Phytochemical Screening and Antimicrobial Activity of Ethanol Extract of *Calotropis procera* Leaves

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KEYWORDS

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ABSTRACT

Medicinal plant secondary metabolites are used to treat a wide range of illnesses. Extensive secondary metabolites of therapeutic significance, including carbohydrates, proteins, steroids, tannins, flavonoids, phenolic, saponins, mucilage, fixed oil, and alkaloids, have been identified using literature-based phytochemical screenings. Ethanol extract of *Calotropis procera* leaves was assessed for its antimicrobial including antifungal and antibacterial and nematicidal activities. The zone of inhibition showed strong potential against root rot fungi and common laboratory bacteria. The extract was also analyzed for the phytochemical screening of various constitutes present in plants in the form of secondary metabolites. It is therefore considered that ethanol extracts of *Calotropi*s are a good source to be used in drug as well as agriculture industries.

1. Introduction

Herbal medicine is a field of conventional and indigenous medicine which concentrates on using plant extracts and their applications for medicinal purposes. In order to avoid and treat diseases, it entails the study and utilization of several plant parts, including roots, leaves, seeds, and flowers. This old discipline blends traditional therapeutic techniques with empirical understanding, having its roots in ancient societies. Herbalism has drawn interest of the scientific community recently because of its potential to advance integrative medicine and pharmacology by highlighting the therapeutic qualities of plant-based molecules (Acharya *et al.,* 2008).

The foundation of herbal medicine is the idea that certain plants have inherent compounds that can both prevent and treat diseases (Rajan *et al.,* 2011) Among the main bioactive substances present in plants are tannins, phenolic compounds, alkaloids, and flavonoids. These ingredients contribute to the medicinal effects of the plant and are essential to its therapeutic qualities. Medicinal herbs may have antioxidant properties because they contain phenolic compounds, which have hydroxyl groups that give these the capacity to donate hydrogen (Narayanaswamy and Balakrishnan, 2011). *Calitropis procera* (giant milk weeds) is mostly known for its antibacterial, antivenomic, antifertility, anticonvulsant and depressive properties among other pharmacological properties. Moreover, it possesses anti-inflammatory, anti-tumor, anti-diabetic, and antioxidant properties. It is said to have tannins, glycosides, alkaloids, and flavonoids (Joseph *et al.,* 2013). Numerous studies conducted in lab settings have demonstrated the strong antifungal and insecticidal effects of extracts from a variety of higher plants. These bioactive chemicals are the subject of continuing pharmacological and agricultural sciences study because they present a promising opportunity for creating alternatives that are natural to chemical agents in the management of microbial diseases and pests. Numerous lab studies were conducted which demonstrated the strong antifungal and insecticidal effects of extracts from a variety of higher plants. These bioactive chemicals are the subject of continuing pharmacological and agricultural sciences study because they present a promising opportunity for creating alternatives that are natural to chemical agents in the management of microbial diseases and pests (Satish *et al.,* 2007). Therefore, offer an excellent opportunity to study how the local environmental conditions affect the phytochemical profiles of plants. Indigenous populations have long used *C. procera* in traditional medicine; confirming these traditional uses through scientific research can help close the knowledge gap

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between traditional medicine and modern medical practices. The medicinal qualities of *C. procera* can provide accessible and alternative solutions for common health conditions experienced by the local people. Additionally, the study will improve our knowledge of local biodiversity and plant adaptation mechanisms by adding significant data to the scientific community.

2. Materials and Methods

2.1 Study Area

This study was conducted in tehsil Paharpur, district Dera Ismail Khan, KPK, Pakistan. It is located 32°6'8N latitude and 70°58'12E longitude, approximately 173 m (570 feet) above sea level. It has a hot, arid to semi-arid climate with mild winters. The region receives minimal rainfall. Tehsil Paharpur is a prosperous agricultural tehsil located in Dera Ismail Khan, Khyber Pakhtunkhwa, Pakistan. Numerous vegetables are cultivated in the area due to its fertile soil, which greatly influences the local economy and nutrition.

2.2 Collection of samples

The plants were randomly collected from different zones of tehsil Paharpur and identified by Dr. Hafiza Farhat at Institute of Biological Sciences Gomal University DI Khan. The samples were collected and placed in sterile zipper bags and brought to the chemical sciences laboratory, Gomal University, Dera Ismail Khan. The leaves were washed with sterile water and air dried, kept in a tight zip bag for later use.

2.3 Preparation of crude extracts

For the extraction process ethanol was used as a solvent. *C. procera* powdered leaves (200 g) plant material was processed using the Soxhlet method at 40 °C for 4 h for each extraction. Whatman filter paper No. 42 was used to filter each extract separately. In order to create semi-solid crude extracts, the mixture further concentrated to a thick viscous state by using a rotary evaporator (Heidoph 4001 efficient). The aqueous extract was warmed at 70 °C in a water bath (Tomar *et al.*, 2014).

2.4 Phytochemical analysis

To identify the phytochemicals present in the plant extract, various chemical tests were conducted to identify the presence of bioactive substances such as saponins, flavonoids, alkaloids, protein, and steroids (Donkor *et al.*, 2016). The plant extract stock solution was prepared at 10 mg/mL for testing.

2.4.1 Test for tannins

2.4.1.1 Preparation of 0.1% Ferric Chloride

A solution of 0.1 % ferric chloride was prepared with addition of 0.1 mL ferric chloride reagent to distilled water (99.9 mL).

2.4.1.2 Ferric Chloride test

An aliquot (1 mL) of the sample was mixed with a few drops of 0.1% ferric chloride solution, and the mixture was observed for the appearance of a brownish-green or blue-black coloration.

2.4.2 Test for Saponins

Distilled water (5 mL) is added to 1 mL extract, and the mixture was then strongly agitated. The presence of saponins was indicated by the observed soaking look.

2.4.3 Test for Flavonoids

Concentrated sulfuric acid used along with the walls of the tube after the addition of 5 mL of diluted ammonia to 1 mL extract. Yellow coloration appeared which indicated the presence of flavonoids.

2.4.4 Test for Alkaloids

A 1 mL sample was treated with a few drops of Dragendorff's reagent and observed for the development of an orange-red coloration.

2.4.5 Test for Steroids

To 1 mL filtrate of *C. procera* crude extract, 10 % H_2SO_4 was added. The mixture was then observed for the development of a green coloration.

2.5 Antibacterial activity against pathogenic bacteria

Bacterial lawns of *P. aeruginosa*, *E. coli*, *B. subtilis*, *S. typhimurium*, and *S. aureus* were prepared on 90

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mm petri dishes containing nutrient agar medium, and ethanol extract filtrates (20, 40, and 60 µL/disc) were applied. The samples were loaded onto thick sterile filter paper discs, dried and arranged in a clockwise order by concentration in the Petri plates. Discs containing distilled water served as the negative control, while Streptomycin (20 µg/disc) was used as the positive control. The petri plates were incubated at 30 °C for 2–3 days, and the zones of inhibition were measured in millimeters (Farhat et al., 2022b).

2.6 Antifungal activity against rotting fungi

The ethanol extract, dissolved in a specific solvent at 2 mg/mL was applied to paper discs at concentrations of 20, 40, and 60 µg, dried and arranged in a clockwise order according to concentration on plates containing Czapek's Dox Agar (CDA). Discs of pathogenic fungi, including *Fusarium solani*, *Fusarium oxysporum*, *Rhizoctonia solani*, *Macrophomina phaseolina*, *Aspergillus flavus*, and *Alternaria alternata*, were placed at the center of petri plates. Czapek's Dox broth (CDB) was used as the negative control, while Carbendazim (20 µg/disc) served as the positive control. The plates were incubated at 30 °C for 5–7 days, and zones of inhibition were measured in millimeters (Farhat et al., 2019).

2.7 Nematode culture

Root knot nematode (*Meloidogyne javanica)* Egg masses were picked from infected egg-plant under stereomicroscope and placed at temperature (25-30 °C) in cavity blocks filled with distilled H_2O. Juveniles hatched after 48 h were used for nematicidal activity. Twenty juveniles in 1 mL water were transferred to another cavity block along with 1 mL culture filtrate of test *ethanol extract of C. procera.* Mortality of juveniles was observed after 48 h (Farhat *et al.,* 2022a).

2.8 Statistical Analysis

Data was analyzed through suitable statistical tools including Minitab, SPPS and MS Excel. Descriptive statistics summarized the data and inferential statistics compared the levels of compounds.

3. Results

3.1 Site Area

The survey was conducted at tehsil Paharpur, district Dera Ismail khan, KPK, Pakistan, which is located at 32°6'8N latitude and 70°58'12E longitude, approximately 173 m (570 feet) above sea level. This has a climatic area based on arid and semi-arid regions with slightly hot summers and to some extent mild winters. In case of rainfall, the region was having very little exposure to it. This region was considered to be agriculture land due to the fertile soil that greatly influences the local economy of the region and makes it diverse (Figure 1).

Fig. 1. The map interpreting the geological position of Paharpur located in the Zone of DI Khan, KPK Pakistan

Fig 2. The specimen of *Calotropis procera* in tehsil Paharpur, district Dera Ismail khan, KPK, Pakistan

3.2 Phytochemical analysis

Phytochemical compounds were screened in ethanol extract of leaves of *C. procera* through a qualitative method (Figure 2). The results showed the presence and absence of various pharmacological active chemical constitutes including alkaloids, saponins, tannins, flavonoids and terpenoids. For the identification of tannins in a plant extract, the test of ferric chloride solution was used and the appearance of bluish black color indicated the presence of

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tannins. Saponins were also analyzed in plant extract of *C. procera* by mixing the extract with water then shaking vigorously. The formation of foam indicated the presence of saponins. After adding 5 mL of diluted ammonia to 1 mL of extract, concentrated sulfuric acid was carefully introduced along the tube walls. The appearance of a yellow coloration indicated the presence of flavonoids. In case of alkaloids identification, few drops of Dragendorff's reagents were added in 1mL of plant extract. The appearance of an orange-red coloration indicated the presence of alkaloids. Salkowski test was performed to analyze the terpenoids in a sample. Extract (5 mL) was mixed with chloroform (2 mL). After this concentrated Sulphuric acid (3 mL) was added carefully to form a layer. Presence of terpenoids was confirmed by the reddish-brown coloration of the inter face. It is therefore considered that the presence of phytochemicals indicated the potential of the crude extract of *C. procera* plants. In this study leaves samples were used for qualitative analysis (Table 1).

Table 1. Phytochemical screening of the crude extract of *C. procera* leaves

Phytochemical Screening	Crude extract of C. procera
Tannins	+
Saponin	-
Flavonoids	+
Alkaloids	+
Terpenoids	+

3.3 Antifungal potential of C. procera

To evaluate the antifungal activity of the crude extract prepared in ethanol, discs (thick filter paper) were loaded with varying concentrations of the extract (20, 40, and 60 µL/disc) and dried under sterile conditions in a laminar flow hood. The plates filled with Czapek's Dox Agar were loaded with disc prepared with different concentration of crude extract and placed at the corner of the plates. The fungus used for testing purposes was inoculated in the center of the petri dishes. A disc without broth inoculation was used as the negative control, while a disc containing 20 µg/disc of Carbendazim served as the positive control. The plates were incubated at 30 °C for 6–8 days, after which the distance between the disc and the test fungus was measured.

The pathogenic fungi used against crude extract of *C. procera* plants were *F. solani, F. oxysporum, R. solani, M. phaseolina, A. flavus and A. alternata.* These phytopathogens are also called root rot fungi. I*n vitro* study showed the potential of plants extract prepared in ethanol. Each positive control (Carbendazim disc) showed different zone against common pathogens *M. phaseolina (*6 mm), *R. solani* (5 mm), *F. solani* (7 mm) and *F. oxysporum* (8 mm). However, the concentration of crude extract of *C. procera* exhibited variation in inhibition zone up to 10 mm and more than 20 mm against *F. solani, F. oxysporum, R. solani, M. phaseolina, A. flavus and A. alternata* of 60 µL/disc. The formation of zones by different concentrations of ethanol extract indicates the presence of some sort of secondary metabolites which were responsible for antifungal activity. Therefore, it was considered that the extract of *C. procera* may be used in order to treat infectious disease caused by phytopathogens in agriculture industries. On the other hand, it can also be used in drug industries in order to treat infectious disease caused by pathogenic fungi (Table 2).

Table 2. *In vitro* suppression rate of pathogenic fungi by ethanol extract of *C. procera* leaves

Pathogenic Fungi	Concentrations				
	-ve Control	+ve Control	20 µL/disc	40 µL/disc	60 µL/disc
M. phaseolina	0.0^{a} ± 0.0	6^{b}±0.2	8^{bc}±0.4	11^{c}±0.9	15^{c}±1.4
R. solani	0.0^{a} ± 0.0	5^{b}±0.1	6^{b}±0.2	9^{c}±0.6	13^{c}±1.3
F. solani	0.0^{a} ± 0.0	7^{bc}±0.4	9^{c}±0.6	12^{cd}±1.1	17^{d}±1.5
F. oxysporum	0.0^{a} ± 0.0	8^{bc}±0.4	11^{cd}±0.9	18^{d}±1.5	21^{de}±2.1
A. flavus	0.0^{a} ± 0.0	6^{b}±0.2	5^{b}±0.1	9^{c}±0.6	15^{c}±1.4
A. alternata	0.0^{a} ± 0.0	3^{a}±0.01	7^{bc}±0.4	11^{c}±0.9	18^{d}±1.5

ANOVA was used to determine statistical differences. Values sharing the same letter in each column do not differ significantly ($p < 0.05$), Duncan's multiple range test; ± SD

3.4 In vitro antibacterial potential of crude extract

The crude extracts were used to check their efficacy level regarding antibacterial activity. In order to determine antibacterial activity, bacterial lawn of different common laboratory bacteria prepared in different plates having replicates and discs of –ve control, +ve control and different concentration

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loaded disc of crude extract were placed at different positions in Petri plates having Trypticase Soya Agar (TSA). The disc of penicillin (10 µg/disc) was used as +ve control for the trails.

For antibacterial activity, five pathogenic bacteria were tested, including both Gram-positive and Gram-negative strains: *Escherichia coli*, *Salmonella typhimurium*, *Bacillus subtilis*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa*.The formation of zones of inhibition at different concentrations against specific bacteria indicates the potential of crude extract. If the zone is quite larger it is considered to be effective in order to further utilize that extract for drug formation or on the other hand it also specifies the extreme level of presence of compounds. At different concentrations, different ranges of zones are formed in order to show positive results. Crude ethanol extract of *C. procera* exhibited strong activity against gram positive and negative bacteria at 60 µL/disc. The ethanol extract of *C. procera* exhibited zones of inhibition of 15 mm or greater against *S. aureus*, *S. typhimurium*, *B. subtilis*, *E. coli*, and *P. aeruginosa* at a concentration of 60 µL/disc. The positive control also produced clear inhibitory effects, whereas the negative control (sterile water–loaded discs) showed no activity. Each result was compared with negative control as well as positive control. Positive results were showed by different concentrations of the ethanol extract of *C. procera* leaves (Table 3; Figure 3 & 4).

Table 3. *In vitro* inhibition rate of *E. coli*, *S. aureus*, *P. aeruginosa*, *S. typhimurium* and *B. subtilis* by crude extract of *C. procera*

Pathogenic Bacteria	Concentrations				
	-ve Control	+ve Control	20 µL/disc	40 µL/disc	60 µL/disc
E. coli	0.0^{a} ± 0.0	5^{b}±0.3	7^{c}±0.4	12^{d}±1.4	18^{e}±1.9
S. aureus	0.0^{a} ± 0.0	7^{c}±0.4	6^{b}±0.4	8^{bc}±0.7	16^{d}±1.7
P. aeruginosa	0.0^{a} ± 0.0	8^{bc}±0.7	9^{bc}±0.1	13^{d}±1.5	19^{e}±2.0
S. typhimurium	0.0^{a} ± 0.0	6^{b}±0.4	10^{d}±1.1	18^{e}±1.9	22^{de}±2.3
B. subtilis	0.0^{a} ± 0.0	6^{b}±0.4	5^{b}±0.3	9^{bc}±0.1	15^{d}±1.6

Statistical differences were determined using analysis of variance (ANOVA). Values in each column sharing the same letter do not differ significantly ($p < 0.05$) according to Duncan's multiple range test. Results are presented as mean ± standard deviation

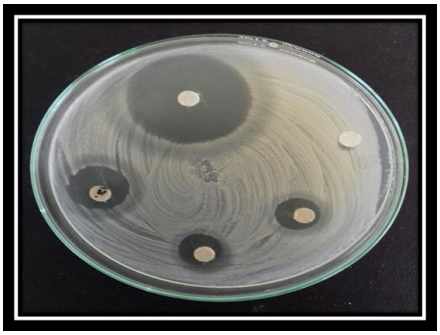

Fig 3: Growth inhibition of *S. typhimurium* by crude extract *of C. procera* in disc diffusion method
A=Control, B=+ve control, C=20µl/disc, D=40 µl/disc, E=60 µl/disc

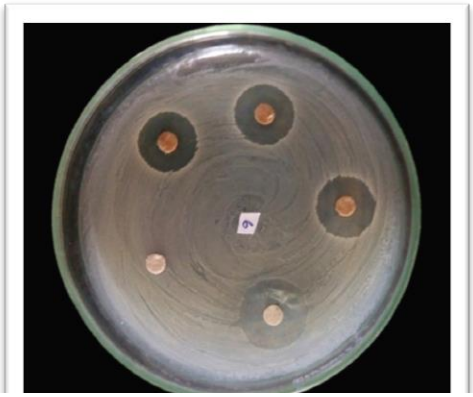

Fig 4: Growth inhibition of *E. coli* by by crude extract *of C. procera* in disc diffusion method
A=Control, B=+ve control, C=20µl/disc, D=40µl/disc, E=60µl/disc

3.5 In vitro nematicidal potential of crude extract

Crude extracts of *C. procera* were evaluated for *in vitro* nematicidal activity. Asuspension containing 10 juveniles per mL was mixed with 1 mL of the crude extract in cavity blocks and incubated at 26 ± 5 °C. Each treatment was replicated three times, and juvenile mortality was recorded at 24- and 48-hour intervals.

Crude extract of *C. procera* exhibited nematicidal effects, juvenile mortality of *M. javanica* occurred at different percentages. The results showed best potential when compared with control one. The crude extract was checked after 24 h and 48 h intervals. 55 % mortality rate of juveniles of *M. javanica* were noticed in 24 h of intervals. After 48 h of intervals, the rate of mortality level increased up to 98 % (Table 4).

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Table 4. Effect of crude extract of *C. procera* leaves on juveniles' mortality of *M. javanica* after 24 and 48 h

Treatments	Juveniles Mortality %	
	24 h	48 h
Control	$8.3^{a}\pm1.6$	$12.5^{a}\pm1.9$
Crude extract	$55^{b}\pm2.6$	$98^{b}\pm3.1$

Statistical differences were determined using analysis of variance (ANOVA). Means within a column followed by the same letter are not significantly different ($p < 0.05$) according to Duncan's multiple range test. Values are expressed as mean ± standard deviation.

4. Discussion

The bioactive secondary metabolites found in medicinal plants are used to treat a wide range of illnesses. Extensive secondary metabolites of therapeutic significance, including carbohydrates, proteins, steroids, tannins, flavonoids, phenolics, saponins, mucilage, fixed oil, and alkaloids, have been identified in using literature-based phytochemical screenings (Pal *et al.,* 2015). Bioactive substances including flavonoids, terpenoids, alkaloids, glycosides, quinines, phenols, tannins, coumarin, and saponins are detected in the methanol extract of *C. procera* leaves (Gandhiraja *et al.,* 2009). It is said that drinking a leaf decoction that has been boiled in water induces diuresis and is applied to treat urinary tract infections. The plant possesses liver-protective, hypolipidemic, antifertility, antihapatotoxic, anticonvulsant, antidepressant, and wound-healing properties. Moreover, plant seeds also exhibited diuretic properties (Krishnaraju *et al.,* 2006).

Traditionally, the entire plant of *C. procera* is used for medicinal purposes. Its flowers, fruits, leaves, stems, and roots are used to treat various diseases including dysentery, leprosy, uterine and vaginal issues, burning sensations, inflammation, leucoderma, asthma, high blood pressure, and fatigue. Additionally, it is effective against amoebic dysentery, diarrhea, bleeding piles, and urinary infections (Meenatchisundaram *et al.,* 2009). Aside from that, numerous studies have documented its anti-inflammatory, antibacterial, anti-venom, anticonvulsant, anti-ulcer, and antihyperglycemic properties. The existence of different plant secondary metabolites, such as bioactive components like alkaloids, terpenoids, glycosides, flavonoids, quinines, tannins, phenols, coumarin, and saponins, are primarily responsible for these activities. The biological activity and phytochemical analysis of *C. procera* has been the subject of numerous studies conducted in Pakistan. However, the literature about these topics is limited in district Dera Ismail Khan. Therefore, the purpose of this study is to carefully investigate the photochemistry and biological activities of *C. procera* in tehsil Paharpur district Dera Ismail Khan. Investigating photochemistry and biological effects of *C. procera* in tehsil Paharpur, is driven by several key reasons. This region is known for its diverse flora and various plants are cultivated in the area due to its fertile soil, which greatly influences the local economy and nutrition. Therefore, offering an excellent opportunity to study how the local environmental conditions affect the phytochemical profile of plants. Indigenous populations have long used *C. procera* in traditional medicine; confirming these traditional uses through scientific research can help close the knowledge gap between traditional medicine and modern medical practices**.**

Numerous studies conducted in lab settings have demonstrated the strong antifungal, antibacterial and insecticidal effects of extracts from a variety of higher plants. These bioactive chemicals are the subject of continuing pharmacological and agricultural sciences study because they present a promising opportunity for creating alternatives that are natural to chemical agents in the management of microbial diseases and pests (Ghani *et al.,* 2003). Roughly 87 % of all recognized human diseases are treated by natural products and medications derived from them. These diseases include a broad spectrum of ailments like cancer, bacterial infections, and immunological problems. Due to their wide range of botanicals, which have therapeutic potential for treating complicated disorders and boosting immune system performance, they have become essential to contemporary drug research. This underscores the crucial importance of sources from nature in both contemporary as well as traditional medicine (Newman *et al.,* 2007).

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References

Acharya, D. (2008). *Indigenous Herbal Medicines:* Tribal formulations and traditional herbal practices. Aavishkar Publishers Distributor, Jaipur-India; pp, 440.

Donkor, A. M., Mosobil, R., & Suurbaar, J. (2016). *In vitro* bacteriostatic and bactericidal activities of *Senna alata*, *Ricinus communis* and *Lannea barteri* extracts against wound and skin disease causing bacteria. *Journal of Analytical & Pharmaceutical Research*, 3(1): 46.

Farhat, H., Urooj, F., Sohail, N., Ansari, M., & Ehteshamul-Haque, S. (2022a). Evaluation of nematicidal potential of endophytic fungi associated with healthy plants and GC-MS profiling of metabolites of endophytic *Fusarium solani*. *South African Journal of Botany*, *146*: 146-161.

Farhat, H., Urooj, F., Sohail, N., Hameedi, S. F., Ali, M. S., & Ehteshamul-Haque, S. (2022b). Evaluation of antibacterial potential of endophytic fungi and GC-MS profiling of metabolites from *Talaromyces trachyspermus*. *South African Journal of Botany*, 150: 240-247.

Farhat, H., Urooj, F., Tariq, A., Sultana, V., Ansari, M., Ahmad, V. U., & Ehteshamul-Haque, S. (2019). Evaluation of antimicrobial potential of endophytic fungi associated with healthy plants and characterization of compounds produced by endophytic *Cephalosporium* and *Fusarium solani*. *Biocatalysis and Agricultural Biotechnology*, *18*: 101043.

Gandhiraja, N., Sriram, S., Meenaa, V., Srilakshmi, J. K., Sasikumar, C., & Rajeswari, R. (2009). Phytochemical screening and antimicrobial activity of the plant extracts of *Mimosa pudica* L. against selected microbes. *Ethnobotanical leaflets*, 13: 618-24.

Ghani, A. (2003). Medicinal plants of Bangladesh. Asiatic Society of Bangladesh. *Dhaka, Bangladesh*, *5*(500), e4.

Joseph, B., George, J., & Mohan, J. (2013). Pharmacology and traditional uses of *Mimosa pudica*. *International Journal of Pharmaceutical Sciences and Drug Research*, 5(2): 41-44.

Krishnaraju, A. V., Rao, T. V., Sundararaju, D., Vanisree, M., Tsay, H. S., & Subbaraju, G. V. (2006). Biological screening of medicinal plants collected from Eastern Ghats of India using *Artemia salina* (brine shrimp test). *International Journal of Applied Science and Engineering*, *4*(2): 115-125.

Meenatchisundaram, S., Priyagrace, S., Vijayaraghavan, R., Velmurugan, A., Parameswari, G., & Michael, A. (2009). Antitoxin activity of *Mimosa pudica* root extracts against *Naja naja* and *Bangarus caerulus* venoms. *Bangladesh Journal of Pharmacology*, *4*(2): 105-109.

Narayanaswamy, N., & Balakrishnan, K. P. (2011). Evaluation of some medicinal plants for their antioxidant properties. *International Journal of PharmTech Research*, 3(1): 381-385.

Newman, D. J., & Cragg, G. M. (2007). Natural products as sources of new drugs over the last 25 years. *Journal of Natural Products*, 70(3): 461-477.

Pal, P., Datta, S., Basnett, H., Shrestha, B., & Mohanty, J. P. (2015). Phytochemical analysis of the whole plant of *Mimosa pudica* (Linn.). *Universal Journal of Pharmaceutical Sciences and Research*, *1*(1): 1-9.

Rajan, S., Thirunalasundari, T., & Jeeva, S. (2011). Anti—enteric bacterial activity and phytochemical analysis of the seed kernel extract of *Mangifera indica* Linnaeus against *Shigella dysenteriae* (Shiga, corrig.) Castellani and Chalmers. *Asian Pacific Journal of Tropical Medicine*, 4(4): 294-300.

Satish, S., Mohana, D. C., Ranhavendra, M. P., & Raveesha, K. A. (2007). Antifungal activity of some plant extracts against important seed borne pathogens of *Aspergillus* sp. *Journal of Agricultural Technology*, 3(1): 109-119.

Tomar, V., Prasad, S., & Kumar, D. (2014). Adsorptive removal of fluoride from aqueous media using *Citrus limonum* (lemon) leaf. *Microchemical Journal*, *112*: 97-103.

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