**Phytochemical and Antibacterial Studies of Lantana camara L. Leaf Fraction and Essential Oil**

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**Abstract**- The study was conducted to determine the antimicrobial activity of varying concentrations of Lantana camara Linn. leaf extracts against Staphylococcus aureus, Bacillus subtilis, Escherichia coli and Salmonella gallinarum. Broth microdilution method was used to determine the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) was evaluated through streaking of bacterial suspension onto Mueller-Hinton agar. Phytochemical tests were done to identify bioactive compounds present in the extracts. Results showed that among extracts used, L. camara leaf ethanolic fraction (EF) and essential oil (EO) demonstrate antibacterial activity. The MIC of essential oil ranges from 312.5μg/mL - 10,000μg/mL better than ethanolic fraction at 1,250μg/mL - 5,000μg/mL. Bacillus subtilis is the most sensitive organism inhibited at 312.5 μg/mL while Salmonella gallinarum showed less sensitivity with MIC starting at 5000 μg/mL. The MIC-MBC ratio revealed that the extracts possess bactericidal activity against test bacteria. Phytochemical evaluation indicates the presence of saponins, tannins and terpenoids in EF and terpenoids in EO. These compounds are believed to be responsible for the broad spectrum activity of the plant extracts.

New strategies should be developed to optimize the usefulness of this plant such as exploring its pharmacologic potential. L. camara besides being inexpensive possesses non-phytotoxic compounds that are found to exhibit inhibitory effect on pathogens. In the Philippines, not only L. camara is known as an ornamental weed but it is also popular in folkloric treatment as cure to various ailments including rheumatism, wound, fever, and asthma. Other studies reported its antitumor, analgesic, antifungal, and hepatotoxic activities.

Our study investigated the antibacterial activity of varying concentrations of L. camara leaf extracts against Bacillus subtilis, Staphylococcus aureus, Escherichia coli, and Salmonella gallinarum. Preliminary antibacterial screening of the plant has been done by other researchers through disc diffusion method but our work demonstrates the minimum inhibitory concentration and minimum bactericidal concentration to partially establish the mode of action of the plant drug. It significantly reports L. camara leaf ethanolic fraction as potential antibiotic. Moreover, we subjected the extract in a series of qualitative phytochemical tests to determine groups of bioactive compounds present in the plant.

**Index Terms**- L. camara, leaf ethanolic fraction, essential oil, phytochemical screening, MIC-MBC

**I. INTRODUCTION**

The number of emerging multi-drug resistant microbial strains is continuously increasing and has become one of the most serious threats to successful treatment of infectious diseases. This increase is mainly attributed to indiscriminate use of broad-spectrum antibiotics. The use of synthetic drugs is not only expensive but often found with adulterations and side effects. New formulations of antimicrobial agents derived from natural plant products is therefore necessitated to address this issue.

The World Health Organization underscores the importance of herbal plants as best source of a variety of drugs and promotes further scientific investigations unto determination of properties, safety and efficacy of plant drugs.

*Lantana camara* (Verbenaceae) is one of the well-known medicinal plants in traditional medicine. Its resilient nature makes the plant invasive and widely distributed in the pantropic. However, this characteristic turns the plant a problem weed because it dominates native species and disrupts biodiversity.

**II. METHODS**

**Plant Collection**

Fresh leaves of *L. camara* were collected at the vicinity of Barangay Pangasugan, Baybay City, Leyte. The leaves were chopped, weighed and air-dried until 20% of moisture content is left.

**Preparation of Extract and Essential Oil**

*L. camara* leaves were sequentially extracted using hexane, ethanol and water. Leaves were first infused in hexane (1:7 v/v) for 24 hours and filtered in Buchner funnel and Whatman filter paper (No. 54). Leaf residues from previous extraction were infused in ethanol (ethanolic fraction) and water (aqueous fraction). The extracts were filtered and concentrated in rotary evaporator at 40°C until volume reached 10mL. The extracts were placed in clean amber bottle and evaporated to dryness.

Essential oil was extracted using modified steam distillation technique. Collected oil was transferred to sterile bottle until analyzed.

**Test Bacteria**
**Bacillus subtilis**, *Staphylococcus aureus*, *Escherichia coli*, and *Salmonella gallinarum* were obtained from the Microbiology Laboratory of the College of Veterinary Medicine, Visayas State University, Baybay City, Leyte. Bacterial inoculum was prepared by inoculating Mueller Hinton broth with each organism and turbidity was adjusted to 1.5 x 10^5 CFU/mL Mc Farland standard.

**Tube Preparation of Leaf Extract and Essential Oil**

Ten tubes were pipetted with 1 mL of Mueller Hinton broth. The first tube was dispensed with 1 mL of *L. camara* leaf extract and essential oil obtained from stock solution (20,000 μg/mL). Subsequently, 1 mL of the content from the first tube was transferred to the second tube and continued until 10 tubes were filled. The final concentration ranged from 10,000 μg/mL to 19.53 μg/mL.

**Antibacterial Activity**

Antibacterial activity of *L. camara* crude hexane extract, ethanolic fraction, aqueous fraction, and essential oil was evaluated through minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC). MIC, which is the lowest concentration with clear content, was evaluated in two-fold microdilution method. Briefly, 100 μg/mL each of the prepared extract concentration and bacterial inoculum were mixed with Mueller-Hinton broth in microtiter plates. Wells with clear content were streaked into Mueller-Hinton agar to determine MBC which is the first concentration without visible growth in the agar. Distilled water was used as negative control and penicillin (gram positive) and streptomycin (gram negative) were reference control. All experiments were repeated three times and incubated at 37°C for 24 hours.

**Phytochemical Profiling of Lantana Leaf Extracts with Antibacterial Activity.**

From the above assay, only those extracts with potential antibacterial reactions (*L. camara* leaf ethanolic fraction and essential oil) were profiled using thin layer chromatography (TLC) and qualitative phytochemical tests. An aliquot (1 μL) of each extract was blotted separately onto TLC plate and soaked in a chamber containing 5 mL of the benzyl and chloroform (1:1 v/v). The plate was visualized at daylight, UV light and ferric chloride tests (tannin) froth and hemolysis tests (saponin), Dragendorff and Mayer’s tests (alkaloid), and Bate-Smith and Metcalf tests (flavonoids). The screening tests determine the components available in the plants with bioactivity or ethno-medical applications.

**Statistical Analysis**

The modal MIC was determined by selecting at which point among 10 concentrations of each leaf extract most likely shows inhibition of bacterial growth.

**III. RESULTS AND DISCUSSION**

**Antibacterial Activity**

Notably, only leaf ethanolic fraction and essential oil among four extracts of *L. camara* leaves showed antibacterial activity against *B. subtilis*, *S. aureus*, *E. coli*, and *S. gallinarum*. Table 1 summarizes the MIC and MBC of *L. camara* leaf ethanolic fraction and essential oil. The MIC of essential oil ranges from 312.5 μg/mL – 10,000 μg/mL better than leaf ethanolic fraction at 1,250 μg/mL – 5,000 μg/mL. The lowest MIC of essential oil was against *B. subtilis* (gram positive) at 312.5 μg/mL and 2,500 μg/mL against *E. coli* (gram negative). Conversely, it takes an MIC of 1,250 μg/mL and 5,000 μg/mL for leaf ethanolic fraction to inhibit the same organisms. *B. subtilis* is remarkably the most susceptible organism and *S. gallinarum* is least susceptible for both extracts. Gram positive bacteria are more sensitive to the extracts than their counterpart.

The reference antibiotics generally showed better MIC values than *L. camara* extracts with lowest MIC at 156.25 μg/mL against gram positive and 625 μg/mL against gram negative bacteria. This reaction is apparent for commercial antibiotics which action is potentiated and maximized. MBC was not done since the mode of action of the antibiotics is pre-determined.

Table 1. MIC and MBC of *L. camara* leaf ethanolic fraction, essential oil and reference control against test bacteria.

<table>
<thead>
<tr>
<th>Concentration (μg/mL)</th>
<th>Test bacteria</th>
<th>Leaf ethanolic fraction</th>
<th>MIC</th>
<th>MBC</th>
<th>MIC</th>
<th>MBC</th>
<th>MIC</th>
<th>MBC</th>
<th>MIC</th>
<th>MBC</th>
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<tr>
<td></td>
<td><em>B. subtilis</em></td>
<td><em>S. aureus</em></td>
<td><em>E. coli</em></td>
<td><em>S. gallinarum</em></td>
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</tr>
<tr>
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<tr>
<td>5,000</td>
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<tr>
<td>1,250</td>
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<td>+</td>
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<td>+</td>
<td>+</td>
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<td>+</td>
<td>+</td>
<td>+</td>
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<tr>
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<td>+</td>
<td>+</td>
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<td>19.53</td>
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<td>+</td>
<td>+</td>
<td>+</td>
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<td>+</td>
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<table>
<thead>
<tr>
<th>Reference control</th>
<th>Penicillin</th>
<th>Streptomycin</th>
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<tbody>
<tr>
<td>10,000</td>
<td>-</td>
<td>-</td>
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<tr>
<td>5,000</td>
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</tr>
<tr>
<td>2,500</td>
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<tr>
<td>1,250</td>
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<td>625</td>
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<td>312.50</td>
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<tr>
<td>19.53</td>
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</table>

The MBC determines the nature of the antibacterial activity of the extract through the MIC and MBC ratio. Figure 1 and 2 demonstrate the MIC-MBC ratio of leaf ethanolic fraction and essential oil. In the ethanolic fraction, the MBC is equal to MIC in *B. subtilis*, four-fold higher in *E. coli* and two-fold higher in *S. aureus* and *S. gallinarum*. The MIC-MBC ratio of essential oil is

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equal in B. subtilis and S. gallinarum, two-fold and four fold higher in E. coli and S. aureus, respectively. Most organisms were inhibited at 5,000μg/mL and were killed at 10,000μg/mL. The antibacterial activity of L. camara extract against test bacteria implies that the plant contained broad spectrum bioactive compounds that can be developed as alternative to synthetic drugs.

Various literatures indicate that an extract has bactericidal activity when the MBC value is the same or generally not more than four-fold higher than the MIC; and bacteriostatic when more than four-folds or many-fold higher than the MIC\(^2\), \(^3\), \(^4\), \(^5\). The closer the MIC to its MBC, the more bactericidal it is\(^6\). From this context, this study demonstrates that L. camara leaf ethanolic fraction and essential oil possess bactericidal compounds against test organisms.

**Phytochemical Screening of Lantana Leaf Ethanolic Fraction and Essential Oil**

The TLC profiling of L. camara leaf ethanolic fraction and essential oil resulted in the presence of a number of phytochemicals with different retention factor (Rf) values reflecting polarity of compounds. From ethanolic fraction, four spots with varying colors and Rf values ranging from 0.10 – 0.35 were seen when viewed in daylight. Six additional spots with Rf values from 0.46 – 0.89 were seen under UV light and eight spots (Rf 0.10-0.72) were viewed after dipping the chromatogram in potassium permanganate (Table 2). The essential oil showed no visible spots under daylight but 10 spots with varying colors and Rf ranging from 0.05 – 0.98 were seen under UV light. In potassium permanganate, seven spots (Rf 0.05-0.54) with varying colors were visible. Subsequently, the profile demonstrated two similar compounds from the extracts with Rf values of 0.46 and 0.81 under UV light. Compound showing high Rf values have low polarity while those with lower Rf values have higher polarity\(^9\),\(^13\). Rf values ranging from 0.3 – 0.9 indicates the presence of less polar compounds such as terpenes or terpenoids\(^7\) which may be the case for the two similar compounds (Rf 0.46 and 0.81) found in both ethanolic fraction and essential oil. This observation is further manifested in the qualitative phytochemical tests of the extract where saponins, tannins and terpenoids were detected in the ethanolic leaf fraction and terpenoids alone in the essential oil (Table 3).

### Table 3. Qualitative phytochemical tests of L. camara leaf ethanolic fraction and essential oil.

<table>
<thead>
<tr>
<th>Extract</th>
<th>Phytochemicals</th>
<th>Detection test</th>
<th>Reaction</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leaf ethanolic fraction</td>
<td>Saponins</td>
<td>Froth test</td>
<td>+</td>
<td>Dense, honeycomb-like froth</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Hemolysis test</td>
<td>+</td>
<td>Hemolyzed area</td>
</tr>
<tr>
<td></td>
<td>Tannins</td>
<td>Gelatin test</td>
<td>+</td>
<td>Trace, cloudy</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ferric chloride test</td>
<td>+</td>
<td>Greenish-brown with orange precipitate</td>
</tr>
<tr>
<td></td>
<td>Alkaloids</td>
<td>Dragendorff’s test</td>
<td>-</td>
<td>No change</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Mayer’s test</td>
<td>-</td>
<td>No change</td>
</tr>
<tr>
<td></td>
<td>Flavonoids</td>
<td>Bate-Smith and Metcalf test</td>
<td>-</td>
<td>No change</td>
</tr>
<tr>
<td></td>
<td>Terpenoids</td>
<td>Salkowski test</td>
<td>+/-</td>
<td>Red-brownish green</td>
</tr>
<tr>
<td>Essential oil</td>
<td>Terpenoids</td>
<td></td>
<td>+</td>
<td>Reddish-brown</td>
</tr>
</tbody>
</table>

Several findings have reported the presence of terpenoids in essential oil\(^20\),\(^22\). The antimicrobial activity of essential oil is attributed to the presence of secondary metabolite of terpenoids present in the extract such as monoterpenes α-pine, β-pine, β-cymene, sesquiterpenes humulene epoxide, and 1,8-cineole\(^21\), \(^27\), \(^22\). Considering the large number of different groups of chemical compounds contained in essential oil, oftentimes the antibacterial activity is not attributable to one specific

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mechanism but that there are several targets in the cell\textsuperscript{14}. Furthermore, it is important to note that the hydrophobicity of essential oil and its components enables partition in the lipids of the bacterial cell membrane and mitochondria that disturbs these structures and renders them more permeable\textsuperscript{1}. Leakage of ions and other cell contents can then occur which causes the death of the organism\textsuperscript{14}. The α-pinene and β-pinene are known to interfere cellular integrity and induce toxic effects on the membrane structure and functions leading to inhibition of ion transport process.

It is important to note that the use of ethanol as organic solvent concentrates more water soluble compounds including saponins and tannins\textsuperscript{22}. Saponin is a major component of plant that acts as antibacterial secondary metabolite\textsuperscript{28}. The mode of action of its antibacterial effects involves membranolytic properties, rather than simply altering the surface tension of the extracellular medium\textsuperscript{1}. Tannins, belonging to a condensed group, are polymeric phenolic derivatives with astringent properties and have the ability to inactivate microbial adhesions, enzymes, and cell envelope transport proteins\textsuperscript{9}.

Gram positive bacteria are generally more susceptible since their outer peptidoglycan layer is not an effective barrier\textsuperscript{7}. Gram negative bacteria, on the other hand, possess tough outer membrane formed by lipopolysaccharide layer along with proteins and phospholipids. This structure hinders the access of most compounds to the peptidoglycan layer of the cell wall\textsuperscript{15}.

**IV. CONCLUSION**

The findings of this study elucidate that *L. camara* leaf ethanolic fraction and essential oil contain phytochemicals responsible for the broad antibacterial spectrum of the plant. The research data provides basis for developing the plant products into useful drug that may yet resolve issues on drug resistance.

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**REFERENCES**


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