Pharmacognostic and Preliminary Phytochemical Evaluation of the Leaves of *Pseuderanthemum Carruthersii (Seem). Guillaumin.* Leaves

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Abstract- ***Various traditional systems of medicine enlightened the importance of the leaves of pseuderanthemum carruthersii (Acanthaceae) to have a great medicinal value. The present study was aimed at pharmacognostic and preliminary phytochemical evaluations of Pseuderanthemum carruthesii leaves. The pharmacognostic investigations were carried out in terms of organoleptic, microscopic and physical parameters. The dried leaves were subjected to maceration using methanol. These solvent extracts were subjected to a preliminary phytochemical screening to detect the different chemical principles present viz., carbohydrates, proteins, amino acids, steroids, glycosides, alkaloids, tannins and phenolic compounds. The phytochemical evaluation revealed the presence carbohydrates, aminoacids, glycosides, flavanoids, tannins and phenolic compounds, alkaloids and saponins and absence of proteins and steroids.***

Indexed Terms- ***Pseuderanthemum carruthesii, Pharmacognosy, Phytochemistry***

I. INTRODUCTION

Plants, which have one or more of its parts having substances that can be used for treatment of diseases, are called medicinal plants. Medicines derived from plants are widely famous due to their safety, easy availability and low cost. Herbal medicines may include whole parts of plant or mostly prepared from leaves, roots, bark, seed and flowers of plants. They are administered orally, inhaled or directly applied in the skin. Medicinal herbs are more significant to the health of individual and community. The medicinal value of these plants lies in bioactive phytochemical constituents that produce definite physiological action on the human body. Some of the most important bioactive phytochemical constituents are alkaloids, essential oils, flavonoids, tannins, terpenoids, saponins, phenolic compounds and many more. These natural compounds formed the foundations of modern prescription drugs as we know today. (Naseem)

The leaves are used to treat skin problems, liver diseases and hepatitis. The decoction of leaves is used as a gargle for sore throat. An infusion is given in jaundice and dysentery. The leaves are also a popular febrifuge and are reported to possess anti tumor properties. The poultice of leaves is applied to cuts and gashes as a scar preventive (16). Today in Brazilian herbal medicine, a leaf decoction of *Pseuderanthemum* is used to treat heartburn and stomach distress caused by spicy foods and as a mild diuretic and mild laxative. It is also used for fevers and malaria and topically, to treat burns. Eight to ten dried leaves are boiled for 10 minutes in 1 liter of water for this popular Peruvian remedy. One cup is drunken warm or cold 3times daily after meals to treat prostate disorders and internal inflammation, arterial hypertension, high cholesterol cystitis, obesity, renal insufficiency, and to eliminate uric acid (13). This decoction is also recommended as a vaginal antiseptic and wound healer, as a wash for skin infections and for liver and stomach disorders. Curanderos (herbal healers) in the Peruvian Amazon squeeze the juice from the fresh leaves and place it in the eye for inflammation and eye infections, and they use the juice of 12 fruits taken twice daily for 5days to treat “epilepsy” (9). The present investigation dealt with the pharmacognostic parameters of the leaves of *Pseuderanthemum*, and also with preliminary

phytochemical evaluation of different solvent extracts of leaves. The leaves were evaluated to observe their organoleptic, microscopic and physical parameters. The successive petroleum ether, chloroform, ethyl acetate, methanol and aqueous extracts of leaf powder of the plant were examined for its phytochemical principles.

II. MATERIALS AND METHODS

- Chemicals: All the chemicals were of highest available purity and were procured from E. Merck, Mumbai, India, HiMedia Laboratories, Mumbai, India and SD Fine Chemicals, Mumbai, India

- Procurement of plant material:

For the present investigation, the leaves of the plant *Pseuderanthemum carruthersi i(Seem.)* Guillaumin. were collected in the month of September 2019 from Bhagathnagar area of Karimnagar district. The plant was identified and authenticated by Botanical Survey Of India, Hyderabad [No.BSI/DRC/2019-20/Tech/Identification/522]. The leaves were collected cleaned and dried in shade. After proper drying they were subjected to grinding to obtain the powder and then sieved to get fine powder.

- Pharmacognostic Evaluation:

Organoleptic evaluation: In organoleptic evaluation, various sensory parameters of the plant material, such as size, shape, color, odor, and taste of the leaves were recorded (5). It includes conclusions drawn from studies resulted due to impressions on organs of senses.

Microscopic evaluation: The microscopic evaluation is essential for powdered crude drugs. Powders of the crude drugs consist of the fragments of cells in the form of recognizable tissues. Another important aspect of microscopic evaluation is the study of surface constants. The leaf constants like stomatal number, stomatal index, and palisade ratio were studied by using camera lucida. These constants are of diagnostic significance and are used for the authentication of leaf drugs or for the detection of their adulterants. Various diagnostic characters of leaves and leaf powder of *P. carruthesii* were studied by microscopic analyses with or without staining.

1. Powder analysis of leaf:

To a little quantity of powder taken onto a microscopic slide, 1–2 drops of 0.1% phloroglucinol solution and a drop of concentrated hydrochloric acid were added, mounted in glycerol, covered with a cover slip and observed under microscope with 10 × 10 magnification. The characteristic features of the powder viz., vascular tissues, xylem fibers, calcium oxalate crystals, starch grains, trichomes etc. were recorded using standard techniques (1, 6, 8, 10, 11). Lignified cells, fibers and stone cells appear pink in color. Presence of starch grains was detected by the formation of blue color on addition of 2–3 drops of 0.01M iodine solution.

2. Determination of stomatal index:

Leaf fragments of about 5 × 5 mm in size were taken in a test tube containing 5 ml of chloral hydrate solution and boiled on water bath until the fragments became clear (~15 min). These fragments were transferred onto microscopic slide, mounted in glycerol and observed under microscope for the presence and quantification of epidermal cells, stomata (type and distribution), palisade cells, vein islet number and veinlet termination number. The slide was examined with 40 × objective and 6 × eye piece to which a camera lucida was attached and recorded the epidermal cells and stomata lying within a selected area. Stomatal index was calculated as the percentage of number of stomata present per number of epidermal cells and each stoma was counted as one cell.

- Physical evaluation:

In physical evaluation, crude fiber, moisture content, ash values viz., total ash, acid insoluble ash and water-soluble ash, and extractive values viz., alcohol soluble extractive value, water soluble extractive and ether soluble extractive values were determined (1, 6, 8, 10, 11, 17). The ash values represent the inorganic salts present in the drug. Extracts obtained by exhausting crude drugs are indicative of approximate measures of certain chemical compounds they contain, the diversity in chemical nature and properties of contents of drug. The determinations were performed in triplicate and the results are expressed as mean ± SD. The percentage w/w values were calculated with reference to the air-dried drug.

1. Estimation of crude fiber (Acid detergent fiber, ADF): The ADF of the leaf composed of cellulose, lignified nitrogen, and alkali-soluble lignin. In a 500 ml Berzelius beaker, 2 g of leaf material was distilled by refluxing with 50 ml ADS (20gm cetyl trimethyl ammonium bromide (cetrimide) in 1 litre of previously standardized N sulphuric acid) by boiling vigorously at first and then more gently. After 1 hr of reflux distillation, the contents of the beaker were transferred to a tared crucible and the contents were allowed to percolate through the sintered glass plates. The residue was repeatedly washed with boiling water until no more foam appeared in the filtered solution. The residue was sucked dry and washed with 3 × 20 ml of acetone and finally sucked dry. The crucibles were kept overnight in a hot air oven at 100 °C, cooled in a desiccator and weighed. The residue which remained insoluble in the hot ADS was the amount of ADF in given sample. The ADF content of leaf powder was calculated.

2. Moisture content (loss on drying): Ten gram of accurately weighed fresh leaves of *P. carruthesii* was placed in a tarred evaporating dish and dried at 105 °C for 5hrs and weighed. Drying and weighing was continued at one hour interval until difference between two successively weighings corresponded to not more than 0.25%. Constant weight was reached when the difference in weight of two consecutive weighings was not more than 0.01gm after drying for 30 minutes and cooling for 30 minutes in desiccator.

3. Determination of total ash: Two gram of leaf powder of *P. carruthesii* was taken in a tared silica crucible and incinerated at a temperature not exceeding 450 °C until free from carbon. The resultant ash was cooled and weighed. The percentage of ash was calculated with reference to the air-dried drug.

4. Acid-insoluble ash: The total ash obtained from 2g of leaf powder was boiled for 5 minutes with 25 ml of dilute hydrochloric acid and the insoluble matter was collected on an ashless filter paper. It was washed with hot water, ignited and weighed. The percentage of acid insoluble ash was calculated with reference to the air-dried drug.

5. Water soluble ash: The total ash obtained from 2g of leaf powder was boiled for 5 minutes with 25 ml of water and the insoluble matter was collected on an ashless filter paper. It was washed with hot water, ignited and weighed. The percentage of water-soluble ash was calculated with reference to the airdried drug.

6. Determination of alcohol-soluble extractive: Accurately weighed powder (5 g) of leaves was taken separately and macerated with 100 ml of 95% alcohol for 24 h. The contents were frequently shaken during the first 6 h and allowed to remain for 18 h. After 24 h, the extract was filtered and 25 ml of the filtrate was evaporated. The extract was dried at 105°C to a constant weight.

7. Determination of water-soluble extractive: Water soluble extractive value was determined using the procedure described for alcohol soluble extractive, except that chloroform water was used for maceration.

8. Determination of ether-soluble extractive: Accurately weighed powder (5 g) of leaves was taken and a thimble pack was prepared. The crude drug in the pack was extracted with solvent ether in a continuous extraction (Soxhlet) apparatus for 6 h. The extract was filtered; the filtrate was evaporated and dried at 105°C to a constant weight.

- Fluorescence Characteristics

The fluorescence nature of Plumeria pudica leaf powder was observed under ultraviolet and visible radiations after treatment with various chemical reagents (19).

- Preliminary Phytochemical Screening:

The leaf powder was subjected to maceration extraction with methanol for one week and the extract were evaporated to dryness. The dried extract was weighed, and percentage yields were calculated. The extract was used for preliminary phytochemical screening with a battery of chemical tests viz., Molisch's, Fehling's, Benedict's and Barfoed's tests for carbohydrates; Biuret and Millon's tests for proteins; Ninhydrin's test for amino acids; Salkowski and Liebermann-Burchard's reactions for steroids; Borntrager's test for anthraquinone glycosides; foam test for saponin glycosides; Shinoda and alkaline tests for flavonoid glycosides; Dragendorff's, Mayer's,

Hager's and Wagner's tests for alkaloids; and ferric chloride, lead acetate, potassium dichromate and dilute iodine tests for tannins and phenolics (3, 7, 12, 14).

III. RESULTS AND DISCUSSION

Pharmacognostic Evaluation:

Organoleptic and microscopic evaluation:

In organoleptic evaluation, appropriate parameters like taste, odor, size, shape and color of the leaves and leaf powder were studied. Macroscopically, the leaf is simple in composition, opposite in arrangement, apex and base are acute, margin is entire, and average leaf size is 7.8 ± 0.6 cm (length) and 4.2 ± 0.5 cm (breadth). Fresh leaves are green in colour and characteristic in odour with a slightly bitter taste. The leaf powder was also green in colour with characteristic odour and bitter taste.

Micromorphological features revealed that the leaf powder contains numerous raphide shaped calcium oxalate crystals and starch grains, both simple and compound. The powder also showed the presence of xylem and phloem. Multicellular, long and covering trichomes seen were lignified.

The quantitative microscopic evaluation of fresh leaves and leaf powder was performed and the results obtained were shown in table 1 and 2 and figures 1, 2 3 4, 5,6,7 and 8. The lower epidermal layers of fresh leaves have shown the presence of stomata with one or more subsidiary cells parallel to the long axis of the pore and guard cells which indicates paracytic arrangement. Upper epidermal layers shown the presence of stomata with two larger guard cells and a smaller subsidiary cells which indicates anisocytic stomata.

- Physical evaluation:

The various physical parameters of leaves and leaf powder viz., crude fiber (acid detergent fiber), moisture content, ash values viz., total ash, acid insoluble ash and water-soluble ash, and extractive values viz., alcohol soluble extractive value, water soluble extractive and ether soluble extractive values were determined.

The results of this study were shown in table 3.

The results of these investigations could serve as a basis for proper identification, collection and investigation of the plant. The macro and micro morphological features of the leaf described, distinguishes it from other members of the genera. Numerical data and quantitative leaf microscopy are parameters that are unique to the plant and are required in its standardization.

- Fluorescence characteristics

It is fast method for the design study of crude drug of unsure specimen, when other methods produce inappropriate results. The plant material may be identified from their adulterants on the basis of fluorescence nature. Results are described in Table 4.

- Preliminary Phytochemical Evaluation:

The leaf powder of *P. carruthesii* was extracted with methanol and the nature and yield of the extract were observed. methanol produced resinous extract in nature. The solvent produced dark green coloured extract from the leaf powder. The yields of the extract were found to be 12.4 % w/w. Methanolic extract of the leaves produced positive tests for flavonoids, steroids, terpenoids, phenolics, tannins, alkaloids and glycosides. These secondary plant metabolites are known to possess various pharmacological effects and might be responsible for the various actions exerted by *P. carruthesii* and the results are shown in Table 5.

P. carruthesii is used in the treatment of various disease conditions. The standardization of a crude drug is an integral part of establishing its correct identity. Before any crude drug can be included in a herbal pharmacopoeia, pharmacognostic parameters and standards must be established. The results of the present investigations could serve as a basis for proper identification, collection and investigation of the plant. The macro-and micromorphological features of the leaf described, distinguishes it from other members of the genera. Numerical data and quantitative leaf microscopy are unique to the plant and are required in its standardization. Phytochemical evaluation revealed the presence of various secondary plant metabolites which have been claimed to be responsible for various pharmacological activities.

CONCLUSION

The pharmacognostic parameters, which are being reported for the first time, could be useful in the identification and standardization of a crude drug. The data produced in the present investigation is also helpful in the preparation of the crude drug's monograph and inclusion in various pharmacopoeias.

TABLE.1: RESULTS OF MICROSCOPY OF FRESH LEAF.

Parameter	Value	
	Upper	Lower
Types of stomata	Anisocytic	Paracytic
Stomata number	15	25
Stomata index	53.57%	55.55%
Vein islet number	19	
Vein termination number	15	
Palisade ratio	9	

TABLE 2: RESULTS OF POWDER MICROSCOPY.

Diagnostic character	Type
Pholemfibers (width)	8.58 – 60µ
Xylem vessels	10.5-74 µ
Trichomes (length)	18-39 µ
Calcium Oxalate crystals (length)	21.65– 52.7µ
Starch grains (diameter)	5.26-47.34 µ

TABLE.3: RESULTS OF PHYSICAL EVALUATION

Physical Parameter	Value (%w/w)
Acid detergent fiber	26.7
Moisture content	65.3
Total ash	6.34
Acid insoluble ash	0.56
Water soluble ash	3.24
Alcohol soluble extract	10.75
Water soluble extract	8.23

Table.4: RESULTS OF FLUORESCENCE ANALYSIS

	Observation (colour developed)		
		Ultraviolet light	
		254nm	366nm
Powder alone	Green	Green	Yellowish green
Powder + Picric acid	Dark green	Dark green	Dark green
Powder + NaOH	Dark green	Dark green	Bluish green
Powder + Glaceialacetic acid	Dark green	Bluish green	Brown
Powder + HCl	Dark green	Dark green	Blackish
Powder + HNO3	Brown	Green	Black brown
Powder + Iodine	Black	Dark green	Blackish
Powder + FeCl3	Dark green	Bluish green	Dark green
Powder + H2SO4	Light brown	Green	Dark green
Powder + Methanol	Dark green	Dark green	Dark green

TABLE 5: PHYTOCHEMICAL EVALUATION OF *P. CARRUTHESII* LEAVES

Chemical tests	Ethanolic extract
Carbohydrates	-
Cardiac glycosides	-
Flavonides	+
Saponins	-
Tannins and phenolic compounds	+

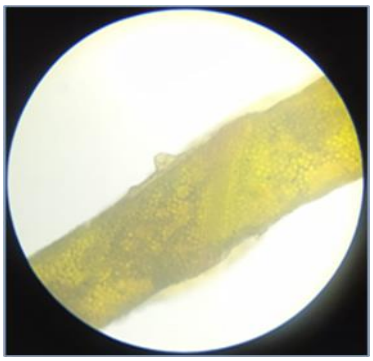

Fig.1: T.S of leaf

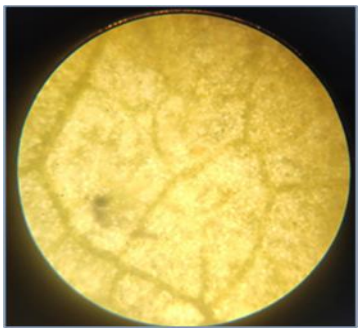

Fig.2: Vein islet number

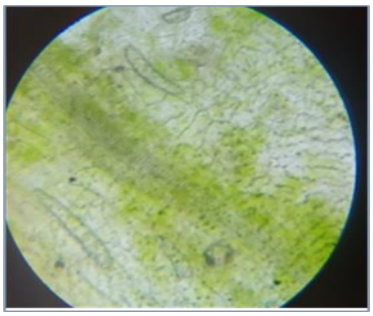

Fig.3: Upper stomata

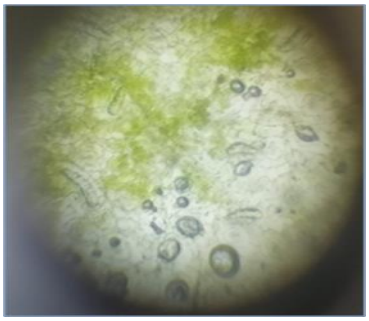

Fig.4: Lower stomata

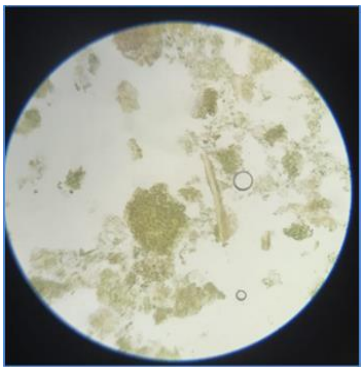

Fig.5: Trichomes

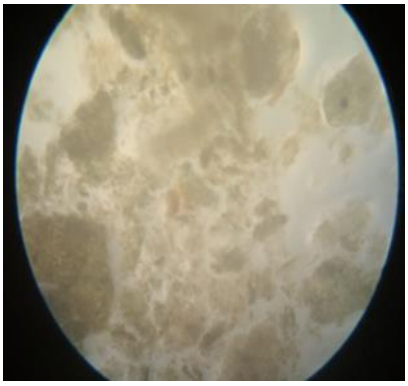

Fig.6: Calcium oxalate crystals

Fig.7: Phloem fibres

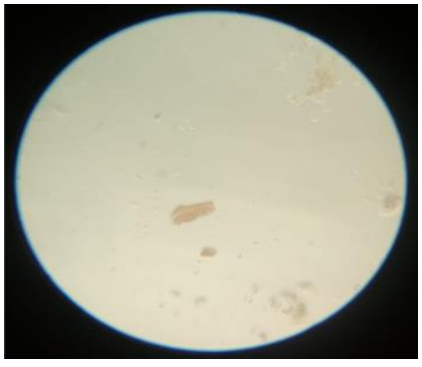

Fig.8: Xylem vessels

REFERENCES

[1] Ansari S.H., Essentials of Pharmacognosy, (Birla Publications, Delhi, 2005–06) 207–594.

[2] Aranez A.T. and Rubio R.O. Genotoxicity of pigments from seeds of Bixa orellana L. (Atsuete) I. Determined by allium test. Philippine J Sci. 125: 259–269 (1996).

[3] Bruneton J., Pharmacognosy, Phytochemistry, Medicinal Plants, (Intercept, Hampshire, 1999) 131–160.

[4] Castello M.C., Phatak A., Chandra N. and Sharon M. Antimicrobial activity of crude extracts from plant parts and corresponding calli of Bixa orellana. Indian J Exp Biol. 40: 1378–1381 (2002).

[5] Evans W.C., Trease and Evans Pharmacognosy, (WB Saunders Ltd, London, 2002) 32, 33, 95–99, 512, 547.

[6] Goyal R.K. and Shah B.S., Practicals in Pharmacognosy, (Nirali Prakashan, Pune, 2005) 128–155.

[7] Harborne J.B., Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis, (Springer Verlag, Berlin, 2005) 107–200.

[8] Heinrich M. and Barnes J., 2004, Fundamentals of Pharmacognosy and Phytotherapy, (Churchill Livingstone, Pune, 2004) 24–29.

[9] Joshi S.G., Medicinal plants, (Oxford and IBH publishing Co Pvt. Ltd, New Delhi, 2000) 99. 10. Khandelwal K.R., Practical Pharmacognosy, (Nirali Prakashan, Pune, 2003) 38–161.

[10] Kokate C.K., Practical Pharmacognosy, (Vallabh Prakashan, Pune, 2002) 107–129

[11] Mukherjee P.K., Quality Control of Herbal Drugs. An Approach to Evaluation of Botanicals, (Business Horizons Pharmaceutical Publications, New Delhi, 2002) 529–533.

[12] Parotta J.A., Healing plants of peninsular India, (CABI Publishing, London, 2001) 176–177. 14. Rangari V.D., Pharmacognosy and Phytochemistry, (Career publications, Nashik, 2004) 129–150.

[13] Srivastava A., Shukla Y.N.M., Jain S.P. and Kumar S., Chemistry, pharmacology and uses of Bixa orellana – a review. J Med Aromat Plant Sci. 21: 1145–1154 (1999).

[14] Anonymous, The Wealth of India (Raw Material), Vol-2B (Council of Scientific and Industrial Research, New Delhi, 1998) 157–160.

[15] Alabi D.A. and Alausa A.A., Evaluation of the mineral nutrients and organic food contents of the seeds of Lablab purpurus, Laucaena leucocephala and Mucua utilis for domestic consumption and industrial utilization, World J Agric Sci. 2: 115–118 (2006).

[16] Naseem Ullah, Muhammad Zahoor, Farhat Ali Khan, Shazeb Khan. A review on general introduction to medicinal plants, its phytochemicals and role of heavy metal and inorganic constituents, LifeSciJ2014;11(7s):520527, 109.

[17] Kokoshi, J., Kokoshi, R., and Salma, FJ., Fluorescence of powdered vegetable drugs under ultraviolet radiation. J Am Pharm Ass, 1958. 47: p. 715.