Research Article

Phytochemical Analysis and Antibacterial Activity of *Tephrosia hookeriana* wight and Arn against human pathogens

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Accepted on: 07-11-2013; **Finalized on:** 31-01-2014.

ABSTRACT

The phytochemical analysis of *Tephrosia hookeriana* leaf, stem and root extract revealed the presence of steroids, triterpenoids, alkaloids, phenolic compounds, flavonoids, saponins and tannins. The antibacterial activity of root extracts was evaluated against some human pathogens. Methanolic *in vivo* root extracts of *T.hookeriana* showed significant antibacterial activity against *Staphylococcus aureus*, *Aeromonas veronii*, *Klebsiella pneumonia, Pseudomonas aeruginosa, and Salmonella typhi.* The antibacterial potential of methanol extract of *T. hookeriana* was tested by using disc diffusion method. The 200mg/ml root extracts showed maximum inhibition against *Pseudomonas aeruginosa* (15mm).

Keywords: Phytochemical Analysis, antibacterial activity, *Tephrosia hookeriana,* human pathogens.

INTRODUCTION

Plants are important source of medicine for thousands of years, the World Health Organization (WHO) estimates up to 80 present 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[1]. 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[2]. The phytochemical screening of plant parts like leaf, stem and root revealed the presence of numerous chemicals including alkaloids, tannins, flavonoids, steroids and saponins. 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 a 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

Plant material was collected from Ayyalur, Dindigul district, Tamil Nadu. The species was identified by Botanical survey of India, Southern regional center at Coimbatore, Tamil Nadu, India and also confirmed by herbarium, St, Josephs college, Tiruchirappalli.

Phytochemical studies

The leaf, stem and root were cut into small pieces and dried under shade condition. The plant extract were obtained by soxhlet apparatus using chloroform, methanol, ethanol and hexane.

Phytochemical Analysis

Plants produce numerous biochemical compounds such as alkaloids, flavonoids, triterpenoids, tannins, and saponins which have physiological as well as therapeutical properties. After collecting the crude extract from the dried powder of plant parts (leaf, stem and root) extract were used for preliminary phytochemical screening[4]. The screening was done to check the presence of steroids, alkaloids, phenolic compound, flavonoids, triterpenoids, tannins and Saponins.

Test for steroids

1g of the test solution was taken, minimum quantity of chloroform mixed with three drops acetic anhydride and one drop of concentration H_2So_4 was added. A change of purple to blue or green colour indicated presence of steroids.

Test for triterpenoids

A small amount of test solution, piece of tin mixed with three drops of Thionyl chloride. A change violet or purple colour formed presence of triterpenoids.

Test for alkaloids

Test solution was taken with 2N concentration HCL. Aqueous layer formed decanted to which one or few drops of Mayer's reagent was added. A white precipitated or turbidity formed presence of alkaloids.

Test for phenolic compounds

Test solution in alcohol, added to one drop of natural ferric chloride (5%) solution. An intense colour developed presence of phenolic compounds.

Test for flavonoids

Alcohol was added to test solution and small pieces of magnesium and one or two drops of concentrate HCL then heated. A red or orange red colour formed presence of flavonoids.

Test for saponins

Test solution was added to distilled water and well shaken. A foamy lather colour formed presence of Saponins.

Test for tannins

Test solution with added distilled water and mixed with lead acetate. A white precipitated developed presence of tannins.

Microbial strains

The clinical isolation of pathogenic bacterial 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 culture. 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[5] (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 jasco 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[6].

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°C for 15 lbs pressure for 20 minutes and allowed to cool[7].

Disc diffusion method[8]

Disc diffusion method provides a simple and reliable test in routine clinical microbiology in order to find out the effect of a particular substance on a specific bacterium. Muller Hinton agar plates were inoculated with test organisms by spreading the bacterial inoculums on the surface of the media. 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 is determined by measuring the inhibition zone (IZ) produced by the diffusion of the antibiotic substances from the disc into the surrounding medium, Whatmann 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 AND DISCUSSION

Phytochemical Analysis

The results of the phytochemical screening of the methanol, ethanol, chloroform and hexane, extract of the leaf, stem and root are presented in (Table 1). Preliminary phytochemical screening of different solvents revealed the presence of Steroids, Triterpenoids, Alkaloids, Phenolic compounds, Flavonoids, Saponins and Tannins.

Antimicrobial assays

The antibacterial activities of methanol, acetone and petroleum ether extracts of *T.hookeriana* were checked by disc diffusion method. The results were recorded in terms of IZ (Zone of inhibition) around disc caused by diffusion of antibacterial compounds from the plant extract impregnated disc into the surrounding medium. The antibacterial activities of methanolic extracts were more effective of other extracts. The root extracts of 150µl and 100µl invariably showed wide range of inhibition, while 50µl had minimum or negligible inhibition. Table 2 clearly shows that the methanolic *in vivo* root extracts of *T.hookeriana* produced inhibitory activities comparable to the reference drug kanamycin. Gram-negative bacteria including *P.aeruginosa* (15mm) and *S. typhi* (10mm) at 150µl indicate the existence of highly promising antibacterial constituents especially against typhoid for which this plant is used in folklore medicine. *Aeromonos veronii* (9 mm) and *Klebsiella pnuemonia* (13 mm). On the other hand, Gram-positive bacteria such as *Staphylococcus aureus* (9mm) which are also resistant to different antibiotics showed higher range of susceptibility to the extracts.

DISCUSSION

There are about 45,000 plant species in India with capacity to produce a large number or organic chemicals concentrated hotspot in the region of Eastern Himalayas of high structural diversity[9, 10]. There are report the presence of different phytochemicals and antibacterial activity with biological activity that can be valuable therapeutic index[11, 12]. The antibacterial potential of methanol extract of Azadirachta indica was tested by using ager well diffusion method[13]. In the present study we have found that the antibacterial activity on some phytochemical were present in over extract of *T. hookeriana*. It is a good responded for activity.

Table 1: Phytochemistry Analysis of leaf, stem and root of *T. hookeriana*

Test	Ethanol			Methanol			Chloroform			Hexane		
	L	S	R	L	S	R	L	S	R	L	S	R
Steroids	-	-	+	+	+	-	+	+	+	+	+	+
Triterpenoids	-	-	-	-	-	+	-	+	+	-	-	+
Alkaloids	+	+	+	+	+	+	-	-	-	+	+	+
Phenolic compounds	+	+	-	+	+	+	+	-	-	+	+	+
Flavonoids	+	+	+	+	+	+	-	-	+	+	+	+
Saponins	+	+	+	+	+	+	-	-	+	-	+	+
Tannins	+	+	+	+	+	+	-	+	-	+	+	+

+presence, -absence

Table 2: Antibacterial activity of *in-vivo* Root extracts of *Tephrosia hookeriana* by agar disc diffusion method

Solvent	Concentration	Zone of inhibition (mm)				
		Sa	Av	Kp	Pa	St
Methanol	50 µl	8 mm	7 mm	6 mm	6 mm	7 mm
	100 µl	9 mm	8 mm	8 mm	9 mm	9 mm
	150 µl	11 mm	12 mm	13 mm	15 mm	10 mm
Acetone	50 µl	6 mm	6 mm	6 mm	7 mm	7 mm
	100 µl	7 mm	7 mm	8 mm	10 mm	8 mm
	150 µl	8 mm	8 mm	9 mm	10 mm	9 mm
Petroleum ether	50 µl	5 mm	5 mm	5 mm	5 mm	6 mm
	100 µl	6 mm	6 mm	6 mm	6 mm	7 mm
	150 µl	7 mm	7 mm	8 mm	7 mm	8 mm

Sa- *Staphylococcus aureus*, Av-*Aeromonas veronii*, Kp-*Klebsiella pneumonia*, Pa-*Pseudomonas aeruginos*, St- *Salmonella typhi.*

CONCLUSION

The phytochemical analysis on *in-vivo* studies of plant part like leaves stem and roots. The screening of extracts revealed the presence of steroids, triterpenoids, alkaloids, phenolic compounds, flavonoids, Saponins and tannins. The antibacterial activity of different polar solvents on ethanol, methanol, acetone and petroleum ether extracts of root of *T.hookeriana* responded very well against the human pathogens. *The research is still in progress on bioactivity guided isolation and* structural elucidation of the bioactive compounds responsible for the observed pharmacological activities.

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Source of Support: Nil, **Conflict of Interest:** None.

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