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TLC profiling and assessment of antibacterial activity of essential oils from three *Lantana* spp

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Abstract: Medicinal plants are important constituents of indigenous traditional medicinal system in India. Various species of *Lantana* have been traditionally used as a medicinal herb to treat various ailments like tetanus, rheumatism, malaria, cough, fevers, cold, rheumatism and asthma. In this study focus has been made on TLC profiling and evaluation of antibacterial activity of volatile fraction of essential oils, extracted from three different *Lantana* species viz. *L. involucrata*, *L. microphylla* and *L. montevidensis*, using hydrodistillation method. The results of antibacterial activity by gaseous contact showed that out of the six bacterial strains tested against the volatile fraction of essential oil of three species of *Lantana*, maximum antibacterial activity was exhibited by *L. involucrata* oil against *S. pyogenes* with minimum inhibitory concentration of 0.26mg/l air and 0.33mg/l air respectively. *P. aeruginosa* was slightly susceptible to essential oil of *L. involucrata* and *L. microphylla*. These results drive new researches with all the three *Lantana* species in order to isolate the constituents responsible for the activity leading to the discovery of novel antibiotics.

Key Words: *Lantana*, essential oil, TLC, gaseous contact, antibacterial activity

1. Introduction

The use of medicinal plants with therapeutic purposes represents a secular tradition in different cultures. Approximately 80% of all established natural products originated from plants and have a significant role in the production of new valuable pharmaceuticals (Phillipson and Wright, 1991; Owolabi et al., 2007). There has been an increasing interest in essential oil research during recent years as an alternative to new antimicrobials due to antibiotic resistance. *Lantana camara* Linn. (Verbenaceae) is a straggling aromatic shrub native of tropical America and Africa. It is cultivated world-wide as an ornamental and decorative plant but now has been regarded as an antagonistic weed. *Lantana* is a genus of about 150 species of perennial flowering plants popularly used as antirheumatic, stimulant, antibacterial and as ornamental plant (Ghisalberti, 2000). The plant has also been shown to have fungitoxic (Saxena and Sharma, 1999), autotoxic (Arora and Kohli, 1993) and antioxidant activity (Romero and Saavedra, 2005) but is also poisonous to grazing animals (Morton, 1994). *Lantana* genus is also known worldwide for its bioactive secondary metabolites and essential oils. The chemical composition and aroma of essential oils from *Lantana* species can provide valuable psychological and therapeutic benefits. The leaf oil of *L. camara* exhibits antimicrobial activities (Saxena and Sharma, 1999). The oil is reported to possess insecticidal (Patil et al., 1997) repellent activities towards bees, mosquitoes and cattle fly (Attri, 1978). Scientists have also studied the chemical composition of essential oil extracted from leaves and flowers of Indian *Lantana* (Khan et al., 2002).

Present study has been focused with TLC profiling and evaluation of antibacterial activity of volatile fraction of essential oils, extracted from three different *Lantana* species viz. *L. involucrata*, *L. microphylla* and *L. montevidensis*, using hydrodistillation method. For studying interspecies variation of their antibacterial activity a set of three gram positive and gram negative bacteria have been employed.

II. Materials and methods

A. Plant sample

Fresh leaves of three different varieties of *Lantana* (*L. involucrata* (L1), *L. microphylla* (L2) and *L. montevidensis* (L3)) were collected from Bhopal city (M. P., India) in August, 2010. The plant materials were further identified by Prof. A.K. Pandey, Botanist, Dept. of Biological Sciences, R. D. University, Jabalpur. A voucher specimen was deposited in the Herbarium of Dept. of Biological Sciences, Jabalpur (India).

B. TLC profiling of pigments

Fresh *Lantana* leaves of each species (0.5 g) were combined with 0.5 g of anhydrous magnesium sulphate and 1.0 g of sand. The mixture was grinded using a mortar and pestle, until it becomes fine, light green powder. To this, 2 ml of acetone was thereby added and the solution was stirred using a stirring bar for 2 minutes. The

mixture was then allowed to sit for 10 minutes. The solid settled to the bottom, leaving a green liquid layer on top. The top green layer was collected and subsequently subjected to TLC.

C. TLC analysis

Silica Gel-G (Sigma) powder was used for preparation of TLC plate. The samples (3-5 μ l) were spotted with a Hamilton syringe on pre-activated TLC plate and subsequently, the plate was developed by means of solvent system consisting of: Petroleum ether: Cyclohexane: Ethyl acetate: Acetone: Methanol (6:1.6:1:1:0.4). After complete run, R_f values of different pigments were calculated.

D. Extraction of Essential Oils

About 100g leaves of each species of *Lantana* were subjected to hydrodistillation for 3-4 hours using Clevenger-type apparatus. The extracted oils were dried over anhydrous sodium sulfate (Na_2SO_4) and were refrigerated in sealed vials prior to analysis (Shibamoto, 1987).

E. TLC profiling of essential oils

To determine the presence of alcohols, esters and carbonyl compounds, the extracted oils were subjected to TLC. Different solvent systems employed were as follows: Alcohols -Benzene: Methanol (10:1); Esters-Benzene (neat); Carbonyl Compounds-n-Hexane: Ether (20:3) (Nigam *et al.*, 1965).

The separated compounds were localized by placing the TLC plate in a chamber saturated with Iodine vapors. Consequently, R_f values were calculated and the spots were identified on the basis of standard values (Nigam *et al.*, 1965).

F. Determination of Antibacterial Activity

Antimicrobial activities of different *Lantana* sp. plants extract individually as well as their combined effect were estimated (equal ratio) by adopting volatile evaluation method (Bocher, 1938). This assay measures the activity of volatile part of a sample extract in a close micro atmosphere between the agar medium and the Petri dish cover in comparison to a control culture. Minimum inhibitory Concentration (MIC) was also determined. Known aliquot of appropriate nutrient medium (NAM for bacteria) was poured into 90mm diameter Petri dish. After the gelation of medium, agar disc of 6mm (dia.) from pre-grown organism (18-24h old bacterial culture) was inoculated in the center of the dish using a sterile cutter and a glass rod. The Petri dishes were turned upside down and a sterile 6mm Whatman filter paper soaked with known concentration of plants extract was placed in the center of the cover. The Petri dishes were then sealed with paraffin tape. In control set, the extract was replaced by sterilized double distilled water or the respective solvents used for extraction purposes. After desired period of incubation, percent inhibitions of growth were calculated by using the following formula.

$$\text{Inhibition \%} = \frac{dc - dt}{dc} \times 100$$

Where:

dc: diameter of radial growth in control plates

dt: diameter of radial growth in test plates

G. Test Bacterial Strains

The following bacterial strains were used as test organisms: *B. subtilis* MTCC 1789, *Staphylococcus aureus* MTCC 87, *S. pyogenes* MTCC, *Escherichia coli* MTCC 443, *Klebsiella pneumoniae* MTCC 2405 and *Pseudomonas aeruginosa* MTCC 934. All the bacterial strains were obtained from Microbial Type Culture Collection Centre, Chandigarh, India. The microorganisms were maintained at 4°C on nutrient agar slants.

III. Results

A. Yield of essential oils

Essential oils were extracted from leaves of different species of *Lantana* using hydrodistillation. The yields of extracted volatile oil were: 1.2 % for *L. involucrata*, 1 % for *L. microphylla* and 0.5 % for *L. montevidensis*.

B. TLC analysis of pigments

The TLC plate after the separation of a number of colorful pigments from leaf extracts is depicted in Figure1 and their respective R_f values are enlisted in Table 1. The solvent system employed was- Petroleum ether: Cyclohexane: Ethyl acetate: Acetone: Methanol (6:1.6:1:1:0.4) which yielded four pigments viz. β -carotene (R_f = 0.95), chlorophyll a (R_f = 0.44), chlorophyll b (R_f = 0.32), and xanthophylls (R_f = 0.16).

C. TLC analysis of essential oils

TLC has been applied successfully to characterize essential oils for the presence of alcohols, esters and carbonyl compounds. Such classes of compounds with different polarity necessitate the use of different developing systems (Nigam *et al.*, 1965). TLC analysis of extracted essential oils of *Lantana* species reveals the presence of different chemical compounds.

D. Detection of Alcoholic components

Alcoholic compounds were separated and identified on the basis of R_f value obtained when compared with standards. Table 2 represents R_f values and their corresponding compounds identified from essential oils of *Lantana* species (Figure 2).

E. Detection of Carbonyl compounds

Different carbonyl compounds isolated from *Lantana* species along with their R_f values are illustrated in Table 3 (Figure 3).

F. Detection of Ester compounds

Major compound geranyl acetate was present uniformly in all the three species of *Lantana* and the results are shown in Table 4 (Figure 4).

G. Results of antibacterial activity

The results of antibacterial activity by gaseous contact showed that out of the six bacterial strains tested against the volatile fraction of essential oil of three species of *Lantana*, maximum antibacterial activity was exhibited by *L. involucrata* oil. The most susceptible strain was *S. pyogenes* followed by *S. aureus* with minimum inhibitory concentration of 0.26mg/l air and 0.33mg/l air respectively. *P. aeruginosa* was slightly susceptible to essential oil of *L. involucrata* (L1), *L. microphylla* (L2). Rest of the volatile fraction and their combination failed to inhibit the growth of *P. aeruginosa*.

IV. Discussion

The R_f values are in agreement with those reported in literature (Stahl, 1965). An advanced experimental method that focuses on extraction and thin layer chromatography of pigments from spinach was developed which clearly resolves chlorophyll a and b from spinach leaves while minimizing the appearance of chlorophyll degradation products (Quach *et al.*, 2004).

The essential oils of some other varieties of *Lantana* have been extracted and studied by researchers. Essential oil was extracted from *L. trifolia* with an oil yield of 0.2% (Juliao *et al.*, 2009). Similarly, hydrodistillation of leaves of *L. camara* Linn afforded pale yellow oil with yield of 0.25% (v/w) based on the dry weight of the plant (Saikia and Sahoo, 2011) whereas, *L. salvifolia* dried leaves gives an average essential oil yield of 0.38% (Ouamba, 2006).

From studies on essential oils and their constituents, R_f data has been calculated for various compounds viz., terpene alcohols, esters and ketones (Nigam *et al.*, 1965). The different polar characteristics of terpenes, alcohols, esters, aldehydes and ketones require the use of different development system. Benzene is found to be a suitable solvent for esters, while a mixture of n-Hexane and ether (20:3) was applied to resolve carbonyl compounds. Alcohols were developed with a benzene-methanol solution (10:1). Iodine vapors were used as location reagent in a non-destructive method. The bioactive properties of compounds isolated from essential oils can be evaluated for therapeutic use. The variability in foliar essential oils among different morphotypes of *Lantana* species complexes and its taxonomic and ecological significance has been also investigated (Love *et al.*, 2009).

Earlier reports show that *S. aureus* was more susceptible (MIC 0.25 mg/L air) to the volatile constituents of the essential oil of *L. montevidensis* Briq. (Sousa *et al.*, 2011). Previous reports verify the antibacterial activity of *L. camara* essential oil against *S. aureus* by direct contact method (Kurade *et al.*, 2010; Costa *et al.*, 2009; Hernandez *et al.*, 2005), but there is no previous report regarding the antibacterial activity by indirect contact. In another study, essential oil of *L. camara* showed antibacterial activity by direct contact against *Arthrobacter protophormiae*, *Micrococcus luteus*, *Rhodococcus rhodochrous* and *S. aureus* with minimal bactericidal concentrations of 50, 25, 12.5 and 200 μ g/mL, respectively (Kurade *et al.*, 2010).

Various species of *Lantana* has been used worldwide to treat a wide variety of disorders and are known for their potential therapeutic values. Due to bacterial resistance to various commercially available antibiotics, therapeutic action of plant essential oils has been widely explored. Investigation of antimicrobial spectrum of bioactive compounds from these three *Lantana* species has to be done more deeply to confirm the correlation between the chemical content of the essential oils and their antibacterial activities. These results are interesting as commercially available antibiotics are poorly active or inactive towards Gram-negative bacteria and in the present research work majority of test bacteria are Gram-negative. Present study indicates the presence of alcoholic, carbonyl and ester compounds in essential oil of *Lantana* species. Thus the remedial action of these compounds could be further investigated to unfold its antibacterial property.

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Table 1: R_f values of different fractions from leaf extracts of *Lantana* species (Bottom to top)

S. No.	R _f values (obtained)			R _f values (Standard)	Pigments
	<i>L. involucrata</i>	<i>L. microphylla</i>	<i>L. montevidensis</i>		
1.	-	-	-	0.16	Xanthophylls
2.	0.36	0.36	0.34	0.32	Chlorophyll-b
3.	-	0.45	-	0.44	Chlorophyll-a
4.	-	-	-	0.95	β- carotenes
5.	0.64	0.58	0.66	0.61	Pheophytin-a
6.	0.52	-	0.51	0.49	Pheophytin-b

Table 2: Alcoholic Compounds isolated from essential oil

S. No.	Plant Name	R _f value (Obtained)	R _f value (Standard)	Compound Name
1.	<i>L. involucrata</i>	0.87	0.86	Citronellol
		0.71	0.72	Geraniol
		0.32	0.31	Limonenediol
2.	<i>L. microphylla</i>	0.97	0.98	Isopiperitenol
		0.91	0.95	Trans-Carveol
		0.83	0.82	Nerol
3.	<i>L. montevidensis</i>	0.97	0.98	Isopiperitenol
		0.87	0.86	Citronellol
		0.82	0.82	Nerol

Table 3: Carbonyl Compound isolated from essential oil

S. No.	Plant Name	R _f value (Obtained)	R _f value (Standard)	Compound Name
1.	<i>L. involucrata</i>	0.87	0.92	γ-Ionone
		0.77	0.77	Piperitone
2.	<i>L. microphylla</i>	0.78	0.78	β-Ionone
3.	<i>L. montevidensis</i>	0.87	0.82	Citral
		0.83	0.86	Citronellol

Table 4: Esters isolated from essential oil

S. No.	Plant Name	R _f value (Obtained)	R _f value (Standard)	Compound Name
1.	<i>L. involucrata</i>	0.89	1.00	Geranyl acetate
2.	<i>L. microphylla</i>	0.90	1.00	Geranyl acetate
3.				
4.	<i>L. montevidensis</i>	0.90	1.00	Geranyl acetate

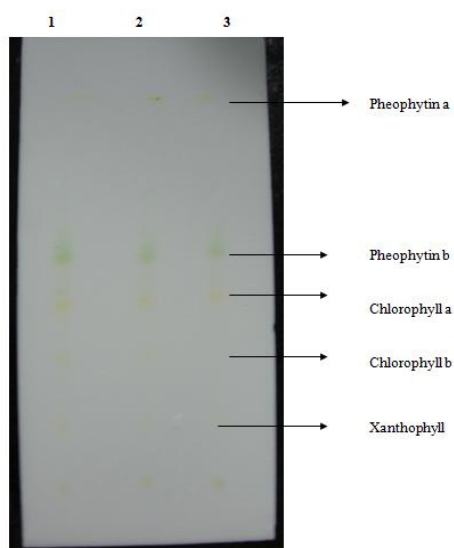


Figure 1

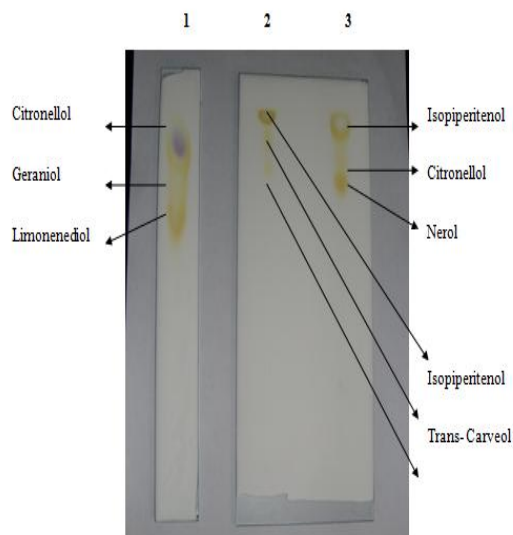


Figure 2

1: *L. involucrata*; 2: *L. microphylla*; 3: *L. montevidensis*

Figure 1: TLC of leaf pigments.

Figure 2: TLC of Essential Oils for Detection of Alcohol
Developing Solvent: Benzene : Methanol (10:1)

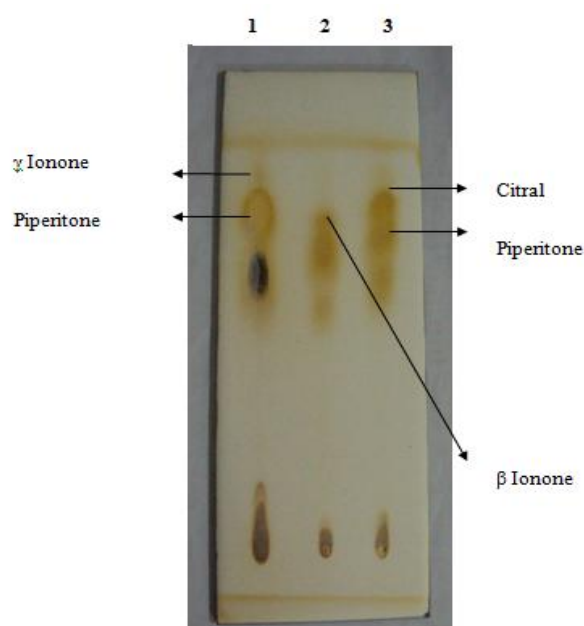


Figure 3

1: *L. involucrata*; 2: *L. microphylla*; 3: *L. montevidensis*

Figure 3: TLC of Essential Oils for Detection of Carbonyl compounds
Developing Solvent: n-Hexane: Ether (20:3)

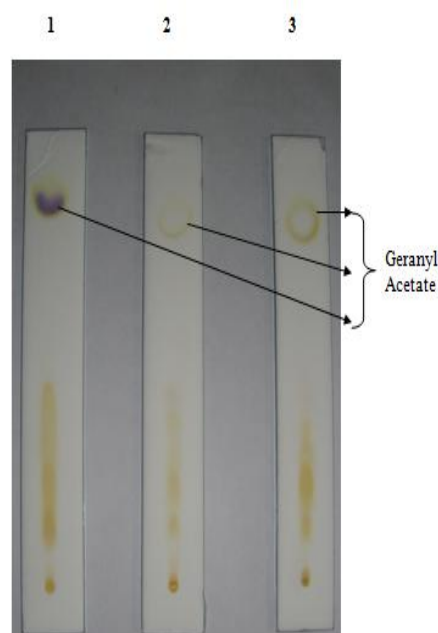
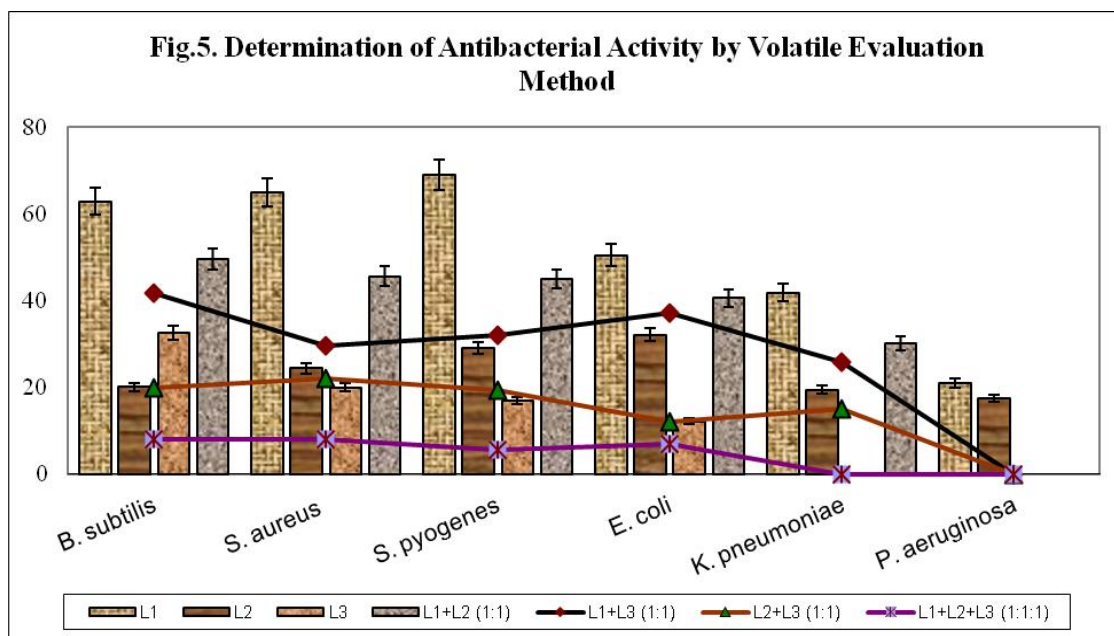


Figure 4

Figure 4: TLC of Essential Oils for Detection of Esters
Developing Solvent: Benzene (neat)



- Data are multiple of the replicate
- Values are mean $\pm$ SEM (Standard Error of Mean)