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ASSESSMENT OF *WATTAKAKA VOLUBILIS* (LINN. F.) BENTH EX. HOOK F. (ASCLEPIDACEAE) FOR ITS BIOTHERAPEUTIC POTENTIAL - A RARE AND THREATENED MEDICINAL PLANT.

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ABSTRACT

The antimicrobial activity of the crude extract of Wattakaka volubilis (Linn. f.) Benth ex. Hook f. (Asclepidaceae) was evaluated against Gram-positive, Gram-negative bacteria and fungi using the disc diffusion test and the agar dilution method and for determining the minimum inhibitory concentration (MIC). The crude methanol extract demonstrated significant antimicrobial activity against all microorganisms tested. Methanol extract has exhibited the maximum inhibitory effect against some bacteria strains like Bacillus subtilis, Bacillus thuringiensis, Salmonella Paratyphi a, Escherichia coli, Salmonella Paratyphi b and Pseudomonas aeruginosa. Fungal strains Aspergillus flavus and Pencillium notatum was showed a maximum activity in methanolic extract and moderate activity against Aspergillus niger, Cladosporium carrionii and Mucor. The MIC of W. volubilis for the Gram-positive, Gram-negative bacteria and fungi varied from7.81 µg/ml to500 µg/ml. Under test conditions the crude methanol extract of W. volubilis exhibited good antibacterial activity against Gram-positive bacteria (MIC = 7.81 µg/ml). Most of Gram-negative bacteria were inhibited with a concentration of 7.81µg/ml. The microorganisms more sensitive were Salmonella paratyphi a and Escherichia coli (MIC = 7.81 µg/ml). Mucor spp. was found to be the most susceptible fungus with minimal inhibitory concentration of 7.81µg/ml.

Key words: *Wattakaka volubilis, disc diffusion, minimum inhibitory concentration, microbial activity.*

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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 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]. Many medicines including strychnine, aspirin, vincristine and taxol are of herbal or plant origin[4]. Antibiotic resistance has become a global concern[5]. 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[6].Numerous studies have identified compounds within herbal plants that are effective antibiotics[7]. Traditional healing systems around the world that utilize herbal remedies are an important source for the discovery of new antibiotics[8]. Some traditional remedies have already produced compounds that are effective against antibiotic-resistant strains of bacteria[9].

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[10]. It is a tall woody climber of 11 m. of height and 9.5 cm. in girth with densely lenticulate branches, occurring throughout the hotter parts of India, ascending to an altitude of 1500 m. The leaves are employed in application for boils and abscesses[11]. This plant is used in the treatment of various ailments since ancient times[12]. Roots and tender stalks are used as emetic and expectorant[11]. 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[11]. A HPTLC method of quandification of Aeridin (2,7-dihydroxy-1, 3-dimethoxy-9, 10-dihydrophenanthropyran), responsible for anti-inflammatory activity [13].

MATERIALS AND METHODS

PREPARATION OF EXTRACTS

10 g of air-dried powder was added to distilled water and boiled on slow heat for 2 h. It was then filtered through 8 layers of muslin cloth and centrifuged at 5000g for 10 min and the supernatant was collected. This procedure was repeated twice. After 6 h, the supernatant collected at an interval of every 2 h, was pooled together and concentrated to make the final volume one-fourth of the original volume[14]. It was then autoclaved at 121 °C temperature and at 15 lbs pressure. This residue stored at 4°C for further use. 10 g of air-dried powder was taken in 100 ml of organic solvents (methanol, ethyl acetate and petroleum ether) in a conical flask, plugged with cotton wool and then kept on a rotary shaker at 190-220 rpm for 24 h. After 24 hours the supernatant was collected and evaporated the solvent to make the final volume one-fourth of the original volume[14] and stored at 4°C in airtight bottles.

TEST MICROORGANISMS

The bacterial and fungal strains were procured from the Department of microbiology, Bharathidasan University, Trichy for the present study. There include *Salmonella paratyphi (*Gram negative)*, Salmonella paratyphi-a* (Gram negative), *Salmonella paratyphi-b* (Gram negative)*, Bacillus*

subtilis (Gram positive), *Escherichia coli* (Gram negative), *Bacillus thuringiensis* (Gram positive) and *Pseudomonas aeruginosa* (Gram negative). The fungal strains like *Aspergillus niger, Rhizopus oryzae, Aspergillus flavus, Cladosporium carrionii, Mucor* spp., *Pencillium notatum* and *Alternaria alternata.*

PREPARATION OF INOCULUM

The gram positive and negative bacteria were pre-cultured in nutrient broth overnight in a rotator shaker at 37°C and centrifuged at 5000 rpm for 10 mins. This pellet was used suspended in double distilled water and the cell density was standardized spectrophotometrically (A_{610}nm). The fungal inoculums were prepared from mother culture grown on potato dextrose agar medium. The petridishes were flooded with 10 ml of distilled water and the conidia were scraped using sterile spatula. The spore density of each fungus was adjusted with spectrophotometer (A_{595}nm) to obtain a final concentration of approximately 10^5 spores/ml.

ANTIMICROBIAL SUSCEPTIBILITY TESTING

DISC DIFFUSION METHOD

The antimicrobial activity of different extracts of *Wattakaka volubilis* was evaluated by agar disc diffusion method[15]. Petridishes were plated with Nutrient agar and potato dextrose agar medium were prepared according to the manufacturers manual was allowed for 30 minutes to solidify. The test organisms were then spread on the surface of the media using a sterile swab stick. The different concentration of the plant extracts were (10mg/ml) was introduced on the disc (0.5 cm) and then allowed to dry. Then the disc was impregnated on the agar plates and tetracycline used as reference drug for the bacteria. The extracts were tested against seven fungal strains and 50µg nystatin used as the reference drug. The plates were then incubated at 37 ºC for 24 h. Microbial growth was determined by measuring the diameter of zone of inhibition. The control zones were subtracted from the test zones and the resulting zone diameter is shown in the graph. The zones of inhibition (mm) were measured and recorded[16]. The experiment was done three times and the mean values are presented.

MINIMUM INHIBITORY CONCENTRATION (MIC)

MIC was determined by the standard method of Van Den Berghe and Vlietinck [17]. Nutrient broth was prepared and sterilized using autoclave. One ml of the prepared broth was dispensed in to the test tubes numbered 1-8 using sterile syringe and needle. A stock solution containing 500µl/ml of the extract was prepared. Then 1 ml of the solution was dispensed into the tubes numbered 1. Subsequently, from tube 1, serial dilution was carried out and 1 ml from tube 1 was transferred up to the tube number 7 and 1 ml from the tube 7 was discarded. The tube 8 was control for sterility of the medium. An overnight culture (inoculums) of each of the test isolates was prepared in sterile nutrient broth. 10µl of the inoculums were transferred into each tube from tube 1 to tube 8. The final concentration of the plant extract in each of the test tubes numbered 1-7 after dilution 500µl, 250µl, 125µl, 62.5µl, 31.25µl, 15.62µl and 7.81µl/ml, were incubated at 37°C for 24 h and examined for growth. The last tube in which growth failed to occur was the MIC tube. The tubes with the extract and broth were inoculated with a micro-organism suspension at a density of 10^5 CFU per ml. The tubes were incubated at 37°C for 24 h and then observed for the Minimum Inhibitory Concentration (MIC).The growths of organisms were observed as turbidity determined by a spectrophotometer (Elico SL177) at 620 nm. Control tubes without the tested extracts were assayed simultaneously. MIC of each extract was taken as the lowest concentration that showed no growth.

RESULTS AND DISCUSSION

Aqueous, petroleum ether, ethyl acetate and methanol extracts of *W. volubilis* (Whole plant) were assessed for antimicrobial activity by using the disc diffusion method by measuring the diameter of growth of inhibition zones with 100mg/ml concentrations (Table 1 and 3). The results showed that among the four extracts, methanol showed significant result of antimicrobial

activity. When compared other extracts, aqueous extract showed minimum level of inhibition. *Salmonella paratyphi* and *E.coli* having maximum MIC shown in methanol extract compared with other extracts. Among the seven bacterial strains maximum zone was 18 ± 0.08, 17 ± 0.13 and 13 ± 0.05 observed in the following bacterial strains such as *Salmonella paratyphi a, Bacillus subtilis* and *B. thuringiensis* respectively. For instance, methanol treated bacterial strains expressed maximum inhibition. *Pseudomonas aeurginosa* found highly susceptible to methanol extract. The inhibition zone of methanol plant extract was similar to that of the control tetracycline (Fig 1).

In vitro antifungal studies of different solvent extracts of *W. volubilis* revealed that the methanol extract had significant activity against most of the organism, while the ethyl acetate extract possessed moderate activity (Table 2 and 4, Fig 2). Methanol extract exhibited the maximum inhibitory effect against fungal strains *Aspergillus flavus* and *Pencillium notatum* and moderate activity against *Aspergillus niger, Cladosporium carrionii and Mucor* spp. Whereas, in aqueous extract has no activity. The positive control nystatin showed highest activity against all organisms presently used. However, the methanol extract had similar activity as in positive control against *Mucor* spp. and *Penicillium notatum*. While, *Alternaria alternata* has more susceptible to all the extracts. *Mucor* spp. was found to be the less susceptible fungal strain with minimal inhibitory concentration of 7.81µg/ml for the methanol extract and moderate activity against *Aspergillus niger, Rhizopus oryzae* with minimal inhibitory concentration of 15.63µg/ml. *Cladosporium carrionii* and *Alternaria alternata* have more susceptible with MIC of 250 µg/ml. Similar results were shown in the medicinal plant *Sarcostemma secomone*[18]. Antimicrobial properties of substances are desirable tools in the control of harmful microorganisms especially in the treatment of infectious diseases and in food spoilage. The active components usually interfere with growth and metabolism of microorganisms and prevent them from contamination[19, 20].

Various workers have already shown that Gram positive bacteria are more susceptible towards plants extracts as compared to Gram negative bacteria[21]. These differences may be attributed to fact that the cell wall in Gram positive bacteria is of a single layer, whereas the Gram negative cell wall is multilayered structure[22]. Alternatively, the passage of the active compound through the Gram negative cell wall may be inhibited. It is thought that observed differences may result from the doses used in this study. In addition, microorganisms show variable sensitivity to chemical substances related to different resistance levels between strains[23]. A variety of compounds are accumulated in plants accounting for their constitutive antimicrobial activities. Antimicrobial compounds are also produce in plants in response to microbial infections[24]. Several scientific reports demonstrated a presence of antibacterial activity in many higher plants[25, 26] considering those facts and also the rich biodiversity of Indian medicinal plants. It is expressed that screening and scientific evaluation of plant extract may provide novel antibacterial compounds.

The antibacterial activity of an extract from *Hemidesmus indicus* was tested against various twelve human pathogenic bacteria using standard disc diffusion method. Moreover, the methanol and petroleum ether extracts were active against most of the tested organisms as they showed potential phytochemical constituents. The antimicrobial activities of the extracts were compared with their respective reference antibiotics as minimum inhibitory concentrations (MIC). Among the twelve bacterial species maximum inhibition zone was observed in the following bacteria such as *E. coli*, *P. mirabilis* and *S. typhimurium* respectively[27].

In the present study methanol extract of *Wattakaka volubilis* showed the most remarkable antimicrobial activity. This plant could be further subjected to isolation of the therapeutic antimicrobials for the further pharmacological evaluation and is to be screened for all the bioactive compounds in the plant and formulated

into appropriate dosage for the treatment of infectious diseases.

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