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A Comparative Study: Antimicrobial Activity of Methanol Extracts of *Lantana camara* Various Parts

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ABSTRACT

Finding new resources of antibiotics based on natural products used by traditional practitioners was the main aim to study extracts of root, stem, leaf, flower and fruit of *Lantana camara* L. (Verbenaceae), a medicinal plant available in Malaysia. A panel of organisms including 10 bacteria and 5 fungi were treated by *L. camara* extracts of different parts based on disk diffusion method and broth microdilution technique. The leaf extract presented the highest antibiotic effect among all parts of plant especially against Gram positive *Bacillus cereus* (zone of inhibition 13.0 ± 0.0 mm, MIC/MBC 9.4 ± 4.4 mg/ml) and Gram negative *Salmonella typhi* (zone of inhibition 13.5 ± 2.1 mm, MIC/MBC 12.5 ± 0.0 mg/ml). In conclusion, this study may support the conventional use of leaf extract of *L. camara* in some infectious gastroenteritis disorders, a potential subject to further isolation and identification as a supply of antibacterial substances.

Keywords: Antimicrobial Activity, Broth Microdilution, Gastrointestinal Diseases, *Lantana camara,* Minimum Inhibitory Concentration, Zone of Inhibition.

INTRODUCTION

Resistant bacterial strains have emerged and have spread throughout the world because of the remarkable genetic plasticity of the microorganisms, heavy selective pressures of use, and the mobility of the world population. The underlying problems are largely economic and societal, and no ready solutions are available (1). Then, there is still a need to explore prospective antibiotic compounds capable to control pathogens.

Folk healers in Asia and South America have used lantana species including *Lantana camara* L. (Verbenaceae) for centuries to treat various human ailments such as dermatological and gastrointestinal diseases, tetanus, malaria and tumors (2–5).

At the present study, antimicrobial potency of different parts of *Lantana camara* (*L. camara*) was compared by implementing disk diffusion method and broth microdilution technique on a panel of microorganisms which some of them cause gastrointestinal complications to appraise the ethnopharmacological aspect of this plant and to discover novel progenitors of antimicrobial agents. In addition, the most susceptible microorganisms to the active parts of the plant were recognized.

MATERIALS AND METHODS

Plant Material Extraction

Mature *L. camara* was collected in a random way from Sungai Petani, Kedah, Malaysia, on July 2008. *Lantana*

camara (L.), genus Lantana L., family Verbenaceae was identified by Dr. S. Sudhakaran, associate professor in Faculty of Applied Sciences, AIMST University, Kedah, Malaysia. A voucher numbered as 11008 was deposited in the herbarium of Biology School, Universiti Sains Malaysia, Penang, Malaysia.

Being washed by tap water, each part of plant namely root, stem, leaf, flower, and fruit was separated and transferred to oven at 50 °C for 4 days. Grinded by blender, the powders were extracted by maceration in methanol for 4 days and filtered by filter paper. After breaking up the solvent by rotary evaporator, the samples were positioned into glass Petri dishes, inside oven at 60 °C to dry to a semisolid matter on the surface.

Panel of Microorganisms

A board of organisms comprising 6 Gram negative bacteria, *Escherichia coli, Enterobacter aerogenes, Klebsiella pneumoniae, Proteus mirabilis, Pseudomonas aeroginosa, Salmonella typhi*, 4 Gram positive bacteria, *Bacillus cereus, Bacillus subtilis, Bacillus thuringinsis, Staphylococcus aureus*, 5 fungi, *Aspergillus flavus, Aspirgilus niger, Penicillium* sp., *Rhizophus* sp., and *Candida albicance*, were selected to test *L. camara* extracts ability to inhibit the growth by disk diffusion method. All strains were provided from subculturing local isolations. The sensitive microbes were used for further analysis by microdilution assay.

Disk Diffusion Method

The extracts of different parts of *L. camara* were tested by disc diffusion method according to the National Committee for Clinical Laboratory Standards (6). Twenty ml nutrient agar (HiMedia Laboratories, India) for bacteria or Sabouraud dextrose agar (Difco, Becton, Dickinson and Compang, France) for fungi, sterilized in a flask and cooled to 45–50 °C, were transferred to sterilized Petri dishes with a diameter of 9 cm. Inoculum suspensions were prepared from 18- to 24-h cultures grown on nutrient agar (for fungus, Sabouraud dextrose agar) and adjusted to equal a 0.5 McFarland standard solution (containing 1.5×10^8 CFU/ml). A 1/1000 dilution was used for fungi strains inocula. Each plate was streaked using a cotton swab that was dipped in the suspension and from which excess fluid was expressed. The filter paper discs (6 mm in diameter) were individually impregnated with 10 µl of the *L. camara* extracts (concentration 100 mg/ml) and then placed onto the agar plates which had previously been inoculated with the test microorganisms (within 15 min). The Petri dishes were kept at 4 °C for 2 h before incubation at 37 °C for 24 h and at 30 °C for 48 h for the fungi. The diameters of the inhibition zones were measured in millimeters. All the tests were performed in duplicate. The standard antimicrobial agent, chloramphenicol (Phyto Technology Laboratories, 30 µg per disk, for bacteria) and Nystatin (Phyto Technology Laboratories, 30 µg per disk, for fungi) were employed as positive controls.

Broth Microdilution Method

Andrews J M method (7) was used to determine minimum inhibitory concentration (MIC). Seventy five µl sterile nutrient broth was decanted into each well of a sterile 96-well microplates (MICROTEST 96, FALCON, USA). *L. camara* extract with the highest concentration (200 mg/ml) was added at 75 µl to the first well. After mixing, 75 µl was transferred to the second well; the same procedure for the next wells to attain dilutions of 1/2, 1/4, 1/8, 1/16 and 1/32. Inoculum solution (equal to turbidity of 0.5 McFarland solution) at 1.5 µl was added to every well except negative controls. Being incubated for 20 h at 37 °C in air, the wells were monitored for turbidity as growth and non-turbidity as no growth. The minimum inhibitory concentration (MIC) endpoint was the lowest concentration of antibiotic at which there was no visible growth. Chloramphenicol at concentration 60 µg/ml was used as reference drug control.

The minimum bactericidal concentration (MBC) was measured based on Cosa P. et al. (8) method by plating-out samples of completely inhibited dilution cultures and assessing growth (static) or no-growth (cidal) after incubation. The MBC endpoint was the lowest concentration of antibiotic at which there was no visible growth.

Statistical Analysis

SPSS 16.0.0 (SPSS Inc, TEAM EQX) was utilized to analyze data. Following test of homogeneity of variances, Games- Howell test from one way ANOVA and Kruskal-Wallis test, a non parametric test, were applied to weigh data. Zone of inhibition outputs were expressed as millimeter ± standard deviation; MIC and MBC values as mg/ml ± standard deviation. For executing analysis, the concentration more than 100 mg/ml was considered 200 mg/ml.

RESULTS AND DISCUSSION

While 75% of tested Gram positive bacteria exhibit inhibition response to *L. camara* extracts, 67% of screened Gram negative ones show no sensitivity at all. Deena M.J. and Thoppil J.E. obtained similar outcome whilst working on chloroform and methanol extracts of this plant (9).

Table 1. Zone of Inhibition (mm ± standard deviation) based on Disk Diffusion Method for Different Parts of L. camara (1 mg/disk) and Chloramphenicol (30 µg/disk) on 5 Bacteria.

	Part of *L. camara* and Chloramphenicol			
Microorganism	**Root**	**Leaf**	**Flower**	**Chloramphenicol**
Salmonella typhi	ni	13.5 ± 2.1	10.0 ± 0.0	33.0 ± 2.8
Bacillus cereus	8.5 ± 0.7	13.0 ± 0.0	10.5 ± 2.1	28.5 ± 0.7
Bacillus thuringinsis	7.5 ± 0.7	12.5 ± 0.7	10.0 ± 0.0	30.0 ± 2.8
Enterobacter aerogenes	ni	11.0 ± 0.0	ni	25.0 ± 7.1
Staphylococcus aureus	ni	8.0 ± 0.0	ni	29.5 ± 2.1

No inhibition has been symbolized as ni.

Table 2. Ranking Effective Parts of L. camara and Chloramphenicol by Kruskal- Wallis.
Non-parametric Test based on Disk Diffusion Method on 5 Bacteria Strains.

Part of *L. camara*	**Number of Samples**	**Mean Rank.**
Chloramphenicol	10	25.50
Leaf	10	13.60
Flower	6	9.92
Root	4	3.62

$p < 0.05$.

Unlike Inada A. *et al.* study (10), there isn't an inhibition activity on 5 selected fungi in the current research.
Table 1 shows zone of inhibition for susceptible microorganisms.

The highest amount of inhibition is observed from leaf extract that is significant based on Kruskal-Wallis test without considering type of organism (Table 2).

This research is not along with Deena M.J. and Thoppil J.E. investigation, recording inhibition of *P. aeruginosa* by methanol extracts of *L. camara* (9). On the other side, the screening results are in line of Barre J.T. et al finding that 22 beta-Acetoxylantic acid isolated from *L. camara* demonstrated antimicrobial activity against *Staphylococcus aureus* and *Salmonella typhi* (*S. typhi*) (11).

Table 3 displays that the best activity of leaf is against *Bacillus cereus* (*B. cereus*) but it's not significantly different from other bacteria in the list based on Games- Howell test. The MBC figure in this case is the same as MIC (9.4 ± 4.4 mg/ml). The two next vulnerable bacteria are *B. thurengensis* and *S. typhi* with MIC and MBC 12.5 ± 0.0 mg/ml. However, the potency of leaf is much lower (approximately 1/400) than that of chloramphenicol with MIC 0.023 ± 0.01 mg/ml for *B. cereus*. Relative sensitivity of *S. typhi* to the extracts of various parts of *L. camara* is alongside of Barre J.T. *et al.* study (11). Since *B. cereus* and *S. typhi* are two foodborne pathogens causing severe nausea, vomiting and diarrhea (12), it could strengthen the customary usage of *L. camara* to cure the gastroenteritis syndromes (4).

The MBC value of chloramphenicol for all test microorganisms is out of studied dilutions that reflects the

Table 3. Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC), in mg/ml ± standard deviation, for 5 Bacteria Treated by Extracts of Different Parts of L. camara and Chloramphenicol based on Broth Microdilution Method.

	Part *of L. camara* and Chloramphenicol					
	Root		**Stem**		**Leaf**	
Microorganism	**MIC**	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**
Salmonella typhi	75 ±35.4	>100	100 ± 0.0	100 ± 0.0	12.5 ± 0.0	12.5 ± 0.0
Bacillus cereus	>100	>100	100 ± 0.0	100 ± 0.0	9.4 ± 4.4	9.4 ± 4.4
Bacillus thuringinsis	>100	>100	>100	>100	12.5 ± 0.0	12.5 ± 0.0
Enterobacter aerogenes	>100	>100	>100	>100	100 ± 0.0	>100
Staphylococcus aureus	>100	>100	>100	>100	50 ± 0.0	>100

	Part of *L. camara* and Chloramphenicol					
	Flower		**Fruit**		**Chloramphenicol**	
Microorganism	**MIC**	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**
Salmonella typhi	75 ± 35.4	100 ± 0.0	100 ± 0.0	100 ± 0.0	0.004 ± 0.0	> 0.040
Bacillus cereus	50 ± 0.0	50 ± 0.0	>100	>100	0.023 ± 0.01	> 0.040
Bacillus thuringinsis	>100	>100	>100	>100	0.009 ± 0.008	> 0.040
Enterobacter aerogenes	>100	>100	>100	>100	0.004 ± 0.0	> 0.040
Staphylococcus aureus	>100	>100	>100	>100	0.008 ± 0.0	> 0.040

bacteriostatic characteristic of this antibiotic. Therefore, to practice MBC method, unless toxic concentrations of chloramphenicol are exploited, otherwise a standard bactericidal agent should be substituted as a positive control.

CONCLUSION

No inhibitory effect was observed from different parts of *L. camara* extracts against fungi tested. In term of antibacterial activity, leaf extract of *L. camara* is the most potent part of plant followed by flower and root. Contrasting with chloramphenicol, the microbial inhibition effectiveness of *L. camara* extracts even for leaf is not robust.

As a general, *L. camara* extracts have the best action against Gram negative *S. typhi*, but leaf extract is more specific toward Gram positive *B. cereus*. Efficient growth control of these two bacteria might confirm the folk medicine application of this plant in gastrointestinal diseases.

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