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RESEARCH ARTICLE

FOURIER TRANSFORM INFRARED SPECTROSCOPY ANALYSIS OF PHYTOCHEMICAL CONSTITUENTS OF VARIOUS SOLVENT EXTRACTS FROM *LANTANA ACULEATE* L. AND ITS ANTIBACTERIAL ACTIVITY

Narendhran, S. and *Rajeshwari Sivaraj

Department of Biotechnology, School of Life Sciences, Karpagam University, Eachanari Post, Coimbatore 641 021, Tamil Nadu, India

ARTICLE INFO	ABSTRACT		
<i>Article History:</i> Received 17 th April, 2015 Received in revised form 04 th May, 2015 Accepted 15 th June, 2015 Published online 31 st July, 2015	The present study is aimed to analyze the antibacterial activity and phytochemical profile of methanol, chloroform, ethanol and aqueous leaves extracts of <i>lantana aculeata</i> L. through FT-IR spectroscopy method. The antibacterial activity was evaluated according to the well diffusion method by using bacterial pathogens (<i>Staphylococcus aureus, Staphylococcus saprophiticus, Escherichia coli</i> and <i>Pseudomonas aeruginosa</i>). This study shows that methanolic leaf extract of <i>lantana aculeata</i> L. inhibit maximum growth of <i>Staphylococcus aureus</i> and <i>Pseudomonas auroginosa</i> , Minimum zone of		
<i>Key words:</i> Lantana aculeate L., Antibacterial Activity, FTIR, Phytochemicals.	inhibition was exhibited for <i>S. saprophiticus</i> . Phytochemical analysis shows the presence of carbohydrate, glycoside, flavonoids, saponin and phenolic compound is present in most of the solvent extracts and aqueous, which possesses antibacterial activity. The FT-IR analyses revealed different characterization absorption peak values with various functional groups in the extracts. The IR results indicate the presence of phenols, carboxylic acids, alkanes, ketones, alkyl halides and primary amine compounds in all the extracts of solvent.		

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INTRODUCTION

The plant Lantana aculeata Linn, family Verbenaceae is a shrub available throughout central and south India. It is now the major exotic weed, spreading rapidly in wastelands and agricultural fields. Lantana is a heavily-branched, scrambling, thicket-forming shrub, usually ranging from 2-4 m in height (Rajendiran et al., 2014). It is a serious invader of disturbed ecosystems including national parks and reserves. The weed can form a dense understory competing with native flora and limiting natural regeneration and can survive drought conditions by dropping its leaves. Dry lantana can appear to be dead but will reshoot from the base of the stem after rain. Frost affected lantana can also reshoot after spring rains. Previously, L. aculeata has been extensively investigated for the preliminary phytochemical screening. Several triterpenoids, napthaquinones, flavonoids, phenol compounds, alkaloids and glycosides were isolated from this plant and are known for diverse biological activities including cytotoxic and anticancer properties (Ghisalberti, 2000). There has been an increasing incidence of multiple resistances in human pathogenic microorganisms in recent years, largely due to commercial

Department of Biotechnology, School of Life Sciences, Karpagam University, Eachanari Post, Coimbatore 641 021, Tamil Nadu, India antimicrobial drugs commonly employed in the treatment of infectious diseases (Afolayan, 2003). The screening of plant extracts for antibacterial and anticancer activity has shown that *L. aculeata* represents a potential source of novel antibiotics. It also facilitates pharmacological studies leading to synthesis of more potent drugs (Ebana *et al.*, 1991). The present study has been conducted to screen the major phyto constituents of the plants and further analysis has been undertaken to explore the antibacterial potentials of common Verbenaceae weed.

MATERIALS AND METHODS

Collection and authentication of plant

Plants were collected from vadavalli region (11.0100° N, 76.9000° E), Coimbatore, Tamil Nadu, India. The sample was authenticated by Botanical Survey of India, Coimbatore (BSI/SRC/5/23/2014-15/Tech/1418). The leaves were collected from the plants, air dried under room temperature for 10 days and powered in a mixer grinder (Bajaj Model GX 11, Mumbai, India) and stored for further analyses.

Test Organism

Four strains of bacteria were used as test microorganisms. The bacterial strains include gram positive *Staphylococcus aureus*

^{*}Corresponding author: Rajeshwari Sivaraj

and *Staphylococcus saprophiticus*, gram negative *Escherichia coli* and *Pseudomonas aeruginosa*. The selected bacterial strains were obtained from the Department of Microbiology, Karpagam University, Coimbatore. All the bacterial strains were maintained on freshly prepared Muller and Hinton Agar (Hi-media) slant and stored at 0^{0} C.

Extraction procedure

The dried leaves (5 g) were extracted with various solvents (100 mL) such as water, methanol, chloroform and ethanol at room temperature under shaking conditions. The crude extract was filtered using a Whatman filter paper (50 mm; Sigma, Bangalore, India) and the resulting liquid was dried using heating plate at 60° C to get semisolid residue. All the four residues of *Lantana aculeata* Linn leaves with different solvents were used for further experiments. The reagents were of analytical grade, obtained from Merck (Mumbai, India).

Phytochemical analysis

One gram dried residue of methanol, ethanol, chloroform, and aqueous extracts of *lantana aculeata* Linn leaves were dissolved in 100 ml of its own mother solvent to obtain a stock of concentration 1% (v/v). The extracts thus obtained were subjected to phytochemical screening using standard procedures (Trease and Evans, 1989, Harborne, 1993).

Antibacterial activity

The antibacterial activities of various solvent extracts of *Lantana aculeata* Linn leaves were assessed by using bacterial pathogens by well diffusion method (Panda *et al.*, 2009). The bacteria were cultured in Nutrient broth and incubated at 37° C for 12 h. A 100 µl of broth bacterial culture was prepared and spread on Muller Hinton agar plates. After that, plates were allowed to stand for 10 min to allow for culture absorption. The 5 mm size wells were punched into the agar with help of sterile gel puncher. A 100 µl (25 µg/ml) of various solvent extracts and (10 µg/ml) positive control (tetracycline for bacteria) were poured into wells on all plates using micropipette. The plates were incubated in the upright position at 37 °C for 24 h (bacteria). After incubation, the size of zone of inhibition diameter in millimeter was measured and the mean values were recorded.

FTIR analysis

The functional groups of various solvent residue were analyzed by using Fourier trans-form infrared spectroscopy (Perkin-Elmer 1725x). The infrared spectra of the samples were recorded using KBr pellet technique in the region 4000–400 cm⁻¹ (Ragavendran *et al.*, 2011).

RESULT AND DISCUSSION

Phytochemical analysis of the alcoholic and aqueous extracts showed the presence of gylcoside, saponins, tannins, phenols and flavonoids which could be the active principles present in the leaves (Table 1). Maluventhan viji *et al.*, 2010 studied that ethanol, chloroform and aqueous extract of *Cardiospermum halicacabum* leaves showed the presence of flavonoids, tannins, steroids and glycosides. Antibacterial activity of *Cardiospermum halicacabum* was studied and was reported as alcoholic extract was active against *Steptococcus aureus* followed by *Salmonella typhi, E. coli* and *P. aeroginosa*. The presence of these phytochemical components may be responsible for the observed antimicrobial activity of the plant leaf extract, similar constituents were found to exhibit antiprotozoal and anticancer activities (Anyanwu and Dawet, 2005).

 Table 1. Phytochemical screening of various solvent extracts of

 Lantana aculeata
 L. Leaves

Test of extract	Ethanol	Methanol	Chloroform	Aqueous extract
Carbohydrate	-	+	+	+
Aminoacid	-	-	-	-
Glycosides	+	+	-	+
Alkaloids	-	-	-	-
Flavonoids	+	-	+	+
Saponin	-	+	+	+
Tannins	-	+	-	-
Phenolic	-	+	-	+
compound				

The Saponin is a glycoside occurring in most of plants. They are abundant in many food consumed by man. Many pharmacological activities like antifungal, antiviral and antulcer have been reported (Soetan *et al.*, 2009). Antibacterial effects of saponins seem to involve the surface tension of the extracellular medium, thus being influenced by microbial population density (Killen *et al.*, 1998).

Table 2. IR absorption frequencies of functional group in various solvent extracts of Lantana aculeata L. leaves

Frequency (cm ⁻¹)	Functional group			
Methanol	Chloroform	Ethanol	Aqueous	
611.43	611.43	613.36	605.65	C-Br stretch
678.94	678.94	680.87	675.09	
839.03	839.03	839.03	839.03	
920.05	920.05	921.97	916.19	O-H bend
1039.63 1074.35	1037.7 1076.28	1039.63 1076.28	1037.7 1068.56 1165	C-O stretch
1166.93 1257.59	1166.93 1257.59	1166.93 1257.59	1232.51 1257.59	
1458.18	1458.18	1458.18	1462.04	C-H bend
1618.28	1618.28	1618.28	1616.35	N-H bend
1708.93	1708.93	1708.93	1708.93	C=O stretch
2854.65 2924.09	2854.65 2922.16	2856.58 2924.09	2852.72 2922.16	C-H stretch
3400.5 3691.75	3402.43	3400.5 3691.75	3398.57 3662.82	O-H stretch

Flavonoids have an ability to complex with cell wall (Prashant Tiwari *et al.*, 2011) and have greater potential benefit to human health (Jouad *et al.*, 2001). Flavonoids are phenolic structures containing one carbonyl group since they are known to be synthesized by plants in response to microbial infections. They have been found to be effective *in vitro* antimicrobial substances against a wide range of micro organisms (Dixon and Dey Pand Lambc, 1983).



Fig. 1. Antibacterial activity of *Lantana aculeate* L. leaf extract using various solvents A: Methanol, B: Chloroform, C: Ethanol, D: Aqueous extract, '+' – positive control, '-'– Negative control

Imaran et al., 2010 studied the phytochemical analysis of Azadiracta indica leaves by using different solvents such as petroleum ether, chloroform and methanol which showed the presence of triterpenes, glycosides and fatty acids. Luseba et al., (2007) reported five plant species (Family: Asphodelaceae) to have good MIC values against S. aureus, E. coli and P. aeruginosa. They attributed the activity to the presence of compounds such as tannins and flavonoids. Extract of Lantana aculeata L. leaves in methanol exhibited good antibacterical and inhibited growth of all the isolates used. The aqueous extract was effective against all the bacterial cultures except Staphylococcus saprophiticus. In this study, methanol and chloroform extract recorded significant antibacterial activities against all tested bacterial strains, while the aqueous extract recorded minimum activity and less significant results were recorded in ethanol extract (Fig. 1).

The study showed that the methanol extracts of *Lantana* aculeata *L*. exhibited potential antibacterial activity against the tested pathogens, suggesting that methanol is recommended for the large scale extraction of active principles (Taous *et al.*, 2005, Nkere and Iroegbu, 2005). The methanol extracts of *Lantana aculeata* L. leaf extracts showed the maximum inhibitory effects against *Staphylococcus aureus*, *E.Coli* and *Pseudomonas auroginosa* with a measured value of zone of inhibition of 15-17 mm. The minimum inhibitor zone of 8 mm was observed in *Staphylococcus saprophiticus*. Nima and Mossa (1983) studied the methanolic extract of bulbs of *Allium cepa* and showed pronounced activity against *Bacillus subtilis*

and Pseudomonas aeruginosa, high activity against Proteus vulgaris, while being inactive against Staphylococcus aureus, Escherichia coli and Salmonella typhi. Kumar et al., 2006 studied the antibacterial activity of dichloromethane: methanol (1:1 v/v) extracts of Vitex negundo against different bacterial strains (B.subtilis, S.aureus, S.epidermidis, E.coli, and *P.aeruginosa*). They concluded that bacteria were inhibited by dichloro-methane: methanol extracts. Panda et al., 2009 studied the antibacterial activity of V. negundo on bark and leaf of petroleum ether, chloroform and aqueous extracts against B.subtilis, S.aureus, S.epidermidis, S.typhimurium, P.aeruginosa, V.cholerae, and V.alginolyteus had minimum activity.



Fig. 2. FTIR spectrum of alcoholic and aqueous leaf extract of Lantana aculeate L. A: Methanol, B: Chloroform, C: Ethanol, D: Aqueous extract

The IR result (Fig. 2) of various solvent extracts showed the spectrum range at 3300 – 3700 cm⁻¹ corresponding to phenol group (O-H stretch). The band at 1450 - 2800 cm⁻¹ indicates the presence of alkanes (C-H stretch & bend), 900 - 1300 cm⁻¹ refers to carboxylic acid (O-H bend & C-O stretch), 1708.93 cm^{-1} which indicate ketone (C=O stretch), 600 - 900 cm⁻¹ show the presence of alkyl halides (C-Br stretch). Table 2 shows that IR spectrum range of various solvent extracts of Lantana aculeata L. leaf has phenol, carboxylic acid, alkanes, ketone, alkyl halides and primary amine functional group responsible for antibacterial properties. Various solvent and aqueous crude extract of bioactive molecules have a capacity to link with protein and bacterial membrane to from complexes. These result shows that Lantana aculeata L. leaf extract have potential antibacterial activity against enteric bacteria. The FTIR analysis of methanolic and aqueous leaf extracts of Bauhinia racemosa revealed the presence of protein, oil, fats, phenolic compounds, flavonoids, saponins, tannins and carbohydrate as major functional groups (Gaurav kumar et al., 2010). Ragavendran et al., (2011) screened the functional groups of carboxylic acids, amines, amides, sulphur derivatives, polysaccharides, organic hydrocarbons, halogens that are responsible for various medicinal properties of Aerva lanata.

Conclusion

The present study concluded that the *Lantana aculeata* Linn leaf extract exhibited pronounced activity against all the tested bacteria. Methanol leaf extract of *Lantana aculeata* L. showed the potential antibacterial activity against *Staphylococcus aureus*, *Staphylococcus saprophiticus*, *Escherichia coli* and *Pseudomonas aeruginosa* when compared to chloroform, ethanol and aqueous leaf extract. The presence of phyto constituents in the leaf extracts may be responsible for the antibacterial activity.

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