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Research article

ANTIBACTERIAL ACTIVITY OF *TEPHROSIA HOOKERIANA* WIGHT AND ARN LEAF EXPLANTS

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ABSTRACT: The antibacterial activity of *Tephrosia hookeriana* leaf extract was tested against pathogenic bacteria like *Staphylococcus aureus*, *Aeromonas veronii*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and *Salmonella typhi*, at a dose of 500µg by using disc diffusion method. Various solvents such as methanol, acetone, petroleum ether, and aqueous were used for extracts. The results reveal that, methanol at a dose of 500µg has showed significant activity against. The methanol extract showed that maximum inhibitory activity against *Pseudomonas aeruginos (*13.67±0.33*)*. The zone of inhibition was measured and compared with standard Kanamycin (10mg). However, in none of the above mentioned extracts the inhibition zone was not more than that found in standard i.e., Kanamycin. This is the first approach in this plant and there are no early reports found.

Key words: Antibacterial activity, *Tephrosia hookeriana*, Kanamycin.

INTRODUCTION

In general, plants something that treats or prevents or alleviates the symptoms of diseases. Once bacteria enter into your body, the immune system recognizes the bacteria as foreign intruder and tries to kill or stop them from multiplying (Renu Solanki., 2010). Antibiotic usage remains one of the most important factors that promote the emergence, selection and dissemination of antibiotic resistant microorganisms in both veterinary and human medicine (Witte, 1998). Plants are important source of medicine for thousands of years, the World Health Organization (WHO) estimates up to 80 percent of people still rely mainly on traditional remedies such as herbs for their medicines. Today, ayurvedic, homeo and unani physicians utilize numerous species of medicinal plants that found their way a long time ago in to Hindu Material Media (Narayana Rao *et al*.,1987). In recent years, there has been a resurgence of interest in the discovery of new compounds from plants with aim of finding novel treatment against a variety of illness. Many medicinal plants that reported to have the potential for medicinal purpose were investigated for useful active compounds (SenthilKumar *et al*.,2013). Many medicinal plants that reported to have the potency of curing variety of illnesses. *Tephrosia hookeriana* Wight & Arn belongs to the family Fabaceae. Vernacular name is Kallu kolingi. It is a perennial shrub found throughout the Indian subcontinent. *T.hookeriana* is a common weed found in all parts of India and has been used as green manure in paddy cultivation. In the present study an attempt was made to preliminary phytochemical screening and antibacterial activities of *Tephrosia hookeriana*.

MATERIALS AND METHODS

The genus *Tephrosia hookeriana* Wight & Arn Plant material was collected from Ayyalur, Dindigul district. The species was identified by Botanical survey of India, Southern regional center at Coimbatore, Tamil Nadu, India.

Microbial strains

The clinical isolation of pathogenic bacteria cultures like *Staphylococcus aureus, Salmonella typhi, Pseudomonas aeruginosa, Klebsiella pneumoniae, Aeromonas veronii* were collected from K.A.P.Viswanathan College of Medical Sciences, Tiruchirappalli. The bacterial isolates were sub-cultured in agar slants in order to obtain pure isolation. They were inoculated into nutrient agar slants and stored at 4°C. Overnight broth culture of the respective bacterial strains were adjusted to turbidity equivalent to 0.5 McFalrand Standards(Tereschuk *et al*., 1997)(0.2ml culture of the organisms was dispensed into 20ml of sterile nutrient broth and incubated for 24h and standardized of 1.5×10^6 $CFUml^{-1}$ by adjusting the optical density (O.D) at 0.1 at 600nm perkin-Elmen UV-Spectrophotometer).

Aseptic conditions

The Laminar air flow chamber (Atlantis Ltd) was cleaned with 70% ethanol and irradiated with short wave UV light, from a lamp.

Inoculums preparation

Bacterial culture were sub-cultured in nutrient broth of 37°C for 24 h and used for the experiments.

Preparation of culture media

Composition of Nutrient Broth

Peptone - 5.0gm
Beef extract - 3.0gm
Yeast extract - 3.0 gm
Sodium chloride - 5.0gm
Distilled water - 1000ml
pH - 7.0

Preparation of Nutrient Agar Medium

Nutrient agar (NA) medium is one of the most commonly used medium for several routine bacterial purposes. It contains peptone-5.0gm; beef extract-3.0gm; yeast extract-3.0gm; sodium chloride 5.0gm; agar-18gm, distilled water 1000ml and pH-7.0. After adding all the ingredients into the distilled water, it were boiled to dissolve them in the medium completely and sterilized by autoclaving at 121^{o}C for 15 lbs pressure for 20 minutes and allowed to cool (Prabakaran *et al*., 2011).

Disc diffusion method (V**.** Berghe and A .Vlietinck., 1991)

Disc diffusion method provides a sample and reliable test in routine clinical biotechnology in order to find out the effect of a particular substance on a specific bacterium. This method consists of impregnation small circular disc of standard filter paper with given amount of culture medium previously spread with a bacterial inoculums be tested. After incubation, the degree of sensitivity in determined by measuring the inhibition zone (IZ) produced by the diffusion of the antibiotic substances from the disc into the surrounding medium, Whatmenn filter paper (no.1) disc of 6mm diameter were impregnated with 10µm of the solution of crude extract (200mg/L^{-1}) prepared using methanol solvent. The disc was evaporated at 37°C for 24h.

Antibiotic sensitivity test on microbes (Positive control)

The antibiotic sensitivity test using standard antibiotics Kanamycin (10µg) were used to gram-positive bacteria respectively as reface antibiotics.

RESULTS

The present study carried out to evaluate antibacterial activity of *T.hookeriana* using methanol, acetone, petroleum ether and aqueous as solvents. All the extracts were tested the antibacterial efficiency against pathogenic bacteria like *Staphylococcus aureus, Salmonella typhi, Pseudomonas aeruginosa, Klebsiella pneumoniae, Aeromonas veronii* at a dose of 100µg, 250µg and 500µg, Kanamycin used as standard drug for comparison inhibition of zone level. The leaf extract showed moderated to high activity against both gram positive and gram negative bacteria. The methanol extract showed higher inhibition zone at a higher dose on 500µg. Overall the methanolic extracts showed greater inhibition of all pathogenic organisms used when compared to acetone, petroleum ether aqueous extracts. The extracts of acetone at the dose level of 500µg showed the inhibition zone of *Pseudomonas aeruginosa* (9.33±0.33), *Klebsiella pneumoniae* (8.00±0.00) *Salmonella typhi* (8.00±0.00), *Staphylococcus aureus* (8.33±0.33) *Aeromonas veronii* (7.00±0.00). Whereas the extracts of petroleum ether at the dose level of 500µg showed the inhibition zone of *Pseudomonas aeruginosa (*8.66±0.33*), Klebsiella pneumoniae* (6.00±0.00) *Salmonella typhi* (5.33±0.33), *Staphylococcus aureus* (5.33±0.33) *Aeromonas veronii* (3.00±0.00). The extracts of aqueous at dose level of 20mg/ml showed the inhibition zone of *Pseudomonas aeruginosa* (2.33±0.33), *Klebsiella pneumoniae* (2.00±0.00) *Salmonella typhi* (2.00±0.00), *Staphylococcus aureus* (2.00±0.00) *Aeromonas veronii* (1.66±0.33). The extract of methanol at the dose level of 500µg showed the diameter of zone of inhibition of *Pseudomonas aeruginosa* (13.67±0.33), *Klebsiella pneumoniae* (11.26±0.33) *Salmonella typhi* (10.67±0.33), *and Staphylococcus aureus* (9.66±0.33) *Aeromonas veronii (*8.66±0.33*).* This study finally concluded that this plant extract have good efficiency against the human pathogenic bacteria's.

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Table: 1 Antibacterial activity of *in-vivo* Leaf extracts of *Tephrosia hookeriana* by agar disc diffusion method

Solvent	Conc.	Zone of inhibition (mm)				
		G^{+ve} bacteria		G^{-ve} bacteria		
		Sa	*Av*	*Kp*	*Pa*	*St*
Methanol	100µg	5.33±0.33	5.33±0.33	5.66±0.33	4.00±0.00	5.33±0.33
	250µg	7.33±0.33	6.33±0.33	7.66±0.33	5.33±0.33	7.66±0.33
	500µg	9.66±0.33	8.66±0.33	11.26±0.33	13.67±0.33	10.67±0.33
Acetone	100µg	6.33±0.33	5.66±0.33	5.66±0.33	6.33±0.33	5.33±0.33
	250µg	7.33±0.33	7.00±0.00	7.00±0.00	9.00±0.00	6.00±0.00
	500µg	8.33±0.33	7.00±0.00	8.00±0.00	9.33±0.33	8.00±0.00
Petroleum ether	100µg	2.33±0.33	1.67±0.33	3.00±0.00	4.66±0.33	2.00±0.00
	250µg	4.66±0.33	2.00±0.00	4.00±0.00	1.66±0.33	3.00±0.00
	500µg	5.33±0.33	3.00±0.00	6.00±0.00	8.66±0.33	5.33±0.33
Aqueous	100µg	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
	250µg	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
	500µg	2.00±0.00	1.66±0.33	2.00±0.00	2.33±0.33	2.00±0.00
Kanamycin	Control	10.00±0.57	8.55±0.60	10.00±0.00	12.00±0.35	10.00±0.00

Sa-Staphylococcus aureus, A- *Aeromonas veronii, Kp-Klebsiella pneumoniae* Pa-*Pseudomonas aeruginosa, St-Salmonella typhi.*

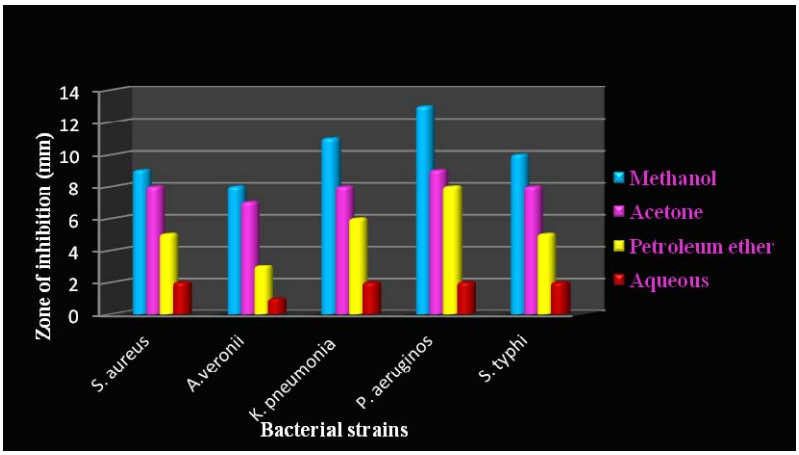
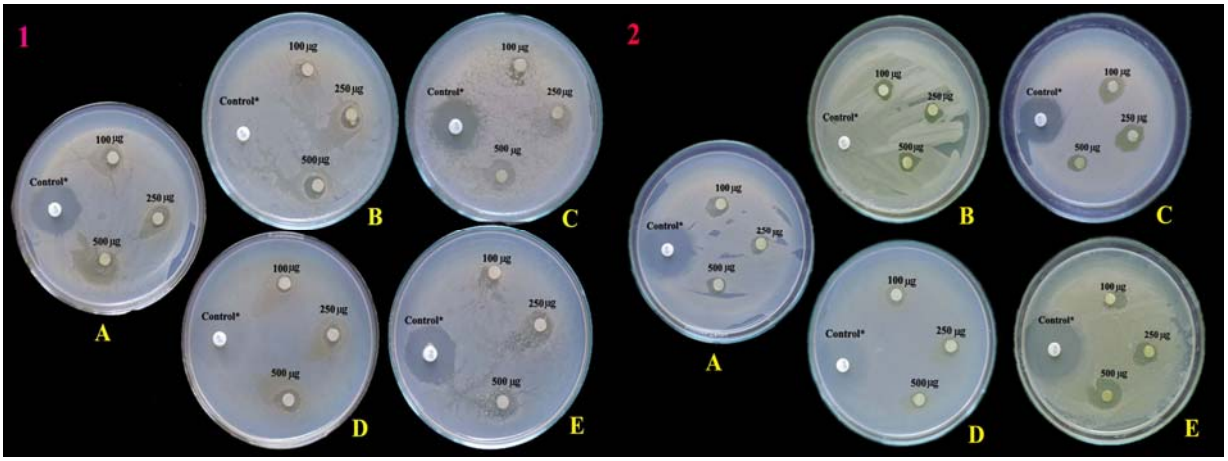
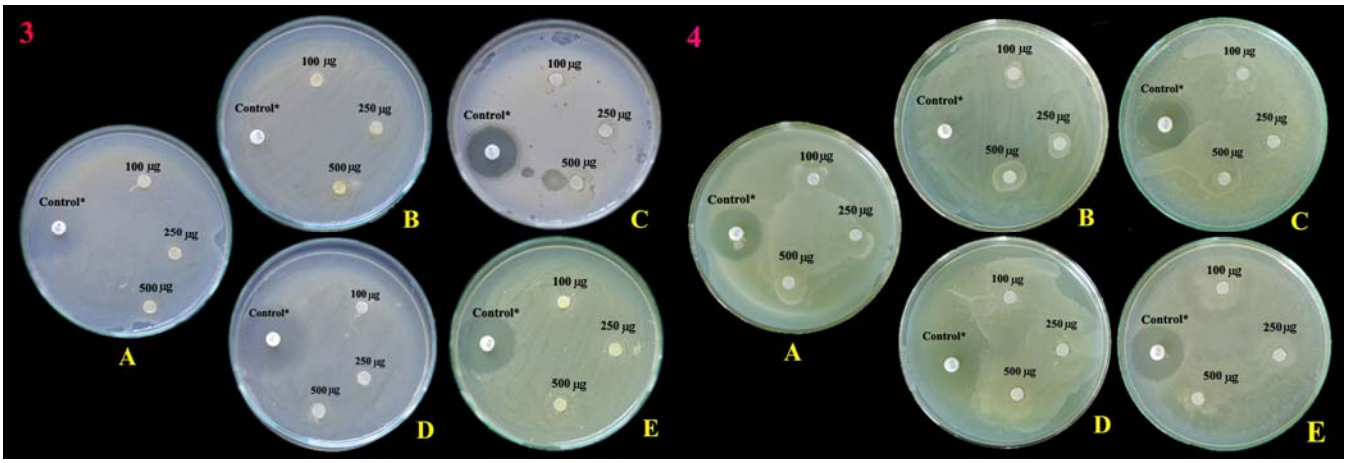


Figure 1: Antimicrobial Activity of Leaf Extract of Tephrosia hookeriana



A) *Aeromonas veronii*
B) *Klebsiella pneumoniae*
C) *Pseudomonas aeruginosa*
D) *Salmonella typhi*
E) *Staphylococcus aureus*



Fugier-2 : Plate-1.Acetne, Plate-2 Methanol, Plate-3.Petroleum ether, Plate-4. Aqueouse.

F) *Aeromonas veronii*
G) *Klebsiella pneumoniae*
H) *Pseudomonas aeruginosa*
I) *Salmonella typhi*
J) *Staphylococcus aureus*

DISCUSSION

In this present study the methanol extract, has shown high zone of inhibition against all the bacterial strains like *Staphylococcus aureus (Gram positive bacteria), Aeromonas veronii, Klebseilla pnemoniae, Pseudomonas aureginosa and Salmonella typhi (Gram negative bacteria).* Acetone extract has shown a high zone of inhibition against *Pseudomonas aureginosa* and *Klebsiella pneumonia* but moderate zone of inhibition in *Staphylococcus aureus, and Aeromonas veronii.* Petroleum ether extract as shown high zone of inhibition against *Staphylococcus aureus, Klebseilla pnemoniae* and *Salmonella typhi* but moderate zone against *Pseudomonas aeruginosa and Aeromonas veronii*. When compared the zone of inhibition with the standard drugs like kanamycin the plant extracts have shown almost equal to the standard drug. The above parameter supports the strong scientific basis for the use of these plants in traditional treatment of microbial diseases (*Kaveri et al*., 2013). The antimicrobial activity of the extracts and their potency was quantitatively assessed by the presence or absence of inhibition zone and zone diameter. Only alcoholic extract was found to be a better solvent for extraction of antimicrobial active substances compared to water and hexane (Ahmad *et al*., 1998). Agarry *et al*., (2005) compared the antimicrobial activities of the gel and leaf of *Aloe vera* against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Trichophyton mentagraphytes*, *T. schoeleinii*, *Microsporium canis* and *Candida albicans*. The antimicrobial analysis it was confirmed that this plant leaf extracts showed positive results against bacterial species such as *Klebsiella pneumoniae, Staphylococcus aureus and Escherichia coli* and fungi *Aspergillus niger* and *candida.* Hence, it can be concluded that the leaf extracts of this plant can effectively act as an antimicrobial agent which have ability to replace most of medium medicines of this era.

CONCLUSION

The present study has reported the importance of natural compounds to control antibiotic resistant bacteria, which have been a menace to human health. This study has revealed the presence of many secondary metabolites in the leaves of *T.hookeriana.* It has the further confirmed that the plant extracts could be used for the treatment of various infections including skin transmitted infections. The results provide credence to the folkloric use, if this plant in treating microbial infection and shows that *T.hookeriana* could be exploited for new potent antimicrobial agents.

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