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ANTIBACTERIAL ACTIVITY OF THE MEDICINAL PLANT *WRIGHTIA TINCTORIA* (Roxb.) R.Br.

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ABSTRACT

The antibacterial activity of *Wrightia tinctoria* (Roxb.) R. Br. callus extract was studied against selected pathogenic bacterial strains, *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Bacillus subtilis* by Agar disc diffusion method. Among the three solvents and water were used , methanol extract of callus showed higher inhibition zone against *Pseudomonas aeruginosa* (27 mm), *Staphylococcus aureus* (22 mm), *Bacillus subtilis* (20 mm) and *Escherichia coli* (07 mm). The level of inhibitory activity varies widely with different bacterial species for different solvents and water extract.

KEY WORDS: Antibacterial activity, Callus extract, Pala indigo plant.

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INTRODUCTION

The medicinal plants are a source of great economic value all over the World. Nature has given us a very rich botanical wealth and large number of diverse types of plants growth in different parts of the country. Ayurveda, Unani and Siddha are systematically used nearly 1,500 plants in indigenous systems of medicine [1]. Medicinal plants represent a rich source of antimicrobial agents. Plants are used medicinally in different countries and are a source of many potent and powerful drugs. Medicinal plants contain large varieties of chemical substances which possess important therapeutic properties that can be utilized in the treatment of human diseases. Most of the synthetic antibiotic now available in the market has major setback due to the multiple resistance developed by pathogenic microorganisms[2]. Hundreds of medicinal plant species Worldwide are used in the traditional medicine as a treatment for skin diseases caused by bacteria, fungi and viruses [3]. *Wrightia tinctoria* (Roxb.) R.Br. belonging to family *Apocynaceae*, is a small deciduous tree, generally up to 1.8 m tall and often under 60 m girth, sometimes up to 7.5 m high, distributed all over India [4]. The five flavonoid compounds, Indigotin, Indirubin, Tryptanthrin, Isatin and Vutin were isolated and identified from the leaves [5]. The plant has been reported for antimicrobial in psoriasis, wound healing and hepatoprotective activity [6, 7, 8]. The leaves of this tree yield a blue dye called 'palo indigo' [9]. It is commonly known as 'Indrajav'. It has got a very important place traditional healing and also is widely recognized medicinal plants [10]. The crushed fresh leaves when filled in the cavity of delayed tooth relieve tooth ache. A decoction of the leaves and bark is taken as a stomachic and in the treatment of abdominal pain. The bark and seeds are effective against psoriasis, bilious infections, leprosy, asthma, various skin diseases and non-specific termatitis [11]. In folk medicine, the dried and powdered roots of *Wrightia tinctoria* along with *Phyllanthus amarus* and *Vitex negundo* is mixed with milk and orally administered to women for improving fertility. The aim of the study is to investigate the antibacterial activity of *Wrightia tinctoria* (Roxb.) R. Br. callus extracts against selected bacterial pathogens.

MATERIALS AND METHODS

The extensive and intensive explorations conducted in the forests of Kudimiyanmalai hills during 2013 – 2014, based on the information recorded from local people this species are being used for skin diseases. The ethnobotanical information regarding the drug-yielding plants was recorded using the standard methods. The voucher specimens were identified with the help of regional floras[12,13] and the same were deposited at Department of Botany, J J College of Arts and Science, Pudukkottai. The nodal explants of sample specimens were cultured using Murashige and Skoog medium (MS medium) for callus induction purpose.

Preparation of plant extracts

The callus was shade dried, powdered (about 50g) and successively extracted with the organic solvents such as chloroform, methanol, acetone, and water using a Soxlet apparatus for 6 h (Fig. 1 a & b). The extracts were filtered and concentrated under reduced pressure, below 40°C to dryness.

Microbial strains

The microbial culture, such as *Bacillus subtilis*, *Escherichia coli, Pseudomonas aeruginosa* and *Staphylococcus aureus* were used to study antimicrobial spectrum on selected plants. These strains were obtained from Research & Post Graduate Department of Microbiology, JJ College of Arts and Science(A), Pudukkottai. The test organisms were subculture at 37°C and maintained on nutrient agar media for further studies.

Antimicrobial assay

The antibacterial activity of different extracts of the plant sample was evaluated by using disc diffusion method [14]. Petri plates containing 20 ml of respective media were seeded with selected microbial strains. Five milliliters of nutrient broth were inoculated with a loop (6 mm) of bacteria and incubated at 35°C for 6 h. One milliliter of broth was taken at 0.6 optical density and inoculated the nutrient agar (sterile) and transferred to 180 mm x 20 mm Petri dishes. The sterile What man No.1 filter paper discs of 6 mm diameter were

impregnated with 1000 – 5000 µg of concentrated plant extract and placed on the surface of the freshly inoculated medium. Standard antibiotic discs viz. Streptomycin (30 µg/disc) obtained from Hi-Media, Mumbai were used as positive control. Methanol and water alone served as negative controls. The assessment of antibacterial activity was based on measurement of inhibition zones formed around the discs. The media were incubated for 24 h at 37°C and the diameters of the inhibition zones were recorded. Three independent trials were conducted for each concentration.

RESULTS AND DISCUSSION

This study reports the antibacterial activity of selected medicinal plant in Kudumiyanmalai of Pudukkottai region against two gram positive bacteria and two gram negative bacteria, including *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Bacillus subtilis***.** The results of the antibacterial activity of the investigated extracts are shown in Table 1. The extracts showed poor activity against *E.coli.* All the water fractions showed moderate inhibition against all the bacteria tested in this study. Generally, among the investigated extracts the methanol fractions exhibited the higher antibacterial effect followed by the acetone extracts. The methanol extract of *W. tinctoria* callus tissue has shown good antibacterial properties for all the bacterial strains tested in this work. The most pronounced activity with inhibition zones of more than 15.0 mm was shown by methanol extract (inhibition zone 27.0 mm against *Pseudomonas aeruginosa* at 500 mg/ml-1. The acetone fraction also showed significant antibacterial activity against *Staphylococcus aureus* and *Bacillus subtilis* with inhibition zones 9.5 and 12.7 mm respectively. The chloroform fraction showed inhibition against *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Bacillus subtilis* with inhibition zones 14.1, 6.0, 8.0 mm respectively at 500 mg ml-1. When the concentration of the extracts was decreased from 500 – 50 mg ml-1 slight decrease in inhibition zones were observed. The total inhibition rate for *Staphylococcus aureus* can be comparable to Gentamycin used as control. For plant pathogenic bacteria *Bacillus subtilis*, 75% total inhibition of growth was noticed at 500 µg/ml. This total inhibition was comparable to control. At concentration 500 mg ml-1 the methanol and acetone extracts of Wrightia tinctoria showed inhibition against *Pseudomonas aeruginosa* and *Staphylococcus aureus*. The methanol and acetone extracts of *Wrightia tinctoria* also showed modest inhibition against *Bacillus subtilis* at both concentrations of 500 and 50 mg ml-1 (Fig. 1 c & d). Antibacterial activity of this study a small inhibition zone of 3.0 mm against *E.coli* at 500 mg ml -1 (Fig. 1 e & f). *Wrightia tinctoria* exhibit some degree of antibacterial activity towards *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Bacillus subtilis*. Thus, it shows that some of the medicinal plants used in traditional medicine are potentially effective antibacterial agents. The result supports traditionally using flowers and leaf against various groups of bacteria and fungi. Similar results were also reported for the methanol extract of fruit of *Cucumis anguria* at 500 µg/ml [15]. Earlier reports using leaf and fruit methanol extracts (250 µg/ml) of *Wrightia tinctoria* supports the present study findings [16, 17]. The potential for developing antimicrobials from higher plants appears rewarding as it will lead to the development of a phytomedicine to act against microbes. Further research is necessary to identify the new antibacterial compounds from with in this plant. However the present studies of *in vitro* antibacterial evaluation of the selected plant forms a primary platform for further phyto chemical, pharmacological and elucidate the molecular basis studies.

Fig.1 Antibacterial activity of Callus extract of *Wrightia tinctoria*

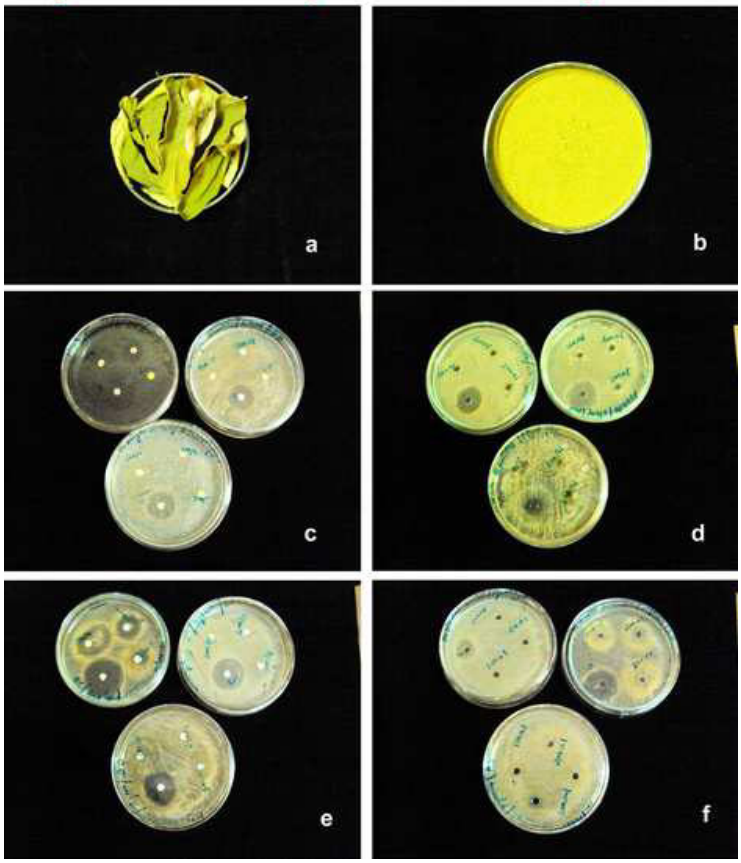

Figure 1
Antibacterial activity of callus extract of wrightia tinctoria

Table 1
Antibacterial activity of Wrightia tinctoria callus extracts

Bacterial Strains	Methanol Extract (µg/ml)					Acetone Extract (µg/ml)					Aqueous Extract (µg/ml)					Chloroform Extract (µg/ml)				
	100	200	300	400	500	100	200	300	400	500	100	200	300	400	500	100	200	300	400	500
Escherchia coli	3.2	3.0	3.7	4.2	3.6	4.0	4.8	5.8	6.7	7.1	5.2	4.6	4.	5.8	5.2	5.5	6.0	6.4	6.7	6.9
Stapylococcus aureus	19.6	19.9	18.6	18.4	19.2	9.5	9.6	9.0	9.7	9.9	10.5	11.7	11.0	10.9	11.6	6.9	6.4	5.0	6.2	6.8
Bacillus subtilis	10.9	10.5	11.8	12.1	11.4	9.9	9.5	10.8	11.1	10.4	8.2	9.4	9.1	9.6	8.8	9.8	9.6	8.2	9.0	9.5
Pseudomonas aeruginosa	27.6	26.8	27.4	28.3	28.8	13.6	12.7	13.4	12.0	11.9	18.2	17.6	16.5	19.6	18.4	14.1	14.7	14.2	13.2	13.9

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