PHARMACOGNOSTIC AND PHYTOCHEMICAL STUDIES ON
OPERULINA TURPETHUM (L.) SILVA MANSO STEM

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ABSTRACT

*Operculina turpethum* (L.) Silva Manso is a highly traded medicinal plant belonging to the family Convolvulaceae. The present study was an attempt to analyse morphological and anatomical features, phytochemical composition and total phenol and flavanoid content of *Operculina turpethum* stem according to the standard procedures. The morphological and anatomical features observed in the study matches with the early reports. The winged nature of stem is an important identification feature of the plant. Anatomical study revealed the presence of starch grains, flavonoids and secretory cells. The dry powder of the stem was subjected to sequential soxhlet extraction with solvents in the order petroleum ether (50°C), chloroform (75°C), methanol (65°C) and water (100°C). The qualitative tests performed on extracts confirmed the presence of steroids, cardiac glycosides, phenols, flavonoids and terpenoids. Quantitative analysis found 42 ± 0.08 and 15 ± 0.00 mg equivalents of respective standards of phenol and flavanoid respectively. This can be taken as a good indication of the hidden antioxidant and antimicrobial potential of the stem of the plant. The study thus forms a platform for the bioprospecting of the stem of *O. turpethum* for the discovery of potential pharmaceutical agents.

KEYWORDS: *Operculina turpethum*, morphology, anatomy, secondary metabolites, phenol, flavanoid.

INTRODUCTION

*Operculina turpethum* (L.) Silva Manso is a large perennial twiner of the family Convolvulaceae. The plant is commonly known as Indian Jalap in English, trivrit in Sanskrit and Thrikolpakonna in Malayalam. It is native to Asia, Africa and Australia while is
naturalized in West Indies.\(^1\) It is commonly found in N. Circars, Godavari, Deccan, Carnatic to South Travencore\(^2\) and the banks of Cauvery or Kolli dam.\(^3\) In Kerala, the plant is found in degraded forest areas and plains.\(^4\) Today \textit{O. turpethum} falls under the threatened category due to overexploitation.\(^5\)

As a potent medicinal plant, it has been used for many medicinal purposes. It has been included in the group of ten purgative herbs, ten antidote herbs, ten herbs supportive for therapeutic enema\(^6\); group of colon cleanser, antitumor and antidote herbs and in the group of herbs eliminating the toxins from the lower half of the body.\(^7\) Root paste is used in skin disorders such as vitilago and for other diseases such as cervical lymphadenitis, haemorrhoids, fistulas, ulcers and cancers. The use of root powder against paralysis, flatulence, rheumatism, scorpion sting and snake bite was also proven.\(^8\) The root powder was also found to be useful for treating hematemeses, tuberculosis and herpes and fresh juice of leaves is found to be effective for treatment of corneal opacity and conjunctivitis.\(^9\) Young leaves and stem of the plant is used as vegetable.\(^10\) The antimicrobial potential of the stem was also studied.\(^11\) But the systematic studies regarding the traditional uses of stem of \textit{O. turpethum} is not available. So the present study was conducted to understand the basic phytochemical constituents in the stem of the plant.

MATERIALS AND METHODS

Procurement of plant material
The plant material for the present study was obtained from Kozhikode district of Kerala, India. The material was verified and confirmed as \textit{Operculina turpethum} by Dr. G. Valsala Devi, Curator, Department of Botany, University of Kerala, Kariavattom. The voucher specimen was deposited in the Department Herbarium (KUBH 8400).

Morphological and anatomical study
The stem characters such as colour, texture and nature of twisting was studied. Thin free hand sections of the stem was taken and stained with different staining agents to study the general anatomy and localization pattern of various cellular components.

Preparation of extracts
The mature, healthy stem of the plant was thoroughly washed and shade dried for one month. The properly dried material was ground to fine powder with the help of a blender. About 15 g of the fine powder was subjected to sequential soxhlet extraction with different solvents in
the ascending order of their polarity ie., petroleum ether- chloroform- methanol- water for 48 hours and soxhlet extraction with methanol alone. Then the extracts were concentrated on a rotary evaporator, dried, weighed and kept at 50°C until use.

**Qualitative phytochemical screening**

The extracts were qualitatively tested for the presence of various classes of secondary metabolites by reaction with different chemicals according to the standard procedures.\[^{12}\]

**Quantification of phenols and Flavonoids**

The total phenol and flavonoid content of the stem was estimated from the pure methanol extract by spectrophotometric method.

Total phenol was estimated according to standard procedure.\[^{13}\] To 1 ml of 1% sample extract, 0.5ml of Folin-Ciocalteu reagent and 2ml of sodium carbonate (20%) was added sequentially and warmed for 1 minute in a waterbath. Then cooled and absorbance was measured at 650 nm against a blank. A standard calibration plot was generated at 650 nm using known concentrations of catechol. The concentrations of phenols in the test samples were calculated from the calibration plot and expressed as mg catechol equivalent of phenol/g of sample.

The aluminum chloride method was used for the determination of total flavonoid content of the sample extracts.\[^{14}\] For analysis, 1ml of 1% extract solution was taken in a test tube and made up the volume to 3ml with methanol. Then 0.1ml AlCl3 (10%), 0.1ml Na-K tartarate and 2.8 ml distilled water were added sequentially. The test solution was vigorously shaken and absorbance at 415 nm was recorded after 30 minutes of incubation. A standard calibration plot was generated at 415 nm using known concentrations of quercetin. The concentrations of flavonoid in the test samples were calculated from the calibration plot and expressed as mg quercetin equivalent /g of sample.

**RESULTS**

*O. turpethum* produces long slender branches from the axils of leaves. Stem was found to be weak, green, winged and smooth at young stage (Fig. 1). Colour of stem gets darkened as the plant grows and finally it became woody and brownish with rough surface in older portions. Right hand (clockwise) twisting was observed in stem. Hairs were found to be present in young stem. In cross section, it appears as two semi circles joined together at two points. An
The outermost layer of epidermis followed by chlorenchymatous hypodermis and parenchymatous cortex was noticed in the cross section of stem. The vascular tissue was found to be arranged in a circular fashion with phloem on outside and xylem inner to the phloem. Single layered endodermis and a large parenchymatous pith was present (Fig. 2). In the cortex and pith region certain specialized cells with secretion were present at regular intervals (Fig. 3a and 3b). Staining with phloroglucinol- HCl reagent revealed the presence of lignin in the vascular and endodermis region of the stem (Fig. 4). Presence of flavonoid was also noticed in the vascular tissues of the stem (Fig. 5). Starch grains were visualized in the pith region with the help of iodine- potassium iodide reagent (Fig. 6).

The phytochemical characteristics of stem of plant tested were summarized in tables 1 to 3. The results revealed the presence of medicinally active compounds in the extracts. Percent yield and colour of all extracts were noted and tabulated in table 1. Alkaloids were found to be absent in the stem but the presence of phenol, flavonoid, steroid, terpenoid and cardiac glycoside were confirmed. The result is presented in table 2. The total phenol and flavonoid content in the methanol extract of the plant is presented in table 3.

**Table 1: CHARACTERISTICS OF EXTRACTS**

<table>
<thead>
<tr>
<th>Extract</th>
<th>Colour of extract</th>
<th>Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Petroleum ether</td>
<td>Light green</td>
<td>38.0</td>
</tr>
<tr>
<td>Chloroform</td>
<td>Light brown</td>
<td>33.9</td>
</tr>
<tr>
<td>Methanol</td>
<td>Blood red</td>
<td>33.9</td>
</tr>
<tr>
<td>Aqueous</td>
<td>Dark brown</td>
<td>14.30</td>
</tr>
</tbody>
</table>

**Table 2: RESULT OF PHYTOCHEMICAL SCREENING**

<table>
<thead>
<tr>
<th>Metabolite group</th>
<th>Test</th>
<th>Petroleum ether</th>
<th>Chloroform</th>
<th>Methanol</th>
<th>Water</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaloids</td>
<td>1. Hager’s test</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>2. Mayer’s test</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>3. Wagner’s test</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Phytosterols</td>
<td>Salkowski’s test</td>
<td>++</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Phenols</td>
<td>1. Lead acetate test</td>
<td>-</td>
<td>-</td>
<td>+++</td>
<td>++</td>
</tr>
<tr>
<td></td>
<td>2. Ferric chloride test</td>
<td>-</td>
<td>-</td>
<td>+++</td>
<td>++</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>1. Alkaline reagent test</td>
<td>-</td>
<td>-</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>2. Ferric chloride test</td>
<td>-</td>
<td>-</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>Terpenoids</td>
<td>Salkowski’s test</td>
<td>+</td>
<td>+</td>
<td>++</td>
<td>+++</td>
</tr>
<tr>
<td>Cardiac glycosides</td>
<td>Keller- Killani test</td>
<td>+++</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>
Table 3: TOTAL PHENOL AND FLAVONOID CONTENT

<table>
<thead>
<tr>
<th>Test</th>
<th>Mean ± S.E</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total phenol content</td>
<td>42 ± 0.08</td>
</tr>
<tr>
<td>(mg catechol equivalent/g dry material)</td>
<td></td>
</tr>
<tr>
<td>Total flavanoid content</td>
<td>15 ± 0.00</td>
</tr>
<tr>
<td>(mg quercetin equivalent/g dry material)</td>
<td></td>
</tr>
</tbody>
</table>

DISCUSSION

Plant morphology and anatomy are the very important areas for phytochemical research as they play important role in the correct identification and characterization of a plant or part of the plant that is used as drug. Anatomical investigation of the medicinal plants is very important for the authenticity and quality control of the drugs. World Health Organization (WHO) has laid much emphasis on the micro and macro characteristics study of a drug.
before proceeding to any test.\textsuperscript{[15]} The morphological and anatomical characters observed for \textit{O. turpethum} in the present study matches with the previous descriptions.\textsuperscript{[16]}

Plants are recognized for their ability to produce a wealth of secondary metabolites. The secondary metabolites are naturally synthesized in all parts of the plant body especially bark, leaves, stem, root, flower, fruits and seeds. Secondary metabolites have some valuable biological properties like antioxidant, antibacterial, antifungal, anticonstipative, spasmolytic and anticancer activities; modulation of detoxification enzymes; immune system stimulation; decreased platelet aggregation and hormone metabolism modulation.\textsuperscript{[17]}

For the discovery of novel drugs, the essential information regarding the chemical constituents is generally provided by the qualitative phytochemical screening of plant extracts. In the present study, qualitative tests conducted in four extracts showed significant indication about the presence of various classes of secondary metabolites such as phenol, flavonoid, phytosterol, terpenoid and cardiac glycosides in the stem of \textit{Operculina turpethum}. This result matches with the observation of the study carried out in the pure aqueous and methanolic extracts of stem of the plant.\textsuperscript{[18]} The methanolic extract was found to be rich in flavonoids followed by aqueous extract. All the tested extracts showed the presence of terpenoids but more intensity was observed in aqueous extract. While steroids were found to be present in all extracts except aqueous extract. The petroleum ether and chloroform extracts showed the presence of cardiac glycosides. This indicates the presence of more non-polar functional groups in the cardiac glycosides of the plant.

The result of spectrophotometric determination of total phenol and flavanoid also found to be a positive sign for the discovery of potent antioxidants and antimicrobials from the stem of the plant. Phenolic compounds are considered to be major contributors to the antioxidant capacity of plants.\textsuperscript{[19]} Flavonoids as one of the most diverse and widespread group of natural compounds are probably the most important natural phenols. These compounds possess a broad spectrum of chemical and biological activities including radical scavenging properties.\textsuperscript{[20]} Therapeutically, terpenoids are reported to exhibit antiviral, antibacterial, antitumour, antiseptic, diuretic and analgesic activities.\textsuperscript{[21]}

**CONCLUSION**

The stem of \textit{O. turpethum} was found to be a source of phytochemicals such as phenol, flavonoid, phytosterol, terpenoid and cardiac glycosides. The quantitative analysis revealed the presence of fairly good amount of antioxidant and antimicrobial groups of secondary
metabolites called phenols and flavonoids. Thus this study points out the relevance of using the stem of *O. turpethum* in pharmaceutical research for the betterment of human life.

**ACKNOWLEDGEMENT**

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**REFERENCES**


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