF READING

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

FIELD SURVEY & PLANT IDENTIFICATION

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

WRITING & SCIENTIFIC CONTRIBUTION

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

DESIGNING

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

OTHER CONTRIBUTION

Mr. Pradeep Badola
Mr. Shivam Walia
Mr. Ashu

Classical Ayurvedic Insight

Boerhavia diffusa L.

Vedic name : (Punarnavakaḥ raktakāṇḍaḥ) (पुनर्नवक: रक्तकाण्ड:)

Botanical name : *Boerhavia diffusa* L.

Utpatti sthānam

Upoṣṇakaṭibandhīye tathoṣṇe tatra sindhutaḥ.

Tathā'ntye bhāratād deśāllaṅkāyāmapi bhārate.

Samastabhāgataḥ prāyo dakṣiṇāt keraleṣu ca.

Pañcāle cā'samaprānte himādriśṛṅkhalāsvapi (Saumitreya Mahodadhiḥ: 1-2)

Distribution

Around the world it is found in tropical and subtropical Asia, Africa, America, Baluchistan, Sri Lanka and Singapore. Throughout India it found from the Punjab to Assam and South to Kerala, ascends in the Himalayan valleys up to an elevation of 2100 m. [1-5]

Vānaspatika vivaraṇam

1. Kāyika lakṣaṇam

Sahasravyāmakocchrāyabhāge bhāti punarnavā.

Bhūstāriṇī ca śākīyā śākhitā ca visāriṇī.

Cikkaṇā mṛduromāḍhyā vidyate bahuvārṣikī.

Mūlaṃ śaṅkunibhaṃ sthūlaṃ kāṣṭhīyañcāpi tiṣṭhati.

Kāṇḍo vyāmonnataḥ prāyo nīlaḥ saḥ parvatastathā.

Sthūlaśca tanukaḥ sūkṣmaromaśo mudgarākṛtiḥ.

Athavā'romaśaścāpi naikaśākhābhirapyuta.

Bhūstāriṇībhirābhāti patrantu saralantathā.

Pratiparvāpi vaiṣamyayuktayugmeṣu tat punaḥ.

Viparītaṃ bhavet tāvad dīrghapatrantu tatra vai.

Dvyaṅgulalambi taddhrasvamekāṅgulavilambitam.

Prāyaśaḥ samatulyañca sthūlamaṇḍanibhaṃ punaḥ.

Āyataṃ prāyaśo golaṃ rekhitaṃ hṛdayākṛti.

Śīrṣañca vartulaṃ vā'tha kuṇṭhāgramūrdhvapṛṣṭhataḥ.

Aromaśaṃ haridvarṇamadhaḥpṛṣṭhantu tasya vai.

Sādhāraṇatayā śvetaṃ sūkṣmaśalkīyameva ca. (Saumitreya Mahodadhi: 3-10)

Botanical Characters

1. Vegetative Characters

A perennial, very variable, diffusely branched, pubescent or glabrous, prostrate herb. Root fusi-form, stout, woody. Stem creeping, often purplish, up to 1.2 m long, swollen at the nodes, slen-der, cylindric, minutely pubescent or nearly glabrous with many procumbent branches. Leaves simple, opposite, at each node in unequal pairs, the larger 2.5-5 cm and smaller 1.3-2 cm long, both nearly as broad as long, thick, broadly ovate or suborbicular, linear, ovate-oblong or oblong-cordate, apex rounded, acute or obtuse, green and glabrous above, usually white, with minute scales beneath, margins entire, often pink coloured, somewhat undulate, base rounded or subcor-date. Petioles nearly as long as the blade or shorter, slender. [1-5]

2. Puṣpīya lakṣaṇam

Upāntaḥ pūrṇataḥ prāyaḥ pāṭalaśca taraṅgitaḥ.

Ādhāre vartulo vā'tha hṛttulyo'pi vibhāvyate.

Parṇānāṃ paṭalaḥ parṇavṛnte samatayoditaḥ.

Hrasvaśca tanutopetaḥ puṣpaṃ tasya tu lohitam.

Pāṭalābhaṃ kvacicchvetaṃ bhūyohrasvavapustathā.

Alpataḥ saṃvṛtaṃ prāyo vṛntahīnaṃ samuṇḍitam.

Catupañcaṣaguccheṣu daśaparyantataḥ punaḥ.

Kakṣīyatastathā'ntasthe tanugucche tathaiva ca.

Hrasvamuṇḍeṣu cābhāti vyavasthitatayoditam.

Paritodalapuñjantu sthitamādhāratastathā.

Āśupātīni caikatve sahapatrāṇi santi ca.

Kuntābhāni yavaprāyalambīni sahapatrikā.

Hrasvā kuntanibhā cāpi tīkṣṇāgrā parilakṣyate.

Paritodalapuñjantu triyavonnatavigraham.

Vārtākuraktavarṇāttu raktapāṭalamasti vā.

Prāyaśaḥ śvetavarṇaṃ vā ghaṇṭābhaṃ ca yavocchritam.

Dvitrāḥ puṅkesarāstatra svalpodvardhitayā matāḥ.

Puntantu kośikāyukto viṣamatvena dṛśyate.

Tiryaḍuṇḍāśayaścāpicchatrikāyuk ca vidyate.

Vartikāgraṃ punastatracchatrikākārakāyakam.

Phalaṃ tu triyavocchrāyaṃ romaśaṃ picchilaṃ tathā.

Granthimayaṃ sadā pañcaśirāyuktañca dīrghataḥ.

Āyatañcāṇḍatulyañca svalpamudgarakākṛti.

Śīrṣe tu vartulaṃ bhāti svabhāvenā'sphuṭadvapu.

Bījaṃ tu tanutopetaphalabhittiyutaṃ bhavet.

Sumanaḥphalakālaśca varṣāhemantamadhyataḥ. (Saumitreya Mahodadhi: 11-23)

2 Floral Characters

Flowers red, pink or white, very small, shortly stalked or nearly sessile, capitate, 4-10 together, borne in small heads arranged in axillary or terminal slender panicles. Bract at base of perianth quickly deciduous, single, lanceolate, 0.8-1 mm. Bracteoles small, lanceolate, acute. Perianth 3 mm long, purplish red to reddish pink or nearly white, campanulate beyond constriction, 1-1.5 mm long. Stamens 2-3, slightly exserted. Filaments capillary, unequal. Ovary oblique, stipitate. Stigma peltate. Fruit ovate, oblong, 0.3 cm long, five-ribbed, pubescent, highly viscid, glandular, shortly clavate, top rounded, indehiscent. Seed single, with thin pericarp. Flowering/Fruiting spring-autumn. [1-5]

Plant Anatomy

Prayojyāṅgam

Punarnavakanāmātra raktakāṇḍo hi pādapaḥ.

Prayojyāṅgaṃ mataṃ vaidyairantaḥsaṃracanocyate. (Saumitreya Mahodadhi:1)

Plant Parts

Whole plant

Antaḥsaṃracanekṣaṇe

(a) Kāṇḍabhāgaḥ

Adhicarmastaro naikakośikīyo'dhikāṇḍakam.

Tvacāromavrajo naikagranthika ekalo mataḥ.

Navato dvādaśa jjñeyā vṛntīyakośikā iha.

Dvikalpakastaro majjakośikāyāṃ vilokyate.

Mṛdutakaḥ sa vijjñeyo'spaṣṭā cāntastvacā matā.

Parirambhaprabhāgo'tra dvikalpakastarīyakaḥ.

Sthūlabhittīyakaścāsau prāyaḥ prakīrṇatantukaḥ.

Rambhabhāga ihāneke saṃvahanakapūlakāḥ.

Ekasmin paridhau sarve vyavasthitā bhavanti ca.

Saṃvahanakapūlāśca pratatā bharaṇotake.

Antaḥpūlīyakā tatra caidhikā parilakṣyate.

Upasthiteti kāṇḍīyāntāracanā pracoditā. (Saumitreya Mahodadhi: 2-7)

Microscopic Characters

Stem

Stem shows epidermal layer containing multi-cellular, uniserite glandular trichome consisting of 9-12 stalked cells. Cortex consists of 1-2 layers of parenchyma. Endodermis indistinct. Pericycle 1-2 layered, thick-walled often containing scattered isolated fibres. Stele consists of many small vascular bundles arranged in a ring. Large vascular bundles are many scattered in ground tissue. Intrafascicular cambium present. [1, 6-7]

(b) Mūlam

Mūle tvakprotakavyūhastanubhittīyako mataḥ.

Sparśarekhīyakā lambā bhittayo babhruvarṇikāḥ.

Tadbāhyastarakairetā dṛśyante pravinirmitāḥ.

Dvikalpakastarīyātra śalkaidhikā matā tathā.

Tanubhittīyasaṃjjñābhiḥ kośikābhirvinirmitā.

Dvitīyakākhyake majjakośikānicaye tathā.

Dvitrastarasamāyuktā dṛśyā mṛdulakośikāḥ.

Pañcato dvādaśa jjñeyaḥ starabhāgo'tra vīkṣaṇe.

Mūlabhāgāntare majjakośikānicayasya vai.

Bahubhujīyakā aṇḍākārāśca kośikā matāḥ. [

Tanubhittīyakāstā nyagvāhotakavrajasya ca.

Kendritapaṭṭikāmadhye mṛdulotakabhūmiṣu.

Tā ekāntaritā dṛśyā nyagvāhakastathā punaḥ.

Vāhikābhiravāgvāhavāhinikīvrajairasau.

Tantūnāṃ nicayaiścāpi vinirmito'bhidṛśyate.

Dvita ihāṣṭaparyantamarīyasamavāyake.

Vāhikā atra dṛśyante saralā gatikāstathā.

Jālikāvatsamāyuktāḥ sthūlanaiḥ sūkṣmadarśane.

Mūlasya madhyamaṃ kṣetraṃ saṃvahanakapūlakaiḥ.

Prāthamikaiḥ samāyuktaṃ cūrṇātukṣārakasya ca.

Pragucchā vividhā dṛśyāstathā maṇḍakaṇā api.

Saralā yuktarūpāśca catuḥkalpatadaṃśake.

Bahulaṃ labhyate majjakośikānicayastathā.

Punarbhūmūlagetyuktāntāracanā samīkṣaṇe. (Saumitreya Mahodadhi: 8-19)

Root

Root shows a cork composed of thin walled tangentially elongated cells with brown walls in the outer few layers. Cork cambium is 1-2 layered and thin-walled cells. Secondary cortex consists of 2-3 layers of parenchymatous cells. Cortex is composed of 5-12 layers of thin-walled, oval to polygonal cells, several concentric bands of xylem tissue alternating with wide zone of parenchymatous tissue present. Xylem consists of vessels, tracheids and fibres. Vessels are found in groups of 2-8 in radial rows, having simple pits and reticulate thickening. Central regions of root consists primary vascular bundles, numerous raphides of calcium oxalate. Starch grains are simple and compound having 2-4 components found in abundance in cortical cells. [1, 6-7]

(c) Patrabhāgaḥ

Aniyatā hi randhrālī patrobhayastare matā.

Catuḥparyantakaṃ tasyā laghuromastaravrajaḥ.

Kośikīyo'bhidṛśyo'tra stambhotakakadambakam.

Ekastarīyakaṃ bhāti tathā mṛdutakāḥ punaḥ.

Spañjino'tra catuḥkalpastīrayakā bhavanti ca.

Laghvavakāśasaṃyuktā vicitrā atha kośikāḥ.

Maṇibhaguhikāyuktā parṇamadhyotake tathā.

Cūrṇātukṣārakasyātra kaṇā apyavalokitāḥ.

Nāgaraṅgasamāḥ śoṇā rālāmayāḥ padārthakāḥ.

Dṛśyante sūkṣmikāyatre punarnavādalekṣaṇe. (Saumitreya Mahodadhi:20-24)

Leaves

Leaf shows anomocytic stomata on surfaces, a few short hairs, 3-4 celled, present on the margin. Palisade are single layered. Spongy parenchyma is 2-4 layered with small air spaces. Idioblasts contains raphides. Crystal of calcium oxalate and orange-red resinous matter found in mesophyll. [1, 6-7]

Medicinal Uses

Cephalic disorders

Punarnavāyārajasogaṣhītvānasyaṃpunaḥpāvakamṛnato nu.

Niḥsaṃṣayaṃsaptabhirevaghasraiprayātiśaṃkhabhaṣkuṭīvyathā'stam.

(Si.Bhe.Ma. Caturthaguccha, Netraroga Ci.:43)

Cephalic disorders- Snuff of *Boerhavia diffusa* (Hog weed) root bark powder is useful in frontal pain and temporal pain.[8] (Sd.Bh.Mm. Ch.Nt.Rg. :43)

Ophthalmic disorders

Śvetādrikarṇyāsapunarnavāyāmūlaiprapṛṭairyavacūrṇayuktaiḥ.

Vilocanaṃpūritamambuyuktairvimucyatepuṣpakṛtopasargāt. (Rā.Mā. 3:13)

Corneal opacity- Application of collyrium prepared from an equal quantity of *Clitoria ternatea* (Butterfly pea) root, *Boerhavia repens* (Creeping spiderling) root and *Hordeum vulgare* (Barley) is useful in corneal opacity. [9] (Ra.Ma. 3:13)

Dugdhenakaṇḍūṃkṣodreṇanetrasrāvaṃ ca sarpiṣā.

Puṣpaṃtailenatimiraṃkāñjikenaniṣāndhatāg.

Punarnavājayedāṣubhāskarastimiraṃyathā.

(Bhā.Pra.Ci. 63:210)(Śā.Sa.Ut.Kha. 13:100-101)

Ophthalmic disorders- Collyrium of *Clitoria ternatea* (Butterfly pea) root paste pounded with cow milk is useful in eye itching. Collyrium of *Boerhavia diffusa* (Hog weed) root paste mixed with honey is useful in epiphora. Collyrium of *Boerhavia diffusa* (Hog weed) root paste mixed with *Ghrita* (Clarified butter) is useful in corneal opacity. Collyrium of *Boerhavia diffusa* (Hog weed) root paste mixed with oil is useful in cataract. Collyrium of *Boerhavia diffusa* (Hog weed) root pounded with sour gruel is useful in xerophthalmia. [10] (Bh.Pr.Ci. 63:210), [11] (Śā.Sa.Ut.Kha. 13:100-101)

Ghṛtenamūlakaṃcāsyāhyaṃjitaṃhantipuṣpakam.

Madhunāsahamūṃtuhyajitaṃsrāvanāśakam.

Aṃjitaṃmārkavarasairnetrakaṇḍūnivāraṇam.

Kevalenajalenaivahyaṃjitaṃtimirāpaham.

Jalenagoṣakṛtācapippalyācāṃjitaṃyadā.

Rātryāṃdhyaṃnaṣyatetenacoṣṇaḥparṇarasaḥsmṛtaḥ.

(Śā.Ni.Guḍūcyādivarga, Pṛṣṭhaḥ 321)

Collyrium of *Boerhavia repens* (Creeping spiderling) root mixed with *Ghrita* (Clarified butter) is useful in ophthalmic disorders. Collyrium of *Boerhavia diffusa* (Hog weed) root paste mixed with honey is useful in subconjunctival hemorrhage. Collyrium of *Boerhavia diffusa* (Hog weed) root juice mixed with *Eclipta alba* (False daisy) juice is useful in eye itching. Collyrium of *Boerhavia diffusa* (Hog weed) root juice mixed with *Piper longum* (Long pepper) and cow milk is useful in xerophthalmia. [12] (Sh.Gr.Ng. Gd.Vg. pp:321)

Abdominal disorders

Mūṃsamaṃtaṇḍuladhāvanenaprapeṣitaṃśvetapunarnavāyāḥ.

Pītaṃbhavetplīhavināśahetu (Rā.Mā.7:5)

Gastrosplenic disorders- Intake of *Boerhavia repens* (Creeping spiderling) root powder alongwith rice water is useful in splenomegaly. [9] (Ra.Ma. 7:5)

Harītakīnāgaradevadārupunarnavāchinnaruhākaṣāyaḥ.

Saguggulurmūtrayutṛcapeyaḥśothodarāṇāṃpravaraḥprayogaḥ.(Vṛ.Mā.38:1)

Punarnavāmṛtādārupathyānāgarasādhitaḥ.

Gomūtragugguluyutaḥkvāthaḥśothodarāpahaḥ.(Śā.Saṃ.Ma.Kha.2:120-121)

Abdominal disorders- Intake of decoction prepared from *Terminalia chebula* (Black myrobalan), *Zingiber officinale* (Dry ginger root), *Tinospora cordifolia* (Tinospora), *Boerhavia diffusa* (Hog weed), *Cedrus deodara* (Deodar) and *Berberis aristata* (Indian barberrry) mixed with *Commiphora mukul* (Gum guggul) and cow urine is useful in abdominal distention and other abdominal disorders. [13] (Vr.Md. 38:1), [11] (Sh.Sm.Md.Kh. 2:120-121)

Kālaśākalavaṇauṣadhānvitaṃbhaktapakvamathasarpiṣānvitam.

Mūlamasyatipunarnavodbhavaṃgulmatodamacireṇaniścitam.(Vai.Ma.8:19)

Gulma- Intake of vegetable prepared from *Boerhavia diffusa* (Hog weed) root and *Corchorus olitorius* (JewÕs mallow) mixed with rock salt is useful in *Gulma* and pin pricking pain. [13] (Vd.Mn. 8:19)

Punarnavāvallijabāṇapuṃkhāviśvāgnipathyācirabilvabilvai.

Kṛtaḥkaṣāyaḥṣamayedaśeṣāndurnāmagulmagrahaṇīvikārān. (Vai.Jī.Dvitīyavilāsaḥ 18)

Intake of decoction prepared from *Boerhavia diffusa* (Hog weed), *Piper nigrum* (Black pepper), *Tephrosia purpurea (*Wild indigo), *Zingiber officinale* (Dry ginger root), *Plumbago indica* (White leadwort), *Terminalia chebula (*Black myrobalan), *Derris indica* (Smooth leaved ponga) and *Aegle marmelos* (Bengal quince) pulp is useful in hemorrhoids, *Gulma* and sprue. [14] (Vd.Ji.Dw.Vi. :18)

Ano-rectal disorders

Parisrāveśaṣṭaṃkṣīraṃsavaṣṣcīrapunarnavam.

Ākhuparṇikayāvā'pitaṇḍulīyakayuktayā.(Ca.Si.10:32)

Parisrāva- Enema of milk processed with *Boerhavia repens* (Creeping spiderling) and *Boerhavia diffusa* (Hog weed) paste is useful in *Parisrāva* (disorder caused due to excessive use of enema). [15] (Ca.Si. 10:32)

Hepatosplenic disorders

Punarnavātrivaṣdvyoṣaviḍaṅagadārucitrakam.

Kuṣṭhaṃharidretriphalādantīcavyaṃkaliṅagakāḥ.

Tāḥpāṇḍurogānplīhānarmāṃsiviṣamajvara.

Ṣvayathuṃgrahaṇīdoṣaṃhanyukuṣṭhaṃkrimīṃstathā.(Ca.Ci. 16:93-96)

Anaemia- Intake of *Punarnavādi Manḍūra* alongwith buttermilk is useful in anaemia, splenomegaly, hemorrhoids, malarial fever, inflammation, sprue and worm infestation. [15] (Ca.Ci. 16:93-96)

Renal and Urinary bladder disorders

Punarnavā'mṛtābhīrusakṣāralavaṇatrayai.

Ṣaṭīkuṣṭhavacāmustarāsnākaṭphalapauṣkarai.....

Kaṭyūruvastimeḍhrasyakukṣivakṣaṇasaṃyutam.

Kaphavātāmaśūlaghnamatravaddheṣcanāśanam.(Bhā.Pra.Ci. 37:95-99)

Calculus- Intake or enema of *Punarnavādi taila* (oil) is useful in diabetes, calculus, pain, dysuria and pain caused due to *Vāta* and *Kapha*. [10] (Bh.Pr.Ci. 37:95-99)

Reproductive system disorders

Apāmārgapatradvayaṃyonimadhyenivişṭaṃkṣaṇādyayoniśūṃnihanti.

Sudurvāramapyevamevaprayuktoviddhyādrasastatrapaunarbhavo'pi.

(Ga.Ni.Kau.Taṃ. 6:15)

Vaginal pain- Insertion of vaginal suppositroy prepared from *Boerhavia diffusa* (Hog weed) paste or juice alleviates vaginal pain. [16] (Gd.Ni.Kau.T. 6:15)

Mūṃpunarnavāyāḥsatailamīṣatkṛṃguhye.

garbhaṃpravepamānaṃsahasātrīṇāṃbahiḥkurute.(Ga.Ni.Kau.Taṃ. 4:38)

Abnormal presentation of fetus- Topical application or insertion of vaginal suppositroy prepared from *Boerhavia diffusa* (Hog weed) root paste mixed with oil promotes normal delivery. [16] (Gd.Ni.Kau.T. 4:38)

Musculoskeletal disorders

śākaṃpaunarnavaṃhitam.(Vaṃ. Se. Āmavātarogādhikāraḥ20)

Rheumatoid arthiritis- Intake of *Boerhavia diffusa* (Hog weed) vegetable is useful in rheumaoid arthritis. [17] (Vg.Sn.Am.Vt. :20)

Punarnavānāgaradārūpathyābhallātakacchinnarūhākaṣāyaḥ.

Daṣāṃghimiśraḥparipeyaurūsbheṃ'thavāmūtrapuraprayogaḥ. (Vai.Jī. Caturthavilāsa : 33)

Thigh stiffness- Intake of decoction prepared from *Boerhavia diffusa* (Hog weed), *Zingiber officinale* (Dry ginger root), *Cedrus deodara* (Deodar), *Terminalia chebula (*Black myrobalan), *Semecarpus anacardium* (Marking nut tree), *Tinospora cordifolia* (Tinospora) and *Dashmoola* (group of roots of ten medicinal herbs) is useful in thigh stiffness. [14] (Vd.Ji.Ch.Vi. :33)

Disorders of extremity

Punanarvāyāḥśvetāyāstailaṃmūlenasādhayet.

Vātakaṇṭakamāhanyātpādābhyaṅagenamardanāt. (Vaṃ.Se. Vātavyādhyidhikāraḥ140)

Calcification of soft tissues- Massage of oil processed with *Boerhavia repens* (Creeping spiderling) root paste and decoction is useful in calcification of soft tissues. [17] (Vg.Sn.Vt.Vy. :140)

Dermatological disorders

.....punarnavācetikuṣṭhinolepāḥ.

Dadhimaṇḍayutāḥsarvedeyāḥ.....(Ca.Ci.7:125)

Leprosy- Topical application of *Boerhavia diffusa* (Hog weed) root pounded with curd water is useful in *Vātaja* and *Kaphaja* leprosy. [15] (Ca.Ci. 7:125)

Punarnavāvarūṇayokvātho'ntarvidradhīñjayet.(Śā.Saṃ.Ma.Kha. 2:128)

Internal abscess- Intake of decoction prepared from *Boerhavia diffusa* (Hog weed) and *Crataeva nurvala* (Three leaved caper) bark alleviates internal abscess. [11] (Sh.Sm.Md.Kh. 2:128)

Punarnavā'bhayānimbadārvītiktāpaṭolakai.

Guḍucīnāgarayutaikvāthogomūtrasaṃyutaḥ.

Pāṇḍukāsodaraśvāsaśūlasarvāṃṅgaśothahāḥ.(Śā.Saṃ.Ma.Kha. 2:77-78)

Inflammation- Intake of decoction prepared form *Boerhavia diffusa* (Hog weed), *Terminalia chebula* (Black myrobalan), *Azadirachta indica* (Margosa tree) bark, *Berberis aristata* (Indian barberrry), *Tinospora cordifolia* (Tinospora) and *Zingiber officinale* (Dry ginger root) mixed with cow urine is useful in anaemia, cough, abdominal disorders, dyspnea, pain and anasarca. [11] (Sh.Sm.Md.Kh. 2:77-78)

Punarnavādārūniśāniśāśuṇṭhīharītakī.

Guḍucīcitrakobhārṅgīdevadārū ca taiśraṣṭaḥ.

Pāṇipādodaramukhaprāptaṃśophaṃnivārayet.(Śā.Saṃ.Ma.Kha. 2:122-123)

Intake of decoction prepared from *Boerhavia diffusa* (Hog weed), *Berberis aristata* (Indian barberrry), *Curcuma longa* (Turmeric), *Zingiber officinale* (Dry ginger root), *Terminalia chebula* (Black myrobalan), *Tinospora cordifolia* (Tinospora), *Plumbago indica* (White leadwort), *Clerodendrum serratum* (Green witch's tongue) and *Cedrus deodara* (Deodar) is useful in hand, feet, oral and abdominal inflammation. [11] (Sh.Sm.Md.Kh. 2:122-123)

Punarnavāṃdārūśuṇṭhīṃsiddhārthaṃśigrumeva ca.

Piṣṭvācaivāranālenapralepaḥsarvaśothajit.(Śā.Sa.U.Kha. 11:3)

Topical application of paste prepared from *Boerhavia diffusa* (Hog weed), *Cedrus deodara* (Deodar), *Zingiber officinale* (Dry ginger root), *Brassica campestris* (Yellow mustard) and *Moringa oleifera* (Drumstick tree) bark pounded with sour gruel is useful in inflammation. [11] (Sh.Sm.Ut.Kh.11:3)

Generalised body disorders

Punarnavākvāthakalkasiddhaṃśothaharaṃghṛtam. (Ca.Da.Ci. 39:30)

Inflammation- Intake of *Ghrita* (Clarified butter) processed with *Boerhavia diffusa* (Hog weed) decoction and paste is useful in inflammation. [18] (Ck.Dt.Ci. 39:30)

Punarnavācitrakadevadārupañcoṣaṇakṣāraharītakīnām.

Kalkenapakvaṃdaṣamūlatoyeghṛtottamaṃśothanişūdanañca. (Ca.Da.Ci. 39:29)

Intake of *Ghrita* (Clarified butter) processed with *Pancakola* (group of five pungent medicinal herbs), *Dolichos biflorus* (Horse gram) decoction and *Boerhavia diffusa* (Hog weed) paste is useful in inflammation. [18] (Ck.Dt.Ci. 39:29)

Rasevipācayetsarpipaṃcakolakulatthayo.

Punarnavāyāḥkalkenaghṛṃśothavināśanam. (Ca.Da.Ci. 39:31)

Intake of *Boerhavia diffusa* (Hog weed) root powder mixed with *Ghrita* (Clarified butter) is useful in inflammation. [18] (Ck.Dt.Ci. 39:31)

Punarnavānimbapaṭolaśuṇṭhītiktāmṛtādārvyabhayākaṣāyaḥ.

Sarvāṃṅgaśophodarakāsaśūlaśvāsānvitaṃpāṇḍugadaṃnihanti. [18] (Vṛ.Mā.38:3)

Punarnāvāmagadhajā ca kaṭutrayaṃ ca nimbābhayā ca kaṭukā ca paṭoladārvī.

Kvāthaḥsukhoṣṇakvathitastuvipācanenaśophojahātijaṭharaṃ ca narasyaśīgham.

(Hā.Saṃ. 3.25:13)

Intake of lukewarm *Punarnavādi* decoction prepared from *Boerhavia diffusa* (Hog weed), *Piper longum* (Long pepper), *Trikaću* (group of three pungent medicinal herbs), *Zingiber officinale* (Dry ginger root), *Piper nigrum* (Black pepper), *Azadirachta indica* (Margosa tree), *Terminalia chebula* (Black myrobalan), *Picrorhiza kurroa* (Picrorhiza), coral ash and *Berberis aristata* (Indian barberrry) is useful in anasarca, abdominal disorders, dyspnea, pain and anaemia. [13] (Vr.Md. 38:3), [19] (Ha.Sm. 3.25:13)

Punarnavānāgaramustakalkānprasthenadhīraḥpayasā'kṣamātrān.(Ca.Ci. 12:23)

Inflammation- Intake of cow milk processed from an equal quantity of *Boerhavia diffusa* (Hog weed) root, *Zingiber officinale* (Dry ginger root) and *Cyperus scariosus* (Umbrella sedge) is useful in *Vātaja* inflammation. [15] (Ca.Ci. 12:23)

Punarnavedve ca bale sapāṭhedaṃtīṃguḍūcīmathacitrakaṃ ca……

Kṣipraṃjayedvarṇabalāyurojastejo'nvitomāṃsarasānnabhojī.(Ca.Ci. 12:34-38)

Intake of *Punarnavādi Ariṣṭa* is useful in cardiac disorders, anaemia, inflammation. It also enhances rejuvenating properties. [15] (Ca.Ci. 12:34-38)

Teṣupaunarnavaṃśākaṃviśeṣācchophanāśanam.(Su.Sū. 46:255)

Intake of *Boerhavia diffusa* (Hog weed) vegetable is useful in inflammation. [20] (Su.Su. 46:255)

Punarnavādāruśuṇṭhīkvāthemūtre'thakevale.

Daṣamūlarasevā'piguggulaḥśothanāśanaḥ. (Vṛ.Mā. 39:13)

Intake of decoction prepared from *Boerhavia diffusa* (Hog weed), *Commiphora mukul* (Gum guggul plant), *Berberis aristata* (Indian barberrry) and *Zingiber officinale* (Dry ginger root) is useful in inflammation. [13] (Vr.Md. 39:13)

Harītakīnāgaradevadārusukhāmbuyuktaṃsapunarnavaṃvā.

Sarvaṃpibettriṣvapimūtrayuktaṃsnātṛcajīrṇepayasā'nnamadyāt. (Ca.Ci.12:22)

Intake of powder prepared from an equal quantity of *Terminalia chebula* (Black myrobalan), *Zingiber officinale* (Dry ginger root), *Cedrus deodara* (Deodar) and *Boerhavia diffusa* (Hog weed) root alongwith cow urine alleviates inflammation. [15] (Ca.Ci. 12:22)

Punarnavāguḍūcī ca gugguluṃsamakalkitam.

Śophadoṣāṃścagulmañcahantyudaraṃkaphāmayam.(Hā.Saṃ.3.25:14)

Intake of paste prepared from an equal quantity of *Boerhavia diffusa* (Hog weed), *Tinospora cordifolia* (Tinospora) and *Commiphora mukul* (Gum guggul) paste is useful in inflammation, *Gulma*, abdominal disorders and *Kaphaja* disorders. [19] (Ha.Sm. 3.25:14)

Sitapunarnavāmūṃpītañcagosalilenanihanti.

Śothaṃsarvasamutthamudarāṇi ca dustarāṇya'cirāt.(Vaṃ.Se.Śothādhikāraḥ74)

Intake of *Boerhavia diffusa* (Hog weed) paste alongwith cow urine is useful in inflammation and abdominal disorders. [17] (Vg.Sn.Sh. :74)

Cūrṇapaunarnavaṃraktālitaṇḍulaṣarkaram.

Raktṛṭhīvīpibetsiddhaṃdrākṣārasapayoghṛtai.(Ca.Ci. 11:26)

Chest injury- Intake of *Ghrita* (Clarified butter) and milk processed with *Boerhavia diffusa* (Hog weed) root powder, *Vitis vinifera* (Dry grapes) juice and *Oryza sativa* (Rice) alongwith sugar is useful in hemoptysis caused due to chest injury. [15] (Ca.Ci. 11:26)

Siṃhovyāghīsiṃhamukhīkākamācīpunarnavā.

Vārttākīnāṃ ca mūlānāṃkvāthonidrākaronaṣṇām.(Hā.Saṃ.3.15:5)

Insomnia- Intake of decoction prepared from *Solanum indicum* (Indian night shade) , *Solanum surattense* (Yellow berred night shade) , *Adhatoda vasica* (Malabar nut) , *Solanum nigrum* (Garden nightshade), *Boerhavia diffusa* (Hog weed), *Solanum melongena* (Eggplant) root is useful in insomnia. [19] (Ha.Sm. 3.15:5)

Rejuvenation and Virilification

Punarnavasyārdhapalaṃnavasyapṛṃpibedyaḥpayasā'rddhamāsam.

Māsadvayaṃ tat triguṇaṃsamāṃvājīrṇo'pibhūyaḥsapunarnavaḥsyāt.(A.Hṛ.U. 39:154)

Rejuvenation- Regular intake of *Boerhavia diffusa* (Hog weed) root paste till 15 days enhances rejuvenating properties. [21] (As.Hr.Ut. 39:154)

Alexipharmic treatment

Śvetāṃpunarnavāñcāsyadadyāddhuttūrakāyutām.(Su.Ka. 7:24)

Rabies- Intake of powder prepared from *Boerhavia diffusa* (Hog weed) root, *Datura metel* (DevilÕs trumpet) seed mixed with *Sesamum indicum* (Sesame) oil, *Calotropis procera* (Calotropis), milk and jaggery alleviates poisonous effects caused due to rabies. [20] (Su.Kl. 7:24)

Lihyānmadhuyutāṃśvetāṃśvetāñcāpipunarnavām. (Su.Ka. 7:24)

Rat poisoning- Intake of *Boerhavia repens* (Creeping spiderling) root powder mixed with honey alleviates rat poisoning. [20] (Su.Kl. 7:24)

Dhavalapunarnavajaṭayātaṇḍulajalapītayā ca puṣyarkṣe.

Apaharativiṣadharaviṣopadraṃmāsaṃvatsaraṃpuṃsām.(Ca.Da.Ci. 65:4)

Yaḥpibatipuṣyadivasejalapṛṃsitapunarnavāmūlam.

Tatsannidhaunavarṣaṃvṛścikabhujagāḥprasarpanti.(Rā.Mā. 29:1)

Poisoning- Intake of *Boerhavia diffusa* (Hog weed) root paste alongwith rice water is useful in poisoning. [18] (Ck.Dt.Ci. 65:4), [9] (Ra.Ma. 29:1)

Punarnavāyāḥśvetāyāḥsiphāṃtandulavāriṇā.(Ga.Ni.Viṣa. Tantra. 3:10)

Snakebite- Intake of *Boerhavia diffusa* (Hog weed) root pounded with rice water alleviates poisonous effects caused due to snakebite. [16] (Gd.Ni.Vs.T. 3:10)

Parts used

Root, leaf, whole plant.

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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 Nāḍāivraṇa 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 in the form of 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 river 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 Uttarkashi 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 starts. The river has a total length of about 2600 km from its main source Gomukh.

The river flows in the hills up to Haridwar, then it enters the plains area. From here it flows southwards, passing through the plains of Uttar Pradesh starting from Bijnor. 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 which is known as Bhagirathi, continues to flow south through West Bengal. Its name changed to Hooghly when it flows in the west and south-west of Kolkata. The Hooghly at

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

Among all the medicinal plants the *Boerhavia diffusa* was considered in this study. It is commonly known as 'Punarnava' because it has the renewing ability of the cells. It is a creeper with small fleshy leaves and small reddish-pink flowers. It has a cooling effect and has high medicinal values mainly the rejuvenating ability of the whole body. Regular use of this makes a person young and energetic, full of vigour and vitality (Vikash and Pankaj, 2023). It is very helpful in curing digestive ailments, kidney, and cardiac disorders along with these it is also useful to treat respiratory distress, anemia, and hernia. On this basis, the current chapter is focused on the morphology, taxonomy, anatomy, and distribution assessment of *B. diffusa* around the Ganga River.

Genus *Boerhavia* vaill. ex L.

Boerhavia Vaill. ex L. is a genus of about 50 species that are widely distributed in tropical and subtropical areas of the world (Fosberg, 1999; Spellenberg, 2003; Douglas and Spellenberg, 2010; Mabberley, 2017). The genus belongs to the family Nyctaginaceae. Mostly the species of this genus grow in the coastal areas and spread in sand, gravelly plains, and rocky slopes. The polymorphism is very common in this genus. Due to the polymorphism, the hybrids between species always create complexity in the taxonomy. The genus is characterized by elongate stems with prominent nodes, slender forking cymose or rarely racemose or spicate inflorescences with small, scale-like free bracts, campanulate small flowers, cylindric, funnel-shaped perianth limb separated by a constriction from the sulcate, enlarged lower portion of the perigone, and anthocarps that are clavate or oblong, often glandular (Fosberg, 1978).

In India, the genus is represented by four species *i.e.*, *B. crispa* F.Heyne ex Hook.f., *B. diffusa* L., *B. erecta* L., and *B. rubicunda* Steud. ex Heimerl. In the flora of British India, this genus treated with a total of six species (*B. repens* L., *B. crispa* F.Heyne ex Hook.f., *B. repanda* Willd., *B. verticillata* Poir., *B. fruticosa* Dalz., and *B. elegans* Chois.) and two varieties (*B. repens* var. *procumbens* (Banks ex Roxb.) Hook.f., and *B. repens* var. *diffusa* (L.) Hook.f.) (Hooker, J.D. 1885). After that various worker revised this genus time to time (Kanjilal, 1940; Gamble, 1956; Cooke, 1958; Duthie, 1960; Prain, 1963; Babu, 1977; Bennet, 1979; Matthew, 1981; Deb, 1981; Manilal, 1988; Chandrabose and Nair, 1988; Saxena and Brahmam, 1995; Panigrahi and Murti, 1999; Paria and Chattopadhyay, 2000; Singh and Singh, 2006). Debasmita et al. (2015) revised the Indian *Boerhavia* and treated it with a total of four species *B. crispa* Heyne, *B. diffusa* L., *B. erecta* L., *B. rubicunda* Steud. Recently, Ravichandran et al. (2020) studied and elaborated a total of six species under this genus as *B. coccinea* Miller, *B. crispa* Heyne ex Hook f., *B. diffusa* L., *B. erecta* L., *B. procumbens* Banks ex Roxburgh, and *B. rubicunda* Steud.

Etymology

The name 'Boerhavia' was given in honor of an eighteenth-century Dutch botanist, humanist, and physician 'Hermann Boerhaave' (1668–1738). In earlier literature, the name was treated as *Boerhaavia* (Bojer, 1842; Hiruma et al., 2000) but after Linnaeus, the adopted spelling is Boerhavia because he Latinized the earlier name to *Boerhavius* (Sprague, 1928; Bogle, 1974; Struwig and Siebert, 2013).

Habitat and Distribution

The genus *Boerhavia* Vaill. ex L. is widely distributed throughout the world mainly in tropical and subtropical areas. It is mainly distributed by birds and human activity. Mostly its distribution is recorded in the warmer parts up to an altitude of 1000 m in disturbed

areas, waste places, roadsides, dry pinelands, among scrub on tropical reefs (Spellenberg, 2004). This is native to India and Brazil (Patil and Bhalsing, 2016). According to the Plants of the World Online database (POWO, 2023), this genus is native to the Afghanistan, Alabama, Aldabra, Algeria, Andaman Is., Angola, Argentina Northeast, Argentina Northwest, Arizona, Arkansas, Aruba, Assam, Bahamas, Bangladesh, Belize, Benin, Bolivia, Borneo, Botswana, Brazil Northeast, Brazil South, Brazil Southeast, Brazil West-Central, Burkina, Burundi, California, Cambodia, Cameroon, Cape Provinces, Cape Verde, Caroline Is., Cayman Is., Central African Repu, Chad, Chagos Archipelago, Chile Central, Chile North, China South-Central, China Southeast, Christmas Is., Cocos (Keeling) Is., Colombia, Comoros, Congo, Cook Is., Costa Rica, Cuba, Desventurados Is., Djibouti, Dominican Republic, Ecuador, Egypt, El Salvador, Eritrea, Ethiopia, Fiji, Florida, French Guiana, Gabon, Galápagos, Gambia, Georgia, Ghana, Gilbert Is., Guatemala, Guinea, Guinea-Bissau, Gulf of Guinea Is., Gulf States, Guyana, Hainan, Haiti, Hawaii, Honduras, Howland-Baker Is., India, Iran, Ivory Coast, Jamaica, Japan, Jawa, Kazan-retto, Kenya, KwaZulu-Natal, Laccadive Is., Laos, Lebanon-Syria, Leeward Is., Lesser Sunda Is., Liberia, Libya, Line Is., Louisiana, Madagascar, Malawi, Malaya, Maldives, Mali, Maluku, Marcus Is., Marianas, Marquesas, Marshall Is., Mauritania, Mexican Pacific Is., Mexico Central, Mexico Gulf, Mexico Northeast, Mexico Northwest, Mexico Southeast, Mexico Southwest, Mississippi, Morocco, Mozambique, Mozambique Channel Is., Myanmar, Namibia, Nansei-shoto, Nepal, Netherlands Antilles, Nevada, New Guinea, New Mexico, New South Wales, Nicaragua, Nicobar Is., Niger, Nigeria, Norfolk Is., North Carolina, Northern Provinces, Northern Territory, Ogasawara-shoto, Oklahoma, Oman, Pakistan, Palestine, Panamá, Paraguay, Peru, Philippines, Phoenix Is., Pitcairn Is., Puerto Rico, Queensland, Rwanda, Samoa, Saudi Arabia, Senegal, Seychelles, Sierra Leone, Society Is., Socotra, Solomon Is., Somalia, South Australia, South Carolina, South China Sea, Southwest Caribbean, Spain, Sri Lanka, Sudan, Sulawesi, Sumatera, Suriname, Swaziland, Taiwan, Tanzania, Texas, Thailand, Togo, Tokelau-Manihiki, Trinidad-Tobago, Tuamotu, Tubuai Is., Turks-Caicos Is., Tuvalu, Uganda, Uruguay, Utah, Vanuatu, Venezuela, Venezuelan Antilles, Victoria, Vietnam, Wake Is., West Himalaya, Western Australia, Western Sahara, Windward Is., Yemen, Zambia, Zaïre, and Zimbabwe. While it is introduced in the Bermuda, Brazil North, East Himalaya, Easter Is., Free State, Italy, Maryland, Mauritius, Missouri, Niue, Rodrigues, Réunion, Sicilia, Sinai, and Virginia (Fig. 1).

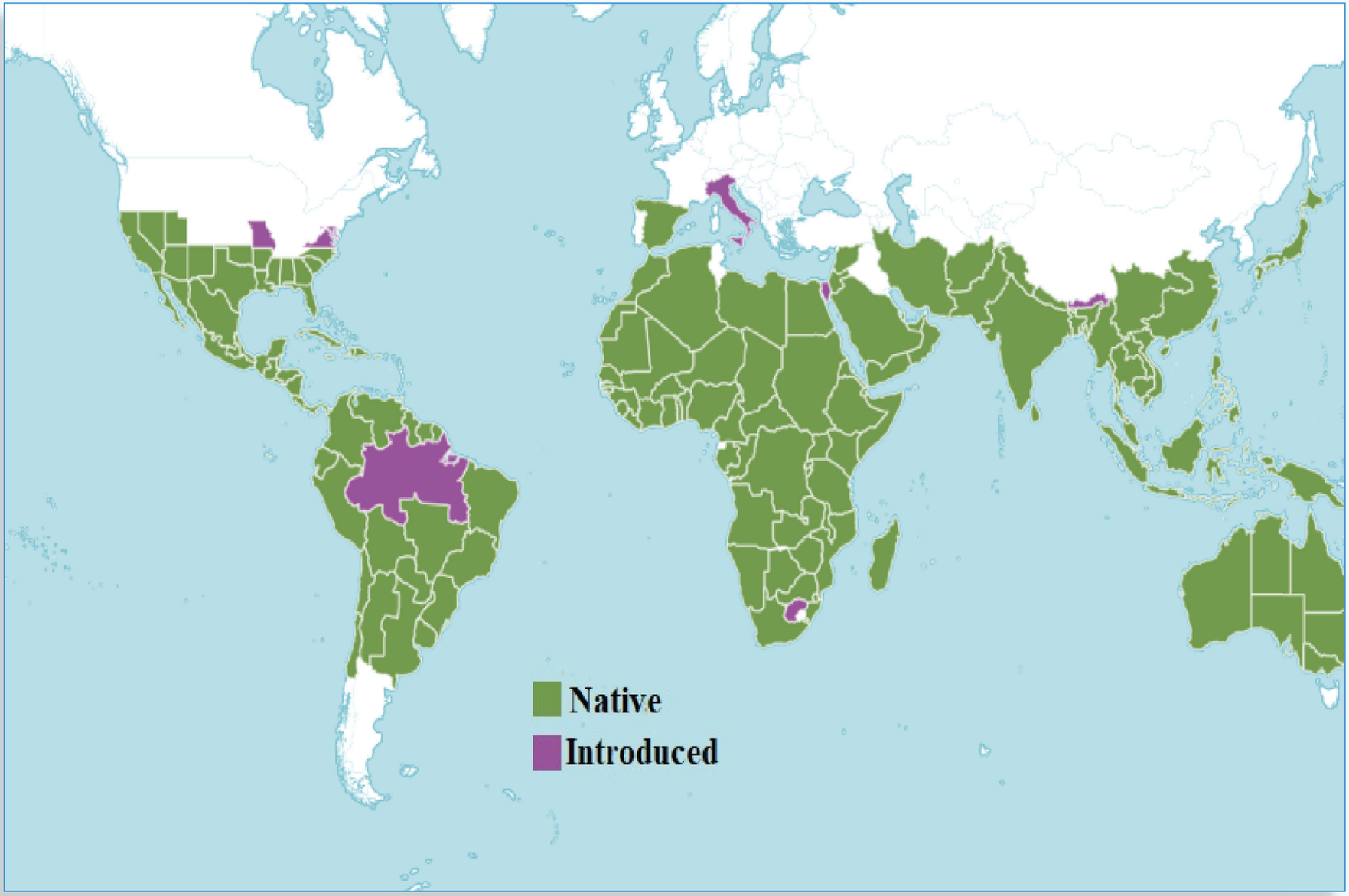

Fig. 1 Global distribution of *Boerhavia*

Botanical Characteristics

Annual or perennial herbs, rarely vine-like, creeping, or scrambling. Stems slender, erect, decumbent, diffuse, or prostrate rarely somewhat woody at the base, glabrate to pubescent, with glandular hairs. Root enlarged. Leaves opposite, petiolate, simple, entire to sinuate or repand lobed, estipulate, obtuse to apiculate, base attenuate to truncate, margins entire or sometimes undulate, glabrous to pubescent. Inflorescence is a much-branched terminal or axillary compound cyme or umbel, glabrous to puberulous, bracteate. Flowers are small at the end of smaller branches in capitate, glomerate, umbellate, or irregular clusters, 1-5 mm long, bisexual, and shortly pedicillate. Involucral bracts subtending ultimate partial inflorescences not connate; perianth of a single completely united series of parts termed a perigone, this separated into two parts by a strong transverse constriction, lower part 1-2 mm long, green sometimes reddish, coriaceous, constricted above the ovary, ovoid to clavate, 3–5-winged or 5-ribbed, glabrous or with glandular hairs, persistent; upper part 1-4 mm long, campanulate, sometimes with a basal tube, petaloid, pink, purple, maroon or white, caducous after anthesis, at maturity closely investing the fruit, termed an anthocarp; stamens basally attached, 1-2, exserted, filaments free, anthers dithecous. pistil 1, ovary unilocular, ellipsoid, ovule 1, basally attached, style filiform, stigma capitate, usually somewhat exserted. Anthocarp 3-5 × 1-3 mm, 3-5-winged or 5-ribbed sulcate, glandular, apices rounded or conic, indehiscent, containing one erect seed. Seed oblong, brown, surface smooth; endosperm scanty; embryo hooked (Fosberg, 1978; Struwig and Siebert, 2013).

Ethnomedicinal Importance

The *Boerhavia* genus has important species that have a broad spectrum of ethnomedicinal uses, used worldwide by various indigenous communities with *B. diffusa* being widely documented. In India, it is known as 'Punarnava' in ancient literature where it is mentioned as 'Rakt Punarnava' and 'Shweta Punarnava' which are botanically known as *B. diffusa* and *B. erecta* respectively. Comparatively, 'Rakt Punarnava' is known to possess more medicinal importance than 'Shweta Punarnava' (Patil and Bhalsing, 2016). The whole plant as well as fruits, roots, leaves, leafy twigs of species like *B. diffusa, B. coccinea, B. elegans, B. erecta, B. procumbens, B. repens* are used in treatment of urinary disorders, hepatitis, cystitis, ascites, beriberi, blenorrhagia (Cruz, 1985), anemia, abdominal tumors, palpitations, jaundice, yaws, tumor, gonorrhoea, nephritis, oedema, gastroenteritis problems, prolapsed uterus, mumps, laryngitis, burns, toothache, problems of liver, gastrointestinal issues, infertility, against fungal infections, convulsions in children, diarrhea, leprosy, syphilis, filarial infection, albuminuria, erysipelas, hysteria, epilepsy, dropsy (Muzila, 2008), asthma, internal inflammation, piles, leucorrhea, rheumatism, stomachache, elephantiasis, seminal weakness, blood

pressure (Mitra and Gupta, 1997), sprain, cholera, malaria (Bouquet and Debray, 1974; N'Guessan et al., 2009), pneumonia (Jiofack et al., 2009), measles (Austin, 2010), urinary disorders, intestinal infections, dysmenorrhea, (Ramazani et al., 2010), ophthalmia, eye wounds, joint pain (Najam et al., 2008), muscular pain, lumbago, scabies, delivery-aid (Sandhu et al., 2011), splenomegaly (Krishna and Shanthamma, 2004; Donald, 2012; Rameshkumar and Ramakritinan, 2013), ulcers and ripen abscesses (Krishna and Shanthamma, 2004), microbial infection (Hilou et al., 2013), swelling and scorpion stings (Katewa et al., 2004). The plant is used in the form of decoction, infusion, smoke of the leaf pulp, paste, juice of the root, poultice, in several indigenous communities of India, Pakistan, Nigeria, Ghana, Cote d'Ivoire, Brazil, Iran, Mexico, Benin, Kenya, Niger and Burkina Faso. It also acts as vermifuge, analgesic, anti-inflammatory, appetizer, and diuretic agent, vasoprotective, emetic, anti-cancer, fish poison antioxidant (Belkacem et al., 2007; Aviello et al., 2011; Goel et al., 2011).

Chemical Constituents

The genus *Boerhavia* is reported to possess a variety of phytochemicals. The phytochemical studies of this genus seem to be of great importance and have been identified from the extract and hold immense medicinal potential. In view of the technological advancement, several studies on this genus revealed a number of important phytochemicals in different parts (Table 1). Although it has many species among them some species have been evaluated for their phytochemical. As per its medicinal importance and demand all the species need to be evaluated which can be a future approach. As in the literature survey, only *B. diffusa, B. repens, B. procumbens,* and *B. erecta* are evaluated for their phytochemicals with most of the work done on the *B. diffusa*. The phytochemicals belong mainly to alkaloids, phenolics, terpenes, saponin, lignans, and some other classes of compounds.

Only four alkaloids punarnavine, betanin, isobetanin, and neobetanin have been identified in this genus. The punarnavine identified from the roots of *B. diffusa, B. repens,* and *B. procumbens* (Manu and Kuttan, 2009; Abbasi et al., 2012 while betanin, isobetanin snd neobetanin found in the stem bark of *B. erecta* (Stintzing et al., 2004; Maurya et al., 2007; Hilou et al., 2013).

The phenolic compounds, especially the flavonoids, are responsible for their traditional uses. In the study in *B. diffusa* the total flavonoid content was found to be 5.651 g/100 g (5.6%) while the alkaloid content was only 0.2%; which means the flavonoids were many folds higher than the alkaloids (Patil and Bhalsing, 2016). To date, about 97 phenolic compounds having flavonoids, rotenoids, phenolic acids, and phenol glycosides have been identified in this genus (Ma et al., 2014). Punarnavoside, ferulic acid and vanillin from *B. diffusa* are also reported (Tacchini et al., 2015; Patil and Bhalsing, 2016).

Another important group is rotenoids which are the isoflavonoids are used as bioinsecticides (Wagner et al., 2012). They can be linked with the isoflavonoids as biosynthesis of isoflavones; it is a successive migration of six carbon atoms in rotenone to twelve carbon atoms (Crombie, 1992). The best-known naturally occurring rotenoid is rotenone (Belkacem et al., 2007). Another rotenoid *i.e.,* boeravinone G is also reported in the genus (Belkacem et al., 2007; Aviello et al., 2011).

Terpenoids are another important compound in plants commonly they are 'flavor fingerprints' because they are recognized by animals and humans; they are a modified form of terpenes called 'terpenoids' (Pereira et al., 2009). They are formed from the isoprene units which are five carbon compounds. They are the components of essential oil hence having important biological activities. The lignins are important biomolecules present in the plants. Liriodendrin and syringaresinol mono-ß-D-glucose has been detected in the *B. diffusa* (Lami et al., 1991). Various saponins are also recognized in this genus. Although different saponins were detected in other members of Nyctaginaceae only oleanolic acid heteroside is still known as saponin in this genus, which has been detected from the roots of *B. diffusa* (Oliver-Bever, 1986; Patil and Bhalsing, 2016).

Table 1. Different chemical compounds in various species of *Boerhavia*

***Boerhavia* species**	**Part**	**Chemical Compounds**
B. diffusa	Root	**Alkaloid –** Punarnavine **Phenolics –** Kaempferol; Quercetin; 5,7-dihydroxy-6-8-dimethoxy flavones (Borhavone); Quercetin 3-O-robinobioside; 3,4-dihydroxy-5-methoxycinnamoylrhamnoside; Quercetin 3-O-(2′- rhamnosyl)-robinobioside; Kaempferol 3-O-(2′-rhamnosyl)-robinobioside; 3,5,4′-trihydroxy-6,7-dimethoxyflavone; 3-O-galactosyl(1-2)glucoside [eupalitin 3-O-galactosyl(1-2)glucoside]; Kaempferol 3-O-robinobioside; Eupalitin 3-O-galactoside; Punarnavoside; Alkamide; N-trans-feruloyltyramine; Caffeoyltartaric acid; Boeravinone A, B, C, D, E, F, G, H, I, M P, Q, R, S; 9-O-methyl-10-hydroxycoccinone B; 10-demethylboeravinone C; 2′-O-methylabroisoflavone; 6-O-demethylberavinone H; Coccineon B, E; Boeravinone J **Terpenes –** Boerhavisterol; Camphor; Isomenthone; Limonene; Menthol; Phellandrene; Safranal; α-Pinene; Geranylacetone; cis 4-Hexen-1-ol; trans 2-Octanalc; 2-Nonen-1-olc; 2-Decen-1-ol; Methylpyrrole; 3-Phenyl-2-(20-pyridyl)-indole; Indole; Eugenol; ß-Cyclocitral; ß-Ionone; Dihydroactinidiolide; Stigmasterol; Campesterol; ß-sitosterol; α-2-sitosterols; Ursolic acid **Saponin -** Oleanolic acid heteroside **Lignans -** Liriodendrin; Syringaresinol mono-ß-D-glucose; hypoxanthine 9-L-arabinose; myricyl alcohol; myristic acid; ß-ecdysone; Boerhadiffusene; Diffusarotenoid; boerhavilanastenyl benzoate; Boerhavine; Borhavone; Vanillin
	Leaves, aerial parts	**Phenolics –** Kaempferol; Quercetin; Quercetin 3-O-robinobioside; 3,4-dihydroxy-5-methoxycinnamoylrhamnoside; Quercetin 3-O-(2′- rhamnosyl)-robinobioside; Kaempferol 3-O-(2′-rhamnosyl)-robinobioside; 3,5,4′-trihydroxy-6,7-dimethoxyflavone; 3-O-galactosyl(1-2)glucoside [eupalitin 3-O-galactosyl(1-2)glucoside]; Kaempferol 3-O-robinobioside; Eupalitin 3-O-galactoside; Phenol, 4, 6-di (1, 1-dimethylethyl)-2-methyl-; Ferulic acid; Syringic acid; Gentisic acid; O-coumaric acids; Caffeoyltartaric acid; Boerhaavic acid; Boeravinone P **Terpenes –** Camphor; Isomenthone; Limonene; Menthol; Phellandrene; Safranal; α-Pinene; Geranylacetone; cis 4-Hexen-1-ol; trans 2-Octanalc; 2-Nonen-1-olc; 2-Decen-1-ol; Methylpyrrole; 3-Phenyl-2-(20-pyridyl)-indole; Indole; Eugenol; ß-Cyclocitral; ß-Ionone; Dihydroactinidiolide; 3-acetoxy-α-amyrin; 4, 10-dihydroxy-8-methoxyguai-7(11)-en-8,12-olide

Boerhavia species	Part	Chemical Compounds
		Others - Myo-lnositol,4-C-methyl-1,14-Tetradecanediol; 1-pentadecyne; Phytol; 3,5-Bis(trimethyl-silyl)-2,4,6-cycloheptatrien-1-one; Androstane-11, 17-dione, 3-[(trimethylisilyl)oxy]-17- [O-(phenyl-methyl)oxime], (3a,5a); Allantoin; Sophorophenolone; N-trans-feruloyl-3-Methyldopamine; (+)-zedoalactone A; Ciwujiatone; 1-ß-D-glucopyranosyloxy-3,5-dimethoxy-4- hydroxybenzene; 1-ß-D-glucopyranosyloxy-3,4-dimethoxybenzene; 1-ß-D-glucopyranosyloxy-1-phenylmethane; 1-ß-D-glucopyranosyloxy-2-pheny-lethane; 1- ß-D-glucopyranosyloxy-2-methoxy-4- ethanoylben-zene; Potassium nitrate; Vitamin E acetate; Oxalic acid; Pyruvic acid; Ketoglutaric acid; Fumaric acid; Quinic acid
	Whole plant	**Phenolics –** Eupalitin; Eupalitin-3-O-ß-D-galactopyranoside; Eupatilin-7-O-ß-D-galactopyranoside; Eupatilin 7-O-α-L-rhamnopyranosyl (1-2) α-Lrhamnopyranosyl (1-6)- ß-D-galactopyranoside; Quer-cetin-3-O- ß-D-glucopyranoside- 7-O- ß-Dglucopyranoside; Quercetin- 3-O-α-L-rhamnopyranosyl (1-6) - ß-Dgalactopyranoside; 3,3′,5-Trihydroxy-7-methoxyflavone; 3,4-dimethoxyphenyl-1-O-ß-D-glu-copyranoside; 4′,7-dihydroxy-3′-methylflavone; Eupalitin 3-O-ß-D- galactopyranosyl-(1 ′- 2)-O-ßD-galactopyranoside; 3,4-Dimethoxyphenyl-1-O-ß-D-apiofuranosyl-(1 ′3)-O-ß-D-galactopyrano-side hexaacetate **Terpenes –** ß-amyrin; ß-amyrin acetate **Others -** Palmitic acid; Heptadecyclic acid; Oleic acid; Strearic acid; Arachidic acid; Behenic acids; Sterol esters; Hentriacontane; Triacontanol; Quinic acid; Fumaric acid; Ketoglutaric acid; Pyruvic acid; Oxalic acid
B. repens	Root	**Alkaloid –** Punarnavine **Phenolics –** Boeravinone F
	Whole plant	**Phenolics –** Eupalitin-3-O-ß-D-galactopyranoside; Eupalitin 3-O-ß-D-galactopyranosyl-(1-2)-ß-Dg-lucopyranoside; 6-methoxykaempferol 3-O-ß-D-(1-6)-robinoside; 5,7,3'-trihydroxycoumaronochro-mone; Repenone; Repenol; Boerharotenoid B; Boerharotenoid A; ß-sitosterol **Others -** Squalene
B. pro-cumbens	Root	**Alkaloid –** Punarnavine **Phenolics –** Kaempferol; Quercetin

***Boerhavia* species**	**Part**	**Chemical Compounds**
	Whole plant	**Phenolics –** Catechin; Rutin; Myricetin; Kaempferol; Quercetin **Others -** Caeffic acid
B. erecta	Stem, bark	**Alkaloid –** Betanin; Isobetanin; Neobetanin **Phenolics –** Epicatechin; Quercetin diglycoside; Quercetin 3-O-rutinoside; Kaempferol diglycoside; Isorhamnetin diglycoside; Isorhamnetin 3-O-rutinoside; Isorhamnetin 3-O-glucoside; 3-methoxybenzoic acid 4-O-ß-glucoside; 3-methoxyacetophenone 4-O-ß-glucopyranoside; Isorhamnetin-3-O-rutinoside-7-O-ß-glucopyranoside; Isorhamnetin-3-O-rutinoside; 2,3-dihydroxypropyl-benzoate 3-O-ß-[4-methoxy] Glucuronide **Others -** Procyanidin B1, B2; Dimeric procyanidin
	Leaves, aerial parts	**Phenolics –** Kaempferol; Catechin; [(-)-epicatechin]; Isorhamnetin; Rutin; Narcissin; Procyanidins; Isoquercitrin; isorhamnetin; Isorhamnetin-3-O-ß-D-glucopyranoside; Isorhamnetin 3-O-α-L-rhamnopyranosyl-(1- 6)-ß-Dglucopyranoside; Isovitexin; Boeravinone C; Boeravinone G, K, M, N; 10-demethylboeravinone C; Cucumegastigmane; Kaempferol 3-O-rutinoside; Quercetin 3-O-ß-D-glucopyranoside; Boeravinone O, J, L
	Whole plant	**Phenolics –** 3′,4′,5,7-tetrahydroxyflavone-3-O-α-D-rhamnopyranosyl (1-6)O-ß-D-glucopyranoside; 4′,5,7-tetrahydroxy-3′-methoxy flavones-3-O-α-Drhamnopyranosyl(1-6)O-ß-D-glucopyranoside; **Terpenes –** ß-sitosterol

Species

Genus *Boerhavia* is known to contain a total of 60 taxonomically accepted species (POWO 2023) that are as-

1. *B. acutifolia* (Choisy) J.W.Moore in Occas. Pap. Bernice Pauahi Bishop Mus. 10(19) 6 (1934)
2. *B. alamasona* Rose in Contr. U.S. Natl. Herb. 1: 110 (1891)
3. *B. alata* S.Watson in Proc. Amer. Acad. Arts 24: 69 (1889)
4. *B. albiflora* Fosberg in Smithsonian Contr. Bot. 39: 11 (1978)
5. *B. angustifolia* L. in Syst. Nat., ed. 12. 2: 52 (1767)
6. *B. anisophylla* Torr. in W.H.Emory, Rep. U.S. Mex. Bound. 2(1): 171 (1859)
7. *B. bracteosa* S.Watson in Proc. Amer. Acad. Arts 20: 370 (1885)
8. *B. burbidgeana* Hewson in Fl. Australia 4: 318 (1984)
9. *B. chrysantha* Barneby in Leafl. W. Bot. 10: 264 (1966)
10. *B. ciliata* Brandegee in Univ. Calif. Publ. Bot. 4: 270 (1912)
11. *B. coccinea* Mill. in Gard. Dict., ed. 8.: n.° 4 (1768)
12. *B. cordobensis* Kuntze in Revis. Gen. Pl. 3(3): 264 (1891)
13. *B. coulteri* (Hook.f.) S.Watson in Proc. Amer. Acad. Arts 24: 70 (1889)
14. *B. crispa* F.Heyne ex Hook.f. in Fl. Brit. India 4: 709 (1885)
15. *B. crispifolia* Fosberg in Smithsonian Contr. Bot. 39: 15 (1978)
16. *B. deserticola* Codd in Bothalia 9: 119 (1966)
17. *B. diandra* L. in Sp. Pl.: 1194 (1753)
18. *B. dichotoma* Hochst. ex Walp. in Ann. Bot. Syst. 3: 298 (1852)
19. *B. diffusa* L.in Sp. Pl.: 3 (1753), nom. cons.
20. *B. dominii* Meikle & Hewson in Fl. Australia 4: 318 (1984)
21. *B. elegans* Choisy in A.P.de Candolle, Prodr. 13(2): 453 (1849)
22. *B. erecta* L. in Sp. Pl.: 3 (1753)
23. *B. fistulosa* Fosberg in Brittonia 40: 57 (1988)
24. *B. gardneri* Hewson in Fl. Australia 4: 318 (1984)
25. *B. glabrata* Blume in Bijdr. Fl. Ned. Ind.: 733 (1826)
26. *B. gracillima* Heimerl in H.G.A.Engler & K.A.E.Prantl, Nat. Pflanzenfam. 3(1b): 26 (1889)
27. *B. graminicola* Berhaut in Bull. Soc. Bot. France 100: 50 (1953)
28. *B. herbstii* Fosberg in Smithsonian Contr. Bot. 39: 17 (1978)
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30. *B. hitchcockii* Standl. in N.L.Britton & al. (eds.), N. Amer. Fl. 21: 206 (1918)
31. *B. hualienensis* Shih H.Chen & M.J.Wu in Taiwania 52: 337 (2007)
32. *B. linearifolia* A.Gray in Amer. J. Sci. Arts, ser. 2, 15: 322 (1853)
33. *B. litoralis* Kunth in F.W.H.von Humboldt,

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35. *B. megaptera* Standl. in Contr. U.S. Natl. Herb. 12: 379 (1909)
36. *B. mutabilis* R.Br. in Prodr. Fl. Nov. Holland.: 422 (1810)
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38. *B. paludosa* (Domin) Meikle in Fl. Australia 4: 319 (1984)
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44. *B. punarnava* Saha & K.H.Krishnam. in J. Sci. Indust. Res., C, Biol. Sci. 21: 253 (1962)
45. *B. purpurascens* A.Gray in Amer. J. Sci. Arts, ser. 2, 15: 321 (1853)
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47. *B. repleta* Hewson in Fl. Australia 4: 319 (1984)
48. *B. schinzii* Heimerl in Verh. Bot. Vereins Prov. Brandenburg 31: 222 (1890)
49. *B. schomburgkiana* Oliv. in Hooker's Icon. Pl. 13: t. 1225 (1877)
50. *B. spicata* Choisy in A.P.de Candolle, Prodr. 13(2): 456 (1849)
51. *B. stellata* Bojer in Rapp. Annuel Trav. Soc. Hist. Nat. île Maurice 2: 43 (1831)
52. *B. tarapacana* Phil. in Anales Mus. Nac. Santiago de Chile 2: 71 (1892)
53. *B. tetrandra* G.Forst. in Fl. Ins. Austr.: 5 (1786)
54. *B. torreyana* (S.Watson) Standl. in Contr. U.S. Natl. Herb. 12: 385 (1909)
55. *B. traubae* Spellenb. in Madroño 46: 208 (1999 publ. 2000)
56. *B. triquetra* S.Watson in Proc. Amer. Acad. Arts 24: 69 (1889)
57. *B. verwbenacea* Killip in J. Washington Acad. Sci. 16: 567 (1926)
58. *B. weberbaueri* Heimerl in Notizbl. Bot. Gart. Berlin-Dahlem 11: 460 (1932)
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The recent database of World Flora Online (WFO, 2023) mentioned it with a total of 105 accepted species as

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2. *B. africana* Lour., Fl. Cochinch. 1: 16 (1790)
3. *B. alamosana* Rose, Contr. U.S. Natl. Herb. 1: 110 (1891)
4. *B. alata* S.Watson, Proc. Amer. Acad. Arts 24: 69 (1889)
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17. *B. chrysantha* Barneby, Leafl. W. Bot. 10: 264 (1966)
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25. *B. decipiens* (Meikle) Govaerts, World Checkl. Seed Pl. 2(1–2): 12 (1996): (1996)
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28. *B. dichotoma* Hochst. ex Walp., Ann. Bot. Syst. (Walpers) 3(2): 298 (1852)
29. *B. diffusa* L., Sp. Pl. 1: 3 (1753)
30. *B. discolor* Kunth, Nov. Gen. Sp. [H.B.K.] 2: 215 (1818)
31. *B. elegans* Choisy, Prodr. [A. P. de Candolle] 13(2): 453 (1849)
32. *B. erecta* L., Sp. Pl. 1: 3 (1753)
33. *B. fallacissima* Heimerl ex Schinz, Verh. Bot. Vereins Prov. Brandenburg xxxi. (1890) 223
34. *B. fistulosa* Fosberg, Brittonia 40(1): 57 (1988)
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39. *B. graminicola* Berhaut, Bull. Soc. Bot. France 100: 50 (1953)

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41. *B. greenwayi* (Meikle) Govaerts, World Checkl. Seed Pl. 2(1–2): 12 (1996): (1996)
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43. *B. helenae* Roem. & Schult., Mant. 1 (Schultes) 73 (1822)
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49. *B. hitchcockii* Standl., N. Amer. Fl. 21(3): 206 (1918)
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86. *B. schomburgkiana* Oliv., Hooker's Icon. Pl. 13: t. 1225 (1877)
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105. *B. xanti* S.Watson, Proc. Amer. Acad. Arts 24: 69 (1889)

Boerhavia diffusa L.

B. diffusa belong to the tribe Nyctagineae of the Nyctagineaceae family. In the earlier texts the generic name mentioned as *Boerhaavia* but now recent texts use *Boerhavia*. However, its correct taxonomy is still unclear. Some authors treat it with *B. repens* as synonym. Some authors suggest these species are more like the *B. procumbens* hence, considered all three as synonyms of each other (Stemmerik, 1964; Singh, 1988). Cooke (1958) referred to it as a 'protean' plant, due to its changing appearance according to environmental conditions. The plant has two types, white and pink flowers; while only the white flowered plants are medicinal (Nadkarni, 1976). The plant parts have high degree of variation from region to region due to ecological factors.

This plant is propagated mainly by root stocks while the seed account only 21% of reproduction. The flowers and fruits persist throughout the year and the first flowers may appear 4 weeks after seed germination (Rojas-Sandoval, 2022). It grows as a weed in ruderal areas, preferring sunny sites, sandy soils, and a slightly seasonal climate, cultivated land, grazing pasture, etc. with a soil pH range from 6.6 to 7.8 (Rojas-Sandoval, 2022).

It has been documented in ancient Indian texts such as *Charaka Samhita*, *Sushrita Samhita*, *Bhaishajya Ratnaavali*, *Ashtaanga Hridaya*, and *Chakradatta*. The methods and mode of formulation were well documented in these books as the treatment of chronic alcoholism mentioned in *Chakradatta*; oedema and haematinic in *Bhaishajya Ratnaavali*; stimulation of urinogenital systems in *Ashtaang Hridaya*; etc. (Khare, 2004). In ancient literature it has been mentioned as "Punarnava" mainly the red-flowered 'Punarnava'; while another Punarnava *i.e.*, 'shweta Punarnava' is also mentioned in the ancient literatures. It has now been confirmed that the 'shweta Punarnava' is the *B. erecta* (Patil and Bhalsing, 2016).

Classification

Kingdom	-	Plantae
Subkingdom	-	Tracheobionta
Super Division	-	Spermatophyta
Division	-	Magnoliophyta
Class	-	Magnoliopsida
Subclass	-	Caryophyllidae
Order	-	Caryophyllales
Family	-	Nyctaginaceae
Genus	-	*Boerhavia*
Species	-	*diffusa*

Common Names

Pananua, Ponounua, Punarnava, Gadapushpa, Punarnova, Hogweed, Hogweed Pigweed, Satodi, Hin Gadahpurna, Biskhapara, Gadahpurna, Gadha-cand, Shothagni, Thavizhama, Ghetula, Ghetoli, Punarnava, Mookarattai, Pigweed Punarnava, Shothagni, Sarandai Sukuaetti.

As Revealed in Vedic Literature

In *Charaka Samhita* this plant is classified as *kaashara* (anti-tussive), *vayasthapana* (rejuvenator), *swedopaga* (an adjuvant to sweating therapy), and *anuvasanupaga* (an adjunct to oleating therapy). Its root is prescribed to treat *kushtha* (skin disorders) when combined with *dahi maanda* (Shukla and Tripathi, 2005).

In *Sushruta Samhita* this plant was classified as *vidharigandhadi gana* (Sharma, 2004a) and *shaak varga* (Sharma, 2004b). The roots (*mool*) are recommended for use in treating *pittaj ashmari* (renal stones) (Sharma, 2004c), *shotha* (anti-inflammatory) with boiled milk (Sharma, 2004d), *mooshaka vish* (rat bite) with *shunthi* (Sharma, 2004e), *alarka visha* (rabied dog bite) with honey (Sharma, 2004e), all types of *jwara* (fevers) with dhatura seed powder (Vaidya, 2005), and have antitoxin activity (Sharma, 2004f).

In *Ashtanga Sangraha* it is a component of both *Kaashara mahakshaya* (as antitussive) (Tripathi, 2003a) and *Vayasthapana* mahakshaya (decoction effective as rejuvenator) (Tripathi, 2003b). Its root powder is combined with milk and *madhuyashti kalka* to make a *rasayana* (rejuvenator) decoction (Vaidya, 2005). The plant has also been used as *shaak* (as vegetable diet) (Tripathi, 2003c).

In *Bhavprakash Nighantu* the two varieties of 'Punarnava' have been illustrated separately as *shweta* (white) and *rakta* (red) 'Punarnava'. The white variety is reported to have a *katu* (pungent) and *kashaya* (astringent) flavour and is used to treat anemia, diminished digestive fire, abdominal ailments, and *vayu* (flatus), *vish* (toxins), and *kapha* (phlegm) (Chunekar and Pandey, 2004). The red variety is *katu vipaka, sheetal* (coolant), light, and enhances *vata* in the body (Chunekar and Pandey, 2004). It also contains *tikta* (bitter) *rasa*. It functions as an astringent and is helpful for *kapha, pitta,* and *rakta* problems.

In *Adarsha Nighantu* the *Dravyaguna* has been discussed in detail. The 'Punarnava' has been addressed in the *Punarnavadi varg*, and this is the first plant from that *varg*. This works well against illnesses like *pandu* (anemia). This has been referred to as *Varshabhu* (because the plant grows green on its own every year) and *Vishakha* (because of the widely spreading stems). Every component of the plant is utilized. It has been suggested that the dose for *jalodara* (ascites) and *shotha* (inflammation) is 1-2 tola (12–24g), while for other illnesses, it is 0.25–0.5 tola (3-6g). *Vipaka* (*katu*), *virya* (*ushna*), the *rasa* (*katu, tikta, kashaya*), and *doshakarma* (*kaphavata shamaka*) have all been extensively addressed (Vaidya, 2005).

The *Sharangdhar Samhita* is a contemporary Ayurvedic text that dates to the 13th century A.D. This text gives a description of *Punarnava rasanjana*. The *mool* (root) is said to be rubbed and used with various substances and is effective in treating a variety of disorders, including *netra kandu* (eye itching), *netrasrava* (watery eyes), timir roga (pre-glaucomatic condition and glaucoma), and *kanj*i in *rataundhi* (night blindness) (Tripathi, 2010).

The *Ras Tantra Sar V Sidh Prayog Sangrah*

is a well-known contemporary ayurvedic book from the 19th century AD that contains several formulas. The numerous herbo-mineral preparations of *Punarnava Mandur* have been explored in this work. 2-4 tablets twice daily with gud (jaggery) and water as *anupana* are the doses listed in the text. A constipated person with dyspepsia is advised to mix it with *haritaki* powder, and *yograj guggul* may also be used for immediate relief. This formulation is beneficial in disorders like *shotha* (inflammations), *pandu* (anemia), *kamala* (jaundice), *udarroga* (abdominal disorders), *shoola* (painful conditions), *shwasa* (difficulty in breathing), *kaasa* (cough), *jvara* (fever), *arsha* (piles), *sangrahini* (irritable bowel syndrome), *krimi* (worm infestation), *vaatrakta* (gout) and *kushtha* (skin disorders). It has been specifically emphasized to be employed in *pandu* (anemia) and *kamala* (jaundice) (Krishan, 2003). The *Rasa Tarangini* recently referenced the 'punarnava' with the same as *Ras Tantra Sar V Sidh Prayog Sangrah* in the 20th century (Shastri, 2004).

In *Ayurveda Sara Sangrah* the various formulations of 'punarnava' as *Punarnava arka* (Sharma, 2005a), *Punarnava kshaar* (Sharma, 2005b), *Punarnava arishta* (Sharma, 2005c), *Punarnavadi taila* (Sharma, 2005d), *Punarnavashtaka kwatha* (Sharma, 2005e), *Punarnavadi kwatha* (Sharma, 2005f) have been mentioned. The *Punarnava ashtaka* is the most commonly used formulations which is made up of 1 tola (12g) of powder heated with 16 times quantity of water until one-fourth behind. Filtered and make decoction with 1-2 tola (12-24g) cow milk. This decoction is given to the patient (2-3 times a day) suffering with hepato-splenomegaly, inflammation, joint pains etc.

The *Ayurvedic Pharmacopeia of India* is the official text which has discussed the *rakta punarnava*. The various common names and synonyms have been compiled and the morphological traits have been elaborated. This text included its microscopic examinations of roots, stem, and leaves as well as composition, purity, and strength of crude drug have been mentioned. In various formulations, the chemicals like alkaloid (Punarnavine) have been mentioned as P*unarnavasava, Punarnavashtaka kwatha, Punarnavadi mandur, Sukumara ghrita* and *Shothaghna lepa.* In *shotha* (inflammation) and *pandu* (anemia) 20-30g drug have been recommended (The Ayurvedic Pharmacopeia of India, 2010).

Global Distribution

The *B. diffusa* is pantropical in distribution. The flora like India, Malaysia, and West Pakistan mentioned this plant. It is well distributed in Africa, tropical and temperate Asia, north and south America, central America, the West Indies, and pacific (Hyde et al., 2023; USDA, 2023). Currently, according to the Plants of the World Online Database (POWO, 2023) (Fig. 2) it is native of Afghanistan, Aldabra, Argentina Northeast, Argentina Northwest, Assam, Bahamas, Bangladesh, Belize, Benin, Bolivia, Botswana, Brazil Northeast, Brazil South, Brazil Southeast, Brazil West-Central, Burkina, Burundi, Cambodia, Cameroon,

Cape Provinces, Cape Verde, Cayman Is., Central African Repu, Chad, Chile Central, Chile North, China South-Central, China Southeast, Christmas Is., Cocos (Keeling) Is., Colombia, Comoros, Congo, Costa Rica, Cuba, Desventurados Is., Djibouti, Dominican Republic, Ecuador, Egypt, El Salvador, Eritrea, Ethiopia, Florida, French Guiana, Gabon, Gambia, Georgia, Ghana, Guatemala, Guinea, Guinea-Bissau, Gulf of Guinea Is., Gulf States, Guyana, Hainan, Haiti, Honduras, India, Iran, Ivory Coast, Jamaica, Japan, Kazan-retto, Kenya, KwaZulu-Natal, Laccadive Is., Laos, Lebanon-Syria, Leeward Is., Lesser Sunda Is., Liberia, Malawi, Malaya, Maldives, Mali, Maluku, Mauritania, Mexico Central, Mexico Gulf, Mexico Northeast, Mexico Southwest, Mozambique, Mozambique Channel Is., Myanmar, Namibia, Nansei-shoto, Nepal, Netherlands Antilles, Niger, Nigeria, Northern Provinces, Ogasawara-shoto, Oman, Pakistan, Palestine, Paraguay, Peru, Philippines, Puerto Rico, Rwanda, Saudi Arabia, Senegal, Sierra Leone, Socotra, Solomon Is., Somalia, South Carolina, South China Sea, Southwest Caribbean, Spain, Sri Lanka, Sudan, Sulawesi, Suriname, Swaziland, Tanzania, Thailand, Togo, Trinidad-Tobago, Turks-Caicos Is., Uganda, Uruguay, Venezuela, Venezuelan Antilles, Vietnam, West Himalaya, Windward Is., Yemen, Zambia, Zaïre, Zimbabwe; and introduced in Alabama, Andaman Is., Angola, Brazil North, Mauritius, Nicaragua, Nicobar Is., Niue.

Distribution in India

It is found in warmer parts of the country up to 1000 m. It commonly occurs in waste places, ditches, and marshy places during rainy season. It is reported from the Andaman & Nicobar Islands, Andhra Pradesh, Assam, Bihar, Gujrat, Haryana, Himanchal Pradesh, Jammu, Kashmir, Jharkhand, Karnataka, Kerala, Madhya Pradesh, Maharashtra, Odisha, Rajasthan, Tamil Nadu, Tripura, Uttarakhand, Uttar Pradesh, West Bengal (Debasmita et al., 2015). In some reports, it has also been cultivated in West Bengal (Agarwal and Dutt, 1936; Basu et al., 1947; Surange and Pendse, 1972; Mahesh et al., 2012). In has been mentioned in several regional floras as Chennai (Gamble, 1957), Tamil Nadu Carnatic (Matthew, 1983), Goa, Diu, Daman and Nagar Haveli (Rao, 1986), Tamil Nadu (Henry et al., 1987), Cannanore (Ramachandran and Nair, 1988) and Eastern Karnataka (Singh, 1988).

Selection of the Sites and Characteristics Studied

The distribution, morphological variations, and association of *B. diffusa* 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 which two sites ranging between around 100 km in distance while the whole distance is about 2600 km (Table 2). The plant diversity was assessed upto the 10 km on both sides in each site. The plants were identified and deposited in the

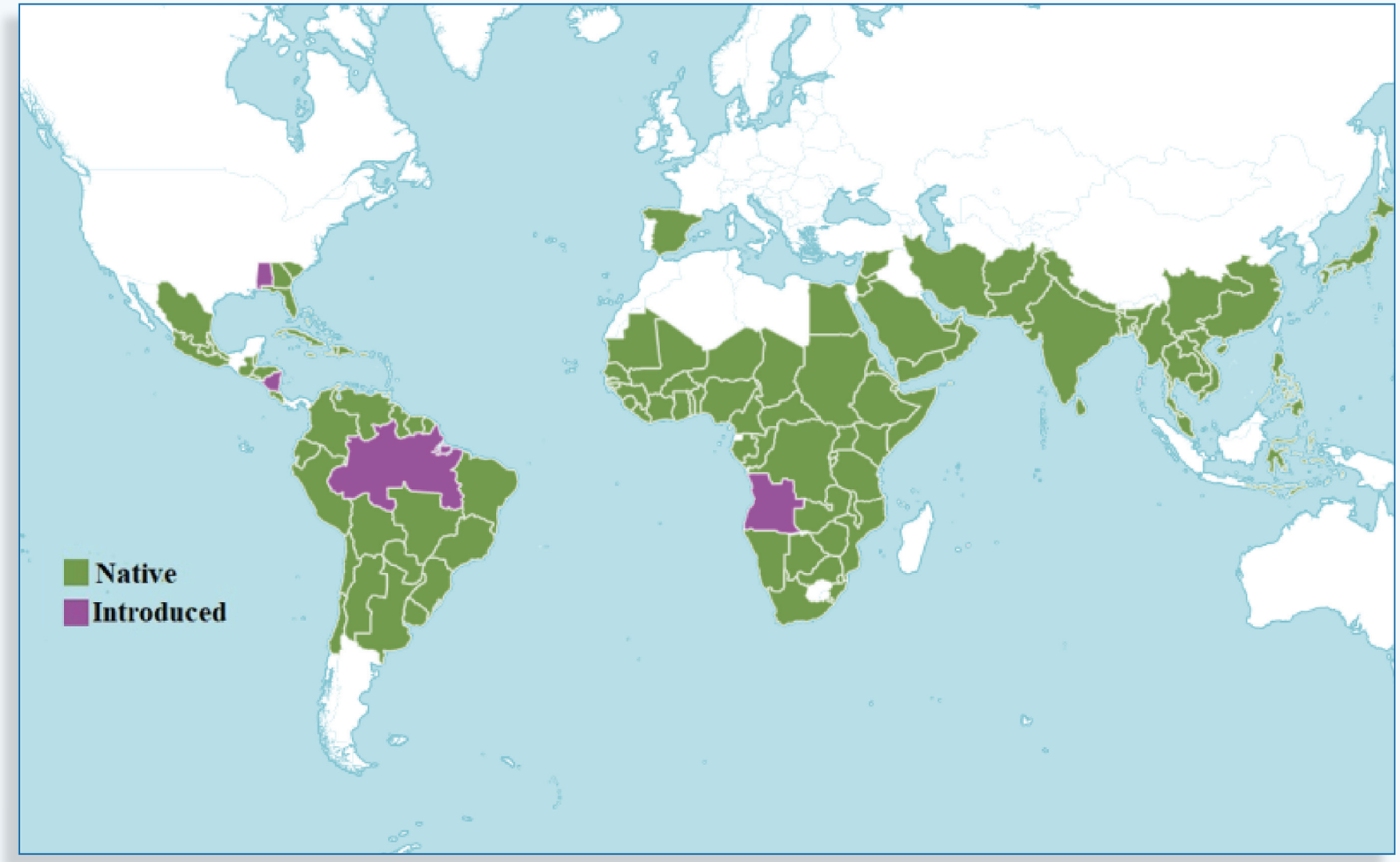

Fig. 2 Global distribution of genus *Boerhavia diffusa*

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

Table 2 Different studied sites and their GPS coordinates along with *B. diffusa* 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	Present
S8	Budaun	164	28.05	79.12	Present
S9	Farrukhabad	151	27.37	79.63	Present
S10	Bithoor	126	26.61	80.27	Absent
S11	Dalmau	115	26.07	81.03	Absent
S12	Prayagraj	98	25.45	81.85	Absent
S13	Mirzapur	80	25.15	82.58	Absent
S14	Varanasi	81	25.32	83.01	Absent
S15	Ballia	67	25.76	84.15	Absent
S16	Revelganj	52	25.78	84.67	Present
S17	Patna	53	25.61	85.14	Absent
S18	Barh	47	25.48	85.72	Present
S19	Bahachouki	55	25.30	86.36	Present
S20	Farka	42	25.23	87.09	Absent
S21	Sahebganj	16	25.25	87.65	Present
S22	Farakka Barrage	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	Absent
S26	Gangasagar	4	22.19	88.19	Absent

Local Occurrence

The plant was recorded in all the sites, but it was collected for the analytical studies only from the sites 4, 5, 6, 7, 8, 9, 16, 18, 19, and 21, as these sites being the source of its bulk collection. The remaining sites as 1, 2, 3, 10, 11, 12, 13, 14, 15, 17, 20, 22, 23, 24, 25, and 26, it was only representative in these sites; hence, it may be concluded that these sites require more conservation of this species.

Taxonomic Treatment

B. diffusa L., Sp. Pl.: 3. 1753; Hook.f., Fl. Brit. India 4: 709. 1885; Duthie, Fl. Gangetic Plain (repr. ed.) 2: 127. 1960; Haines, Bot. Bihar Orissa (Repr. ed.) 2: 795.1961; Uniyal & al., Fl. Pl. Uttarakhand, Checkl.: 203. 2007; Sinha & Sukla, Fl. Uttar Pradesh 2: 364.2020.

Synonyms

The Plants of the World Online Database mentioned it with its three homotypic and twenty-four heterotypic synonyms (POWO, 2023) as:

Homotypic Synonyms

1. *B. diffusa* var. *vulgaris* Domin in Biblioth. Bot. 22: 646 (1926), not validly publ.
2. *B. diffusa* var. *vulgaris* Domin in Biblioth. Bot. 22(89): 92 (1926), not validly publ.
3. *B. repens* var. *diffusa* (L.) Hook.f. in Fl. Brit. India 4: 709 (1885)

Heterotypic Synonyms

1. *Axia cochinchinensis* Lour. in Fl. Cochinch.: 36 (1790)
2. *Boerhavia adscendens* Willd. in Sp. Pl., ed. 4. 1: 19 (1797)
3. *B. africana* Lour. in Fl. Cochinch.: 16 (1790)
4. *B. caespitosa* Ridl. in J. Straits Branch Roy. Asiat. Soc. 45: 214 (1906)
5. *B. coccinea* var. *leiocarpa* (Heimerl) Standl. in Publ. Field Mus. Nat. Hist., Bot. Ser. 11: 108 (1931)
6. *B. decumbens* Vahl in Enum. Pl. 1: 284 (1804)
7. *B. diffusa* var. *leiocarpa* (Heimerl) C.D.Adams in Mitt. Bot. Staatssamml. München 8: 115 (1970)
8. *B. diffusa* var. *pubescens* Choisy in A.P.de Candolle, Prodr. 13(2): 453 (1849)
9. *B. diffusa* f. *subhirsuta* Heimerl in Bull. Herb. Boissier 5(App. 3): 67 (1897)
10. *B. friesii* Heimerl in Oesterr. Bot. Z. 56: 253 (1906)
11. *B. laxa* Pers. in Syn. Pl. 1: 36 (1805), nom. superfl.
12. *B. obtusifolia* Steud. in Nomencl. Bot., ed. 2, 1: 213 (1840)
13. *B. paniculata* Lam. in Tabl. Encycl. 1: 10 (1791)
14. *B. paniculata* Rich. in Actes Soc. Hist. Nat. Paris 1: 105 (1792), nom. illeg.
15. *B. paniculata* f. *esetosa* Heimerl in Annuaire Conserv. Jard. Bot. Genève 17: 225 (1913)
16. *B. paniculata* var. *guaranitica* Heimerl in Oesterr. Bot. Z. 56: 252 (1906)
17. *B. paniculata* f. *leiocarpa* Heimerl in Oesterr. Bot. Z. 56: 252 (1906)

18. *B. paniculata* var. *leiocarpa* (Heimerl) Heimerl in Annuaire Conserv. Jard. Bot. Genève 17: 225 (1913)
19. *B. paniculata* f. *multiglandulosa* Heimerl ex Parodi in Revista Fac. Agron. Veterin. 7: 197 (1930)
20. *B. paniculata* f. *setosa* Heimerl in Annuaire Conserv. Jard. Bot. Genève 17: 225 (1913)
21. *B. rottleri* Steud. in Nomencl. Bot., ed. 2, 1: 213 (1840), not validly publ.
22. *B. rugosa* Rottler ex Wall. in Numer. List: n.° 6771 (1832), not validly publ.
23. *B. xerophila* Domin in Biblioth. Bot. 22(89): 92 (1925), pro syn.
24. *Commicarpus africanus* (Lour.) Dandy in F.W.Andrews, Fl. Pl. Sudan 1: 152 (1950)

While another database i.e., World Flora Online (WFO, 2023) mentioned it with a total of following twenty-one synonyms

1. *Axia cochinchinensis* Lour., Fl. Cochinch. 1: 36 (1790)
2. *Boerhavia adscendens* Willd., Sp. Pl., ed. 4 [Willdenow] 1(1): 19 (1797)
3. *B. caespitosa* Ridl., J. Straits Branch Roy. Asiat. Soc. 45: 214 (1906)
4. *B. ciliatobracteata* Heimerl, Jahresber. K. K. Staats-Ober-Realschule Steyr xxiii. repr. 25 (?1897)
5. *Boerhavia coccinea* var. *leiocarpa* Standl., Publ. Field Mus. Nat. Hist., Bot. Ser. 11: 108 (1931)
6. *B. coccinea* var. *paniculata* Moscoso, Cat. Fl. Domingensis 1: 180 (1943)
7. *B. diffusa* var. *leiocarpa* (Heimerl) C.D.Adams, Mitt. Bot. Staatssamml. München 8: 115 (1970)
8. *B. diffusa* var. *pubescens* Choisy, Prodr. [A. P. de Candolle] 13(2): 453 (1849)
9. *B. friesii* Heimerl, Oesterr. Bot. Z. 56: 253 (1906)
10. *B. paniculata* Rich., Actes Soc. Hist. Nat. Paris 1: 105 (1792)
11. *B. paniculata* f. *esetosa* Heimerl, Annuaire Conserv. Jard. Bot. Genève 17: 225 (1913)
12. *B. paniculata* var. *guaranitica* Heimerl, Oesterr. Bot. Z. 56: 252 (1906)
13. *B. paniculata* f. *leiocarpa* Heimerl, Oesterr. Bot. Z. 56: 252 (1906)
14. *Boerhavia paniculata* var. *leiocarpa* Heimerl, Annuaire Conserv. Jard. Bot. Genève 17: 225 (1913)
15. *B. paniculata* f. *multiglandulosa* (ex Parodi) Heimerl, Revista Fac. Agron. Veterin. 7: 197 (1930)
16. *B. paniculata* var. *subacuta* Choisy, Prodr. [A. P. de Candolle] 13(2): 451 (1849)
17. *B. repens* var. *diffusa* (L.) Hook.f., Fl. Brit. India [J. D. Hooker] 4(pt. 12): 709 (1885)
18. *B. xerophila* Domin, Biblioth. Bot. lxxxix. 92 (1925)
19. *B. diffusa* var. *paniculate* Kuntze Rev. Gen. 533. (1891)
20. *B. diffusa* var. *diffusa* L.
21. *B. diffusa* var. *mutabilis* (R.Br.) Heimerl Jahresber. K. K. Staats-Ober-Realschule Steyr 23: 27 (1897)

Botanical Description

Prostrate or ascending diffuse herb, about 50 cm long, many-branched from a taproot; twigs cylindrical, glabrous, thickened at the nodes. Leaves simple in unequal pairs; blades 1.0-6.5 × 1.1-4.5 cm, ovate to orbicular ovate, chartaceous, sparsely pilose or woolly, especially on veins, lower side glaucous, apex rounded to acute, shortly apiculate, base rounded, truncate to nearly cordate, margins wavy, ciliate; petioles pilose, about 1 cm long. Flowers nearly sessile, very small, dark pink, 2-7 in terminal, subcapitate or subumbellate clusters on axillary racemes or terminal panicles; axes glabrous; bracts and bracteoles lanceolate, deciduous, lancelike. Calyx base 0.5-1.7 mm, puberulent, limb funnel-shaped, red, violet, purplish red, reddish pink, 0.6-1 mm long, bell-shaped beyond the constriction; stamens 2-3, slightly exserted. Anthocarp sessile, green, glandular hairy, sticky, short club-shaped, 2-2.5 mm long, 5-ribbed (Fig. 3 A, B).

Fig. 3A Plant habit

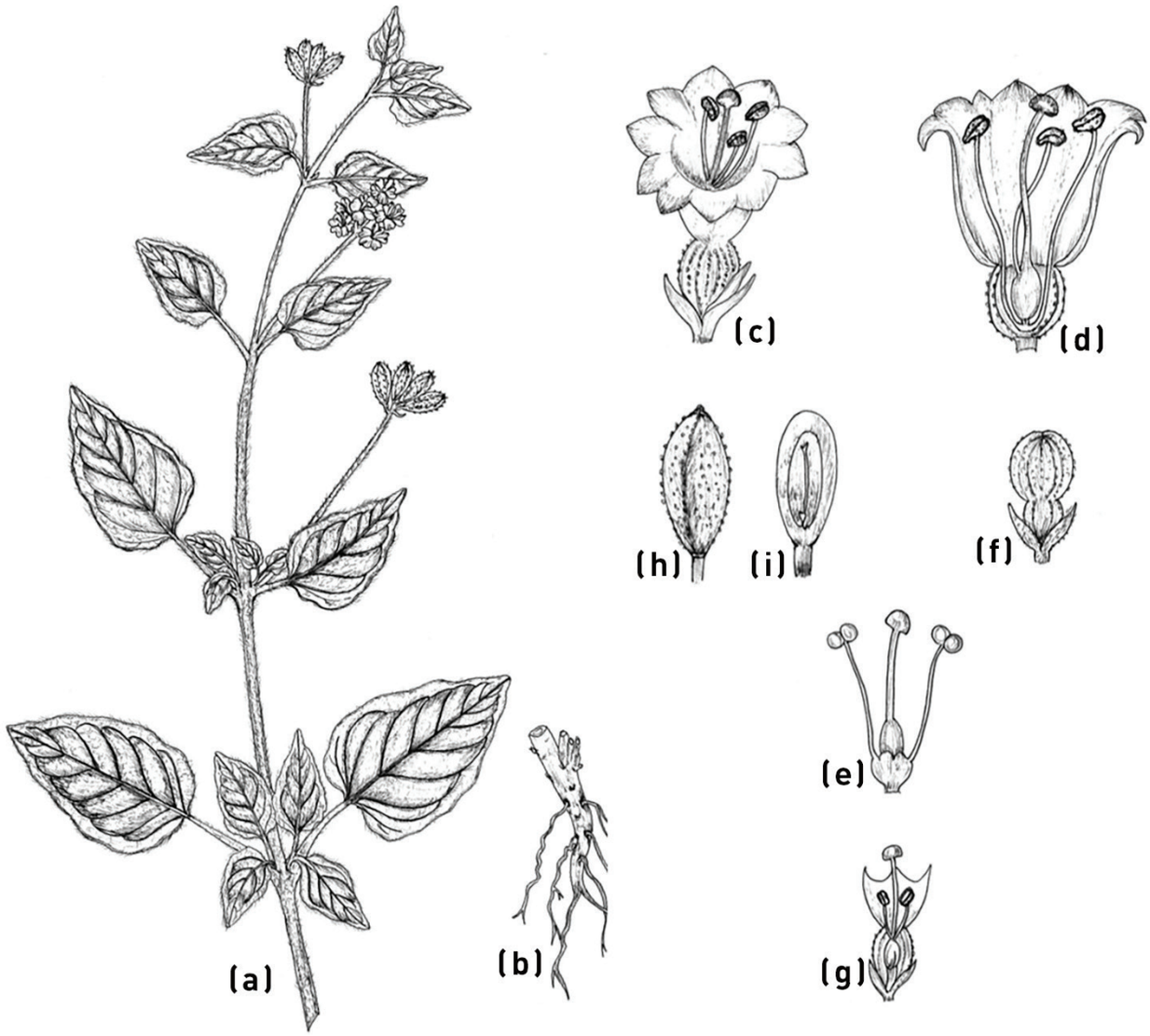

Fig. 3B (a)Plant twing (b) Root (c) L.S of flower (e) Pistil with two stamens (f) Flower bud with bracteole (g) L.S of Flower bud (h) Fruit (i) L.S. of fruit

Chromosome Number

The basic chromosome numbers are 13 (x=14) (Srivistava and Misra, 1966; Gill and Abubakar, 1975). Among these 6 are submetacentric and 7 are metacentric (Majumder et al., 2021). Polyploidy is commonly recorded in several studies. Carr (1978) recorded 26 and 27 haploid number of chromosomes (n=26 & 27). Thrombre (1959) recorded 58 haploid number of chromosomes (n=58). Bala et al. (2011) studied the population of *B. diffusa* and recorded two cytotypes at different levels of polyploidy; one with pinkish flowers is having n=26 and the purple colored morphotype has n=52.

Anatomical Features

Structure of Leaf

The leaf is dorsi ventral, with a thick lamina and a less prominent midrib. The midrib is around 500 µm thick, has a narrow semicircular abaxial section, and a shallow concavity on the abaxial side. The epidermis is composed of spherical cells with thick walls. The parenchyma cells of the ground tissue are angular and compact. Its thickness is 470 µm. The stomata are found in both the surface *i.e.,* amphistomatic. The epidermal layer is thick and comprises of fairly thick cells. There is a thin cuticle layer on the outside. The mesophyll layer is found inside the epidermis and is composed of a single adaxial row of short cylindrical palisade cells and lobed, loosely distributed spongy parenchyma cells. the lateral veins in the top section are round. A rosette of Kranz-cells with noticeable chloroplasts surrounds the vein's tiny group of xylem and phloem components. The marginal part of the lamina is bluntly conical and measures 300 µm in thickness. The epidermal cells of margin consist of elliptical thick-walled cells. The mesophyll tissue near the edge lacks differentiation from the centre portion. The calcium oxalate crystals known as raphides are found in the mesophyll cells. These raphide measure around 150 × 40 µm and are a spindle-shaped bundle of many, thin, sharp needles. Three vascular bundles are arranged in an arc along the midrib (Fig. 4).

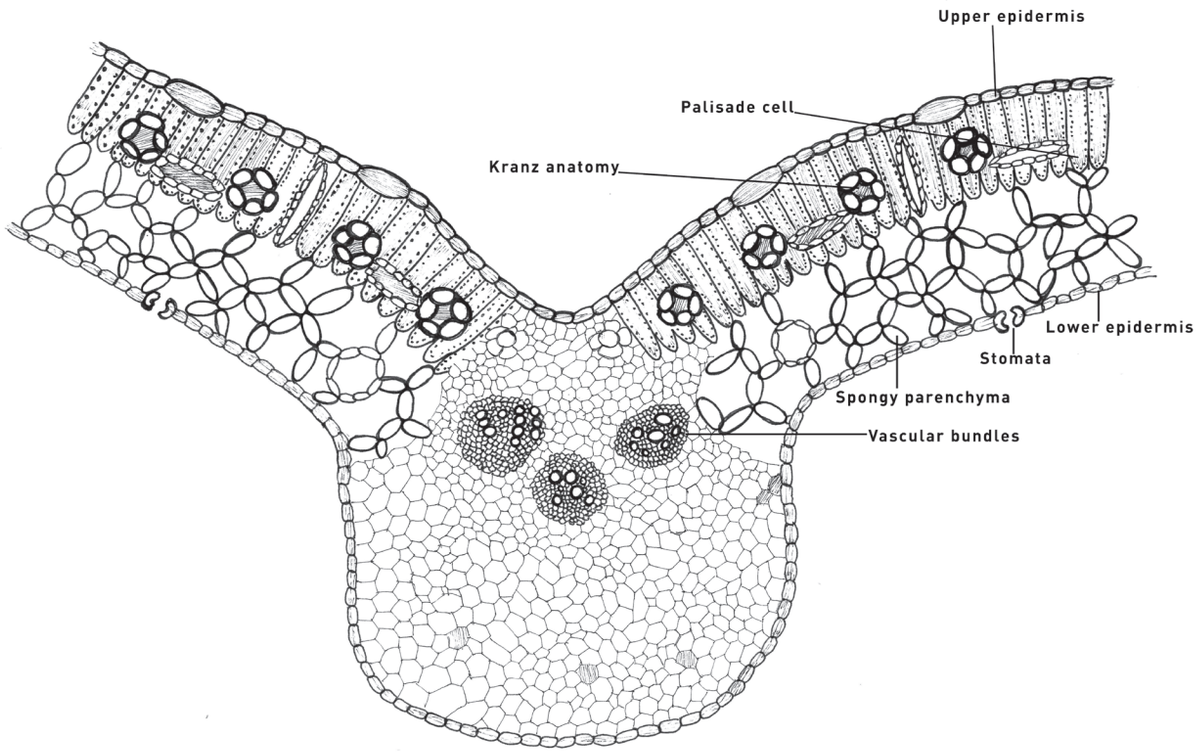

Fig. 4 TS of the leaf

Structure of Petiole

The cross section of petiole shows a groove. The epidermis is outermost layer that follows the several layers of cortex internally. Six vascular bundles are present in an open arc, with two interspersed ones and accessory vascular bundles (Fig. 5).

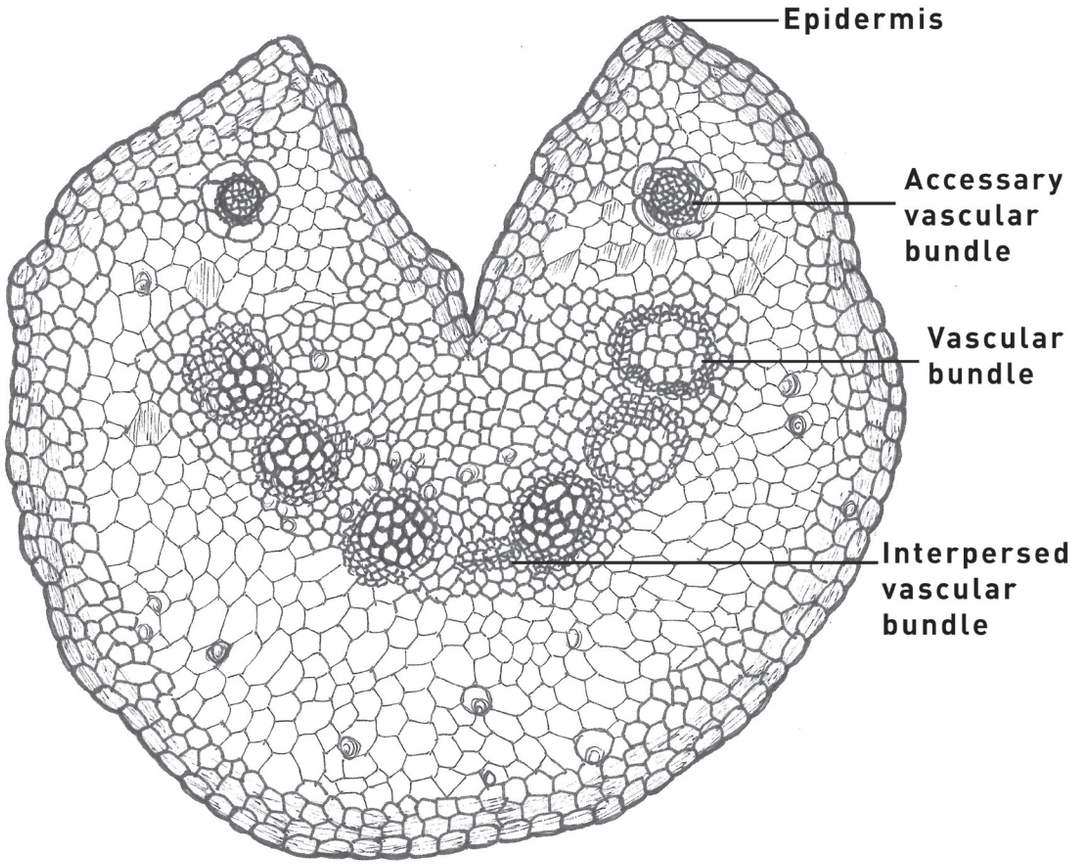

Fig. 5 TS of the petiole

Structure of Stem

The outermost epidermal layer is thin and made up of isodiametric cells which is covered with prominent cuticle. Numerous multicellular hairs are present in epidermis. Below it about six layers of cortex is present which is made up of rectangular thin-walled parenchymatous cells. The mature stem contains raphides in the cortical region. The stem shows an abnormal secondary growth with a unique conformation. It has a central group of discrete vascular bundles called medullary bundles which are surrounded by a thick continuous secondary xylem. The vascular bundles are arranged in three rings, with about twenty smaller bundles in the outer ring, about fourteen medium-sized bundles in the centre, and two larger bundles in the inner ring. All the bundles are conjoint and open that have primary xylem and limited amount of secondary xylem. The intrafascicular cambium functions for a short period and

produces secondary xylem and secondary phloem. Then the cambium activity stops. A new cambium originates from the outer part of cortical zones which produces new secondary xylem and secondary phloem. The secondary xylem consists of thick cylindrical xylem fibres in which radial groups of wide circular solitary vessels occur. The vessels towards the center are small as compare with the those towards the peripheral region (Fig. 6).

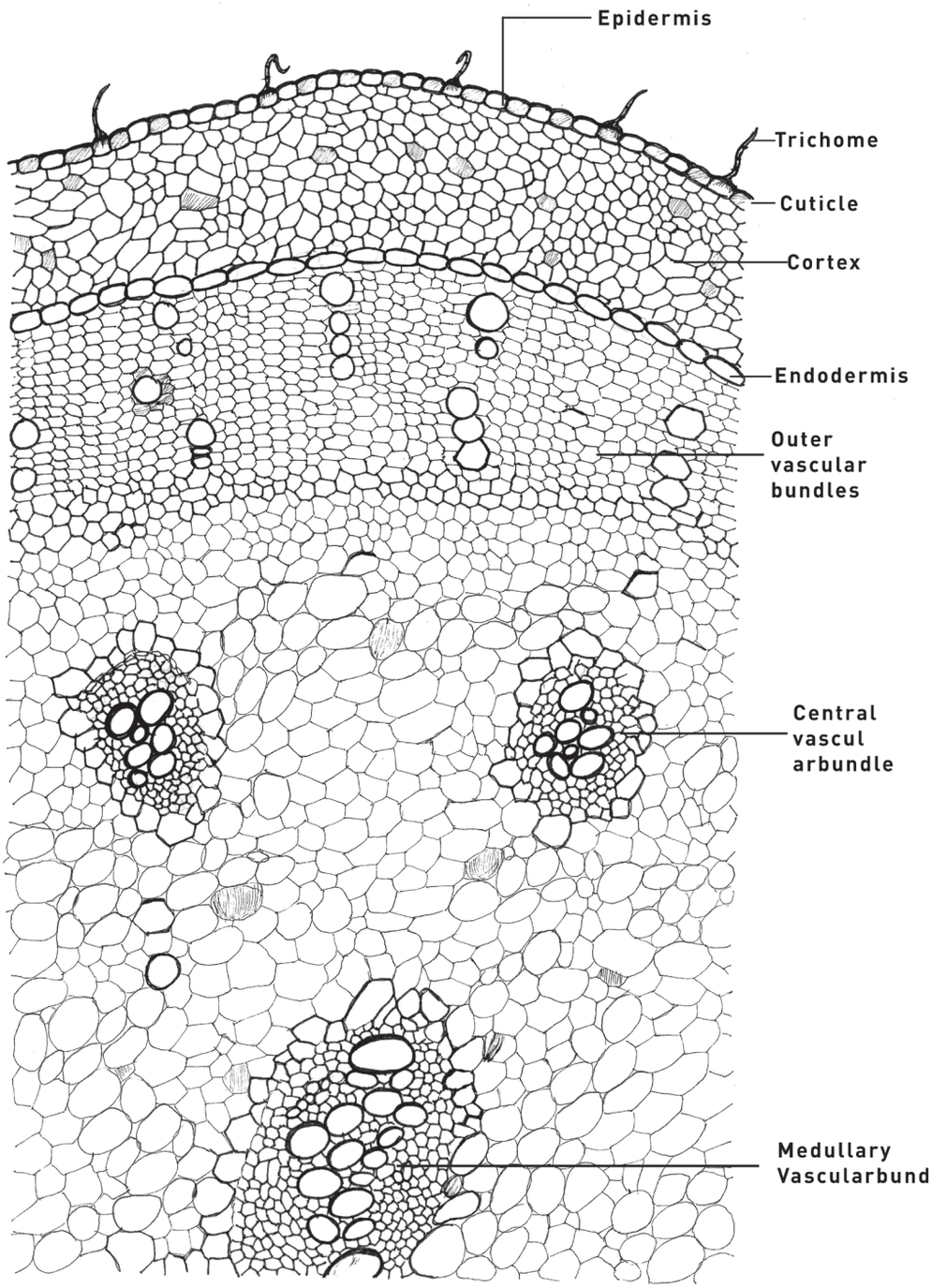

Fig. 6 TS of the stem

Structure of Root

The transverse section of root shows well developed spongy tissue of periderm with small fissures. The cortical region has the raphides. The vascular bundles show clear structures of anomalous secondary growth that having the successive cambium rings in patches because of the formation of interfascicular parenchyma. The xylem and phloem arranged one above in a circular ring. The cells of parenchyma have starch grains above the phloem region. Most of the vessel elements are distributed in the external region of the xylem (Fig. 7).

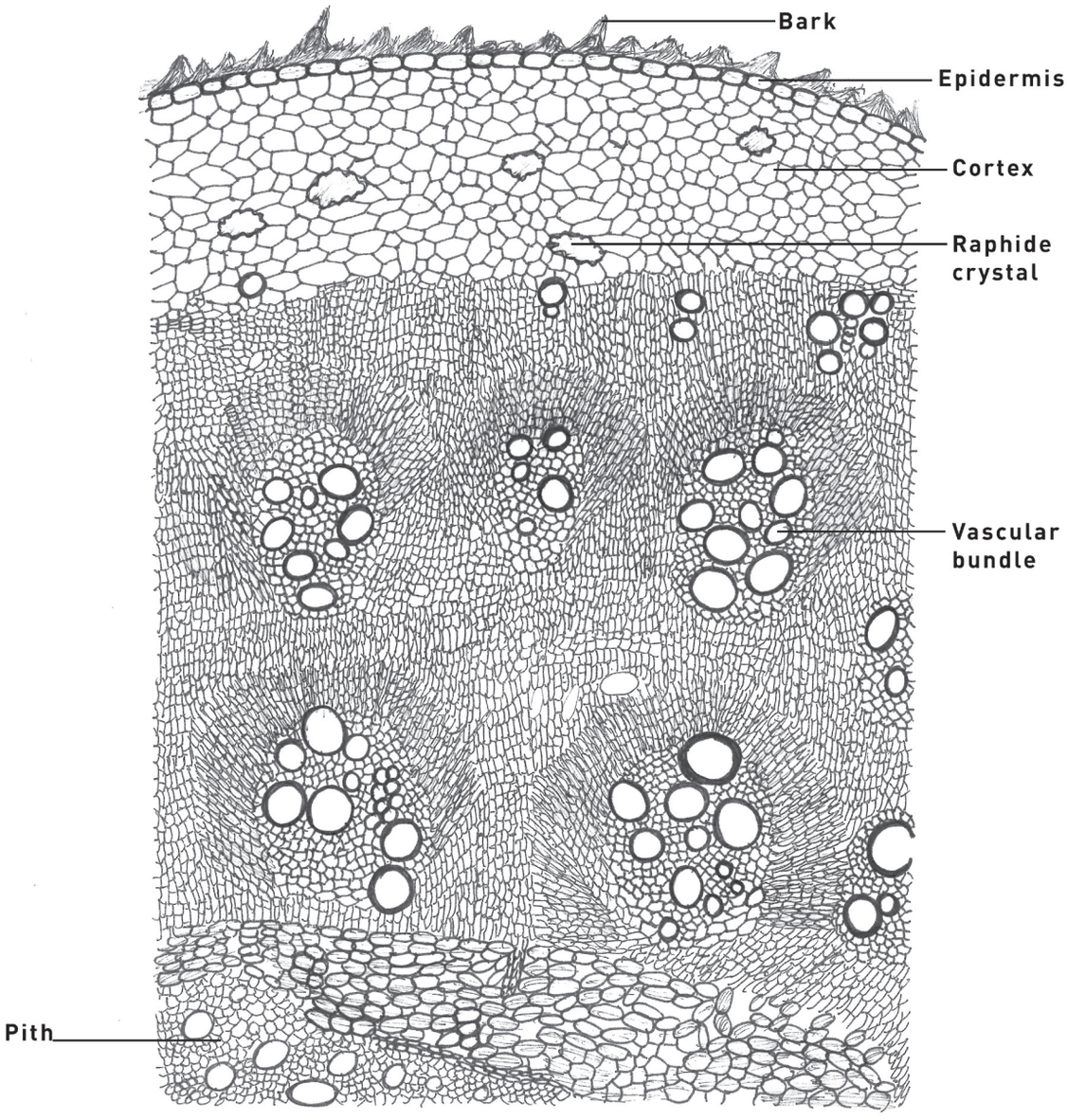

Fig. 7 TS of the root

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CHAPTER

02

Bibliometric Analysis

INTRODUCTION

Boerhavia diffusa recognized as "Punarnava" or "Red Spiderling," has garnered substantial attention within the scientific community owing to its extensive relevance in traditional medicine systems such as Ayurveda, Siddha, and Unani(Patil and Bhalsing, 2016). Studies on *B. diffusa* have explored its phytochemical composition, biological activities, therapeutic potential, ecological adaptations, and traditional uses across different cultures (Kaur, 2019; Gaur et al., 2022). Its pharmacological properties, including anti-inflammatory, antioxidant, diuretic, hepatoprotective, and anti-cancer activities, have been of particular interest to researchers seeking novel therapeutic agents (Mishra et al., 2014; Rani et al., 2022). This plant has been utilized in traditional medicinal practices for treating conditions such as edema, urinary disorders, jaundice, fever, and skin diseases, among others. Such multifaceted attributes have spurred widespread interest among researchers, leading to a considerable body of scholarly literature exploring its therapeutic potential and applications across various health domains.The burgeoning interest in *B. diffusa* has prompted a need for a comprehensive bibliometric analysis to delve into the depth and breadth of research devoted to this plant species.

Bibliometric analysis has transformed into an essential tool in comprehending the intricate network of research landscapes within scientific investigation. This approach offers a systematic and quantitative method to assess the path of academic output in specific topic areas, uncovering trends, patterns, and influential works that shape the dissemination of information (Liang et al., 2020). A meticulous examination of the scholarly output, trends, collaborations, and impact of publications related to *B. diffusa* will offer invaluable insights into the evolving research landscape surrounding this medicinal plant. This research focuses on *B. diffusa*, a significant plant species known for its ecological importance, pharmacological traits, therapeutic uses, and diverse medicinal applications. The aim is to comprehensively analyze the current research trends, major contributors, and collaborative networks associated with this botanical species by systematically examining scholarly literature devoted to it. By systematically

analyzing the publications, identifying key contributors, mapping collaborations, and assessing the impact of research efforts, this bibliometric analysis aims to present a holistic understanding of the current state and prospects within the realm of *B. diffusa* research.

Data Sources and Analytical Tools

The bibliometric analysis of research pertaining to *B. diffusa* presents valuable insights into the scholarly production, impact, and trends within academic literature along with collaboration among the different indices.This study conducted a bibliometric analysis using the exact keyword '*Boerhavia diffusa*' within titles and abstracts across multiple articles sourced from dimensions.ai.The analysis was performed using Microsoft Excel, and VosViewer (Version 1.6.19), not only promises to reveal the current state of awareness regarding *B. diffusa* but also to provide strategic insights into potential directions for future research endeavors, ultimately enhancing our comprehension of its ecological significance and therapeutic potentials.The main field of study was identified through the analysis of extracted data utilizing the integrated tools within lens.org.

Comprehensive Analytical Insights

Examining the bibliometric analysis from 2000 to 2023 unveiled an enduring interest that persisted consistently throughout the past two decades. A collection of 247 documents was sourced from 234 distinct publications, encompassing books and journals, contributing to this comprehensive analysis. The significance and impact of research on *B. diffusa* were underscored by an average citation rate of 6.91 per document. The study identified 959 authors who contributed to the field of *B. diffusa* research, signifying widespread engagement in this area. A total of 17 authors independently published documents, showcasing their specialized competence and enduring interest in this subject matter. This study indicated that research on this species was actively progressing and remained a pertinent subject in contemporary scientific conversations.

Temporal Evolution and Growth Analysis

This comprehensive analysis encompassed a total of 247 documents that were sourced from the bibliographic database known as Dimensions.ai. These scholarly publications comprised various document types, including 234 articles, 8 chapters, 3 preprints, and 2 proceedings, collectively portraying a rich diversity in the arena of research on *B. diffusa*. The study primarily focused on showcasing the advancements made in *B. diffusa* research spanning from the year 2000 to 2023. A modest increase in publications between 2012 and

2021 suggested that interest was developing (Fig. 1). The peak in scholarly publications occurred in 2021, with 37 publications, followed by 32 in 2022, and a slight decline to 25 and 23 publications in 2020 and 2023, respectively. A significant temporal exponential growth in publications was observed from 2000 to 2023, denoted by an R-square value of 0.8244. This exponential trend suggested a substantial increase in research output and interest in *B. diffusa* over the years. Moreover, the study emphasized the diverse range of strategies employed for disseminating *B. diffusa* research. Research articles were identified as the primary mode of communication, supplemented by proceedings, chapters, and preprints. This diverse dissemination strategy underlined the multifaceted nature of research output in this domain. Overall, the findings underscored the growing significance and interest of the scientific community in *B. diffusa* research.

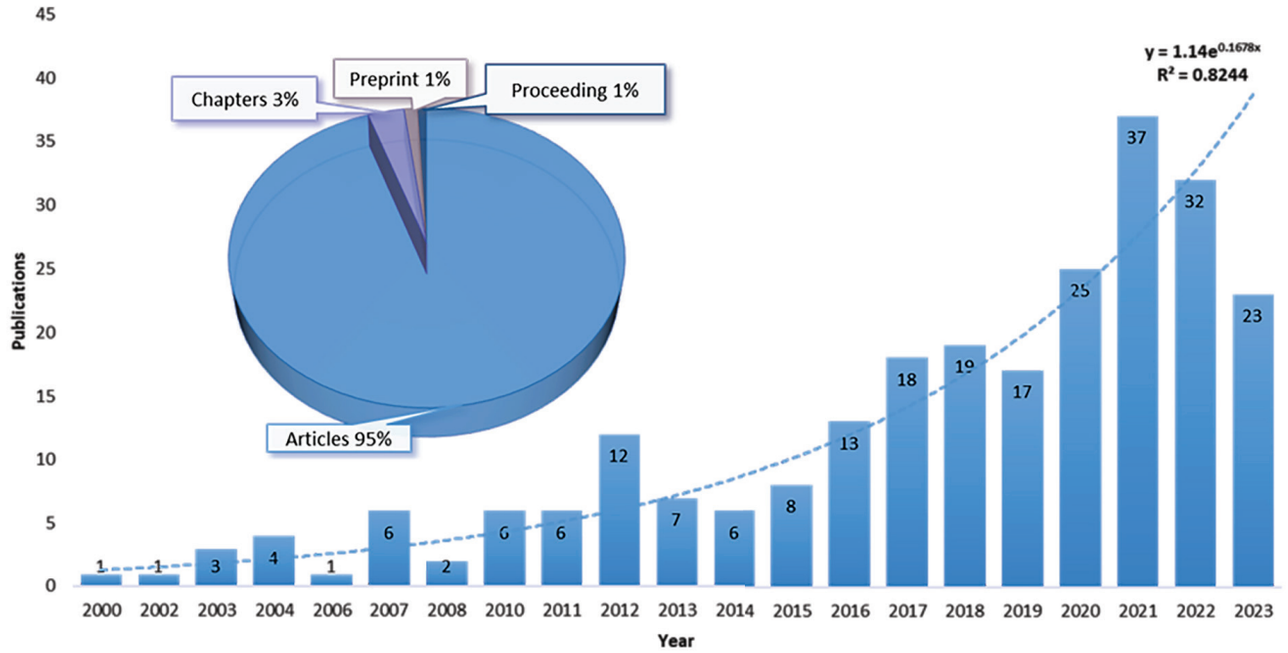

Fig. 1 Publication trends and distribution types in *B. diffusa* research

Citation Analysis

This study conducted a citation analysis of studies concerning *B. diffusa* from 2000 to 2023, exploring the scientific literature to uncover significant trends and variations in research interest regarding this plant. The analysis highlighted a significant turning point in 2004, marked by a surge in *B. diffusa* citations to 102, signifying increased scientific interest and attention towards the plant and its potential applications. The year 2012 stood out as the peak period in citation analysis, with the highest recorded citations at 225, likely reflecting a substantial volume of research conducted (Fig. 2). Subsequently, there was a decline in the number of citations noted between 2016 and 2023. This decrease might

have been attributed to the dataset's cutoff rather than a decline in interest or research activity related to *B. diffusa*. This period might not have accurately reflected the actual trend, as the data collection ceased in October 2023. Overall, the data reflected fluctuations in citation numbers, highlighting key moments of heightened attention and significant contributions to the *B. diffusa* field, while variations indicated shifts in research focus and popularity over time.

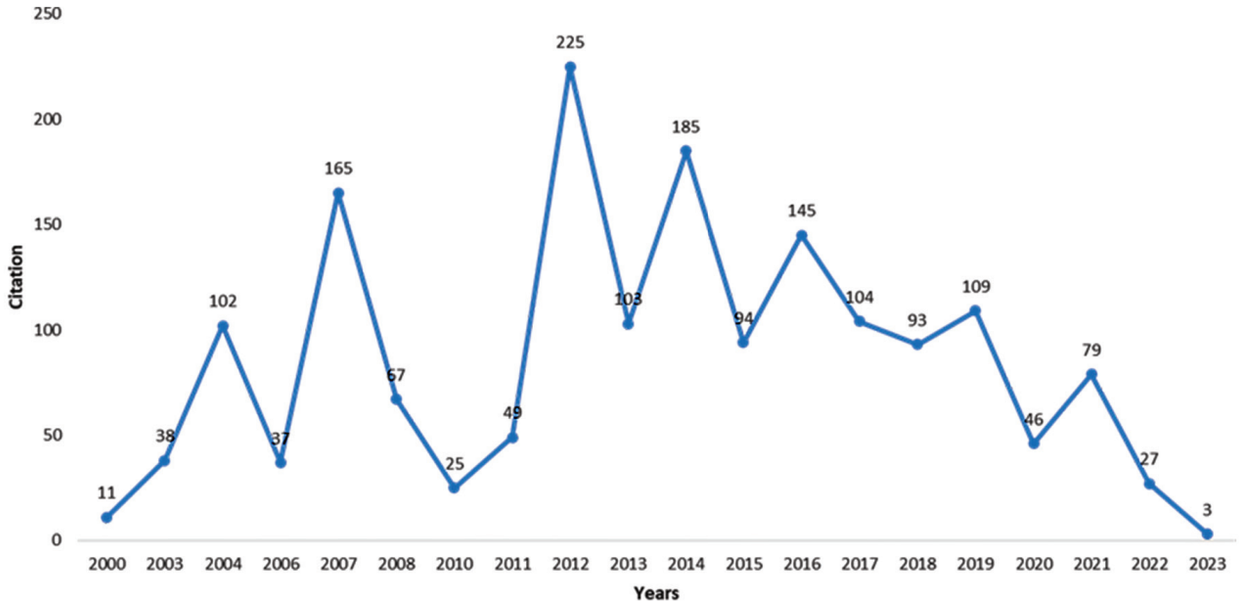

Fig. 2 Citation trends for *B. diffusa* research over time

Country-wise Publication Analysis

The comprehensive analysis demonstrated the extensive distribution of research findings on *B. diffusa* across 39 nations. India emerged as a prominent contributor with 76 documents and 882 citations, boasting an average citation rate of 11.61 per document, signifying substantial scholarly impact. Following closely, countries like Brazil and Saudi Arabia presented fewer documents of 14 and 12, respectively, and their citation averages were moderate, with Brazil averaging 3.21 citations per document and Saudi Arabia averaging 5.42 citations per document. South Africa, despite a lower document count of 7, recorded a remarkable citation count of 93, resulting in an impressive average of 13.29 citations per document, showcasing significant influence within the field. Nigeria and South Korea exhibited fewer documents (6 each) and relatively lower citation counts of 23 and 19, respectively. Their average citation rates of 3.83 and 3.80 per document, respectively, denoted a comparatively lower impact in terms of citations within this research domain (Fig. 3). Overall, while India maintained the highest publication volume, countries like South Africa, Pakistan, and Bangladesh showed significant impact with fewer documents but significantly higher citation averages, indicating their considerable scholarly influence in the domain of *B. diffusa* research.

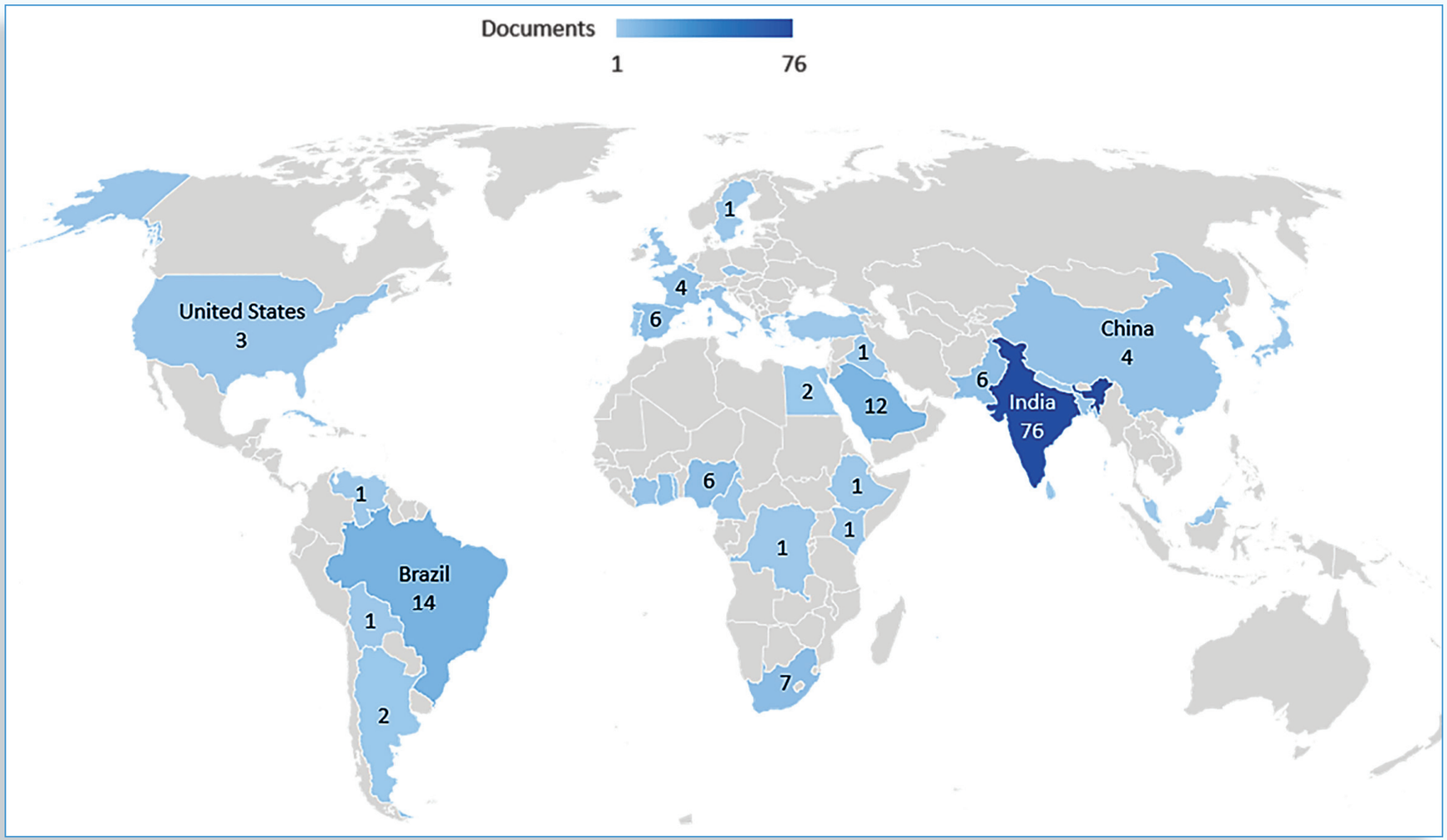

Fig. 3 Global distribution of research published on *B. diffusa*

Most Prominent Authors

In the study, the publication counts, citations received, and citation per document ratios of several authors were examined, providing insight into their scholarly impact through bibliometric analysis.Out of all the authors, Andréia Mitsa Paiva Negreiros had the highest number of articles published (6) during the examined period.Josep Armengol stood out with a high citation per document ratio of 7, indicating a significant impact relative to the number of documents published. Sumeet Gairola's exceptional citation per document ratio of 56, despite a smaller number of publications, highlighted the remarkable influence of their work within their field. Authors like Andréia Mitsa Paiva Negreiros, Rui Sales Júnior, and Acharya Balkrishna exhibited respectable citation per document ratios ranging between 3.67 and 5, suggesting a moderate impact with a decent number of citations per published document. Conversely, Márcia Michelle De Queiroz Ambrósio, Ram Chand Bhatti, Rainer W. Bussmann, Rupinder Kaur, and Abhishek Kumar had lower citation per document ratios, ranging from 0.67 to 1.5, indicating comparatively fewer citations received per document published(Fig. 4). This analysis provided valuable insights into the varying levels of impact and recognition of authors' work within their respective academic domains, emphasizing the significance of citation metrics in evaluating scholarly contributions.

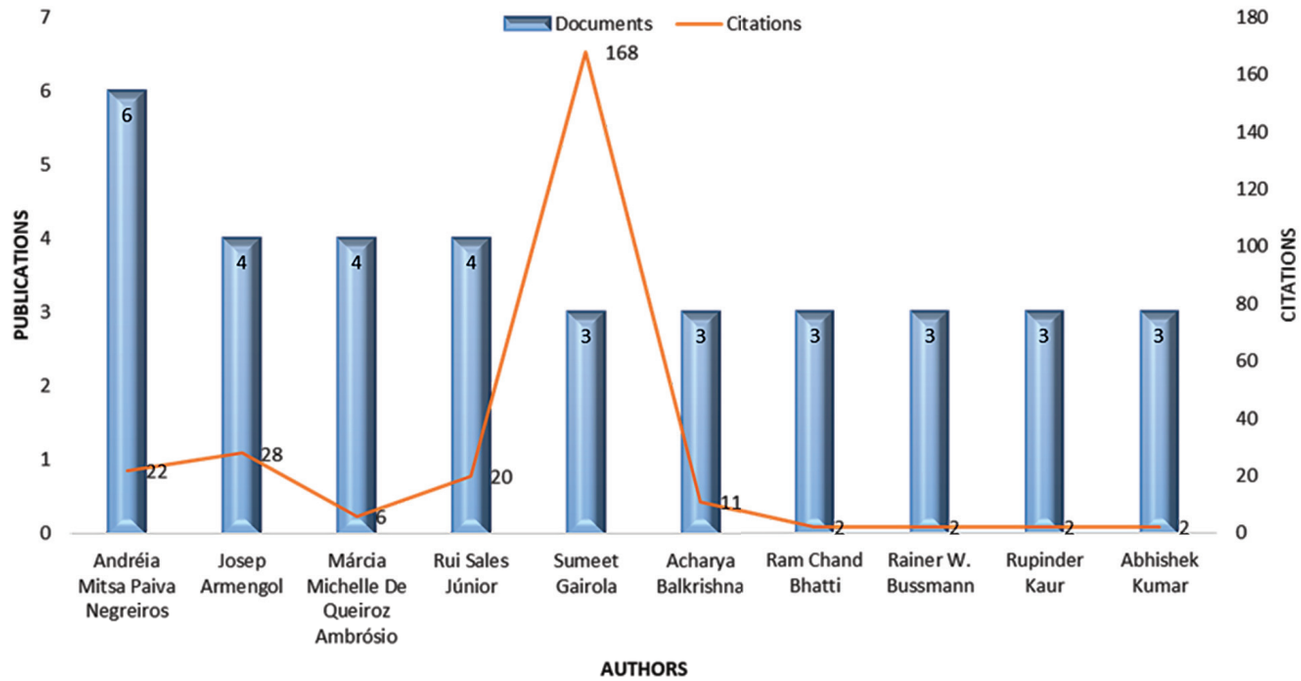

Fig. 4 Most prominent authors based on research contributions to *B. diffusa*

Highly Cited Articles

In the study, the most cited article was Mishra et al. (2014), published in BioMed Research International, with an impressive 120 citations, highlighting its significant impact on the field. Sharma et al.'s contributions in the Journal of Ethnopharmacology in 2012 and 2013 gathered 87 and 81 citations, respectively. Additionally, significant contributions included Ishtiaq et al.'s (2007) work in the Pakistan Journal of Biological Sciences with 60 citations, Satheesh et al.'s (2004) research in the Indian Journal of Experimental Biology with 52 citations, and Miech et al.'s (2016) study in the Journal of Insects as Food and Feed, also with 52 citations, emphasizing the enduring impact in their respective domains.Ranjitkar et al.'s (2014) publication in Ecological Modelling with 51 citations, Okoli et al.'s (2007) work in the Pakistan Journal of Nutrition with 46 citations, and Aladesanmi et al.'s (2007) article in the African Journal of Traditional Complementary and Alternative Medicines with 43 citations, all signified significant influence within their focused areas. Lastly, Selvaraj et al.'s (2012) article in Genomics Proteomics & Bioinformatics with 39 citations further highlighted its impact in the realms of genomics, proteomics, and bioinformatics (Table 1). Collectively, these highly cited articles illustrated their substantial contributions and enduring relevance within their respective scientific domains.

Table 1 Top 10 highly cited research article published on *B. diffusa*

Rank	Titles	Citations	Years	Journal	References
1	Phytochemical, Therapeutic, and Ethnopharmacological Overview for a Traditionally Important Herb: *Boerhavia diffusa* Linn.	120	2014	BioMed Research International	Mishra et al., 2014
2	The treatment of jaundice with medicinal plants in indigenous communities of the Sub-Himalayan region of Uttarakhand, India	87	2012	Journal of Ethnopharmacology	Sharma et al., 2012
3	Ethnomedicinal plants used for treating epilepsy by indigenous communities of sub-Himalayan region of Uttarakhand, India	81	2013	Journal of Ethnopharmacology	Sharma et al., 2013
4	An Ethnomedicinal Survey and Documentation of Important Medicinal Folklore Food Phytonims of Flora of Samahni Valley, (Azad Kashmir) Pakistan	60	2007	Pakistan Journal of Biological Sciences	Ishtiaq et al., 2007

Rank	Titles	Citations	Years	Journal	References
5	Antioxidant effect of *Boerhavia diffusa* L. in tissues of alloxan induced diabetic rats.	52	2004	Indian Journal of Experimental Biology	Satheesh et al., 2004
6	Growth and survival of reared Cambodian field crickets (*Teleogryllus testaceus*) fed weeds, agricultural and food industry by-products	52	2016	Journal of Insects as Food and Feed	Miech et al., 2016
7	Ensemble forecast of climate suitability for the Trans-Himalayan Nyctaginaceae species	51	2014	Ecological Modelling	Ranjitkar et al., 2014
8	Medicinal Herbs Used for Managing Some Common Ailments among Esan People of Edo State, Nigeria	46	2007	Pakistan Journal of Nutrition	Okoli et al., 2007
9	Antimicrobial And Antioxidant Activities of Some Nigerian Medicinal Plants	43	2008	African Journal of Traditional Complementary and Alternative Medicines	Aladesanmi et al., 2008
10	DNA Barcode ITS Effectively Distinguishes the Medicinal Plant *Boerhavia diffusa* from Its Adulterants	39	2012	Genomics Proteomics & Bioinformatics	Selvaraj et al., 2012

Most Active Journals

The current study unveiled the leading journals in *B. diffusa* research, assessing their publication frequency, citations, and average citations per article. The top-ranking journal, "Journal of Pharmaceutical Research International" had presented 10 documents with 10 citations, highlighting its prominence. However, the pivotal findings emphasized the significance of esteemed publications, notably positioning the "Journal of Ethnopharmacology" at the forefront in the past, with 6 documents, 294 citations, and an impressive average of 49 citations per item, underscoring its substantial impact. The "Asian Journal of Pharmaceutical and Clinical Research" held the third position, showcasing 5 documents, 8 citations, and an average of 1.6 citations per article (Table 2). Moreover, the "Pharmaceutical Biology" Journal presented 3 documents with 78 citations and an average of

26 citations per article. Additionally, the "African Journal of Traditional Complementary" had demonstrated significant influence previously, with an average of 18 citations per article across 3 documents. These findings served as a guiding reference for readers and researchers seeking pertinent and valuable resources for their exploration of *B. diffusa* during that period.

Table 2 Top journals in *B. diffusa* research having the highest published document and citations

Rank	Journals	Publisher	Impact factor	Documents	Citations	Average citations per document
1	Journal of Pharmaceutical Research International	-	-	10	10	1
2	Journal of Ethnopharmacology	Science Direct	5.4	6	294	49
3	Asian Journal of Pharmaceutical and Clinical Research	-	-	5	8	1.6
4	Ethnobotany of Mountain Regions	Springer	0.2	5	2	0.4
5	Revista Caatinga	-	1.1	4	7	1.75
6	Pharmaceutical Biology	Taylor & Francis	3.98	3	78	26
7	African Journal of Traditional Complementary and Alternative Medicines	-	0.5	3	54	18
8	Molecules	MDPI	4.92	3	34	11.3
9	Advances In Traditional Medicine	Springer	2.48	3	19	6.3
10	3 Biotech	Springer	2.83	3	5	1.6

Top Productive Organizations

The contributions of various organizations to *B. diffusa* research highlighted varying impacts and output. The study's findings list the top organizations according to how much research they have done on *B. diffusa* with 8 publications, 33 citations, and an average of 4.1 citations per piece, the Universidade Federal Rural Do Semi-Árido dominated the field of study. Each of the three institutions had a significant influence, with 5 documents, 35, 35

and 60 citations, and average citation counts of 7.0, 6.4 and 12.0 for the King Saud University, Universitat Politècnica De València and University of Cape Coast respectively. Jamia Hamdard from India exhibited exceptional impact despite a smaller document count, amassing 206 citations across 4 documents, resulting in an average of 51.5 citations per paper. Contrastingly, Panjab University, also from India, had the lowest impact, with 4 documents yielding only 2 citations, averaging a mere 0.5 citations per document. Other Indian institutions such as the Indian Institute of Integrative Medicine, Annamalai University, and the National Institute for Interdisciplinary Science and Technology also demonstrated significant impact through their research, with high average citations per document 33, 31, and 21 respectively. The findings indicated that while the number of publications mattered, the distinct impacts per document, as measured by citations, revealed the varied contributions and importance of dissemination strategies for research findings in the domain of *B. diffusa*.

Table 3 Top organizations' contributions to *B. diffusa* research

Rank	Organization	Country	Documents	Citations	Average citations per document
1	Universidade Federal Rural Do Semi-Árido	Brazil	8	33	4.1
2	King Saud University	Saudi Arabia	5	35	7
3	UniversitatPolitècnica De València	Spain	5	32	6.4
4	University of Cape Coast	Ghana	5	60	12
5	Jamia Hamdard	India	4	206	51.5
6	King Khalid University	Saudi Arabia	4	24	6
7	Panjab University	India	4	2	0.5
8	Indian Institute of Integrative Medicine	India	3	99	33
9	Annamalai University	India	3	93	31
10	National Institute for Interdisciplinary Science and Technology	India	3	63	21

Top Field of Study

In the study, traditional medicine stood out as the most frequently mentioned field, appearing in 188 documents, closely followed by biology, which had 187 documents. Particularly, the mention of "Boerhavia" in 144 documents underscored its significance within this context, likely indicating a specialized area or focus within the broader landscape of study (Fig. 5). The prominence of medicine (126), chemistry (109), medicinal plants (65), and pharmacology (63) signified their substantial roles in this domain. Botany, phytochemical, and antioxidant, with 61, 51, and 38 document mentions respectively, reflected a specific emphasis on plant-based research and chemical compounds, suggesting an intersection between disciplines such as botany, chemistry, and medicine. The co-occurrence of terms across these fields indicated potential interdisciplinary connections, particularly in the exploration of medicinal properties of plants (medicinal plants, phytochemical, antioxidant) within biology, botany, chemistry, pharmacology, and traditional medicine.

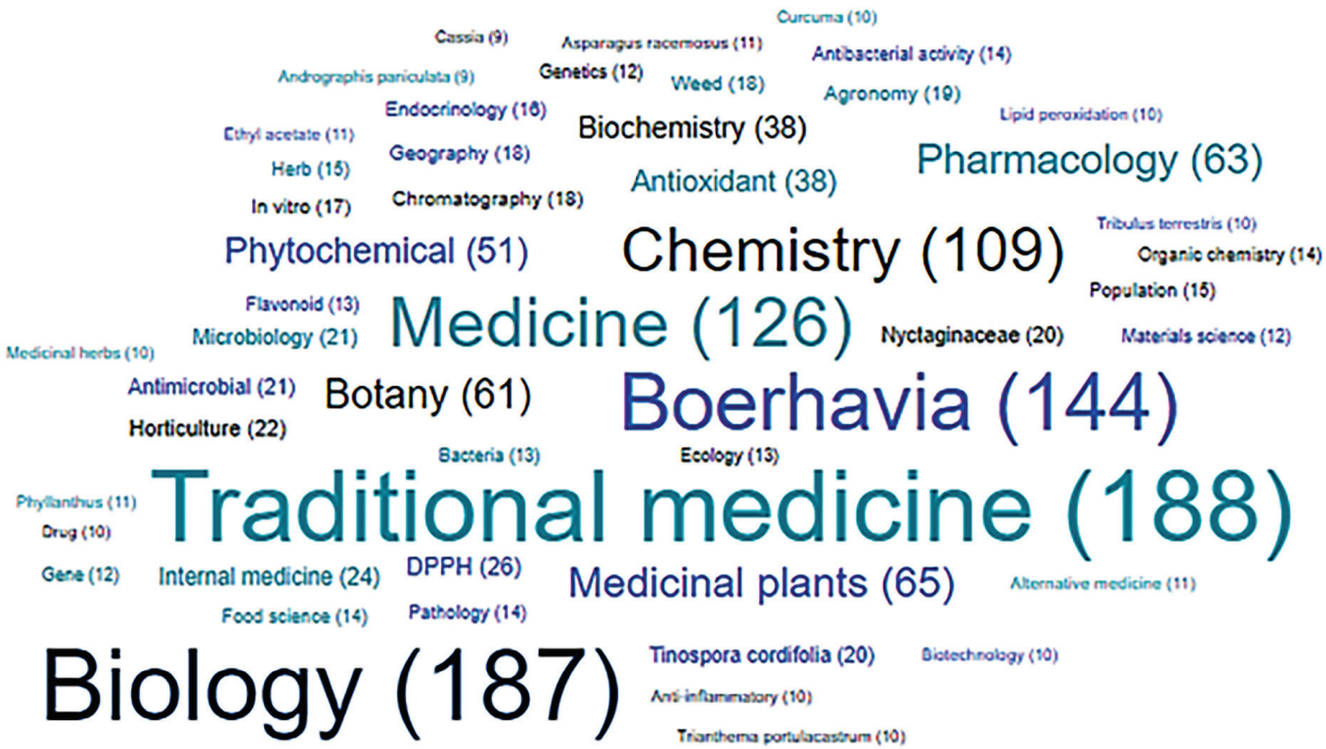

Fig. 5 Analysis of term co-occurrences in leading fields of study

Collaboration Network Analysis

Authors Collaboration Assessment

The study conducted an extensive examination involving 959 authors, uncovering a significant presence of researchers within the subject domain. Co-authorship was used as a metric to assess the similarity between authors based on shared citations. The network consisted of authors who contributed at least one document on *B. diffusa* and met a minimum citation threshold of 1 citation per document. From this analysis, 6 distinct clusters emerged, representing interconnected groups of collaborating authors. Cluster 1 emerged as the largest, comprising 6 authors, while clusters 2 and 3 consisted of 5 authors each (Fig. 6). In the assessment of author collaboration, cluster 4 comprised 4 authors, while clusters 5 and 6 consisted of 3 and 2 authors, respectively. Andréia Mitsa Paiva Negreiros emerged as the most prominent author, exhibiting the highest total link strength of 26. Following closely were Josep Armengol and Rui Sales Júnior, with respective total link strengths of 21 and 18. These findings highlight a diverse research community characterized by varying degrees of specialization and collaborative efforts.

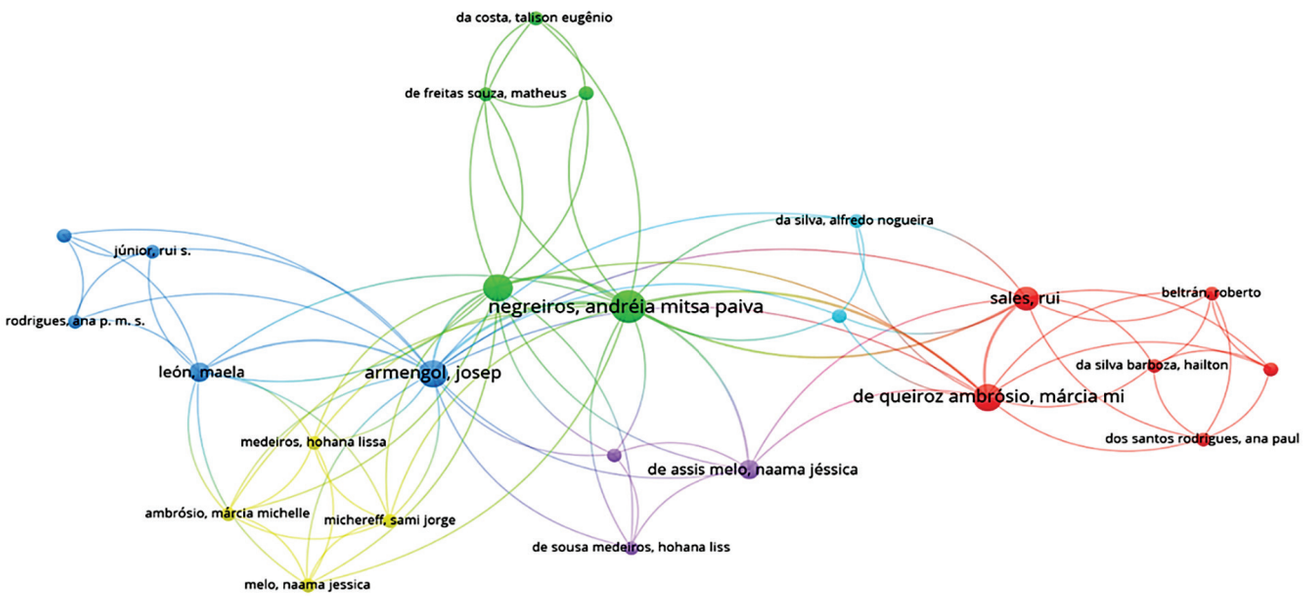

Fig. 6 Collaborative network among the authors on *B. diffusa*

Journal-wise Collaboration Network

In this investigation, a journal-wise network analysis was utilized to scrutinize collaborative dynamics among 199 journals involved in *B. diffusa* research. Seven interlinks were discerned by mapping connections through bibliographic coupling, unveiling shared areas of interest and potential opportunities for collaborative members. The network encompassed journals that had contributed at least three documents on *B. diffusa* and had met a minimum citation threshold of three citations per document. Cluster 1 comprised four journals, whereas Cluster 2 encompassed three journals, each demonstrating distinctive thematic concentrations within the domain (Fig. 7). Molecules as a journal exhibited robust connection strengths (32), positioning itself as a pivotal hub for fostering cooperative initiatives. Furthermore, Advances in Traditional Medicine achieved a total link strength of 31, while Pharmaceutical Biology Journal attained a total link strength of 28 with other journals.

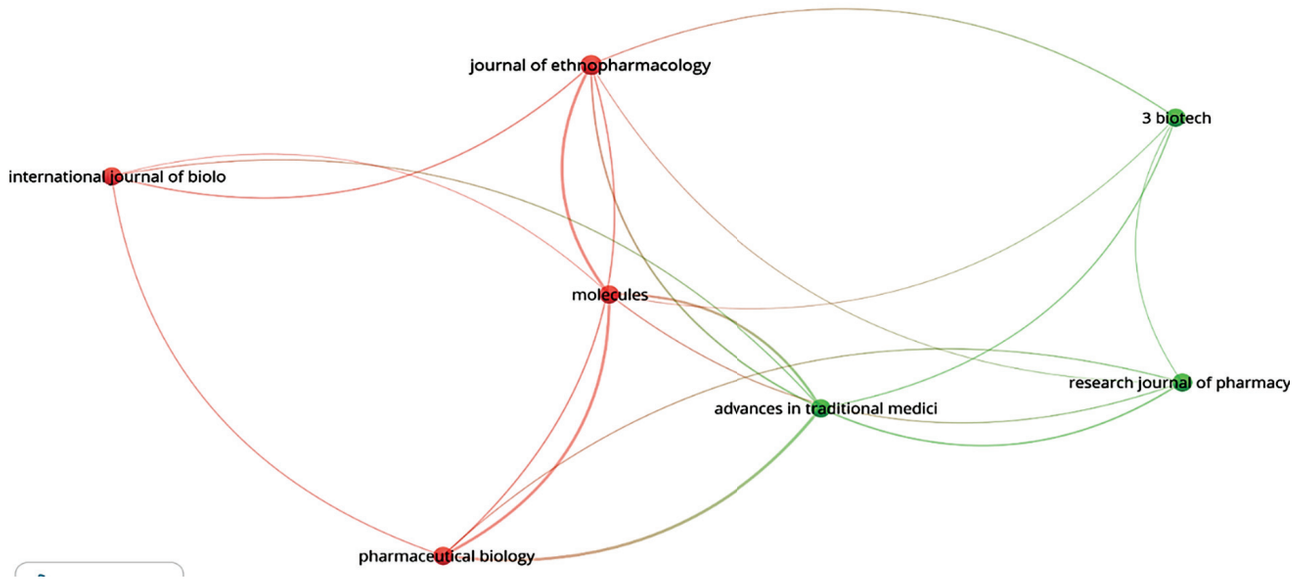

Fig. 7 Journal-wise collaboration network analysis on *B. diffusa* research

Organizational Collaboration Network

In this study, bibliographic-coupling analysis was used to examine the network of organizational collaboration in the *B. diffusa* study. Within a pool of 192 organizations, connections among 13 were unveiled, based on meeting specific criteria of having a minimum of three documents on *B. diffusa* and meeting a citation threshold of at least 3 citations per document. There are 3 clusters in the network of organizational collaboration

(Fig. 8). Cluster 1 and 2 were the largest, consisting of 5 organizations, and had a high level of inter-organizational interaction. Cluster 3 is composed of three organizations that are closely related to each other. Universidade Federal Rural Do Semi-Árido and UniversitatPolitècnica De València showed the highest total link strength of 254 for each. The King Khalid University also showed significant dominance within this collaborative network, leading the field with an overall connection strength of 249. This understanding can serve as a catalyst for fostering meaningful partnerships and collaborations, thereby advancing the research landscape concerning *B. diffusa*. Such collaborations hold the promise of significant contributions to the field, benefiting scientific advancements and enriching the collective knowledge base.

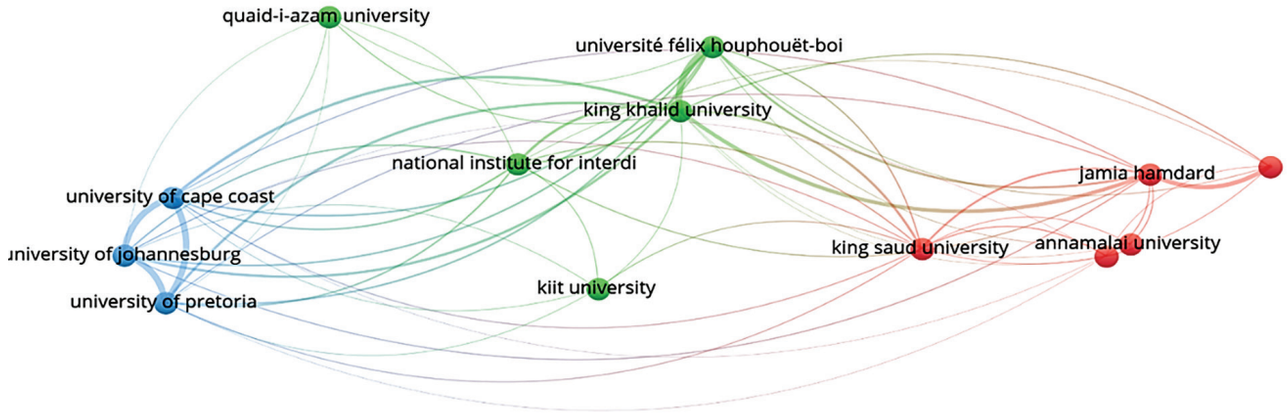

Fig. 8 Clusters of collaborative institutions for *B. diffusa* research

Country-wise Collaboration Network

The current study analyzed 39 countries to identify 16 connections within country-wise collaborative networks. The analysis employed specific criteria where each country needed a minimum of three documents on *B. diffusa* and had to meet a citation threshold of at least 3 citations per document. Using co-authorship as a type of analysis, the study delineated 4 distinct clusters signifying varying levels of collaboration and research focus. Clusters 1, 2, and 3 had 4 countries each within their collaborative network, while cluster 4 consisted of only 2 countries in its collaborative network. Saudi Arabia topped the list with a total connection strength of 16, followed by India with a total connection strength of 13. Moreover, South Africa and South Korea showed a total connection strength of 7 for each (Fig. 9). The study exhibited the diversity and interdependence of global research on species, providing significant insights into the global research arena.

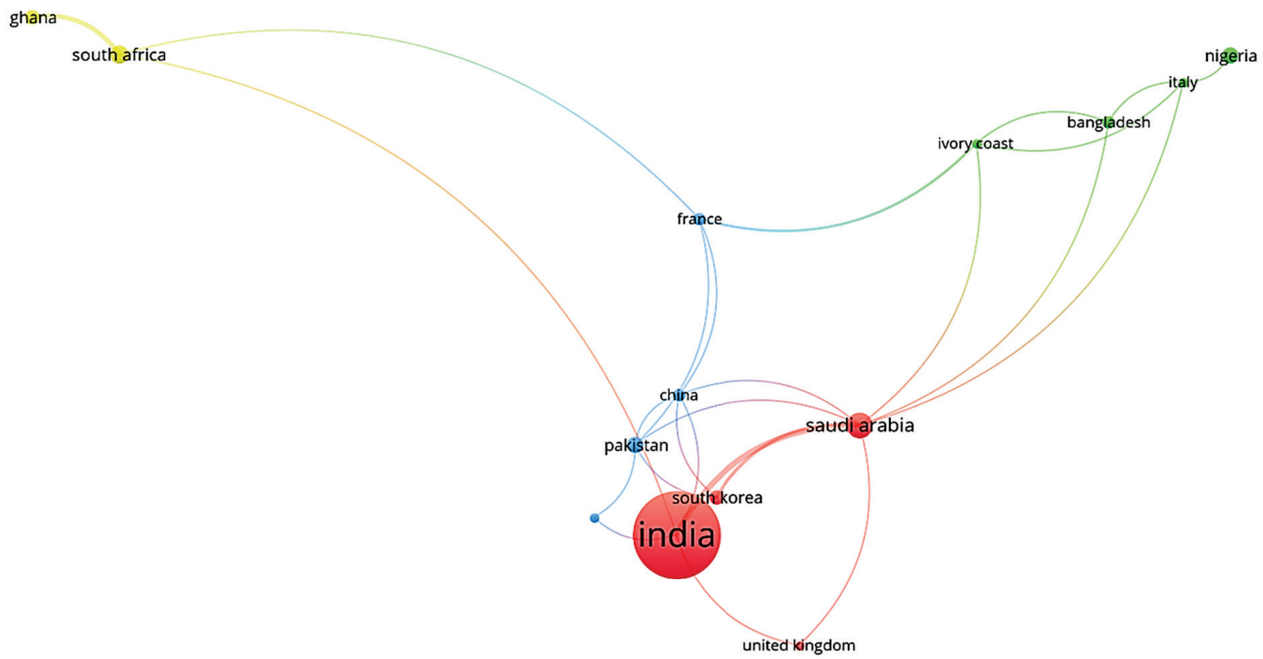

Fig. 9 Country-wise collaboration network in *B. diffusa* research

Conclusion

This study delved into various aspects of bibliometric analysis on *B. diffusa* research, focusing on publishing growth, citations, and national publications.According to the findings, *B. diffusa* showed a significant increase in published articles in 2021, with the peak citations reaching 225 in 2012. India led in active contributions with 76 publications, while Universidade Federal Rural Do Semi-Árido displayed high productivity. The Journal of Pharmaceutical Research International was the most active journal with the highest published articles of 10. Andréia Mitsa Paiva Negreiros authored the highest number of articles (6), and Mishra et al.'s (2014) research article was found remarkably highly cited. These findings highlighted evolving research trends and influential contributions in the *Boerhavia diffusa* research domain. Investigations into collaboration networks among journals, nations, authors, and organizations offered insights into clustering tendencies and collaborative dynamics within the research landscape. The study covered publishing patterns, influential works, active authors, and collaborative networks. Despite the increasing research growth, *B. diffusa* citations have declined. Leading organizations and authors maintain significant impact, while collaborative networks demonstrate complex dynamics and varying levels of specialization. This comprehensive information has the potential to advance *B. diffusa* research, informing researchers, decision-makers,

and stakeholders about the field's current state and identifying promising collaborative opportunities. It encourages further exploration, interdisciplinary collaborations, and improvements in this research domain.

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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.

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 on 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. 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. They showed that total phenolic and flavonoid content increased because of Rhizobium inoculation in soil as compared to inoculated 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 (PGPR) 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 understand plant physiology and the best possible conditions for the cultivation of medicinal plants. In the current study, we have attempted to study 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 soils) 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
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 site 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%

Soil 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

Soil 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. The majority of 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 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 of (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 litres 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

$$\frac{X-Y}{2} \times 0.003 \times \frac{100}{W}$$

Interpretation

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

Available Nitrogen

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

Chemicals and reagents required

1. Potassium permanganate solution ($KMnO_4$): 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 (Merwin and Peech, 1951; Black, 1965; Jackson, 1973).

Chemicals and reagents required

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

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 one 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 50 ml volumetric flasks and volume make up to the mark.
6. Samples were vortexed properly and run of ICP-MS against the linearity.

Calculations

Calculate the concentration of the elements as follows.

$$\text{Sample Conc.} = \frac{(\text{Sample reading - reagent blank reading}) \times \text{dilution factor}}{\text{Sample Wt.}}$$

Micronutrient in Soil

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 0.01M $CaCl_2.2H_2O$ 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 25°C.
4. The contents were then filtered through Whatman No. 42 filter paper to obtain a clear solution

5. Also, a blank was run with only DTPA solution without the soil.

Calculations

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

1. Different growth medium is used to grow different types of microorganisms is given in Table 3.
2. The medium is sterilized in an autoclave.
3. Petri dishes are used to hold the growth media.
4. A small number of bacteria is needed to inoculate the growth media.
5. 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.
6. The inoculated growth media is incubated at the optimal temperature and conditions for the bacteria to grow.
7. 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

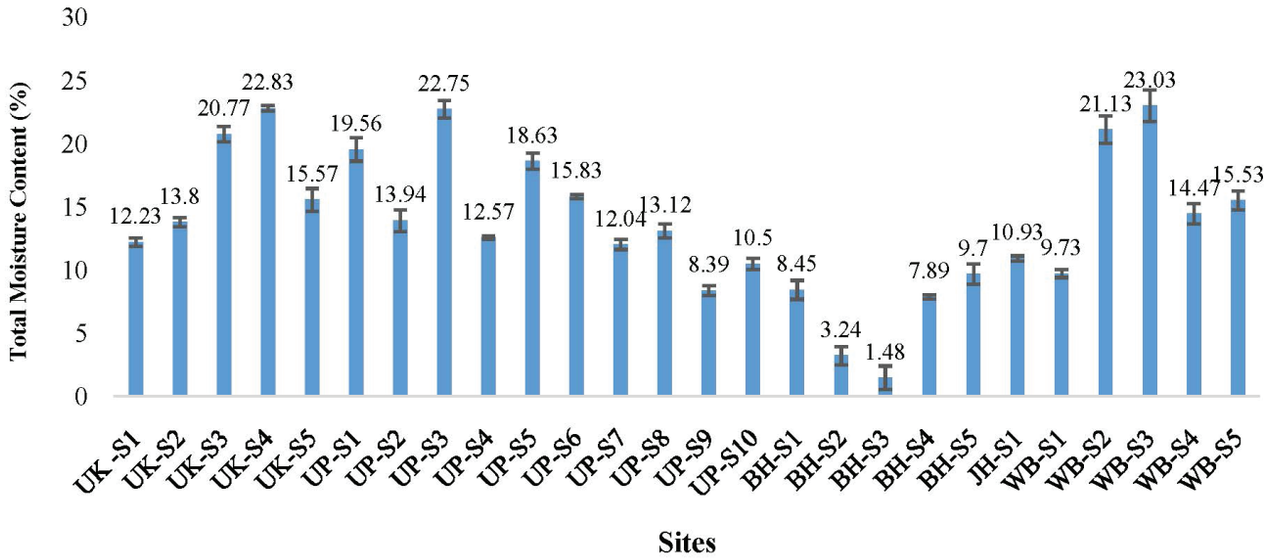


Fig. 1 Total moisture content of different sampling sites

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. Soil bulk density 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

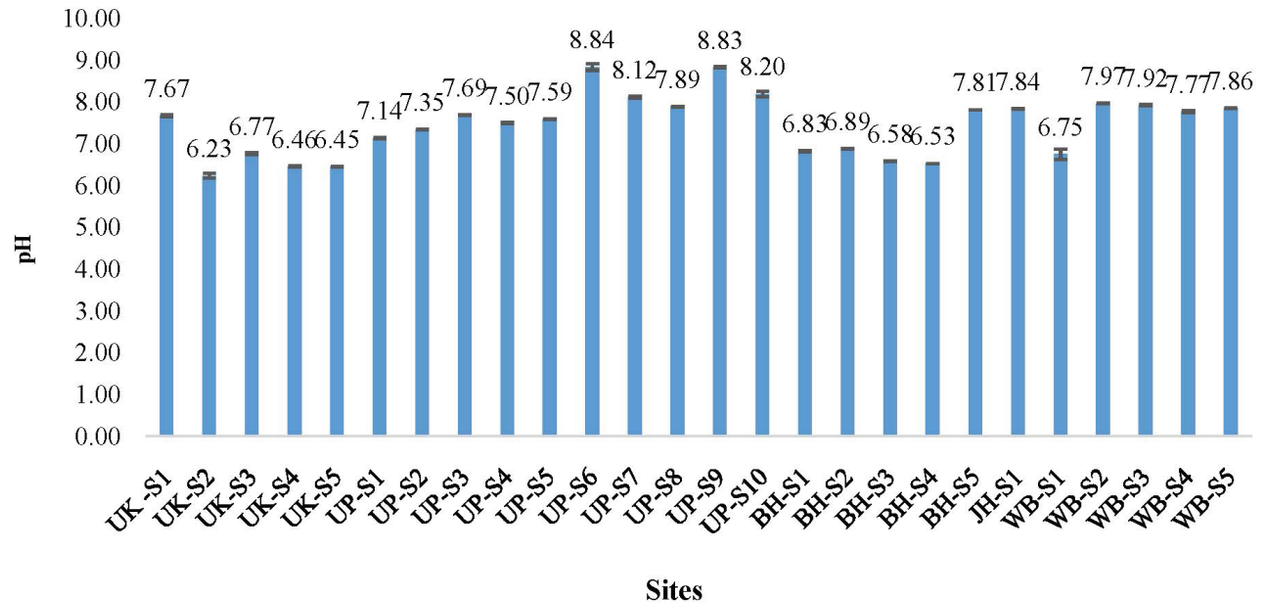


Fig. 3 Soil pH of different sampling sites

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

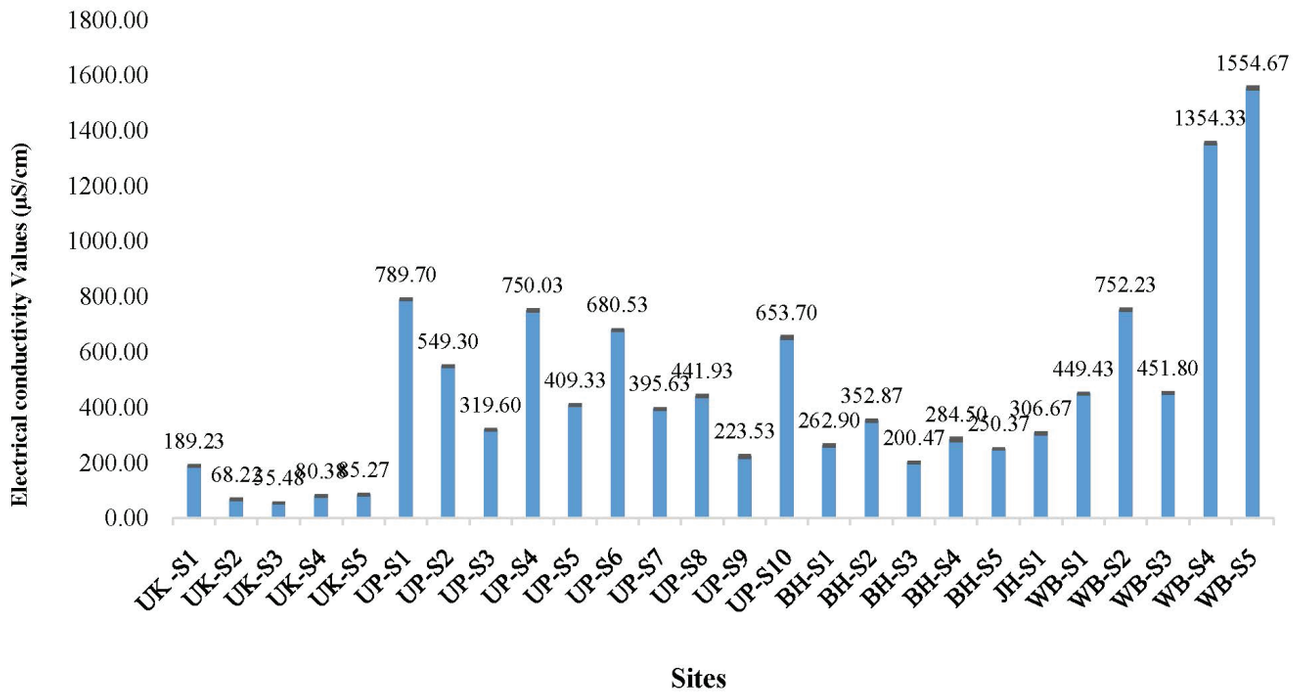


Fig. 4 Soil electrical conductivity at different sampling sites

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

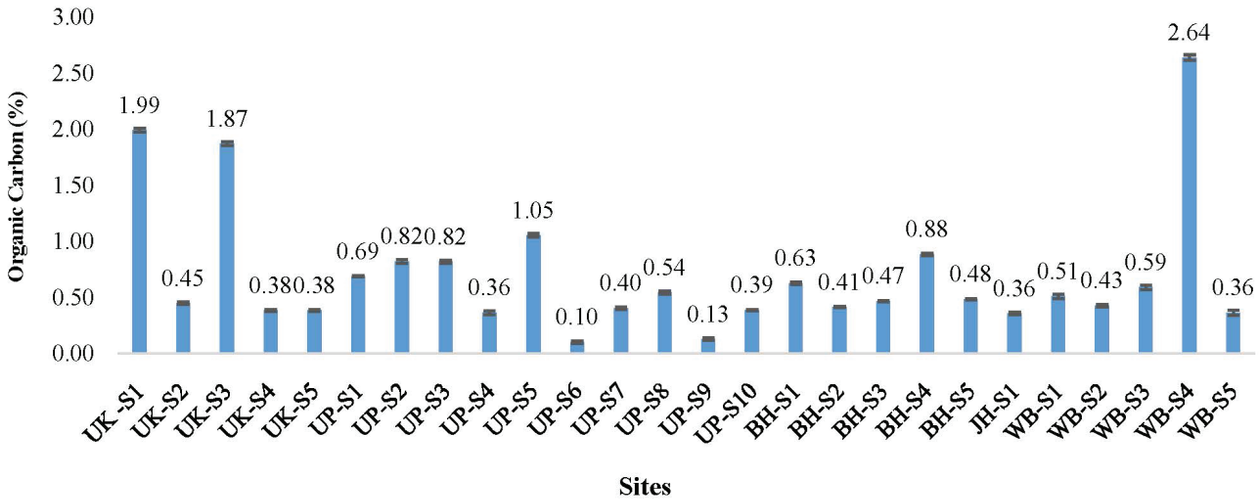


Fig. 5 Soil organic carbon content at different sites

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.

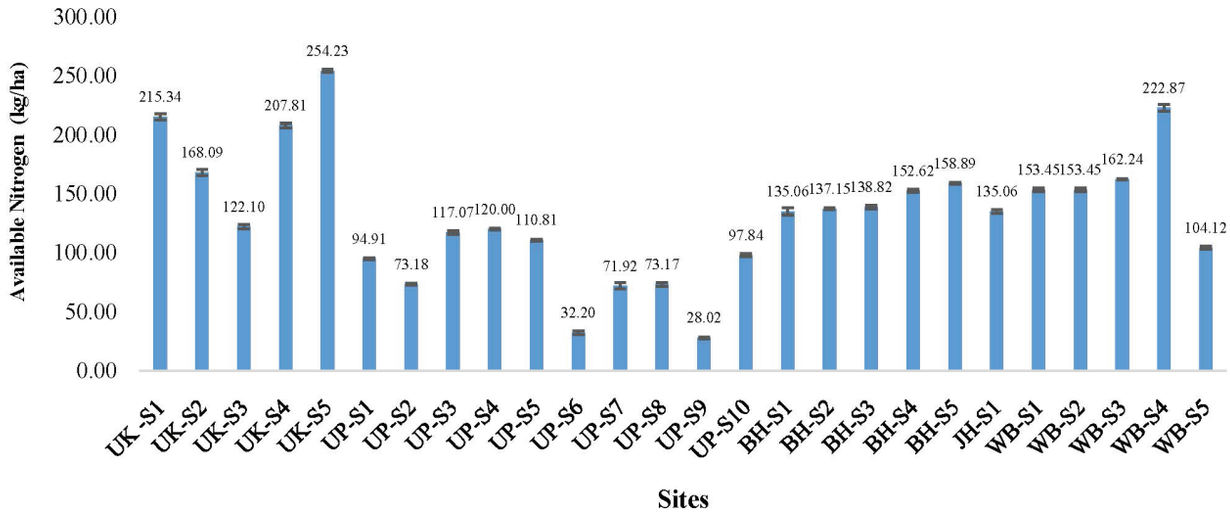


Fig. 6 Soil available nitrogen content at different sites

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

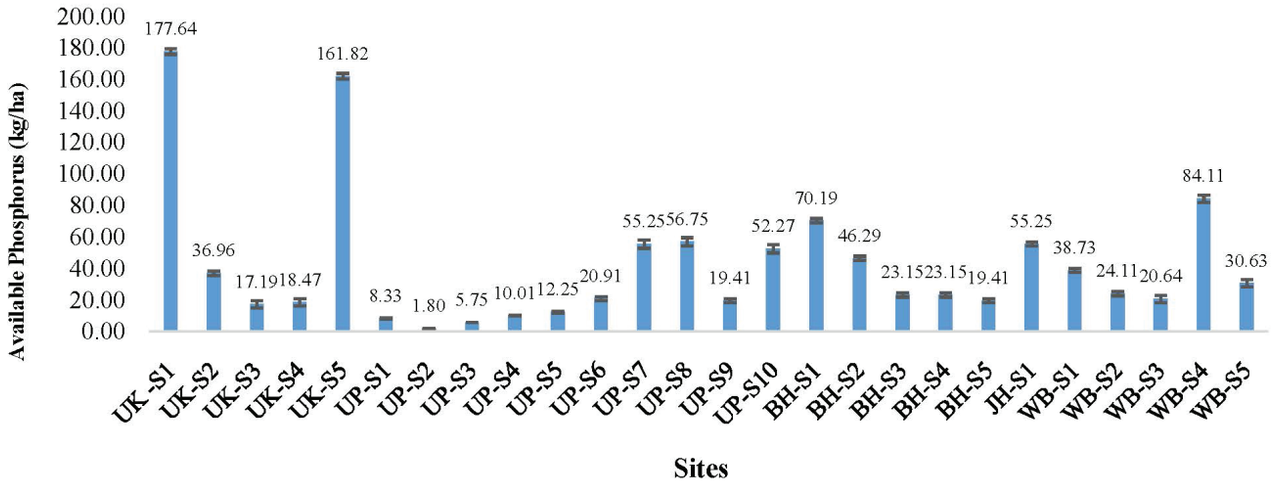


Fig. 7 Soil available phosphorus content at different sites.

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. 4 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.

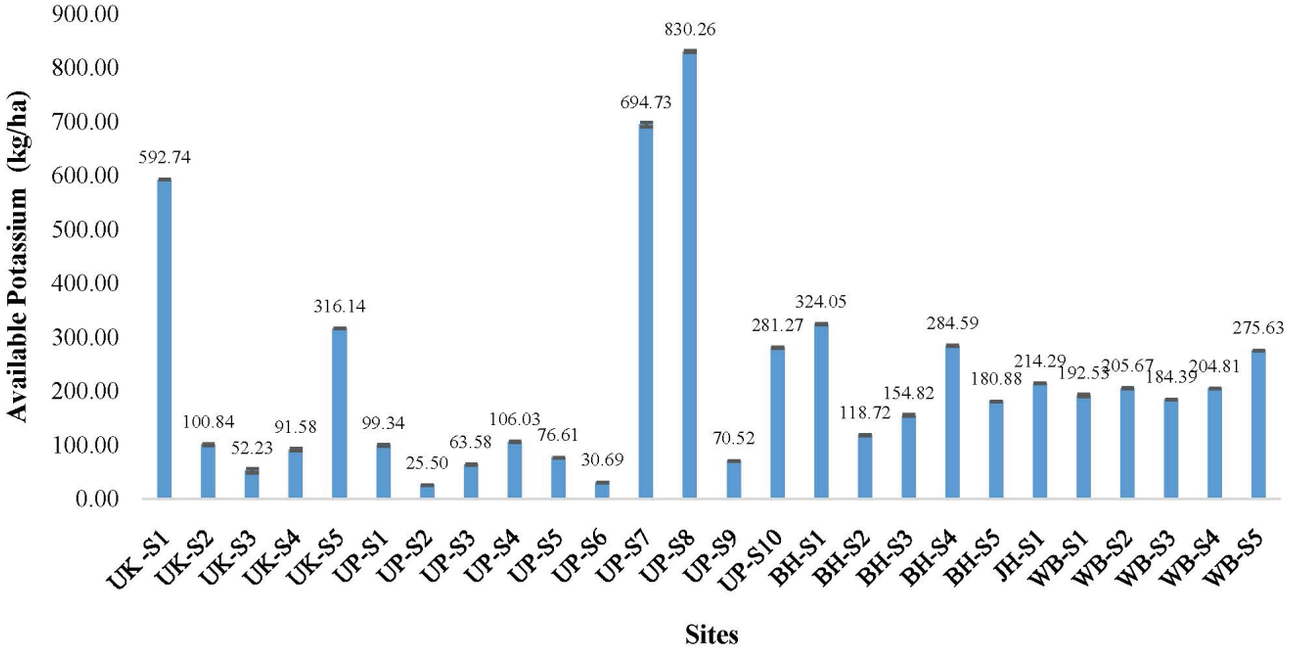


Fig. 8 Soil available potassium content at different sites

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 soils of the 10 sites is shown in Fig. 9.

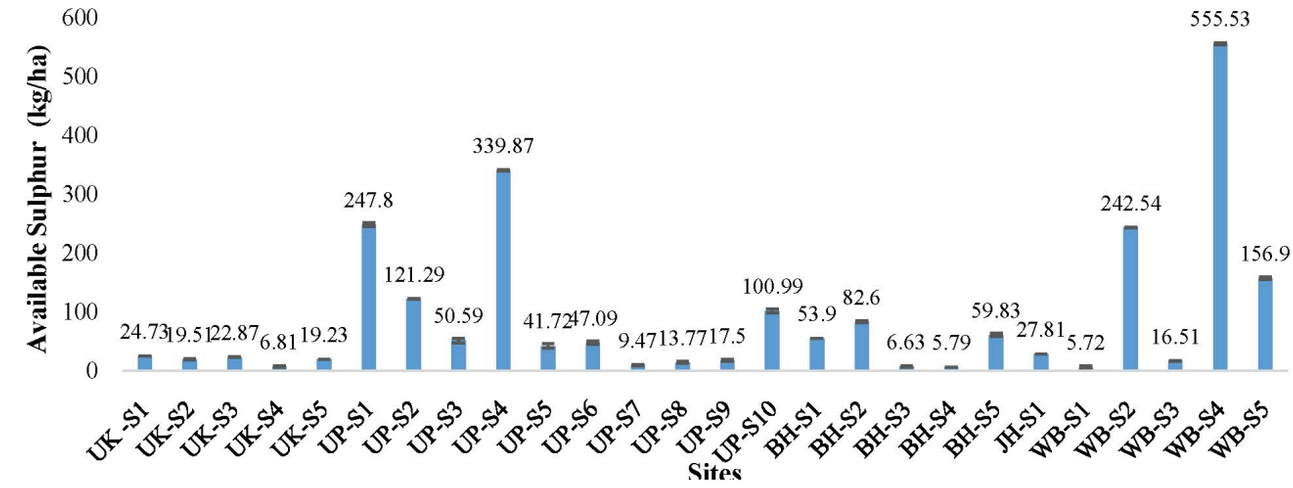


Fig. 9 Soil available sulphur content at different sites

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 seven 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.

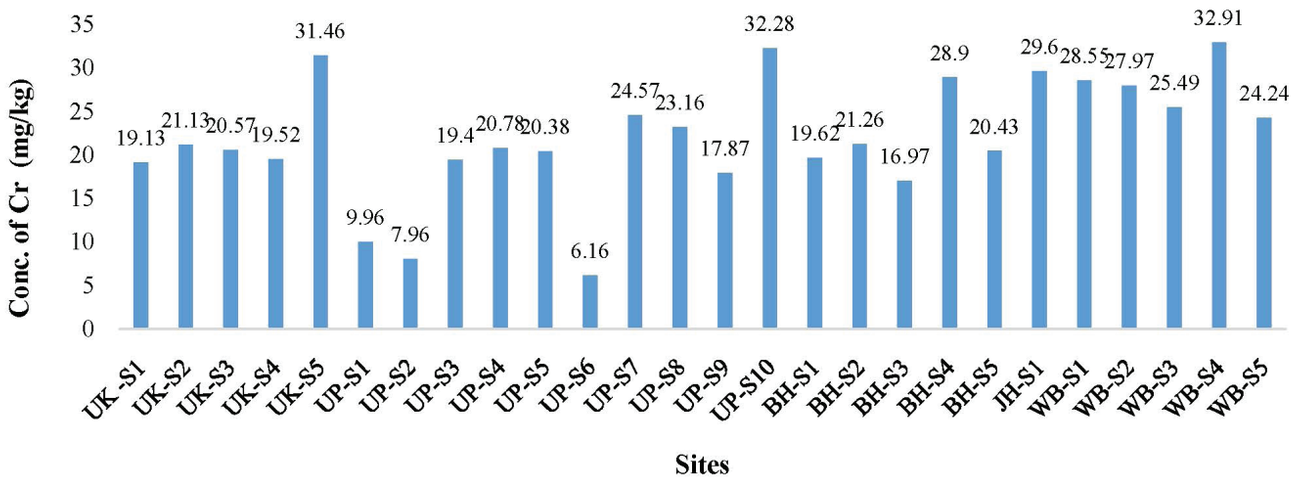


Fig. 10a Concentration of chromium at different sites

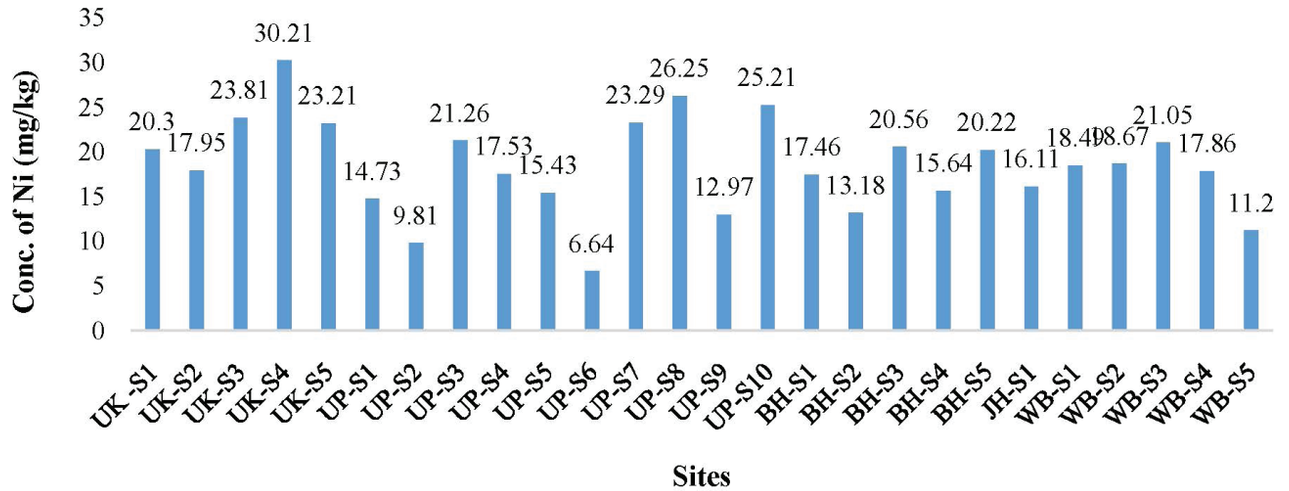


Fig. 10b Concentration of nickel at different sites

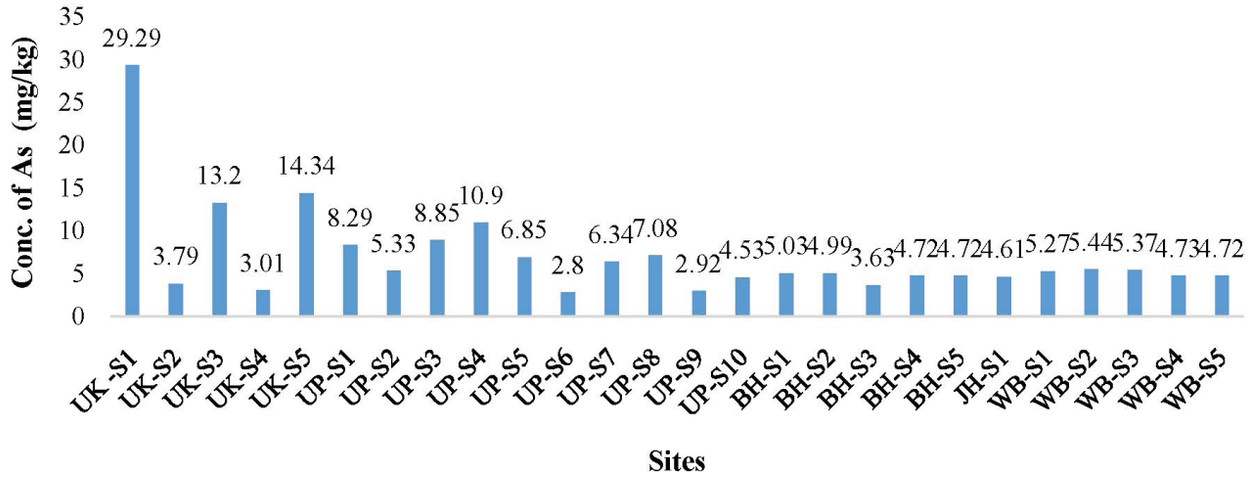


Fig. 10c Concentration of arsenic at different sites

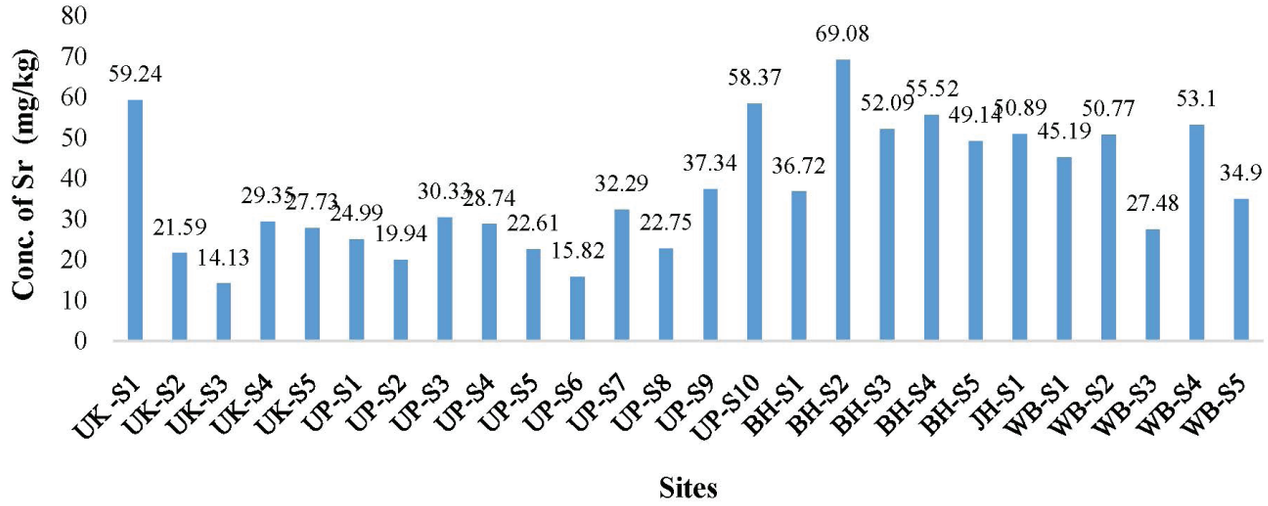


Fig. 10d Concentration of strontium at different sites

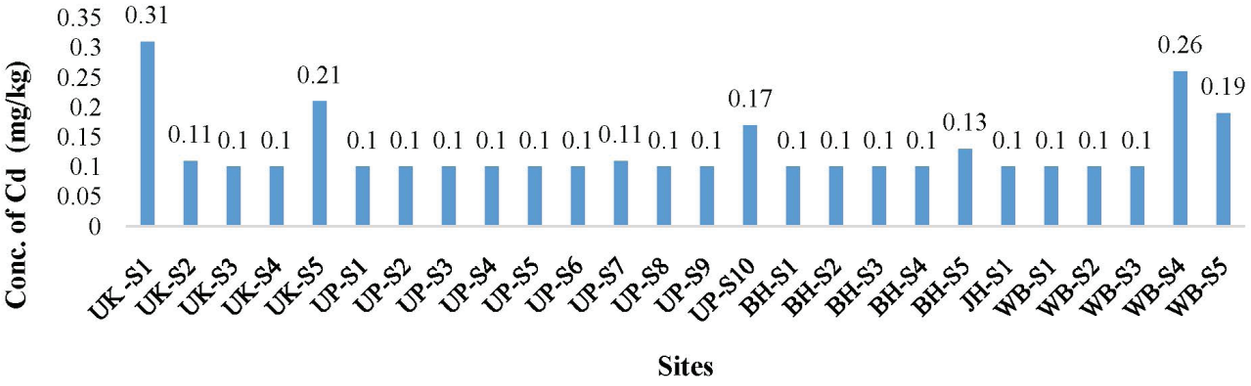


Fig. 10e Concentration of cadmium at different sites

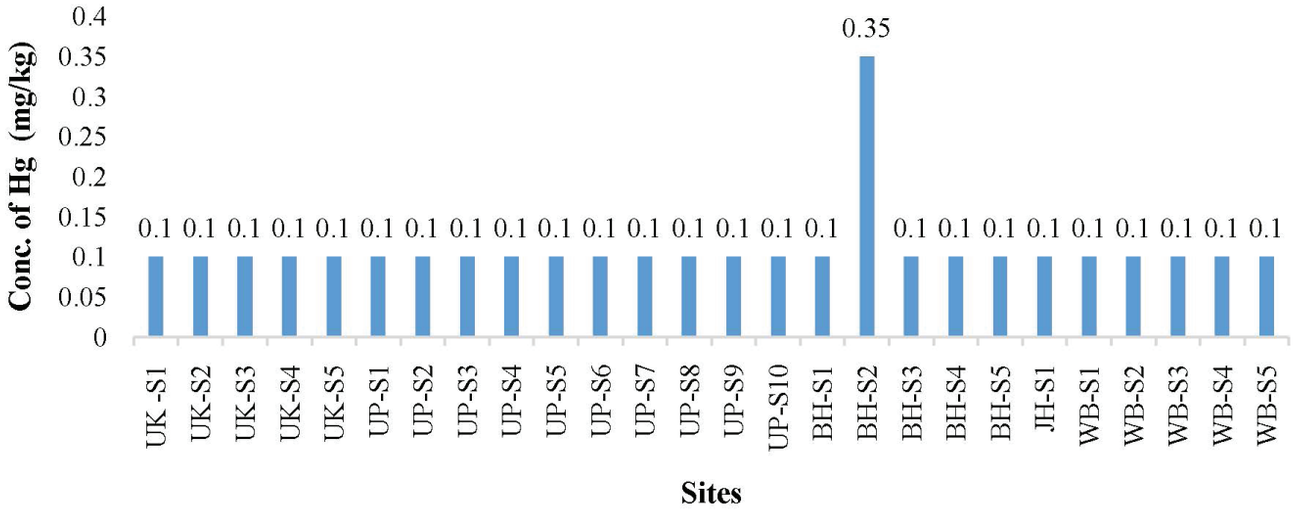


Fig. 10f Concentration of mercury at different sites

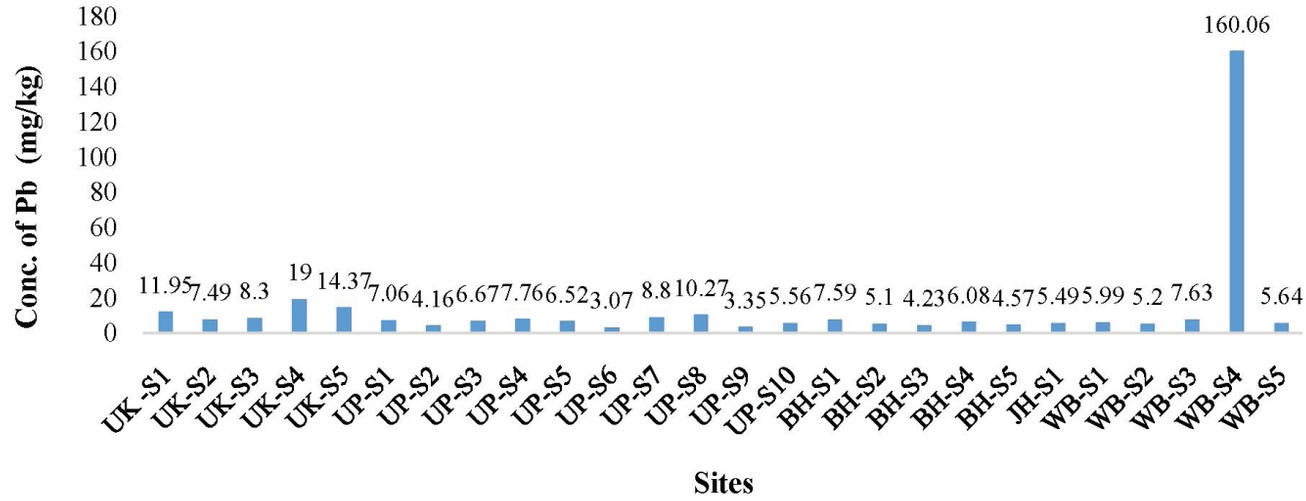


Fig. 10g Concentration of lead at different sites

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 10 sites are represented in Fig. 11 a, b, c and d.

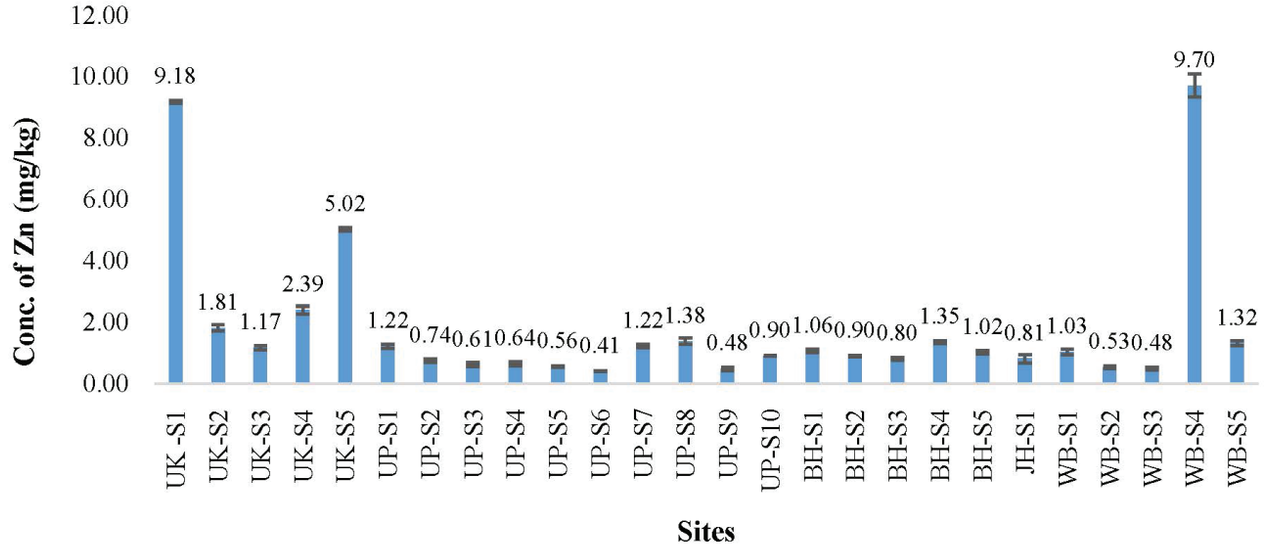


Fig. 11a Concentration of zinc at different sites

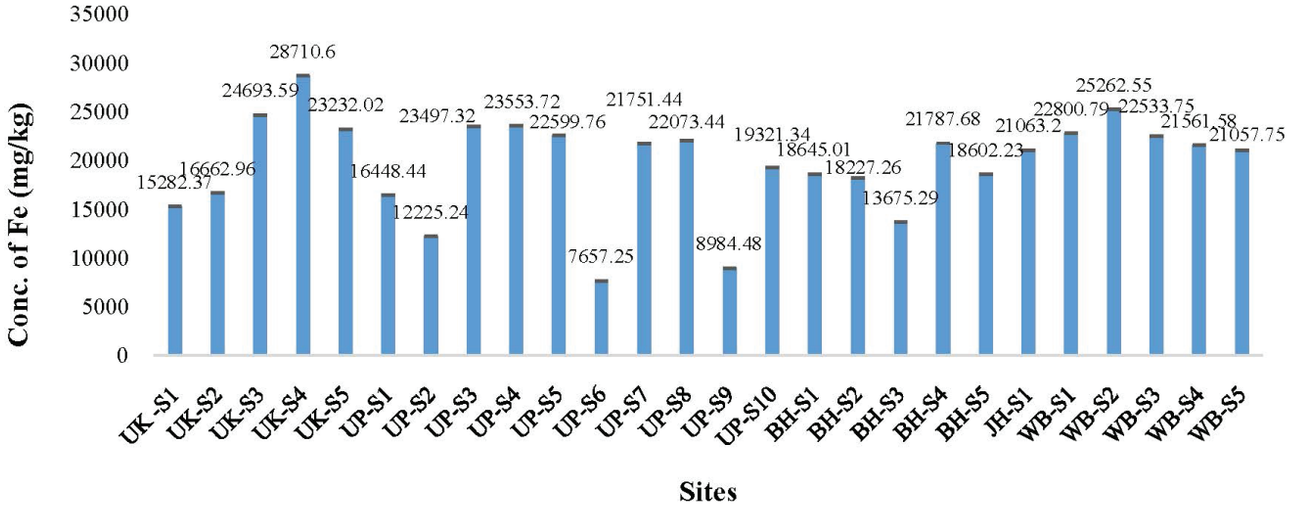


Fig. 11b Concentration of iron at different sites

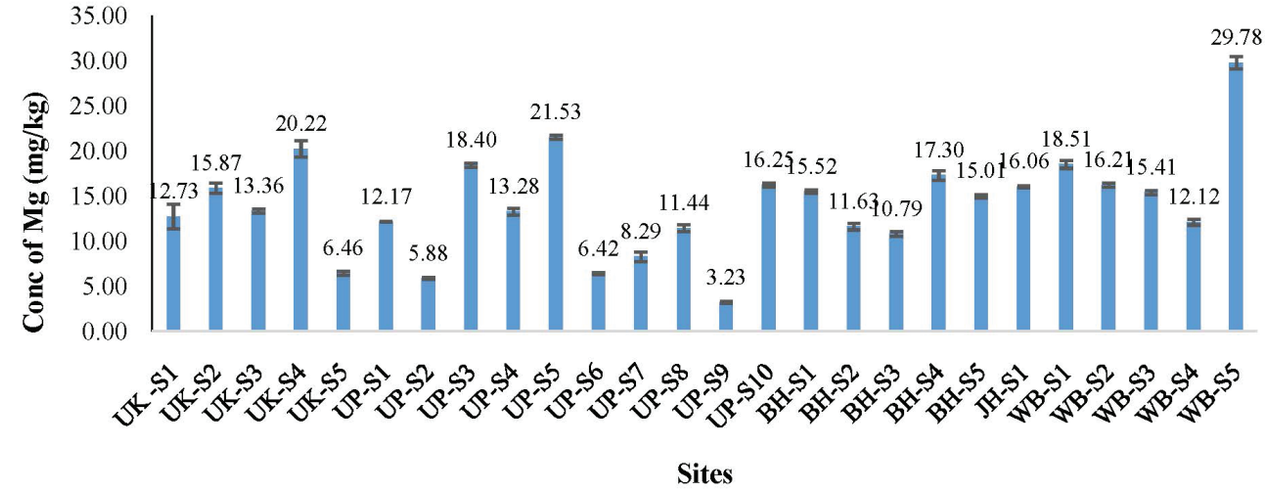


Fig. 11c Concentration of manganese at different sites

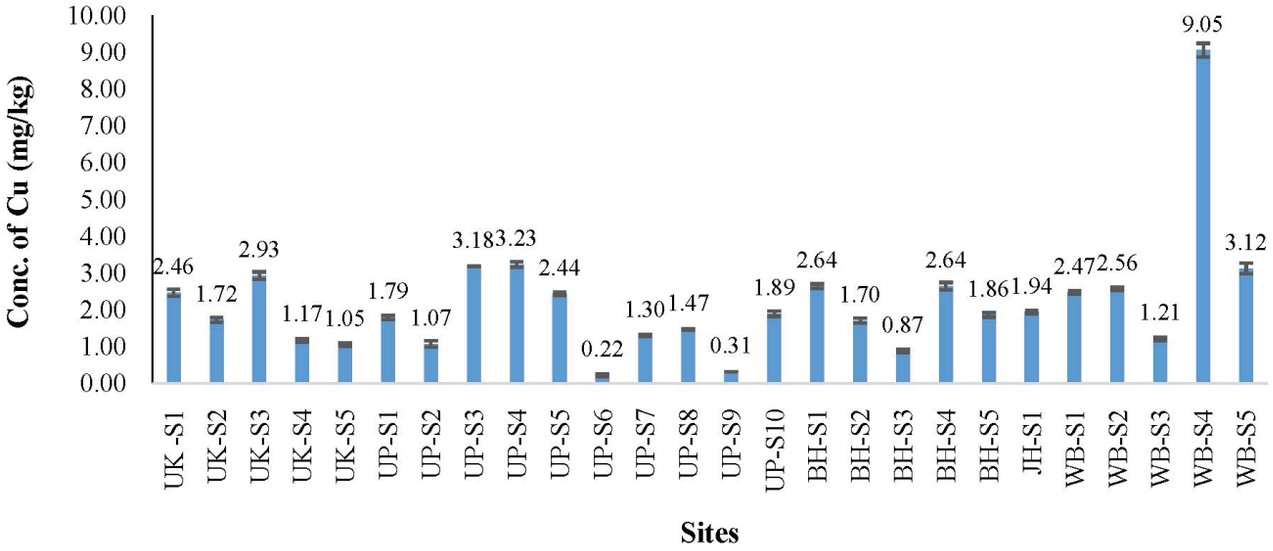


Fig. 11d Concentration of copper at different sites

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. The Table 5 shows the status of soil at the different sites with respect to the micronutrient concentration.

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 *Boerhavia diffusa* L.in different locations shown in Fig. 12

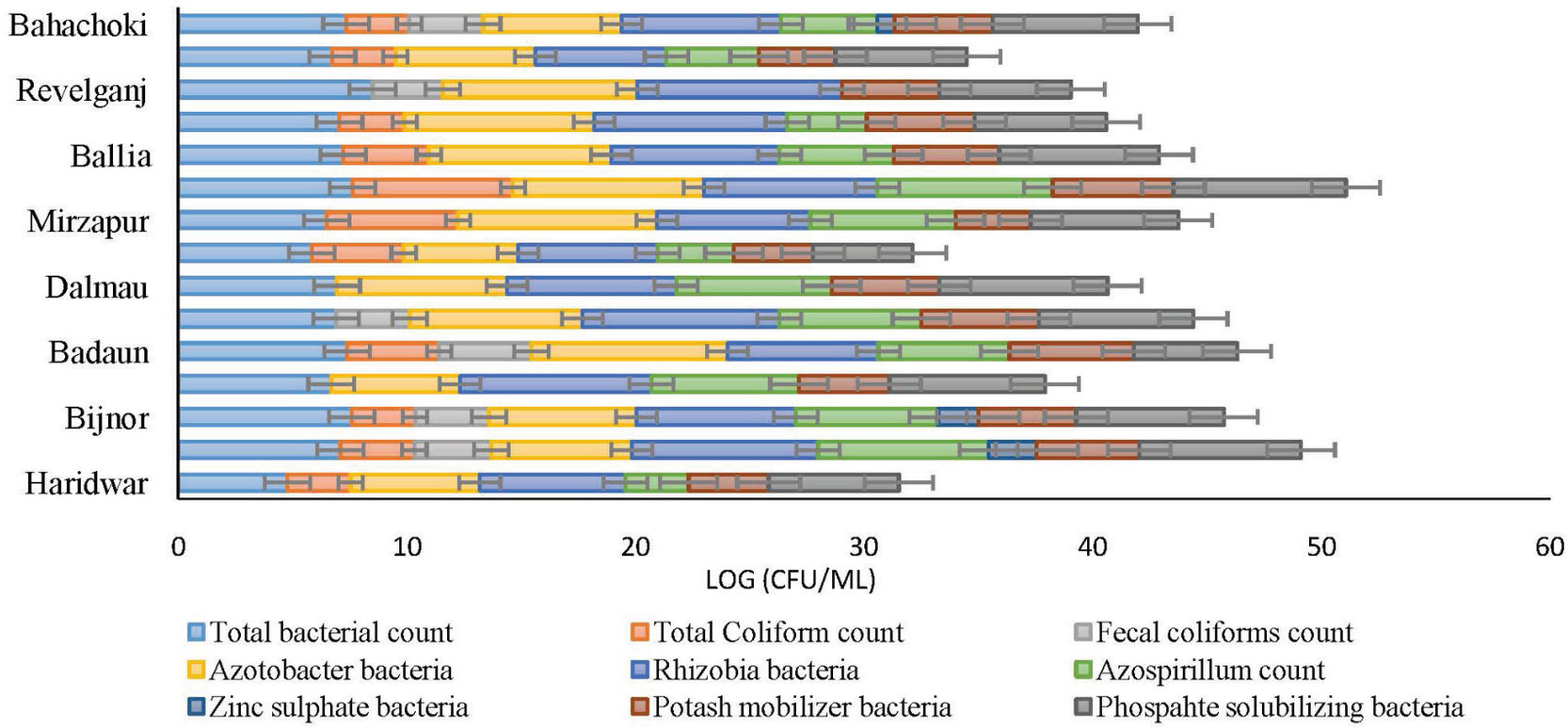


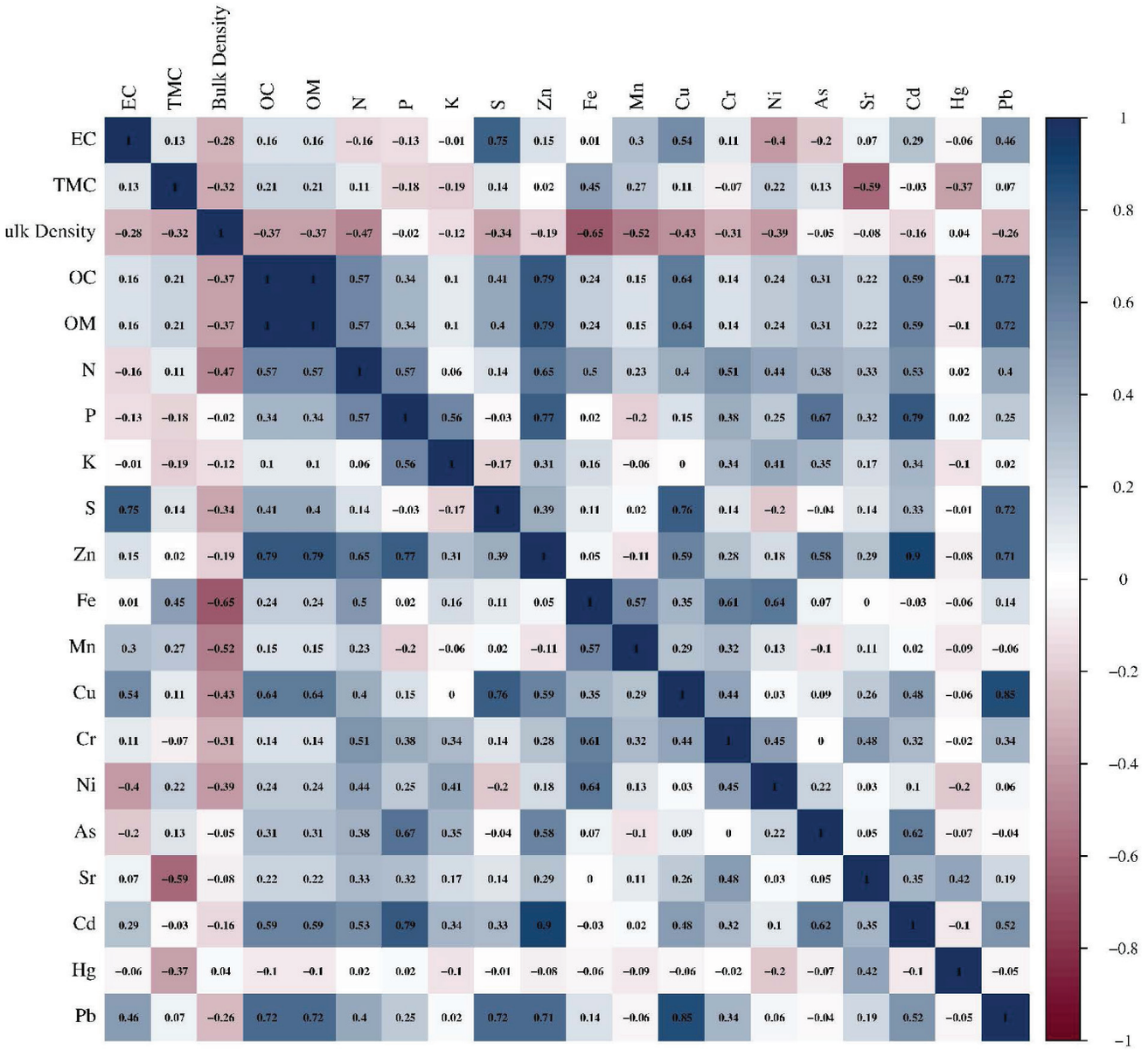
Fig. 12 Total and differential bacterial count

The total rhizosphere microbial test is performed for the identification of total bacterial count which is found highest in Revelganj 8.5022 cfu/ml and lowest in Haridwar is 4.7839 cfu/ml. Whereas the total faecal and coliform count is highest in Badaun and Varanasi is 4.0246 and 7.0166 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 4 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 colouring 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	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	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	AR	AR	BR	WR	WR	WR	WR	WR	WR	WR	WR	WR	WR	WR
UP-S8	AB	WR	WR	BR	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	AR	WR	WR	WR	WR	WR	WR	WR	WR	WR	WR	WR	WR	WR
BH-S1	WR	WR	WR	BR	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

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

In the above table the following abbreviation is used represents the following indicative statement: CSSC (Critical for Salt-Sensitive Crops); WR (Within Range); AR (Above Range); BR (Below Range) the recommended range of the parameters has been adopted fromKatyal 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

The herbs play a crucial role in our daily lives. When medications were first introduced, they were the most efficient source (Ekor et al., 2014). With fewer adverse effects than synthetic pharmaceuticals, herbs are as important to contemporary medicine today (Sen and Chakraborty, 2019). The extraction and development of numerous medications and chemotherapeutics from these plants, in addition to conventionally used rural herbal remedies, led to the developing belief in using medicinal plants in developed nations (Kim et al., 2019). Different kinds of herbs can be used to prevent illnesses affecting different organs in humans and animals (Chaughule and Barve., 2023). Native species were discovered to have qualities employed to create customized medications to save lives. Many important herbal medicines have been created based on traditional folk healers for the treatment of illnesses. Furthermore, plants are the first line of treatment for several diseases (Bent et al., 2004). The tribal societies still rely primarily on plants for their medical needs; as a result, they use them to treat various conditions. It is also a component of their beliefs and rituals, thus they have maintained a treasure of traditional knowledge and successfully transferred it generation after generation (Patil and Patil, 2012).

The extremely polymorphic genus *Boerhavia* Vaill. ex L., also known as *Boerhaavia*, belongs to the Nyctaginaceae family. This family is sometimes known as the "four-o'clock family" since most of its species open their flowers four hours after noon or in the early evening or morning (Fosberg et al., 1978; Levin et al., 2001). *B. diffusa* (*Punarnava*) was given its name in the 18th century in honor of Dutch physician Hermann Boerhaave (Banjare et al., 2012). The genus *Boerhavia* contains a variable number (20-40) of subtropical or pantropical herb species (synonyms and homonyms) because of taxonomic conflict and ideas (Fosberg et al., 1978; Douglas and Manos et al., 2007). It thrives in a variety of terrestrial environments, including vast forest gaps, managed grasslands, wastelands, and agroecosystems. In tropical and subtropical areas like India, Brazil, Africa, Australia, China, Egypt, Pakistan, Sudan, Sri Lanka, the United States, Iran, and numerous Middle Eastern nations, the plants of this genus also thrive as weeds. Six of these *Boerhavia*

species, including *B. diffusa*, *B. chinensis*, *B. erecta*, *B. repens*, *B. rependa*, and *B. rubicunda*, are said to exist in India (Thakur et al., 1989). *Boerhavia diffusa* L., a herbaceous member of the family Nyctaginaceae, is widely dispersed throughout the tropics and subtropics (Dhar et al., 1978). Out of the 180 compounds from the *Boerhavia* genus that were isolated, *B. diffusa* shared about 131 of them alone, and it is now the only source for most of them. Additionally, numerous pharmacological actions of *B. diffusa* have been described, and information about *B. diffusa* has been included in certain publications (Prathapan et al., 2011; Patil and Bhalsing, 2016; Gaur et al., 2022). It is a perennial creeping herb and is also referred to as "Punarnava" in Indian medicine. Its roots are administered to treat anasarca, ascites, and jaundice because they are believed to be diuretic and laxative (Rawat et al., 1997). Numerous substances, including flavonoids, alkaloids, steroids, punarnavine, triterpenoids, lipids, lignins, carbohydrates, proteins, and glycoproteins are also extracted from this species (Surange et al., 1972). The herb contains 15 amino acids, where 6 are important ones while there are 14 in the roots, where 7 are essential ones (Chopra et al., 1923). In various regions of India, the plant's leaves are frequently consumed as a green vegetable. They are typically employed to lessen discomfort as well as to safeguard and enhance vision. Further, its leaves and roots are used to treat a variety of illnesses, including asthma, urinary disorders, rheumatism, and pain. The ancient Ayurvedic doctors had a thorough understanding of digestion and metabolism.

Traditional and Ayurvedic Benefits

Medicinal plants have been utilized to treat a wide variety of illnesses, according to the knowledge of many generations of doctors and traditional medical practices from many ethnic communities. Ayurveda, an Indian system of medicine that relies on plant resources and treats the whole person, is essential in managing and controlling a variety of medical conditions (Seth et al., 1986; Nandini and Siddamallayya, 2013). However, these functions can be damaged or destroyed by factors like food, activity, the environment, and stress. Ayurveda uses a variety of strategies, such as suggestions for diet and exercise, herbal preparations, purifying procedures (*panchakarma*), and surgical techniques (*shalya chikitsa*), to restore the body's normal physiological processes (Kumar et al., 2017). Old Indian medical texts like the 'Charaka Samhita' and 'Sushrita Samhita' mention the usage of *punarnavastaka kvath*, *punarnava kshar*, and *punarnava taila*, three Ayurvedic medicines manufactured from *punarnava*, for treating a variety of diseases. *Punarnava* is also listed in 'Sushruta Samhita' under *Vidarigandhadi Gana*, *Samshamniya Varga*, and *Tikta Varga*, as well as in compound formulations for the treatment of various ailments in the form of *Taila*, *Ghrita Nasya*, *Lepa*, etc (Chopra, 1969). There are several ayurvedic formulations in classical texts for the management of various diseases as discussed in **Table 1.**

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

S. No.	Diseases/ Conditions	Mode of Administration/Uses	References
1.	Cephalic disorders	**Punarnavāyārajasogaṣhītvānasyaṣpunaṣpāvakamṣnato nu. Niṣsaṣṣayaṣsaptabhirevaghasraiprayātiśaṣkhabhaṣkuṣīvya thā'stam-** root bark powder, useful in frontal and temporal pain	Bhatta, 2008
2.	Ophthalmic disorders	**Śvetādrikarṣyāsapunarnavāyāmūlaip-rapṣṣairyavacūrṣayuktaiṣ.Vilocanaṣpūritamambuyuktairvi-mucyatepuṣpakṣtopasargāt-**collyriumpreparedfromanequal quantity of *Clitoria ternatea* (Butterfly pea) root, *Boerhavia repens* (Creeping spiderling) root and *Hordeum vulgare* (Barley), useful against corneal opacity; **Dugdhenakaṣṣūṣkṣodreṣanetrasrāvaṣ ca sarpiṣā.Puṣpaṣtailenatimiraṣkāñjikenaniṣāndhatāg.Punarnavājayedāṣubhāskarastimiraṣyathā-** collyrium of *Clitoria ternatea* (Butterfly pea) root	Mishra, 2009a; Misra and Vaisya, 2011; Vaisya, 2012a

S. No.	Diseases/ Conditions	Mode of Administration/Uses	References
		paste, pounded with cow milk, recommended in eye itching, collyrium of *B. diffusa* root paste, mixed with honey, useful in epiphora, collyrium of root paste mixed with *Ghrita* (Clarified butter), useful in corneal opacity, collyrium of root paste, mixed with oil, used in cataract, collyrium of roots pounded with sour gruel, useful in xerophthalmia; **Ghṣtenamūlakaṣcāsyāhyaṣjitaṣhantipuṣpakam. Madhunāsahamūṣtuhyajitaṣsrāvanāśakam.Aṣjitaṣmārkavarasairnetrakaṣṣūnivāraṣam.Kevalenajalenaivahyaṣjitaṣtimirāpaham.Jalenagoṣakṣtācapippalyācāṣjitaṣyadā. Rātryāṣdhyaṣnaṣyatetenacoṣṣaṣparṣarasaṣsmṣtaṣ-** collyrium of *B. diffusa* (Hogweed) root paste, mixed with honey, useful in subconjunctival hemorrhage, collyrium of *B. diffusa* (Hogweed) root juice, mixed with *Eclipta alba* (False daisy) juice, useful against eye itching, collyrium of *B. diffusa* root juice, mixed with *Piper longum* (Long pepper) and cow milk, useful in xerophthalmia;	
3.	Abdominal disorders	**Mūṣsamaṣtaṣṣuladhāvanenaprapeṣitaṣśvetapunarnavāyāṣ. Pītaṣbhavetplīhavināśahetu.....Gastrosplenic disorders-** root powder along with rice water is useful in splenomegaly; **Harītakīnāgaradevadārupunarnavāchinnaruhākaṣāyaṣ.Saguggulurmūtrayutṣcapeyaṣśothodarāṣāṣpravaraṣprayogaṣ, Punarnavāmṣtādārupathyānāgarasādhitaṣ.Gomūtragugguluyutaṣkvāthaṣśothodarāpahaṣ-** decoction prepared from *Terminalia chebula* (Black myrobalan), *Zingiber officinale* (Dry ginger root), *Tinospora cordifolia* (Tinospora), *B. diffusa* (Hogweed), *Cedrus deodara* (Deodar) and *Berberis aristata* (Indian barberry), mixed with *Commiphora mukul* (Gum guggul) and cow urine, useful in abdominal distention and other abdominal disorders; **Kālaśākalavaṣauṣadhānvitaṣbhaktapakvamathasarpiṣānvitam.Mūlamasyatipunarnavodbhavaṣgulmatodamacireṣaniścitam. *Gulma***- vegetable prepared from *B. diffusa* (Hogweed) root, *Corchorus olitorius* (JewÕs mallow) mixed with rock salt, useful in *Gulma* and pin pricking pain; **Punarnavāvallijabāṣapuṣkhāviśvāgnipathyā**	Mishra, 2009b; Tiwari, 2007; Tripathi, 2013; Sharma, 2012; Sharma, 2013a

S. No.	Diseases/ Conditions	Mode of Administration/Uses	References
		cirabilvabilvai.Kṣtaṣkaṣāyaṣṣamayedaśeṣāndurnāmagulmagrahaṣīvikārān- decoction prepared from *B. diffusa* (Hog weed), *Piper nigrum* (Black pepper), *Tephrosia purpurea* (Wild indigo), *Zingiber officinale* (Dry ginger root), *Plumbago indica* (White leadwort), *Terminalia chebula* (Black myrobalan), *Derris indica* (Smooth leaved ponga) and *Aegle marmelos* (Bengal quince) pulp, useful in hemorrhoids, *Gulma* and sprue.	
4.	Anorectal disorders	**Parisrāveśaṣtaṣkṣīraṣsavaṣṣcīrapunarnavam.Ākhuparṣikayāvā'pitaṣṣulīyakayuktayā. *Parisrāva*-** enema of milk, processed with *B. repens* (Creeping spiderling) and *B. diffusa* (Hogweed) paste, useful in *Parisrāva* (a disorder caused due to excessive use of enema).	Śāstrī and Chaturvedi, 2011a
5.	Hepato-splenic disorders	**Punarnavātrivaṣdvyoṣaviṣaṣagadārucitrakam.Kuṣṣhaṣharidretriphalādantīcavyaṣkaliṣagakāṣ.....Tāṣpāṣṣurogānplīhānarmāṣsiviṣamajvara.ṣvayathuṣgrahaṣīdoṣaṣhanyukuṣṣhaṣkrimīṣstathā. Anemia**- *Punarnavādi Manḍūra* along with buttermilk, useful in anemia, splenomegaly, hemorrhoids, malarial fever, inflammation, sprue and worm infestations.	Śāstrī and Chaturvedi, 2011b
6.	Renal and urinary bladder disorders	**Punarnavā'mṣtābhīrusakṣāralavaṣatrayai.ṣaṣīkuṣṣhavacāmustarāsnākaṣphalapauṣkarai...Kaṣyūruvastimeṣhrasyakukṣivakṣaṣasaṣyutam.Kaphavātāmaśūlaghnamatravaddheṣcanāśanam. Calculus**- intake or enema of *Punarnavādi taila* (oil), useful in calculus, pain, dysuria, and pain caused due to *Vāta* and *Kapha*.	Misra and Vaisya, 2012b
7.	Reproductive system disorders	**Apāmārgapatradvayaṣyonimadhyeniviṣṣaṣkṣaṣādyayoniśūṣnihanti.Sudurvāramapyevamevaprayuktoviddhyādrasastatrapaunarbhavo'pi. Vaginal pain**- insertion of vaginal suppository, prepared from *B. diffusa* (Hogweed) paste or juice, useful in vaginal pain; **Mūṣpunarnavāyāṣsatailamīṣatkṣṣguhye. garbhaṣpravepamānaṣsahasātrīṣāṣbahiṣkurute. Abnormal presentation of fetus-** topical application or insertion of vaginal suppository, prepared from *B. diffusa* (Hogweed) root paste, mixed with oil, to promote normal delivery.	Tripathi, 2012a; Tripathi, 2012b

S. No.	Diseases/ Conditions	Mode of Administration/Uses	References
8.	Musculo-skeletal disorders	**Sākaṣpaunarnavaṣhitam. Rheumatoid arthritis-** *B. diffusa* (Hogweed) vegetable, useful in rheumatoid arthritis;**Punarnavānāgaradārūpathyābhallātakacchinnarūhākaṣāyaṣ. Daṣāṣghimiśraṣparipeyaurūsbheṣ'thavāmūtrapuraprayogaṣ- Thigh stiffness-** decoction, prepared from *B. diffusa*, *Zingiber officinale* (Dry ginger root), *Cedrus deodara* (Deodar), *Terminalia chebula* (Black myrobalan), *Semecarpus anacardium* (Marking nut tree), *Tinospora cordifolia* (Tinospora) and *DaÜmoola* (group of roots of ten medicinal herbs), useful in thigh stiffness.	Tripathi, 2009a; Sharma, 2013b
9.	Disorders of extremity	**Punanarvāyāṣśvetāyāstailaṣmūlenasādhayet.Vātakaṣṣakamāhanyātpādābhyaṣagenamardanāt. Calcification of soft tissues-** massage of oil processed with *B. repens* (Creeping spiderling) root paste and decoction is useful in calcification of soft tissues.	Tripathi, 2009b
10.	Dermatological disorders	**Punarnavācetikuṣṣhinolepāṣ.Dadhimaṣṣayutāṣsarvedeyāṣ.....Leprosy-** topical application of *B. diffusa* root, pounded with curd water, useful in *Vātaja* and *Kaphaja* leprosy; **Punarnavāvarūṣayokvātho'ntarvidradhīñjayet. Internal abscess-** decoction prepared from *B. diffusa* and *Crataeva nurvala* (Three-leaved caper) bark, useful against internal abscess; **Punarnavā'bhayānimbadārvītiktāpaṣolakai.Guṣucīnāgarayutaikvāthogomūtrasaṣyutaṣ.Pāṣṣukāsodaraśvāsaśūlasarvāṣṣgaśothahāṣ. Inflammation-** decoction prepared form *B. diffusa*, *Terminalia chebula* (Black myrobalan), *Azadirachta indica* (Margosa tree) bark, *Berberis aristata* (Indian barberry), *Tinospora cordifolia* (Tinospora), and *Zingiber officinale* (Dry ginger root), mixed with cow urine, useful in anasarca; **Punarnavāṣdārūśuṣṣhīṣsiddhārthaṣśigrumeva ca.Piṣṣvācaivāranālenapralepaṣsarvaśothajit-** paste prepared from *B. diffusa*, *Cedrus deodara* (Deodar), *Zingiber officinale* (Dry ginger root), *Brassica campestris* (Yellow mustard), and *Moringa oleifera* (Drumstick tree) bark, pounded with sour gruel, useful in inflammation.	Śāstrī and Chaturvedi, 2011c; Tripathi, 2013a; Tripathi, 2013b; Tripathi, 2013c

S. No.	Diseases/ Conditions	Mode of Administration/Uses	References
11.	Generalized body disorders	**Punarnavākvāthakalkasiddhaṣśothaharaṣghṣtam. Inflammation-** *Ghrita* (Clarified butter) processed with the plant decoction and paste, used against inflammation; **Punarnavācitrakadevadārupañcoṣaṣakṣāraharītakīnām. Kalkenapakvaṣdaṣamūlatoyeghṣtottamaṣśothaniṣūdanañca-** *Ghrita* (Clarified butter) processed with *Pancakola* (group of five pungent medicinal herbs), *Dolichos biflorus* (Horse gram) decoction and *B. diffusa* (Hogweed) paste, useful in inflammation; **Punarnavānimbapaṣolaśuṣṣhītiktāmṣtādārvyabhayākaṣāyaṣ.Sarvāṣṣgaśophodarakāsaśūlaśvāsānvitaṣpāṣṣugadaṣnihanti; Punarnāvāmagadhajā ca kaṣutrayaṣ ca nimbābhayā ca kaṣukā ca paṣoladārvī. Kvāthaṣsukhoṣṣakvathitastuvipācanenaśophojahātijaṣharaṣ ca narasyaśīgham-** decoction prepared from the plant, *Piper longum* (Long pepper), *Trikatu* (group of three pungent medicinal herbs), *Zingiber officinale* (Dry ginger root), *P. nigrum* (Black pepper), *Azadirachta indica* (Margosa tree), *Terminalia chebula* (Black myrobalan), *Picrorhiza kurroa* (Picrorhiza), coral ash and *Berberis aristata* (Indian barberrry), useful in anasarca, abdominal disorders, dyspnea, pain, and anemia; **Punarnavedve ca bale sapāṣhedaṣtīṣguṣūcīmathacitrakaṣ ca... Kṣiprasjayedvarṣabalāyurojastejo'nvitomāṣsarasānnabhojī-** *Punarnavādi Ariqqa*, useful in cardiac disorders, anemia, inflammation and also enhances rejuvenating properties; **Punarnavāguṣūcī ca gugguluṣsamakalkitam. Śophadoṣāṣṣcagulmañcahantyudaraṣkaphāmayam-**paste prepared from an equal quantity of *B. diffusa* (Hog weed), *Tinospora cordifolia* (Tinospora) and *Commiphora mukul* (Gum guggul) paste, useful in inflammation, *Gulma*, abdominal disorders and *Kaphaja* disorders; **Cūrṣapaunarnavaṣraktālitaṣṣulaṣarkaram.Raktṣṣhīvīpibetsiddhaṣdrākṣārasapayoghṣtai-** *Ghrita* (Clarified butter) and milk, processed with *B. diffusa* root powder, *Vitis vinifera* (Dry grapes) juice and *Oryza sativa* (Rice) along with sugar, useful in hemoptysis caused due to chest injury; **Siṣhovyāghīsiṣhamukhīkākamācīpunarnavā.Vārttākīnāṣcamūlānāṣkvāthonidrākaronaṣṣām.**	Tripathi, 2010a; Tripathi, 2010b; Pandey, 2010a; Pandey, 2010b; Pandey, 2010c; Śāstrī and Chaturvedi, 2011d; Śāstrī and Chaturvedi, 2011e

S. No.	Diseases/ Conditions	Mode of Administration/Uses	References
		Insomnia- decoction prepared from *Solanum indicum* (Indian night shade) , *S. surattense* (Yellow berred night shade), *Adhatoda vasica* (Malabar nut), *S. nigrum* (Garden nightshade), *B. diffusa*, *S. melongena* (Eggplant) roots, useful against insomnia.	
12.	Rejuvenation and Virilification	**Punarnavasyārdhapalaṣnavasyapṣṣpibedyaṣpayasā'rddhamāsam.Māsadvayaṣ tat triguṣaṣsamāṣvājīrṣo'pibhūyaṣsapunarnavaṣsyāt-** regular intake of the root paste, till 15 days, to enhance rejuvenating properties.	Tripathi, 2011
13.	Alexipharmic treatment	**Śvetāṣpunarnavāñcāsyadadyāddhuttūrakāyutām. Rabies-** powder prepared from *B. diffusa* root, *Datura metel* (DevilÕs trumpet) seed, mixed with *Sesamum indicum* (Sesame) oil, *Calotropis procera* (Calotropis), milk, and jiggery, alleviates poisonous effects caused due to rabies; **Yaṣpibatipuṣyadivasejalapṣṣsitapunarnavāmūlam.Tatsannidhaunavarṣaṣvṣścikabhujagāṣprasarpanti-** root paste along with rice water, useful against poisoning.	Chunekar and Yadava, 2005; Mishra, 2009c

Therapeutic Potential

The discovery of the bioactive compounds in medications and their advancements begins with the use of herbs. Native species must be preserved since they frequently develop to withstand environmental factors. Herbs are the first line of defense in the fight against many diseases (Dwivedi et al., 2007; Dwivedi et al., 2008; Veeresham, 2012). The knowledgeable tool for many activities and talents is traditional medicine. These are based on numerous ideas, concepts, and benefits as well as their own experiences and knowledge of their culture. They are used to maintain and improve health as well as to diagnose and treat physical and mental issues. Alternative medicine is a word that has been accepted by the public for traditional medicine (Gour, 2021; Vandana et al., 2007). The most crucial herb for life is called *B. diffusa*, or *Punarnava* in Sanskrit. It is known to exhibit diuretic, anti-diabetic, antioxidant, and hepatoprotective properties (Olaleye et al., 2010). Additionally, the entire plant and its parts, such as the leaves, stem, and roots, are believed to have medicinal effects (Sahu et al., 2008; Nayak and Thirunavoukkarasu, 2016; Ghosh, 2018). For instance, the roots, have been employed for gastrointestinal, hepatoprotective, and gynecological purposes across India as well as in other areas of the world [Govindarajan et al., 2005]. Similarly, the

whole plant is useful against asthma, renal disorders, blood disorders, urinary ailments, venereal diseases, etc (Vanila et al., 2008; Kumar et al., 2011; Bagartee et al., 2020). The detailed description is summarised in **Table 2.**

Table 2 Medicinal importance along with their preparation and mode of administration of *B. diffusa* L.

S. No.	Parts Used	Preparations	Diseases Treated and Therapeutic Uses	References
1.	Whole plant	Powder, paste	Jaundice, kidney problems, snake-bites, asthma, stomach problems, fever, diabetes, anemia, stomachache, cough, cold, expectorant, laxative	Bagartee et al., 2020; Aslam et al., 1996
		Extract	Albuminuria, beriberi, cystitis, edema, gonorrhea, guinea worms, hepatitis, hypertension, liver disorders, renal disorders, snakebites, enlarged spleen, urinary disorders, gallbladder problems	Cruz et al., 1985
		-	Liver complaints	Vanila et al., 2008
		Decoction	Gonorrhea, nephritis, edema	Zagari et al., 1992
		Infusion	Asthma, bronchitis	Kumar et al., 2011
2.	Roots	Paste	Eye irritation	Bagartee et al., 2020;
		Powder mixed with onion juice	Night blindness	Bagartee et al., 2020;
		Boiled infusion	Sleeplessness	Bagartee et al., 2020;
		Infusion	Heart disease	Bagartee et al., 2020;
		Spongy decoction	Cirrhosis, jaundice, hepatitis	Bossard et al., 1996

S. No.	Parts Used	Preparations	Diseases Treated and Therapeutic Uses	References
		Decoction	Diuretics, urinary disorders, albuminuria, gallstones, hepatitis, jaundice, cystitis, ascites, beriberi, blenorrhagia, anemia, yaws, heart troubles, palpitations, asthma, internal inflammation disorders, piles, Leukorrhea, rheumatism, stomachache, elephantiasis, seminal weakness, blood pressure, sprains, rheumatism, diffuse pains, maux of reins	Bossard et al., 1996; Cruz et al., 1985; Muzila et al., 2008; Mitra et al 1997; Bouquet et al., 1974
		Tea	Prolapsed uterus	Muzila et al., 2008
		Sap	Mumps, laryngitis, burns	Muzila et al., 2008
		Boiled	Gastro-enteritic problems,	Muzila et al., 2008
		Powder with butter and oil	Abdominal tumours	Muzila et al., 2008
		Powder boiled with water or milk	Stomachache due to lifting heavy objects, muscular and joint pain due to rheumatoid arthritis	Semwal et al., 2021
		-	Jaundice, ascites, anasarca, sentry urine, internal inflammations, asthma, piles, energy tonic, diuretic, laxative, expectorant	Gupta et al., 1962; Kumar et al., 2011; Aslam et al., 1996
3.	Stem	Powder mixed with giloy and tulsi	Cough, vomiting, indigestion, snakebites	Bagartee et al., 2020;
4.	Leaves	Infusion and juice	Liver diseases, fever, cough	Bagartee et al., 2020;
		Decoction	Anticonvulsant, antiasthmatic, expectorant, emetic, cholera, paludism	Adesina et al., 1982; Guessan et al., 2009

S. No.	Parts Used	Preparations	Diseases Treated and Therapeutic Uses	References
		Infusion	Appetizer, joint pain	Zagari et al., 1992
		Juice/decoction	Analgesic, anti-inflammatory	Hiruma et al., 2000
		-	Childbirth, appetizer, alexiteric	Aslam et al., 1996; Vanila et al., 2008
		Extract	Eye complaints	Kumar et al., 2011
5.	Seeds	-	Tonic, expectorant, carminative, lumbago, scabies, scorpion-sting, blood purifier, muscular pain, energizer, digestion	Gupta et al., 1962; Aslam et al., 1996
6.	Roots and leaves	Boiled	Expectorant, febrifuge, emetic	Whitehouse et al., 1996
7.	Aerial parts	Decoction	Gastro-intestinal pains, convulsions, intestinal worms, menstrual problems	Whitehouse et al., 1996
8.	Stem and leaves	Infusion	Arthritis, cramps, joint pain, rheumatism, kidney pain, anti-inflammatory	Bossard et al., 1996

Ethnomedicinal and Folk treatments along the Ganga Basin of India

About 70% of the world's population uses the traditional healthcare system, which is considered as the most effective in treating illnesses [World Health Organization, 2019]. Traditional medicine has demonstrated its superiority over contemporary biomedicine in numerous situations, such as chronic illnesses. Numerous studies have demonstrated that herbal remedies are deemed safe while synthetic medications have numerous adverse effects, both acute and chronic. Additionally, herbal medicine is often believed to be more affordable for everyone than synthetic medications. As a result, due to its affordability and safety, people all over the world are turning back to herbal therapy (Semwal et al., 2019). To treat various ailments that are passed down from generation to generation, various indigenous cultures have distinct methods of preparation and occasionally the same. Many rural and indigenous people in the country still cannot access medical facilities today. These populations have little other option but to use traditional or folk remedies because

they are affordable, accessible, and generally well-tolerated. A few therapeutic herbs also have phytoestrogenic or phytoprogesteronic properties (Pala et al., 2019; Lawal et al., 2013; Van Andel et al., 2014).

The millions of residents of the Indian Himalayan Region (IHR) as well as those who reside in other areas of India have long relied on this region as a source of medicine (Dhar et al., 2000). The Indian state of Uttarakhand is renowned for its wide variety of medicinally significant plants and related traditional knowledge. The rural populations in Uttarakhand's remote Chamoli region have their distinct ways of interacting with one another on a social and cultural level. Regardless of how simple or complex they are, many societies have extensive traditional knowledge of medicinal plants (Semwal et al., 2010). Ethnomedicinal research revealed that traditional knowledge is still widely used in many Himalayan regions, especially by tribes, the elderly, and traditional medicine healers. Paste of the roots of *B. diffusa* is majorly used by different traditional healers for the management of bloody dysentery (Sharma et al., 2022). Likewise, powder of the roots is recommended at 3 g orally by the Tharu community for the treatment of epilepsy (Sharma et al., 2013). Furthermore, the juice of the fresh roots is used as an eye drop, against liver ailments, as well as asthma (Dwivedi et al., 2019).

Located in the northwest of the nation, Uttar Pradesh is the fourth largest and most populous state in India. Uttar Pradesh shares borders with the following states: Madhya Pradesh to the south and south-west, Bihar to the east, Uttarakhand to the northeast, Himachal Pradesh to the north-west, Haryana to the west, Rajasthan to the south-west, and Delhi to the north (Sachan et al., 2015). *B. diffusa* is commonly referred to as "Gajpunna" in eastern Uttar Pradesh. The plant's strong, fusiform root system, which penetrates deeply into the soil, contains its medicinal qualities. In rural sections of the region, plant roots are used to treat jaundice (Singh, 2007). Moreover, in some regions of Bihar, juice of the fresh roots is used for treating jaundice by the traditional healers, while a decoction of the roots with kutki (*Picrorhiza kurroa*), chirayata (*Swertia chirata*) and sonth (*Zingiber officinale*) is used against ascites [Singh et al., 2013].

Conclusion and Future Perspectives

Popularly used in many traditional medical systems like Ayurveda and Unani, *B. diffusa* is a well-known medicinal herb. Over the ages, the *B. diffusa* plant has evolved into a remarkable medicinal herb with an abundance of chemical components beneficial for treating a wide range of ailments. This plant is very important to traditional herb-based treatments all around the world. All its parts- the fruit, seed, stem, leaf, and root have therapeutic benefits. For conditions of the liver, kidney, spleen, and bronchi, it is an extremely useful resource. It is one of the more heavily used medicinal plants that can be found in the wild. 'Punarnava' is a

strong herbal medication used in Ayurveda that can be taken as a single herb, a combination of herbs, or a herbal-mineral mixture. The traditional literature informs us of its numerous qualities and formulations that are applied to a range of problems, including joint discomfort, anemia, jaundice, inflammations, kidney stones, and eye disorders. Also, from the ethnomedicinal point of view, this plant is widely used by different communities for treating several ailments making it a blessing for humanity. Further, owing to its immense use it can be implemented into agricultural practices as a potential agro-crops.

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CHAPTER

05

Phytochemical Analysis

INTRODUCTION

Boerhavia diffusa, commonly known as 'Punarnava', is a plant of significant importance in traditional Indian medicine and beyond. This herb belongs to the Nyctaginaceae family and is renowned for its diverse medicinal properties. It is a well-known *rasayana* herb in Ayurveda, attributed with antiaging, rejuvenating, and disease-preventive qualities. Its roots, leaves, and stems have been extensively used for various health conditions, including gastrointestinal issues, hepatoprotection, and gynecological disorders. The plant is rich in phytochemicals such as rotenoids, flavonoids, xanthones, purine nucleosides, lignans, and steroids, which contribute to its therapeutic potential (Mishra et al., 2014; Das et al., 2023). Geographically, *B. diffusa* thrives in tropical and subtropical regions worldwide, often found as a weed during rainy seasons in the Indian subcontinent, Northern and Southern America, and Southeastern Africa.

The genus *Boerhavia* encompasses around 40 species, with *B. diffusa* being a prominent member known for its medicinal value. The plant has various names across different regions due to its widespread distribution (Patil and Bhalsing, 2016). Traditional uses of this species have been documented in Ayurvedic texts with over 35 formulations incorporating it as a key ingredient. Its classification as a *rasayana* herb underscores its role in promoting longevity, enhancing vitality, and fortifying the body against illnesses. The ethnopharmacological significance of this species extends to its reported benefits in reproductive disorders, gastrointestinal ailments, respiratory issues, urinary complications, liver conditions like jaundice, cardiovascular health, and even cancer (Mishra et al., 2014). *B. diffusa* stands out as a botanical treasure with a rich phytochemical profile and a long history of traditional use across diverse cultures for its therapeutic properties that address a wide array of health concerns.

Sampling Sites

Table 1 Sampling sites for the *Boerhavia diffusa*, collected from various state-specific locations spread over the Gangetic course

S. No.	Sampling sites
1.	Farrukhabad, Uttar Pradesh
2.	Prayagraj, Uttar Pradesh
3.	Mirzapur, Uttar Pradesh
4.	Varanasi, Uttar Pradesh
5.	Ballia, Uttar Pradesh
6.	Barh, Bihar
7.	Sahibganj, Jharkhand
8.	Gaumukh, Uttar Pradesh

Phytochemical Analysis

The quantification of phytochemical constituents, including tannin (via titration), total saponins (via gravimetry), total polyphenols, and total flavonoids (via UV-visible spectrophotometer), was conducted following established protocols and standard methodologies. 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 1–10 g sample was used in the method, which also required adding 50 ml of milli-Q water, stirring, and sonicating it for 30 minutes. Following that, the sample was filtered, and its volume adjusted to 100 ml. Ten milliliters of this was then extracted and mixed with 750 ml of milli-Q water. Twenty-five milliliters of indigo sulphonic acid was then added and agitated. Potassium permanganate solution (0.1N) was added to the solution and titrated until the endpoint for color turned golden yellow. A blank was run without the sample.

Determination of Saponin Content

A 5 g sample was taken and combined with 50 ml of a 1:1 methanol and water solvent mixture. The solution was refluxed for 1 hour, cooled, and filtered. This process was repeated three times. The filtrates were then combined, concentrated, and evaporated to dryness. Following this step, 25 ml of petroleum ether was added, and the solution was refluxed for 10 minutes. The solution was then cooled, and the ether layer was decanted. Next, 10 ml of methanol and 100ml of acetone were added and the solution was filtered. The filter paper containing residue was dried at 80˚C for 1 hour and weighed.

Determination of Total Polyphenol Content

A 1 ml sample was taken in a test tube and combined with 1 ml of Folin–Ciocâlteu reagent, then incubated for 5 minutes. Following this, 1 ml of 10% sodium carbonate solution was added. The mixture was then kept in the dark for 1 hour before absorbance was taken at 760 nm using a UV-visible spectrophotometer. This process was repeated for gallic acid to establish linearity.

Determination of Total Flavonoid Content

A 1 ml sample was placed in a test tube and combined with 0.4 ml of 10% aluminum chloride, 0.4 ml of sodium acetate, and 3 ml of ethanol. The mixture was then left at room temperature for 30 minutes before measuring the absorbance at 450 nm using a UV-visible spectrophotometer. These steps were repeated for quercetin dihydrate to establish linearity.

HPTLC Fingerprinting

The application of 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 *Boerhavia* 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 patterns, generally refer as “fingerprints”, are used for the identification of phytochemicals. The bands or spots obtained during the test are characteristic of a particular herb. A color image of a typical TLC fingerprint provides a clearer guide to the users.

Sample Preparation

About 1 g of sample from each batch was individually dissolved in 10 ml of methanol. The samples were shaken and sonicated for 20 min. The solution was then centrifuged

for 5 min at 5000 rpm. A clear solution thus obtained was used for analysis.

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} aluminum sheet (1.0554.0007)
Mobile phase	Ethyl acetate: toluene: formic acid (7.5: 2.5: 1)
Saturation time	15 minutes
Migration distance	70 mm
Band length	8 mm
Injection volume	25 µl
Visualization	254 nm and 366 nm; without derivatization

High-Performance Liquid Chromatography (HPLC)

Sample and Standard Preparation

Sample Preparation: About 1 g of sample from each batch individually was diluted with up to 25 ml of hydromethanol (20:80). Then it was sonicated for 4 hours, centrifuged for 5 min at 9000 rpm, and filtered using 0.45 µm nylon filter. The filtered solution was used for analysis.

Standard preparation: A standard stock solution of 1000 µg/ml was prepared by dissolving standard boeravinone B in methanol. Furthermore, 100 µg/ml of the standard working solution was obtained using 0.02 ml of this stock.

Analytical and Instrumentation Condition

Analysis was performed by Prominence-XR UHPLC system (Shimadzu, Japan) equipped with a quaternary pump (NexeraXR LC-20AD XR), DAD detector (SPD-M20 A), auto-sampler (Nexera XR SIL-20 AC XR), degassing unit (DGU-20A 5R) and column oven (CTO-10 AS VP). 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 consisted of water (solvent A) and acetonitrile (solvent B). The flow was set at 1.0 ml/min during the analysis. Twenty microliters of standard and test solution were injected. The wavelength was set at 272 nm.

Gradient program used

Time (Min)	A %	B%
0	90	10
25	10	90
35	90	10

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

The objective of this analysis was to identify the major compounds in *Boerhavia diffusa* test sample (PRF/CHI/1223/1304).

Sample Preparation

Five hundred milligrams 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 × 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 35 °C throughout the analysis and sample temperature was kept at 20°C. Detection was carried out by Xevo G2-XS QToF. Two microliters of test solution was injected in UPLC/MS-QToF and chromatograms were recorded in positive and negative ionization mode.

Gradient program

Time (min)	Flow (ml/min)	Mobile phase A%	Mobile phase B%
0	0.3	95	5
5	0.3	95	5
10	0.3	85	15
35	0.3	65	35
50	0.3	40	60

Time (min)	Flow (ml/min)	Mobile phase A%	Mobile phase B%
55	0.3	20	80
56	0.3	95	5
60	0.3	95	5

Xevo G2-XS QToF Parameters

Parameter	Polarity (+ve)	Polarity (-ve)
Ionization 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 En-kaphalin)	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 phytochemical analysis of *Boerhavia diffusa* samples collected from various locations revealed significant variations in the concentration of tannins, saponins, total polyphenols, and total flavonoids (Table 2). These phytochemicals are known for their medicinal properties and play a crucial role in the plant's therapeutic potential (Miean and Mohamed, 2001; Ullah andKhan, 2008; Hala et al., 2011; Wenfeng,2011).

1. **Tannins**: The tannin content ranged from 0.020% to 0.062%, with the highest concentration found in the Prayagraj sample (0.062%). Tannins are known for

their antioxidant properties and contribute to the astringent taste of plants. They play a role in protecting plants from microbial infections and can have various health benefits, including anti-inflammatory and anticancer activities.

2. **Saponins**: The saponin content varied widely, from 3.900% to 7.916%, with the highest concentration observed in the Prayagraj sample (7.916%). Saponins have been reported to possess a range of pharmacological properties, including cholesterol-lowering, immune-boosting, and anticancer effects. The high saponin content in the Prayagraj sample suggests its potential for significant therapeutic applications.
3. **Total Polyphenols**: The total polyphenol content ranged from 0.179% to 0.561%, with the Ballia sample exhibiting the highest concentration (0.561%). Polyphenols are well-known antioxidants and play a vital role in reducing oxidative stress in the body. Their presence in high concentrations can enhance the medicinal value of the plant, providing protection against cardiovascular diseases and certain types of cancer.
4. **Total Flavonoids**: The flavonoid content varied from 0.184% to 0.229%, with the highest concentration found in the Sahibganj sample (0.229%). Flavonoids are known for their anti-inflammatory, antiviral, and anticancer properties. They contribute to the overall antioxidant capacity of the plant and can improve health by modulating cell signalling pathways.

Table 2 Phytochemical analysis of *Boerhavia diffusa* collected from different locations

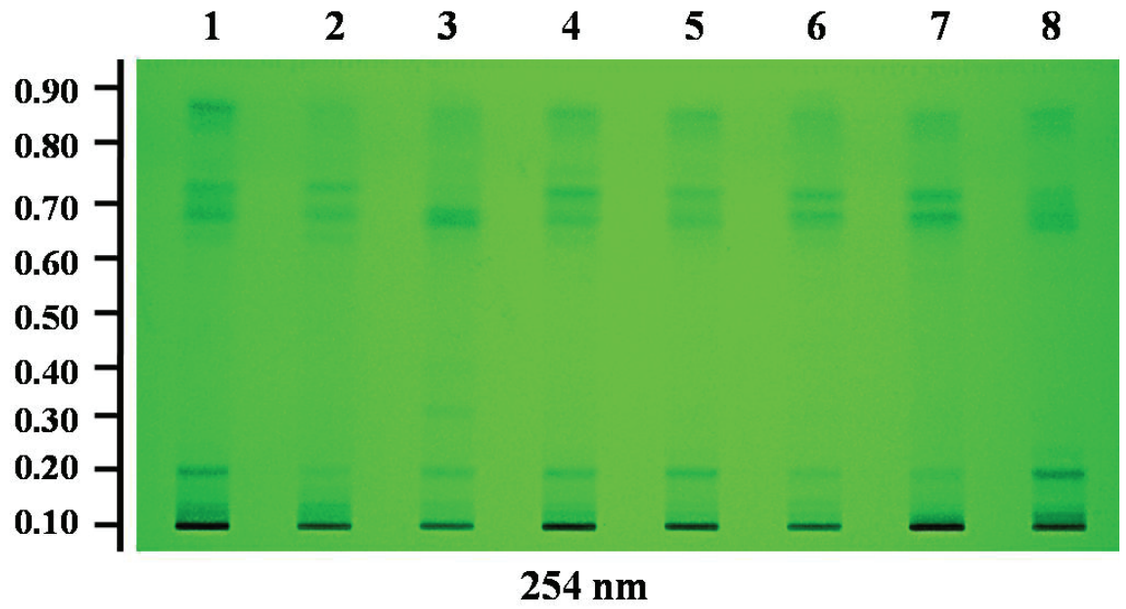
S. No.	Sample code	Tannin content (%w/w)	Saponin content (%w/w)	Total polyphenol content (%w/w)	Total flavonoid content (%w/w)
1.	Farrukhabad, Uttar Pradesh	0.061	3.900	0.179	0.214
2.	Prayagraj, Uttar Pradesh	0.062	7.916	0.208	0.218
3.	Mirzapur, Uttar Pradesh	0.020	6.250	0.469	0.184
4.	Varanasi, Uttar Pradesh	0.040	4.498	0.220	0.211
5.	Ballia, Uttar Pradesh	0.040	5.890	0.561	0.222
6.	Barh, Bihar	0.041	6.423	0.488	0.227
7.	Sahibganj, Jharkhand	0.020	4.372	0.421	0.229
8.	Gaumukh, Uttar Pradesh	0.040	4.273	0.448	0.228

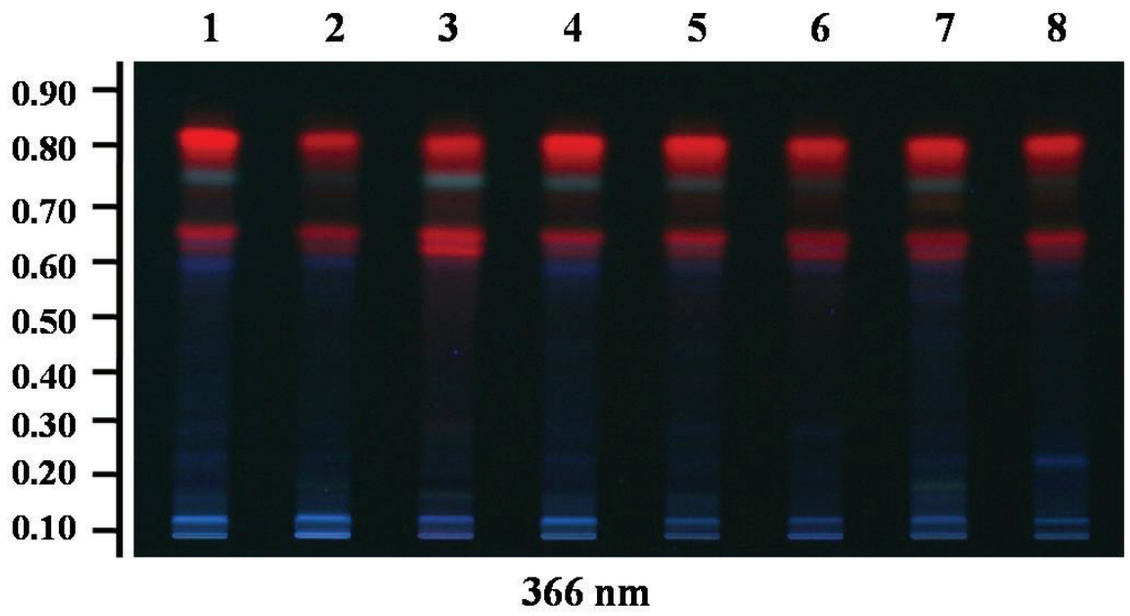
The quantitative differences in these phytochemicals among the samples could be attributed to variations in geographical conditions, soil composition, and climatic

factors, which influence the biosynthesis of these compounds.

HPTLC Fingerprint Analysis

The HPTLC fingerprint analysis provided a visual representation of the phytochemical composition of *Boerhavia diffusa* samples. Distinct patterns were observed in the chromatograms at different wavelengths (254 nm, 366 nm, and white light). The Prayagraj sample exhibited relatively low band intensity, indicating a lower concentration of certain phytochemicals compared to other samples (Fig.1). This analysis helps in the standardization and quality control of herbal medicines by providing a chemical profile unique to each sample.





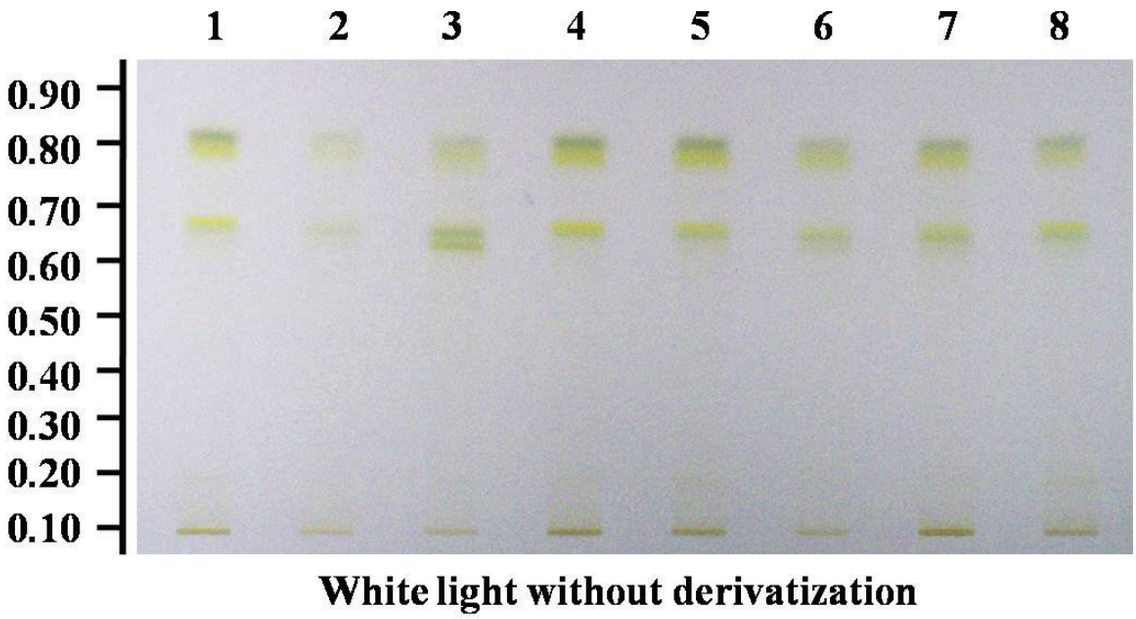


Fig. 1 HPTLC fingerprinting of *Boerhavia diffusa* at (a) 254 nm(b) 366nm and (c) underwhitelight without derivatization

HPLC Analysis

The high-performance liquid chromatography (HPLC) analysis focused on boeravinone B, a significant phytochemical in *Boerhavia diffusa*. The quantitative results of the HPLC analysis indicated substantial variability in the concentration of boeravinone B across different samples. The Farrukhabad sample exhibited the highest concentration of boeravinone B at 0.182 µg/mg, whereas the Mirzapur sample showed the lowest concentration at 0.019 µg/mg (Table 3). Such variations can be attributed to environmental factors, soil quality, and cultivation practices specific to each location.

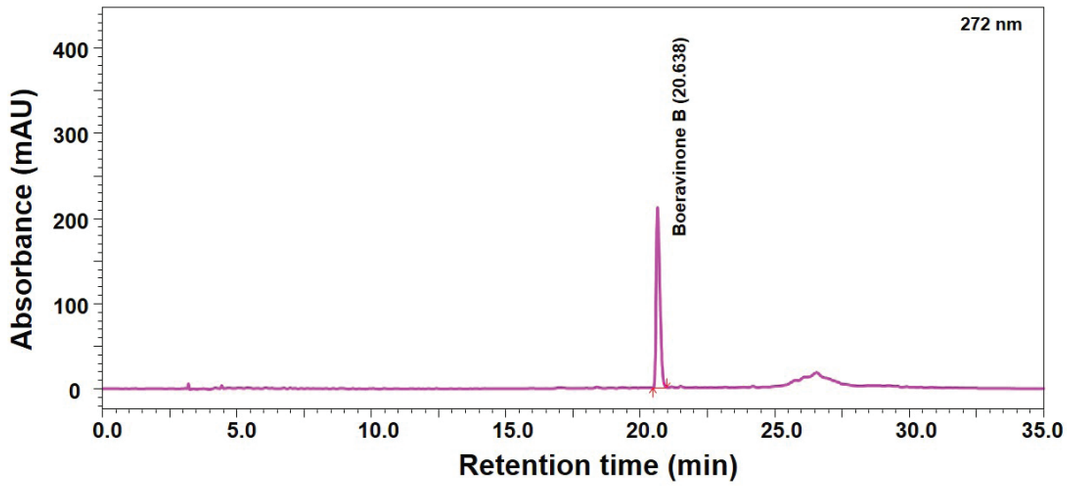


Fig 2. HPLC chromatogram of standard Boeravinone B at 272 nm

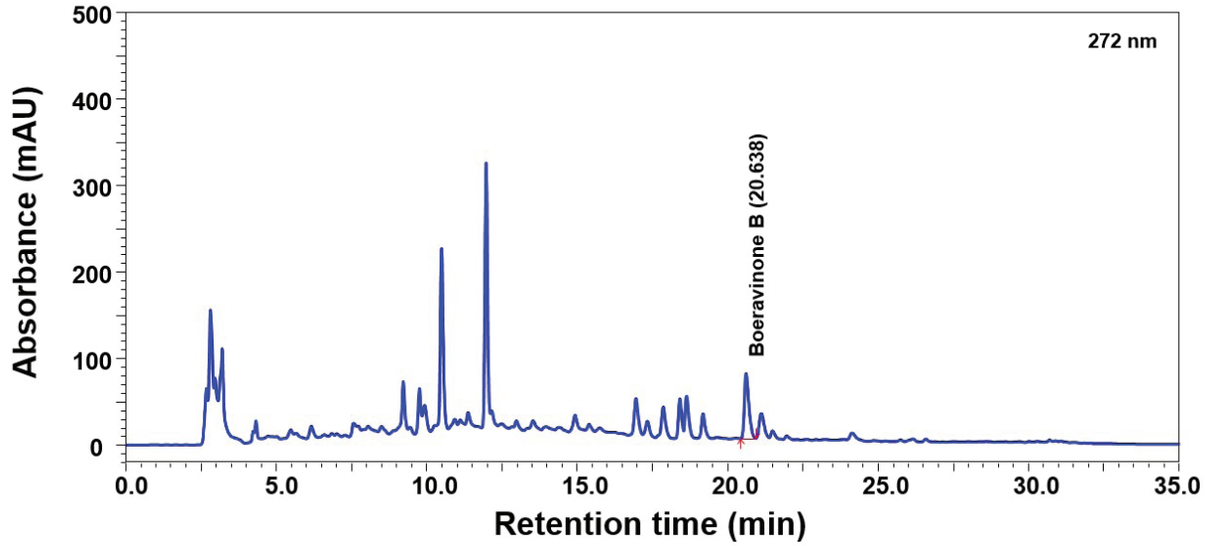


Fig 3. HPLC chromatogram of *Boerhavia diffusa* (PRF/CHI/1223/1304) at 272 nm

Table 3 Amount of phytochemical presentin *Boerhavia diffusa* collected from different locations

S.No.	Internal code	External code	Boeravinone B (µg/mg)
1	PRF/CHI/1223/1304	UP1P10S4	0.182
2	PRF/CHI/1223/1305	UP2P10S7	0.060
3	PRF/CHI/1223/1306	UP2P10S8	0.019
4	PRF/CHI/1223/1307	UP2P10S9	0.084
5	PRF/CHI/1223/1308	UP2P10S10	0.079
6	PRF/CHI/1223/1309	BHP10S3	0.028
7	PRF/CHI/1223/1310	JHP10S1	0.064
8	PRF/CHI/1223/1311	UKP10S1	0.094

Boeravinone B is known for its potent anti-inflammatory and anticancer properties, making it a crucial compound for therapeutic applications. The high concentration of boeravinone B in the Farrukhabad sample suggests a higher medicinal value, making it a preferred source for herbal preparations targeting inflammation and cancer. Conversely, the lower concentration in the Mirzapur sample highlights the necessity for selecting appropriate geographical sources to maximize therapeutic benefits.

These findings underscore the importance of geographical and environmental

conditions in influencing the phytochemical content of *Boerhavia diffusa*. They also highlight the need for standardized cultivation and harvesting practices to ensure the consistent quality and efficacy of herbal products derived from this plant.

UPLC/MS-QToF Analysis

The analysis resulted in a fast, high-resolution separation with high sensitivity. The qualitative (identification of compounds), and relative quantification (response curve) were obtained for a variety of substances within the complex mixture. The TIC chromatogram as well as the list of identified compounds and their associated parameters in both positive and negative modes have been presented below. A total of 34 compounds were consequently identified in the test sample,showcasing the plant's rich and diverse phytochemical composition.

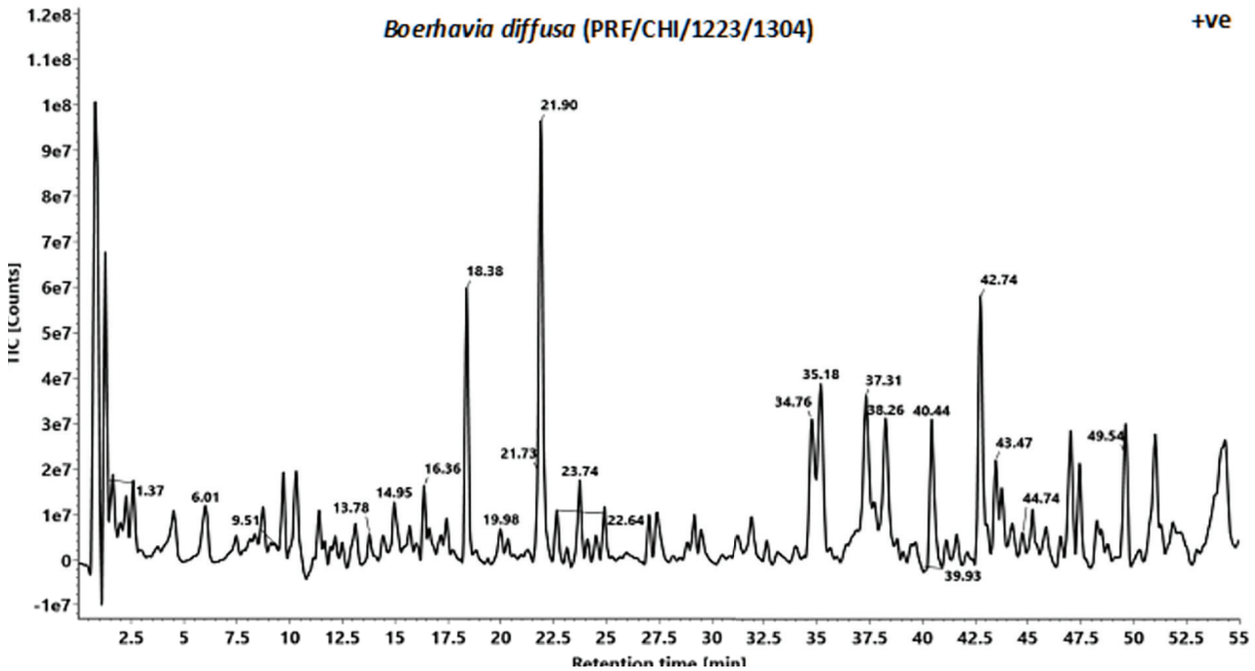


Fig 4. TIC chromatogram of TIC chromatogram of *Boerhavia diffusa* (PRF/CHI/1223/1304) in positive ionization mode

Some of the key compounds identified include:

1. **Adenosine**: Known for its role in cellular energy transfer and neurotransmission, adenosine also possesses anti-inflammatory and cardioprotective properties.
2. **Tryptophan**: An essential amino acid, tryptophan is a precursor to serotonin and melatonin, influencing mood regulation and sleep.
3. **Catechin**: A type of flavonoid with strong antioxidant properties, catechin helps in

reducing oxidative stress and has potential anticancer effects.

4. **Ferulic Acid**: This phenolic compound is recognized for its antioxidant, anti-inflammatory, and antimicrobial activities.
5. **Flavonoids**: Various flavonoids, including quercetin, rutin, and isorhamnetin derivatives, were identified. These compounds are known for their extensive pharmacological activities, including antioxidant, anti-inflammatory, antiviral, and anticancer effects.

The detailed molecular composition provided by UPLC/MS-QToF analysis enhances the understanding of the therapeutic potential of *Boerhavia diffusa*. The identification of these compounds supports the traditional uses of the plant in treating various ailments and provides a scientific basis for its medicinal properties.

Table 4 Compound-dependent parameters of analytes/identified compounds in *Boerhavia diffusa* (PRF/CHI/1223/1304) in positive ionization mode

S. N.	Component name	Formula	Neutral mass (Da)	Observed m/z	Mass error (mDa)	RT (min)	Response	Adducts
1	Adenosine	$C_{10}H_{13}N_5O_4$	267.0968	268.1062	2.2	1.37	976374	+H
2	Tryptophan	$C_{11}H_{12}N_2O_2$	204.0899	205.0981	0.9	6.01	729868	+H
3	Catechin	$C_{15}H_{14}O_6$	290.0790	291.0886	2.2	9.51	407757	+H
4	Ferulic acid	$C_{10}H_{10}O_4$	194.0579	195.0662	1.0	13.78	102249	+H
5	Quercetin 3-O-robinobioside	$C_{27}H_{30}O_{16}$	610.1534	611.1599	-0.8	14.54	117870	+H
6	Rutin	$C_{27}H_{30}O_{16}$	610.1534	611.1597	-1.0	14.80	186671	+H
7	Isorhamnetin 3,7-O-di-ß-D-glucopyranoside	$C_{28}H_{32}O_{17}$	640.1640	641.1703	-1.0	14.95	1429601	+H
8	Patuletin-3-O-glucoside	$C_{22}H_{22}O_{13}$	494.1060	495.1139	0.6	15.70	956318	+H
9	Isorhamnetin-7-O-rhamnose-3-O-glucoside	$C_{28}H_{32}O_{16}$	624.1690	625.1750	-1.3	16.36	1992158	+H
10	Kaempferol-3-O-rutinoside	$C_{27}H_{30}O_{15}$	594.1585	595.1655	-0.3	16.81	256379	+H
11	Isorhamnetin 3-O-rutinoside	$C_{28}H_{32}O_{16}$	624.1690	625.1758	-0.5	17.19	428564	+H

S. N.	Component name	Formula	Neutral mass (Da)	Observed m/z	Mass error (mDa)	RT (min)	Response	Adducts
12	Monoxerutin	$C_{29}H_{34}O_{17}$	654.1796	655.1855	-1.3	18.38	7174089	+H
13	11-O-Syringyl-bergenin	$C_{23}H_{24}O_{13}$	508.1217	509.1299	1.0	19.98	719385	+H
14	N-feruloyltyramine	$C_{18}H_{19}NO_4$	313.1314	314.1403	1.6	21.73	2336419	+H
15	Eupalitin 3-galactoside	$C_{23}H_{24}O_{12}$	492.1268	493.1347	0.7	21.90	10442528	+H
16	Eupalitin-3-O-b-D-glucoside	$C_{23}H_{24}O_{12}$	492.1268	493.1351	1.0	22.64	1128292	+H
17	Diosmin	$C_{28}H_{32}O_{15}$	608.1741	609.1805	-0.9	23.14	308265	+H
18	N-trans-Feruloyl 4-O-methyldopamine	$C_{19}H_{21}NO_5$	343.1420	344.1511	1.8	23.74	3052609	+H
19	Boeravinone E	$C_{17}H_{12}O_7$	328.0583	329.0672	1.6	34.76	8047232	+H
20	Eupalitin	$C_{17}H_{14}O_7$	330.0740	331.0828	1.5	35.18	7881619	+H
21	Coccineone B	$C_{16}H_{10}O_6$	298.0477	299.0563	1.2	37.31	5314160	+H
22	Boeravinone O	$C_{17}H_{12}O_7$	328.0583	329.0672	1.6	38.26	4398868	+H
23	Boeravinone J	$C_{16}H_{10}O_6$	298.0477	299.0563	1.3	39.93	218525	+H
24	Boeravinone G	$C_{18}H_{14}O_7$	342.0740	343.0825	1.3	40.44	4375736	+H
25	Boeravinone B	$C_{17}H_{12}O_6$	312.0634	313.0718	1.1	42.74	9408759	+H
26	Boeravinone I	$C_{18}H_{14}O_7$	342.0740	343.0822	1.0	43.47	3583316	+H
27	Boeravinone K	$C_{17}H_{12}O_6$	312.0634	313.0716	1.0	44.74	623160	+H
28	Boeravinone H	$C_{19}H_{16}O_7$	356.0896	357.0971	0.2	48.28	783720	+H
29	Boeravinone A	$C_{18}H_{14}O_6$	326.0790	327.0865	0.2	48.47	1319642	+H
30	Coronaric acid	$C_{18}H_{32}O_3$	296.2351	297.2408	-1.6	49.54	1592123	+H

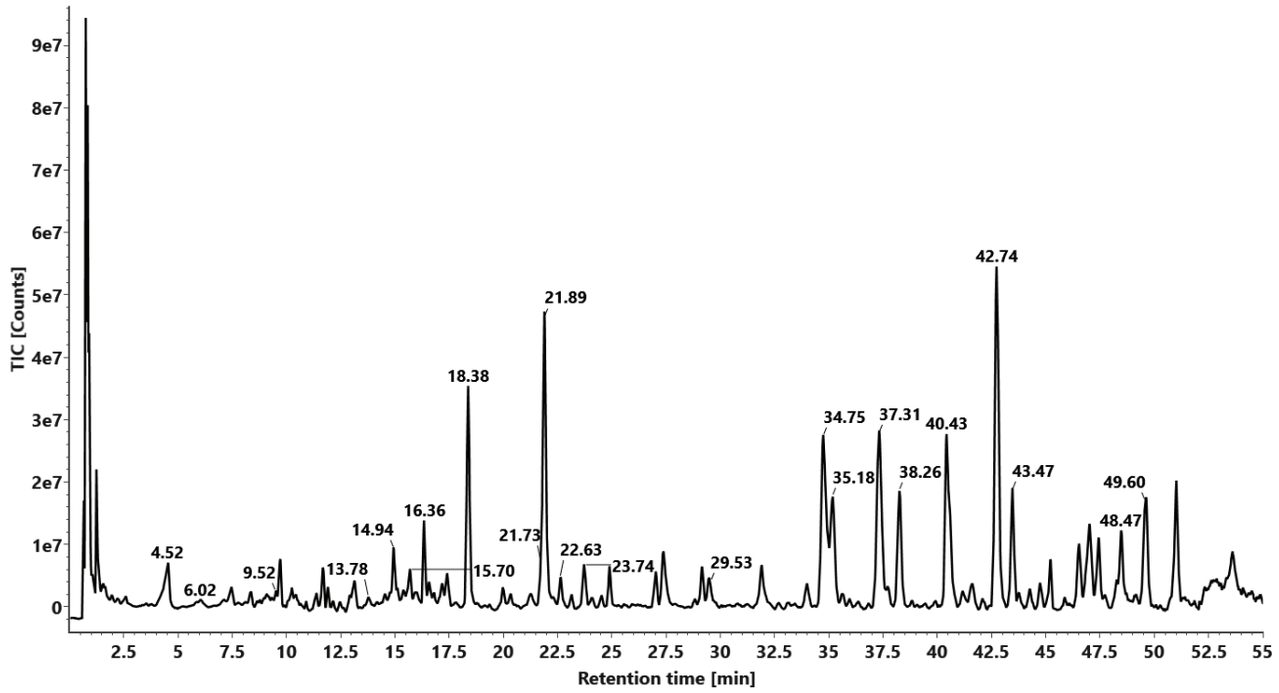


Fig 5. TIC chromatogram of *Boerhavia diffusa* (PRF/CHI/1223/1304) in negative ionization mode

Table 5 Identified compounds in *Boerhavia diffusa* (PRF/CHI/1223/1304) in negative ionization mode

S. N.	Component name	Formula	Neutral mass (Da)	Observed m/z	Mass error (mDa)	RT (min)	Response	Adducts
1	Cornoside	$C_{14}H_{20}O_8$	316.1158	315.1066	-1.9	4.52	1338112	-H
2	Tryptophan	$C_{11}H_{12}N_2O_2$	204.0899	203.0814	-1.2	6.02	178377	-H
3	Catechin	$C_{15}H_{14}O_6$	290.0790	289.0701	-1.7	9.52	341934	-H
4	(-)-Epicatechin	$C_{15}H_{14}O_6$	290.0790	289.0699	-1.9	11.29	162790	-H
5	Ferulic acid	$C_{10}H_{10}O_4$	194.0579	193.0495	-1.1	13.78	100227	-H
6	Quercetin 3-O-robinobioside	$C_{27}H_{30}O_{16}$	610.1534	609.1466	0.5	14.53	145396	-H
7	Rutin	$C_{27}H_{30}O_{16}$	610.1534	609.1467	0.6	14.79	210498	-H
8	Isorhamnetin 3,7-O-di-ß-D-glucopyranoside	$C_{28}H_{32}O_{17}$	640.1640	639.1577	1.0	14.94	1455074	-H
9	Patuletin-3-O-glucoside	$C_{22}H_{22}O_{13}$	494.1060	493.0979	-0.9	15.70	631431	-H
10	Isorhamnetin-7-O-rhamnose-3-O-glucoside	$C_{28}H_{32}O_{16}$	624.1690	623.1624	0.6	16.36	2567901	-H

S. N.	Component name	Formula	Neutral mass (Da)	Observed m/z	Mass error (mDa)	RT (min)	Response	Adducts
11	Kaempferol-3-O-rutinoside	$C_{27}H_{30}O_{15}$	594.1585	593.1516	0.4	16.80	305599	-H
12	Isorhamnetin 3-O-rutinoside	$C_{28}H_{32}O_{16}$	624.1690	623.1622	0.5	17.18	394893	-H
13	Monoxerutin	$C_{29}H_{34}O_{17}$	654.1796	653.1734	1.1	18.38	8015928	-H
14	11-O-Syringyl-bergenin	$C_{23}H_{24}O_{13}$	508.1217	507.1141	-0.3	19.98	608694	-H
15	N-feruloyltyramine	$C_{18}H_{19}NO_4$	313.1314	312.1226	-1.5	21.73	727425	-H
16	Eupalitin 3-galactoside	$C_{23}H_{24}O_{12}$	492.1268	491.1191	-0.4	21.89	10577908	-H
17	5,6,7,3′-Tetrahydroxy-4′-methoxyisoflavone	$C_{16}H_{12}O_7$	316.0583	315.0493	-1.7	22.30	255583	-H
18	Eupalitin-3-O-b-D-glucoside	$C_{23}H_{24}O_{12}$	492.1268	491.1189	-0.6	22.63	897581	-H
19	Diosmin	$C_{28}H_{32}O_{15}$	608.1741	607.1674	0.6	23.13	402645	-H
20	N-trans-Feruloyl 4-O-methyldopamine	$C_{19}H_{21}NO_5$	343.1420	342.1326	-2.1	23.74	1178044	-H
21	5,7,8,3'-Tetrahydroxy-3,4'-dimethoxy flavone	$C_{17}H_{14}O_8$	346.0689	345.0599	-1.7	29.53	404030	-H
22	Boeravinone E	$C1_7H_{12}O_7$	328.0583	327.0493	-1.7	34.75	8458691	-H
23	Eupalitin	$C_{17}H_{14}O_7$	330.0740	329.0649	-1.8	35.18	4886534	-H
24	Coccineone B	$C_{16}H_{10}O_6$	298.0477	297.0388	-1.6	37.31	6102940	-H
25	Boeravinone O	$C_{17}H_{12}O_7$	328.0583	327.0494	-1.6	38.26	2343155	-H
26	Boeravinone J	$C_{16}H_{10}O_6$	298.0477	297.0385	-2.0	39.93	181402	-H
27	Boeravinone G	$C_{18}H_{14}O_7$	342.0740	341.0647	-2.0	40.43	5364634	-H
28	Boeravinone B	$C_{17}H_{12}O_6$	312.0634	311.0544	-1.7	42.74	10642541	-H
29	Boeravinone I	$C_{18}H_{14}O_7$	342.0740	341.0649	-1.8	43.47	2618465	-H
30	Boeravinone K	$C_{17}H_{12}O_6$	312.0634	311.0541	-2.0	44.74	704797	-H
31	Boeravinone A	$C_{18}H_{14}O_6$	326.0790	325.0695	-2.3	48.47	1848611	-H
32	Coronaric acid	$C_{18}H_{32}O_3$	296.2351	295.2262	-1.7	49.60	3711082	-H

Conclusion

In conclusion, our phytochemical study of *Boerhavia diffusa* samples collected from various locations along the Gangetic course revealed significant variations in the content of metabolites across different sites. Through meticulous testing, including phytochemical evaluation of tannins, saponins, flavonoids, and polyphenols, as well as advanced techniques like HPTLC and HPLC for boeravinone B, we gained insights into the phytochemical diversity of these samples. Our findings demonstrated consistent presence of tannins and flavonoids across all analysed samples, while saponin content showed variation, with maximum levels in Prayagraj and minimum in Farrukhabad, Uttar Pradesh. Likewise, total polyphenol content exhibited notable differences, being highest in Ballia and lowest in Farrukhabad. Furthermore, HPTLC revealed a diverse array of phytochemicals in the samples, with boeravinone B content ranging from minimum in Mirzapur to maximum in Farrukhabad. Lastly, the study also employed advanced hyphenated techniques, UPLC/MS-QToF, for the identification and quantification of a total of 34 compounds. This advanced methodology allowed for a highly detailed and accurate characterization of the chemical constituents present in the species.

This comprehensive analysis underscores the importance of geographical location in influencing the phytochemical composition of *B. diffusa*, thus providing valuable insights for further research and potential applications in pharmaceutical and herbal medicine industries.

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CHAPTER

06

Insilico Analysis Against Lung Cancer

INTRODUCTION

Lung cancer has become a pervasive health concern among people worldwide. According to GLOBOCAN 2020, lung cancer has occupied the spot of second most prevalent cancer with 2,206,771 cases and the highest mortality incidences of 1,796,144. Lung cancer is histologically divided into two categories, the non-small cell lung carcinoma (NSCLC) and small-cell lung carcinoma (SCLC). Among these two subtypes, only NSCLC reflects 80 to 90% of all diagnosed lung cancer incidences (Woodard et al., 2016; Schabath and Cote, 2019). Albeit for the novel ventures in diagnostic and treatment methodologies, most of the cases of lung cancer are diagnosed only at advanced stages, which is one of the reasons for delayed prognosis (Tao, 2019). Currently available treatment options for lung cancer are frequently associated with cardiac and extra-cardiac complications. For instance, traditionally used anthracyclines, tyrosine kinase inhibitors (TKIs), alkylating agents and anti-metabolites can lead to heart failure (HF), coronary artery disease (CAD), myocarditis, arrhythmias, and vascular and metabolic disturbances (Shyam Sunder et al., 2023). The epidermal growth factor regulator (EGFR) belongs to the family of tyrosine kinase type I receptors, the gene for which is localized at short arm of human chromosome 7.24. The 28 exons in EGFR give rise to a protein that binds epidermal growth factor or heparin-binding EGF, an event that regulates cell growth. In NSCLC, EGFR presents deletions in exon 19 and substitutions in exon 21 L858R. Development of TKIs in order to target EGFR have been found an efficient strategy in combating NSCLC (Yuan et al., 2019). However, as mentioned above, TKIs can lead to cardiac complications (Shyam Sunder et al., 2023), which leaves a gap for the research into safer options with minimal side-effects.

Plant-based medicines have built an important niche for themselves in the present century owing to their financial affordability, availability, and minimal side effects (Nguyen et al., 2021). *Boerhavia diffusa* is an herbaceous plant, belonging to the family Nyctaginaceae (Gour, 2021). This rejuvenating herb is also famous as 'Punarnava', red spiderling, spreading hogweed, tarvine (Pandey et al., 2019). *B. diffusa* is a native to India and Brazil, but it also enjoys distribution in tropical, subtropical, and temperate

regions of American continents, Mexico, Africa, Asia, Indian Ocean islands, Pacific Island, Australia, and Southeast Africa. However, it is still commonly sourced from wild or wastelands, typically in rainy season (Rutwick Surya and Praveen, 2021; Gaur et al., 2022). *Vaidyas*, herbal experts and tribal people of India have utilized this herb to treat various maladies, making it an integral part of Ayurveda, Unani, Siddha, homeopathy and modern pharmaceutical industries (Ali et al., 2022; Gupta et al., 2022). For instance, the whole plant is employed in ayurvedic and Unani medicinal systems to ease the symptoms of bacterial infection, stress, congestive heart failure and diabetes (Prathapan et al., 2011). Moreover, the literature mentions the plant as an immunomodulatory, antifibrinolytic, hepatoprotective, antidiabetic, antioxidant, anti-convulsant, and antioxidant agent (Juneja et al., 2020). The present evidence in support of the plant prompted us to investigate its anti-cancer potential against the persisting problem of lung cancer.

As a result of the previously mentioned issue, a comprehensive *in silico* study was conducted by targeting the EGFR protein, which is a preferred target for therapeutic interventions, in the pursuit of novel drug design and development. Furthermore, our results have established a strong candidature named liriodenine to be used as a drug-like molecule to alleviate lung cancer. To elucidate their dynamic and thermodynamic properties and to support the molecular docking results, molecular dynamics simulation was performed on liriodenine. In addition, to ensure liriodenine's safety and efficacy in the treatment, its drug-likeness, physicochemical properties, and ADMET were investigated.

Etiology of Disease

The occurrence and mortality associated with lung cancer is intimately linked with the indulgence in smoking among all genders. Apart from direct smoking, passive smoking, E-cigarettes, occupational exposures, domestic biomass fuels COPD and other pulmonary conditions, ambient air pollution and other environmental exposures, diet and nutrition, and genetic factors also contributes towards surfacing of lung cancer (Barta et al., 2019; Corrales et al., 2020). At genetic level, various genes have been found to be heavily mutated in lung cancer cells. The most common genetic alteration witnessed in the cases of NSLC and SCLC are p53 mutations. In addition, RB gene and p16 gene, which are involved in progression of cell cycle are frequently inactivated in the case of lung cancer. Furthermore, loss of heterozygosity on chromosome 3p is seen in various cases, which leads to inactivation of several genes, including FHIT, RASSF1, and SEMA3B. Inactivation of tumour suppressor genes, such as PTEN, SMAD4/TGFßRII, and PPP1R3 has been found in both SCLC and NSCLC whereas, K-ras has been found mutated mostly in 15% cases of NSCLC. In rare instances, mutations resulted in activation of ß-catenin gene (Yokota and Kohno, 2004).

Reported Therapeutic Uses

B. diffusa is one of the renowned medicinal plants with numerous therapeutic properties. As supported by several studies, *B. diffusa* is a promising anti-diabetic agent. In one such study, methanolic root extract and its partially purified bioactive fraction managed to lower the fasting blood glucose and glycated hemoglobin in rats (Akhter et al., 2019). Further, the hydro-methanolic extract of the extract showed significant anxiolytic effect in mice during elevated plus maze and open field tests (Kumari et al., 2023). The plant extract exhibited anti-cancer potential, as displayed by the alleviation of skin carcinogenesis in treated mice (Bharali et al., 2003). The whole plant and roots are a preferred remedy against jaundice. In addition, the root juice is utilized to manage asthma, leukorrhea, urinary disorders, rheumatism, and encephalitis (Ghosh, 2022). In addition, the ethanolic extract of the plant showed promising effects towards prevention of pathological hypertrophy and cardiac abnormalities in rats (Prathapan et al., 2017). Table 1 elaborates the therapeutic potential of the *B. diffusa*.

Table 1 List of therapeutic uses of *B. diffusa*

Plant part	Therapeutic use	Therapeutic use identifiers	References
Aerial part, Root, Whole plant	Liver diseases	MESH:D008107, UMLS:C0023895, DOID:409, ICD-11:SA0Z; MESH:D008107, UMLS:C0023895, DOID:409, ICD-11:SA0Z; MESH:D008107, UMLS:C0023895, DOID:409, ICD-11:SA0Z; MESH:D008107, UMLS:C0023895, DOID:409, ICD-11:SA0Z	ISBN:9788172362089; ISBN:9770972795006, ISBN:9788172362089;ISBN:9770972795006; ISBN:9770972795006, ISBN:9788172360481
Flower, Root, Seed	Contraceptive agents	MESH:D003270, UMLS:C0009871, MESH:D003270, UMLS:C0009871, MESH:D003270, UMLS:C0009871	ISBN:9788172360481, ISBN:9770972795006, ISBN:9788172360481
Fruit, Leaves, Root, Whole plant	Diuretics	MESH:D004232, UMLS:C0012798, ICD-11:XM4D06;MESH:D004232, UMLS:C0012798, ICD-11:XM-4D06;MESH:D004232, UMLS:C0012798, ICD-11:XM4D06;MESH:D004232, UMLS:C0012798, ICD-11:XM4D06; MESH:D004232, UMLS:C0012798, ICD-11:XM4D06	ISBN:9770972795006;IS BN:9770972795006, ISB-N:9788172360481;A Report on Medicinal Plants of Kachchh (Gujarat), ISBN:9770972795006, ISBN:9788172361266, ISBN:9788172363130, Me-dicinal Plants of Nagpur and Wardha Forest Divisions (Maha-rashtra);ISBN:9770972795006, ISBN:9788172360481; ISBN:9770972795006, ISBN:9788173717024

Plant part	Therapeutic use	Therapeutic use identifiers	References
Leaves, Root, Seed	Abdominal pain	MESH:D015746, UMLS:C0000737, SYMP:0000457, ICD-11:MD81.4; MESH:D015746, UMLS:C0000737, SYMP:0000457, ICD-11:MD81.4; MESH:D015746, UMLS:C0000737, SYMP:0000457, ICD-11:MD81.4; MESH:D015746, UMLS:C0000737, SYMP:0000457, ICD-11:MD81.4	ISBN:9770972795006, ISBN:9789327275590; Contribution to the Medico-Botany of East Godavari and West Godavari Districts of Andhra Pradesh, ISBN:9789327275590; ISBN:9789327275590; Contribution to the Medico-Botany of East Godavari and West Godavari Districts of Andhra Pradesh, ISBN:9770972795006, ISBN:9788172360481
Leaves	Analgesics	MESH:D000700, UMLS:C0002771, ICD-11:XM49F7	ISBN:9770972795006
Leaves, Root, Seed	Anemia	MESH:D000740, UMLS:C0002871, DOID:2355, ICD-11:3A9Z; MESH:D000740, UMLS:C0002871, DOID:2355, ICD-11:3A9Z; MESH:D000740, UMLS:C0002871, DOID:2355, ICD-11:3A9Z; MESH:D000740, UMLS:C0002871, DOID:2355, ICD-11:3A9Z	ISBN:9789327275590; ISBN:9770972795006, ISBN:9788172361266, ISBN:9789327275590, Medicinal Plants of Nagpur And Wardha Forest Divisions (Maharashtra), The Ayurvedic Pharmacopoeia of India Part-1 Volume-9; ISBN:9789327275590; ISBN:9770972795006, ISBN:9788171360536, ISBN:9788173717024

Plant part	Therapeutic use	Therapeutic use identifiers	References
Leaves	Antifungal agents	MESH:D000935, UMLS:C0003308, ICD-11:XM83G4	ISBN:9770972795006, ISBN:9788172362089
Leaves	Antihypertensive agents	MESH:D000959, UMLS:C0003364, ICD-11:XM2PT6	ISBN:9770972795006
Leaves, Root, Whole plant	Anti-inflammatory agents	MESH:D000893, UMLS:C0003209, ICD-11:XM7XD1;MESH:D000893, UMLS:C0003209, ICD-11:XM7XD1; MESH:D000893, UMLS:C0003209, ICD-11:XM7XD1; MESH:D000893, UMLS:C0003209, ICD-11:XM7XD1	ISBN:9788172360481; ISBN:9770972795006; ISBN:9788172360481; ISBN:9770972795006, ISBN:9788172360481, ISBN:9788173717024
Leaves	Antineoplastic agents	MESH:D000970, UMLS:C0003392; MESH:D000970, UMLS:C0003392	ISBN:9770972795006; ISBN:9788172360481
Leaves, Root	Antirheumatic agents	MESH:D018501, UMLS:C0003191, ICD-11:XM95N2; MESH:D018501, UMLS:C0003191, ICD-11:XM95N2	ISBN:9770972795006, ISBN:9788172360481; ISBN:9770972795006
Leaves, Root	Appetite stimulants	MESH:D019167, UMLS:C0376447; MESH:D019167, UMLS:C0376447; MESH:D019167, UMLS:C0376447	ISBN:9770972795006; ISBN:9770972795006, ISBN:9788172361266; ISBN:9770972795006
Leaves, Root, Seed	Arthralgia	MESH:D018771, UMLS:C0003862, ICD-11:ME82; MESH:D018771, UMLS:C0003862, ICD-11:ME82; MESH:D018771, UMLS:C0003862, ICD-11:ME82	ISBN:9789327275590; ISBN:9789327275590; ISBN:9789327275590

Plant part	Therapeutic use	Therapeutic use identifiers	References
Leaves, Root	Arthritis	MESH:D001168, UMLS:C0003864, DOID:848, ICD-11:FA2Z; MESH:D001168, UMLS:C0003864, DOID:848, ICD-11:FA2Z	ISBN:9788172362089; ISBN:9770972795006
Leaves, Root, Seed	Asthma	MESH:D001249, UMLS:C0004096, DOID:2841, ICD-11:CA23; MESH:D001249, UMLS:C0004096, DOID:2841, ICD-11:CA23; MESH:D001249, UMLS:C0004096, DOID:2841, ICD-11:CA23; MESH:D001249, UMLS:C0004096, DOID:2841, ICD-11:CA23	ISBN:9770972795006, ISBN:9789327275590; ISBN:9770972795006, ISBN:9788172361266, ISBN:9789327275590, Medicinal Plants of Nagpur AndWardha Forest Divisions (Maharash-tra); ISBN:9789327275590; ISBN:9770972795006
Leaves, Root, Seed	Astringents	MESH:D001252, UMLS:C0004110, ICD-11:XM0VK6; MESH:D001252, UMLS:C0004110, ICD-11:XM0VK6; MESH:D001252, UMLS:C0004110, ICD-11:XM0VK6; MESH:D001252, UM-LS:C0004110, ICD-11:XM0VK6	ISBN:9789327275590; ISBN:9789327275590; ISBN:9789327275590; ISBN:9770972795006
Leaves	Common cold	MESH:D003139, UMLS:C0009443, DOID:10459, ICD-11:CA00	ISBN:9770972795006
Root, Seed	Cystitis	MESH:D003556, UMLS:C0010692, DOID:1679, ICD-11:GC00; MESH:D003556, UMLS:C0010692, DOID:1679, ICD-11:GC00	ISBN:9789327275590; ISBN:9789327275590

Plant part	Therapeutic use	Therapeutic use identifiers	References
Leaves	Digestive system diseases	MESH:D004066, UMLS:C0012242, ICD-11:DE2Z	ISBN:9770972795006
Leaves, Root, Seed	Dyspepsia	MESH:D004415, UMLS:C0013395, ICD-11:DD90.3; MESH:D004415, UMLS:C0013395, ICD-11:DD90.3; MESH:D004415, UMLS:C0013395, ICD-11:DD90.3; MESH:D004415, UMLS:C0013395, ICD-11:DD90.3	ISBN:9770972795006, ISBN:9789327275590; ISBN:9789327275590; ISBN:9789327275590; ISBN:9788171360536, ISBN:9788173717024
Leaves	Eczema	MESH:D004485, UMLS:C0013595	ISBN:9770972795006, ISBN:9788172360481
Leaves, Root	Edema	MESH:D004487, UMLS:C0013604, ICD-11:MG29.1; MESH:D004487, UMLS:C0013604, ICD-11:MG29.1; MESH:D004487, UMLS:C0013604, ICD-11:MG29.1	ISBN:9770972795006, ISBN:9788172360481; A Report on Medicinal Plants of Kachchh (Gujarat), ISBN:9770972795006, ISBN:9788172361266, Medicinal Plants of Nagpur And Wardha Forest Divisions (Maharashtra); ISBN:9770972795006, ISBN:9788172360481, ISBN:9788172363130

Plant part	Therapeutic use	Therapeutic use identifiers	References
Leaves, Root, Seed	Endophthalmitis	MESH:D009877, UMLS:C0014236, DOID:4692, ICD-11:9C21; MESH:D009877, UMLS:C0014236, DOID:4692, ICD-11:9C21; MESH:D009877, UMLS:C0014236, DOID:4692, ICD-11:9C21; MESH:D009877, UMLS:C0014236, DOID:4692, ICD-11:9C21	ISBN:9770972795006, ISBN:9788172360481, ISBN:9789327275590; ISBN:9770972795006, ISBN:9789327275590; ISBN:9789327275590; ISBN:9788173717024
Leaves, Root, Seed	Expectorants	MESH:D005100, UMLS:C0015314; MESH:D005100, UMLS:C0015314; MESH:D005100, UMLS:C0015314; MESH:D005100, UMLS:C0015314	ISBN:9789327275590; ISBN:9770972795006, ISBN:9788172361266, ISBN:9788172363130, ISBN:9789327275590; ISBN:9770972795006, ISBN:9789327275590; ISBN:9770972795006, ISBN:9788173717024
Leaves, Root, Seed	Flatulence	MESH:D005414, UMLS:C0016204, ICD-11:ME08; MESH:D005414, UMLS:C0016204, ICD-11:ME08; MESH:D005414, UMLS:C0016204, ICD-11:ME08	ISBN:9789327275590; ISBN:9789327275590; ISBN:9770972795006, ISBN:9789327275590
Leaves, Root, Seed	Gallbladder emptying	MESH:D016473, UMLS:C0085085; MESH:D016473, UMLS:C0085085; MESH:D016473, UMLS:C0085085	ISBN:9789327275590; ISBN:9789327275590; ISBN:9789327275590

Plant part	Therapeutic use	Therapeutic use identifiers	References
Leaves, Root, Seed	Gonorrhea	MESH:D006069, UMLS:C0018081, DOID:7551, ICD-11:1A7Z; MESH:D006069, UMLS:C0018081, DOID:7551, ICD-11:1A7Z; MESH:D006069, UMLS:C0018081, DOID:7551, ICD-11:1A7Z; MESH:D006069, UMLS:C0018081, DOID:7551, ICD-11:1A7Z	ISBN:9770972795006, ISBN:9788172360481; ISBN:9770972795006; ISBN:9770972795006; ISBN:9770972795006, ISBN:9788172363130
Leaves, Root, Seed	Heart diseases	MESH:D006331, UMLS:C0018799, DOID:114, ICD-11:BC4Z; MESH:D006331, UMLS:C0018799, DOID:114, ICD-11:BC4Z; MESH:D006331, UMLS:C0018799, DOID:114, ICD-11:BC4Z; MESH:D006331, UMLS:C0018799, DOID:114, ICD-11:BC4Z	ISBN:9789327275590; ISBN:9770972795006, ISBN:9789327275590; ISBN:9789327275590; ISBN:9770972795006

MeSH ID: Medical Subject Headings database ID of National library of medicine in National Institutes of Health; UMLS ID: Unified Medical Language System database ID of National library of medicine in National Institutes of Health; ICD-11: International Classification of Diseases 11th Revision database ID of World Health Organization.

After an extensive study of the reported traditional medicinal uses of B. diffusa, it was found that this plant exhibits efficacy against a variety of ailments and holds potential for future exploration. Consequently, we have opted to utilize B. diffusa in addressing lung cancer, aiming to contribute to the alleviation of the impact of malignant diseases.

Materials and Methods

Protein Structure Retrieval and Preparation

The crystal structure of EGFR (PDB ID: 5HG8), with mutations at L858R, T790M, V948R, obtained via X-RAY diffraction method at a resolution of 1.42 Å was retrieved from RCSB protein data bank (https://www.rcsb.org/) (Fig. 1). To generate accurate predictions, any of the associated water molecules or heteroatoms were removed from the structures with the assistance of PyMol (https://pymol.org/2/). Further, the protein structures were repaired using protein repair and analysis server (https://www.protein-science.com/) by adding missing hydrogens, side chains and secondary structure elements. The final structure was saved in PDB format for further studies.

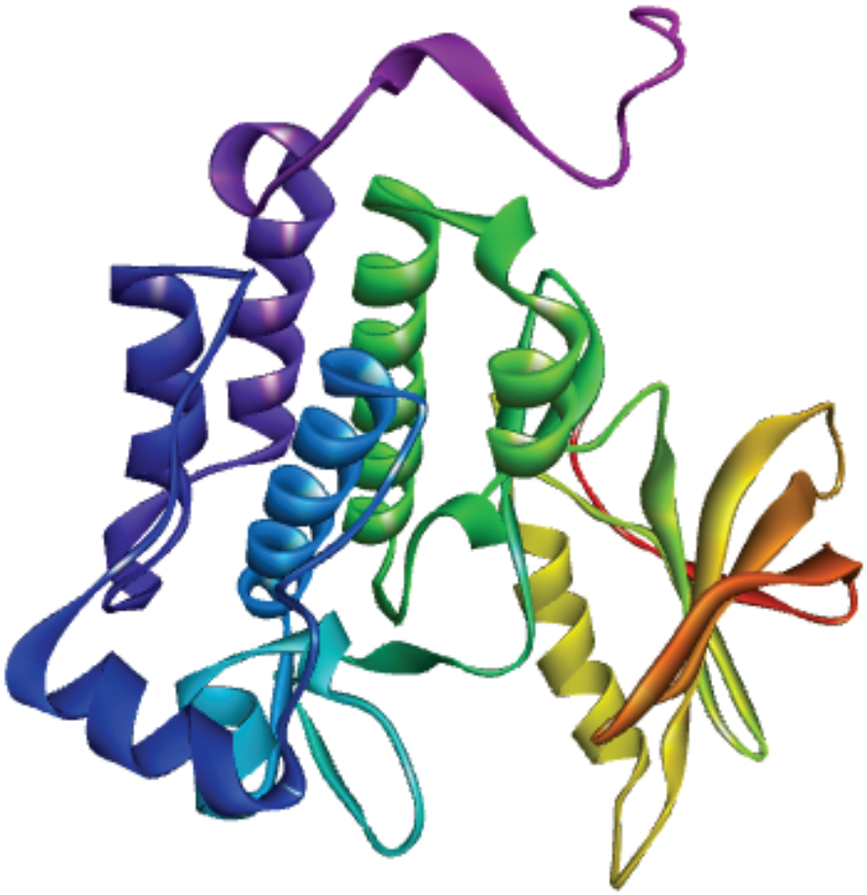

Fig. 1 Protein structure of EGFR

Phytochemical Library Preparation

Extensive literature survey was performed to obtain a list of 70 phytoconstituents present in *B. diffusa*. The three-dimensional structures of the compounds in SDF format were retrieved from PubChem database (https://pubchem.ncbi.nlm.nih.gov/). In addition, the SDF files were converted into PDB format by utilizing Open Babel GUI (https://openbabel.org/wiki/Main_Page), to facilitate the process of molecular docking.

Rule of Five (RO5)

For initial prescreening of phytoconstituents, an online web-based platform, ADMET lab 2.0 (https://admetmesh.scbdd.com/) was utilized to predict the drug-likeliness of the compounds. For small molecules, it is an empirical rule of thumb to assess their physicochemical aspects, to refine their drug ability. In the present study, we analyzed several properties including molecular weight (MW), partition coefficient (LogP), number of hydrogen bond acceptors (nHA) and number of hydrogen bond donors (nHD). As these attributes form the foundation for the Lipinski rule of five, any molecule deviating from the threshold values was eliminated from further analysis (Lipinski, 2004).

Molecular Docking

In the current study, PyRx software (https://pyrx.sourceforge.io/) was utilized for the high-throughput molecular docking study. The prepared PDB structures of target proteins and plant phytoconstituents were imported into the software and were processed to generate the respective PDBQT files. Blind molecular docking was performed with grid box sizes x = 47.670, y = 62.115 and z = 61.470. Moreover, the protein-ligand complexes were prepared using PyMol. These complexes were imported to Discovery Studio Visualizer v21.1.0.20298 (https://discover.3ds.com/discovery-studio-visualizer-download) for visualization of various interactions formed between the target and phytoconstituents.

Bioavailability Radar, Bioactivity Score and PASS Prediction

Drug-likeliness of screened candidate with best binding energy was analysed in a comprehensive way using SwissADME web-based tool (http://www.swissadme.ch/index.php). To do so, physiochemical properties such as size (MW), lipophilicity (XLOGP3), polarity (TPSA), solubility (LogS), flexibility (fraction Csp3), and saturation (rotatable bonds) were investigated and a bioavailability radar diagram representing these parameters was obtained (Lipinski et al., 2012). The region highlighted in pink delineates the optimal parameter values, and substantial deviations from these values on a significant scale imply that the ligand may not possess favourable oral bioavailability (Daina et al., 2017). The bioactivity scores were determined by using Molinspiration software (http://www.molinspiration.com/). Parameters analysed

include ion channel modulator (ICM), nuclear receptor ligand (NRL), protease inhibitor (PI), enzyme inhibitor (EI), G protein-coupled receptor ligand (GPCR ligand). In addition, to validate the lead compound as a suitable drug candidate, we performed prediction of bioactivity spectra for biologically active substances, using PASS online (http://www.way2drug.com/passonline/) webserver. PASS online utilizes the structural formula of a compound to estimate its biological activities. The probability for active compound (Pa) and probability for inactive compound (Pi) lie between 0 to 1, with Pa > 0.7 indicating high chances of finding the experimental activity (Filimonov et al., 2014).

In silico ADMET analysis

After molecular docking studies of all the compounds with targeted protein of ZIKV, pharmacokinetics parameters such as ADMET (absorption, distribution, metabolism, excretion and toxicity) of the best candidate were investigated using ADMETlab 2.0 (https://admetmesh.scbdd.com/). ADMET constitutes a crucial aspect of drug design and development, encompassing the assessment of significant pharmacokinetic parameters (Olasupo et al., 2020). ADMET properties include absorption (P-glycoprotein substrate, P-glycoprotein inhibitor, human intestinal absorption, Caco-2 permeability, MDCK permeability, human oral bioavailability 20%, and human oral bioavailability 30%); distribution: (plasma protein binding, blood-brain barrier penetration, volume distribution, and fraction unbound in plasms); metabolism (CYP 1A2/2C19/2C9/2D6/3A4 inhibitor and CYP 1A2/2C19/2C9/2D6/3A4 substrate); excretion (clearance of a drug and half-life of a drug); toxicity (skin sensitization, carcinogenicity, respiratory toxicity, rat oral acute toxicity, eye irritation/corrosion, Ames test for mutagenicity and maximum recommended daily dose) (Xiong et al., 2021).

Molecular Dynamics Simulation

On the basis of molecular docking analysis, top phytochemical of *B. diffusa* was further selected for molecular dynamics (MD) simulation to examine its stability and intermolecular interactions using Desmond molecular dynamics study software (Bowers et al., 2006). Using the protein preparation wizard of the Desmond-maestro interface, all protein-ligand contacts were pre-processed and improved. The system was configured for each complex using the SPC model of solvent, an orthorhombic box simulation box was calculated using the buffer method with a distance of 10 × 10 × 10 Å. Later, counter ions (Na^+ or Cl^-) were added for neutralization, system's concentration was set to 0.15 M and OPLS3e force field was applied to the system. This generated system was simulated for 100 ns in a minimized system in the NPT ensemble. After simulation, the trajectory was analysed using parameters such as root mean square deviation (RMSD), root mean square fluctuation (RMSF), protein-ligand interactions, radius of gyration of the lead compound, and ligand torsion analysis using the simulation interaction diagram utility of the Desmond.

Results and Discussion

Despite of cutting-edge research and development taking place to produce innovative solutions to combat cancers, lung cancer has become the most common cancer, second only to the breast cancer (GLOBOCAN 2020). Mutations in the epidermal growth factor receptor (EGFR) gene are a well-established driver of NSCLC progression, particularly in adenocarcinomas, and represent a significant area of therapeutic focus (Canale et al., 2022). EGFR is a transmembrane protein that plays a critical role in cell growth, proliferation, differentiation, and survival. Following ligand binding, EGFR undergoes activation through a process known as phosphorylation, which initiates downstream signalling pathways that regulate these cellular processes (Passaro et al., 2021). Mutations in the EGFR gene can lead to constitutive activation, even in the absence of ligand binding, promoting uncontrolled cell growth and the development of malignancies (Dong et al., 2021). The sole objective of this study is to identify potential compounds from *B. diffusa* with good binding affinity against lung cancer protein EGFR, using diverse computational tools.

Preliminary Screening of Drug-likeness

In the pursuit of creating a highly effective drug with minimal adverse effects, preliminary screening on the basis of drug-likeness is a crucial step. Drug-likeness refers to the physicochemical properties that influence a molecule's ability to become a successful drug (Kar and Leszczynski, 2020). Molecules with poor drug-likeness characteristics are more likely to exhibit unfavourable pharmacokinetic properties, such as poor absorption, distribution, metabolism, and excretion, leading to failure in later development stages (Agamah et al., 2020). To conduct this preliminary screening, Lipinski's rule of five was utilized for evaluating all the phytoconstituents derived from *B. diffusa.* A compound demonstrates favourable oral bioavailability when its molecular weight (MW) ⩽ 500 Da, hydrogen bond donors (HbD) ⩽ 5, hydrogen bond acceptors (HbA) ⩽ 10, and an octanol-water partition coefficient log P ⩽ 5 (Lipinski, 2004). Compounds that violated more than one parameter were excluded, while the remaining compounds were identified as ligands for the subsequent docking study. As illustrated in Table 2, out of the total 97 phytoconstituents, 79 candidates successfully passed Lipinski's rule and were carried forward for further analysis. Lipinski's rule of five provides a valuable initial filter for prioritizing drug candidates with a higher probability of success in preclinical and clinical development. Compounds violating these rules may still exhibit good activity but are more likely to encounter challenges in formulation, absorption, or clearance (Tinworth and Young 2020).

Table 2 Screening of phytochemicals based on Lipinski's rule

Phytochemical	LogP	MW	nHA	nHD	nV
Caffeoyltartaric acid	-0.368	312.05	9	5	0
N, N'-Ethylenebis(2-[2-hydroxy-phenyl])glycine	-1.404	360.13	8	6	1
Ethylene glycol	-1.149	62.04	2	2	0
3,4-Dihydroxy-benzyl alcohol	-0.105	140.05	3	3	0
4-Methylcatechol	1.505	124.05	2	2	0
D-Pinitol	-2.423	194.08	6	5	0
Ferulic acid	1.803	194.06	4	2	0
Caffeic acid	1.43	180.04	4	3	0
1-Monopalmitin	5.812	330.28	4	2	0
trans-2-Decen-1-ol, trifluoroac-etate	4.805	252.13	2	0	0
Cholesta-4, 6-dien-3-ol, benzo-ate, (3.beta.)-	7.969	488.37	2	0	1
Cholest-5-en-3-ol (3.beta.)-, propanoate	7.85	442.38	2	0	1
Stigmasterol	7.436	412.37	1	1	1
.beta.-D-Glucopyranose, 1,6-an-hydro-	-1.943	162.05	5	3	0
Phen-1,5-diol, 2-[3-oxododeca-noyl]-	4.555	306.18	4	2	0
Methyl 4-o-methyl d-arabinopy-ranoside	-1.397	178.08	5	2	0
Oxirane, tetradecyl-	6.795	240.25	1	0	0
2-Hexadecene, 3,7,11,15-te-tramethyl-, [R-[R*,R*-(E)]]-	8.708	280.31	0	0	1
3,7,11,15-Tetramethyl-2-hexade-cen-1-ol	7.764	296.31	1	1	1
Phthalic acid, 4-bromophenyl octyl ester	6.599	432.09	4	0	1

Phytochemical	LogP	MW	nHA	nHD	nV
Oxirane, octyl-	3.869	156.15	1	0	0
2-(Dimethylamino)ethyl 2-methylacrylate	1.18	157.11	3	0	0
1,2-Benzenedicarboxylic acid	1.187	166.03	4	2	0
Cholesta-6,22,24-triene, 4,4-dimethyl-	8.016	394.36	0	0	1
Methyl 10,12-pentacosadiynoate	9.429	388.33	2	0	1
Urs-12-ene	8.657	410.39	0	0	1
Squalene	12.248	410.39	0	0	1
Cholesta-8, 24-dien-3-ol, 4-methyl-, (3beta, 4alpha)-	7.225	398.35	1	1	1
1-Hexanesulfonic acid, methyl ester	1.91	180.08	3	0	0
Ethyl iso-allocholate	2.981	436.32	5	3	0
beta-Sitosterol	7.663	414.39	1	1	1
beta-Sitosterol acetate	7.973	456.4	2	0	1
Phthalic acid, monoamide, N,N-diheptyl, pentyl ester	8.33	431.34	4	0	1
Xanthine, 8-[3-iodocyclopentyl]-1,3-dipropyl-	4.22	430.09	6	1	0
Benzamide, N,N-didecyl-4-methoxy-	9.329	431.38	3	0	1
14-Acetyldictyocarpine	1.291	535.28	10	1	1
Vobtusine, anhydrode(methoxycarbonyl)-	3.456	642.36	7	0	1
6-Aza-5,7,12,14-tetrathiapentacene	6.192	354.96	1	0	1
Benzeneethanamine,	1.285	121.09	1	2	0
4-(2-diethylaminosulfonyll-4-nitrophenyl)-	1.649	386.16	9	1	0
Amodiaquine	4.714	355.15	4	2	0

Phytochemical	LogP	MW	nHA	nHD	nV
bis(.eta.-5-piperidinylcyclopenta-dienyl)-	2.667	153.15	1	0	0
beta-Lumicolchicine	2.102	399.17	7	1	0
6,6'-Bis(phenylethynyl)-2,2'-bi-pyridine	6.181	356.13	2	0	1
alpha-Tocopherol	9.624	430.38	2	1	1
Eupomatilone-3	3.141	430.16	8	0	0
Irieol	5.895	446.08	1	1	1
9-Deacetoxy-14,15-deepoxyxe-niculin	4.799	402.24	5	0	0
Kanzonol M	5.67	398.17	6	2	0
Oxirane	-0.149	44.03	1	0	0
cis-Vaccenic acid	7.131	282.26	2	1	1
Oxacycloheptadec-8en-2-one, (8Z)	5.719	252.21	2	0	0
9,10 anthracenedion	3.414	208.05	2	0	0
cis-13-Eicosenoic acid	7.93	310.29	2	1	1
Boerhavin A	3.651	300.16	3	0	0
4,25-Secoobscurinervan	2.485	486.27	8	1	0
Quercetin	2.155	302.04	7	5	0
3,5,7,2,5 Penta hydroxyl flavone	2.118	302.04	7	5	0
Boeravinone A	2.53	326.08	6	2	0
Boeravinone B	1.887	312.06	6	3	0
Liriodenine	3.606	275.06	4	0	0
Eupalitin	2.806	330.07	7	3	0
boeravinone F	2.706	326.04	7	3	0
Boerhavisterol	8.02	414.39	1	1	1
Boerhadiffusene	10.903	414.42	0	0	1
Diffusarotenoid	3.776	396.12	7	2	0

Phytochemical	LogP	MW	nHA	nHD	nV
3,3',5-Trihydroxy-7-methoxyflavone	3.344	300.06	6	3	0
4',7-Dihydroxy-3'-methylflavone	3.671	268.07	4	2	0
Boeravinone D	2.21	342.07	7	3	0
Boeravinone E	1.123	328.06	7	4	0
Boeravinone C	2.856	344.09	7	3	0
D-Glucuronic Acid	-2.767	194.04	7	5	0
Repenone	1.686	372.05	9	3	0
Repenol	1.344	388.04	10	4	0
1-Triacontanol	12.664	438.48	1	1	1
Ursolic acid	6.083	456.36	3	2	1
Lunamarine	3.331	309.1	5	0	0
20-Hydroxyecdysone	1.317	480.31	7	6	1
Boeravinone G	2.48	342.07	7	2	0

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

Molecular Docking Analysis

Molecular docking plays a vital role as a computational tool in the field of drug discovery. It was performed on the ligands that had undergone the pre-screening. In the current study, the selected targeted protein is docked against screened phytochemicals to examine their binding affinity. The docking results of top 10 lead compounds against each studied protein are detailed in Table 3. On the basis of Lipinski violation and docking results evaluation, it was found that liriodenine was able to bind the target protein with highest binding affinity of -10.1 kcal/mol and 2 hydrogen bonds with residue MET A:793 at distances of 1.67 and 2.26 Å (Table 4; Fig. 2). In addition, liriodenine showed 0 Lipinski violations, making it a suitable candidate for further analysis. In a related study focused on identifying anti-cancer agents targeting EGFR, alliin, a compound from *Allium sativum*, demonstrated a binding energy of -7.4 Kcal/mol (Sarkar et al., 2022). Similarly, liriodenine from *Goniothalamus elegans* exhibited a binding affinity of -9.7 Kcal/mol towards EGFR (PDB ID: 1XKK) and formed two hydrogen bonds with the residues Lys745 and Cys797 (Tran et al., 2022). Liriodenine, a cytotoxic isoquinoline alkaloid found in various plant genera, is known for its broad-spectrum pharmacological properties (Chang et al., 2004). The anti-cancer effects of liriodenine have been widely documented in scientific

literature, highlighting its potential against lung cancer, ovarian cancer, breast cancer, colon cancer, and other malignancies (Chang et al., 2004; Chen et al., 2012; Nordin et al., 2015; Li et al., 2017). Based on these findings, liriodenine was selected for further evaluation of additional drug-likeness parameters.

Table 3 Molecular docking results of top 10 phytoconstituents

Phytochemical	Binding Affinity (kcal/mol)
Liriodenine	-10.1
Vobtusine anhydrode(methoxycarbonyl)-	-9.8
Ursolic acid	-9.6
Cholesta-6,22,24-triene-4 4-dimethyl-	-9
Boeravinone F	-9
Urs-12-ene	-8.9
9,10 anthracenedion	-8.8
Repenone	-8.8
6,6-Bis(phenylethynyl)-2 2-bipyridine	-8.7
Boeravinone A	-8.7

Table 4 Hydrogen bond interactions formed by Liriodenine

Phytochemical	Hydrogen bonds	Residue	Distance (Å)
Liriodenine	2	MET A:793	1.67, 2.26

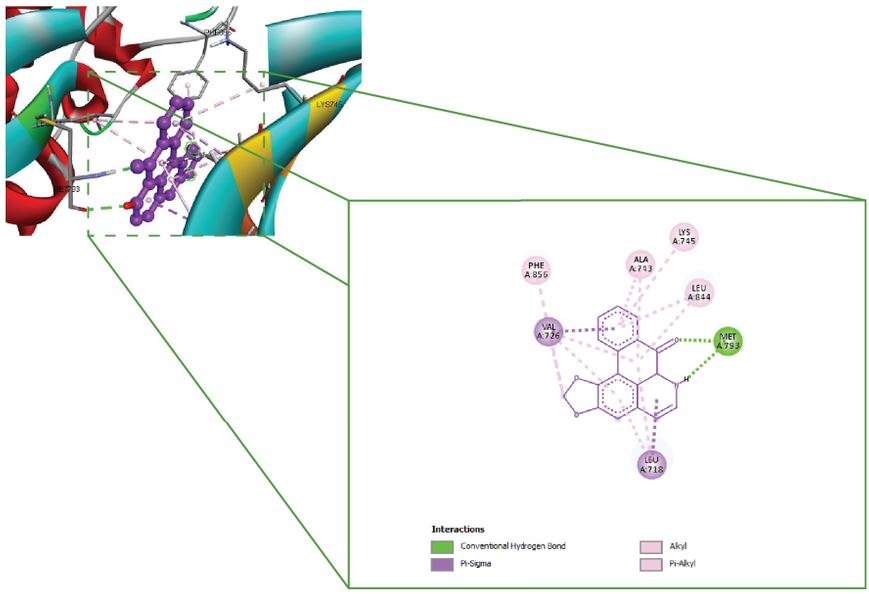

Fig. 2 Illustration of EGFR protein with Liriodenine

Bioavailability Radar, Bioactivity score and PASS prediction

Bioavailability is an important parameter of the drug development process (Wei et al., 2022). In this study, bioavailability was evaluated for candidate with best binding affinity *i.e.* ursolic acid. As shown in the Fig. 3, the compound depicted complete rigidity and insaturation (fraction Csp3) of 0.06, apart from this, all of the bioavailability parameters were within the prescribed range (Daina et al., 2017). Overall, the compound was found to possess acceptable bioavailability with a score of 0.55.

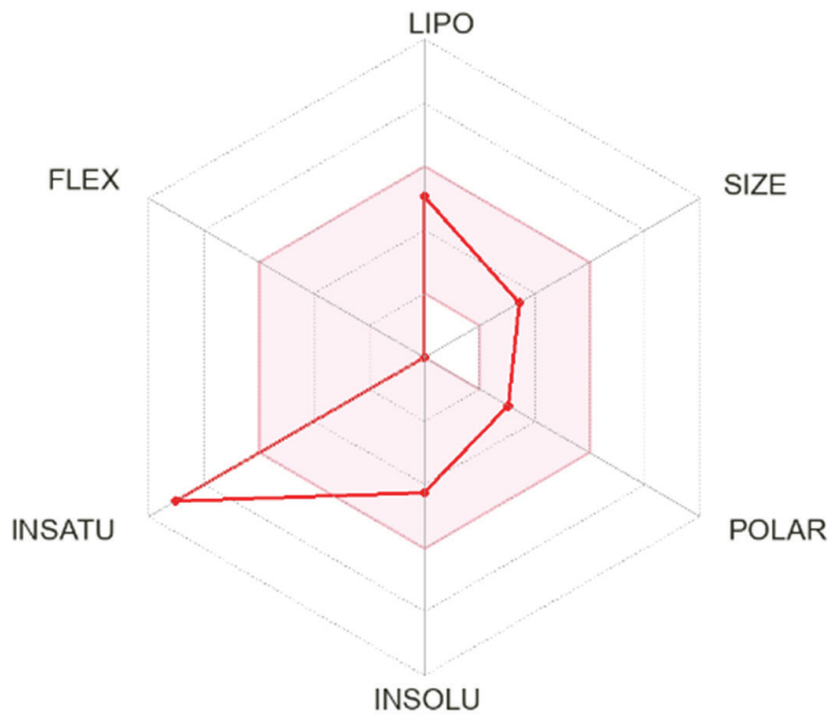

Fig. 3 Bioavailability radar of Liriodenine

In addition, presence of various bioactivities, including G protein coupled receptor (GPCR) ligand, ion channel modulator, kinase inhibitor, nuclear receptor ligand and protease inhibition were assessed for liriodenine using Molinspiration online tool. In general, organic molecules are considered active when bioactivity score is >0, moderately active if score is between -5.0 to 0.0 and inactive when the score is <-5.0 (Kulkarni et al., 2021). As elaborated in the Fig. 4, the lead compound possessed potent kinase inhibition and enzyme inhibition activities. Kinase inhibition activity has emerged as a promising therapeutic strategy in lung cancer treatment. Kinases are enzymes that play a crucial role in cell signalling pathways, regulating various cellular processes like

growth, proliferation, and survival. In lung cancer, mutations or abnormal activation of specific kinases can contribute to uncontrolled cell growth and tumour formation (Qin et al., 2020). Moreover, moderate GPCR ligand, ion channel modulation, nuclear receptor ligand inhibition and protease inhibition activities were also found.

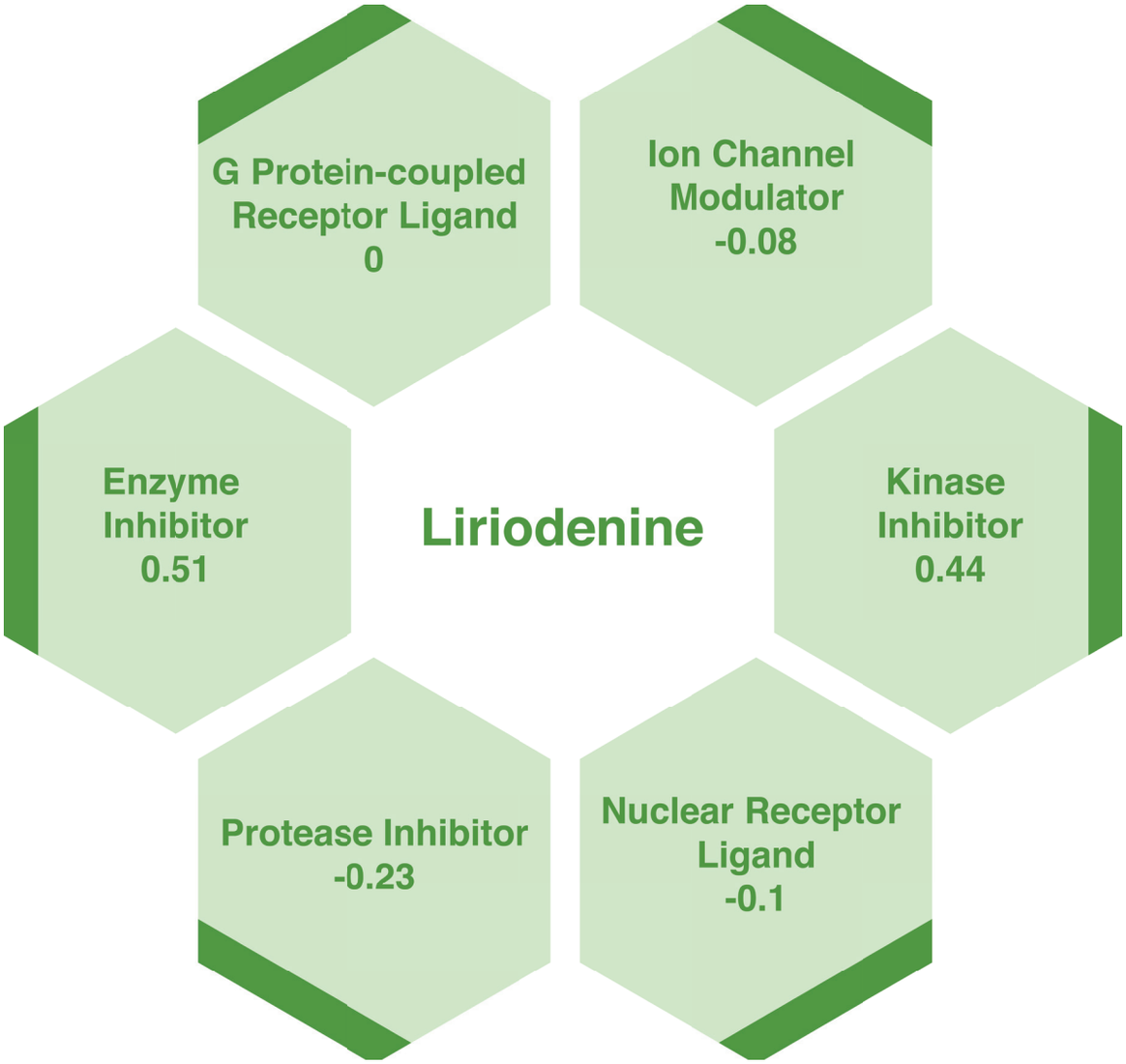

Fig. 4 Bioactivities of Liriodenine

Furthermore, Table 5 presents the top ten outcomes of PASS prediction. Liriodenine possess, the desired antineoplastic activity against lung cancer with Pa value of 0.656. Apart from this, anti-neoplastic activity against colorectal and colon cancers were also detected.

Table 5 PASS prediction of Liriodenine

Pa	Pi	Activity
0.889	0.002	Neurotransmitter uptake inhibitor
0.784	0.014	Antineoplastic
0.763	0.008	Caspase 3 stimulant
0.71	0.015	Alkane 1-monooxygenase inhibitor
0.689	0.005	Antineoplastic (colorectal cancer)
0.678	0.006	Antineoplastic (colon cancer)
0.68	0.014	Kinase inhibitor
0.656	0.007	Antineoplastic (lung cancer)
0.638	0.003	Antineoplastic alkaloid
0.629	0.005	Caspase 8 stimulant

In silico ADMET Analysis

In the drug discovery pipeline, a critical bottleneck lies in identifying promising lead compounds with favourable pharmacokinetic (ADME) and toxicological (T) properties. Traditionally, these properties are assessed through extensive *in-vitro* and *in-vivo* experiments, which are time-consuming, resource-intensive, and often require large quantities of the candidate drug. *In silico* ADMET analysis offers a powerful alternative approach to evaluate these properties computationally, playing a crucial role in modern drug discovery (Pradeepkiran et al., 2021). Table 6 exhibits results of ADMET prediction of lead compound liriodenine. The information in the Table has been analysed on the basis of explanation given on the ADMETlab 2.0 webserver. Liriodenine depicted excellent probability of being a Pgp-inhibitor, as well as substrate. P-glycoprotein (P-gp) is a transmembrane efflux pump expressed in various tissues throughout the body, including the intestinal epithelium and the blood-brain barrier. It plays a critical role in limiting drug absorption and distribution by actively transporting xenobiotics, including many therapeutic drugs, out of cells. Whereas a P-gp substrate is a molecule recognized and actively transported by P-gp. This leads to reduced intracellular drug accumulation at its site of action, potentially compromising its therapeutic efficacy (Seelig, 2020). Furthermore, the compound displayed potent HIA, F (20%), Caco-2 permeability and MDCK permeability whereas, the F (30%) was found to be medium. Under the parameters of distribution, the compound showed excellent VDss and BBB however, the PPB and Fu were found to be lower. VDss represents the theoretical volume in which a drug would need to be uniformly distributed to achieve its observed plasma concentration. A low VDss indicates high concentration in blood plasma, likely due to high plasma protein binding

or water solubility. A high VDss suggests extensive tissue distribution, possibly due to high lipid solubility or tissue binding (Smith et al., 2015*). In silico* models can predict the likelihood of a drug crossing the BBB based on its properties. A drug that readily crosses the BBB is more likely to reach its target in the central nervous system but may also lead to unwanted central nervous system side effects (Pandit et al., 2020). PPB refers to the fraction of a drug bound to plasma proteins like albumin, with only the unbound drug being pharmacologically active, meaning a high PPB results in less free drug available to exert its effect and may lead to a longer duration of action. In addition, FU is the unbound fraction of the drug in plasma, representing the pharmacologically active portion (Di, 2021). To explain the metabolism of liriodenine, the Table 6 depicts probabilities of being CYP1A2, CYP2C19, CYP2C9, CYP2D6, CYP3A4 inhibitors and substrates, with values ranging from 0 to 1. A CYP substrate is a compound that is metabolized by a specific CYP enzyme whereas, A CYP inhibitor is a drug or compound that can decrease the activity of a specific CYP enzyme (Deodhar et al., 2020). Finally, the lead compound displayed short half-life and moderate clearance, among the excretion properties. Lastly, the lead compound depicted acceptable toxicity parameters.

Table 6 In silico ADMET analysis of Liriodenine

Property		Liriodenine
Absorption	Pgp-inh	0.198
	Pgp-sub	0
	HIA	0.003
	F(20%)	0.002
	F(30%)	0.389
	Caco-2	-4.863
	MDCK	1.90E-05
Distribution	BBB	0.294
	PPB	97.98%
	VDss	0.853
	Fu	1.58%
Metabolism	CYP1A2-inh	0.976
	CYP1A2-sub	0.169
	CYP2C19-inh	0.879
	CYP2C19-sub	0.099
	CYP2C9-inh	0.628

Property		Liriodenine
	CYP2C9-sub	0.879
	CYP2D6-inh	0.74
	CYP2D6-sub	0.821
	CYP3A4-inh	0.82
	CYP3A4-sub	0.135
Excretion	CL	11.173
	T12	0.121
Toxicity	Ames	0.92
	ROA	0.053
	FDAMDD (maximum recommended daily dose)	0.87
	SkinSen	0.131
	Carcinogenicity	0.946
	EC	0.004
	EI	0.651
	Skin Sensitization	2

Molecular Dynamics Simulations

On the basis of molecular docking, bioavailability, bioactivity, and ADMET analysis, liriodenine fulfilled all the parameters and was therefore, subjected to molecular dynamics simulations for 100 ns. The dynamic behaviours or molecular stability of EGFR-liriodenine complex was observed through root-mean square deviation (RMSD), root mean square fluctuation (RMSF), protein-ligand interaction analysis, and Ligand torsion analysis.

RMSD of EGFR- Liriodenine Complex

RMSD analysis gives an idea whether the simulation has equilibrated or not, with acceptable fluctuations ranging between 1 to 3 Å. Fig. 5 depicts RMSD plots for simulated protein- liriodenine complex. As clear from the figure, the trajectory of c-alpha showed increase in the beginning of the reaction, the RMSD stabilized up to 28.80 ns. Post that, there was a slight increase in C-alpha RMSD, followed by stabilization up to 65.40 ns. The c-alpha trajectory again showed fluctuations from 65.40 to 90.70 ns before finally attaining equilibrium from 97.10 ns to the end of the simulation period. In addition, the figure gives insights on the ligand-bound protein RMSD plot, where the complex displayed an initial increase in RMSD trajectory, followed by achievement of equilibrium from 6.30 ns

to 65.90 ns. After that, the trajectory depicted slight fluctuations and overlapping of RMSD trajectories of c-alpha and ligand-bound protein from 75.70 to 79.90 ns and 90.20 to 92.60 ns, which indicate formation of a stable complex.

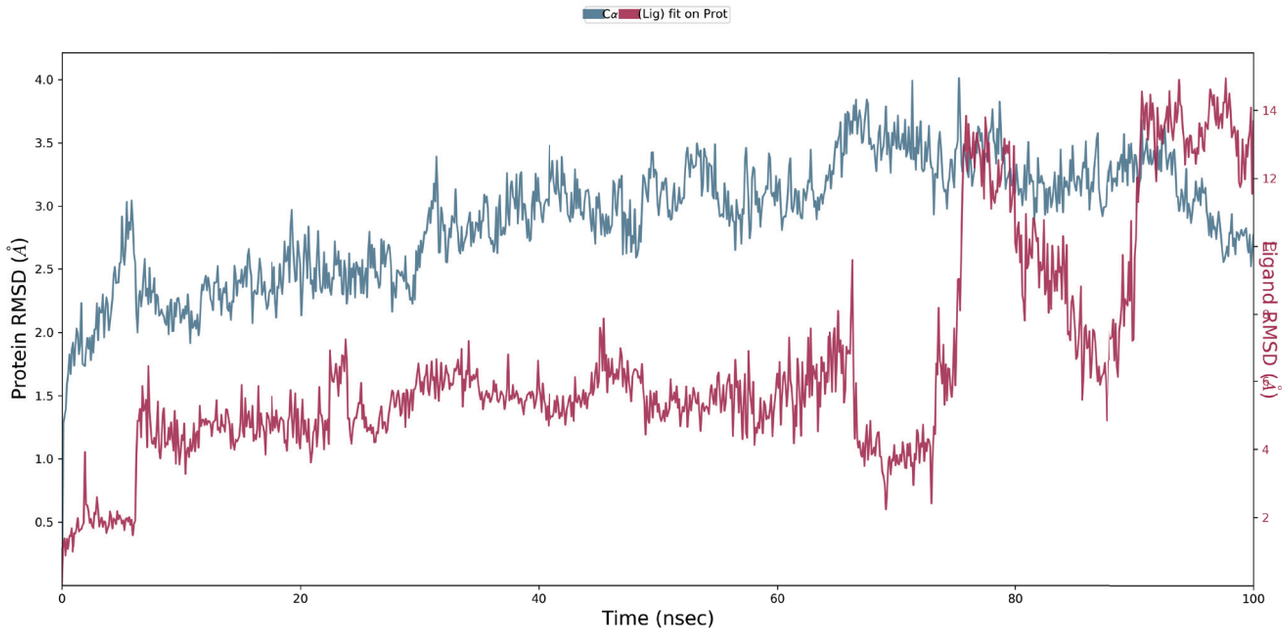

Fig. 5 RMSD trajectory for EGFR-Liriodenine complex

RMSF of EGFR- Liriodenine Complex

RMSF is a measure of the displacement of the reference structure over the number of atoms. In Fig. 6, peaks indicate areas of the protein that fluctuate the most during the simulation. Typically, the tails (*N*- and *C*-terminal) fluctuate more than any other part of the protein. Secondary structure elements like alpha helices and beta strands are usually more rigid than the unstructured part of the protein, and thus fluctuate less than the loop regions. The protein residues that interact with the ligand are marked with green-coloured vertical bars. Generally, RMSF value >2.5 Å points towards less stable bonds (Fatriansyah et al., 2022). As shown in figure, the RMSF plot of c-alpha depicts fifteen peaks, exceeding the threshold value of 2.5 Å. These peaks are occupied by ASN 700, GLY 735, LYS 737, THR 751, LYS 754, LYS 757, GLU 758, SER 921, SER 924, GLU 928, GLU 985, PRO 992, THR 993, ASN 996 and TYR 998. In addition, the residues responsible for making protein-ligand contacts showed RMSF values within the permissible range, depicting stable bonds.

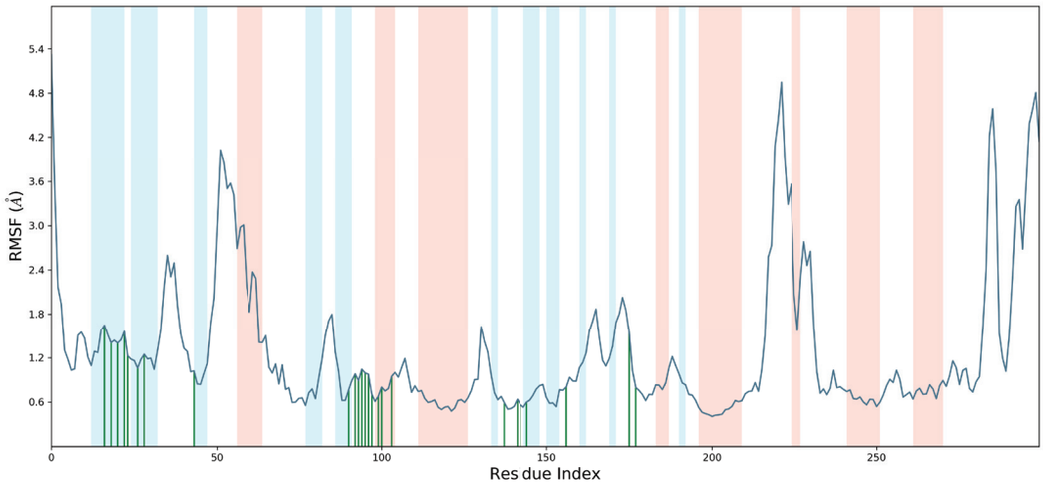

Fig. 6 RMSF for EGFR-Liriodenine complex

Protein-Ligand Contacts

Protein interactions with the ligand can be monitored throughout the simulation. These interactions can be categorized into four types: hydrogen bonds, hydrophobic, ionic and water bridges. Consideration of hydrogen bonds is importance due to their strong influence on drug specificity. As shown in Fig. 7, the majority of the significant ligand-protein interactions determined with MD are water bridges, hydrogen bonds, hydrophobic interactions. Four residues participated in formation of hydrogen bonds i.e. ASP 800, CYS 797, MET 793 and SER 720, where ASP 800 showed the highest interaction fraction of 0.338. The protein-ligand interactions maintained for more than 30% of the simulation time are showed in Fig. 7.

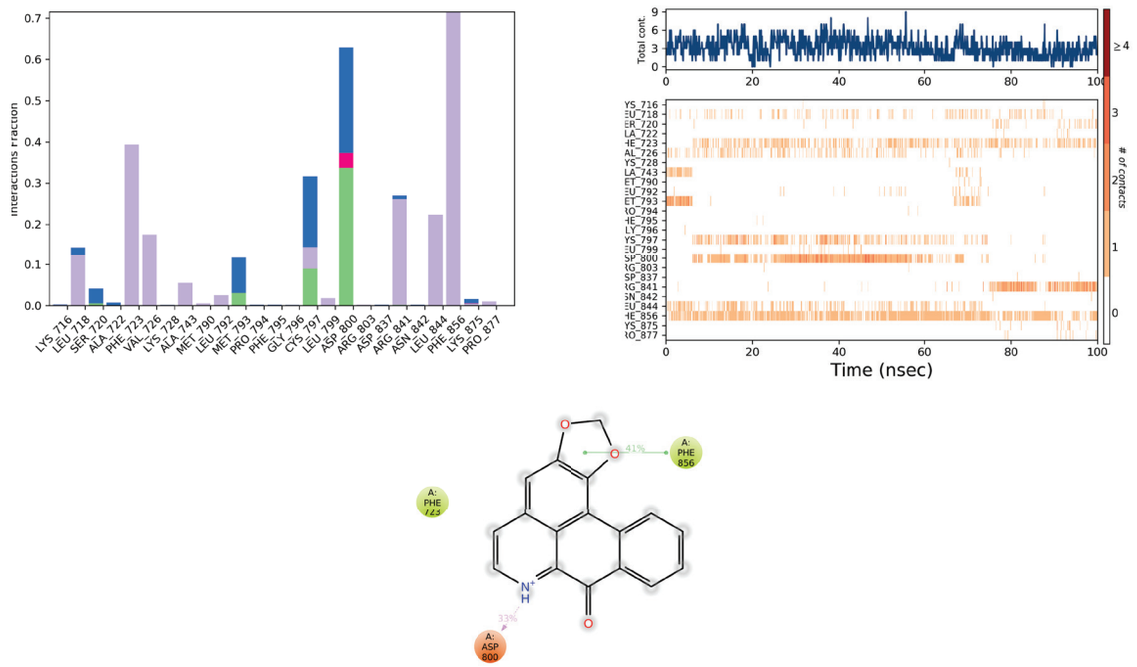

Fig. 7 Illustration of protein-ligand contacts in EGFR-Liriodenine complex

Ligand Torsion Analysis

The ligand, liriodenine was completely rigid without any flexible bonds. Hence, no results were obtained for ligand torsion analysis.

Conclusion

In conclusion, this comprehensive computationalstudyhasunveiledthesubstantial potential of bioactive phytochemicals derived from the medicinal plant *B. diffusa* in targeting the EGFR protein. Through the adept utilization of virtual screening, encompassing molecular docking analysis and molecular dynamics simulations, we have provided evidence of the therapeutic promise of liriodenine in combating lung cancer. Our molecular docking analysis has underscored the robust binding affinity of liriodenine with the target protein, indicating its potential as an inhibitor for EGFR-mediated lung cancer progression. Furthermore, liriodenine exhibited favourable pharmacokinetic properties, bioavailability, and bioactivities, affirming its safety and suitability as a prospective drug candidate. The insights gained from molecular dynamics simulations have further substantiated the stability of the liriodenine-EGFR protein complex, shedding light on their potential as an effective therapeutic strategy against lung cancer. In essence, this research has shed light on the promising potential of liriodenine as a natural therapeutic agent targeting EGFR, a pivotal player in the pathogenesis of lung cancer. These findings provide a solid foundation for future investigations and the development of targeted therapies for lung cancer, fostering optimism for improved treatment options and enhanced outcomes for patients afflicted with this devastating disease.

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CHAPTER

07

Reported Pharmacological Profile

INTRODUCTION

Boerhavia diffusa L. (Nyctaginaceae) is a well-known medicinal plant in traditional Indian medicine as well as in other regions of the world, such as Southern America and Africa (Govindarajan et al., 2005). The genus '*Boerhavia*' is named after Hermann Boerhaave, a distinguished 18th-century Dutch physician, while the species '*diffusa*' is named for the plant's distinctive diffuse branching (Mishra *et al.,* 2014). *B. diffusa* is a well-known medicinal plant that is used to cure a wide range of human illnesses as mentioned in 'Ayurveda', 'Charaka Samhita', and 'Sushrita Samhita'. More than 35 distinct types of ayurvedic medicines use it as a significant component. This species has been classified as a *rasayana* herb, which is said to have anti-aging, reestablishing youth, strengthening life and brain power, and disease prevention properties, all of which imply that they increase the body's resistance to any onslaught, in other words, providing hepatoprotection and immunomodulation (Govindarajan et al., 2005). Because of its regeneration qualities during the rainy season, *B. diffusa* is known as 'Punarnava' in traditional medicine. It is a creeping perennial plant with drooping branches that may grow up to 1 m long. During rains, it is plentiful in ditches, marshy areas, and waste areas (Pooja *et al.,* 2017). The plant in whole or its peculiar parts (aerial parts and roots) have a numerous medicinal property and are used by endemic and tribal people in India and Unani medicine in Arab countries to show anti-bacterial, anti-nociceptive, hepatoprotective, hypoglycaemic, anti-estrogenic, anti-inflammatory, anti-stress, and anti-metastatic activities and, also in treatment of stress, dyspepsia, abdominal pain, inflammation, jaundice, etc. Furthermore, animal investigations and clinical trials have verified the presence of actions such as immunomodulation, anti-fibrinolysis, anti-cancer, and diuresis.

Pre-clinical Study

Pre-clinical studies often begin with a phytochemical analysis to identify and isolate the active compounds present in *B. diffusa.* Various classes of phytochemicals, such as alkaloids, flavonoids, saponins, and triterpenoids, have been identified in the plant. *B. diffusa* has been the subject of various pre-clinical studies to investigate its potential medicinal properties. Pre-clinical studies typically involve research conducted on animals or *in vitro* (outside the human body) to assess the safety and efficacy of a substance before moving on to clinical trials. Further research, including clinical trials, is typically needed to confirm these effects and determine safe and effective dosages for medicinal use. Several pre-clinical potential of *B. diffusa* were depicted in Fig. 1.

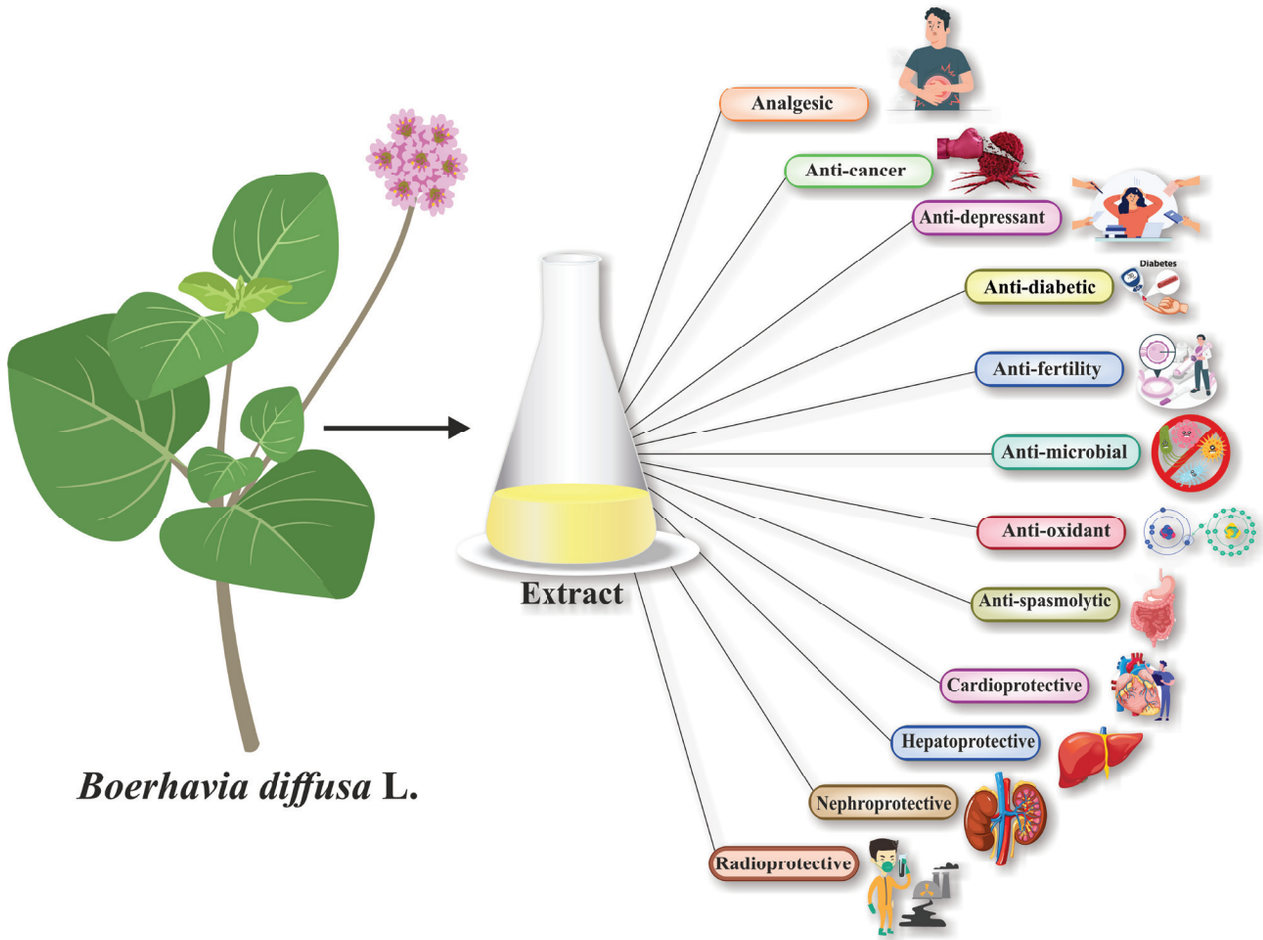

Fig. 1. Schematic representation of various pharmacological properties of *B. diffusa*

Analgesic and Anti-inflammatory Activities

Hiruma-Lima et al. (2000) studied the analgesic activity of decoction (DE) or juice (JE) of leaves of *B. diffusa* against pentobarbital sodium-induced sleep and acetic acid-induced writhing in mice as well as hot plate test. Both JE and DE at 1000 mg/kg dose exert significant inhibition with 100, 50 and 47% for acetic acid-induced abdominal writhing in mice. In hot-plate test, JE exhibit significant increase in latency during the observation time. Only DE raised the pain thresholds during the first period (30 min) of observation.

Bairwa and Jachak (2015) reported the anti-inflammatory effect of rotenoid-rich fraction (RRF, at 100 mg/kg p.o.) from roots of *B. diffusa* against carrageenan-induced rat paw edema in Sprague-Dawley (SD) rats. RRF exhibited 48.53% inhibition of edema in rats when compared ibuprofen used as standard (50.41%) for tested model.

Bairwa et al. (2012) evaluated the anti-inflammatory potential of ethanol extract and rotenoid-rich fraction along with boeravinone A, B, E, L, M, and N from *B. diffusa* roots against COX-1 and COX-2 inhibition assays. All samples attained inhibitory effect with IC_{50} ranged in between 21.7 to 33.1 and, 25.5 to 31.4 µM for both inhibition assays, respectively. Whereas, only boeravinone B showed significant effect (56.6% at 50 mg/kg) better than ibuprofen used as positive control (50.40% at 100 mg/kg), followed by extract and fraction (34.3 and 48.5% at 100 mg/kg p.o.) in carrageenan-induced rat paw edema model.

Anthelmintic Activity

Rajagopal (2013) evaluated the anthelmintic effect of ethanol extract from *B. diffusa* leaves against Indian earthworm *Pheretima posthuma*. The extract showed anthelmintic effect at 100 mg/ml, as well as paralysis and death at similar concentrations.

Anti-angiogenic Activity

Saraswati et al. (2013) studied the anti-angiogenic activity of punarnavine of *B. diffusa* on human umbilical vein endothelial cells (HUVECs) *in vitro* and Ehrlich ascites tumor *in vivo*. The compound inhibited endothelial cell migration and invasion and capillary structure formation of HUVECs. Also, inhibited MMP-2 and MMP-9 expression at 50 µM in HUVECs, neovascularization in sponge implant assay. The compound (15 mg/kg, b.w./d) decreased the ascitic fluid volume by 60.94% and, tumor volume by 86.40% in Ehrlich ascites model along with reduction in peritoneal angiogenesis by compound treatment.

Anti-cancer Activity

Sinan et al. (2021) evaluated the anti-cancer activity from methanolic extract of *B. diffusa* aerial parts against MDA-MB-231 breast cancer cell line using MTT assay. The extract exhibited inhibitory effect with IC_{50} of 582.9 and 304.7 µg/ml for 24 and 48 h, respectively.

Sreeja and Sreeja (2009) studied the anti-cancer activity of methanol extract (20-320 µg/ml) from *B. diffusa* against MCF-7 breast cancer cell lines. The extract exert moderate

to very strong growth inhibition in MCF-7 cell lines. The extract competed with [3H]-estradiol for binding to estrogen receptor (ER) with IC_{50} of 320 µg/ml. Further, the extract reduced mRNA expression of pS2 indicating the antiestrogenic action. The extract showed an increase in number of MCF-7 cells in the G0-G1 fraction from 69.1 to 75.8%, with a reciprocal decrease of cells in all other phases indicating cell cycle arrest at G0-G1 phase.

Srivastava et al. (2011) evaluated the anti-cancer effect of methanol-chloroform fraction (BDF 5) from *B. diffusa* roots on growth of human cervical (HeLa) cancer cells. The fraction at 200 µg/ml significantly reduced cell proliferation with visible morphological changes in HeLa cells. The fraction BDF 5 caused cell death *via* apoptosis as evident from DNA fragmentation and caspase-9 activation. BDF 5 at 200 µg/ml showed decrease in G1 phase cells from 52 to 48% in treated cells and, decrease in the G1 phase was accompanied by increase in the population of the G2+M phase from 13% in control to 16% in treated cells.

Anti-diabetic Activity

Pari and Satheesh (2004) evaluated the anti-hyperlipidemic effect of aqueous extract (200 mg/kg, b.w.) of *B. diffusa* leaves against alloxan-induced in diabetic rats. The oral administration of extract exhibits a significant ($p<0.05$) reduction in serum and tissue cholesterol, free fatty acids, phospholipids, and triglycerides for tested model.

Satheesh and Pari (2004) studied the anti-diabetic activity of aqueous extract from *B. diffusa* leaves at 200 mg/kg dose which exhibit reduced blood glucose level and, a significant reduction in thiobarbutric acid reactive substance and hydroperoxides, as well as increase in reduced glutathione, super dioxide dismutase, catalase, glutathione peroxidase and glutathione-S-transferase in liver and kidney of alloxan-induced diabetic albino rats.

Singh et al. (2011) examined the anti-diabetic effect of ethanolic leaves extract from *B. diffusa* against alloxan-induced in diabetic albino Wistar rats. The oral administration of extract at 500 mg/kg dose exerts a significant decrement in blood glucose level, improved glucose tolerance curve and decreased area under curve. The extract also decreased serum urea and creatinine levels. Both extract and metformin attained reversal levels of serum ionic levels and, renal Na^+-K^+ ATPase activity of treated rats. In addition, the extract and metformin were equally effective in decreasing the levels of lipid peroxidation in diabetic rats.

Pari and Satheesh (2003) investigated the effects of daily oral administration of aqueous solution of *B. diffusa* leaves extract (200 mg/kg) exert a significant decrease in blood glucose, glycosylated haemoglobin and, an increase in plasma insulin level as well as total hemoglobin level in normal and alloxan-induced diabetic rats. Also, significantly increased hexokinase and glucose-6-phosphatase, but fructose-1,6-bisphosphatase significantly decreased and, improvement in glucose tolerance in treated rats were observed.

Anti-fertility Activity

Jain et al. (2016) examined the anti-fertility effect of methanolic root extract of *B. diffusa* at 400 mg/kg and observed that it inhibited pregnancy with mean number of implants to be 4.47±0.23 in female albino rats. The oral administration of extract (200 and 400 mg/kg) caused a significant increase in the uterine weight of immature rats. The uterotropic changes such as diameter of uterus ($p<0.001$) and, thickness of endometrium significantly ($p<0.001$) increased in rats.

Anti-genotoxic Activity

Aher et al. (2013) evaluated the anti-genotoxic activity of punarnavine (500-2000 mg/kg) from roots of *B. diffusa* against cyclophosphamide-induced DNA damage in bone marrow cells of Swiss mice. The compound decreased (26 to 52%) DNA damage by cyclophosphamide in treated mice. Also, the compound exert reduction in the frequency of micronucleated polychromatic erythrocytes and did not show any chromosomal aberration.

Anti-leishmanial Activity

Kaur et al. (2014) investigated the anti-leishmanial effect of whole plant extract of *B. diffusa* against *Leishmania donovani* infection in BALB/c mice. Extract at 100 mg/kg showed 55.2% reduction in hepatic parasite load in *L. donovani* infected BALB/c mice. The extract showed significant reduction in levels of SGOT, SGPT, serum bilirubin, serum urea, BUN and creatinine as well as higher levels of IgG2a over IgG1.

Anti-metastatic Activity

Manu and Kuttan (2009) evaluated the anti-metastatic potential of punarnavine (40 mg/kg) isolated from *B. diffusa* against B16F-10 melanoma cells in C57BL/6 mice. The compound reduced pulmonary metastases formation of B16F-10 melanoma cells. The compound significantly increased the survival rate of metastatic tumor-bearing mice and, elevated g-GT level was significantly reduced to 31.185±4.94 U/l (prophylactic) and 38.61±4.1 U/l (simultaneous) modalities. In mice with developed tumor, the serum gamma-glutamyl transferase was reduced to 54.945±4.46 U/l by the compound. The elevated level of serum vascular endothelial growth factor (VEGF) was significantly ($p<0.05$) reduced. But VEGF expression was only down regulated in treated mice with compound after tumor development. The compound down regulated and suppressed the MMP-2 and MMP-9 protein expressions when treated prophylactically and simultaneously. Also, it significantly reduced the level of sialic acid in serum.

Leyon et al. (2005) investigated the anti-metastatic effect of aqueous methanol (3:7) extract from *B. diffusa* showed reduction in the metastases formation of B16F10 melanoma cells. The prophylactic administration of extract at 0.5 mg/dose inhibited the metastases formation by about 95 and 87% in the lung metastases formation in syngenic C57BL/6 mice. The extract significantly reduced ($p<0.001$) lung collagen hydroxyproline, hexosamines and uronic acid levels, and reduced levels of serum sialic

acids and g-glutamyltranspeptidase. The extract enhanced the survival of animals and inhibited the cell proliferation as estimated by ^{3}H-thymidine uptake and, inhibited the expression or activity of gelatinases A and B (MMP-2 and MMP-9).

Anti-microbial Activity

Kaviya et al. (2022) evaluated the anti-microbial activity of ethanolic extract of leaves, stems, and roots of *B. diffusa* against *Pseudomonas aeruginosa* and *Staphylococcus aureus*. The extract inhibited the growth of *P. aeruginosa* and *S. aureus* with zones inhibitions were 8 and 20 mm at 200 µg concentration, respectively.

Apu et al. (2012) examined the anti-microbial effect of n-hexane, ethyl acetate and methanol extracts of aerial parts of *B. diffusa* against *Staphylococcus aureus*, *Shigella dysenteriae* and *Candida albicans*. All extracts showed anti-microbial effect with zone inhibitions ranged from 7.33 to 7.90 mm at 1000 µg/disc for *C. albicans*, while only methanol extract showed 9.77 and 7.43 mm at 1000 µg/disc for other microorganisms.

Nair et al. (2007) reported the anti-microbial activity of aqueous and ethanol extracts from *B. diffusa* against *Pseudomonas testosteroni*, *Staphylococcus epidermidis*, *Proteus morganii*, *Bacillus subtilis*, *Micrococcus flavus*, and *Klebsiella pneumonia* using disc diffusion assay. The ethanol extract exhibited effectiveness with inhibitions ranging in between ~4 to 5 mm for *M. flavus,* followed by aqueous extract ~1 to 4 mm for all tested microorganisms except *Ps. testosteroni* and *K. pneumonia*.

Bose et al., (2017) performed the anti-viral activity of boeravinone H from *B. diffusa* herb against Hepatitis C virus (HCV) infection. The compound inhibited 63% binding and, entry of HCV-like particles (HCV-LPs) in hepatoma cells. Further, the compound inhibited the initial phase of HCV entry probably by acting directly on the viral particle. Importantly, the compound prevents (>95%) HCV entry and infection in cell culture (*ex vivo*).

Antioxidant Activity

Sinan et al. (2021) evaluated the antioxidant activity from different extracts of *B. diffusa* aerial parts using ABTS, DPPH, CUPRAC, FRAP, metal chelating and phosphomolybdenum assays. In ABTS and DPPH assays, the methanol extract showed strongest ability (91.62 and 103.56 mg TE/g), followed by infusion (58.89 and 71.04 mg TE/g), dichloromethane (37.99 and 26.09 mg TE/g) and ethyl acetate (27.61 and 19.84 mg TE/g). In metal chelating assay, the infusion attained best ability with 20.32 mg EDTAE/g, followed by methanol and ethyl acetate extracts (6.80 and 1.55 mg EDTAE/g).

Kaushik et al. (2017) investigated the antioxidant potential of *swaras* and *hima* extracts of *B. diffusa* using DPPH, nitric oxide, and hydroxyl radical scavenging assays. The extracts showed inhibitory effect with IC_{50} of 53.43 and 48.38 µg/ml and, 123.53 and 145.13 µg/ml for DPPH and hydroxyl assays as compared to gallic acid (44.96 and 156.58 µg/ml) and, weak effect for nitric oxide assay (310.27 and 204.54 µg/ml). However, both extracts did not exhibit any reducing power activity.

Apu et al. (2012) examined the antioxidant effect of crude n-hexane, ethyl acetate and methanol extracts of aerial parts of *B. diffusa* using DPPH, and nitric oxide radical assays. All extracts exhibited scavenging effect with IC_{50} ranged from 8.18 to 43.81 and, 95.16 to 132.47 µg/ml as comparison to ascorbic acid (76.11 and 34.06 µg/ml) used as standard for both assays.

Rathor and Pandey (2018) performed the antioxidative stress activity of boeravinone B (BOB) from *B. diffusa* roots against oxidative stress resistance and lifespan extension in *Caenorhabditis elegans*. BOB extends lifespan of *C. elegans*, reducing accumulation of reactive oxygen species (ROS). BOB was found to recover the shortened lifespan of oxidative stress prone mutants *mev*-1 and *gas*-1 with 14.75 and 16.11%, respectively. BOB (25 µM) significantly increased the expressions of target genes such as superoxide dismutase (SOD-3), glutathione-S-transferase (GST-4) and heat shock protein (HSP-16.2) fused to green fluorescent protein (GFP), and modulating the stress related signalling pathways (SEK-1) and transcription factors (SKN-1/Nrf, DAF-16/Foxo). Moreover, BOB (25 µM) exert changes of age-dependent biomarkers such as pharyngeal pumping, body bend, locomotor activity and lipofuscin accumulation concluding that it retards the aging.

Anti-spasmolytic Activity

Borrelli et al. (2005) evaluated the anti-spasmolytic effect of methanolic extract (1 to 1000 µg/ml), its fractions along with boeravinone E, G and I isolated from *B. diffusa* against acetylcholine-induced in guinea-pig ileum. The extract significantly inhibited ($p>0.001$) acetylcholine contractions in pigs. All compounds were able to reduce acetylcholine-induced contractions with IC_{50} of 11, 4.22 and 5.46 µg/ml as compared to papaverine (2.1×10^{-5} µg/ml), used as standard.

Cardioprotective Activity

Prathapan et al. (2018) performed the cardioprotective effect of ethanolic extract (5-100 µg/ml) of *B. diffusa* against apoptosis in angiotensin II (Ang II)-stimulated hypertrophic cardiac cells (H9c2). The extract reduced the up-regulation of Bax and cytosolic Cytochrome-C by Ang II as well as, reduced the degree of Ang II- induced down-regulation of Bcl-2. The extract exert reduction in caspase-3 (33.77%) and TNF-α in treated cells stimulated with Ang II. Further, the up-regulation of phospho-p38 MAPK was attenuated by extract treatment.

Prathapan et al. (2013) evaluated the cardioprotective effect of ethanolic extract of *B. diffusa* against angiotensin II-induced hypertrophy in H9c2 cardiac myoblast cells. Treatment with extract reduced cell volume, protein content and concentrations of atrial natriuretic peptide (ANP) and B-type natriuretic peptide (BNP) ($p<0{\cdot}05$) in H9c2 cells. Also, it reduced intracellular reactive oxygen species generation, lipid peroxidation and protein carbonyls in cells. In addition, the extract reduced ($p<0{\cdot}05$) expression of NF-κB and transforming growth factor ß1 and

genes. The extract also inhibits angiotensin-converting enzyme and xanthine oxidase with EC_{50} of 166·12 and 60·05 mg/ml, respectively.

Prathapan et al. (2014) studied the cardioprotective effect of ethanolic extract from *B. diffusa* leaves against mitochondrial dysfunction in angiotensin II (Ang II)-induced hypertrophy in H9c2 cardiomyoblasts. The extract prevented the generation of intracellular reactive oxygen species (ROS) and mitochondrial superoxide radicals as well as, protected the mitochondria by preventing dissipation of ΔΨm, opening of mPTP, mitochondrial swelling and enhanced the activities of respiratory chain complexes and oxygen consumption rate in H9c2 cells. Also, it significantly reduced ($p<0.05$) activities of aconitase and thioredoxin reductase in hypertrophied cells along with reduction in the intracellular calcium overload in Ang II treated cells.

Chemo-preventive Activity

Bharali et al. (2003) evaluated the chemopreventive activity of hydroalcoholic extract of *B. diffusa* leaves and stems against 7,12-dimethyl benz(a)anthracene (DMBA)-induced skin papillomagenesis in male albino Swiss mice. The extract showed reduction in the values of tumor incidence in treated mice, and average number of tumors per tumor bearing mouse and papillomas per papilloma bearing mouse (65%, 2.8 and 3.1 in peri-initiational phase (*i.e.* 7 days before and 7 days after the application of DMBA), in post initiational phase (30%, 0.75 and 2.5 in the day of start of croton oil treatment and continued till the end of experiment), continuously at peri- and post-initiational stages with 25%, 0.35 and 1.2 for the 7 days prior to DMBA application and continued till the end of experiment).

Enzyme Inhibitory Activity

Sinan et al. (2021) assessed the enzyme inhibitory effect of different extracts from *B. diffusa* against cholinesterases (AChE and BChE), tyrosinase, amylase and glucosidase. In AChE inhibitory assay, dichloromethane and methanol extracts were found active on the enzyme and, the best action was determined in dichloromethane (5.01 mg GALAE). The highest tyrosinase inhibitory effect was provided by the methanol extract with 81.57 mg KAE/g, followed by ethyl acetate (41.55 mg KAE) and dichloromethane (35.62 mg KAE/g). Similar to tyrosinase, the strongest glucosidase inhibitory effect observed by methanol extract.

Genoprotective Activity

Aviello et al. (2011) demonstrated the genoprotective effect of boeravinone G, D and H (0.1-1 ng/ml) isolated from roots of *B. diffusa* against Fenton's reagent (H_2O_2/Fe^{2+}, 1 mM)-induced malondialdehyde (MDA)-equivalents production and H_2O_2-induced DNA damage. Boeravinones G, D and H reduced the signal intensity of ESR (electron spin resonance spectroscopy) induced by hydroxyl radicals. Only boeravinone G inhibited both TBARS and ROS formation induced by Fenton's reagent, increased SOD and reduced H_2O_2-induced DNA

damage. However, boeravinone G reduced the levels of pERK1 and phospho-NF-κB p65 (but not of pERK2) increased by Fenton's reagent.

Hepatoprotective Activity

Thajudeen et al. (2022) evaluated the hepatoprotective activity of isolated eupalitin-3-*O*-β-*D*-galactopyranoside from *B. diffusa* leaves against carbon tetrachloride-induced toxicity in HepG2 cells. The compound showed decrease in cell viability of tested cells. In addition, the compound exerts hepatoprotective effect ranged from 62.62 to 70.23% at 500 and 1000 µg/ml which was compared to silymarin (80.2 to 86.94%) at 100 to 500 µg/ml used as standard drug.

Kaushik et al. (2017) examined the hepatoprotective effect of *swaras* and *hima* extracts from whole plant of *B. diffusa* against paracetamol induced hepatotoxicity in Swiss mice. Both extracts significantly ($p<0.01$) reduced SGOT and ALP levels. The oral administration of both extracts significantly ($p<0.001$) attenuated serum cholesterol, triglyceride, LDL, creatinine, urea, and uric acid concentrations and increased the HDL concentrations.

Bano et al. (2019) evaluated the hepatoprotective effect of ethanolic extract of *B. diffusa* against oxaliplatin-induced hepatotoxicity in albino Wistar rats. The extract decreased the levels of alanine aminotransferase, aspartate aminotransferase and gamma glutamyl transferase, cholesterol, LDL, and total lipid content as well as, triglycerides levels were significantly ($p<0.001$) increased.

Immunomodulatory Activity

Mehrotra et al. (2002) evaluated the immunomodulatory effect of ethanolic extract of *B. diffusa* roots, that inhibit human NK cell cytotoxicity, LPS-induced production of nitric oxide (NO) in mouse macrophage cells at 100 and 500 µg/ml, PHA-stimulated IL-2 as well as LPS-induced TNF-α in human PBMCs. Intracytoplasmic IFN-g and cell surface markers such as CD16, CD25, and HLA-DR was not affected by extract.

Manu and Kuttan (2009) evaluated the immunomodulatory effect of punarnavine from *B. diffusa* on the immune system using BALB/c mice. The compound increased the bone marrow cellularity and number of α-esterase positive cells. The compound also enhanced proliferation of splenocytes, thymocytes and bone marrow cells both in the presence and absence of specific mitogens. Also, the compound reduced LPS-induced elevated levels of proinflammatory cytokines such as TNF-α, IL-1ß, and IL-6 in mice.

Pandey et al. (2004) performed the immunomodulatory potential of chloroform and ethanolic extracts of *B. diffusa* roots and reported that it inhibited ($p<0.01$, $p<0.05$) PHA stimulated proliferation of peripheral blood mononuclear cells, two-way MLR, NK cell cytotoxicity as well as LPS-induced NO production by RAW 264.7. The compound Bd-I (eupalitin-3-O-h-D-galactopyranoside) inhibited production of PHA stimulated IL-2 at the protein and mRNA transcript levels and LPS-stimulated TNF-α production in human PBMCs ($p<0.01$, $p<0.05$), also blocked the

activation of DNA binding of nuclear factor-κB and AP-1 and expression of the IL-2 and IL-2R gene.

Nephroprotective Activity

Sawardekar and Patel (2014) investigated the nephroprotective effect of aqueous extract of *B. diffusa* roots in gentamicin-induced nephrotoxicity in Wistar rats. The extract exhibited reduction in the blood urea nitrogen and serum creatinine levels, significantly reduced the malondialdehyde levels and, maintained the glutathione (GSH) level as comparable to α-lipoic acid. The extract did not show any significant improvement in *Para*-aminohippurate clearance.

Kalaivani et al. (2021) evaluated the nephroprotective activity of ethyl acetate fraction of *B. diffusa* against tacrolimus (TAC)-induced nephrotoxicity in MDCK cell lines. The fraction increased the survivability of MDCK cells, reduced LDH release, ROS ($p<0.05$), LPO and NO ($p<0.001$), decreased collagen accumulation ($p<0.001$) with increase in the cell survival of tubular epithelial cells.

Neuroprotective Activity

Ayyappan et al. (2016) evaluated the neuroprotective effect of ethanolic extract from *B. diffusa* against quinolinic acid (QA), 3-nitropropionic acid (NPA), sodium nitroprusside (SNP), and Fe (II)/EDTA complex-induced oxidative stress in rat brain homogenates. The extract decreased the production of TBARS ($p<0.05$) and, increased catalase and superoxide dismutase along with increased concentration of non-enzymatic antioxidant, reduced glutathione (GSH). Similarly, the extract caused decrease in the lipid peroxidation (LPO) in cerebral cortex. The extract also exhibited inhibitory potential with IC_{50} of 38.91 µg/ml against deoxyribose degradation.

Dhingra and Valecha (2014) evaluated the antidepressant activity of aqueous extract of *B. diffusa* roots on immobility period of Swiss mice using tail suspension and force swimming tests. The extract decreased the immobility periods in both tests as well as, reduced brain MAO-A levels.

Dhingra and Valecha (2014) studied the effect of ethanol extract along with punarnavine from *B. diffusa* root against depression in albino Swiss mice using tail suspension (TST) and forced swim tests (FST). The ethanol extract exhibited anti-depression effect with ED_{50} of 26.30 mg/kg (FST) and 33.11 mg/kg (TST); as well as punarnavine with 15.14 mg/kg (FST) and 17.38 mg/kg (TST). However, the extract and punarnavine significantly reduced mouse brain monoamine oxidase (MAO)-A levels.

Radioprotective Activity

Manu et al. (2007) examined the radioprotective effect of whole-plant extract of *B. diffusa* against gamma radiation-induced damage in albino Swiss mice. The extract reduced the elevated levels of ALP and GPT in both serum and liver. It also reduced the DNA fragmentation in treated mice. The extract exhibit significant reduction in lipid peroxidation levels of treated mice.

Renoprotective Activity

Pareta et al. (2011) reported the renoprotective effect of aqueous extract of *B. diffusa* roots against ethylene glycol-induced hyperoxaluric oxidative stress and renal injury in albino Wistar rat kidney. Treatment with extract significantly ($p<0.001$) reduced level of MDA and improved the activity of antioxidant enzymes (SOD, CAT, GST, and GPx), followed by reduction in BUN and serum creatinine. It also reduced the number of calcium oxalate (CaOx) monohydrate crystals in the urine. In histological analysis, the extract inhibited deposition of CaOx crystal and renal cell damage.

Thrombolytic Activity

Apu et al. (2012) reported the thrombolytic effect of crude n-hexane, ethyl acetate and methanol extracts of aerial part of *B. diffusa* on *in vitro* clot lysis of human blood. The methanol extract showed percentage of clot lysis of 10.26%, followed by other extracts (6.60 and 7.12%) as compared to streptokinase (40.40%) used as standard.

Wound-healing Activity

Juneja et al. (2020) demonstrated the wound healing activity of methanol extract (10% w/v) of *B. diffusa* leaves on cell viability and wound scratch assays as well as *in vivo* excision wound assays in albino Wistar rats. The extract enhanced viability and migration of human keratinocyte cells (HaCaT). In excision wound model, the extract decreased the wound area by 91% on 14th day.

Yahaya et al. 2018 evaluated the wound healing effect of n-hexane, ethyl acetate, methanol, and water extracts from whole plant of *B. diffusa* against 2,2'-azo-*bis*(2-amidinopropane) dihydrochloride (AAPH)-induced oxidation and mediate cell migration after artificial wound generation in C2C12 myoblast cells using scratch wound assay. Pretreatment with all 100 µg/ml of the extracts reduced AAPH-induced oxidation to 1.70-fold of the untreated controls. Both methanol and water extracts attained 18.08 and 20.76% higher wound closure in C2C12 myoblasts.

Clinical Study

Oburai et al. (2015) reported a clinical evaluation on the effectiveness of *B. diffusa* hydroalcoholic root extract (500 mg p.o. per dog daily) on 20 dogs of mixed breeds suffering from canine chronic renal failure (CRF) from 1 to 2 months. The extract significantly ($p<0.05$) reduced both systolic and diastolic blood pressure by day 30. Also, it exerted a significant reduction in serum creatinine, urea nitrogen, phosphorus, urinary protein, ALP, and GGT, as well as Hb values were comparable to normal in 60 days. However, potassium levels were normalized by root extract treatment on day 30. Thus, the efficacy of extract was comparable to standard *i.e.*, enalapril treatment of CRF in dogs.

Toxicological Profile

B. diffusa is a plant that has been used traditionally in various systems of medicine, including Ayurveda and traditional Chinese medicine, for its potential medicinal properties. It is often used for its diuretic, anti-inflammatory, and antioxidant effects. While there is interest in its potential health benefits, it's essential to consider the potential toxicological aspects. Benil et al. (2020) assessed the toxicity of aqueous extract (25-200 mg/ml) of *B. diffusa* roots against toluene induced reproductive and developmental toxicity in *Drosophila melanogaster*. The extract significantly prevented the deterioration of reproductive and developmental toxicity parameters of larval period, pupal period, percentage of egg hatching, morphometric characters of larva, pupa and adult, fertility, fecundity, and lifespan in *Drosophila.* Also, the drug significantly elevated the levels of antioxidant enzymes. Orisakwe et al. (2003) evaluated the acute and sub chronic toxicity of aqueous leaves extract of *B. diffusa* in albino mice and rats. The extract increased the food and fluid intake in rats. The lethal dose (LD_{50}) was found to be greater than 2000 mg/kg in mice and rats. The extract increased the body weight of rats. Apu et al. (2012) reported the toxicity of crude n-hexane, ethyl acetate and methanol extracts of aerial part of *B. diffusa* against brine shrimp lethality bioassay. All extracts showed cytotoxicity with LC_{50} value of 140.55, 141.89 and 163.75 µg/ml, in comparison to positive control (potassium dichromate, 13.23 µg/ml).

Summary And Future Outlook

B. diffusa, commonly known as 'Punarnava', is a medicinal plant that has traditional significance in Ayurveda and traditional Chinese medicine. The pharmacology of *B. diffusa* is characterized by its diverse range of bioactive compounds, which contribute to its therapeutic properties. Its pharmacology encompasses a wide range of potential health benefits, many of which have been explored through scientific research. In conclusion, *B. diffusa* has a rich pharmacological profile with various potential health benefits, ranging from anti-inflammatory and antioxidant effects to anti-microbial and hepatoprotective properties. While there is substantial evidence supporting many of its pharmacological effects, it's important to note that further research is needed to fully understand the mechanisms of action, identify specific active compounds, and validate its efficacy in clinical settings. Additionally, safety considerations and potential interactions with medications need to be thoroughly investigated. Its outlook involves in-depth research to unlock its full potential, identify active compounds, conduct clinical trials, explore new therapeutic applications, and ensure its safe and effective use in various medical and pharmaceutical contexts. A multidisciplinary approach,

combining traditional knowledge with modern scientific methods, will contribute to a better understanding of this plant's medicinal properties and its role in improving human health.

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