International Journal of Research in Engineering and Bioscience ISSN: 2321-743X
Special Issue: National conference on "**Drug discovery, Threatened medicinal plants and strategies for sustainable use**" EDITED BY, S. DOMINIC RAJKUMAR & J. K. LAL
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IJREB

ISSN 2321-743X
International Journal of Research in
Engineering and Bioscience
Volume 1 (Special Issue 1)
Journal home page: www.ijreb.org

ANTIMICROBIAL AND PHYTOCHEMICAL SCREENING OF *TRAGIA INVOLUCRATA* L. USING UV-VIS AND FTIR

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ABSTRACT

India is endowed with a rich wealth of medicinal plants. Some of these plants are the potential source of antibiotics for various infections. The antimicrobial activity of *Tragia involucrata* L. stem and leaf extracts was determined against 7 pathogenic bacteria and fungi by disc diffusion method. The Chloroform stem extract and Ethanol leaf extract has showed higher inhibition in bacterias like *Pseudomonas aeruginosa* and *Vibrio cholerae*. Ethanol stem extract and Methanol stem extract has more effect against fungi like *Aspergillus niger* and *Rhizopus arrhizus*. There was no activity against *Penicillium chrysogenum*. Preliminary phytochemical screening was carried out for qualitative determination of plant extracts. The secondary metabolites such as Alkaloids, Flavonoids, Steroids, Lignin's, Quinines and Triterpenoides. The different peaks were obtained in the UV-VIS profile and by the FTIR, it was confirmed that the presence of Alcohol, Amides, Phosphines, Anhydrides, Carboxylic acids, Alkenes, Sulfoxides and Alkyl halides.

Keywords: *Tragia involucrata* L, Antimicrobial activity, Phytochemical analyzes, UV-Vis, FTIR.

INTRODUCTION

Medicinal plants constitute an important natural wealth of a country. India is endowed with a rich wealth of medicinal plants. Plants have been an essential part of human society since the start of civilization. Medicinal plants are a boon of nature to cure a number of ailments of human beings. Ayurveda and Unani systems of medicine regularly employ a large number of Indian medicinal plants as antibiotic agents. Our country represents a store house of genetic diversity of plants (Perumal *et al*., 2004). In India around 20,000 medicinal plants have been recorded recently, but more than 500 traditional communities use about 800 plant species for curing different diseases (Kalaivani *et al*., 2012).

Uses of antimicrobial agents against harmful pathogen are known to have been common practice at least 2000 years. In order to promote the use of medicinal plants as potential sources of antimicrobial compounds, it is important to thoroughly investigate their composition and activity and thus validate their use (Nair *et al*., 2006). Some Phytochemicals produced by plants have antimicrobial activity and used for the development of new antimicrobial drugs (Sahaya Sathish *et al*., 2012). Higher plants are much more important in the production of economically important organic compounds and pharmaceuticals. The rising incidence of multidrug resistance amongst pathogenic microbes has further necessitated the need to search for newer antibiotic sources. The use of plant extracts and Phytochemicals with known antimicrobial properties can be of great significance in therapeutic treatments (Sahaya Sathish *et al*., 2011).

In recent years attempts are being made to screen out the efficiency of better known plants which have medicinal values, especially to find out their antimicrobial properties against different pathogenic microbes. Several countries have already done analytical studies on better known medicinal plants for controlling diseases with this motive. The screening of plants extracts and natural products for antimicrobial activity has revealed the potential of higher plants as a source of the new anti-infective agents as well as serving drug discovered from natural products for primary and secondary compounds. Antimicrobial agents include all classes of secondary metabolites. These principles play an important role in the biochemical resistance against pathogenic organisms. Some of these plants are the potential source of antibiotics for various

infections. Keeping all this in mind, in the present investigation an attempt was made in the medicinal plant to study the antimicrobial activity and phytochemical constituents of *T. involucrata* L. using UV-VIS, FTIR.

MATERIAL AND METHODS

Collection and Preparation of different Plant extracts

Healthy, disease free and mature plant collected from Madurai. Madurai district, Tamil Nadu, India. Successive solvent extraction-the *T. invocrata* L. plant were taken and subjected to successive solvent extraction. The plant leaves and stem were collected, washed with distilled water and dried under shed. The dried samples were grinded to fine powder. 30 gm of powdered sample was mixed with 50 ml of solvent (Ethanol, Methanol, Chloroform and Aqueous) kept undisturbed for 3 days. Finally the solvents were filtered to collect the plant extract.

Tested Microorganisms

Tested Microorganisms were *Bacillus subtilis,* (Gram positive bacteria), *Pseudomonas aeruginosa, Salmonella typhi, Vibrio cholerae* (Gram negative bacteria) and *Penicillium chrysogenum, Aspergillum niger, Rhizopus arrhizus* (Fungus). The Microorganisms were obtained collected from Govt. Medical College, Tiruchirappalli, Tamil nadu, India.

Antibacterial activity assay

The antibacterial activity of the plant extracts were tested by the modified disc diffusion method (Baur *et al.,* 1966). The bacterial inoculums (20 h broth) were uniformly spread over the agar plates using a glass L-rod. A total of 0.2ml of each extract was aseptically added to the discs (0.5mm diameter) and allowed to dry before being placed on the top of the agar plate. The plates were incubated at 37°C for 24h and the diameter of growth inhibition zone was recorded. A standard antibiotic for Bacteria, Chloramphenicol and Fungi, Gentamicine was used as positive controls.

Phytochemical analysis

The Ethanol leaf extract was selected for Preliminary Phytochemical screening because of their better antimicrobial activity. Compounds identification by chemical tests. Tests for Steroids, Triterpenoides, Glycosides, Carbohydrate, Alkaloids, Phenolic compound, Catachins, Flavanoids, Saponins, and Tannins were carried out according to the methods of (Brindha *et al.,* 1981).

UV-VIS and FTIR Spectroscopic analysis

The extracts were examined under visible and UV light for proximate analysis For UV-VIS and FTIR spectrophotometer analysis, the extracts were centrifuged at 3000 rpm for 10 min and filtered through Whitman No. 1 filter paper by using high pressure vacuum pump. The sample is diluted to 1:10 with the same solvent. The extracts were scanned in the wavelength ranging from 300-1100 nm using Perkin Elmer Spectrophotometer and the characteristic peaks were detected. FTIR analysis was performed using Perkin Elmer Spectrophotometer system, which was used to detect the characteristic peaks and their functional groups. The peak values of the UV-VIS and FTIR were recorded. Each and every analysis was repeated twice for the spectrum confirmation.

RESULTS AND DISCUSSION

In Ethanolic leaf extract, the antimicrobial activity in *P. aeruginosa, B. subtilis, V. cholerae, A. niger, R. arrhizus* showed higher inhibition zone (1.63cm, Table-1). The inhibitory action was observed in terms of inhibition zone formed around each disc caused by the diffusion of antimicrobial substances from the paper disc into the surrounding media. Chloroform stem extract showed the higher inhibition zone (1.26cm) in *P. aeruginosa, B. subtilis, V. cholerae, and R. arrhizus* (Table-2). Hence the antimicrobial activity of various solvent extracts of leaf and stem on selected microorganisms clearly revealed that the aqueous extract was found to possess significant inhibitory property. Both aqueous extracts showed the less inhibition on pathogenic strains (0.7cm). The antimicrobial activity was maximum of Ethanolic extract while minimum activity is in Aqueous extract (Table-1 & 2). However *P. chrysogenum* are resistance to all the solvent extracts.

The qualitative UV-VIS spectrum profile of *T. invocrata* L. Ethanolic stem extract was selected at wavelength from 350 to 1100 nm due to sharpness of the Ethanolic peaks and proper baseline. The profile showed the peaks at 350 and 975nm with the absorption of 353.68 and 974.14 respectively (Fig. 1; Table- 5) and UV-VIS profile of Ethanolic leaf extract of *T. invocrata* L. was chosen at a wavelength of 400 to 1100 nm and the profile showed the peaks at 423.26, 617.64 and 676.47nm with the absorption 425.52, 616.18 and 674.77 respectively (Fig. 2; Table-5). Performing the next advanced phytochemical analysis technique of FTIR,

the presence of various functional group of different compound was found. Every solvent had its respective functional group like Alcohol, Amides, Phosphines, Carboxylic acids, Alkenes, Sulfoxides, Alkyl halides. Hence, the crude extracts subjected to UV-VIS and FTIR analysis is used for the identification of chemical constituents present in *T. invocrata* L (Fig.3; Table-4). In addition, UV-VIS and FTIR spectroscopy is proved to be a reliable and sensitive method for detection of biomolecular composition. (Komal Kumar *et al.*, 2011).

Table: 1. Antimicrobial activity of leaf extract of *T. involucrata* L.

Selected Microbes (Bacteria & Fungi)	Zone of Inhibition (cm)*				
	Ethanol extract	Methanol extract	Chloroform extract	Aqueous extract	Standard antibiotics
P. aeruginosa	1.2±0.28	1.26±0.15	0.76±0.15	_	2
B. subtilis	1.0±0.95	0.86±0.75	0.56±0.51	0.53±.0.47	2.1
S. typhi	_	_	0.93±0.32	_	2
V. cholerae	1.63±0.20	1.26±0.05	1.16±0.25	_	2
P. chrysogenum	_	_	_	_	1.9
A. niger	0.86±0.30	0.86±0.30	_	0.83±0.15	2
R. arrhizus	1.5±0.20	1.3±0.20	0.96±0.56	0.73±0.20	2.1

Note: *values are the mean of triplicate, Standard antibiotics (Bacteria Chloramphenicol, Fungi Gentamicine), ± standard deviation

Table: 2. Antimicrobial activity of stem extract of *T. involucrata* L.

Selected Microbes (Bacteria & Fungi)	Zone of Inhibition (cm)*				
	Ethanol extract	Methanol extract	Chloroform extract	Aqueous extract	Standard antibiotics
P. aeruginosa	1.06±0.45	0.95±0.15	1.3±0.26	0.8±0.43	1.9
B. subtilis	_	_	1.03±0.55	0.7±.0.35	2.1
S. typhi	0.83±0.46	1.16±0.46	0.93±0.35	_	2
V. cholerae	_	_	0.93±0.55	0.83±0.11	1.9
P. chrysogenum	_	_	_	_	1.9
A. niger	0.8±0.3	0.8±0.3	_	0.63±0.15	2
R. arrhizus	1.13±0.20	1.26±0.20	0.96±0.56	0.66±0.20	1.9

Note: *values are the mean of triplicate, Standard antibiotics (Bacteria Chloramphenicol, Fungi Gentamicine), ± standard deviation

Table: 3. Phytochemicals screenings of *T. involucrata L.* Ethanol leaf extract.

S. No	Phytochemicals	Ethanol leaf extract
1	Steroids	+
2	Triterpenoides	+
3	Glycosides	+
4	Carbohydrate	+
5	Alkaloids	+
6	Phenolic compound	-
7	Catachins	-
8	Flavanoids	+
9	Saponins	-
10	Tannins	-

Note: + represent as present; - represents as absent

Table: 4. FTIR peak values of Ethanol leaf extracts of *T. involucrata L.*

Function group	Molecular motion	Wave number (cm^{-1})
Alcohol	O-H stretch	~3696.72
Amides	N-H stretch	~3434.77
Phosphines	P-H stretch	~2355.95
Anhydrides	C=O stretch	1813.91
Carboxylic acids	C=O stretch	1613.87
Alkenes	C=C stretch (conjugated)	1613.87
Alkenes	C-H in –plane bent	1404.51
Sulfoxides	S=O stretch	1030.40
Alkyl halides	C-CI stretch	776.33
Alkynes	Acetylenic C-H bent	685.24

Table 5: UV-VIS peak values of Ethanolic stem & leaf extracts of *T. involucrata* L.

S. No	Ethanolic stem extract		Ethanolic leaf extract	
	nm	Absorption	nm	Absorption
1.	350.00	353.69	425.52	423.26
2.	506.25	505.22	617.64	616.18
3.	525.00	535.89	676.47	674.77
4.	606.25	605.86	__	__
5.	656.25	661.94	__	__
6.	975.00	974.14	__	__

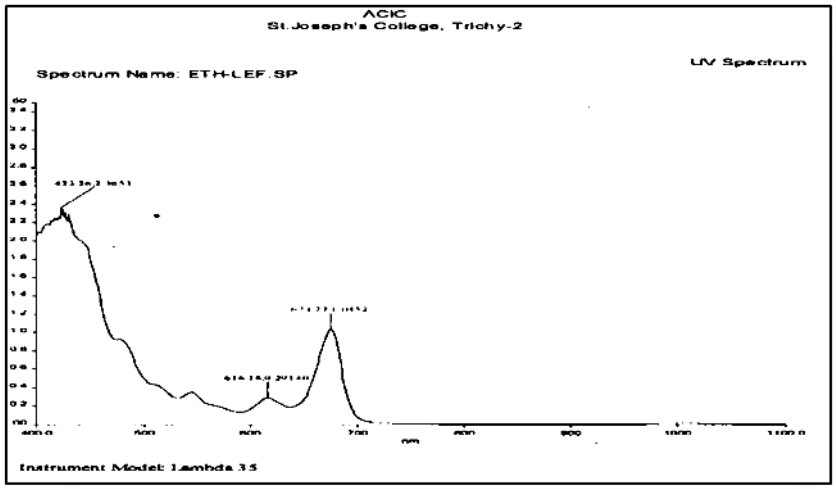

Fig. 1: UV-VIS Spectrum of Ethanolic stem extracts of *T. involucrata L*

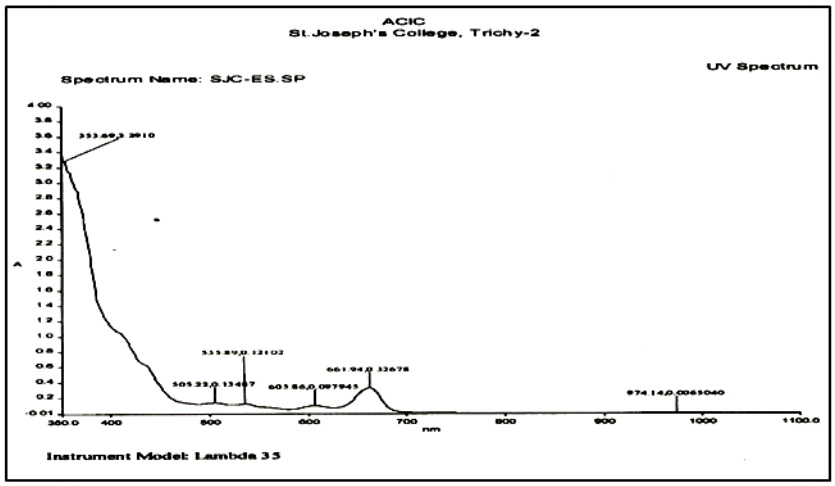

Fig. 2: UV-VIS Spectrum of Ethanolic leaf extracts of *T. involucrata L.*

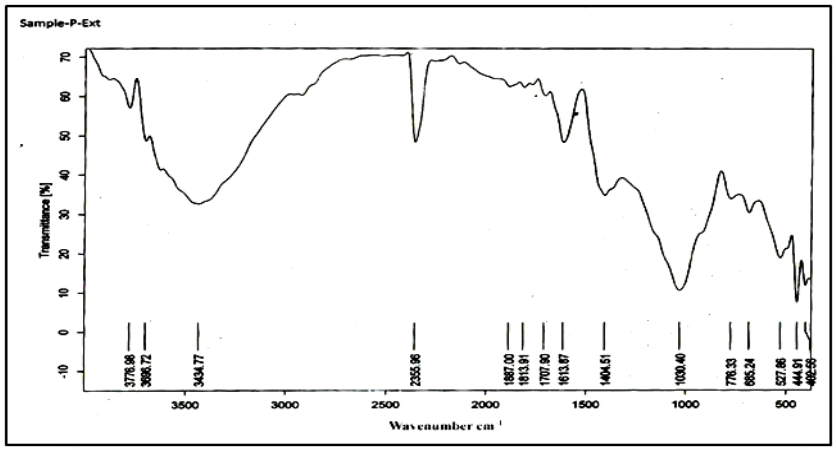

Fig. 3: FTIR Spectrum of Ethanolic leaf extracts of *T. involucrata* L.

SUMMARY AND CONCLUSION

The phytochemical analysis and antimicrobial activity was performed in the leaf and stem of *T. involucrata* L. the antimicrobial activity was studied by disc diffusion method in the 3 different solvent extract and Aqueous extract of the plant *T. involucrata* L. leaf and stem. There was antimicrobial activity observed in the all the 4 tested bacterial and 2 fungal species. The Chloroform stem extract and Ethanol leaf extract has showed higher inhibition in *P. aeruginosa* (1.3cm) and *V. cholera* (1.6cm). Ethanol stem extract and Methanol stem extract has more effect against *A. niger* (1.5cm) and *R. arrhizus* (1.26cm). There was no activity against *P. chrysogenum*. On taking leaf extract, preliminary phytochemical analysis (Brindha *et al.*, 1981) was performed which enabled to indentify the presence of different secondary metabolites and principle bioactive compounds. Further studies comprising of phytochemical investigation of the taken plant *T. involucrata* L. was done by UV-VIS and FTIR. The peaks for the UV-Vis and FTIR for Ethanolic leaf extract were obtained and further interpretation is done. In FTIR, the peaks were analyzed and presences of different group of chemical compounds were found. From the phytochemical analysis of *T. involucrata* L is to be concluded that this plant have bioactive compounds like Steroids, Triterpenoides, Glycosides, Carbohydrate, Alkaloids, Phenolic compound, Catachins, Flavanoids, Saponins, and Tannins form the characteristic nature for its medicinal uses. These compounds also possess antimicrobial activity against pathogenic microbes as well. This Indian herb serves to cure many diseases an also used for various other herbal treatment.

ACKNOWLEDGEMENT

I heartily thankful to Arch Bishop Casimier Instrumentation center, St. Joseph's College (Autonomous), Tiruchirappalli, Tamil Nadu, India for providing the instrumentation facilities for UV-Vis and FTIR analysis.

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