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PHARMACOGNOSTICAL EVALUATION AND ANTIBACTERIAL ACTIVITY OF BARK OF *Plumeria alba* LINN.

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ABSTRACT

In the present study the bark of *Plumeria alba* Linn. (Apocynaceae) was evaluated for pharmacognostical and phytochemical parameters and methanolic extract for antimicrobial activity by determining its MIC (by checker board method) and zone of inhibition (by cup plate method).Pharmacognostical parameters of the bark of *Plumeria alba* Linn. (Apocynaceae) were studied with the aim of drawing the pharmacopoeial standards for this species. The present study was also designed to evaluate the antimicrobial efficacy of the methanolic extract of the bark of *Plumeria alba* Linn. (Apocynaceae) against various diarrhea and dysentery causing drug resistant microorganisms isolated from patients admitted in hospitals. This study has pointed to the potential application of *P. alba* as a bactericide and fungicide.

KEYWORDS: Bactericide, MIC, fungicide, diarrhea.

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INTRODUCTION

Plumeria genus of family Apocynaceae comprises of about 8 species that occur in India. It is genus of lactiferous trees and shrubs, native of tropical America. *Plumeria alba* Linn. is a deciduous, small tree, mainly grown as ornamental tree, particularly in the vicinity of religious places and burial grounds throughout the India. The leaves are lanceolate to oblanceolate. The flowers are white with yellow centre, sometimes pink-flushed outside, in terminal panicles, fragrant, in corymbose fascicles. The follicles are divaricate and brownish black; seeds are oblong, winged, with pappus. The plant is propagated through cuttings. The vernacular names of the plant is '*Chameli*' in Hindi, '*Perungalli*' in Tamil, '*Khairchampa*' in Marathi, '*Frangipani*' in English and '*Dalanaphula*' in Bengali[1].

Figure. 1
Flowers and Leaves of Plumeria alba Linn.

Traditionally, many parts of the plant considered medicinal. The bark is used as purgative, emmenagogue and febrifuge. The latex of the plant has rubefacient and purgative properties. The fruit is edible, latex is applied to ulcers, herpes and scabies and seeds possess haemostatic properties. Moreover its bark is bruised and applied as plaster over hard tumors[2], whereas the latter taxon finds use as purgative, cardiotonic, diuretic and hypotensive. The plant is reported to contain amyrinacetate, mixture of amyrins, ß-sitosterol, scopotetin, the iriddoids isoplumericin, plumieride, plumieride coumerate and plumieride coumerate glucoside[2&3]. Bioactive richness of these active constituents were present in the plant.The extract of the bark shows antifungal activity against *Helminthosporium sativum*. The bark extract also showed antifilarial activity [4].Thus, establishment of the pharmacognostical profile of the bark of *P. alba* will assist in standardization of the latter that can assure quality, purity and identification of the samples.The present study also focused on the antimicrobial properties of the methanolic extract of the bark. The minimum inhibitory concentration (MIC) of the extract was determined against various microorganisms by serial dilution checker board technique. The agar dilution or cup plate method was used to determine the zone of inhibition against the sensitive strains at MIC so determined.

MATERIALS AND METHODS

Plant Material

The flowers and leaves of *P. alba* were collected from the surrounding areas of Greater Noida in the month of March. The plant was identified and authenticated (voucher no. NHCP/NBPGR/2010-18 dated 13th April' 2010) by Dr. Anjula Pandey, Principal Scientist, NBPGR, Pusa Campus, New Delhi. A voucher specimen has been retained in the Dept. of Pharm. Technology, N.I.E.T., Greater Noida, for future references. The coarse powder of the bark (50 gm) was extracted in a soxhlet

apparatus with methanol and the solvent was removed by evaporation on a heating mantle by taking care that the temperature did not rise above 60ºC. A semisolid dark viscous crude extract (yield 4.93% w/w) thus obtained was tested for its antibacterial and antifungal potentiality.

Test microorganisms

The test bacteria used were *Shigella flexneri* type 36 NK 381, *Sh. flexneri* type 6B 999, *Sh. flexneri* type BCH 995, *Sh. boydii* 22461, *Sh. boydii* 16552, *Sh. boydii* 8, *Sh. soneii* BCH 397, *Sh. soneii* E08869, *Sh. soneii* NK 840, *Sh. soneii* BCH 937, *Sh. soneii* I, *Sh. soneii* DN3, *Sh. soneii* F11001, *Sh. soneii* NK 29, *Sh. dysenteriae* 1, *Sh. dysenteriae* 9, *Vibrio cholerae* 1023, *V. cholerae* BD 1/81, *V. cholerae* 1341, *V. cholerae* 452, *V. cholerae* 1033, *V. cholerae* 575, *V. cholerae* 765, *V. cholerae* 1311, *V. cholerae* 756, *V. cholerae* DN6, *V. cholerae* A 26, *E. coli* AP600, *E. coli* 383, *E. coli* RH 07/12, *E. coli* 18/9, *E. coli* 597, *E. coli* 798, *E. coli* 35B, *E. coli* 306, *E. coli* K88, *E. coli* 872, *Enterobacter* spp. AP596 , *S. typhii* Type 2, *S. aureus* ML 267, *S. aureus* ATCC 6538, *S. aureus* MTCC 96, *S. aureus* 381, *B. subtilis* MTCC 441, *B. cereus* MTCC 1305, *B. pumilus* 8241, *Pseudomonas putida* MTCC 2252, *P. aeuriginosa* AP585 NLF, *Klebsiella pneumoniae* and *Proteus vulgaris* AP679 NLF.The test fungi used were *Candida albicans* ATCC 10231, *Candida albicans* 5, *Aspergillus niger* MTCC 281, *Penicillium chrysogenum* MTCC 2725, *Phaenorochaete chrysporium* MTCC 787 and *Ralstonia entropha* MTCC1255. These microbial strains included various drug resistant hospital isolates collected and characterized in the Department of Pharmaceutical Technology, Jadavpur University, India. All strains were maintained on Nutrient Agar (NA) for bacteria and Sabourauds's Dextrose Agar (SDA) slants for fungi at 4ºC prior to use for antibacterial and antifungal tests respectively.

Macroscopic and Microscopic Analysis

The macroscopy and microscopy of the stem were studied according to the method of Brain and Turner[5]. For the microscopical studies, cross sections were prepared and stained.

Physicochemical Analysis

All parameters were applied on bark only physicochemical analysis i.e., percentage of ash values and extractive values, were performed according to the official methods prescribed in Indian Pharmacopoeia, 1996 and the WHO guidelines on quality control methods for medicinal plant materials (WHO/QCMMPM guidelines). Fluorescence analysis was carried out according to the method of Chase and Pratt[6].

Preliminary Phytochemical Screening

Chemical tests were performed in the preliminary phytochemical screening to identify various secondary metabolites such as tannins and phenols, carbohydrates, glycosides, saponins, alkaloids, flavonoids and sterols using standard methods[7&8].

Determination of MIC by Serial Dilution technique[9, 10&11]

The bark extract (stock solution) was reconstituted with a minimum amount of dimethyl sulfoxide (DMSO). This solvent did not possess any antimicrobial activity of its own. Calculated volumes of this stock solution were dispensed in a series of McCartney bottles previously containing calculated volume of sterile cooled molten nutrient agar media (40-45ºC) to prepare final volume of 30 ml each with dilutions of 5, 10, 25, 50, 100, 200, 400, 800 and1000 µg/ml. The stock solution were dispensed into molten SDA to prepare varying dilutions of 100, 200, 400, 800, 1500 and 2000 µg/ml while determining the MIC against the fungi. Then these molten media containing varying concentration of extract were poured aseptically in pre-sterilized Petri dishes (70 mm) to give sterile nutrient agar plates with varying dilution of extract. These plates were then kept in refrigerator at 4ºC for 24hrs to ensure uniform diffusion of the extract. Then these plates were dried at 37ºC for bacteria and 25ºC for fungi for 2 hours before spot inoculations. One loopful (loop diameter: 3mm) of an overnight grown

bacterial strain suspension (10^5 CFU/ml) was added in each quadrant as marked by checker board technique. The spotted plates were incubated at 37ºC and 25ºC for 24 hours for bacteria and fungi respectively, in an incubator and MIC values were obtained.

Determination of zones of inhibition by Disc Diffusion method [9, 10&11]

The stock solution (each of 10μg/ml) of both extract and ciprofloxacin were prepared. From these stock solutions two sets of four dilutions (200, 400, 800, 1000 μg/ml) each of bark extract (solvent: DMSO) and ciprofloxacin (solvent: sterile distilled water) were prepared in sterilized McCartney bottles. Sterile agar plates were prepared and incubated at 37ºC for bacteria for 24 hours to check for the presence of any sort of contamination. Then each sterilized agar plates were flooded with liquid culture of the strains, dried for 30 minutes at 37ºC for bacteria. The sterile Whatmann filter paper disc (4 mm diameter) were soaked in four different dilutions of the crude extract and placed in appropriate position of the plates marked as quadrant at the back of Petri dishes. All the flooded plates with corresponding paper discs soaked with appropriate dilutions of extract were incubated for 24 hours and diameter of zone of inhibition were measured in mm. Similar procedure was adopted for reference standard drug and corresponding zone diameters were measured and compared accordingly.

Determination of Mode of Action of the Extract [10&11]

To determine whether the extract was bacteriostatic or fungistatic and bactericidal or fungicidal in nature, plugs from the zone of inhibition were taken out and reincubated into fresh media which were then examined for their growth after 96 hours incubation at 37ºC and 25ºC in an incubator, respectively.

RESULTS AND DISCUSSION

Macroscopic Characters

Plumeria alba was found to grow as a spreading shrub or small tree to a height of 7-8 m (20-25 ft) and similar width. It has a thick succulent trunk and sausage-like blunt branches covered with a thin grey bark. The branches were found to be somewhat brittle and when broken, oozed a white latex, or sap, which could be irritating to the skin and mucous membranes.

Microscopic Characters

The outer portion of the bark consisted of cortex, which were two layered parenchymatous cells. The next layer consisted of phelloderm which is having 4-6 layered cells. They were followed by loosely packed parenchymatous cells consisting of pericyclic fibres.

1(a) **1(b)**

Figure. 1(a) & (b)
Outer portion of the bark of Plumeria alba

The vascular tissues formed a wide bowl shaped outline. Phloem was in continuous line. Apart from the outer phloem, there were numerous scattered irregular masses of phloem strands distributed along the inner boundary of the xylem arc. Distal part of the bark had two thick cylindrical adaxial wings and wide, shallow adaxial semicircular round surface.

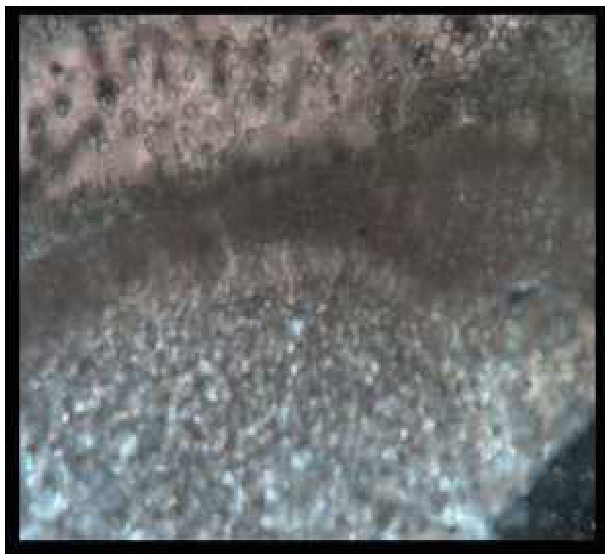

Figure. 2
Vascular bundle of P. alba bark

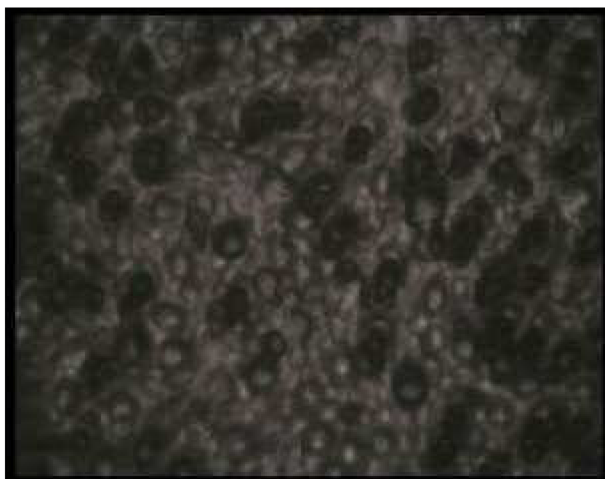

Figure. 3
Mucilage cells of P. alba bark

Powder Microscopy

The bark powder was found to be brownish green in color with an unpleasant order and bitter taste. On microscopical examination the powder showed numerous calcium oxalate crystals. They were prismatic as well as arranged in sheath form. The cork cells were found to be stratified with broken ends. The xylem fibers were of libriform type. They had thick lignified walls and wide lumen. The central portion of the fiber was wider and two ends of the fiber were tapering. Some fibers were non-lignified cylindrical shaped. Laticifers were found to be quite abundant in the stem powders. The vessels were of reticulate type and starch grains were absent.

Physicochemical Studies

Ash value of a drug gives an idea of the earthy matter or the inorganic composition and other impurities present along with the drug. Extractive values are primarily useful for the determination of exhausted or adulterated drugs. The alcohol soluble extractive was high in bark of *P. alba*. The results of physicochemical constants of the drug powder are presented in (Table 1).

Table 1
Physicochemical Constants

S.No.	Parameters Values	% (w/w)
1.	Total ash	10.25
2.	Acid insoluble ash	2.5
3.	Water Soluble ash	4.7
4.	Loss on drying	2.8
5.	Extractive Values	
(i)	Water soluble extractive	1.29
(ii)	Alcohol soluble extractive	3.5

Fluorescence Analysis of Powder Drug

Fluorescence analysis of drug powder treated with acids was studied at day light and the observations are presented in (Table 2).

Table 2
Fluorescence analysis of powdered drug of P. alba

Treatments	Color developed under UV light	
	Short (254 nm)	Long (365 nm)
Powder as such	Greenish brown	Brown
Powder + 1N HNO_3	Reddish brown	Reddish brown
Powder + NaOH (Aqueous)	Reddish brown	Yellowish brown
Powder + 1N HCl	Dark brown	Dark brown
Powder +Iodine	Dark brown	Dark brown
Powder + 50%H_2SO_4	Dark greenish brown	Yellowish brown
Powder + $FeCl_3$	Dark brown	Dark brown
Powder + Acetic acid	Dark brown	Light brown
Powder +Methanol	Green	Greenish brown

Preliminary Phytochemical Screening

Preliminary phytochemical screening of the extract revealed the presence of tannins, saponins, flavonoids, steroids, terpenoids, cardiac glycosides and reducing sugar in it (Table 3).

Table 3
Preliminary Phytochemical screening of bark extracts of Plumeria alba using various solvents

S.No	Plant constituents	Petroleum ether extract	Chloroform extract	Acetic Acid extract	Acetone extract	Methanol extract	Aqueous extract	Benzene extract
1	Alkaloids	-	-	+	-	-	-	+
2	Carbohydrates	-	+	-	-	+	-	-
3	Glycosides	-	+	-	-	+	-	+
4	Saponins	+	-	-	-	+	-	-
5	Phenolic compds & Tannins	_	-	-	-	+	-	-
6	Flavonoids	+	+	-	-	+	+	-
7	Phytosteroides	-	+	+	-	+	+	-

Antibacterial Activity

The result in Table 4 depicted the MIC values of the methanolic extract of the bark of *Plumeria alba* Linn. against various tested bacterial pathogens.

Table 4
Determination of MIC of the bark extracts of P. alba Linn. against different bacterial strains

S.No.	Name of m/o	Bacterial growth in different concentrations (µg/ml) of bark extract of *P. alba*									
		0*	5	10	25	50	100	200	400	800	1000
1	*Shigella flexneri* type 36 NK 381	+	+	+	+	+	+	+	+	+	+
2	*Sh. flexneri type* BCH 995	+	+	+	+	+	+	+	+	+	+
3	*Sh. flexneri* type 6BCH 999	+	+	+	+	+	+	+	+	+	+
4	*Sh. boydii* 22461	+	+	+	+	+	+	+	+	+	+
5	*Sh. boydii* 16552	+	+	+	±	±	±	±	±	±	±
6	*Sh. boydii* 8	+	+	±	-	-	-	-	-	-	-
7	*Sh. sonnei* BCH 397	+	+	IC	IC	IC	IC	-	-	-	-
8	*Sh. sonnei* E08869	+	+	+	+	+	±	-	-	-	-
9	*Sh. sonnei* NK 840	+	+	+	±	±	±	±	-	-	-
10	*Sh. sonnei* BCH 937	+	+	+	+	+	+	+	+	+	+
11	*Sh. sonnei* 1	+	+	+	+	±	±	±	±	±	±
12	*Sh. sonnei* DN3	+	+	+	+	+	+	+	+	+	+
13	*Sh. sonnei* F11001	+	+	+	+	+	+	+	+	+	+
14	*Sh. sonnei* NK 29	+	+	+	+	+	+	-	-	-	-
15	*Sh. dysenteriae* 1	+	+	+	+	±	±	±	±	±	±
16	*Sh. dysenteriae* 9	+	+	+	+	+	+	+	+	+	+
17	*Vibrio cholerae* 1023	+	+	+	+	+	-	-	-	-	-
18	*V. cholerae* BD 1/81	+	+	+	-	-	-	-	-	-	-
19	*V. cholerae* 1341	+	+	+	±	±	±	±	-	-	-
20	*V. cholerae* 452	+	+	+	+	+	+	+	+	+	+
21	*V. cholerae* 1033	+	+	+	+	+	+	±	±	±	±
22	*V. cholerae* 575	+	+	+	+	+	-	-	-	-	-
23	*V. cholerae* 765	+	+	+	+	+	+	+	+	+	+
24	*V. cholerae* 1311	+	+	+	±	±	±	-	-	-	-
25	*V. cholerae* 756	+	+	+	IC	IC	IC	-	-	-	-
26	*V. cholerae* DN6	+	+	+	+	+	+	+	+	+	+
27	*V. cholerae* A 26	+	+	+	+	+	+	+	+	+	+
28	*Escherichia coli* AP600	+	+	+	+	+	+	+	+	+	+
29	*E. coli* 383	+	+	+	+	+	+	+	+	+	+
30	*E. coli* RH 07/12	+	+	+	IC	IC	IC	-	-	-	-
31	*E. coli* 18/9	+	+	+	-	-	-	-	-	-	-
32	*E. coli* 597	+	+	+	+	+	+	-	-	-	-
33	*E. coli* 798	+	+	+	+	+	+	+	+	+	+
34	*E. coli* 35B	+	+	+	+	+	+	+	+	+	+
35	*E. coli* 306	+	+	+	+	+	+	+	+	+	+
36	*E. coli* K88	+	+	±	±	±	±	±	-	-	-
37	*E. coli* 872	+	+	+	+	+	+	+	+	+	+
38	*Enterobacter* spp. AP596	+	+	+	+	±	±	±	±	±	±
39	*Salmonella typhii* Type 2	+	+	+	+	+	+	+	+	+	+
40	*Staphylococcus aureus* ML 267	+	+	+	-	-	-	-	-	-	-
41	*S. aureus* ATCC 6538	+	+	+	+	+	±	-	-	-	-
42	*S. aureus* MTCC 96	+	+	+	-	-	-	-	-	-	-
43	*S. aureus* 381	+	+	+	+	+	-	-	-	-	-
44	*Bacillus subtilis* MTCC 441	+	+	+	+	±	±	-	-	-	-
45	*B. cereus* MTCC 1305	+	+	+	+	+	+	+	+	+	+
46	*Pseudomonas putida* MTCC 2252	+	+	+	+	+	+	-	-	-	-
47	*Ps. aeruginosa* AP585 NLF	+	+	+	+	-	-	-	-	-	-
48	*B. pumilus* 8241	+	+	+	+	±	±	±	±	±	-
49	*Klebsiella pneumoniae*	+	+	+	+	-	-	-	-	-	-
50	*Proteus vulgaris* AP679 NLF	+	+	+	+	+	+	+	+	+	+

It was evident from the results shown in the Table 4 that the extract was highly active against *Shigella boydii*, *Sh. soneii*, *Vibrio cholera*, *E. coli*, *S. aureus*, *Ps. aeruginosa* and *K. pneumoniae*.The result of determination of zone of inhibition of the crude extract of the bark of the plant and their comparison with those of standard antibacterial agent Ciprofloxacin against the tested bacterial strains is recorded in Table 5.

Table 5
Determination of diameter of zone of inhibition (in mm) produced by the methanolic extract of the bark of Plumeria alba and its comparison with that of Ciprofloxacin against selected sensitive bacterial strains*

S.No.	Name of Bacteria	Extract (µg/ml)				Ciprofloxacin (µg/ml)			
		200	400	800	1000	200	400	800	1000
1.	*Sh. boydii* 8	7.5	9.0	11.0	12.5	9.5	11.0	12.5	14.0
2.	*Sh. sonnei* BCH 397	9.5	10.5	12.0	13.0	12.0	12.5	13.0	15.0
3.	*Sh. sonnei* NK 29	8.0	8.5	9.0	11.0	10.0	10.5	11.0	13.0
4.	*V. cholerae* BD 1/81	9.0	9.5	11.5	12.0	10.5	11.5	13.0	14.5
5.	*V. cholerae* 756	7.0	7.5	9.0	10.0	9.0	9.5	11.0	13.0
6.	*E. coli* 18/9	8.5	9.0	10.0	11.5	11.5	12.5	13.0	13.5
7.	*E. coli* RH 07/12	8.5	9.0	10.5	11.5	10.5	11.0	12.5	13.5
8.	*Ps. aeruginosa* AP585 NLF	7.0	7.5	9.5	11.0	9.0	10.5	11.5	12.5
9.	*S. aureus* MTCC 96	8.0	8.5	10.0	10.5	11.0	12.0	13.5	14.5
10.	*Klebsiella pneumoniae*	9.0	9.5	11.0	12.0	11.0	11.5	12.5	15.0

(* Average of two plates)

The sensitivity pattern of the bacterial organisms to the extract was found to decrease in the following order: *Sh. sonnei* BCH 397, *Sh .boydii* 8, *V. cholerae* BD 1/81, *Klebsiella pneumonia*, *E. coli* RH 07/12, *E. coli* 18/9, *Ps. aeruginosa* AP585 NLF, *Sh. sonnei* NK 29, *S. aureus* MTCC 96 and *V. cholerae* 756, as evident from the results shown in Table 4 and 5.

Antifungal Activity

The observation suggested that antifungal principles in the extract had a broad spectrum of activity. The sensitivity pattern of the fungal organisms to the extract was found to decrease in the following order: *Aspergillus niger* MTCC 281, *Candida albicans* 5, *Penicillium chrysogenum* MTCC 2725, *Candida albicans* ATCC 10231 and *Ralstonia entropha* MTCC 1255, as evident from Table 6.

Table 6
Determination of MIC of the bark of P.alba Linn. against different fungal strains

S.No.	Name of Fungi	Growth of tested fungi in various concentrations (µg/ml) of the methanolic extract of the bark of *P. alba*							
		0*	100	200	400	800	1000	1500	2000
1	*Candida albicans* 5	+	+	+	+	-	-	-	-
2	*Aspergillus niger* MTCC 281	+	+	+	-	-	-	-	-
3	*Penicillium chrysogenum* MTCC 2725	+	+	+	+	-	-	-	-
4	*Phaenorochaete chrysporium* MTCC 787	+	+	+	+	+	+	±	±
5	*Candida albicans* ATCC 10231	+	+	+	+	+	±	-	-
6	*Ralstonia entropha* MTCC1255	+	+	+	+	±	±	±	-

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CONCLUSION

The pharmacognostical and phytochemical evaluation of *Plumeria alba* (Apocynaceae) bark provided useful information for identification and authentication of the plant. The antibacterial study of the methanolic extract of the bark of *P. alba* showed the maximum activity against *Shigella boydii*, *Sh. soneii, Vibrio cholerae*, *E. coli*, *S. aureus*, *Ps. aeruginosa* and *K. pneumoniae*. The extract also provided the potential antifungal effect against *Aspergillus niger* MTCC 281, *Candida albicans* 5, *Penicillium chrysogenum* MTCC 2725, *Candida albicans* ATCC 10231 and *Ralstonia entropha* MTCC 1255. The results of phytochemical analysis and antimicrobial activity studies of the plants extracts confirmed its therapeutic usage, as depicted in the literature. The active plant extract may be further subjected to biological and pharmacological investigations for isolation of antibacterial and therapeutic compounds.

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