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RESEARCH ARTICLE

Pharmacognostic Studies on Leaf of *Operculina turpethum* (L.) Silva Manso

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Abstract

Operculina turpethum (L.) Silva Manso is a perennial medicinal plant of the family Convolvulacae. Since ages, the plant has been used for the treatment of various diseases. Use of the plant for treating various types of diseases is mentioned in Ayurvedic treatise like Charaka Samhita. But there were no sufficient reports regarding the pharmacognostic characteristics of leaf of the plant. So the present work was designed and conducted to evaluate the pharmacognostic properties of leaf of *Operculina turpethum*. Study of macroscopic characters of the leaf revealed the presence of polymorphic leaves with respect to both shape and size. Leaves were found to be simple, amphistomatic with ciliate margin and reticulate venation. Paracytic type of stomata was noticed in the plant. Powder analysis of the leaf revealed the presence of mineral depositions of various shapes. In fluorescent study, the leaf powder exhibited different colours with different reagents. Various physicochemical parameters studied for the dried leaf powder gave satisfactory results. Soxhlet extraction of the dried leaf powder was carried out with non-polar to polar solvents. Preliminary phytochemical screening of extract revealed the presence of alkaloids, terpenoids, steroids, cardiac glycosides, phenols and flavonoids. The study opened a window to the bioprospecting of leaf of *O. turpethum* for various medicinal principles as well as properties.

Introduction

The genus *Operculina* is one of the main genera of morning glory family Convolvulaceae and it comprises 15 species all over the world. *Operculina aequisepala* (Domin) R. W. Johnson, *Operculina brownii* Ooststr, *Operculina hamiltonii* (G. Don) D. F. Austin & Staples, *Operculina pinnatifida* (Kunth) O'Donell, *Operculina pteripes* (G. Don) O'Donell and *Operculina turpethum* Silva Manso (Indian Jalap) are the most common species of the genus. Out of the 15 species, only *Operculina turpethum* is present in India. *O. turpethum*, commonly known as Indian Jalap in English and Thrikolpakonna in Malayalam is a large perennial twiner with milky white exudation. It is pantropical in distribution and in India it is found in N. Circars, Godavari, Deccan, Carnatic to South Travencore (Gamble, 1993) and the banks of Cauvery or Kollidam rivers (Mathew,1983).

In ayurvedic system of medicine root of the plant is widely used in the trade name TRIVRIT. Trivrit is a composite of six rasas (sarvanubhuti) and effective for treating all types of doshas. Hence it is called Tribhandi (Sivarajan and Balachandran, 1994). Trivrit has been included in the group of ten purgative herbs, ten antidote herbs, ten herbs supportive for therapeutic enema (Brahmanand, 2008); group of colon cleanser, anti-tumor and antidote herbs and in the group of herbs eliminating toxins from the lower half of the body (Anantaram, 2008). Root paste is used in skin disorders such as vitilago and for other diseases such as cervical lymphadenitis, haemorrhoids, fistulas, ulcers and chancres. The use of root powder against paralysis, flatulence, rheumatism, scorpion sting and snake bite is also

proven (Nadkarni and Nadkarni, 2007). The root powder is also found to be useful for treating hematemesis, tuberculosis and herpes (Balpal, 2005). Leaves are reported to possess antibacterial activities and there are reports about the use of fresh juice of leaves for the treatment of corneal opacity and conjunctivitis.

There are reports regarding overexploitation of the plant in India (Sebastinraj et al., 2013). A recent national level trade study undertaken by Foundation for Revitalisation of Local Health and Traditions in Kerala reported this as an endangered medicinal plant with high trade value. As the environment influences the characters of a plant a lot, it is very necessary to set the pharmacognostic standards for various plant parts in different geographic locations. The objective of the present study is to set pharmacognostical standards for leaf of *O. turpethum* in Kerala.

MATERIALS AND METHODS

Procurement of plant Material

Plant material for the present study was collected from Feroke of Calicut district in Kerala ($11^0$11'13.8834" N and $75^0$47'59.9994" E). The specimen was examined and authenticated by Dr. G Valsaladevi, Curator, Department of Botany, Kariavattom. Fresh leaves and dried powder of leaves were subjected to various studies.

Macroscoic evaluation

To ensure quality of the drug, characters such as shape, colour, odour, taste, margin, apex, base, surface and venation of leaves were studied with the help of sense organs and size was measured with the help of a ruled scale.

Microscopic evaluation

Epidermal study

Epidermal features were studied according to the method of Glory et al. (2011). Small pieces of dried leaves were boiled in water and kept in a test tube containing nitric acid for 5hours to separate the epidermal layers. The leaf pieces were removed from the acid with a spatula and washed in distilled water. The upper and lower epidermal layers were separated with the help of a spatula, treated in 70, 85 and 100% ethyl alcohol and later stained with saffranin O for two minutes. Then the peels were mounted in glycerin and the microphotographs were taken using image Analyser (Olympus-BX51TF, Japan).

Abaxial and adaxial epidermal peels prepared were observed for the characters such as presence, type and distribution of stomata and trichomes, shape of epidermal cells, stomatal number and stomatal index.

Powder microscopy

Fine powder of the leaf was placed on a clear grease free microslide and one drop each of phloroglucinol and HCl was poured to it. Then it was covered with a coverslip and observed under microscope to record various anatomical features of the plant.

4. Fluorescence Analysis

Fluorescence Analysis of the leaf powder was done as per the method of Pratt and Chase (1949) and Kokoski et al. (1958). A small quantity of leaf powder was trated with 1-2 drops of freshly prepared reagent solutions of different kinds. After 2 minutes, the slide was placed inside the UV transilluminator chamber and viewed in visible light and ultraviolet radiations to study the fluorescence behavior of powder.

5. Physico-chemical analysis

Physicochemical parameters such as loss on drying, pH, water soluble extractive and alcohol soluble extractive values were determined according to the well established procedures (Renjumol, 2011).

6. Phytochemical screening

Screening for various phytoconstituents in leaf was conducted using extracts prepared in different solvents. Five gram of plant powder was subjected to serial extraction in a soxhlet extractor with petroleum ether, chloroform, methanol and water respectively. Solvents were get evaporated and concentrated extracts were kept at 4^0C until use. Preliminary screening was carried out using the standard procedures described by Khandelwal (2004).

RESULTS

Occurrences of polymorphic leaves during different stages of growth and also during different seasons were noticed in the plant. In general, the shape is cordate but under stress conditions such as drought it produces hastate or lanceolate leaves as an adaptation to reduce water loss. The observed morphological characters are recorded in table1. Leaves are found to be amphistomatic with paracytic or Rubiaceous type of stomata (figure 1) on both surfaces. The qualitative and quantitative epidermal features studied were presented in table 2. Powder analysis revealed the presence of starch grains and druses, raphides and prism type crystals (figures 2-9). In Fluorescent

analysis, plant powder treated with different chemical reagents produced different colours under visible and UV radiations (table 3). Result of physicochemical analysis was summarized in table 4.

The amount of extract obtained, its colour and nature varied among four extracts. Petroleum ether extract was cowdung green in colour and it corresponded to only 3% w/w of the sample taken for extraction. Chloroform, methanol and distilled water extracts appeared respectively dark green, cowdung green and dark brown in colour. Maximum yield was obtained from the solvent methanol and it was 11.8% w/w of sample. Respective weight of chloroform and aqueous extracts were 4% and 9% w/w of the sample taken for extraction. The extracts evaluated for the presence of major secondary metabolites revealed the presence of alkaloids, terpenoids, steroids, glycosides, cardiac glycosides, tannins, saponins, phenols and flavonoids. The result is presented in table 5.

Table 1: Macroscoic features of the leaf

Leaf character	Observation
Type	Simple
Attachment	Petiolate
Arrangement	Alternate
Shape	General- Cordate
Size	12.9cm long; 8.1cm wide
Margin	Entire or lobed at base
Apex	Apiculate or Mucronate
Base	Cordate
Venation	Reticulate
Surface	Pubescent; Ciliate
Colour	Green
Odour	Characteristic
Taste	Tasteless

Table 2: Epidermal features

Epidermal character	Adaxial	Abaxial
No.of epidermal cells	47.6±1.53	40.4±0.74
No.of stoma	5.2±0.66	12.8±0.73
Stomatal index	9.83±1.29	23.98±0.81
Shape of epidermal cells	Polygonal	Irregular
Nature of epidermal cell wall	Straight	Sinuous
Type of stoma	Paracytic	Paracytic

Table 3: Flourescent analysis of powder

Reagent	Colour observed		
	Visible	Short UV	Long UV
1N NaOH	Light green	Light green	Black
1N KOH	Light green	Light green	Black
5% $FeCl_2$	Light green	Light green	Black
Ammonia	Light green	Light green	Black
Glacial Acetic acid	Green	Green	Black
Con. HCl	Light green	Light green	Black
Con. H_2SO_4	Greenish brown	Green	Black
Conc. HNO_3	Light green	Light green	Black

Table 4: Physicochemical evaluation

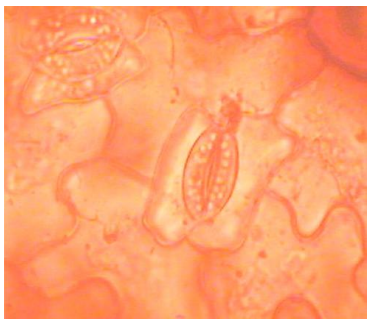
Physicochemical parameter	Observed value
Moisture content	13%
pH	5.6
Water soluble extractives	50%
Alcohol soluble extractives	79.5%

Table 5: Preliminary phytochemical screening of extracts

Test	Petroleum ether extract	Chloroform extract	Methanol extract	Aqueous extract
Alkaloids Mayer's reagent- creamy white precipitate	-	+	-	-
Terpenoids Keller kiliani test- blue ring at interface	++	+	++	++
Steroids Salkowski Test- greenish yellow fluorescence	+	+	+	+
Glycosides Borntrager'sTest- rose pink colour	+	+	+	+
Cardiac glycosides Keller kiliani test- reddish brown colour at interface and bluish green fluorescence at upper layer	++	+	+++	-
Phenols Lead acetate test- bluish black colour	+	+	-	-
Flavonoids Alkaline reagent test- intense yellow colour	++	+++	-	+
Tannins Ferric chloride test- brownish green colour	-	-	-	+
Saponins Froth test- persistent froth	-	-	-	+

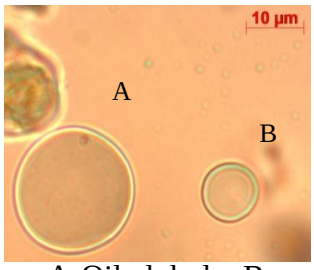
Figure 1: Rubiaceous type stomata

Figures 1-8: Result of



powder analysis

Fig. 2

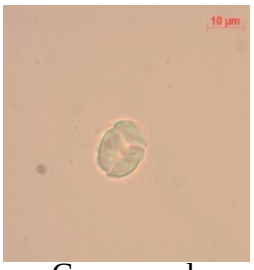


A-Oil globule; B-concentric starch

Fig. 3

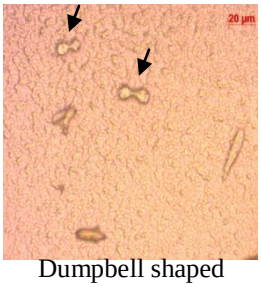
Feather shaped starchgrains

Fig. 4



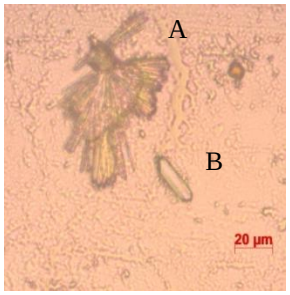
Compound starchgrain

Fig. 5



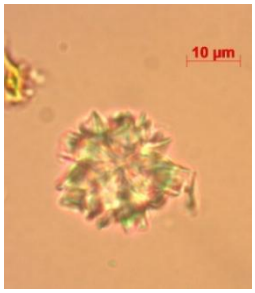
Dumpbell shaped starch grains

Fig. 6



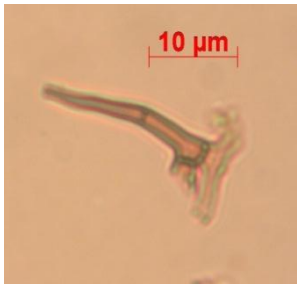
A-bundle raphides; B- prismatic crystal

Fig. 7



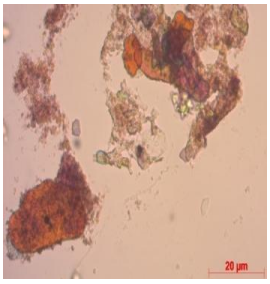
Druses type crystal

Fig. 8



Multicellular trichome

Fig. 9



Brown coloured deposits

DISCUSSION

There is a need for documentation of research work carried out on traditional medicines (Dahanukar, 2000) and also it becomes extremely important to make an effort towards standardization of the plant material to be used as medicine. The process of standardization can be achieved by stepwise pharmacognostic studies. These studies help in identification and authentication of the plant material. Correct identification and quality assurance of the starting materials is an essential prerequisite to ensure reproducible quality of herbal medicine which will contribute to its safety and efficacy. The present study uses simple pharmacognostic tools such as morphology (macro and micro-morphology), powder microscopy, fluorescent analysis, physicochemical analysis and phytochemistry for the documentation of *O. turpethum*, an important traditional medicine.

The macroscopic characters observed in this study matches with the observations reported by Gamble (1993) and Sharma and Singh (2012). As reported by Tayade and Patil (2011), most of the Convolvulaceous plants have amphistomatic leaves. In the present investigation, *O. turpethum* also found to have amphistomatic leaves. The highest stomatal index on abaxial surface matches with their observations on other members in the family.

Presence of crystals noticed in the study is consistent with that described for several members of the Convolvulaceae (Metcalfe and Chalk, 1950). As the occurrence of calcium oxalate crystals is rare among plants, the drusses observed in the powder analysis can be considered as a very important feature for the identification of the plant (Evans, 1997). Result of fluorescent studies conducted for the qualitative evaluation of the drug can be used as a reference data for checking purity of the drug. The moderate pH, and good extractive values observed for the plant will be a good sign for extracting phytoconstituents from the plant. The secondary metabolites give the characteristic medicinal property to plants and it was the main ingredient in treatment of diseases all over the world by traditional as well as modern medical systems (Ross et al., 2001). The phytochemicals like alkaloids, phenolics, terpenes, glycosides and flavonoids are reported to have many properties including anti-cancerous activity (Prasad et al., 2005). The occurrence of majority of secondary metabolites in leaf of the plant is a promising sign of discovery of new medicinal principles from it.

So, the various pharmacognostic properties described in this paper can be used as standards for identification of the plant *O. turpethum* and proper studies on leaf regarding pharmacological activities of various compounds are very much needed for the discovery of potent drugs.

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