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ANTIBACTERIAL ACTIVITY OF DIFFERENT SOLVENT EXTRACT OF *OPERCULINA TURPETHUM* (L.) SILVA (ROOT) AGAINST IMPORTANT SPECIES OF BACTERIA

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ABSTRACT

Antibacterial activity of different solvent extracts viz., petroleum ether extract, benzene, chloroform, methanol and ethanol extracts of root of *Operculina turpethum* (L.) was tested against six bacterial species viz., *Escherichia coli, Enterobacter aerogenes, Klebsiella oxytoca, Proteus vulgaris, Bacillus cereus and Staphylococcus aureus* at 500, 1000, 1500 and 2000ppm concentration. Among the five solvent extract tested, significant activity was recorded in methanol extract (25 to 30mm inhibition) compared to synthetic antibiotic Gentamycein at 25mg concentration. Methanol extract was followed by ethanol and recorded (8mm to 11mm inhibition) moderate activity in all the test bacterial species tested.

KEYWORDS: *Operculina turpethum,* Antibacterial activity, synthetic bacteria.

INTRODUCTION

In the present time multiple drug resistance in microbial pathogens become a serious health problem to humankind worldwide (Peng *et al.,* 2006). Nowadays multiple drug resistance has developed due to the indiscriminate use of commercial antimicrobial drugs commonly used in the treatment of infectious disease. In addition to this problem, antibiotics are sometimes

associated with adverse effects on the host including hypersensitivity, immune-suppression and allergic reactions. This situation forced scientists to search for new antimicrobial substances (Joshi *et al*., 2009). Plants are rich source of wide variety of secondary metabolites viz. tannins, terpenoids, alkaloids, and flavonoids, which possess enormous antimicrobial properties (Suresh *et al*., 1992). Green plants are an important source of various chemical compounds which are used for mankind for thousands of years to cure diseases. It contains many chemical compounds such as alkaloids, flavonoids, glycosides, phenols, resins, steroids, saponins, tannins and volatile oils which were deposited in their specific parts such as flowers, fruits, bark, leaves, root and seeds etc. (Tonthubthimthong *et al*., 2001; Digambar and Sahera, 2018). Approximately 25 to 50% of current pharmaceuticals are derived from plants. Most of them were found effective against many pathogenic bacteria (Bilgrami *et al.*, 1992). Various medicinal plants have been used for years in daily life to treat disease all over the world. The use of traditional plant extracts as well as other alternative forms of medical treatments have been getting momentum since the 1990s (Cowan, 1999).

The medicinal use of plant species outnumbered (~10%) its use as food and feed (Moerman, 1996). Biologically active compounds present in the medicinal plants have always been of great interest to scientists working in this field. In recent years this interest to evaluate plants possessing antibacterial activity for various diseases is growing (Clark and Hufford, 1993; Panthi and Chaudhary, 2006). In the present study , different solvent extracts of root of *O. turpethum* belongs to family Convolvulaceae were evaluated for antibacterial activity against six bacterial species in *in vitro* condition.

MATERIALS AND METHODS

Plant Material

Healthy roots of *O. turpethum* free from diseases were collected and the roots were washed thoroughly 2-3 times. The washed root materials was then air dried on a sterile blotter paper under shade and used for further extraction.

Solvent extraction

25 grams of shade dried powder of roots of *O. turpethum* was filled in the thimble and extracted successively with petroleum ether, benzene, chloroform, methanol and ethanol in a Soxhlet extractor for 48 hours. Solvent extracts were concentrated under reduced pressure. After complete evaporation, 1 gram of each concentrated solvent extracts were dissolved in 9 ml of methanol and used for antibacterial assay (Lalitha *et al*., 2011).

Test pathogens

Six bacteria viz., *Escherichia coli, Enterobacter aerogenes, Klebsiella oxytoca, Proteus vulgaris, Bacillus cereus and Staphylococcus aureus* were collected from research center, Pooja Bhagavat Memorial Mahajana P.G. Centre, K.R.S. Road, Metagalli, Mysore. The obtained cultures were sub-cultured on nutrient agar medium and incubated at 37°C for 24 hours. After incubation, the cultures were preserved aseptically in lower temperature until further use.

Preparation of Inoculum

All the test bacterial species were inoculated into 2 ml nutrient broth and incubated at 37^{o}C for 24 hours till the growth in the broth was equivalent with Mac-Farland standard (0.5%) as recommended by WHO.

Antibacterial assay

One gram of each of the solvent extract of *O. turpethum* was dissolved in 10ml of respective solvents, which served as the mother solvent extracts. Nutrient agar medium with different concentration of each of the solvent extracts viz., 500ppm, 1000ppm, 1500ppm and 2000ppm were prepared. Nutrient agar medium in petridishes was uniformly smeared with test bacterial culture. For each treatment ten replicates were maintained. Respective solvents served as control. Standard antibiotic Gentamicin (25mg), was used to compare the efficacy of solvent extract against test organisms (Joshi *et al.*, 2009).

Statistical Analysis

The data were subjected to Tukey's HSD analysis. Data on percentages were transformed to arcsine and analysis of variance (Anova) was carried out with transformed values. The means were compared for significance using Tukey's HSD (P=0.05).

RESULT

Antibacterial activity of solvent extract: Among the five solvent extract viz., petroleum ether extract, benzene, chloroform, methanol and ethanol tested, significant activity was recorded in methanol extract tested at 500, 1000, 1500 and 2000ppm concentration. Maximum activity was observed in *E.coli* and showed 30.0mm inhibition, *E.aerogenes* recorded 29.0mm, *K.oxytoca* recorded 23.0mm, *P.vulgaris* recorded 24.0mm and *B.cereus* 29.0mm and *S. aureus* recorded 25.0mm. significant activity was also observed in 500, 1000 and 1500ppm concentration and the percent inhibition was in the range of 12.0mm to 27.0mm respectively

compared to synthetic antibiotic Gentamycein at 25mg concentration. Methanol extract was followed by ethanol and recorded moderate activity in all the test bacterial species tested. Maximum inhibition was recorded in *E.coli* followed by *E.aerogenes*, *P.vulgaris*, *K.oxytoca* and *S. aureus*. The inhibition percentage was in the range of 10mm to 15mm against all the test fungi tested (Table 1).

Table 1: Antibacterial activity of Benzene and methanol extract of *Operculina turpethum* (L.) Silva root.

Bacteria	Solvent extract								Standard Antibiotics Gentamycin 25mg
	Methanol				Ethanol				
	Concentration of the plant extract								
	500 ppm	1000 ppm	1500 ppm	2000 ppm	500 ppm	1000 ppm	1500 ppm	2000 ppm	
E. coli	23.0^{a} ±0.1	27.0^{b} ±0.1	29.0^{c} ±0.0	30.0^{d} ±0.1	4.0^{a} ±0.0	7.0^{b} ±0.0	11.0^{c} ±0.0	15.0^{d} ±0.1	30.0^{i} ±0.0
E.aerogenes	20.0^{a} ±0.0	22.0^{b} ±0.1	27.0^{c} ±0.1	29.0^{d} ±0.0	3.0^{a} ±0.0	6.0^{b} ±0.0	10.0^{c} ±0.1	13.0^{d} ±0.0	24.0^{g} ±0.0
K. oxytoca	13.0^{a} ±0.1	16.0^{b} ±0.1	18.0^{c} ±0.0	23.0^{d} ±0.1	2.0^{a} ±0.1	5.0^{b} ±0.1	7.0^{c} ±0.0	10.0^{d} ±0.1	25.0^{h} ±0.0
P.vulgaris	16.0^{a} ±0.0	20.0^{b} ±0.0	22.0^{c} ±0.1	24.0^{d} ±0.0	3.0^{a} ±0.0	5.0^{b} ±0.0	8.0^{c} ±0.0	11.0^{d} ±0.0	29.0^{h} ±0.0
B. cereus	24.0^{a} ±0.0	26.0^{b} ±0.1	27.0^{c} ±0.0	29.0^{d} ±0.1	2.0^{a} ±0.0	3.0^{b} ±0.1	5.0^{c} ±0.0	10.0^{d} ±0.1	32.0^{i} ±0.0
S. aureus	12.0^{a} ±0.1	18.0^{b} ±0.0	22.0^{c} ±0.1	25.0^{d} ±0.0	40.0^{a} ±0.1	6.0^{b} ±0.0	6.0^{c} ±0.0	8.0^{d} ±0.0	32.0^{i} ±0.0

- Values are the mean of five replicates, ±standard error.
- The means followed by the same letter (s) are not significantly different at P 0.05 when subjected to Tukey's HSD.
- Pattern of percentage inhibition increase is not uniform for all the microorganisms

DISCUSSION

There is a continuous and urgent need to discover new antimicrobial compounds with diverse chemical structures and novel mechanisms of action because there has been an alarming increase in the incidence of new and re-emerging infectious diseases. Another big concern is the development of resistance to the antibiotics in current clinical use (Rajendran and Ramakrishnan, 2009.) Infectious diseases caused by bacteria, fungi, viruses, and parasites remain a major threat to public health, despite tremendous progress in human medicine. Their impact is particularly great in developing countries because of the relative unavailability of medicines and the emergence of widespread drug resistance (Prashith et al., 2010; Okeke,

2005). Root of *O. turpethum* showed a promising result when tested with different solvent extract. Among five solvent used, methanol extract recorded a maximum inhibition of the test bacteria. Hence the root of *O. turpethum* is a potent medicinal plants for future drug isolation to manage all the pathogenic microorganisms.

CONCLUSION

From the above observation, it was concluded that, root of *O. turpethum* showed a significant and moderate activity against all six bacterial species tested in methanol and ethanol extract. A further investigation is necessary to isolate a bioactive principle and structural elucidation from the roots of *O. turpethum* by adopting standard procedure.

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