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RESEARCH ARTICLE

Pharmacognostic, physicochemical evaluation and preliminary phytochemical screening of a neglected weed from Mizoram, India

Anima Debbarma[1,2], Probin Kr Roy[1], Sonjit Das[1], Lalhlenmawia Hauzel[1], Lalhriatpuii Tochhawng[1,*]

[1]Department of Pharmacy, Regional Institute of Paramedical and Nursing Sciences (RIPANS), Zemabawk, Aizawl, Mizoram-796017
[2]School of Medical and Paramedical Sciences, Mizoram University, Aizawl, Mizoram-796004

Chromolaena odorata (CO) is a commonly available weed in Mizoram, India, and has been used by traditional healers for a long time in treating wounds, cuts, and infections. However, due to the presence of morphologically similar-looking invasive weed species, there might be a high chance of misidentification, which might result in compromising its therapeutic safety and efficacy. The present study provides a comprehensive pharmacognostic profile of the CO leaf found in Mizoram, India via its detailed macroscopic and microscopic characteristics, along with physicochemical evaluation and phytoconstituent screenings. This study not only provides the evidence of its therapeutic claims but also provides the parameters for its quality control analysis.

Keywords : *Chromolaena odorata*, quality control, traditional medicine, Pharmacognostic evaluation, wound healing

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*For correspondence:
tclalhriatpuii@ripans.ac.in

Contact us:
mizoacadsci@gmail.com

Introduction

Chromolaena odorata (L.) R.M. King & H. Rob. (CO) belonging to the Asteraceae family, is a perennial shrub commonly known as Siam weed, Communist weed, Bitter bush, Siam marijuana, and Devil weed. CO is native to tropical and subtropical regions of the United States of America. However, recently it has spread to other country regions including Africa, southern Asia, as well as Australia, and has become one of the dominant agricultural weed species. Due to its rapid-growing capabilities, it has been classified among the world's most invasive weed species.[1] Fresh leaf juice of CO has been traditionally used in various developing countries, such as Thailand, Vietnam, and India, to treat ailments including leech bites, diabetes, periodontitis, wounds and wound infections, rashes, and as an insect repellent.[2] Traditional healers from Mizoram, India, used this plant as a paste to cure fresh cuts and snake bites. This plant has been reported to have various pharmacological activities, including antimicrobial, antifungal, anti-inflammatory, and anti-malarial properties.[3,4] The present study was carried out to provide evidence-based detailing of the CO leaf found in Mizoram, India, via Pharmacognostic, physicochemical evaluation, and screening of phytochemical constituents.

Materials and methods

Drugs and chemicals

Petroleum ether (60-80°C), extra pure, chloroform, and methanol were analytical grade and obtained from Merck Pvt. Ltd. All other chemicals, reagents, and solvents used were of analytical grade.

Collection and authentication of the plant

CO leaves were collected in the months of March-June 2022, from Aizawl, Mizoram, India. The

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plant was authenticated from the Botanical Survey of India (BSI), Shillong, with reference No: BSI/ ERC/Tech/2022-23-163 .

Preparation of extract

CO plant leaves were shade-dried and coarsely powdered. Successive extraction methods were carried out using a Soxhlet apparatus. Powdered leaves were subjected to extraction using petroleum ether, chloroform, followed by methanol. Extracts were filtered, concentrated and stored at 4°C for further evaluation. Methanolic extracts of CO were used to perform preliminary phytochemical screening.

Pharmacognostic studies

Various types of Pharmacognostic studies were performed for the CO leaf and are described below:

Organoleptic and macroscopic studies

Fresh leaf of CO was subjected to macroscopic and organoleptic evaluation, such as colour, odour, taste, and texture. Approximately 40 leaves were collected to measure the average lamina length and width.

Qualitative and quantitative microscopy

Fresh leaves as well as coarsely powdered dried leaves of CO were taken for morphological and histological studies. The transverse section of the leaf midrib was sectioned using a blade for the morphological and histological studies, and the sections were mounted in glycerine and stained with safranin under a microscope fixed with a camera (Primo star, Zeiss), and the photographs were taken at 10X and 40X magnification. Thin section of the lamina was observed for various types of cells under a microscope, and photographs were taken to calculate the stomata index and stomatal number. Dried leaves of CO were crushed into powders, and the microscopic evaluation of the powder was done, and the photographic contents were taken.[5]

Physicochemical analysis

Physicochemical parameters like loss on drying, total ash, acid insoluble ash, water soluble ash and extractive yield values for the CO leaf were determined. The percentage yield was calculated as per the WHO and Indian Pharmacopoeia guidelines for quality control of medicinal plants with modifications.[6,7]

Preliminary Phytochemical screening

Preliminary phytochemical screening of major phytoconstituents such as alkaloids, flavonoids, tannins, phytosterols, glycosides, triterpenoids, saponins and quinones for methanolic extracts of CO leaves was performed by following standard procedures.[8,9]

Results & Discussion

The organoleptic and macroscopic characteristics of CO leaves were presented in Table 1, and the fresh leaf pictures of the plant are shown in Fig. 1.

Prepared thin sections of fresh CO leaf lamina showed the presence of anomocytic stomata

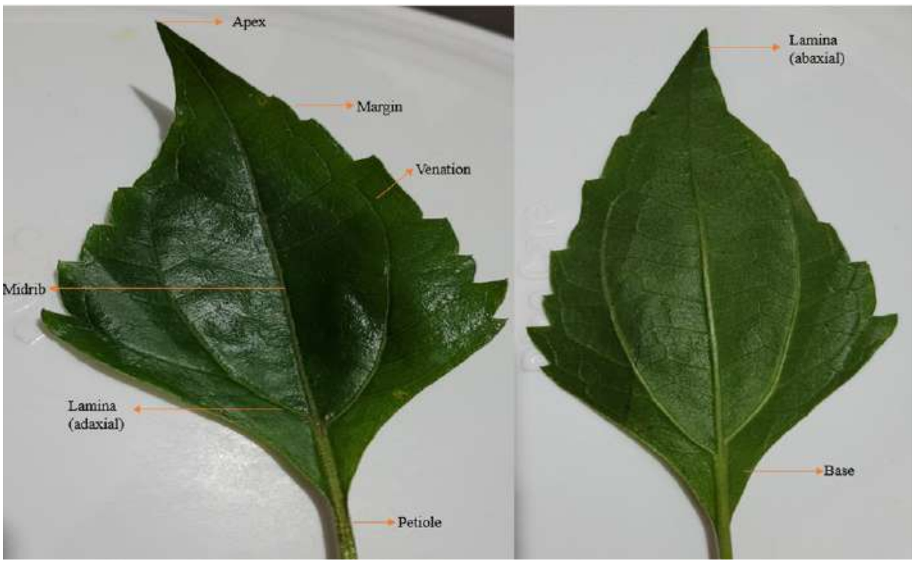

Figure 1. Picture of fresh leaves of *Chromolaena odorata*

Table 1. Organoleptic, macroscopic characteristics and leaf constants of CO

Parameter	CO Leaf sample
Type	Simple
Shape	Deltoid
Margin	Denate
Apex	Acuminate
Base	Hastate
Petiolation	Petiolate
Texture	Velvety hairs
Venation	Reticulate
Colour (adaxial)	Dark green
Colour (abaxial)	Dark green
Surface	Pubescent
Leaf arrangement	Opposite
Odour	Characteristics
Taste	Bitter
Lamina length (mean± SD, N=40)	11.65±1.10 cm
Lamina width (mean±SD, N=40	6.17±0.81 cm
Stomata Index	24-78
Stomatal number	26-32

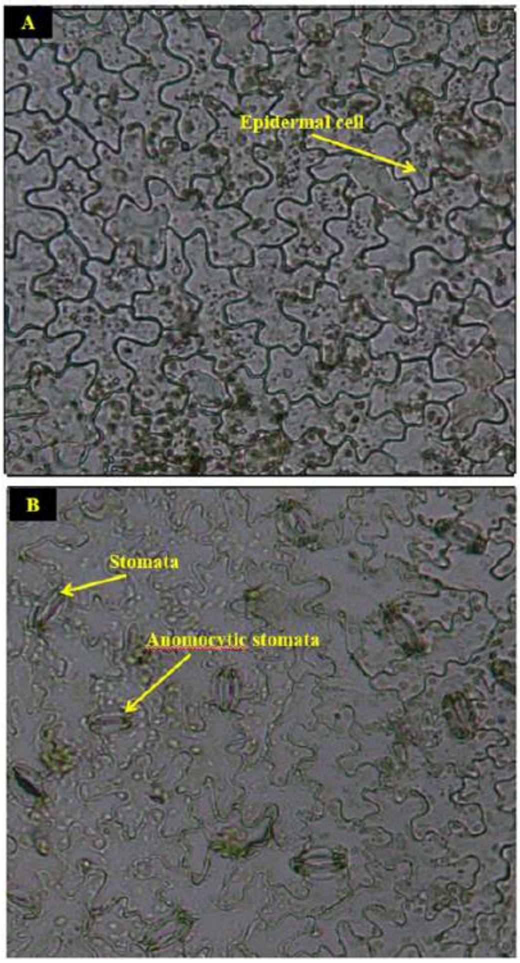

Figure 2. Representing microscopic images of CO fresh leaves at 10X **A.** Upper epidermis of CO **B.** Lower epidermis of CO

gathered by sinuous anticlinal-walled epidermal cells on both abaxial and adaxial surfaces (Fig 2. A & B). The transverse section of the CO mid-rib revealed the presence of covering trichomes on the upper epidermal surface, whereas the lower epidermis shows the presence of glandular trichomes. The microscopic image shows the presence of collenchyma and spongy parenchyma beneath epidermal cells, and the vascular bundle comprises xylem and phloem (Fig 3. A & B). Quantitative microscopic analysis for both plant leaves was performed to determine the total number of stomata index and stomatal number, and the results obtained were presented in a tabulated format in Table 1. Unstained powder microscopy of CO revealed the presence of stomata, unicellular trichomes and calcium crystals (Fig 3. C & D).

Various physicochemical parameters were represented in tabulated format (Table 2) for authentication of the quality control studies of CO. Preliminary phytoconstituents screening of CO methanolic plant extracts revealed the presence of various major phytochemicals given in Table 3.

Worldwide, the demand for herbal medicines is increasing due to their ability to target multiple pharmacological pathways, sustainability, less cost and fewer side effects, etc. However, adulteration of herbal drugs often leads to ineffective therapy or loss or compromised quality of drugs. Adulteration is the main reason for compromised herbal product quality. Due to the lack of proper quality control methods and compromised product quality, herbal drug products for medicinal purposes are limited. Therefore, proper identification and the quality of the medicinal plants need to be standardised, which indeed helps to study the quality control of herbal drugs. Pharmacognostic study of plants plays a crucial role in the identification and authentication of

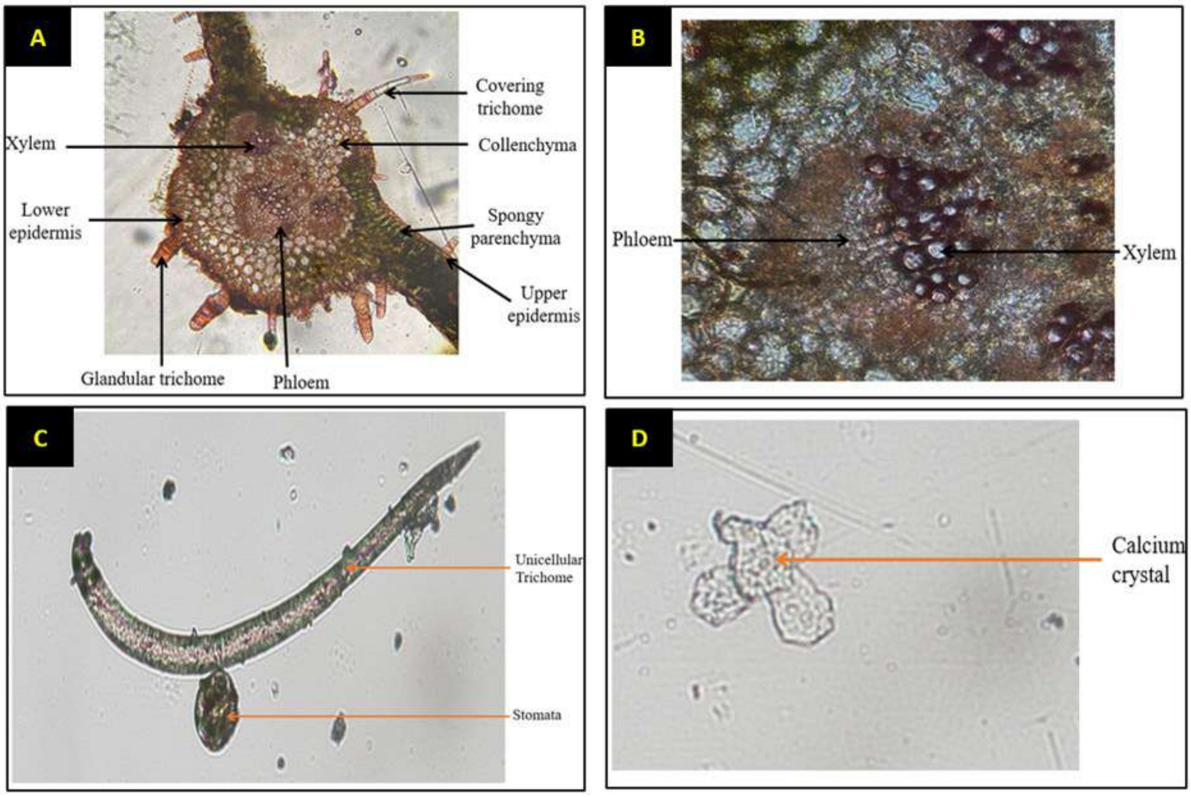

Figure 3. Representing the transverse section of midrib and the powder microscopy of CO **A & B.** Transverse section of CO at 10X and 40X **C & D.** Powder microscopy of CO

Table 2. Physicochemical properties of the CO leaf

Parameter		CO ± SD
Percent ash value (gm)	Total ash	10.44 ± 0.09
	Acid-insoluble ash	9.44 ± 0.35
	Water-soluble ash	8.64 ± 0.16
Percent loss on drying (gm)	-	12.95 ± 0.21
Percent extractive yield (gm)	Methanol	13.65 ± 1.00

herbal drug products by comparing the macroscopic and microscopic profiles of the plants. Microscopic evaluation of powder is one of the simplest ways to determine adulterations in herbal products. Ultimately, pharmacognostic studies help in maintaining the quality of the medicinal plants and help ensure consistent quality products.[10]

In our study, we have demonstrated the macroscopic and microscopic parameters of CO, which may further be utilised for quality control of CO-based products. Pharmacognostic study of CO revealed the qualitative organoleptic and macroscopic characters that can be easily used for the identification of the plant. The microscopic studies of the fresh and dried leaves revealed the

Table 3. Phytoconstituents present in methanolic leaf extracts of CO

Phytoconstituents	CO Methanolic leaf extracts
Alkaloids	+
Flavonoids	+
Phenolic compounds and tannins	+
Glycosides	+
Phytosterols	+
Saponins	+
Triterpenoids	+
Quinones	-

histological or internal cellular representation of the plant, to distinguish it from other similar-looking plant species.[11] Various physicochemical parameters like total ash, extractive value and loss on drying were usually studied for determining the quality and the purity of the crude plant materials. The total ash value provides the information regarding the inorganic materials or other impurities that might be present along with the crude drug. Extractive value and loss on drying also give an idea about the nature of the phytoconstituents extracted and their solubility in a specific solvent, and the amount of volatile constituents present along with water. Therefore, the lower the amount of these parameters, more the purity of the crude drugs.

The preliminary phytochemical screening is another parameter that helps in giving a basic idea regarding the nature of the phytoconstituents present in the crude extract, which in future could be used in drug discovery.[12] CO plant extracts showed the presence of various secondary plant metabolites like alkaloids, flavonoids, Phenols, tannins, phytosterols, glycosides, triterpenoids and saponins in preliminary phytoconstituents analysis. Many studies revealed the role of the above-mentioned phytoconstituents in treating various wounds. For example, a study suggested the involvement of alkaloids in treating subcutaneous wounds by collagen and fibroblast proliferation and promoting anti-inflammation.[13] These fibroblasts are the key components in wound closure as they help in fibrin clot breakage and produce extracellular matrix and collagen.[14] Flavonoids are one of the great sources of antioxidants and have multiple pharmacological activities, like reducing inflammation and bacterial growth. Flavonoids also prevent bacterial growth by lysis and damaging cell walls.[15,16] Phenolic compounds are also rich in antioxidants and antimicrobial properties. Another study suggested the involvement of phenols in inhibiting wound infection causing various pathogens to accelerate the healing process.[17] Tannins help in wound closure by helping scar formation by fibroblasts, which will lead to cell formation at the injury sites. Other than this activity, tannins also possess antibacterial and antioxidant properties, which are also the crucial properties of a plant extract which help in faster healing.[18,19] Another study revealed that glycosides are a type of triterpenoid which helps in wound healing by stimulating collagen during wound contraction, which helps in skin regeneration.[20,21] Saponins possess antibacterial properties as well as play a vital role in the wound healing process via endothelial cell migration, formation of new blood vessels, maintaining oxidation to inhibit cell apoptosis and pathways responsible for wound healing like Glycogen Synthase Kinase (GSK)-3β/β-catenin pathways.[22,23] This GSK-3 is a protein which helps in wound healing via modulating endothelin-1; therefore, targeting GSK-3 will control the wound healing process. A study by Fayed et al.[24] revealed the involvement of phytosterols in targeting GSK-3 for promoting wound healing.

Conclusion

Chromolaena odorata is a commonly available weed in Mizoram and has been utilised by traditional healers for a long time in treating wounds, cuts and infections. Locally, it is known as Tlang-sam. However, due to the presence of morphologically similar-looking invasive weed species, there might be a high chance of misidentification, which might result in compromising its therapeutic safety and efficacy. The present study provides a comprehensive Pharmacognostic profile of the CO leaves via its detailed macroscopic and microscopic characteristics, along with physicochemical evaluation and phytoconstituent screenings. These findings help in saving the plant from misidentification, adulteration and compromising its therapeutic efficiency. The phytochemical analysis of the plant extracts gives confirmation of the presence of various secondary plant metabolites, which are responsible for its therapeutic claims. This study not only provides the evidence of its therapeutic claims but also provides the parameters for its quality control analysis. However, extensive *in-vitro* and *in-vivo* analysis is required for a better understanding of its mechanism of action and future use in natural formulation development.

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RESEARCH ARTICLE

Biochemical characterization of *Bacillus paramycoides* DW1, a bacterial isolate from dairy waste water

Jugami Boro, Mintu Medhi, Asahan Ali, Nchebui Zeliang, Tovikali Zhimo, Jyotirmoy Bhattacharya*

Department of Biotechnology, School of Life science, Mizoram University, Aizawl-796004, Mizoram, India

Bacillus paramycoides DW1 (hereafter *B. paramycoides* DW1), an isolate from dairy waste water is a gram-positive, rod-shaped, endo-spore forming bacterium. The aim of this study was to assess the ability of this bacterium to hydrolyze lactose, proteins, and lipids (major organic constituents of dairy waste water), and its potential to grow using lactose, glucose, and galactose as sources of carbon, and ability to utilize other complex carbohydrates. The results suggest that lactose is a good source of carbohydrate for *B. paramycoides* DW1 growth. Although the best growth of the bacterium was observed in the presence of 2% lactose, it was able to grow even at higher concentration of lactose (5%) usually encountered in dairy waste water, and could also utilize a variety of other carbohydrates, such as xylose, cellobiose, saccharose, trehalose, glucose, and galactose. Concomitant with its growth using lactose, *B. paramycoides* DW1 displayed lactose-hydrolyzing intracellular β-D-galactosidase activity irrespective of concentrations of lactose with the highest being at 2%. This activity was inhibited in bacterial cells grown in the presence of glucose and galactose and by a metal ion chelator ethylene diamine tetraacetic acid (EDTA) suggesting that the enzymatic reaction is catabolite repressible and requires metal ions for its activity. Further biochemical characterization revealed that a neutral pH (7.0) and mesophilic temperature (37°C) was optimum for β-D-galactosidase activity in *B. paramycoides* DW1. Additionally, the bacterium was also endowed with extracellular protease (caseinolytic) and lipase activities. Overall, the findings reveal that the potential to hydrolyze lactose, protein, and lipid probably enables *B. paramycoides* DW1 to grow and survive in nutritionally complex dairy waste water environment and implies that such a bacterium may be a significant contributor to reduction of organic pollutant load in dairy waste water .

Keywords : *Bacillus paramycoides* DW1, β-D-galactosidase, Dairy waste water, Lipase, Protease

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*For correspondence:
mzut078@mzu.edu.in

Contact us:
mizoacadsci@gmail.com

Introduction

Bacteria are vital sources of numerous important enzymes. The gram-positive, rod-shaped, sporulating, ubiquitous firmicutes belonging to the genera *Bacillus* are worth notable in this regard.[1] The *Bacillus* spp. produce highly useful enzymes belonging to the hydrolase class, and include amylase, cellulase, protease, and lipase, among others. Such enzymes are not only of high industrial value, but are also critical to growth and survival of *Bacillus* in nutritionally diverse natural environments.[2,3] The dairy waste waters are one of many examples of nutritionally complex environment which harbor a large number of *Bacillus* spp. These include *Bacillus subtilis* VUVD001, *Bacillus pumilus*, *Bacillus amyloliquefaciens*, *Bacillus cereus*, *Bacillus anthracis*, *Bacillus thuringiensis*,

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