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Micrococca Mercurialis Benth– Pharmacognostic Analysis and Antimicrobial Activity of an Important Folk Medicinal Plant

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Abstract
The drug evaluation and bioassay of traditional herbs of various herbal systems is now getting more momentum throughout the world. It is the high time now to evaluate scientifically the information stored in different herbal medicine systems of the world in terms of their pharmacognostic, phytochemical and pharmacological characterization.The evaluation of quality and purity of crude drugs by means of various parameters is the most important aspects of pharmacognosy. The present study deals with different pharmacognostic parameters of *Micrococca mercurialis*, an ethnomedicinally important plant of the family Euphorbiaceae. The leaves are traditionally used by the tribal people to cure old sores, rheumatic pain, constipation, etc. The parameters like micromorphological, organoleptic evaluation, physical and chemical evaluation, antimicrobial activity studies, etc. of powder drugs have been considered for the pharmacognostical evaluation of the plant. The plant bears amphistomatic leaves and stomata are paracytic type with occurrence of few anisocytic and anomocytic types. Palisade ratio is 8 and stomatal index is 13 and 17 on the upper and lower surfaces respectively. Dried leaf powder is dark olive in colour and gives characteristic colouration under UV light. Methanolic extract of the leaf indicates the presence of alkaloids, flavonoids, reducing sugars, gums, tannins and saponins, etc. Ash value and moisture content of the leaves are 28.48% and 72.59% respectively. This plant seems to be very potent against different human pathogenic bacteria as the leaf extracts in different solvents (methanol, water, ethyl acetate extract) gave characteristic inhibition zone (*Bacillus subtilis* ATCC 11778– 16mm. in methanolic extract; *Escherichia coli* NCTC 10418– 11mm. in water extract; *Enterococcus faecalis* ATCC 19433– 40mm. in ethyl acetate extract, etc.). This study will throw some scientific knowledge to herbalists and pharmacologists for proper evaluation and validation of folk drug.

Background
Medicinal plant research has now got a momentum among the scientists of the world. The scientific evaluation of ethnomedicinally important plants is now being done thoroughly covering various aspects of their study like efficacy of the crude drugs, chemistry of active principles, different pharmacognostic parameters, etc. Chemical analyses and biological assay of medicinal plants are the important factors for identification of novel bioactive phytochemicals and drug discovery. Use of micromorphology and anatomy is now a recognised tool in the field of plant systematics. Importance of epidermal characters in general and those of trichomes in particular are widely recognised in taxonomic consideration of angiosperms **[1, 2, 3, 4]**; Ontogeny and structure of stomata are now also considered as an important taxonomic character for many of the angiospermic taxa **[5, 6, 7, 8, 9, 10].** The members of different genera and families of angiosperm have been studied anatomically by various workers with special emphasis on leaf epidermal micromorphology **[11, 12, 13, 14, 15].** Only to some extent, the ontogeny, structure of stomata and phytochemical studies of different members of Euphorbiaceae have been studied by different workers **[16]**. But the detailed foliar epidermal characters including trichomes, stomata, chemical analysis, physical evaluation, antimicrobial activity, etc. of many members of the family Euphobiaceae have not yet been studied. Chemical analysis and biological assays are very important aspects in pharmacognostic evaluation of medicinal plants **[17]**.

Materials And Methods

Plant material

***Micrococca mercurialis* Benth.**
(Family: Euphorbiaceae)

Common name: Badam, Desei badam.
Botanical Characteristics: Erect, 2.5-5.0 x 1.25-2.5 cm, ovate to ovate- lanceolate, rounded or acute at base, glabrous. Flowers few, distant, in slender racemes longer than the leaves; female flowers usually solitary, with several males. Sepals glabrous. Capsules sub-globose, of three hairy cocci. Seeds globose deeply ovulate, pale-brown.
Flowering and fruiting time: August to October.
Distribution: In India and Sri Lanka; also in Burma, Arabia and Tropical Africa..
Habit and habitat: Annual, erect herb. Terrestrial, wild, common, grows mainly along the shady wall sides; in waste places and along railway tracks.
Useful part: Leaf.
Medicinal use: Purgative.
Chemical constituents: Information not available.

Methods

For the study of foliar epidermis, leaf samples were cleared by Bokhari's method (1970) [5]. The cleared leaf samples were then mounted on the slide with a drop of 10% glycerine and 1% aqueous safranin solution and observed under compound light microscope. The drawings of the leaf epidermal micromorphological characters were made with the camera lucida and measurements were taken with standardized ocular micrometer in each cases. Finally, the leaf powders were extracted (soxhlet extraction) with 90% methanol and these extracts were used for different chemical colour reaction tests for identification of different phytochemical groups. Screening of antimicrobial activity was carried out by agar diffusion method. Nutrient agar medium was prepared by suspending nutrient agar (Merk) 20g/L in distilled water. The pH value of the media was adjusted to 7.0, autoclaved, and allowed to cool up to 45^{O}C. 50 µl of extract was poured in 6mm wells punched in test culture seeded assay plates. 24 h old bacteria cultures in nutrient broth were used to seed the assay plates containing NAM. Assay plates seeded with bacterial cultures were incubated at 37° C, for 24 hours. After incubation antimicrobial activity was determined by measuring the zone of inhibition. The selected bacteria eight common human pathogenic bacterial strains are *Bacillus subtilis* ATCC 11778, *Enterococcus faecalis* ATCC 19433 , *Escherichia coli* NCTC 10418, *Klebsiella aerogenes* W70, *Micrococcus luteus* NCTC2665, *Pseudomonas aeruginosa* NCTC10662, *Salmonella typhi* ATCC 19430 and *Streptococcus faecalis* MTCC 439.

Results

Macromorphology

i) Leaf: Herbaceous, 2.5-5.0 x 1.25-2.5 cm, ovate to ovate-lanceolate, rounded or acute at base, glabrous.
ii) Stem: Erect, branched, glabrous, hairy.

Micromorphology

General description and measurement of the epidermal cells, stomata, trichomes and crystals of the investigated plant are represented in Tables 1, 2, 3 and 4.
1. Epidermis:
Epidermal cell walls are irregular in shape and the out lines are wavy on both the surfaces. Epidermal cell size is 26.64 µm x 53.28 µm on the upper surface and 30.64 µm x 96.57 µm on the lower surface. Frequency is 903.08 /mm2 on the upper surface and 814.97 /mm2 on the lower surface. Palisade ratio is 08.23 (Table-1; Fig. I- A, B,

C, D).

2. Stomatal Complex:
Leaves are amphistomatic i.e. stomata are present on both the surfaces. On the upper surface paracytic stomata are predominant with few anomocytic types. On the lower surface mainly paracytic stomata are mixed with few anisocytic and anomocytic types. Size of the stomata is 24.97 µm x 16.65 µm on the upper surface and 28.30 µm x 19.98 µm on the lower surface. Frequency of the stomata is 132.16 /mm2 and 210.61 /mm2 on the upper and lower surfaces respectively. Stomatal index is 17.39 (Table-2; Fig. I- A, B, C, D).

3. Trichomes:
Nonglandular, unicellular, straight trichomes with pointed apex are present on both the epidermal layers. Size is 266.40 µm x 10.82 µm and frequency is 11.01 /mm2. Trichome index is 01.71 (Table-3; Fig. I- E, F, G, H, I).

4. Crystals:
Small spheroidal Ca-oxalate crystals are found on both the epidermal layers (Table-4).

Organoleptic Features of the Crude Drug
Colour: Dark olive; Odour: No specific odour; Taste: No specific taste; Texture: Herbaceous, glabrous (in fresh form).

Microchemical Evaluation of the Powdered Drug
Through the phytochemical tests of the methanolic extract of leaf, the detected phytochemical groups are alkaloids, flavonoids, reducing sugars, gums, tannins and saponins, etc. (Table 5).

Physical Evaluation
[I] PHYSICAL CONSTANT
i) Ash Value:
a) Total ash - 28.48% b) Water soluble ash- 09.23 c) Acid insoluble ash- 11.56%
ii) Moisture Content- 72.59 % (in fresh form).
[II] FLUORESCENCE ANALYSIS
Here in this study it is observed that drug powder treated with different chemical reagents gives characteristic colourations when seen under UV light and it is compared with the colourations observed under ordinary light. In some cases there are marked differences in colour (Table 6).

Antimicrobial Activity
Methanolic and ethyl acetate soluble foliar extracts of *Micrococca mercurialis* exhibited extreme potency against the selected bacteria. All the selected eight bacteria were successfully inhibited by the methanolic and ethyl acetate soluble foliar extracts. Water soluble extract was inhibitory against Escherichia coli only.

Methanolic extract exhibited maximum inhibitory activity against *Bacillus subtilis* ATCC 11778 (inhibition zone, 16 mm). Water extract showed 11 mm of inhibition zone against *Escherichia coli* NCTC 10418. Ethyl acetate extract exhibited maximum inhibitory effect to *Enterococcus faecalis* ATCC 19433 (inhibition zone, 40 mm).

Tables

Table1: EPIDERMAL CELL CHARACTERS OF THE INVESTIGATED PLANT *

Plant	Leaf Surface	Cell Shape	Cell Length (µm)	Cell Width (µm)	Cell Frequency /mm^2	Cell Wall Outline	Palisade Ratio
Micrococca mercurialis	Upper	Irregular	26.64	53.28	903.08	Wavy	8
	Lower	Irregular	30.64	96.57	814.97	Wavy	

Table2:
STOMATAL FEATURES OF THE INVESTIGATED PLANT*

Plant	Leaf Surface	Stomatal Type	Stomatal Length (µm)	Stomatal Width (µm)	Stomatal Index (%)	Stomatal Frequency /mm^2
Micrococca mercurialis	Upper	Mainly paracytic; few anomocytic	24.97	16.65	17	132.16
	Lower	Mainly paracytic; few anomocytic and anisocytic	28.30	19.98		210.61

Table 3: TRICHOME FEATURES OF THE INVESTIGATED PLANT *

Plant	Leaf Surface	Types	Trichome Length (µm)	Trichome Width (µm)	Trichome Frequency /mm^2	Trichome Index %
Micrococca mercurialis	Upper	Nonglandular, unicellular, straight	266.40	10.82	11.01	1
	Lower	Nonglandular, unicellular, straight	266.40	10.82	11.01	

Table- 4: CRYSTAL FEATURES OF THE INVESTIGATED PLANT *

Plant	Surface	Types	Dissolved in
Micrococca mercurialis	Upper	Small spheroidal	HCl
	Lower	Small spheroidal	HCl

** Data presented in the tables are averages of 20 observations*

Table 5: MICROCHEMICAL TESTS OF LEAF AND STEM EXTRACTS OF THE INVESTIGATED PLANT

Tests/ Reagents	Tests For	Nature of Changes	Degree of Changes
Dragendroff's reagent	Alkaloids	Orange brown ppt	+
Wagner's reagent	Alkaloids	_	_
Shinoda's tests	Flavonoids	Magenta colour	+
10% NaOH	Flavonoids	Magenta colour	+
Salkowski test	Steroids and triterpenoids	_	_
Benedict's reagent	Reducing sugars	Brick red ppt	+++
Fehling's reagent	Reducing sugars	Brick red ppt	+++
Molish's test	Gums	Red-violet ring	+
10% aqueous potassium dichromate solution	Tannins	Yellowish-brown ppt	_
10% aqueous lead acetate solution	Tannins	Yellow ppt	++
5% aqueous ferric chloride solution	Tannins	Greenish-black colour	+++
1% lead acetate	Saponins	White ppt	+++
Borntrager's test	Anthraquinones	_	_

+ = Present; - = Absent

Table 6: UV FLUORESCENCE NATURE OF THE INVESTIGATED PLANT

Materials and treatment	In fluorescence light	In ordinary light
Powder as such	Black green	Dark olive
Treated with dilute nitric acid	Orange	Orange
Treated with sodium hydroxide in water	Light yellowish pink	Light reddish brown
Treated with hydrochloric acid	Brilliant yellow green	Yellowish green
Treated with dilute sulphuric acid	Very pale blue	Blackish red
Treated with antimony trichloride	Greyish purplish blue	Yellowish brown

Table 7: ZONE OF INHIBITION (mm) FOR *Micrococca mercurialis* LEAF EXTRACTS AGAINST SOME HUMAN PATHOGENIC BACTERIA *

SELECTED BACTEIA	INHIBITION ZONE (mm)		
	METHANOLIC EXTRACT	WATER EXTRACT	ETHYL ACETATE EXTRACT
	72 Hrs	72 Hrs	72 Hrs
Bacillus subtilis ATCC 11778	16±0.05	-	33±0.04
Enterococcus faecalis ATCC 19433	12±0.03	-	40±0.02
Escherichia coli NCTC 10418	14±0.02	9±0.01	17±0.03
Klebsiella aerogenes W70	11±0.02	-	15±0.03
Micrococcus luteus NCTC2665	10±0.023	-	35±0.03
Pseudomonas aeruginosa NCTC10662	14±0.03	-	36±0.04
Salmonella typhi ATCC 19430	15±0.02	-	34±0.02
Streptococcus faecalis MTCC 439	13±0.032	-	30±0.03

*** = Values are mean of three replicates**
- = No inhibition zone
± = Standard error

DISCUSSION

Pharmacognosy implies a particular knowledge of methods of identification and evaluation of crude drugs obtained from plants which include macromorphology, phytochemical and pharmacological studies. The major problem of the commercial supply of crude drugs is identification of genuine drug. Crude drugs may easily be adulterated or substituted by or confused for other ones because neither it has any trade name printed on it nor it carries any identifying structure for the easy identification of it by the plant taxonomists, rather drug samples supplied are shrunken, rolled, twisted, deformed and discoloured. So, pharmacognostic evaluation of crude drugs with macromorphology, micromorphology, microchemical colour reaction tests, organoleptic tests, ash value UV fluorescence study, etc will help in identifying genuine drugs and thus in checking adulteration, because the tests are very specific for a particular drug.

Leaf micromorphology of this investigated plant show the general features of *Micrococca mercurialis* which conform to the characters reported in earlier works [17, 18]. Importance of epidermal characters in general is widely recognized in taxonomic considerations of angiosperm [19, 20, 21] and in many cases they have successfully used in identification of taxa at genus as well as species levels. Leaves are amphistomatic i.e. stomata are present on both the surfaces. On the upper surface paracytic stomata are predominant with few anomocytic types. Studies in stomata can have a great taxonomic as well as pharmacognostic value in proper identification of different plant taxa including medicinal plants [22, 23]. Size of the stomata is 24.97 µm x 16.65 µm on the upper surface and 28.30 µm x 19.98 µm on the lower surface. Trichome features are also very

important in proper identification of the plants and considered as one of the valuable taxonomic marker now. Here nonglandular type of trichomes is found. Small spheroidal crystals are present.

In case of crude drug evaluation, ash value plays a very important role which includes % of total ash, water soluble ash and acid insoluble ash [24]. Ash value gives a maker character for identification of crude drugs obtained from the investigated taxa. Here ash value of *Micrococca mercurialis* is 28.48% which is very distinct and will be successfully used in evaluation of drug quality of the plant. Similarly fluorescence characters of the crude drugs are considered very important marker in making distinction among the drugs. Here some fluorescence features have been identified which are very much distinctive in identifying the respective drug obtained from this plant. Resistance to antimicrobial agents is emerging in a wide variety of microorganisms and multiple drug resistant organisms pose serious threat to the treatment of infectious diseases [25]. Keeping this problem in mind various workers are actively involved in bioprospection of phytochemicals as potent antimicrobials from the ethnomedicinal plants with the help of ethnobotanical leads [26, 27, 28, 29, 30, 31]. Kirby-Bauer (1966) elaborately showed the methods of detecting antimicrobial activity of certain specimens[32]. Recently floral and foliar extracts are screened by different workers against several pathogenic and non-pathogenic microorganisms [33]. In this context, antimicrobial activity of the selected plant has been done. The leaf extracts in different solvents (methanol, water, ethyl acetate extract) gave characteristic inhibition zone.

[V] CONCLUSION

The results obtained from this investigation are very authentic and can be used as an effective tool in proper identification of the crude drugs produced from this plant. It will also be very useful in quality control of the genuine drugs plant and for detection of adulterants. The information generated in this study will enrich the data base of Indian Pharmacopoeia by incorporating the pharmacognostic information of these selected ethnomedicinal plants. The information regarding the ethnomedicinal uses of these respective plants will also be included in the Indian national ethnomedicinal inventory. Such findings will have a great value not only to the botanists, ethnobilogists, chemists but also to the rural poor people of our country fot their economic development if these plants are cultivated commercially. Finally it will highlight some promising areas for further investigations to the researchers in the fields of ethnobotany, phytochemistry, pharmacology, pharmaceutical science and molecular biology.

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