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PHARMACOGNOSTIC AND PHYTOCHEMICAL SCREENING IN LEAF DRUG OF MEDICINAL PLANT CELESTRUS EMARGINATA (GRAH.) COLLECTED FROM THE FOREST AREA OF ADILABAD DISTRICT, TELANGANA STATE, INDIA

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ABSTRACT

The present study has given more importance to the leaf drug of the plant *Celestrus emarginata* of to family Celestraceae and was collected from the forest areas of Adilabad District. The study has revealed that some medicinally important plants have been used by Gondu tribal community. The plant leaf extract is given to increase male sex vigour in human beings, which opened a new scope to take up the present research. In the process of research in order to standardize the leaf drug of the plant, the leaves of the plant were studied for micro morphological studies of the epidermal, stomatal, costal cells and trichomes if any, colour characterization, heavy metal analysis and phytochemical screening by using the standard Pharmacognosy protocols. The study revealed that the shapes Of the epidermal cells are polygonal aniso diametric non-linear on both adaxial and abaxial surface further; stomata were observed to be anisocytic type. The leaf powder residue in 10% H_2SO_4 and in alcohol are observed to be similar in colour. The results of heavy metal analysis are much lower than the WHO prescribed standards. The phytochemicals like alkaloids, glycosides, phenols, resins and quinones are maximum in their presence. Considering the above characteristic specific features of the plant there is an utmost importance to standardize the leaf drug for checking the adulteration and for further drug formulation research.

KEY WORDS: *Celestrus emarginata*, medicinal plant, Phytochemistry, phytochemistry micro morphology, Adilabad District.

INTRODUCTION

Medicinal plants are being used by all cultures throughout the world since time immemorial; even today people in developing countries use traditional medicines for their health care. Medicinal herb is considered to be a chemical factory as it contains multitude of chemical compounds like alkaloids, glycosides, saponins, resins, oleoresins, sesquiterpene, lactones oils etc.,[1] Herbs are mine of medicinal agents and a large number of medicinal herbs are found to be efficacious, cheap and safe in preventing various diseases. [2] The knowledge of medicinal plants has been accumulated in the course of many centuries based on different medicinal systems such as Ayurveda, Unani Siddha. In India, it is reported that traditional healers use 2500 plant species and 100 species of plants serve as regular sources of medicine. [3] Some Biomolecules from the plant *Celestrus emarginata* has been reported to be active against HIV-Protease[4], Carcinoma and leukemia[5], Ulcers[6] and Multi Drug Resistance.[7] Various parts of this plant contain immense medicinal properties. *Celestrus emarginata* (*syn. Maytenus emarginata.* Willd), a large shrub, sometimes a small tree, usually armed with long straight thorns. Leaves coriaceous, broadly obovate-cuneate, rounded or very shortly and bluntly acuminate at the apex, crenulate, dark green glabrous and polished above, pale beneath, reticulately veined, the veins not prominent on the underside; petioles long, stout. Flowers greenish-white, in short-peduncle or subsessile cymes. Calyx glabrous, divided nearly to the base, lobes broadly ovate or triangular, with membranous ciliolate margins. Petals ovate-oblong, obtuse, the margin entire or faintly denticulate. Stamens shorter than the petals; filaments flattened. Seeds 6, ellipsoid with a small basal aril, brownish orange, smooth, shinning. Flowers: April-June [8] ("Fig.1"). The present study was conducted with the objective to investigate the Pharmacognostic and Phytochemical studies of the plant leaf to standardize and identify the leaf drug in whole form or cut or powdered material, further it helps in designing the new potent drug.

MATERIALS AND METHODS

Collection and Authentication of Plant Materials

The leaves of the plant species were collected wildly from the forest areas of Seethagondi, Adilabad District, Telangana State with the help of local tribes (GPS- Ele: 1088 ft, N: $19^0$35.535', E: $078^0$31.334'). The collected plants were identified using available published literature. [9], [10]

Fixation of the Material: Mature plant parts (leaves) were fixed in the Carney's fixative consisting of alcohol and glacial acetic acid 3:1. After two days the fixative was replaced by 70% alcohol for preservation of the material. [11]

Micropreparations: Several techniques were attempted such as double treatment method which gave satisfactory results. The peels were prepared from five places of the leaf i.e., apex, base, midvein, margins and lamina. [12]

Micrometry: For calculating the epidermal cell frequency, the epidermal cells as well as stomatal subsidiaries were counted together. Epidermal cell frequency(E. C. F), epidermal index (E. I), Stomatal frequency (S. F), Stomatal index (S. I) was calculated in five places on both adaxial and abaxial surfaces of the leaf. The values are averages derived from ten readings and calculated by standard method. [13]

Powder Analysis: The ground leaf powders were dissolved in water, alcohol, acetone, chloroform, 10% sulphuric acid, 10% sodium hydroxide and were observed under ordinary light and UV light immediately and after 24 hours for their colour characterization. The exhibited colours were compared with standard Dictionary of colours. [14]

Metal Analysis: The leaf powders were analyzed for concentration of accumulation of elements in the used plant part collected from the forest area at Adilabad district. Samples were prepared by acid digestion method and calculated using Atomic Absorption Spectrum (1950). Analyses for zinc, magnesium, cadmium, copper, nickel, cobalt were screened for their concentrations. The results thus obtained were correlated with the WHO standards.

Phytochemical Screening: Chemical tests for the screening and identification of bioactive chemical constituents in the medicinal plant under study were carried out in extracts as well as powder specimens using the standard procedures as described. [15], [16], [17], [18]

Statistical Analysis: To improve visualization of data and for better understanding of the results, all the statistical analyses were computed with XLstat software.

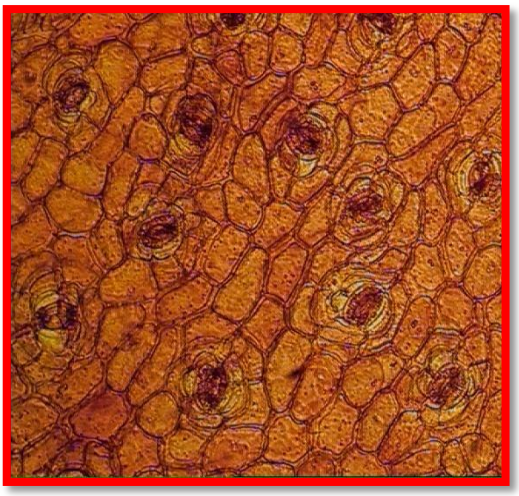
RESULTS AND DISCUSSIONS

Leaf Micro Morphological Characters

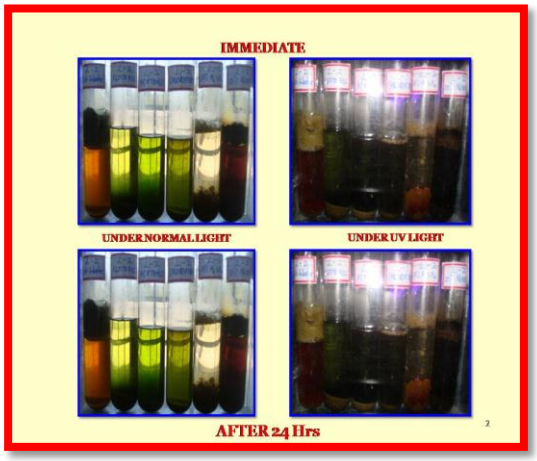
The shape of the epidermal cell in present studied taxa is Polygonal anisodiametric non linear on both the surfaces. The nature of the cells can be said as flat outer wall, straight anticlinal

walls, with scanty cytoplasm in the cells and the cells were variously oriented with irregular arrangement to the main axis of the plant. The epidermal cell frequency differ on both the surfaces and also within the same surface at various locations of the same leaf i.e., at leaf base, midrib, apex, lamina and margin. The epidermal frequency on ad axial surface was maximum at leaf base as 10918.03 cm^{-2} and minimum is observed at leaf lamina as 9426.29 cm^{-2}, whereas on abaxial surface it is maximum at leaf base as 11008.19 cm^{-2} and minimum at leaf midrib as 9385.24 cm^{-2}. Similarly epidermal index on adaxial surface it is maximum at leaf base as 88.07 cells per unit area and minimum at leaf lamina as 82.14 cells per unit area, while on abaxial surface it is maximum at leaf lamina as 85.45 cells per unit area and minimum at leaf midrib as 77.55 cells per unit area. (Table1). Costal cells are absent. Stomata were reported to be Anisocytic; it is circular, narrowly elliptic in nature on both the surfaces. The positions of the guard cells are sunken. Subsidiary cells are tricyclic in nature. Free type (f-type), where a subsidiary of stoma does not abute either a subsidiary or guard cell of another stoma was observed. Amphistomatic condition was seen, where the stomata are present on both the sides of leaf. Stomatal frequency on adaxial surface is maximum at leaf apex as 27049.18 cm^{-2} and minimum at leaf base as 14754.09 cm^{-2}, but on abaxial surface is maximum at leaf midrib as 27049.18 cm^{-2} and minimum at leaf lamina as 18032.78 cm^{-2}. The Stomatal index on adaxial surface it is maximum at leaf apex as 19.76 cells per unit area and minimum at leaf base as 11.92 cells per unit area but on abaxial surface it is maximum at leaf midrib as 22.44 cells per unit area and minimum at leaf base as 14.64 cells per unit area. Trichomes are absent on both the surfaces. ("Fig. 2") (Table 2).

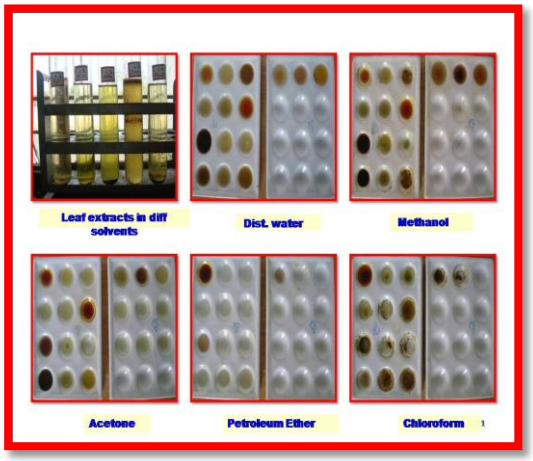
"Fig. 1"- *Celestrus emarginata*- Habit



"Fig. 2"- Epidermal and stomatal cells



"Fig. 3"- Powder analysis in OL and UV



"Fig. 4"- Phytochemical screening

Table-1 Statistics of Leaf Epidermal Frequency and Epidermal Index on Adaxial and Abaxial Surface of Plant *Celestrus emarginata.*

READING	LEAF BASE		LEAF APEX		LEAF MID RIB		LEAF LAMINA		LEAF MARGIN	
	Adaxial	Abaxial	Adaxial	Abaxial	Adaxial	Abaxial	Adaxial	Abaxial	Adaxial	Abaxial
MEAN	133.2	134.3	134	134	116	114.5	115	117	118.3	117
S.D	2.82	3.12	2.86	3.33	3.12	3.43	3.33	3.26	2.94	2.78
S.E	0.89	0.98	0.90	1.05	0.98	1.08	1.05	1.03	0.93	0.88
E.C.F (cm^{-2})	109180.32	110081.96	109836.06	109836.06	95081.96	93852.45	94262.29	95901.63	96967.21	95901.63
E.I	88.07	85.37	80.23	84.81	81.69	77.55	82.14	85.45	83.68	84.17

Table-2 Statistics of Leaf Stomatal Frequency and Stomatal Index on Adaxial and Abaxial Surface of Plant *Celestrus emarginata.*

READING	LEAF BASE		LEAF APEX		LEAF MID RIB		LEAF LAMINA		LEAF MARGIN	
	Adaxial	Abaxial	Adaxial	Abaxial	Adaxial	Abaxial	Adaxial	Abaxial	Adaxial	Abaxial
MEAN	18	23	33	24	26	33	25	33.1	23	22.3
S.D	1.33	1.69	1.82	1.49	1.56	2.02	2.10	1.52	1.88	1.49
S.R	0.42	0.53	0.57	0.47	0.49	0.64	0.66	0.48	0.59	0.47
S.F (cm^{-2})	14754.09	18852.45	27049.18	19672.13	21311.47	27049.18	20491.80	27049.18	18852.45	18032.78
S.I	11.92	14.64	19.76	15.18	18.30	22.44	17.85	22	16.31	15.82

Table-3 Colour Characterisation of Leaf Powder in Different Solvents under OL and UV **Lights.**

IMMEDIATE				
	UNDER ORDINARY LIGHT		**UNDER ULTRAVOILET LIGHT**	
SOLUTION/ SOLVENTS	**NAME OF THE COLOUR**		**NAME OF THE COLOUR**	
	Sup.	**Res**	**Sup.**	**Res**
Dist. Water	Mikado	Tarragona	Red feather	Sea foam
Alcohol	Chalcedony	Locarno Gr.	Aqua Gr.	Reed Gr.
Acetone	Viridine Y	Oriental Gr.	Eden Gr.	Grape Gr.
Chloroform	Sea foam Gr.	Fern	Civette Gr.	Laurel Gr+
10% H_2SO_4	Butterfly	Eucalyptus Gr.	Sky blue	Vanilla
10% NaOH	Brazil R	Kettledrum morored+	Clove Manchu	Bison-
AFTER 24 HOURS				
	Sup.	**Res**	**Sup.**	**Res**
Dist. Water	Tokyo	Doubloon roman ochre	Hydrangea red	Chalcedony Y.
Alcohol	Reed Y.	Meadow Gr.	Calliste Gr.	Eucalyptus Gr.
Acetone	Sea foam	Winter Gr.	Paradise Gr.	Grape Gr.
Chloroform	Sky Gr.	Monticello Gr.	Cossaek Gr.	Palm
10% H_2SO_4	Amber white	Certosa	Eucalyptus Gr.	Rose Amber
10% NaOH	Moroccan	Bronze brown	Castor	Pelt + pampas

Table-4 Phytochemical Screening of Leaf Powder in Different Solvents

S.No	**PHYTOCHEMICAL CONSTITUENTS**	**DISTILLED. WATER**	**METHANOL**	**ACETONE**	**P.ETHER**	**CHLOROFORM**
1	Flavonoids	++	+	-	-	-
2	Alkaloids	++	++	+++	++	++
3	Glycosides	+	+	+++	-	+
4	Steroids	++	+	++	+	-
5	Phenols	++	+++	+	-	-
6	Terpenoids	+	+	-	++	+
7	Saponins	+	-	-	++	++
8	Resins	+	+	+++	+	-
9	Tannins	+	++	-	-	-
10	Cardiac Glycosides	++	+	+	+	++
11	Carboxylic acid	-	-	-	+	-
12	Coumarins	+	++	++	-	-
13	Quinones	+++	+++	+++	-	-
14	Xanthoproteins	+	-	-	++	+

Powder Analysis: The immediate aqueous extract colours of the leaf powders shown Mikado under O.L and Red feather under U.V and after 24 hours the colours are Tokya under O.L and Hydrangea red under U.V light. Immediate colours of aqueous residues are Tarragona

under O.L and Sea foam under U.V and after 24 hours the colours are Doubloon roman ochre under O.L and Chalcedony Y. under U.V light. The colours of alcoholic extractions immediately are Chalcedony under O.L and Aqua Gr. under U.V and after 24 hours the colours are Reed Y. under O.L and Calliste Gr. under U.V light. The immediate colours of alcoholic residue are Locarno Gr. under O.L and Reed Gr. under U.V and after 24 hours the colours are Meadow Gr. unde O.L and Eucalyptus Gr. under U.V light. The immediate colours of acetone extracts are Viridine Y. under O.L and Eden Gr. under U.V and after 24 hours the colours are Sea foam under O.L and Paradise Gr. under U.V light. The colours of acetone residues are Oriental Gr. under O.L and Grape Gr. under U.V. light and after 24 hours the colours of residue are Winter Gr. under O.L and Grape Gr. under U.V light. The immediate colours of chloroform extracts are Sea foam Gr. under O.L and Civette Gr. under U.V and after 24 hours the colours are Sky Gr. under O.L and Cossaek Gr. under U.V. The colours of chloroform residue are Fern under O.L and Laurel Gr+ under U.V. and after 24 hours the colours of residue are Monticello Gr. under O.L and Palm under U.V. The immediate sulphuric acid extract colours are under O.L and Sky blue under U.V. and the sulphuric acid residue colours are Eucalyptus Gr. under O.L and Vanilla under U.V, after 24 hours sulphuric acid extract colours are Amber white under O.L and Eucalyptus Gr. under U.V. and colors of residue are Certosa under O.L and Rose Ambers under U.V. The immediate colours in 10% NaOH are Brazil R. under O. L and Clove Manchu under U.V. and the colours of the residue are Kettledrum moro red+ under O.L and Bison- under U.V, after 24 hours colours of extracts are Moroccan under O.L and Castor under U.V. and the colours of the residue are Bronze brown under O.L and Pelt+ damps under U.V. ("Fig. 3") (Table 3).

METAL ANALYSIS

In the leaf powders of the studied plant *Celestrus emarginata* the concentration of Zinc metal is 0.474 mg/kg., the Magnesium is 6.597 mg/kg., while Cadmium is 0.016 mg/kg., Copper is 0.118 mg/kg and the metal Nickel is 0.677 mg/kg., but Cobalt is not detectable.

PHYTOCHEMICAL SCREENING

In *Celestrus emarginata*, alkaloids, glycosides, resins and quinones are maximum in acetone extracts, phenols are maximum in methanol extracts and quinones are maximum in aqueous, methanol and acetone extracts. Saponins are absent in methanol and acetone extracts, but are adequately present in petroleum ether and chloroform extracts. Tannins are absent in acetone, petroleum ether and chloroform extracts but are adequately present in methanol and aqueous

extracts. Coumarins are adequately present in aqueous, methanol and acetone extracts but completely absent in petroleum ether and chloroform extracts. ("Fig. 4") (Table 4)

CONCLUSION

From the present investigations of leaf micro morphology, it is clear that the characteristic features exhibited by the studied plant can be used to identify the plant species internally, externally and helps to check the adulteration of the plant drug. The differences in the colours produced by the leaf powder helps in identifying the taxa even if it is in powder form and also help in checking the adulteration of the drug preparation. Further, the study revealed that the amount of the metals present in the plant part. The data thus obtained is correlated with the standards given by WHO, which are below the toxic levels, from this, it is clear that the plants collected from unpolluted area are least contaminated and safe for drug administration and for new formulations. Abundant production of Phytochemicals in the studied plant can be used in the pharmaceutical industries for producing a potent drug.

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