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IN VITRO EVALUATION ANTIBACTERIAL POTENTIALITY OF AQUEOUS EXTRACT OF *OPERCULINA TURPETHUM* (L.) SILVA (ROOT) AGAINST IMPORTANT SPECIES OF BACTERIA

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ABSTRACT

Aqueous extract of *Operculina turpethum* root was evaluated against six bacterial speies viz., Escherichia coli, Enterobacter aerogenes, Klebsiella oxytoca, *Proteus vulgaris*, *Bacillus cereus* and *Staphylococcus aureus* tested at 10, 20, 30, 40, 50, 60, 70, 80, 90 and 100% concentration respectively. Maximum inhibition was observed in *Staphylococcus aureus* (37.0mm at 100% concentration) followed by *Bacillus cereus*, *Proteus vulgaris* and *E.coli* and recorded 34.0mm at 100% concentration. *E.aerogenes* and *K.oxytoca* recorded 33.0 mm at 100% concentration. Significant inhibition was also observed from 10 to 90% concentration against all the test bacterial species. Compared to standard antibiotic Gentamycin at 25mg concentration, *Operculina turpethum* showed significant activity.

KEYWORDS: *Operculina turpethum*, Aqueous extract, Antibacterial activity, Gentamycin.

INTRODUCTION

Infectious disease is the number one cause of death accounting for approximately one-half of all deaths in tropical countries. Death from infectious diseases ranked 5th in 1981, has become the 3rd leading cause of death in 1992, with an increase 58%.[1] Bacterial diseases accounts for high proportion of health problems in the developing countries. To manage the bacterial diseases, many synthetic antibiotics are regularly used. Due to indiscriminate use of synthetic antibiotics, Bacteria have developed resistance to many antibiotics and as a result, immense clinical problem in the treatment of infectious diseases has been created.[2] Medicinal plants represent a rich source of antimicrobial agents. It has been estimated that only 5 to 15 percent of the 250,000 to 750,000 existing species of higher plants has been surveyed for biologically active components. During the screening, natural compounds useful as new drugs for other ailments or conditions. Traditional healing systems around the world that utilize herbal remedies are an important source of discovery of new antibiotics.[3,4] Thus it appears that plant kingdom has received little attention as a resources of potentially useful bioactive compounds.[5] Plants contain active constituents that are used in the treatment of many human diseases. Plants are rich sources of ecologically developed secondary metabolites, which are potential remedies for different ailments. Extreme interest in plants with antibacterial activity has revived as result of current problems such as resistance associated with the use of antibiotics obtained from microorganisms.[6,7] In the present study, aqueous and solvent extracts of leaf of *Tribulus terrestris* L. belongs to family Zygophyllaceae were evaluated for antibacterial activity against five different human pathogens. In the present study, aqueous extract of root of *Operculina turpethum* (L.) Silva belongs to family Convolvulaceae were evaluated for antibacterial potentiality against six bacterial species in vitro condition.

MATERIALS AND METHODS

Plant Material: Healthy roots of *O. turpethum* free from diseases were collected from Mysore. The roots were washed thoroughly 2-3 times with running tap water and once with sterile distilled water, root material was then air dried on a sterile blotter under shade and used for extraction.

Aqueous extraction: 50 grams of thoroughly washed roots of *O. turpethum* were macerated with 50 ml of sterile distilled water in a Waring blender (Waring International, New Hartford, CT, USA) for 10min. The macerate was first filtered through double-layered muslin cloth and then centrifuged at 4000 g for 30 minutes. The supernatant was filtered through Whatman No.1 filter paper and sterilized at 120^0 C for 15 minutes. The extract was preserved aseptically in a brown bottle at 5^{0}C until further use.[8]

Test pathogens: Six pathogenic bacteria namely *Escherichia coli* (Gram Negative), *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 subcultured 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

Preparation of standard culture inoculums of test organism: All the test bacterial species were inoculated into 2 ml nutrient broth and incubated at 37 ^{0} C for 24 hours till the growth in the broth was equivalent with Mac-Farland standard (0.5%) as recommended by WHO.[9]

Antibacterial assay

Agar cup diffusion method: An overnight culture of *Escherichia coli*, *Enterobacter aerogenes*, *Klebsiella oxytoca*, Proteus vulgaris, Bacillus cereus and *Staphylococcus aureus* were inoculated into petri plates containing nutrient agar medium. The culture medium was allowed to set. Thereafter, a sterile cork borer of 5.0 mm diameter was used to punch wells in the seeded nutrient agar. Five wells were made in the petriplate containing media (One in centre and Four at the border), the agar plugs were removed with a flamed and cooled wire loop. For each well 50 µl of different concentrations (10, 20, 30, 40, 50, 60, 70, 80, 90 and 100% concentration) of the aqueous extract was added. The plates were incubated at 37°C for 24 hours and the zone of inhibition was measured in millimeter. For each treatment ten replicates were maintained. The same procedure were followed for standard antibiotics Gentamicin (25mg) to compare the efficacy of aqueous extract against test organisms.[10]

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

Among the six bacterial species tested, *S. aureus* showed a maximum inhibition and recorded 37.0mm inhibition at 100% concentration, 32.0mm in 90% and 26.0mm in 70% concentration. *S. aureus* was followed by *B. cereus* and recorded 34.0mm, 32.0mm, 30.0mm and 27.0mm inhibition at100, 90, 80 and 70% concentration respectively. *B. cereus* was followed by *E.coli* and recorded 34.0, 30.0, 27.0 and 24.0mm inhibition at 100, 90, 80 and 70% concentration. *E. aerogenes* and *K. oxytoca* recorded 33.0mm and 33.0mm at 100% concentration, 30.0 and 29.0mm at 90% concentration and 28.0 and 27.0mm at 80% concentration respectively. *P. vulgaris* recorded 34.0, 33.0 and 30.0mm inhibition at 100, 90 and 80% concentration. Significant inhibition was also observed in 10 to 50% concentration against all the test bacterial species. Standard antibiotic gentamycin was compared against all the test bacterial species at 25mg concentration and recorded *E. coli* (30.0mm), *E. aerogenes* (24.0mm), *K. oxytoca* (25.0mm), *P. vulgaris* (29.0mm), *B. cereus* (32.0mm) and *S. aureus* (32.0mm).

Table 1: Antibacterial activity of aqueous extract of *Operculina turpethum* (L.) Silva (root) against important species of bacteria.

Bacteria	Inhibition(mm)										
	Concentration of the Aqueous extract										Standard Antibiotics
	10%	20%	30%	40%	50%	60%	70%	80%	90%	100%	Gentamycin 25mg
E. coli	12.0^a ±0.0	14.0^b ±0.0	16.0^c ±0.0	19.0^d ±0.0	20.0^e ±0.0	23.0^f ±0.0	24.0^g ±0.0	27.0^h ±0.0	30.0^i ±0.1	34.0^j ±0.0	30.0^i ±0.0
E.aerogenes	8.0^a ±0.1	12.0^b ±0.1	14.0^c ±0.0	16.0^d ±0.0	19.0^e ±0.0	22.0^f ±0.1	26.0^h ±0.0	28.0^i ±0.0	30.0^j ±0.1	33.0^k ±0.0	24.0^g ±0.0
K. oxytoca	12.0^a ±0.0	15.0^b ±0.0	17.0^c ±0.0	18.0^d ±0.0	20.0^e ±0.0	20.0^f ±0.1	22.0^g ±0.0	27.0^i ±0.0	29.0^j ±0.1	33.0^k ±0.0	25.0^h ±0.0
P.vulgaris	10.0^a ±0.0	12.0^b ±0.0	15.0^c ±0.1	17.0^d ±0.0	18.0^e ±0.1	22.0^f ±0.1	25.0^g ±0.1	30.0^i ±0.0	33.0^j ±0.0	34.0^k ±0.1	29.0^h ±0.0
B. cereus	12.0^a ±0.1	13.0^b ±0.0	17.0^c ±0.0	20.0^d ±0.0	22.0^e ±0.0	24.0^f ±0.0	27.0^g ±0.0	30.0^h ±0.1	32.0^i ±0.0	34.0^j ±0.1	32.0^i ±0.0
S. aureus	12.0^a ±0.1	14·0^b ±0.0	15.0^c ±0.0	17.0^d ±0.0	20.0^e ±0.0	21.0^f ±0.0	24.0^g ±0.0	26.0^h ±0.1	32.0^i ±0.0	37.0^j ±0.0	32.0^i ±0.0

- **Values are the mean of ten 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.**

DISCUSSION

Medicinal plants constitute an important natural wealth of India and man plant species possess diverse medicinal properties. These medicinal plants play a vital role in providing primary health care services to rural people. There is a urgent need to identify and discover novel

bioactive 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. Also there is a threatening concern is the development of resistance to the antibiotics in current clinical use. In recent years, drug resistance to human pathogenic bacteria has been commonly reported from all over the world. Recently scientific interests in medicinal plants has burgeoned due to the increased efficiency of plant derived drugs and raising concern about the side effects of modern medicine. Therefore, the searches for new drugs from plants continue to be a major source of commercial drugs. Plant based antimicrobials represent a vast untapped source of medicines even after their enormous therapeutic potential and effectiveness in the treatment of infectious disease. hence, further exploration of plant antimicrobials need to occur. From the observation it can concluded that, the *Operculina turpethum* (L.) Silva (root) is a potent source as antibacterial agent. Many bioactive compounds were observed during the process of isolation procedure. Thus a further work is needed to isolate the bioactive compounds and evaluating its antibacterial activity against different human and plant pathogens.

CONCLUSION

From the above observation, it was noted that root of *O. turpethum* showed a significant and moderate result against six bacterial species tested. In the present study, aqueous extract were evaluated and observed a maximum inhibition at in all the test concentration tested. A further evaluation of solvent extracts is needed against different bacterial species and standardization of protocol for isolating the bioactive compound, its characterization and structural elucidation is needed.

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