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PHARMACOGNOSTIC AND PHYTOCHEMICAL INVESTIGATIONS OF *OPERCULINA TURPETHUM* LINN. ROOT

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ABSTRACT:

Objective: To study detailed Pharmacognosy of the root of *Operculina turpethum* (Convolvulaceae) well known Ayurvedic drug (trivrit).

Method: Macroscopy, microscopy, physicochemical analysis, preliminary phytochemical testing of the root and other WHO recommended methods for the standardization were done.

Results: The periderm cells are tabular in shape; the phloem cells are homogeneous, tabular in shape and have suberized walls. The phelloderm cells are also tabular in shape and have cellulose walls. Irregular masses of squarish sclerenchyma cells are seen inner to the periderm and with outer cortical zone. Sclereids are also seen in small masses in the interior portion of the cortex. The cortical cells are densely loaded with starch grains and sparsely distributed calcium oxalate crystals. Secondary xylem is the thick and dense central cylinder comprising wide, circular thin walled vessels and thick walled lignified fibers. The vessels are solitary and diffuse in distribution. The xylem parenchyma cells are thick, tangential layered and they are apotracheal layered type. The parenchyma layers are away from the vessels.

INTRODUCTION: *Operculina turpethum* (Convolvulaceae) found throughout India and cultivated in Ceylon, tropical America, Mauritius, Philippines, tropical Africa and Australia.

This plant is also known as "Trivrit" in Sanskrit and "Nishottara" in Marathi.

Trivrit has two varieties as Aruna or Shweta (i.e. having whitish or reddish colored root) and Shyama (i.e. having blackish root). The botanical name of Aruna or Shweta trivrit is *Operculina turpethum* (L.) Silva Manso (syn. *Ipomoea turpethum*), and Shyama is *Ipomoea petaloides* Choisy. Aruna or Shweta Trivrit is the best amongst the herbs used for Virechana (therapeutic purgation). Shyama, with its drastic purgative action, can treat the conditions like intoxication & abdominal tumors. However, Shyama is inferior in properties and can cause fainting, burning sensation, giddiness, confusion, chest pain and roughness of throat and hence is rarely used in medicine.

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Operculina turpethum has been used for centuries as a medicinal plant in ayurvedic medicines because of its resin content (10 %) known as turpethin, jalapine and convolvuline glycoside and essential oil contents. *Operculina turpethum* is perennial plant with milky juice, root are long, slender, fleshy, much branched. Stems are very long, twining and much twisted together, angled and winged, pubescent, tough and brown when old¹⁻⁴.

But the present investigation reports focus on more details pharmacognostic, phytochemical study profiles of *Operculina turpethum* root which was not mentioned in previous histological and physicochemical analysis study.

MATERIALS AND METHODS:

Plant material: Root of *Operculina turpethum* was Purchased from Shri Shail Medicinal Plants Farms (Supplier), Nagpur and authenticated by Dr. A.S. Upadhye, Agarkar Research Institute, Pune, where a sample specimen (voucher number: R-148) has been deposited.

Macroscopic and Organoleptic studies: The macroscopic study of a medicinal plant was helpful in rapid identification of plant material and also plays an important role in standardization of drug. The fresh leaves were subjected to macroscopic studies which comprised of organoleptic characters viz., color, odor, appearance, taste, texture etc.

1. Microscopic studies:

- i. **Root Microscopy:** For qualitative microscopic evaluation, the collected *Operculina turpethum* root were fixed in FAA solution (Formalin-Aceto-Alcohol: Formalin, Acetic acid each 5 ml, in 90ml of 70% ethanol) for 24 hrs then dehydrated with graded series of tertiary-butyl alcohol and castled in paraffin blocks. Later, the paraffin embedded specimens were subjected for sectioning with the help of rotary microtome and de-waxed the sections. These sections were stained with safranin and observed under compound microscope at projection 10X and 40X⁵.

- ii. **Powder Microscopy:** To study the presence or absence of various types of tissues or structures, the dried of *Operculina turpethum* root are powdered using electric grinder, passed through sieve No. 60 and then subjected for microscopic studies. The powder microscopy was performed according to the methods of Kokate⁶ and Khandelwal⁷.

Physicochemical parameters: Physicochemical values such as the percentage of ash values and extractive values were determined according to the official methods^{8,9} and as per WHO guidelines on quality control methods for medicinal plant materials^{10,11}.

- i. **Determination of ash values:** For determining ash content of drug, about 3 g of powder was spread in a pre-ignited and weighed silica crucible. Then the crucible was incinerated gradually to make the crucible free from carbon. After cooling, crucible was weighed to get the total ash content and then the ash was subjected for determining the acid insoluble and water soluble ash. The percentage of total ash was calculated by taking the air dried sample as standard.
- ii. **Determination of extractive values:** Considering the diversity and chemical nature of drug, five different solvents viz. petroleum ether, chloroform, alcohol and water were used for determination of extractive values. About 5 g of powdered material was subjected continuous Soxhlet extraction with 100 ml of petroleum ether, chloroform, alcohol as solvents while using maceration process water extraction done. Determination of extractive values of a crude drug is beneficial in its evaluation process wherever evaluation of chemical components is applicable. After extraction, the extracts are concentrated in rota vaporizer and dried in vacuum desiccator. Then the extractive values are calculated as percentage w/w of solvent soluble extractive with reference to the air dried drug.

- iii. **Determination of moisture content:** Moisture content was determined by loss of weight on drying (LOD) method. For this 5gm of drug (powdered root material) was taken and kept in an oven at 105°C till a constant weight was obtained. Amount of moisture present in the sample was calculated as reference to the air dried material.
- iv. **Fluorescence analysis:** Crude drugs show their own characteristic fluorescence when exposed to ultra violet radiation and is dependent on its chemical constituents. This analysis is useful to identify adulterants during crude drug evaluation. In the present study, one gram of crude drug was taken in watch glass and subjected for fluorescent analysis as such and after treatment with different reagents.

Preliminary phytochemical screening: Plants are considered as bioreactors or biosynthetic laboratories as they synthesize wide range of characteristic therapeutically important molecules in the form of secondary metabolites. Thus, a systematic preliminary phytochemical screening of plant material is essential for identifying plant constituents and to establish a chemical profile of a crude drug for its proper evaluation. For preliminary phytochemical extracts were subjected for preliminary screening using standard procedure for identifying various phytoconstituents.

RESULTS: A systematic approach is necessary in pharmacognostic study which helps in confirmation and determination of identity, purity and quality of a crude drug. This detailed and systematic pharmacognostic study will give valuable information for future research work.

Macroscopic and Organoleptic Studies: The root is thick and wood. The surface of the root is irregularly fissured; the fissures are shallow. The thickness of the periderm varies along the circumference as shown in **Fig. 1**.



FIG. 1: MORPHOLOGY OF OPERCULINA TURPETHUM L. ROOT

Microscopic Studies:

Root Microscopy:

- a. **Periderm:** In the thicker region of the periderm, it consists of outer zone of about eight layers of phloem cells and inner derivative of about twelve layers of phelloderm cells. The periderm cells are tabular in shape; the phloem cells are homogeneous, tabular in shape and have suberised walls. The phelloderm cells are also tabular in shape and have cellulose walls as shown in **Fig. 2b**. Irregular masses of squarish sclerenchyma cells are seen inner to the periderm and with outer cortical zone. Sclereids are also seen in small masses in the interior portion of the cortex shown in **Fig.2.a**.
- b. **Cortex:** The cortical zone gradually gets transformed into secondary phloem and there is no border or distinct boundary between the cortex and secondary phloem. The cortical cells are densely loaded with starch grains and sparsely distributed calcium oxalate crystals shown in **Fig. 2a** and **2c**. The starch grains are either simple; concentric or exocentric. These are also compound starch grains.
- c. **Secondary xylem:** Secondary xylem is the thick and dense central cylinder comprising wide, circular thin walled vessels and thick walled lignified fibers. The vessels are solitary and diffuse in distribution. There are also xylem parenchyma cells. The xylem parenchyma cells are thick, tangential layered and they are apotracheal layered type. The parenchyma layers are away from the vessels **Fig. 2a & 2b**.

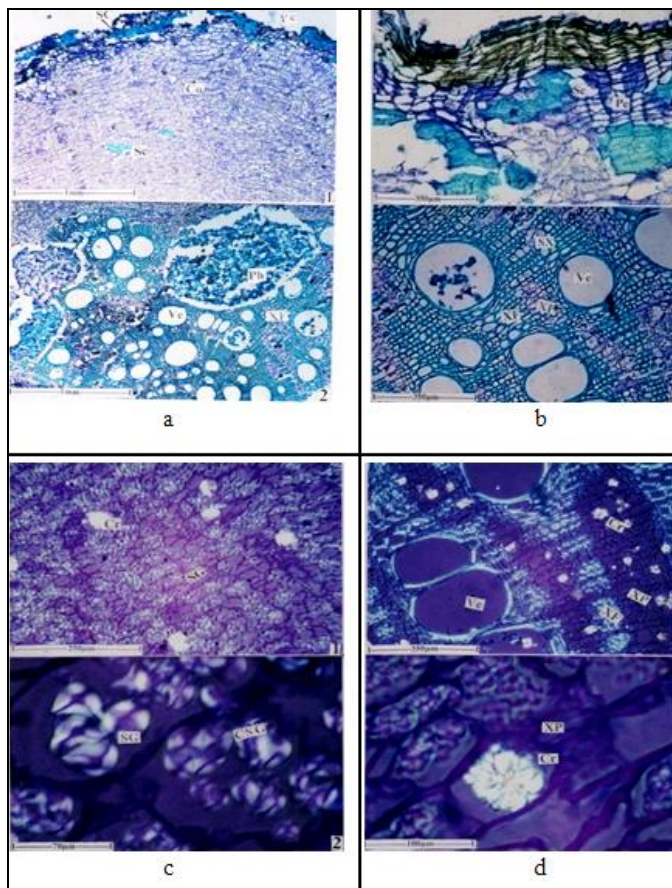


FIG. 2: MICROSCOPIC CHARACTERISTICS OF OPERCULINA TURPETHUM ROOT

a. - T.S. of root showing periderm & cortical zone with dense accumulation of starch grains and few sclerenchymas (1) & secondary xylem with large masses of phloem buried with xylem (2). **b.** - Periderm and Sclereids masses enlarged view (1) & secondary xylem showing the shapes, size and distribution of the vessels (2). **c.** Cortical cells possessing starch grains and crystals as seen under the polarized microscope (1) & Simple and compound starch grains as seen

under the polarized microscope (2). **d.** T.S. of secondary xylem with apotracheal parenchyma with calcium oxalate druses as seen under the polarized microscope (1) & Single calcium oxalate crystal enlarged as seen under the polarized microscope (2).

Where, Pe: Periderm, Co: Cortex, Ph: Phloem, SC: Sclerenchyma mass, Ve: Vessel, SX: Secondary xylem, XF: Xylem fiber, XP: Xylem parenchyma, Cr: Crystal, CSG: Compound starch grains, SG: starch grains.

Powder Microscopy: Secondary xylem is the thick and dense central cylinder comprising wide, circular thin walled vessels and thick walled lignified fibers. The cortical cells are densely loaded with starch grains and sparsely distributed calcium oxalate crystals. The starch grains are either simple; concentric or exocentric (**Fig. 3 a, b & c**).

Physicochemical constants: Determination of physicochemical parameters of a crude drug is essential as it helps in identification and estimation of mishandling, adulteration and also in setting of proper standards. Various physicochemical parameters like ash values, extractive values, moisture content and fluorescence on reaction with various chemical reagents were investigated and the results are presented (**Tables 1-3**).

Ash values of the drug give idea about earthy matter or inorganic composition and other impurities present along with the drug. The extractive values are primarily useful for the determination of the exhausted or adulterated drug.

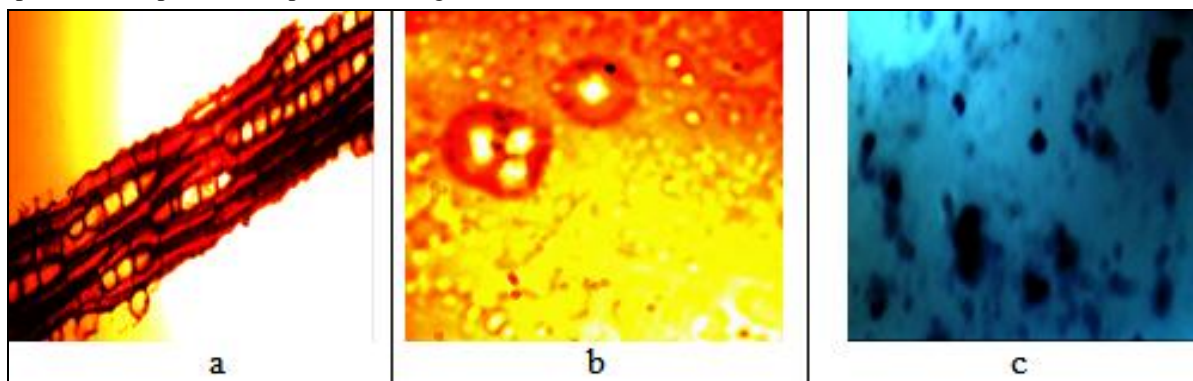


FIGURE 3: POWDER MICROSCOPY OF O. TURPETHUM ROOT. A - XYLEM FIBER AND XYLEM VESSEL; B - CALCIUM OXALATE CRYSTAL; C - STARCH PARTICLE

TABLE 1: PHYSICOCHEMICAL PARAMETERS OF OPERCULINA TURPETHUM ROOT

Physicochemical parameter values	% W/W
Water soluble ash	1.06 % w/w
Acid Insoluble ash	1.20 % w/w
Moisture content	2 % w/w

TABLE 2: SOLVENT EXTRACTIVE VALUES (%W/W) OF *OPERCULINA TURPETHUM* ROOT

Name of extract	Color	Extractive value
Petroleum ether Extract	Light yellow brown	1.2 % w/w
Chloroform Extract	Yellow	3.06% w/w
Methanol Extract	Brown	11.76 % w/w
Water Extract	Brown	13.5 % w/w

TABLE 3: FLUORESCENCE ANALYSIS OF *OPERCULINA TURPETHUM* ROOT

Reagent	UV short (254 nm)	UV long (365 nm)	Visible
Methanol	Yellowish white	White	Faint Yellow
1 N Methanolic NaOH	White	White	Faint Yellow
Ethanol	Yellowish white	Whitish Yellow	Faint Yellow
1 N Ethanolic NaOH	Dark green	White	Yellow
1N HCL	Black	Dark Yellow	Dark Yellow
1N NaOH	Green	Faint Yellow	Dark Brown
50 % H ₂ SO ₄ ,	Brown	Faint Yellow	White
50 % HNO ₃	Faint Yellow	Faint Yellow	White
5 % KOH	Dark Yellow	Faint Yellow	Whitish Yellow
Acetone.	Faint Yellow	White	White

Preliminary phytochemical screening: Extracts obtained by continuous Soxhlet were subjected to be subjected for standard qualitative phytochemical tests to identify the presence of chemical constituents (viz., alkaloids, glycosides, tannins, flavonoids, sterols, fats, oils, phenols and saponins) present in them. Preliminary phytochemical

screening mainly revealed the presence of steroids and triterpenes in petroleum ether extract; steroids, triterpenes and glycosides in chloroform extract; carbohydrate, glycoside, flavonoids and saponins in methanol extract and carbohydrate and glycoside in aqueous extract (**Table 4**).

TABLE 4: PHYTOCHEMICAL ANALYSIS OF *OPERCULINA TURPETHUM* ROOT EXTRACTS

Chemical constituent	Chemical test	Pet. Ether extract	Chloroform extract	Methanol extract	Water extract
Alkaloids	Dragendorff's test	-	-	-	-
	Mayers test	-	-	-	-
Steroids	Salkowski test	++	-	-	-
	Liebermann-Burchard test	++	-	-	-
Triterpenes	Vanillin-sulphuric acid test	+++	+++	-	-
Tannin	Ferric chloride test	-	-	-	-
Glycoside	Keller-killiani test	-	+++	+++	+
Carbohydrate	Molish test	-	-	++	+++
	Fehling's test	-	-	-	-
Flavonoids	Shinoda Test	-	-	++	-
Saponins	Lead acetate test	-	-	++	-
Proteins	Biuret test	-	-	-	-

+: Weak positive test; ++: Low positive test; +++: Strong positive test; -: negative test.

DISCUSSION: Ash values and extractive values are useful in identification and authentication of the plant material. Extractive values are useful to evaluate the chemical constituents of crude drug. Preliminary phytochemical screening mainly revealed the presence of steroids and triterpenes in petroleum ether extract; steroids, triterpenes and glycosides in chloroform extract; carbohydrate, glycoside, flavonoids and saponins in methanol

extract and carbohydrate and glycoside in aqueous extract. The periderm cells are tabular in shape; the phloem cells are homogeneous, tabular in shape and have suberised walls. The phelloderm cells are also tabular in shape and have cellulose walls. Irregular masses of squarish sclerenchyma cells are seen inner to the periderm and with outer cortical zone. Sclereids are also seen in small masses in the interior portion of the cortex.

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CONCLUSION: In the present investigation, a set of pharmacognostical standardization parameter studies were conducted on *Operculina turpethum* root as per pharmacopoeia and WHO guidelines. These studies revealed the presence of various important bioactive compounds and proved that the plant roots are also medicinally important. These results may help in standardization, identification and in carrying out further research in *Operculina turpethum* root based drugs which are used in Ayurveda and modern pharmacopoeia.

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