



EVALUATION OF PRELIMINARY PHYTOCHEMICAL AND ANTIMICROBIAL ACTIVITIES ON *TEPHROSIA TOMENTOSA* PERS

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ABSTRACT

Plants are valuable source of natural products for maintaining human being health. Traditionally plants have proved reliable sources of unbelievable chemical compounds. Medicinal plants have been considered interesting since they are frequently used in different medicinal system as remedies to cure many infectious diseases. Present investigation focused on *Tephrosia tomentosa* has been used in traditional medication for the treatment of diseases and ailments of human beings. The whole plant and its roots are used for medicinal purposes. In the present studies the plant *Tephrosia tomentosa* were subjected to extract the by using different organic solvents like petroleum ether, chloroform, methanol, ethanol and aqueous extracts of leaf, stem, root and whole plant extract of *Tephrosia tomentosa* were analyzed for preliminary Phytochemical and in vitro antimicrobial property by agar disc diffusion method. In the preliminary Phytochemical test were showed positive reaction for phenols, flavonoids, steroids, tannin, glycosides and negative for alkoids, triterpenoids and saponins etc. further the crude extract was focused to analyze antibacterial and antifungal activities. The crude extract of *Tephrosia tomentosa*, the methanol and aqueous whole plant extract showed good antimicrobial activity against the *S. aureus*, *K. pneumonia* (bacteria) and *K. pneumonia*, *A. flavus* (fungal) at different concentrations 50µl, 100µl and 150µl. the obtained results shows that the hexane extract were minimum inhibition zone when compared to other extracts. Whole plant extract of methanol and aqueous has showed good antimicrobial activity against the all the microbial activities. An overall result reveals that as increase in the concentration increase the inhibition zones.

KEYWORDS: *Tephrosia tomentosa*, Phytochemical, Antibacterial and Antifungal activities.

INTRODUCTION

Plants are valuable source of natural products for maintaining human being health. Plants are used in traditionally proved reliable sources of antimicrobial compounds. Medicinal plants have been considered interesting since they are frequently used in medicinal system as remedies to cure many infectious diseases. Since from ancient times plants have provided a source of inspiration for novel drug compounds, as plant derived medicines have made large contributions to human health and well being.^[1, 2] With the rising prevalence of microorganisms developing resistance to antibiotics, there is an urgency to develop new antimicrobial compounds. Being nontoxic and easily affordable, there has been resurgence in the consumption and demand for medicinal plants.^[3,4] Though many Indian medicinal plants are used in various Indian systems of medicine like Ayurveda, Siddha, Unani and Homeopathy, Still almost over half of the Indian medicinal plants are not exploited fully for the therapeutic activity or pharmaceutical aid^[4]. *Tephrosia tomentosa* belongs to the family Fabaceae. It is a perennial herb found throughout the Indian subcontinent. The plant is 30-

50cm height. Leaves are bipinnate, the flowers are effectively used as medicines are leaves, stems and roots. According to Ayurveda used for healing all types of wounds, jaundice.^[4,5,6] *Tephrosia tomentosa* is a common weed found in all parts of India and has been used as green manure in paddy cultivation. In the present study to determine the antimicrobial activity of the different parts of *Tephrosia tomentosa* on different pathogenic bacteria and fungi by using different solvent extract.

Tephrosia is a genus of flowering plants in the pea family, Fabaceae. The generic name is derived from the Greek word (tephros), meaning "ash-colored," referring to the greyish tint given to the leaves by their dense trichomes.^[7] Hoarypea is a common name for plants in this genus.^[8] Many species in the genus are poisonous, particularly to fish, for their high concentration of rotenone. The black seeds of *Tephrosia* species have historically been used by indigenous cultures as fish toxins.^[9,10] In the last century, several *Tephrosia* species have been studied in connection with the use of rotenon as an insecticide and pesticide. The plant *Tephrosia vogelii* is also one of the many beneficial nitrogen-fixing

legumes that can be used in a permaculture forest gardening system as a source of living 'chop and drop' mulch.^[11]

MATERIALS AND METHODS

Plant material was collected from Gulbarga University, Kalaburagi, Karnataka, India. The plant was identified with the help of the Flora of Presidency of Madras^[12] and the Flora of Gulbarga district.^[13] The voucher specimens were deposited in the Botany department herbarium, Gulbarga University, Kalaburagi.

Phytochemical studies

The leaf, stem and root and whole plant were cut into small pieces and dried under shade condition. The shade dried plant materials were powdered using mixer grinder and subjected to Soxhlet extraction using petroleum ether, chloroform, methanol, ethanol and distilled water. The obtained extracts were condensed and used for preliminary phytochemical tests by adopting standard methods.^[14,15,16,17,18]

Phytochemical Analysis

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

Extraction of plant material

The plant *Tephrosia tomentosa* was washed with acetone and shade dried, coarsely powdered and extracted with petroleum ether, chloroform, ethanol (Hi-media, Mumbai) and distilled water sequentially in Soxhlet for 36h each. The extracts were evaporated to dryness in vacuo, percentage of extracts colour and odor was calculated.

Preliminary phytochemical tests

The extracts of *Tephrosia tomentosa* were dissolved in their respective organic solvents and were subjected for preliminary phytochemicals test^[14] to screen the presence of various secondary metabolites like alkaloids, flavonoids, phenols, glycosides, sterols, saponins, terpenoids, lignin and tannins.

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 H₂SO₄ 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.

Antimicrobial Activity Assay

Antimicrobial activity of organic solvent extracts was determined by cup diffusion method on nutrient agar medium⁶, for bacterial and potato dextrose agar (PDA) medium for fungi. Cups were made in nutrient agar plate using sterile cork borer (5 mm) and inoculums containing 10⁶ CFU/mL of bacteria were spread on the solid plates with a sterile swab moistened with the bacterial suspension. Then 50 µL each of all solvent extracts were placed and the cups medium inoculated plates. The treatments also included 50 µL of DMF separately which served as control. Streptomycin sulphate and Nistatin 1 µg/mL at their respective recommended dosage were treated for comparative efficacy. The plates were incubated for 24 and 38 h for bacterial and fungi, temperature was maintained 37 °C and 27 °C respectively. The zone of inhibition around the wells was measured in mm.

Microbial strains

The isolation of pathogenic bacterial cultures like *Klebsiella pneumonia*, *Escherichia coli*, *Staphylococcus aureus*, *Salmonella typhi*, *Vibrio cholera* and fungal strains like *Aspergillus flavus*, *Aspergillus niger*, *Aspergillus terreus*, *Candida albicans* were collected from Department of Microbiology, Gulbarga University, Kalaburagi. 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 McFarland Standards, (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 CFU/ml by adjusting the optical density (O.D) at 0.1 at 600nm UV-Spectrophotometer. For each treatment five replicates were maintained. The data was subjected to statistical analysis using Graphpad Instat 3.1 for windows software.

Aseptic conditions

The Laminar air flow chamber was cleaned with 70% ethanol and irradiated with short wave UV light, from a lamp.^[19]

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, disc diffusion method.^[20]

Antibiotic Sensitivity Test on Microbes (Positive control)

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

RESULTS

Phytochemical Analysis

The results of the phytochemical screening of the hexane, chloroform, methanol, ethanol and aqueous, extract of the leaf, stem, root and whole plants were presented in (Table 1). Preliminary phytochemical screening of different solvents extracts were revealed the presence of Phenolic compounds, Flavonoids, Triterpenoids, Saponins, Steroids, Alkaloids, and Saponins, Tannins, lignin were observed very less. It was observed that the whole plant extract has showed positive results hence further the whole plant extracts selected to determine the antimicrobial activities in different organic solvent extracts such as hexane, chloroform, methanol, ethanol and aqueous using successive soxhlet extracts.

ANALYSIS OF ANTIMICROBIAL IN DIFFERENT MICROORGANISMS

Antibacterial activities using different solvent extracts

The antibacterial activities of hexane, chloroform, methanol, ethanol and aqueous extracts of *T. tomentosa* were determined by disc agar well 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 whole plants extracts of 150µl and 100µl invariably showed wide range of inhibition, while 50µl had minimum or negligible inhibition (table-2). The present investigation clearly shows that the methanolic extract in vivo whole plant extracts of *T. tomentosa* produced inhibitory activities comparable to the reference standard drug streptomycin. The whole plants methanolic and aqueous extract of bacteria *S. aureus* (21.18±0.87, 20.18±0.87 mm) at 150µl respectively and in 18 mm at 50µl and 100 µl in both the extracts indicate the existence of highly promising antibacterial constituents especially against different pathogenic microbes for which this plant is used in ayurveda system of medicine. *K. pneumonia* 19.31±1.03mm, 17.93±0.65mm, 16.19±0.31 at 150 µl, 100 µl, 50 µl inhibition zone were observed and next to *S. aureus*. The minimum inhibition zone was observed in Hexane extract of *V. cholera* 13.17±0.99mm 07.12±1.23 mm, 9.13±0.38 mm, at 150 µl, 100 µl, 50 µl. it was observed that increase in the concentration of dose increase the zone of inhibition in all the tested organisms and methanol and aqueous extracts had more inhibition zone than the other extracts.

Antifungal activities using different solvent extracts

The antifungal activities of different organic solvents like hexane, chloroform, methanol, ethanol and aqueous extracts of *T. tomentosa* were determined by disc agar well diffusion method. The results were recorded in terms of IZ (Zone of inhibition) around disc caused by diffusion of antifungal compounds from the plant extract impregnated disc into the surrounding medium. The antifungal activities of methanolic extracts were more effective of other extracts. The whole plant extracts of 150µl and 100µl invariably showed wide range of inhibition, while 50µl had minimum or negligible inhibition (table-3). The present investigation clearly shows that the methanolic in vivo whole plant extracts of *T. tomentosa* produced inhibitory activities comparable to the reference standard drug Nystatin. The whole plants aqueous extract of fungi *A. flavus* (22.17±0.37, 21.01±0.92, 17.08±0.37 mm) and methanolic (21.18±1.32, 20.17±0.58, 19.03±1.23 mm) at 150µl, 100 µl, 50 µl respectively, both the extracts indicate the existence of highly promising antibacterial constituents especially against different pathogenic microbes for which this plant is used in ayurveda system of medicine. The minimum inhibition zone was observed in *A. terreus* 18.16±1.23mm, 17.02±0.77mm, 16.16±0.97mm at 150 µl, 100 µl, 50 µl of aqueous extracts and methanolic extract was 08.12±0.16 mm, 15.13±0.76 mm, 15.16±1.62 mm at 150 µl, 100 µl, 50 µl in *C. albican*. The minimum inhibition zone was observed in Hexane extract of *A. niger* 13.01±0.78 mm, 10.06±0.63 mm, 13.03±1.23 mm, at 150 µl, 100 µl, 50 µl. it was observed that increase in the concentration of dose increase the zone of inhibition

in all the tested organisms and methanol and aqueous extracts had more inhibition zone than the other extracts.

DISCUSSION

There are about 45,000 plant species in India with capacity to produce a large number of organic chemicals concentrated hotspot in the region of Eastern Himalayas of high structural diversity^[21, 22]. There are report the presence of different phytochemicals and antibacterial activity with biological activity that can be valuable therapeutic index on *Tephrosia* species.^[4,23,24,25,26,27] In the present study we have found that the antibacterial activity on some phytochemical were present in over extract of *T. tomentosa*. It is a good responded for activity and still it needs to study in detail for further future use.

CONCLUSION

The phytochemical analysis on in-vivo studies of plant part like leaves stem, roots and whole plant extracts. The screening of extracts revealed the presence of phenolic compounds, flavonoids, steroids, triterpenoids, alkaloids, Saponins and tannins. The antimicrobial activities of different polar organic solvents on , chloroform, methanol, ethanol and aqueous, extract of the leaf, stem, root and whole plants extracts of *T. tomentosa* 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.

Table 1: Preliminary phytochemicals screening tests of secondary metabolites in *Tephrosia tomentosa*

Sl. No.	Test	hexane				chloroform				methanol				ethanol				aqueous			
		L	S	R	WP	L	S	R	WP	L	S	R	WP	L	S	R	WP	L	S	R	WP
1	Alkaloids																				
	a. Mayers Test	+	+	+	-	+	-	-	+	+	+	+	+	+	+	+	+	-	-	+	+
	b. Dragendroff's test	+	-	-	+	+	-	-	+	+	-	+	+	+	+	+	+	-	-	-	+
	c. Wagners	+	+	+	-	+	-	-	+	-	+	+	+	+	+	+	+	-	-	+	+
2	Flavonoids																				
	a. Shinoda test	+	+	+	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	b. Lead acetate	+	+	+	+	-	-	-	+	+	+	+	-	+	+	+	-	+	+	-	+
	c. Pew's test	+	-	+	-	-	-	+	-	+	+	+	+	+	+	+	+	+	-	+	+
	d. NaOH test	+	+	+	+	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+
3	Phenols																				
	a. Ellagic test	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	-	-	+
	b. Phenol test	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	-	+	+
	c. Hot water test	+	+	+	+	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+
4	Glycosides Test																				
	a. Keller's test	-	+	-	+	+	-	+	+	+	+	+	-	+	-	+	+	+	+	+	+
	b. Concn. H ₂ SO ₄	+	-	+	--	+	-	+	+	-	+	+	+	-	+	+	+	+	-	+	+
	c. Molisch test	-	-	-	-	-	-	+	-	+	-	-	+	+	+	+	+	+	+	+	+
5	Steroids																				
	a. Liebermann-Burchard	+	-	+	-	+	-	+	+	-	+	+	+	+	+	+	+	+	+	+	+
	b. Salkowski Test	+	-	-	+	+	-	+	+	+	-	+	+	+	+	+	+	+	+	+	+
6	Saponins																				
	a. Foams test	-	+	+	+	-	-	+	+	+	+	+	+	-	-	+	+	+	+	+	+
7	Tripternoids																				
	a. Liebermann-Burchard	-	-	+	+	-	+	-	+	+	+	+	+	-	+	+	+	+	+	+	+
	b. Salkowski Test	+	-	-	+	+	+	-	+	-	-	+	+	-	+	+	+	+	-	+	+
8	Lignins test																				
	a. Labat test	-	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	b. Lignin test	-	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+
9	Tannins																				
	a. Gelatin Test	-	+	+	+	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+

Table 2: Antibacterial test for different pathogenic organism with different solvents extracts of *Tephrosia tomentosa*

Solvents	Concent ration	Zone of Inhibition(mm)					
		Streptomycin	K. pneumonia	E. Coli	S. aureus	S. typhi	V. cholera
Hexane	50 µl	12.12±1.32	16.03±1.34	12.17±1.16	12.15±0.93	14.05±1.94	9.13±0.38
	100 µl	15.16±1.62	16.07±1.03	14.06±0.83	13.03±0.89	15.18±0.39	07.12±1.23
	150 µl	17.16±1.62	18.16±0.94	16.18±0.17	18.16±0.12	17.12±0.98	13.17±0.99

methanol	50 µl	18.16±1.23	16.19±0.31	14.16±0.93	18.01±0.31	10.13±0.21	07.12±1.23
	100 µl	18.32±0.92	17.93±0.65	15.62±0.17	18.09±1.32	12.18±1.03	13.17±0.99
	150 µl	20.12±0.16	19.31±1.03	18.16±0.91	21.18±0.87	17.93±1.93	16.16±1.32
Ethanol	50 µl	14.32±0.92	16.14±1.07	16.93±0.17	08.16±0.19	10.93±1.93	18.16±0.88
	100 µl	12.01±0.16	16.14±1.07	17.16±0.12	10.12±0.93	15.19±0.33	11.03±0.92
	150 µl	14.17±0.58	17.16±0.96	19.12±0.16	16.16±0.13	17.93±1.60	21.16±1.20
Aqueous	50 µl	17.32±0.82	18.16±0.94	15.18±0.17	18.01±0.31	10.93±1.60	17.17±1.63
	100 µl	19.17±0.37	16.19±0.31	17.16±0.93	18.09±1.32	15.19±0.33	17.89±1.62
	150 µl	22.33±0.58	17.93±0.65	18.62±0.17	20.18±0.87	17.93±1.60	19.13±0.38

*Correlation is significant at the 0.05 level; **correlation is significant at the 0.001 level

Table 3: Antifungal test for different pathogenic organism with different solvents extracts of *Tephrosia tomentosa*

Solvents	Concentration	Zone of Inhibition(mm)				
		Nystatin	A. niger	A. terreus	C. albicans	A. flavus
Hexane	50 µl	13.17±0.98	13.01±0.78	16.19±0.33	14.12±0.32	17.06±1.62
	100 µl	15.12±1.26	10.06±0.63	17.01±0.16	16.12±0.16	19.03±1.23
	150 µl	16.12±0.16	13.03±1.23	19.16±1.62	16.12±0.16	19.03±1.23
methanol	50 µl	19.16±1.76	15.16±1.62	12.18±1.32	08.12±0.16	19.03±1.23
	100 µl	20.12±1.63	15.07±0.16	13.01±0.73	15.13±0.76	20.17±0.58
	150 µl	20.18±1.32	17.12±0.16	19.03±1.23	15.16±1.62	21.18±1.32
Ethanol	50 µl	12.19±1.32	07.17±0.37	10.33±0.58	15.13±0.76	17.17±0.58
	100 µl	15.16±1.09	10.01±0.92	11.07±0.12	15.12±1.26	19.06±0.63
	150 µl	15.13±0.76	11.17±0.58	14.32±0.92	15.33±0.58	23.13±0.76
Aqueous	50 µl	18.18±1.06	16.03±0.83	16.16±0.97	11.16±0.92	17.08±0.37
	100 µl	19.39±0.73	21.12±1.06	17.02±0.77	16.16±1.09	19.01±0.92
	150 µl	22.12±0.16	21.03±1.23	18.16±1.23	21.19±1.32	22.17±0.37

*Correlation is significant at the 0.05 level; **correlation is significant at the 0.001 level

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