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Phytochemical screening and antibacterial activity of different fractions of *Operculina turpethum* root and leaf

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ABSTRACT

In Bangladesh, the herb *Operculina turpethum* is used for otitis media, suppurative sores, burns, conjunctivitis and skin diseases (eczema, abscesses, acne, scabies and warts) although the compounds responsible for the medicinal properties have not been identified. The present study has been undertaken for antibacterial activity of the ethanol, ether and chloroform extract of *O. turpethum* root and leaf. Antibacterial activity has been investigated against *Shigella boydii, Shigella flese, Shigella dysenteriae, Escherichia coli, Proteus vulgaris, Salmonella typhi, Hafnia alvei, Staphylococcus epidermidis, Streptococcus pyogenes, Staphylococcus aureus, Enterococcus faecalis* by disc diffusion and broth macro-dilution assay. The zone of inhibition has been observed with almost all bacteria with some exceptions. Minimum inhibitory concentrations (MIC) of the extracts were found to be significant.

Keywords: *Operculina turpethum*, Phytochemical screening, Antimicrobial activity, MIC

INTRODUCTION

Operculina turpethum (syn. *Ipomoea turpethum*) (L.) (Family:Convolvulaceae) is commonly known as Dudh kalmi in Bangladesh, Sanskrit- Kalameshi, Rechani, Kutarana, Bhandi, English-Turpeth root, Indian jalap. *O. turpethum* is native to Asia (India, Nepal, Bangladesh, Pakistan, Shri Lanka, China, Taiwan, Myanmar, Thailand, Indonesia, Malaysia, Papua New Guinea, & Philippines), Africa (Kenya, Tanzania, Mozambique, Zimbabwe, Madagascar, Mauritius and Reunion) & Australia. Four new dammarane-type saponins, operculinosides A–D (1–4), were isolated from the aerial parts of *O. turpethum* (Wenbing *et al.*, 2011). Root is administered to treat obesity, haemorrhoids, cough, asthma (Sharma and Dravyaguna, 2006), dyspepsia, flatulence, paralysis, gout, rheumatism, melancholia, scorpion sting, and snake bites (Nadkarni, 2007). In constipation, it is an effective laxative. It is used in periodic fevers and in the treatment of anemia accompanied by spenomegaly. It is also used to relieve flatulence and colic and in the treatment of obesity to decrease fat. It is used to treat dropsy, dyspepsia with constipation and flatulence, gout and rheumatism, and other inflammations (Kumar *et al.*, 2006). The root extract of *O. turpethum* has been used as an anti-inflammatory, purgative, and hepatoprotective agent (Riaz, 2009). Ethereal, alcoholic and aqueous extracts of roots of *I. turpethum* (Nishoth) have been screened for their anti- inflammatory activity (Khare *et al.*, 1982). The root of *O. turpethum* is the chief ingredient in the Ayurvedic formulation viz. Avipattikarachurna used for the treatment of gastric ulcer and related gastrointestinal disturbances (Rajashekar *et al.*, 2006). Etheric extract of *O. turpethum* stems showed antioxidant activity (Anbuselvam *et al.*, 2007). The chloroform extract of stem of *I. turpethum* and the crude petroleum ether, chloroform and ethylacetate extracts were screened against thirteen pathogenic bacteria for their antibacterial activities (Rashid *et al.*, 2002).

Although the crude petroleum ether, chloroform and ethylacetate extracts of stem of *I. turpethum* was screened for antibacterial activity, but no literature is currently available to substantiate antimicrobial activities from 95% ethanol, ether and chloroform extract of *O. turpethum* leaves and roots, therefore the present study is a part of our on-going antimicrobial and chemical screening of selected *O. turpethum* leaves and roots and designed to provide scientific evidence for its use as a traditional folk remedy by investigating the antimicrobial activities.

MATERIALS AND METHODS

Plant material: The plant parts of *O. turpethum* was collected from Khulna University, Bangladesh and identified by the experts at Bangladesh National Herbarium, Dhaka, Bangladesh. The necessary plant parts were carefully cleaned and separated from other parts of the plant as well as from undesirable materials. After cutting into small pieces, these were dried under shade with ample aeration. After complete drying, the plant material was grinded into a coarse powder with the help of a suitable grinder. The powdered plant material was weighed using an electric balance, kept in a suitable airtight container and then stored in a dark, cool and dry place for further use.

Extraction: The powdered plant material (crushed roots and leaves) was macerated in 95% ethanol, ether and chloroform respectively for three days with occasional shaking. It was then filtered through a piece of clean, white cloth and then through a cotton plug to remove the plant debris. The filtrate was evaporated using a rotary vacuum evaporator at a temperature of 50^{o} C to yield the crude extract.

Phytochemical screening: The freshly prepared crude extract was qualitatively tested for the presence of chemical constituents, by using the following reagents and chemicals, for example, alkaloids were identified by the dragendorff's reagent, flavonoids with the use of Mg and HCl, tannins with ferric chloride and potassium dichromate solutions, steroids with Libermann-Burchard reagent and reducing sugars with benedict's reagent (Ghani, 1998; Evans, 1989; Harborne, 1984).

Test microorganism: Four Gram-positive bacteria, *S. epidermidis*, *S. pyogenes*, *S. aureus* and *E. faecalis* and seven Gram-negative bacteria, *S. boydii*, *S. flese*, *S. dysenteriae*, *E. coli*, *P. vulgaris*, *S. typhi* and *H. alvei* were taken for the test. The bacterial strains used for this investigation were obtained from the bacterial stocks preserved in animal cell culture laboratory of Bangladesh Council of Scientific and Industrial Research, Dr. Qudrat-E-Khuda Road, Dhaka-1205, Bangladesh.

Antibacterial assay: The antibacterial activity was investigated using two methods: disc diffusion and broth macro-dilution assay (Cruickshank, 1968; Andrews, 2001; Ríos *et al.*, 1988). Reference microorganisms from the stock were streaked onto nutrient agar plates and the inoculated plates were incubated overnight at 37°C. Using a sterile loop, small portion of the subculture was transferred into test tube containing nutrient broth and incubated (2-4 h) at 37°C until the growth reached log phase. Nutrient agar media seeded with standard inoculums suspension was poured in Petri-dishes and allowed to solidify. Discs (BBL, Cocksville, USA) impregnated with extract (250 µg/disc), standard antibiotic disc (Kanamycin 30 µg/disc, Oxoid Ltd, UK) and blank (solvent ethanol) discs were placed on the Petri-dishes with sterile forceps and gently pressed to ensure contact with the inoculated agar surface. Finally the inoculated plates were incubated at 37° C for 18 h and the zone of inhibition was measured in millimeters.

The broth macro-dilution assay was carried out to determine the minimum inhibitory concentration (MIC). Stock suspension of the extract was prepared in nutrient broth with tween-80 concentration not exceeding 5%. Serial dilution of the stock was carried out to obtain seven different concentrations (8, 4, 2, 1, 0.5 and 0.25 mg/ml) in six vials containing 1 ml each. The same procedure was followed for the standard antibiotic solution of ceftriaxone to obtain seven different concentrations (8, 4, 2, 1, 0.5 and 0.25 µg/ml) in six vials containing 1 ml each. Then 1 ml of freshly grown inoculum was added to each vial and incubated at 37° C for 12 h. After incubation period, the vials were checked for turbidity and the lowest concentrations of the extract/standard showing no turbidity were regarded as the MIC of the test substance.

RESULTS

Phytochemical screening of the ethanol extract of roots of *O. turpethum* indicates the presence of carbohydrates, steroids, gums and saponins and ether and chloroform extract of roots of *O. turpethum* indicates the presence of carbohydrates, steroids, gums, flavonoids and saponins and phytochemical screening of the ethanol, ether and chloroform extract of leaves of *O. turpethum* indicates the presence of

carbohydrates, steroids, tannins, gums, flavonoids, alkaloids and saponins (Table 1).

Table 1: Phytochemical constituents of extracts of *O. turpethum*

Extracts	Steroids	Alkaloids	Reducing Sugars	Tannins	Gums	Flavonoids	Saponins
Ethanol extract of O. turpethum root	+	-	+	-	+	-	+
Ether extract of O. turpethum root	+	-	+	-	+	+	+
Chloroform extract of O. turpethum root	+	-	+	-	+	+	+
Ethanol extract of O. turpethum leaf	+	+	+	+	+	+	+
Ether extract of O. turpethum leaf	+	+	+	+	+	+	+
Chloroform extract of O. turpethum leaf	+	+	+	+	+	+	+

+ = Presence of constituents; - = Absence of constituents

In the antibacterial assay the ether and chloroform extract of root and leaf inhibited all bacteria. In disk diffusion assay the ethanol extract of root inhibited all the microorganisms except *P. vulgaris* and *E. faecalis* (Table 2). The highest zone of inhibition was 8.9 mm against *S. aureus*. Zone of inhibition for the standard Kanamycin discs ranged between 24.30 to 33.80 mm (Table 2).

The data obtained from broth macro dilution assay for determining MIC is presented in (Table 3). Minimum inhibitory concentration (MIC) of the ethanol extract of root was 8000 µg/ml for *P. vulgaris*, *S. typhi*, *H. alvei* and *E. faecalis*; 4000 µg/ml for *S. boydii*; 2000 µg/ml for *S. dysenteriae*, *E. coli*, *S. epidermidis*, *S. pyogenes* and *S. aureus*. MIC of the ether extract of root was 8000 µg/ml for *S. flese*, *P. vulgaris*, *S. typhi*, and *E. faecalis*; 4000 µg/ml for *S. boydii* and *H. alvei*; 1000 µg/ml for *E. coli, S. epidermidis, S. pyogenes and S. aureus*. MIC of the chloroform extract of root was 8000 µg/ml for *P. vulgaris* and *E. faecalis*; 2000 µg/ml for *S. boydii*, *S. flese* and *S. typhi*; 1000 µg/ml for *S. dysenteriae*, *E. coli*, *H. alvei* and *S. epidermidis*; 500 µg/ml for *S. pyogenes* and *S. aureus*. MIC of the ethanol extract of leaf was 4000 µg/ml for *P. vulgaris*, *S. typhi*, *H. alvei* and *E. faecalis*; 2000 µg/ml for *S. boydii*, *S. flese, S.* dysenteriae, *E. coli*, *S. epidermidis*, *S. pyogenes* and *S. aureus*. MIC of the ether extract of leaf was 8000 µg/ml for *P. vulgaris*; 4000 µg/ml for *S. typhi*, *H. alvei* and *E. faecalis*; 2000 µg/ml for *S. boydii*, *S. flese*, *S. dysenteriae*, *E. coli*, *S. epidermidis*, *S. pyogenes* and *S. aureus*. MIC of the chloroform extract of leaf was 8000 µg/ml for *P. vulgaris*; 4000 µg/ml for *S. typhi*, *H. alvei* and *E. faecalis*; 2000 µg/ml for *S. boydii*, *S. flese*, *S. dysenteriae*, *S. epidermidis*, *S. pyogenes* and *S. aureus*; 1000 µg/ml for *E. coli*.

Table 2: Disc diffusion assay of different extracts of *O. turpethum* root and leaf

	Diameter of zone of inhibition (mm)						
Bacterial strain	**I**	**II**	**III**	**IV**	**V**	**VI**	**Kanamycin (30 µg/disc)**
Gram negative bacteria							
Shigella boydii	4	4.8	5.7	5.1	5.8	6.1	32.4
Shigella flese	3.6	4.1	5.2	5.4	5.5	5.6	24.3
Shigella dysenteriae	5.1	5.6	6.8	5.6	5.8	5.9	24.3
Escherichia coli	5.6	6.1	7.2	5.9	5.9	6.7	29.3
Proteus vulgaris	0	2	3.2	4.3	3.7	3.9	33.1
Salmonella typhi	3.8	4	5.1	4.7	5.1	4.9	33.4
Hafnia alvei	4.1	4.8	6.3	4.5	5	4.3	33.1
Gram positive bacteria							
Staphylococcus epidermidis	5.9	6.4	7.6	6.2	6.2	6.5	25.8
Streptococcus pyogenes	6.2	7.1	8.6	6.7	6.8	6.3	26.2
Staphylococcus aureus	6.1	7	8.9	6.8	6.3	6	28.3
Enterococcus faecalis	0	1.6	2.9	4.9	4.1	4.3	33.8

I=ethanol extract of root of *O. turpethum* (250 µg/ml), II=ether extract of root of *O. turpethum* (250 µg/ml), III=chloroform extract of root of *O. turpethum* (250 µg/ml), IV=ethanol extract of leaf of *O. turpethum* (250 µg/ml), V=ether extract of leaf of *O. turpethum* (250 µg/ml), VI=chloroform extract of leaf of *O. turpethum* (250 µg/ml).

Table 3: MICs of *O. turpethum* root and leaf extracts

	Minimum Inhibitory Concentration (MIC)						
Bacterial strain	**I**	**II**	**III**	**IV**	**V**	**VI**	**Ceftriaxone (µg/ml)**
Gram negative bacteria							
Shigella boydii	4000	4000	2000	2000	2000	2000	0.5
Shigella flese	8000	8000	2000	2000	2000	2000	1
Shigella dysenteriae	2000	2000	1000	2000	2000	2000	0.25
Escherichia coli	2000	1000	1000	2000	2000	1000	0.25
Proteus vulgaris	8000	8000	8000	4000	8000	8000	2
Salmonella typhi	8000	8000	2000	4000	4000	4000	2
Hafnia alvei	8000	4000	1000	4000	4000	4000	1
Gram positive bacteria							
Staphylococcus epidermidis	2000	1000	1000	2000	2000	2000	1
Streptococcus pyogenes	2000	1000	500	2000	2000	2000	0.5
Staphylococcus aureus	2000	1000	500	2000	2000	2000	0.5
Enterococcus faecalis	8000	8000	8000	4000	4000	4000	2

I=ethanol extract of root of *O. turpethum* (µg/ml), II=ether extract of root of *O. turpethum* (µg/ml), III=chloroform extract of root of *O. turpethum* (µg/ml), IV=ethanol extract of leaf of *O. turpethum* (µg/ml), V=ether extract of leaf of *O. turpethum* (µg/ml), VI=chloroform extract of leaf of *O. turpethum* (µg/ml).

DISCUSSIONS

The ethanol extracts of root and leaf showed moderate antibacterial activity whereas ether and chloroform extracts of root and leaf showed relatively higher antibacterial activity in both the assays. Although the ethanol extract of root did not show antibacterial activity against *E. faecalis* and *P. vulgaris* but inhibited the same microorganism in broth marco dilution assay. However, the MIC was obtained at a higher concentration (8000 µg/ml) than the extract content in the disc (250 µg/ml). Therefore, concentration may play a role for the observed activity in latter experiment. Antibacterial activity offered by non polar compound(s) may also be a reason as it may fail to diffuse in agar media to exhibit antibacterial activity in disc diffusion assay (Anderson *et al.*, 1988). A difference in inoculums size used for the assay can lead to variable results for a given sample. In the present study we adjusted to keep the inoculums size as close to the recommended standard of 5×10^5 CFU/ml (Ríos *et al.*, 1988).

The plant is also reported to contain saponins. There is growing interest in natural saponins caused as much by the scientific aspects extraction and structural analysis of these compounds, as by the fact of their wide spectrum of pharmacological activities; for instance, bactericidal, antiviral, cytotoxic, analgesic, anti-inflammatory, anti-cancer and antiallergic (Attele *et al.*, 1999). Phytochemical constituents such as tannins, flavonoids, alkaloids and several other aromatic compounds of plant that serve as defense mechanisms against predation by many microorganisms, insects and herbivores. The antibacterial activity of flavonoids is probably due to their ability to complex with extracellular and soluble proteins and to complex with bacterial cell walls (Doss *et al.*, 2009; Doss *et al.*, 2011a; Doss *et al.*, 2011b). Several reports are available in support of antimicrobial activity of saponins against bacterial and fungal pathogens (Gopish and Kannabiran, 2008).The alkaloids are known to have antimicrobial and anti-parasitic properties. Verpoorte have reported about 300 alkaloids showing such activity (Verpoorte, 1998). Similar results on antibacterial activity were reported on related species of the genus Mahonia (Duraiswamy, 2006; Livia *et al.*, 2004; Li *et al.*, 2007).

CONCLUSION

The present study provides a rationale for the use of *O. turpethum* in traditional medicine in Bangladesh. Further studies like HPLC and LC-MS can be carried out to confirm whether the observed activity of the root and leaf is due to the presence of (-)-epicatechin-3-*O*-β-glucopyranoside, the compound responsible for the antimicrobial activity of the leaves and root. The present results therefore offer a scientific basis for traditional use of the plant *O. turpethum* against infection by burns or wounds. But *in vivo* studies on the medicinal plant are necessary and should seek to determine toxicity of active

constituents, their side effects, serum-attainable levels, pharmacokinetic properties and diffusion in different body sites. The antibacterial activity could be enhanced if active components are purified and adequate dosage is determined for proper administration. It goes a long way in curbing administration of inappropriate concentration, a common practice among many traditional practitioners. This represents a preliminary report on the antibacterial activity of the medicinal plant *O. turpethum* in Bangladesh and for rational use of the traditional plant it requires further scientific study as necessary on it.

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