

EVALUATION ON PHYTOCHEMICAL, ANTIOXIDANT AND ANTIBACTERIAL POTENTIAL OF THE ROOT OF *OPERCULINA TURPETHUM* (LINN.) SILVA MANSO

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ABSTRACT

Operculina turpethum(Linn.) Silva Manso is an excellent plant having medicinal property for treating constipation and urinary disorders. The roots of the plant have antiulcer and hepatic stimulant activity. Phytochemical, antioxidant and antibacterial activities were traced in present investigation. Using Soxhlet apparatus, the dried root of the plant were extracted with methanol and also water extract were taken. Methanol and water extracts were tested qualitatively for phytoconstituents. These extracts showed the presence of resins, tannins, saponins, phytosterols, lactones, glycosides, anthraquinones

and flavonoids. Antioxidant was assayed by DPPH radical scavenging activity and the plant showed significant antioxidant potential. These extracts were further tested for antibacterial activity against bacterial strains of *Escherichia coli*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Bacillus subtilis*. These bacterial strains are sensitive to the plant extracts. Hence the results validate the role of *Operculina turpethum* in allopathic medicine due to the presence of many different secondary metabolites, antioxidant property and antibacterial efficacy.

KEYWORDS: Antioxidant, anti-ulcer, secondary metabolites, phytoconstituents and antibacterial.

1. INTRODUCTION

Secondary metabolites like alkaloids, glycosides, tannins, flavanoids, volatile oil, fixed oil, etc. which are produced in very small amount in plants for their survival or growth These

secondary metabolites can be isolated by using techniques such as column, paper, gas liquids, and high performance liquid chromatography (Joseph Mathew P, 2006). The ethanolic extract obtained from roots of *Operculina turpethum* were evaluated for hepatoprotective activity (Suresh Kumar *et al.*, 2006). The plant contains (7-8%) resins and volatile oil. Alcoholic soluble portion of the resin is a mixture of gluco-rhamnosides of turpethinic acids like jalapinolic acid and operculonic acid (Mohammed Ali, 1994). The present study deals with phytochemical, antioxidant and antibacterial properties of the dried root of *Operculina turpethum*.

2. MATERIALS AND METHOD

2.1 Collection and identification of sample

The plant *Operculinaturpethum* (Linn.) Silva Manso was collected from ATIC, Medicinal plants, Mannuthy and identified by taxonomist Jacob Abraham pulikkal, St. Thomas college, Thrissur.

2.2 Extraction of the plant material

25g of powdered dried root of *Operculina turpethum* were extracted in methanol using soxhlet apparatus. Also water extracts were taken using water bath. The phytochemical screening, antioxidant and antibacterial activity were carried out after evaporating the solvents using solvent recovery apparatus and reconstituting the residue in the respective solvents (10ml).

2.3 Phytochemical Screening

The quantitative chemical tests were performed on methanol and aqueous extracts of dried root of *Operculina* for the phytoconstituents such as alkaloids, glycosides, tannins, resins, flavonoids, saponins, phytosterols, lactones and anthraquinones. The alkaloids were tested with Dragendorff's reagent; tannins with Ferric chloride; phytosterol with Liebermann-Burchard test; flavanoids with Shinoda test; lactones with legal test, reducing sugar with benedict test.

2.4 Antioxidant assay

The free radical scavenging activity of *Operculinaturpethum* was analyzed by the DPPH assay using a spectrophotometer. The test extract at different concentrations (10, 20, 30, 40 and 50 μ l) each were mixed with 1ml of 0.3 μ g/ μ l DPPH (in methanol) in a cuvette. The absorbance at 517 nm was taken after 20 mins of incubation in the dark at room temperature.

The concentrations were prepared in triplicates and the percentage antioxidant activity (AA) was calculated as follows:

$$\% \text{ AA} = (\text{Control OD} - \text{Sample OD}) / \text{Control OD}$$

A volume of 2ml of methanol plus 1.0 ml of the extract was used as the blank while 1.0 ml of the DPPH solution plus 3.0 ml of methanol was used as the negative control. Ascorbic acid (vitamin C) was used as reference standard (Iwaleva *et al.*).

2.5 Antibacterial assay by disc-diffusion method

In this method, a Petri plate containing an agar growth medium is inoculated uniformly over its entire surface (Fuerst R. 1978). Sterile discs impregnated with methanol and water extracts of *Operculina* were placed on the surface of the agar. Incubate the Petri plates, inverted at 37°C for 24 hours. During incubation, the antimicrobial agents diffuse from the disc, from an area of higher concentration to an area of lower concentration. An effective agent will inhibit the bacterial growth, and measurements can be made of the size of the zones of inhibition around the discs. The zone size is affected by such factors as the diffusion rate of the antimicrobial agents and the growth rate of the organisms (Johnson T.R and Case C.L. 2010). Statistical analysis of the data was found out by one way ANOVA test using MSTATC software.

3. RESULTS AND DISCUSSION

3.1 Phytochemical screening

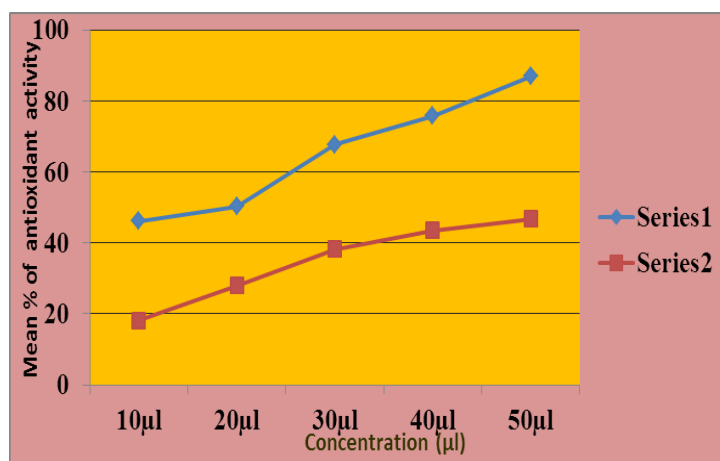
Table 1. Qualitative analysis of *Operculina turpethum*

Tests	Methanol extract	Water extract
Alkaloids -Dragendroff,s reagent	-	-
Tannins- Ferric chloride test	+	+
Phytosterols- Liebermann-Burchard test	+	+
Saponin- Foam test	+	+
Flavanoids-Shinoda test	+	+
Resins- general test	+	+
Glycosides- Keller-Killiani	+	-
Anthraquinones	+	-
Lactones- Legal test	+	-
Reducing sugar- Benedict reagent	+	-
Protein- Biuret test	-	-

+ = present; - = absent

3.2. Determination of in vitro antioxidant using 2,2-diphenyl-1-picrylhydrazyl (DPPH) photometric assay

The extract produced a concentration-dependent increase in the antioxidant assay. The antioxidant activities of *Operculina turpethum* were significantly lower when compared to ascorbic acid. The plant extract showed significant antioxidant activity when assayed using 2,2-diphenyl-1-picrylhydrazyl (DPPH) photometric assay as shown in graph 1. The IC₅₀ value was calculated to be 27.92 ± 1.33 (20µl).



Graph 1: Showing antioxidant activity of *Operculina turpethum*.

3.3 Antibacterial activity

The present study indicates that both the methanol and water extracts of *Operculina turpethum* of under study were effective against all the tested human pathogenic organisms. Among bacterial strains, *Escherichia coli*, *Staphylococcus aureus* and *Klebsiella pneumoniae* showed highly significant activity against all the fruit peels extracts. But *Pseudomonas aeruginosa* showed little resistant to all the extracts. Also the plant extracts showed significant activity against *Bacillus subtilis* as shown in table 2.

Table 2: Antibacterial activity of *Operculina turpethum* against human pathogen.

Sample	Average Zone in mm	P value	Average Zone in mm	P value	Average Zone in mm	P value
<i>Escherichia coli</i>	19.33	<0.01	20.66	<0.01	24	<0.01
<i>Klebsiella pneumoniae</i>	22.8	<0.01	24.66	<0.01	27	<0.01
<i>Pseudomonas aeruginosa</i>	7.6	<0.01	8	<0.01	19	<0.01
<i>Staphylococcus aureus</i>	10.33	<0.01	12.33	<0.01	20	<0.01
<i>Bacillus subtilis</i>	8	<0.01	9	<0.01	25	<0.01

4. CONCLUSION

Operculina turpethum (Linn.) *Silva Manso*, is the most important medicinal plant. The main chemical constituents present are resin, tannins, lactones, anthraquinones, phytosterol, glycosides, flavonoids, saponins and reducing sugars. The root of *Operculina turpethum* possesses antioxidant and antimicrobial property with special effectiveness in treating infections of the gastrointestinal tract and respiratory system. Further studies were recommended for detecting the presence of phyto-mediated silver nanoparticles in the plant as it has the anti-inflammatory, antioxidant and antibacterial properties and also studies can be done for revealing the action of plant extracts against gastric ulcers and stomach cancer as the plant is used for curing ulcer and other stomach disorders.

5. ACKNOWLEDGEMENT

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