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, rheumatisms 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 µl) 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 (\( \text{Na}_2\text{SO}_4 \)) 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 Figure 1 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. \( \beta \)-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 Rf 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 Rf 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. trifolium 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. salviifolia dried leaves gives an average essential oil yield of 0.38% (Ouamba, 2006).

From studies on essential oils and their constituents, Rf 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 μg/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.

Acknowledgements

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References


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**Table 1:** *R* <sub>f</sub> values of different fractions from leaf extracts of *Lantana* species (Bottom to top)

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Plant Name</th>
<th><em>R</em> &lt;sub&gt;f&lt;/sub&gt; value (OBTAINED)</th>
<th><em>R</em> &lt;sub&gt;f&lt;/sub&gt; value (STANDARD)</th>
<th>Pigments</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td><em>L. involucrata</em></td>
<td>0.36</td>
<td>0.34</td>
<td>Chlorophyll-b</td>
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<tr>
<td>2.</td>
<td><em>L. microphylla</em></td>
<td>0.45</td>
<td>0.44</td>
<td>Chlorophyll-a</td>
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<tr>
<td>3.</td>
<td><em>L. montevidensis</em></td>
<td>0.58</td>
<td>0.66</td>
<td>β-carotenes</td>
</tr>
<tr>
<td>4.</td>
<td><em>L. salvifolia</em></td>
<td>0.64</td>
<td>0.71</td>
<td>Pheophytin-a</td>
</tr>
<tr>
<td>5.</td>
<td><em>L. aculeata</em></td>
<td>0.52</td>
<td>0.51</td>
<td>Pheophytin-b</td>
</tr>
</tbody>
</table>

**Table 2:** Alcoholic Compounds isolated from essential oil

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Plant Name</th>
<th><em>R</em> &lt;sub&gt;f&lt;/sub&gt; value (OBTAINED)</th>
<th><em>R</em> &lt;sub&gt;f&lt;/sub&gt; value (STANDARD)</th>
<th>Compound Name</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td><em>L. involucrata</em></td>
<td>0.87</td>
<td>0.97</td>
<td>Citronellol</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.71</td>
<td>0.91</td>
<td>Geranial</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.32</td>
<td>0.83</td>
<td>Limonenediol</td>
</tr>
<tr>
<td>2.</td>
<td><em>L. microphylla</em></td>
<td>0.97</td>
<td>0.97</td>
<td>Isopiperitenol</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.91</td>
<td>0.82</td>
<td>Trans-Cardel</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.82</td>
<td>0.98</td>
<td>Nerol</td>
</tr>
<tr>
<td>3.</td>
<td><em>L. montevidensis</em></td>
<td>0.97</td>
<td>0.97</td>
<td>Isopiperitenol</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.82</td>
<td>0.98</td>
<td>Citronellol</td>
</tr>
</tbody>
</table>

**Table 3:** Carbonyl Compound isolated from essential oil

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Plant Name</th>
<th><em>R</em> &lt;sub&gt;f&lt;/sub&gt; value (OBTAINED)</th>
<th><em>R</em> &lt;sub&gt;f&lt;/sub&gt; value (STANDARD)</th>
<th>Compound Name</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td><em>L. involucrata</em></td>
<td>0.87</td>
<td>0.92</td>
<td>γ-Ionone</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.77</td>
<td>0.82</td>
<td>Piperitone</td>
</tr>
<tr>
<td>2.</td>
<td><em>L. microphylla</em></td>
<td>0.78</td>
<td>0.78</td>
<td>β-Ionone</td>
</tr>
<tr>
<td>3.</td>
<td><em>L. montevidensis</em></td>
<td>0.87</td>
<td>0.87</td>
<td>Citral</td>
</tr>
</tbody>
</table>

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Table 4: Esters isolated from essential oil

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Plant Name</th>
<th>R&lt;sub&gt;f&lt;/sub&gt; value (Obtained)</th>
<th>R&lt;sub&gt;f&lt;/sub&gt; value (Standard)</th>
<th>Compound Name</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>L. involucrata</td>
<td>0.89</td>
<td>1.00</td>
<td>Geranyl acetate</td>
</tr>
<tr>
<td>2.</td>
<td>L. microphylla</td>
<td>0.90</td>
<td>1.00</td>
<td>Geranyl acetate</td>
</tr>
<tr>
<td>3.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4.</td>
<td>L. montevidensis</td>
<td>0.90</td>
<td>1.00</td>
<td>Geranyl acetate</td>
</tr>
</tbody>
</table>

Figure 1: TLC of leaf pigments.

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

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

Figure 4: TLC of Essential Oils for Detection of Esters
Developing Solvent: Benzene (neat)
Data are multiple of the replicate
Values are mean ± SEM (Standard Error of Mean)