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.
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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[8]. This increase is mainly attributed to indiscriminate use of broad-spectrum antibiotics[24]. 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[5,11]. 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[12].

Lantana camara (Verbenaceae) is one of the well-known medicinal plants in traditional medicine[6]. 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[18]. 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[26]. In the Philippines, not only *L. camara* is known as an ornamental weed but is also popular in folkloric treatment as cure to various ailments including rheumatism, wound, fever, and asthma[17]. Other studies reported its antitumor, analgesic, antifungal, and hepatotoxic activities[14].

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.

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 w/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^{o}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 Mueler Hinton broth with each organism and turbidity was adjusted to $1.5x10^8$ CFU/mL Mc Farland standard.

Tube Preparation of Leaf Extract and Essential Oil

Ten tubes were pipetted with 1mL of Mueller Hinton broth. The first tube was dispensed with 1mL of *L. camara* leaf extract and essential oil obtained from stock solution (20,000µg/mL). Subsequently, 1mL 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 10,000µg/mL to19.53µg/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 (1µl) of each extract was blotted separately onto TLC plate and soaked in a chamber containing 5mL of the benzyl and chloroform (1:1v/v). The plate was visualized at daylight, UV light and $KMNO_4$ and retention factor (R_f) value was recorded. Furthermore, extracts were subjected to Salkowski test (test for terpenoids), gelatin 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 312.5µg/mL – 10,000µg/mL better than leaf ethanolic fraction at 1,250µg/mL – 5,000µg/mL. The lowest MIC of essential oil was against *B. subtilis* (gram positive) at 312.5µg/mL and 2,500µg/mL against *E. coli* (gram negative). Conversely, it takes an MIC of 1,250µg/mL and 5,000µg/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.

Concentration (µg/mL)	Test bacteria							
	B. subtilis		*S. aureus*		*E. coli*		*S. gallinarum*	
Leaf ethanolic fraction	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
10,000	-	-	-	-	-	-	-	-
5,000	-	-	-	+	-	+	-	+
2,500	-	-	-	+	+		+	
1,250	-	-	+		+		+	
625	+	+	+		+		+	
312.50	+		+		+		+	
156.25	+		+		+		+	
78.12	+		+		+		+	
39.06	+		+		+		+	
19.53	+		+		+		+	
Essential oil								
10,000	-	-	-	-	-	-	-	-
5,000	-	-	-	-	-	+	+	
2,500	-	-	-	-	-	+	+	
1,250	-	-	-	+	+		+	
625	-	-	+		+		+	
312.50	-	-	+		+		+	
156.25	+	-	+		+		+	
78.12	+	+	+		+		+	
39.06	+		+		+		+	
19.53	+		+		+		+	
Reference control	Penicillin				Streptomycin			
10,000	-		-		-		-	
5,000	-		-		-		-	
2,500	-		-		-		-	
1,250	-		-		-		+	
625	+		-		-		+	
312.50	+		-		+		+	
156.25	+		-		+		+	
78.12	+		+		+		+	
39.06	+		+		+		+	
19.53	+		+		+		+	

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

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.

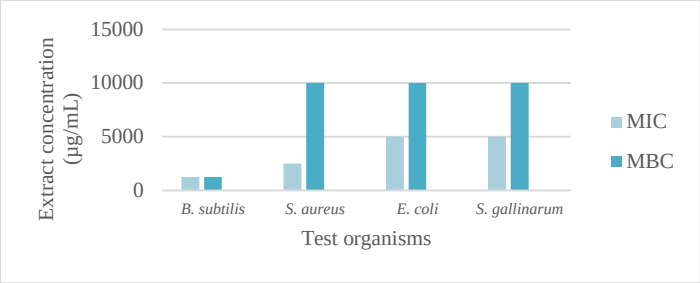

Figure 1. MIC-MBC ratio of *L. camara* leaf ethanolic fraction.

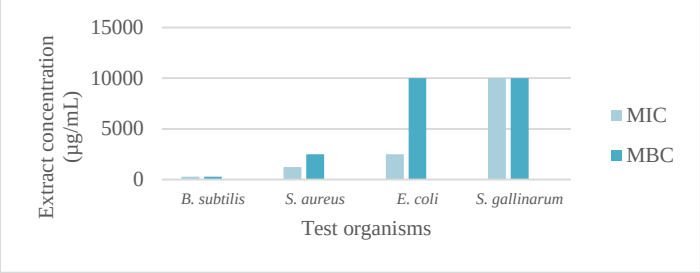

Figure 2. MIC-MBC ratio of *L. camara* essential oil.

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, 23, 16, 25]. The closer the MIC to its MBC, the more bactericidal it is[10]. 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[9] which may be the case for the two similar compounds (Rf 0.46 and 0.81) found

Table 2. Phytochemical profiles of *L. camara* leaf ethanolic fraction and essential oil in TLC.

Extract	Spot	Rf values (cm)	Visualization method		
			Daylight	UV light	$KMNO_4$
Leaf ethanolic fraction	Spot 10	0.89	None	Yellow	None
	Spot 9	0.81	None	Faint blue violet	None
	Spot 8	0.72	None	Faint blue	Faint yellow
	Spot 7	0.65	None	Faint yellow	Yellow orange
	Spot 6	0.57	None	Faint green	Faint yellow
	Spot 5	0.46	None	Faint blue	Brownish yellow
	Spot 4	0.35	Dark Green	Grayish green	Dark yellow
	Spot 3	0.22	Light green	Yellow	Light Brown
	Spot 2	0.20	Yellow reen	Blue	Light Brown
	Spot 1	0.10	Light ellow	Green	Faint Brown
Essential oil	Spot 11	0.98	None	Dark blue violet	None
	Spot 10	0.91	None	Violet	None
	Spot 9	0.81	None	Faint blue violet	None
	Spot 8	0.74	None	Faint blue	None
	Spot 7	0.54	None	Light gray	Yellow orange
	Spot 6	0.46	None	Faint blue	Brownish yellow
	Spot 5	0.42	None	Faint violet	Brownish Orange
	Spot 4	0.32	None	Faint violet	Orange
	Spot 3	0.24	None	Light blue	Light brown
	Spot 2	0.18	None	Faint violet	Light orange
	Spot 1	0.05	None	Light violet	Light brown

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.

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

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-pinene, β-pinene, p-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

mechanism but that there are several targets in the cell[3]. 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[3]. Leakage of ions and other cell contents can then occur which causes the death of the organism[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[24]. Saponin is a major component of plant that acts as antibacterial secondary metabolite[28]. The mode of action of its antibacterial effects involves membranolytic properties, rather than simply altering the surface tension of the extracellular medium[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[4].

Gram positive bacteria are generally more susceptible since their outer peptidoglycan layer is not an effective barrier[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[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

1. Aliyu, A.B., Musa, A.M., Abdullahi M.S, Ibrahim, M.A., Tijjani, M.B., Aliyu, M.S. & Oyewale, A.O. (2012). Activity of saponin fraction from *Anisopus manni* against some pathogenic microorganisms. *Journal of Medicinal Plant Research*, 5(31):6709-6713.

2. Basri, D.R. & Fan, S.H. (2005). The potential of aqueous and acetone extract of galls and *Quercus infectoria* as antibacterial agents. *Indian Journal of Pharmacology*, 37(1): 26-29.

3. Burt, S. 2004. Essential Oils: Their antibacterial properties and potential applications in foods - A Review. *International Journal of Food Microbiology*, 94: 223– 253.

4. Cowan, M. M. 1999. Plant Products as Antimicrobial Agents. *Clinical Microbiology Reviews Journal*, 12**(4)**: 564.

5. Dabur, R., Gupta, A., Mandal, T.K., Deepak Sigh D., Bajpai, V., Gurav, A.M., and G.S. Lavekar. 2007. Antimicrobial activity of some Indian Plants. African Ethnomedicines, 4(3): 313-318.

6. Dharmagada, V.S.S., Tandonb, M. and P. Vasudevan. 2005. Biocidal activity of the essential oils of *Lantana camara*, *Ocimum sanctum* and *Tagetes patula*. Journal of Scientific & Industrial Research, 64: 53-56.

7. Dubey, M., Sharma, S., Senvar, R., Bhadauria, S., and R.K. Gautam. 2011. *In Vitro* antibacterial activity of *Lantana camara* leaves hydrosol. Journal of Pharmacy and Research, 4(11): 3972-3974.

8. Kalyani, L.**,** Lakshmana Rao, A., and U.S. Mishra. 2011. Antibacterial and analgesic activity of leaves of *Lantana camara*. International Journal of Phytomedicine, 3: 381-385.

9. Lavanya, G. and G.P. Brahmaprakash. 2011. Phytochemical screening and antimicrobial activity of compounds from selected medicinal and aromatic plants. International Journal of Science and Nature, 2(2): 287-291.

10. Levison, M.E. 2004. Pharmacodynamics of antimicrobial drugs. Infectious Disease Clinics of North America, 18:451-465.

11. Maji, S., Dandapat, P., Ojha, D., Halder, S.K., Das Mohapatra, P.K., Pathak, T.K., Pati, B.R., Samanta, A., and K.C. MONDAL. 2010. *In Vitro* antimicrobial potentialities of different solvent extracts of ethnomedicinal plants against clinically isolated human pathogens. Journal of Phytology, 2(4): 57-64

12. Nascimento, G.G.F., Locatelli, J., Freitas, P.C. and G.L. Silva. (2000). Antibacterial activity of plant extracts and phytochemicals on antibiotic resistant bacteria. *Braz. J. Microbiol.*, 31: 247-256.

13. Nigam, M.C., Nigam, I.C. and L. Levi. 1965. Essential oil and their constituents. XXV. Thin layer chromatography. Some chemical and chemotaxonomic applications. Journal of the Society of Cosmetic Chemists, 16: 155-168.

14. Oosterhayen, K., Poolman, B. and E.J. SMID. 1995. S-carvone as a natural potato sprout inhibiting, fungistatic and bacteristatic compound. Industrial Crops and Products, 4:23-31.

15. Pattnaik, S and B. Pattnaik. 2010. A study of *Lantana camara* linn aromatic oil as an antibacterial agent. International research Journal of Pharmacological Science, 1(1): 32-25.

16. Peterson, L.R. and C.J. Shanholtzer. 1992. Tests for Bactericidal Effects of Antimicrobial Agents: Technical Performance and Clinical Relevance. Clinical Microbiology Review, 5: 420-432.

17. Philippine Medicinal Plants. (2015). Kantutay. Retrieved from http://www.stuartxchange.com/Kantutay.html.

18. Priyanska, N. & Joshi, P.K. (2013). A review of Lantana camara studies in India. *International Journal of Scientific and Research Publications*. 3(10):1-11.

19. Ravinder, K.K., Daizy, R., Batish, H.P., Singh, Kuldip, S.D. (2000). Status, invasiveness and environmental threats of three tropical American invasive weeds (*Parthenium hysterophorus L., Ageratum conyzoides L., Lantana camara L.*) in India. *Biological Invasions*. 8:1501–1510

20. Saikia, A. K. and R.K. Sahoo. 2011. Chemical composition and antibacterial activity of essential oil of *Lantana camara* L. Middle-East Journal of Scientific Research. 8(3): 599-602.

21. Saraf, A., Quereshi, S., Sharma, K., and N.A. Khan. 2011. Antimicrobial activity of *Lantana camara* L. Journal of Experimental Sciences, 2(10): 50-54

22. Sousa, E.O., Almedia, T.S., Menez, I.R.A., Rodriguez, F.F.G., Campos, A.R., Lima, S.G., and J.G.M. Da Costa. 2010. Chemical composition of essential oils of Lantana camara L. (Verbenacea) and Synergistic effect of aminoglycosides gentamicin and amikacin. Records of Natural Products, 6(2): 144-150.

23. Sherris, J.C. 1986. Problems in In Vitro Determination of Antibiotic Tolerance in Clinical Isolates. Antimicrobial Agents and Chemotherapy, 30(5):633-637.

24. Suffredini, I.B., Sader, H.S., Goncalves, A.G., Reis, A.O., Gales, A.C., Varella, A.D., and R.N. Younes. 2004. Screening of antibacterial extracts from plants native to the Brazilian Amazon Rain Forest and Atlantic Forest. Brazilian Journal of Medicinal and Biological Research, 37: 279-384.

25. Toumanen, E., Durack, D.T. and A. Tomasz. 1986. Antibiotic Tolerance among Clinical Isolates of Bacteria. Antimicrobial Agents and Chemotherapy, 30(4): 521-527.

26. Vaidya, G.S. and N. Bhattarai. 2009. Antagonistic study of *Lantana camara* (Linn) against with pathogenic bacteria. Scientific World, 7(7): 64-67.

27. Vasquez, E.A., 2000. Pesticidal and Other Biological Activities of Selected Philippine Plant Species. Hieronymus, Muenchen, Germany, 217 pp.

28. Wadhai, V.S. 2012. Antibacterial Activity of and Comparative Phytochemicals Analysis of Flower and Leaves of *Lantana camara.* Bionano Frontier, 5(2-III): 143-145.

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