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Original Article

Pharmacognostical and Phytochemical evaluation of *Ventilago calyculata* Tul. (Bark)

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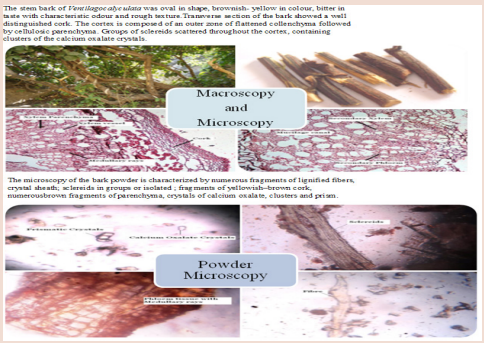
ABSTRACT

Background: *Ventilago calyculata* Tul. ('kevati'), is found throughout India as climbing shrub. It is widely used in various traditional system of medicine. **Objective:** In the present work pharmacognostical standardization has been developed for the systematic identification of the bark of *Ventilago calyculata*. Phenols and flavonoids were also quantified. **Materials and Methods:** Morphological, microscopical and phytochemical studies were performed. Various physicochemical parameters conforming the identity, quality, purity of the bark. The quantity of phenols and flavonoids were estimated. **Results:** The bark was oval, brownish yellow, bitter with characteristic odour and rough texture. The microscopical studies revealed the presence of cork with brownish contents, crimson inner cork, collenchyma, cellulosic parenchyma with cuboidal calcium oxalate crystals and schlereids. The total ash value, acid insoluble ash value and water soluble ash values of stem bark were found to be 15% w/w, 3.4% w/w and 11.6% w/w respectively. The percentage yields, total phenolic content and the total flavonoid content of the petroleum ether, chloroform, ethyl acetate, ethanol and aqueous extracts were 2.77% w/w, 2.66% w/w, 3.8% w/w, 5.8% w/w, 11.0% w/w; 2.16 ± 0.04, 4.16 ± 1.04, 9.12 ± 1.14, 7.16±1.16, 1.16 ±1.02 mg/g (gallic acid equivalent) and 4.5 ± 0.55, 8.20 ± 1.12, 10.1 ± 0. 26, 6.5 ± 1.3, 0.66 ± 1.13 mg/g (rutin equivalent) respectively. **Conclusion:** There was a need to evaluate the extracts of the plant in order to provide scientific proof for its application and to explore the possibility of treating various diseases and disorders. Literature review indicates that very less work has been done on this plant and there is a wide scope for investigation.

Key words: Standardization, Total flavonoid content, Total phenolic content, *Ventilago denticulata* Willd, *Ventilago madraspatana* var. *calyculata* (Tul.) King.

SUMMARY

- *Ventilago calyculata* Tul. (*Rhamnaceae*) commonly known as 'kevati', 'raktavalli' in Sanskrit and 'pappili' in Siddha.
- It is found throughout India in hotter parts as climbing shrubs or lianas, on the trees.
- Different parts of this plant possess multifarious medicinal properties.
- Traditionally, its stem bark is used as a tonic (especially for stomach and liver as it is bitter and stimulates them to release enzymes). It's root, flower, and stem used in fever, night blindness, earache, urine retention, headache, rheumatism, dysuria, diabetes, eye diseases, abortifacient, syphilis, ulcer and stomachache. In Ayurveda, it is an ingredient of "mahā śyonāka taila" which is used in the form of drink, massage, inhalation and enema. This medicated oil is used in looseness of joints, chronic fever, gout, insanity, dysuria, vomiting, trembling etc.
- Until now, no scientific investigations had been carried out for the standardization of *Ventilago calyculata* Tul. bark. Hence, there was a need to provide scientific proof for standardization by the pharmacognostical and phytochemical evaluation of the bark of *Ventilago calyculata* Tul.



PICTORIAL ABSTRACT

Abbreviations used: Tul: Tulasne, var: variety, UV: Ultra Violet, nm: nano meter, SD: Standard deviation, µL: micro liter.

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INTRODUCTION

Herbal medicine is a major element of almost all traditional systems like Ayurvedic, Homeopathic, Naturopathic, Traditional Chinese medicine, and Native American medicine.[1] The commence of medicinal plants is flourishing globally day by day and besides there is also a huge demand of plant drugs all over the world, thus there might be a possibility of adulteration and substitution of the crude drugs with the genuine ones. Hence standardization of medicinal plants and natural products will provide useful information with regards to its correct identity and will help to differentiate from other closely related species as well as from other commercially available crude drugs.

Ventilago calyculata Tul. (*Rhamnaceae*) commonly known as 'Kevati', 'raktavalli' in Sanskrit and 'pappili' in Siddha. It is found throughout India in hotter parts as a climbing shrubs or lianas, on the trees.[2,3] Flowering from October to December and fruiting December to April of following year. Previous phytochemical studies of *Ventilago calyculata* Tul. have reported the presence of several anthraquinones, naphthoquinones, naphthalenes, xanthone and naphthoquinone lactones in root bark.[4-7] Different parts of this plant possess multifarious medicinal properties. In Ayurveda, it is an ingredient of "mahā śyonāka taila" which is used in the form of drink, massage, inhalation and enema. This medicated oil is used in looseness of joints, chronic fever, gout, insanity, dysuria, vomiting, trembling etc.[8] Traditionally, the stem bark powder mixed with sesame oil is used externally for the treatment of skin diseases and sprains. Sap obtained from the bark is utilized for the treatment of deafness. The root bark is used for the atonic dyspepsia, diabetes, mild fever and debility.[3] A paste of root is applied locally to excite granulation in wounds.[9]

Until now, no scientific investigation had been carried out for standardization of *Ventilago calyculata* Tul. Bark. Hence, there was a need to provide scientific proof for standardization by the pharmacognostical and phytochemical evaluation of the bark of *Ventilago calyculata* Tul.

MATERIALS AND METHODS

Plant collection and Authentification

The stem bark of *Ventilago calyculata* were collected from the local area of Bhopal, Madhya Pradesh, in the month of October 2010 and authenticated by Dr. Zia Ul Hassan, Botanist, Saifia College of Science and Education, Bhopal, Madhya Pradesh. The voucher specimen (248/Bot/Saifia/11) was deposited in Department of Pharmacognosy, Technocrats Institute of Technology, Pharmacy, Bhopal, Madhya Pradesh. The barks were washed thoroughly with tap water, shade dried, homogenized to coarse powder and stored in air tight bottle.

Pharmacognostic studies

Macroscopic characteristics

For morphological observations, fresh barks approx. 2-5 cm in lengths was used. The macromorphological features of the bark were observed under magnifying lens.[10]

Fluorescence analysis

Powdered material was analyzed under visible light, short ultra-violet light, long ultra-violet after treatment with organic and inorganic reagents was carried out for the powder.[11]

Microscopic characteristics

Bark sample was fixed and specimens were cast into paraffin blocks.[12] The paraffin embedded specimens were sectioned with the help of rotary microtome. The sections were stained[13] and glycerin mounted slides were observed. Photographs with different magnification were taken with Nikon Lab Photo 2 microscopic units. Powder microscopy was also carried out and the specific diagnostic characteristics were recorded.[15]

Physicochemical parameters

The physicochemical parameters like total ash value, loss on drying, water soluble ash value, acid insoluble ash value were determined as per WHO guidelines.[16] For physicochemical investigation, 10 g of powder was extracted by individual cold percolation method using alcohol and water as solvent. The solvent was evaporated to dryness and the dried crude extracts were stored in air tight bottle.

The dried and coarse powder of stem bark of *Ventilago calyculata* was extracted with the solvents of increasing polarity successively by hot extraction method using Soxhlet apparatus. The percentage yield of stem bark extracts viz. petroleum ether, chloroform, ethyl acetate, ethanol and aqueous extract was calculated.

Phytochemical analysis

The dried and coarse powder of stem bark of *Ventilago calyculata* was subjected to qualitative phytochemical analysis.[17,18]

Quantitative Determination of Phytochemical Constituents

Determination of Total Phenolic Content[19]

For quantitative estimation of total phenolic component Folin-Ciocalteu phenolic reagent was used. 0.5 ml of sample (1mg/ml) solution was mixed with 2.5 ml of Folin Ciocalteu phenolic reagent (10%) and 2 ml of 7.5 % Na_2CO_3 solution and mixed. After that reacting mixture was incubated for 30 min at room temperature in dark condition and measured the absorbance at 760 nm. Here gallic acid was used as standard. The sample was tested in triplicate and a calibration curve for gallic acid was obtained. The results were compared to gallic acid calibration curve and the total phenolic content of extract was expressed as mg of gallic acid equivalents (GAE) per gram of dry extract.

Determination of Total Flavonoids Content[20]

The spectrophotometer assay for the quantitative determination of flavonoid content was carried out as described by Wang *et al*. with minor modifications using rutin as a standard. Briefly, extracts or standard solutions (0.25 mL) were mixed with 1.25 mL distilled water and 75 µL 5% $NaNO_2$. After 6 min, 75 µL of 10% $AlCl_3$ was added. After another 5 min, 0.5 mL of 1 M NaOH was added to the mixture. Immediately, the absorbance of the mixture was determined at 510 nm *versus* prepared water blank. Total flavonoids content was expressed as mg rutin equivalents (RE) per gram dry extract.

Statistical analysis: The data was expressed as mean ± SD. The significance of differences among the group was assessed using one way analysis of variance (ANOVA). $P \leq 0.05$ were considered as significance.

RESULTS AND DISCUSSION

Macroscopic characteristics

The stem bark of *Ventilago calyculata* is more or less oval or rounded in shape, outer bark is brownish yellow with crimson striations. It is bitter in taste with characteristic odour and rough texture. (Figure 1)

Figure 1: Macropscopy of *Ventilago calyculata*.
(A) *Ventilago calyculata* in the natural habitat; (B)Arial part showing the fruits; (C) Stem bark of *Ventilago calyculata*; (D) Powdered bark.

Fluorescence analysis

Fluorescence characteristic of powdered stem bark of *Ventilago calyculata* were observed for resolution of doubtful specimen. Powdered material was analyzed under visible light, short ultra-violet light, long ultra-violet after treatment with organic and inorganic reagents (Table 1).

Microscopic characteristics

Transverse section of the bark showed a well distinguished cork consists of numerous layers of small thin walled flattened cells arranged in radial rows and having yellowish brown contents which give a purple color with alkalies. The cortex is composed of an outer zone of flattened collenchyma followed by a large inner zone of thin walled flattened cellulosic parenchyma. Groups of sclereids scattered throughout the cortex. Some of them contain clusters of the calcium oxalate crystals. The secondary phloem is transversed by 1-5 seriate medullary rays, the sieve tubes are arranged in tangential bands alternating with the phloem fibers and phloem parenchyma. The fibers usually occur in small groups which are surrounded with parenchyma cells, each containing prism of calcium

Table 1: Fluorescence characteristics of dried powder of *Ventilago calyculata*

Powder + Reagent	Color observed in Ordinary light	Color observed under UV Short (254 nm)	Color observed under UV Long (365nm)
Powder as such	Brown	Green	Green
Powder+1N NaOH in water	Dark green	Brownish-green with black spots	Yellow with white spots
Powder +1N HCl	Yellow with pinkish spots	Yellow	Yellow with black spots
Powder+50% H_2SO_4	Brown	Yellow	Yellow with pink spots
Powder + Formaldehyde	White	Brown	Yellow
Powder + 5% Ferric chloride	Brown	Brown	Black
Powder + Iodine	Black	Yellow	Black

oxalate forming a crystal sheath. (Figure 2).

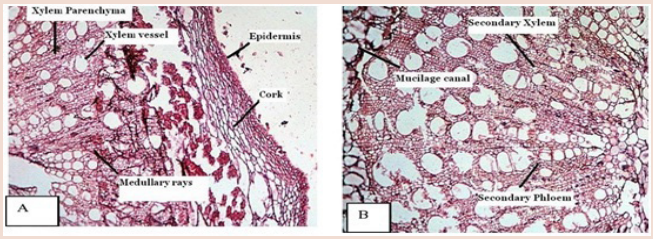

Figure 2: Photomicrograph of Transverse section (T.S.) of stem bark of Ventilago calyculata x400
(A) T.S. showing epidermis, cork, xylem parenchyma, xylem vessel, medullary rays; (B) T.S. showing secondary xylem, mucilage canal, secondary phloem

The powder of the bark powder is characterized by numerous fragments of lignified fibers, fibers in groups accompanied by crystal sheath; sclereids in groups or isolated ; fragments of yellowish–brown cork, numerous brown fragments of parenchyma, crystals of calcium oxalate, clusters and prism. The crystals are cuboidal in shape scattered in the powder. The crystals are in regular vertical fills or strands and vary in shape and sizes (Figure 3).

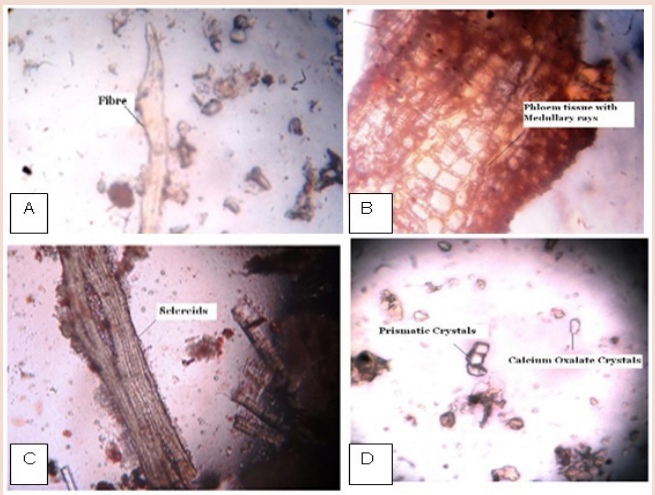

Figure 3: Photomicrograph of powder characteristics of stem bark of *Ventilago calyculata* x400
(A) Fibre; (B) Fragments of phloem tissue and medullary rays; (C) Sclereids; (D) Prismatic crystals of calcium oxalate

Physicochemical parameters

Physicochemical parameters of any drug give an idea of the earthy matter and/or inorganic composition and/or other impurities present along with a drug. The total ash value, acid insoluble ash value and water soluble ash value of stem bark were found to be 15% w/w, 3.4% w/w and 11.61% w/w respectively. The moisture content of the powdered drug was evaluated using loss on drying method and value observed was 3.18% w/w (Table 2).

Table 2: Physiochemical analysis of dried powder of *Ventilago calyculata*

S. No.	Values	Stem bark (% w/w)
Ash values		
1	Total ash value	not more than 11.61
2	Acid insoluble ash	not more than 4.2
3	Water soluble ash	not more than 7.41
Loss on drying		3.18

The dried and coarse powder of stem bark of *Ventilago calyculata* was extracted with the solvents of increasing polarity successively by soxhlet apparatus, while the aqueous extract was obtained by cold maceration method. The percentage yield of stems bark extracts *viz*. petroleum ether, chloroform, ethyl acetate, ethanol and aqueous extract were found to be 2.77% w/w, 2.66% w/w, 3.8% w/w, 5.8% w/w, 8.0% w/w respectively (Table 3).

Table 3: Nature and percentage yield of various extracts of dried powder of *Ventilago calyculata*

Extracts	Colour & consistency of extract	Percentage yield (% w/w)
Petroleum ether	Reddish brown powder	2.77
Chloroform	Brownish powder	2.66
Ethyl acetate	Brownish powder	3.8
Ethanol	Dark brown with syrupy consistency	5.8
Water	Blackish brown powder	8.0

Phytochemical analysis

The dried and coarse powder of stem bark was tested for the presence of phytoconstituents using reported methods mentioned in the standards and results are given in Table 4. The preliminary phytochemical screening of various extracts was carried out and it was found that petroleum ether extract showed the presence of glycosides, flavonoids, phytosterols and phenolic compounds, chloroform extract showed the presence of glycosides, phenolic compounds, ethyl acetate extract showed the presence of alkaloids, flavonoids, glycosides, phenolic compounds and carbohydrates, ethanol extract showed the presence of carbohydrates, glycosides, phenolic compounds and flavonoids and aqueous extract showed the presence of alkaloids, carbohydrates, phenolic compounds,

Table 4: Qualitative chemical tests performed in the various extracts dried powder of *Ventilago calyculata*

Phytochemical tests	Petroleum Ether	Chloroform	Ethyl acetate	Ethanolic	Aqueous
Test for Alkaloids					
Mayer's reagents	-	-	-	-	+
Dragendorff's reagent	-	-	+	-	-
Hager's reagents	-	-	+	-	+
Wagner's reagent	-	-	-	-	+
Test for Carbohydrates					
Molisch's test	+	-	+	+	+
Fehling's test	+	-	+	+	+
Benedict's test	+	-	+	+	+
Barfoed's test	+	-	+	+	+
Test for Glycosides					
Legal's test	+	+	+	-	-
Borntrager's test	+	+	+	-	-
Baljet test	+	-	-	-	-
Keller-Killiani	+	-	-	+	-
Test for Phytosterol					
Libermann Burchard test	+	-	-	-	-
Salkowski test	+	-	-	-	-
Test for saponins					
Foam	-	-	-	-	+
Heamolysis	-	-	-	-	+
Test for Flavonoids					
Shinoda's test	+	-	+	+	+
Test for Mucilage	-	-	-	-	+
Test for Tannins/Phenols					
Lead acetate	+	+	+	+	-
Ferric chloride test	+	+	+	+	+

+ Present – Absent

Table 5: Total phenolic content of extracts of *Ventilago calyculata*

S.No.	Plant extract	Gallic acid equivalent (mg/g)
1	**Petroleum ether**	2.16±0.04*
2	**Chloroform**	4.16±1.04*
3	**Ethyl acetate**	9.12±1.14*
4	**Ethanol**	7.16±1.16*
5	**Aqueous**	1.16±1.02*

*Values are means of triplicate determination ± Standard deviation

Table 6: Total flavonoid content of extracts of *Ventilago calyculata*

S.No.	Plant extract	Rutin equivalent(mg/g)
1	**Petroleum ether**	4.5±0.55*
2	**Chloroform**	8.20±1.12*
3	**Ethyl acetate**	10.1±0.26*
4	**Ethanol**	6.5±1.3*
5	**Aqueous**	0.66±0.13*

*Values are means of triplicate determination ± Standard deviation

saponins and flavonoids. The total phenolic content of the petroleum ether, chloroform, ethyl acetate, ethanol and aqueous extracts were 2.16 ± 0.04, 4.16 ± 1.04, 9.12 ± 1.14, 7.16 ± 1.16 and 1.16 ± 1.02 mg/g gallic acid equivalent respectively (Table 5). The total flavonoid content of petroleum ether, chloroform, ethyl acetate, ethanol and aqueous extracts were 4.5 ± 0.55, 8.20 ± 1.12, 10.1 ± 0.26, 6.5 ± 1.3 and 0.66 ± 1.13 mg/g rut in equivalent respectively (Table 6).

The present study dealt with the pharmacognostic and phytochemical investigation of stem bark of *Ventilago calyculata*. Morphological and microscopic studies of the stem bark will enable to identify the crude drug. Ash values, extractive values can be used as reliable aid for detecting adulteration. The information obtained from preliminary phytochemical screening will be useful in finding out the genuineness of the drug. These simple but reliable standards will be useful to a lay person in using the drug as a home remedy. Also the manufacturers can utilize them for identification and selection of the raw material for drug production.

CONCLUSION

The plant *Ventilago calyculata* is widely used in various traditional system of medicine as a herbal remedy. It has been used since centuries as a tonic, in treatment of diarrhea, liver disorders, inflammation, leucorrhea, urinary tract infections, malarial fever and diabetes. Phytochemical screening assay is a simple, quick, and inexpensive procedure that gives the researcher a quick answer to the various types of phyto-

chemicals in a mixture and an important tool in bioactive compound analysis. Since bioactive compounds occurring in plant material consist of multi-component mixtures, their separation and determination still creates problems. Practically most of them have to be purified by the combination of several chromatographic techniques and various other purification methods to isolate bioactive compound(s). On the basis of physicochemical studies and preliminary phytochemical screening, this plant may give a significant effect against diseases and disorders, literature review indicates that very less work has been done on this plant and there is a wide scope for investigation.

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CONFLICT OF INTEREST

There are no conflicts of interest.

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