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Biopotential activity of a rare medicinal plant - *Wattakaka volubilis* leaf.

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Abstract: The use of medicinal plants for the treatment of various diseases is an old practice in most countries and it still offers an enormous potential source of new anti-infective agents. The present study was conducted to evaluate the antimicrobial activity of *Wattakaka volubilis* leaf extract. Antimicrobial activity of *Wattakaka volubilis* methanol extract is determined by disc diffusion method . The zone of inhibition of *Wattakaka volubilis* leaf extract against bacteria was maximum against *Escherichia coli* followed by *Staphylococcus aureus, Proteus vulgaris*. The least zone of inhibition was recorded against *Pseudomonas aeruginosa* . The Minimum inhibitory Concentration (MIC) ranged from 2 mg/ml to 4 mg/ml. For fungai , the zone of inhibition was maximum against *Aspergillus Niger*, *Aspergillus flavus* and *Rhyzobium* . The least zone of inhibition was recorded against *tricoderma* . The MIC was 0.5mg/ml.

Keywords: Wattakaka *volubilis*, Methanol extract, disc diffusion, Antimicrobial activity**,** Minimum Inhibitory Concentration.

Introduction:

Antimicrobial drugs have been the most effective of all medicines . Their success is reflected by their continued use and the decrease in morbidity and mortality from bacterial infections over the past 50 years. In recent years, however, the increase in the number of multi drug resistant bacteria has led to the prediction that we are reentering the pre–antibiotic era[1]. In reality, the situation will be far worse because today's bacterial strains are not only resistant to commonly available antibiotics , but more importantly may also have acquired virulence genes. As a result even commonly occurring bacteria have been transformed into invasive and non–toxin producing pathogens . This precarious setting has been exacerbated by the cessation, or at the very least downsizing of antibacterial drug discovery efforts at large pharmaceutical companies. At the same time, their activity is generally weak –orders of magnitude less than that of common antibiotics produced by bacteria and fungi. Some plant antimicrobials are produced at high levels and owing to their mechanism of action , need to the present at milimolar concentration to offer adequate production. [2]

The WHO estimates that 80% of the population living in rural areas use or depend on herbal medicine for their health needs.[3] Antibiotic resistance has become a global concern.[4] The increasing use or overuse of antibiotics in the treatment of bacterial infections is bringing on an increase in pathogenic organisms that are resistant to available antibiotics. This has forced scientist to search for new antimicrobial substances from various sources like the medicinal plants. Search for new antibacterial agents should be continued by screening many plant families. Recent work revealed the potential of several herbs as sources of drugs[5]. Numerous studies have identified compounds within herbal plants that are effective antibiotics[6]. Traditional healing systems around the world that utilize herbal remedies are an important source for the discovery of new antibiotics[7]. Some traditional remedies have already produced compounds that are effective against antibiotic resistant strains of bacteria[8].

Wattakaka volubilis (Linn.f.) Stapf belongs to the family Asclepiadaceae , and is commonly known as ' koti p-palai' in tamil . It is a rare and threatened species.[9] It is a tall woody climber of 11m , of height and 9.5 cm in girth with densely lenticulate branches , occurring throughout the hotter parts of India , ascending to an altitude of 1500m. The leaves are employed in application for boil and abscesses[10] . This plant is used in the treatment of various aliments since ancient times[11]. Roots and tender stalks are used as emetic and expoterant [12]. It is reported that an alcohol (50%) extract of the plant showed activity on the central nervous system as well as anticancer activity against sarcoma 180 in mice [13].

Materials And Methods:

Collection of plant material:

The *Wattakaka volublis* was collected from Tiruchirappalli. The plant was identified and voucher specimen was deposited in the Rapinet Herbarium, St.Joseph's college, Tiruchirappalli.

Preparation of extracts:

The plant leaves were air dried and crushed to small piece using mortar and pestle and powdered in an electric grinder. Dried and powdered plant material is extracted using soxlet apparatus with methanol and distilled water. These extracts were concentrated to dryness in flash evaporator under reduced pressure and controlled temperature(50-60°C) for 72 hrs. Methanol extracts put in air tight containers stored in refrigerator.

Test Microorganism

In this study , there are four bacterial strains and the four fungal strains , The bacterias were *Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa, and Proteus vulgaris*. And the fungal strains used were *Aspergillus niger, Aspergillus flavus, Rhizobium, Tricoderma*.

Media Preparation:

Bacterial Medium:

The growth media employed in the present study included nutrient agar and nutrient broth . Nutrient agar is composed of beef extract-3.0g, peptone-5.0g, Agar-15.0g and distilled water-1000ml. The medium was adjusted to ph 7.4 and sterilized by autoclaving at 15lbs pressure (121°C) for 15 mts.

Fungal Medium :

200gm of potato slices were boiled with distilled water . The potato infusion was used as water source of media preparation. 20g of dextrose was mixed with potato infusion and ph adjusted to 5.6. 20g of agar was added as a solidifying agent. These constituents were mixed and autoclaved . Streptomycin sulphate of 50µg/ml was added when pouring into petriplates at molten condition . The solidified plates were pored with 6mm dia cork borer.

Inoculum Preparation:

Bacterial inoculum:

Bacterial inoculums was prepared by inoculating a loopful of test organisms in 5ml nutrient broth and incubated at 37°C for 5 to 8 hrs . Till a moderate turbidity was developed . The turbidity was matched with 0.5 Mcfarland standard (WHO drug information , 1993) and the culture was diluted with sterile distilled water if necessary which corresponds to the cell density of 1.5x10(cfu/ml).

Fungal inoculums:

The *in vitro* method proposed by National committee for Clinical Laboratory Standard for testing Models (NCCLS,1998) was followed for the present study . The fungal stock inoculum suspension was prepared from two day old culture grown on PDA medium . The fungal colonies were covered with 10ml of sterile distilled water and suspensions were made by gently probing the surface with the tip of the pasteur

pipette , The resulting measure of conidia and hyphal fragments were withdrawn and transferred to a sterile tube. Heavy particles were allowed to settle for 5-20 minutes , and the homogenous suspension were collected and mixed with a spectrophotometer at a wave length of 530nm for 80-85% to obtain the standard inoculums.

Preparation of test solution and disc

The test solution was prepared with known weight of crude extracts dissolved in methanol. The sterile filter paper discs (6mm) were impregnated with 20µl of the *Wattakaka volubilis* methanol extract (corresponding to 100, 200 and 300mg/ml of *Wattakaka volubilis* methanol extract) and allowed to dry at room temperature.

Antimicrobial Susceptibility Testing

Disc diffusion method:

The antimicrobial activity of leaf extracts of *wattakaka volubilis* was evaluated by agar disc diffusion method 15. Petri dishes were plated with nutrient agar and potato dextrose agar medium were prepared according to the manufactures manual was allowed for 30 minutes to solidify . The test organisms were then spread on the surface of the media using a sterile swap stick . The different concentration of the plant extracts were (100mg/ml) was introduced on the disc (0.5cm) and then allowed to dry. Then the disc was impregnated on the agar plates and streptomycin used as reference drug for the bacteria . The extracts were tested against four fungal strains and 50µg streptomycin used as the reference drug . Finally the inoculated plates were incubated at 37°C for 24 hours (bacteria), and 28°C for 72-96 hours (mycelial fungi). The zone of inhibition was observed and measured in millimeters. Each assay in this experiment was repeated for four times.

Minimum Inhibitory Concentration (MIC)

The MIC of the *Wattakak volubilis* (leaf) in methanol extract was tested in Muller Hinton Agar for bacteria, and potato Dextrose Agar for mycelial fungi by Broth micro-dilution method. From the stock solution, 0.5ml of Muller Hinton Broth for bacteria, and potato Dextrose broth for mycelial fungi to get a concentration of 64, 32, 16, 8, 4, 2, 1, 0.5, 0.25, 0.125 and 0.6mg/ml. 50µl of standardized suspension of the test organisms were transferred onto each tube. The control tube contained only organisms devoid of *Wattakaka volubilis* (leaf) methanol extract. The culture tubes were incubated at 37°C for 24 hours (bacteria), and 72 hours (mycelial fungi). The lowest concentrations, which did not show any other growth of tested microorganisms after macroscopic evaluation was determined as MIC.

Results And Discussion:

In the modern world multiple drug resistance has developed against many microbial infections 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. Given the alarming incidence of antibiotic resistance in bacteria of medical importance , there is a constant need for new and effective therapeutic agents. Therefore, there is a need to develop alternative antimicrobial drugs for the treatment of infectious diseases from medicinal plants.[14]

In the present study the antibacterial activity of the methanol extract of *Wattakaka volubilis* (leaf) was assayed against various bacterial pathogens and the results were showed in [**Table1].** The zone of inhibition of 300mg of the methanol extract of *Wattakaka volubilis* (leaf) was maximum against Escherichia coli(19.0), followed by staphylococcus aureus (18.3) , Proteous vulgaris (17.1). the least zone of inhibition was recorded against Pseudomonas aeruginosa (11.0). In comparison, the zone of inhibition of methanol extract against of *Wattakaka volubilis* (leaf) against bacteria was more at 300mg/ml concentration when compared to other two concentrations (100mg/ml and 200mg/ml). For the positive control streptomycin, the zone of inhibition ranged from 28.3to 33.01mm. The Minimum Inhibitory Concentration was ranged from 2mg/ml to 4mg/ml.

Table-1: Antibacterial activity of methanol extract in *Wattakaka volubilis* (leaf) Zone of inhibition (mm in dm)

S.NO	Bacteria	100mg/ml	200mg/ml	300mg/ml	Control Streptomycin	MIC mg/ml
1.	Escherichia coli	18.0	18.2	19.0	33.01	4
2.	Proteus vulgaris	15.0	16.5	17.1	30.8	2
3.	Pseudomonas aeruginosa	9.5	10.8	11.0	30.8	2
4.	Staphylococcus aureus	14.5	15.7	18.3	28.3	4

Some studies concerning the effectiveness of extraction methods highlight that methanol extract yields higher antibacterial activity than n-hexane and ethyl acetate[15], Whereas other report that chloroform is better than methanol and benzene[16]. It is clear that using organic solvents provides a higher efficiency in extracting compounds for antimicrobial activities compared to water based method[17]. It was concluded that the solvent methanol able to leach out antimicrobial principle very effectively from the plant than the other solvents. In the present research, the antifungal activity of the methanol extract of *Wattakaka volubilis* (leaf) was assayed against various fungal pathogens and the results were showed in (**Table2**). The zone of inhibition of 300mg of the methanol extract of wattakaka volubilis leaf extract is maximum against *Aspergillus Niger* (20.3), *Aspergillus flavus* (23.3), and Rhyzobium (20.1). The least zone of inhibition was recorded against *tricoderma* (17.6). In comparison, the zone of inhibition of methanol extract in *Wattakaka volubilis* leaf extract against fungai was more at 300mg/ml concentration when compared to other two concentrations (100mg/ml and 200mg/ml). For the positive control streptomycin, zone of inhibition ranging from 21.0 to 23.6 mm, and the Minimum inhibitory Concentration was 0.5mg/ ml.

Table-2: Antifungal activity of methanol extract in *Wattakaka volubilis* (leaf) Zone of inhibition (mm in dm)

S.No	Fungai	100mg/ml	200mg/ml	300mg/ml	Control Streptomycin	MIC mg/ml
1.	Aspergillus niger	19.3	19.6	20.3	21.0	0.5
2.	Aspergillus flavus	19.6	21.8	23.3	23.6	0.5
3.	Tricoderma	16.0	17.1	17.6	23.0	0.5
4.	Rhizobium	16.5	20.0	20.1	22.6s	0.5

Conclusion:

The study of antimicrobial activity of methanol extract of herbal plant *Wattakaka volubilis* showed promising antibacterial activity against bacterial and fungal pathogens. The results also indicated that scientific studies carried out on medicinal plant having traditional claims of effectiveness might warrant fruitful results and this the plant could serve as useful source of new antimicrobial agent. Further study on the isolation of therapeutic substances in *Wattakaka volubilis* for the treatment on infections diseases is to be carried in future.

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