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Crotalaria Multibracteata in Sustainable Therapeutics: Integrating Traditional Knowledge with Green Science

Sachin Nitave [a++], Vrushabh Patil [b#*], Satish Kilaje [b†] and Dhanshree Rajput [b#]

[a] *Dr. J. J. Magdum Trusts Anil Alias Pintu Magdum Memorial Pharmacy College, Dharangutti: Jaysingpur, Kolhapur, Maharashtra, India.*
[b] *Dr J.J.Magdum Pharmacy College Jaysingpur, Kolhapur, Maharashtra, India.*

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ABSTRACT

Introduction: Medicinal plants serve as a crucial reservoir for novel therapeutic agents, with *Crotalaria multibracteata* emerging as a species of significant interest due to its traditional medicinal uses and bioactive potential within the *Crotalaria* genus.
Methods: This review compiles published data on *C. multibracteata*, including its botanical traits, ethnomedicinal uses, phytochemistry, pharmacology, toxicity, and biotechnological potential. Literature was selected from databases such as PubMed, Scopus, Web of Science, and Google

[++] *Principal and Professor;*
[#] *Assistant Professor;*
[†] *Professor;*
**Corresponding author: E-mail: vrushabhpatil555@gmail.com;*

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Scholar using relevant keywords. Only peer-reviewed, English-language studies were included. Extraction and isolation techniques like solvent extraction, chromatography, and spectroscopy are also summarized.

Results: Phytochemical investigations reveal diverse compounds, including flavonoids, alkaloids, terpenoids, and phenolics, which contribute to demonstrated antioxidant, anti-inflammatory, antimicrobial, and anticancer activities validated through in vitro and in vivo studies. However, toxicological data remain scarce, necessitating further investigation.

Conclusion: *Crotalaria multibracteata* exhibits promising pharmacological properties and therapeutic potential, yet comprehensive studies on safety, mechanisms of action, and cultivation practices are required. Interdisciplinary research approaches will be vital to fully harness its medicinal value and translate traditional knowledge into clinical applications.

Keywords: Crotalaria multibracteata; medicinal plants; phytochemicals; pharmacology; toxicology; ethnomedicine.

1. INTRODUCTION

Medicinal plants have played a vital role in the prevention and treatment of various ailments for centuries and continue to be an integral part of traditional and modern healthcare systems. According to the World Health Organization (WHO), more than 80% of the global population relies on plant-based medicines for primary healthcare needs, particularly in developing countries (World Health Organization, 2023). The increasing resistance to synthetic drugs, along with adverse side effects and high treatment costs, has intensified interest in natural product-based therapeutics.

The genus *Crotalaria*, belonging to the family Fabaceae, comprises over 600 species globally, many of which are distributed in tropical and subtropical regions of Asia, Africa, and South America (Polhill, 1982). Species of *Crotalaria* are known for their rich phytochemical profiles, particularly in flavonoids, alkaloids (including pyrrolizidine alkaloids), terpenoids, and phenolics (Singh et al., 2015). Several members of this genus, such as *Crotalaria juncea* and *Crotalaria retusa*, have been extensively studied for their pharmacological activities including anti-inflammatory, antioxidant, antimicrobial, and anticancer properties (Ambasta, 2006; Bhosale & Mote, 2018).

Despite the diversity of this genus, *Crotalaria multibracteata* remains relatively underexplored. Endemic to specific regions in India, it has been traditionally used by local communities for treating skin disorders, wounds, and inflammation (Reddy & Rao, 2011). However, systematic documentation and scientific validation of its phytochemical constituents and pharmacological potential are still lacking.

The primary objective of this review is to provide a comprehensive overview of *Crotalaria multibracteata*, including its botanical characteristics, ethnomedicinal uses, phytochemistry, pharmacological activities, toxicological concerns, and future research prospects. By compiling and analyzing available data, this review aims to highlight the untapped potential of *C. multibracteata* as a source of novel bioactive compounds for therapeutic applications.

2. BOTANICAL DESCRIPTION, ETHNOMEDICINAL USES, CULTIVATION, AND AVAILABILITY

The species is generally found growing in wild or semi-wild conditions, along forest margins, fallow fields, and roadsides, indicating its ecological adaptability to disturbed habitats and poor soils. Morphologically, *C. multibracteata* presents as a small herbaceous plant or undershrub with simple, alternate, and narrowly oblong to ovate-lanceolate leaves. Its most distinguishing feature is the presence of numerous bracts associated with the inflorescence, which is the origin of the epithet “multibracteata” (Wight & Arnott, 1834; Gamble, 1935).

The plant produces bright yellow, papilionaceous flowers arranged in axillary racemes, consistent with the floral morphology typical of the *Crotalaria* genus. The fruit is an inflated, many-seeded pod that produces a characteristic rattling sound when shaken due to loose seeds inside a trait from which the genus name *Crotalaria* is derived (Greek *krotalon*, meaning "rattle"). It possesses a well-developed taproot system, often nodulated, facilitating nitrogen fixation—a common trait in Fabaceae (Gamble, 1935).

In traditional ethnomedicine, *C. multibracteata* has been utilized by local and tribal communities for the treatment of various ailments. Leaves of the plant are often crushed into a paste and applied externally to treat skin infections, itching, wounds, and eczema. Decoctions made from the aerial parts are consumed orally for managing fever and inflammation. Some local healers also report its use in treating ulcers and snake bites, although these claims remain largely anecdotal and unverified by scientific studies (Kirtikar & Basu, 2001; Ignacimuthu & Ayyanar, 2009).

Despite the significant traditional relevance, *C. multibracteata* has not yet been brought into mainstream medicinal plant cultivation. It is primarily collected from the wild, and no large-scale or organized farming systems have been established for its propagation. However, the plant's agronomic traits suggest potential for cultivation. It thrives in semi-arid to dry tropical climates and prefers well-drained sandy or loamy soils with a slightly acidic to neutral pH. Propagation is typically through seeds, which germinate within one to two weeks under warm and moist conditions. The plant follows an annual life cycle, with flowering commencing within two months of germination and continuing for several weeks. This quick growth cycle could be advantageous for short-duration herbal cropping systems (Singh & Duggal, 2016).

Given its ability to fix atmospheric nitrogen, *C. multibracteata* may serve as a beneficial cover crop or intercropping species, contributing to soil fertility. However, there is a lack of agronomic trials and propagation studies specifically targeting this species. Currently, the availability of *C. multibracteata* is limited to local environments where it grows naturally, particularly in tribal regions of southern India. It is not commonly available in commercial nurseries or herbal markets, which restricts its accessibility for research, conservation, and pharmacological use. The absence of standardized cultivation practices and the risk of overharvesting from natural habitats raise concerns about the plant’s long-term sustainability (Ved & Goraya, 2008).

Conservation assessments indicate a need for seed banking, nursery development, and awareness campaigns aimed at promoting its sustainable use. Given its therapeutic promise and ecological resilience, the development of cultivation protocols and inclusion in community herbal farming programs could help in preserving *C. multibracteata* while unlocking its full medicinal potential (Rao & Palanisamy, 2020).

3. NATIVE HABITATS AND GEOGRAPHICAL SPREAD

Crotalaria multibracteata is a plant species endemic to the Indian subcontinent, with its distribution primarily confined to the southern peninsular region. It has been reported from parts of Tamil Nadu, Karnataka, Kerala, and to a lesser extent, Andhra Pradesh and Telangana, where it grows naturally in dry deciduous forests, scrublands, and grassy slopes (Singh et al., 2000). Its growth is often observed along the margins of agricultural fields, roadsides, and disturbed habitats, indicating its adaptability to semi-wild and anthropogenically influenced environments.

The species thrives in warm, subtropical to tropical climates, and it typically prefers well-drained sandy or loamy soils with moderate fertility. It is found at altitudes ranging from 300 to 1200 meters above sea level, though local environmental conditions largely influence its abundance and morphology. It is especially prevalent in Deccan Plateau regions and Eastern Ghats, which provide suitable microclimatic conditions for its growth, including moderate rainfall and seasonal drought (Ramasubbu & Arumugam, 2018).

Despite being largely overlooked in major floristic surveys due to its similarity with other *Crotalaria* species, *C. multibracteata* has been documented in several herbarium records and regional ethnobotanical reports. In Tamil Nadu, its occurrence is noted in districts such as Dharmapuri, Salem, and Erode, while in Karnataka, it is found in the dry plains and hill slopes of Chitradurga, Bellary, and Tumkur regions (Yoganarasimhan, 2000).

Currently, the species is considered to be of least concern in terms of conservation status, primarily because of its wide adaptability and occurrence in various ecological niches. However, habitat fragmentation, unregulated grazing, and seasonal bush fires in semi-arid zones may pose localized threats. Furthermore, the lack of awareness regarding its medicinal potential and underrepresentation in pharmacognostic literature suggest a need for more comprehensive regional surveys and habitat-specific ecological assessments (Reddy et al., 2011).

Efforts to map the distribution of *C. multibracteata* using geospatial tools and digitized herbarium records are still limited, making it a priority for future ethnobotanical and conservation research. Integrating botanical field surveys with local traditional knowledge systems could significantly aid in understanding its biogeography and ecological preferences.

4. ETHNOBOTANICAL AND TRADITIONAL USES

Crotalaria multibracteata has a long-standing presence in traditional and folk medicine practices, particularly among tribal communities in southern India. Its therapeutic applications have been shaped by indigenous knowledge systems passed down through generations, where local healers recognize its potential for treating a variety of ailments, particularly those related to the skin, inflammation, and wounds.

Historically, this plant has been used in tribal medicine systems by communities residing in the Eastern and Western Ghats. Ethnobotanical surveys conducted in Tamil Nadu, Andhra Pradesh, and parts of Karnataka document the use of leaf pastes and decoctions in treating eczema, wounds, skin infections, and itching disorders (Ayyanar & Ignacimuthu, 2005). The leaves are usually crushed fresh and applied topically to affected areas, or in some instances, boiled and used as a wash for infected skin. The antiseptic and anti-inflammatory properties are commonly cited by traditional healers.

In tribal practices, the plant has also been used as a febrifuge (to reduce fever), and decoctions made from aerial parts are ingested to alleviate body aches, internal ulcers, and digestive disturbances (Rani & Rajalakshmi, 2010). Certain ethnic groups have even used the plant in the treatment of snake bites, although such uses remain anecdotal and are not yet scientifically validated (Ragupathy & Newmaster, 2009).

Unlike more commonly cited *Crotalaria* species such as *C. juncea* or *C. retusa*, *C. multibracteata* is not widely referenced in classical Ayurvedic texts, likely due to its limited geographic distribution and the specificity of its traditional use. However, the pharmacological actions ascribed to it by tribal healers wound healing, detoxification, and pain relief closely mirror Ayurvedic principles, suggesting potential for integration into formal herbal pharmacopoeias pending further phytochemical and clinical validation.

In many rural villages, the plant also plays a cultural role, often used in local rituals for cleansing wounds or during postpartum care for women. The easy availability of the plant in dryland fields and forest margins has made it a low-cost and accessible remedy, especially in areas where modern healthcare infrastructure is lacking.

Despite this ethnobotanical importance, scientific literature remains scarce, and systematic pharmacognostic documentation of *C. multibracteata* is minimal. This underscores the urgent need for ethnopharmacological validation, bioactive compound isolation, and toxicological assessment. Preserving the indigenous knowledge surrounding this species could significantly contribute to the development of plant-based, region-specific therapeutic agents and support biocultural conservation efforts (Pushpangadan & George, 2010).

Table 1. Traditional uses of *Crotalaria multibracteata*

Plant Part Used	Form/ Preparation	Traditional Use	Mode of Application	Region/ Community	Ref
Leaves	Crushed into paste	Treatment of eczema, skin rashes, infections	Topical application	Tamil Nadu (Irula tribes)	(Ayyanar & Ignacimuthu, 2005)
Whole plant	Decoction	Fever, internal inflammation	Oral consumption	Sirumalai Hills (Paliyar)	(Rani & Rajalakshmi, 2010)
Leaves	Boiled, used as wash	Wound cleansing and antiseptic purposes	External wash	Andhra Pradesh	(Ragupathy & Newmaster, 2009)
Aerial parts	Decoction	Body pain, digestive issues, ulcers	Oral consumption	Eastern Ghats	(Rani & Rajalakshmi, 2010)
Leaves	Crushed and applied	Snake bite (folk practice, unverified)	Topical application	Tirunelveli tribal region	(Ayyanar & Ignacimuthu, 2005)
Fresh leaves	Applied post-delivery	Postpartum care (believed to aid healing)	Topical, external use	Rural Tamil Nadu	(Pushpangadan & George, 2010)

5. TECHNIQUES FOR EXTRACTION, ISOLATION, AND IDENTIFICATION OF PHYTOCHEMICALS FROM *Crotalaria multibracteata*

Phytochemical exploration of *Crotalaria multibracteata* relies on well-established methodologies used in medicinal plant research. These include sequential extraction, chromatographic separation, and spectroscopic techniques for compound characterization. Although literature on *C. multibracteata* itself is sparse, methodologies employed for related *Crotalaria* species offer a scientific framework that can be extrapolated for future studies.

5.1 Extraction Methods

The initial step in phytochemical investigation involves extraction of bioactive constituents using solvents of varying polarity. Soxhlet extraction, maceration, and ultrasound-assisted extraction (UAE) are the most commonly used techniques.

Soxhlet Extraction: This classical method involves continuous percolation of powdered plant material using organic solvents such as methanol, ethanol, chloroform, or petroleum ether. Methanol and ethanol are particularly favored for their ability to dissolve a broad range of polar and semi-polar compounds like alkaloids, flavonoids, and glycosides (Harborne, 1998).

Maceration: Simpler but time-intensive, maceration involves soaking dried plant material in a suitable solvent at room temperature, typically for 48–72 hours. This method is suitable for heat-sensitive constituents and is often followed by filtration and solvent evaporation under reduced pressure (Wallis, 2004).

Ultrasound-Assisted Extraction (UAE): UAE enhances mass transfer between solvent and plant tissue by employing ultrasonic waves, which create micro-cavitation and disrupt plant cell walls. This technique is advantageous for reducing extraction time and increasing yield, especially for heat-labile flavonoids and polyphenols (Vinatoru, 2001).

The choice of solvent and method depends on the target compound group—nonpolar solvents like hexane are used for lipids and waxes, while polar solvents like methanol or water target phenolics, alkaloids, and tannins.

5.2 Isolation Techniques

Once crude extracts are obtained, fractionation and purification are essential to isolate individual compounds. Several chromatographic techniques are employed:

Column Chromatography (CC): Using stationary phases such as silica gel or alumina, column chromatography separates extract components based on polarity. Elution is typically done using gradients of hexane, ethyl acetate, or methanol. This is a foundational technique for isolating flavonoids, alkaloids, and saponins from *Crotalaria* species (Wagner & Bladt, 1996).

Thin Layer Chromatography (TLC): TLC serves as a quick, cost-effective tool for preliminary analysis and monitoring of compound separation. It helps detect major classes such as flavonoids (e.g., with $AlCl_3$ reagent), terpenoids, and alkaloids (Sasidharan et al., 2011).

Preparative High-Performance Liquid Chromatography (prep-HPLC): For high-resolution separation of pure compounds, prep-HPLC is employed. This technique is particularly suitable for isolating bioactive flavonoids, alkaloids, and rare phytoconstituents from small quantities of extract (Mandal et al., 2007).

Liquid–Liquid Partitioning: Often used prior to chromatography, liquid–liquid extraction helps in partitioning crude extracts into different solvent fractions (e.g., chloroform, ethyl acetate, butanol) to enrich compound classes before purification.

5.3 Identification and Structural Elucidation

Following isolation, structural characterization of phytochemicals is carried out using spectroscopic and spectrometric techniques, including:

UV Visible Spectroscopy: Used for the initial identification of phenolic compounds and flavonoids by analyzing their absorption maxima (Singleton & Rossi, 1965).

Fourier-Transform Infrared Spectroscopy (FTIR): Provides information on functional groups (e.g., OH, COOH, NH_2) present in the compound, aiding in preliminary structure prediction (Silverstein et al., 2005).

Table 2. Summary of extraction, isolation, and identification techniques for phytochemicals from *Crotalaria multibracteata*

Technique	Purpose	Solvents / Tools Used	Target Phytochemicals	Ref
Soxhlet Extraction	Exhaustive extraction of phytochemicals	Ethanol, methanol, chloroform, petroleum ether	Flavonoids, alkaloids, terpenoids	(Harborne, 1998)
Maceration	Gentle extraction for heat-sensitive compounds	Ethanol, water, hydroalcoholic mixtures	Tannins, glycosides, saponins	(Wallis, 2004)
Ultrasound-Assisted Extraction (UAE)	Rapid, high-yield extraction	Ethanol, methanol, water	Polyphenols, flavonoids, essential oils	(Vinatoru, 2001)
Column Chromatography (CC)	Fractionation and purification	Silica gel, gradient solvents	Alkaloids, steroids, flavonoids	(Wagner & Bladt, 1996)
Thin Layer Chromatography (TLC)	Rapid screening and monitoring	Solvent system (e.g., ethyl acetate:hexane)	General compound class identification	(Sasidharan et al., 2011)
Preparative HPLC	High-purity isolation	Reverse phase C18 columns, MeOH/H_2O gradients	Flavonoids, glycosides	(Mandal et al., 2007)
Liquid–Liquid Partitioning	Solvent-based enrichment	Ethyl acetate, butanol, water	Saponins, terpenoids, alkaloids	(Wagner & Bladt, 1996)
UV–Vis Spectroscopy	Preliminary identification	Spectrophotometer	Phenolics, flavonoids	(Singleton & Rossi, 1965)
FTIR Spectroscopy	Functional group analysis	Infrared spectrometer	Broad organic functional groups	(Silverstein et al., 2005)
GC–MS	Analysis of volatile compounds	Gas chromatograph with MS detector	Essential oils, low MW terpenoids	(Dey & Harborne, 1997)
LC–MS	Non-volatile compound identification	Liquid chromatograph with MS detector	Flavonoids, glycosides	(Dey & Harborne, 1997)
NMR Spectroscopy (^{1}H, ^{13}C)	Structural elucidation	NMR solvents ($CDCl_3$, DMSO-d_6, MeOD)	All major phytochemical classes	(Pretsch et al., 2009)

Gas Chromatography–Mass Spectrometry (GC–MS): Particularly useful for volatile constituents and low-molecular-weight phytochemicals. It combines separation and mass analysis to identify unknown compounds using library matching (Dey & Harborne, 1997).

Liquid Chromatography–Mass Spectrometry (LC–MS): Employed for thermolabile and non-volatile compounds, LC–MS enables molecular weight determination and fragmentation analysis.

Nuclear Magnetic Resonance (NMR) Spectroscopy: Both ^{1}H and ^{13}C NMR are used for detailed structural elucidation. NMR provides insight into the molecular framework and stereochemistry of isolated phytochemicals such as flavonoids, alkaloids, and terpenoids (Pretsch et al., 2009).

X-ray Crystallography: For compounds that crystallize well, this technique can offer unambiguous confirmation of molecular structure, though it is less commonly applied due to complexity and cost.

6. FINDINGS AND DISCUSSION

6.1 Phytochemical Constituents of *Crotalaria multibracteata*

Preliminary phytochemical screening and studies on related *Crotalaria* species suggest that *Crotalaria multibracteata* contains a rich diversity of secondary metabolites such as flavonoids, alkaloids, terpenoids, saponins, tannins, and phenolic acids. These bioactive constituents contribute significantly to the plant's traditional therapeutic uses and potential pharmacological value.

6.1.1 Flavonoids

Flavonoids are among the most widely distributed polyphenolic compounds in *Crotalaria* species. They act as potent antioxidants, anti-inflammatory, and antimicrobial agents.

Common types: Quercetin, kaempferol, luteolin derivatives.

Pharmacological role: Flavonoids scavenge free radicals, modulate inflammatory pathways (e.g., COX-2 inhibition), and support capillary integrity and wound healing (Jeyadevi et al., 2012).

Ayurvedic relevance: These effects align with Ayurvedic concepts of balancing Pitta and Raktha dhatu (blood tissue), often associated with detoxifying and cooling actions.

Local use: Leaf pastes used for eczema and wounds are believed to derive efficacy from flavonoid content.

6.1.2 Alkaloids

Alkaloids in *Crotalaria* are structurally diverse and biologically active, although some may be pyrrolizidine alkaloids (PAs), which warrant caution due to hepatotoxic potential in high doses.

Common types: Monocrotaline, retrorsine (noted in other *Crotalaria* spp.)

Pharmacological role: Exhibit antimicrobial, cytotoxic, and vasoactive properties. Some are known to induce apoptosis in cancer cell lines (Fu et al., 2004).

Ayurvedic correlation: Alkaloids contribute to Katu rasa (pungent taste) and are often used in traditional formulations to stimulate digestion and blood circulation.

Local use: Decoctions made for fever and internal pain may be attributed to these compounds.

6.1.3 Terpenoids

Terpenoids are present in moderate quantities and contribute to the plant's fragrance and possible anti-inflammatory and analgesic properties.

Common types: Sesquiterpenes, diterpenes (tentative based on genus profiling).

Pharmacological role: Terpenoids show anti-inflammatory, antioxidant, and antitumor activities by modulating cytokine release and signaling pathways like NF-κB (Rabi & Bishayee, 2009).

Ayurvedic relevance: Their ushna virya (heating potency) helps in breaking down obstructions (Avarana) and alleviating chronic inflammatory conditions.

Local use: Traditionally used for muscular pain and postpartum care.

6.1.4 Phenolic compounds

Phenolic acids and tannins are common across *Crotalaria* species and are known for their antioxidant and antiseptic properties.

Common types: Gallic acid, ferulic acid, caffeic acid.

Pharmacological role: Phenolics help in reducing oxidative stress, inhibiting microbial growth, and promoting wound healing by cross-linking collagen (Rice-Evans et al., 1996).

Ayurvedic relevance: Considered cooling and drying, useful in managing Pitta disorders, ulcers, and skin inflammation.

Local use: External washes for skin infections and heat boils.

6.1.5 Saponins and tannins

These compounds exhibit antifungal, astringent, and immune-modulating properties.

Saponins: Known for surface-active properties, enhancing the absorption of other compounds and contributing to anti-inflammatory activity.

Tannins: Show astringent, antidiarrheal, and anti-infective properties, often linked to their ability to precipitate proteins and form protective layers on mucosa.

6.1.6 Additional compounds

Preliminary reports on *Crotalaria multibracteata* and related species suggest the presence of: Glycosides, associated with cardiotonic and anti-inflammatory actions.

Sterols like β-sitosterol, which may have anti-diabetic and cholesterol-lowering effects (Bhatt et al., 2010).

6.2 Pharmacological Activities of *Crotalaria multibracteata*

6.2.1 Anti-inflammatory activity

Crotalaria multibracteata exhibits significant anti-inflammatory effects attributed primarily to its flavonoids and terpenoid content. In vitro assays have demonstrated inhibition of pro-inflammatory enzymes such as cyclooxygenase (COX) and lipoxygenase (LOX), reducing the synthesis of inflammatory mediators (Kumar & Singh, 2018). In vivo studies on rodent models have shown that extracts of the plant reduce paw edema and inflammation markers, supporting its traditional use in managing inflammatory conditions (Patel et al., 2019).

6.2.2 Antioxidant activity

The high content of flavonoids and phenolic compounds imparts potent antioxidant properties to *C. multibracteata*. In vitro assays such as DPPH radical scavenging and FRAP (ferric reducing antioxidant power) have confirmed its ability to neutralize free radicals and reduce oxidative stress (Reddy & Kumar, 2017). Animal studies further show protection against oxidative damage in liver and kidney tissues, highlighting its potential in preventing oxidative stress-related diseases (Singh & Gupta, 2020).

6.2.3 Antimicrobial activity

Multiple in vitro studies have reported that *C. multibracteata* extracts inhibit the growth of various Gram-positive and Gram-negative bacteria, including *Staphylococcus aureus* and *Escherichia coli* (Sharma & Mehta, 2017). Its antifungal activity against species such as *Candida albicans* has also been demonstrated. The antimicrobial effects are believed to arise from alkaloids and phenolic compounds that disrupt microbial cell walls and interfere with metabolism.

Table 3. Overview of major phytochemicals in *Crotalaria multibracteata* and their therapeutic relevance

Phytochemical Class	Key Compounds	Pharmacological Activities	Ayurvedic Role	Traditional/Local Uses
Flavonoids	Quercetin, Kaempferol, Luteolin	Antioxidant, anti-inflammatory, antimicrobial	Balances Pitta and Raktha dhatu; detoxifying	Used in wound healing, skin disorders
Alkaloids	Monocrotaline, Retrorsine	Antimicrobial, cytotoxic, vasoactive	Katu rasa; digestive stimulant, improves circulation	Used for fever, internal pain relief
Terpenoids	Sesquiterpenes, Diterpenes	Anti-inflammatory, analgesic, antioxidant	Ushna virya (heating potency); reduces obstructions	Used in muscle pain, postpartum care
Phenolic Compounds	Gallic acid, Ferulic acid, Caffeic acid	Antioxidant, antiseptic, wound healing	Cooling, drying; manages Pitta disorders, ulcers	External washes for skin infections
Saponins & Tannins	Various saponins and tannins	Anti-inflammatory, antifungal, astringent, immune modulation	Astringent; supports tissue healing and immune response	Used in diarrhea, infections, and inflammation
Other Constituents	Glycosides, Sterols (β-sitosterol)	Cardiotonic, anti-inflammatory, cholesterol-lowering	Supports heart health and metabolic balance	Used for diabetes and cardiovascular support

Table 4. Summary of pharmacological activities of *Crotalaria multibracteata* with experimental models

Pharmacological Activity	Key Findings	Model/Method	References
Anti-inflammatory	Inhibits COX and LOX enzymes; reduces paw edema and inflammatory markers	In vitro enzyme assays; in vivo rodent paw edema model	(Kumar & Singh, 2018; Patel et al., 2019)
Antioxidant	Strong free radical scavenging activity; protects liver and kidney from	DPPH, FRAP assays; animal oxidative stress	(Reddy & Kumar, 2017; Singh &

Pharmacological Activity	Key Findings	Model/Method	References
	oxidative damage	models	Gupta, 2020)
Antimicrobial	Effective against Gram-positive and Gram-negative bacteria (*S. aureus*, *E. coli*); antifungal activity against *Candida albicans*	In vitro microbial inhibition assays	(Sharma & Mehta, 2017)
Cytotoxic / Anticancer	Selective cytotoxicity on breast and lung cancer cell lines; induces apoptosis and tumor inhibition	In vitro MTT assays; in vivo tumor models	(Das & Roy, 2021)
Antidiabetic	Enhances insulin secretion and glucose uptake in diabetic animal models	In vivo streptozotocin-induced diabetic rats	(Singh & Verma, 2019)
Hepatoprotective	Reduces elevated liver enzymes and oxidative liver damage caused by toxins	In vivo CCl4-induced liver injury model	(Joshi & Kumar, 2018)

6.2.4 Cytotoxic and anticancer effects

Preliminary cytotoxicity screening indicates that certain alkaloids and flavonoids in *C. multibracteata* exhibit selective cytotoxicity against cancer cell lines, including breast and lung carcinoma cells (Das & Roy, 2021). Both in vitro assays (MTT, apoptosis induction) and in vivo tumor inhibition studies have shown promising anticancer potential, although further mechanistic studies are required.

6.2.5 Antidiabetic and hepatoprotective activities

Though limited, some studies suggest *Crotalaria* species possess antidiabetic properties by enhancing insulin secretion and improving glucose uptake (Singh & Verma, 2019). Hepatoprotective effects have been observed in animal models where plant extracts reduce elevated liver enzymes and oxidative damage induced by toxins (Joshi & Kumar, 2018). These effects are attributed to antioxidant flavonoids and sterols.

6.3 Toxicological Profile of *Crotalaria multibracteata*

Certain species within the *Crotalaria* genus are known to contain pyrrolizidine alkaloids (PAs), which have been associated with hepatotoxicity, genotoxicity, and potential carcinogenicity when consumed in high doses or over prolonged periods (Fu et al., 2004). These alkaloids can cause veno-occlusive disease, liver fibrosis, and other toxic effects primarily through metabolic activation in the liver, producing reactive intermediates that damage hepatic tissue (Mattocks, 1986).

In the case of *Crotalaria multibracteata*, phytochemical studies have confirmed the presence of some PAs, although their concentrations tend to vary based on geographic location, plant part, and harvesting time (Chen et al., 2017). Toxicological evaluations using in vitro cytotoxicity assays and in vivo animal models have reported dose-dependent toxicity, with higher doses showing liver enzyme elevation and histopathological alterations (Kumar & Singh, 2019).

However, controlled administration of standardized extracts at recommended doses has shown an acceptable safety margin in acute and sub-chronic toxicity studies. For example, oral doses up to 500 mg/kg body weight in rodents did not produce significant adverse effects over 28 days of exposure, suggesting a relatively safe dosage range for therapeutic exploration (Sharma et al., 2020).

Nonetheless, caution is advised when considering long-term use or high-dose therapies due to the potential risk of PA accumulation. It is recommended that future research focuses on detailed PA profiling, detoxification techniques, and establishing clear therapeutic indices to ensure safe clinical use (Wang et al., 2021).

6.4 Biotechnological and Agronomic Aspects of *Crotalaria multibracteata*

The propagation of *Crotalaria multibracteata* primarily occurs through seeds, which exhibit varying germination rates depending on environmental conditions and seed treatment methods. Techniques such as scarification and soaking in gibberellic acid (GA3) have been employed to enhance germination percentage and speed, facilitating more reliable establishment of seedlings in cultivation systems (Singh et al., 2018). Additionally, in vitro propagation via tissue culture methods including nodal explants and callus induction has been explored to produce disease-free plants and mass propagation of elite genotypes, although these studies remain limited and warrant further optimization (Rao et al., 2012).

From an agronomic perspective, *Crotalaria multibracteata* shows adaptability to a range of soil types but prefers well-drained, sandy loam soils with moderate moisture levels. It thrives under tropical and subtropical climates, with optimal growth reported under full sunlight exposure and temperatures between 20–30°C (Kumar & Singh, 2019). Due to its nitrogen-fixing ability, *Crotalaria* species are often employed as green manure crops in sustainable agriculture, improving soil fertility and structure, thus reducing the need for synthetic fertilizers (Rao & Reddy, 2017). The cultivation potential of *C. multibracteata* for medicinal purposes is promising, yet detailed agronomic studies on yield optimization and harvesting times are sparse, indicating a gap in research that needs addressing for commercial-scale production (Sharma et al., 2021).

In the realm of green synthesis, recent preliminary investigations have utilized *Crotalaria* plant extracts, including *C. multibracteata*, as reducing and stabilizing agents in the biosynthesis of metal nanoparticles such as silver and gold nanoparticles. These biogenic nanoparticles exhibit potent antimicrobial and antioxidant properties, making the plant a valuable resource in nanotechnology applications aimed at environmentally friendly synthesis routes (Gupta et al., 2022). However, systematic studies focusing specifically on *C. multibracteata* remain limited and should be expanded to fully harness its potential in this emerging field.

7. CONCLUSION

Crotalaria multibracteata represents a promising yet underexplored medicinal plant with a diverse range of bioactive compounds, including flavonoids, alkaloids, terpenoids, and phenolics, that contribute to its notable pharmacological activities such as antioxidant, anti-inflammatory, antimicrobial, and cytotoxic effects. Its traditional and ethnomedicinal uses underscore its therapeutic potential, while recent scientific studies have begun to validate these claims through both *in vitro* and *in vivo* research. Despite this progress, significant gaps remain, particularly in detailed toxicological profiling, mechanism-based pharmacological investigations, and large-scale cultivation strategies. Future interdisciplinary research integrating modern biotechnological tools and advanced extraction techniques will be critical to unlocking the full potential of *C. multibracteata*. Furthermore, the exploration of its applications in pharmaceutical, nutraceutical, and green synthesis fields could pave the way for novel therapies and sustainable agricultural practices. In summary, *Crotalaria multibracteata* holds considerable promise as a source of new therapeutic agents, but comprehensive research efforts are essential to translate its traditional knowledge into safe and effective clinical applications.

CONSENT AND ETHICAL APPROVAL

It is not applicable.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of this manuscript.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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