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ANTIMICROBIAL AND CELLULAR METABOLIC EFFECTS OF THE ETHANOLIC EXTRACT OF THE DALLAS RED VARIETY OF LANTANA CAMARA

Clint Cabrido, Cesar G. Demayo*

Department of Biological Sciences, College of Science and Mathematics
MSU-Iligan Institute of Technology Iligan City 9200 Philippines

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ABSTRACT

This study was conducted to determine the antimicrobial potential and cellular metabolic effects of the Dallas red variety of Lantana camara, a variety which has shown resistance to the herbivore Uroplata girardi Pic. The phytochemical and chemical compounds present in the extract were also done to be able to correlate which of the medicinal properties reported these can be attributed to. For antimicrobial screening, the plant extract showed antimicrobial activity in four out of six bacterial strains; K. pneumonia, P. aeruginosa, S. aureus and B. subtilis. Stimulation/Suppression of splenocyte proliferation (MTT) assay revealed that the cells were not significantly affected by the extract. Phytochemical screening revealed the different concentrations of alkaloids (+), saponins (+++), flavonoids (+++), steroids (+++), and tannins (+). Cyanogenic-glycosides (-), anthraquinones (-) and fatty acids (-) were not detected. Gas Chromatography- Mass Spectrum analysis revealed fifteen (15) phytocompounds some of which have antioxidant and antimicrobial properties. The antimicrobial and splenocyte response results may indicate that the plant has shown scientific basis about its ethnomedicinal properties.

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Introduction

Lantana camara is an invasive, flowering ornamental plant documented to be used for the treatment of various diseases in several regions in the world [1,2]. The folk medicinal uses include anti-rheumatic, carminative, antibacterial, antispasmodic, emetic, antifungal and antitumoral activities, bronchopulmonary diseases, malaria, ulcers, cancer, high blood pressure, tetanus, tumors, eczema, cuts, catarrhal infections, atoxy of abdominal viscera, chicken pox, measles, asthma, fevers, sore throat, cough, conjunctivitis, toothache, skin rashes and itchin, headache and colds, diaphoretic, stimulant, and treatment of jaundice [3-9]. Studies have shown L. camara contains several classes of bioactive natural products such as triterpenoids, flavonoids, steroids, iridoide glycosides, oligosaccharides, phenylpropanoid glycosides, and naphthoquinones [10-12]. Likewise, varieties of lead phytomolecules such as oleanolic acid, ursolic acid, lantanoside, linaroside, camarinic acid, verbascoside, umuhengerin and phytol isolated from L. camara were reported to have hepatoprotective, leishmanicidal, anticancer, antibacterial, antioxidant, antimycobacterial, nematicidal, and antiulcer biological activities [7, 9, 13-17]. In Asian countries, leaves were also documented in the treatment of various ailments such of treat cuts, rheumatism, ulcers, vermifuge, leprosy, scabies and gastrointestinal diseases [3].

These above studies however, failed to document which variety of L. camara was used. L. camara have so many variant forms based on the kind of flower the plant produces. In this study, we have evaluated the kind of variety the plant that show resistance to feeding from the common herbivore Uroplata girardi Pic and used this as basis for its selection for the evaluation of its biological properties. It is argued that the plant variety that showed resistance to the herbivore, has the potential of having the bioactive compounds that will have antimicrobial and cellular metabolic properties thus this study was conducted.

Methodology

Corresponding Author: Cesar G. Demayo, Department of Biological Sciences, College of Science and Mathematics MSU-Iligan Institute of Technology, Iligan City 9200 Philippines, Email: cgdemayo@gmail.com

In the selection of the variety to be used in this study, plants showing strong resistance to the common herbivorous insect Uroplata giradi Pic were surveyed in a village where they were abundantly grown. There were 3 general plant types observed in the area. These were documented according to their flower color (White trailing, Purple trailing, and Dallas Red) (Fig. 1). These three varieties were surveyed based on the damage caused by the insect based on the different parts of the plant (flower, leaves, and stem). The damage was rated by comparing the extent of damage based on feeding marks.

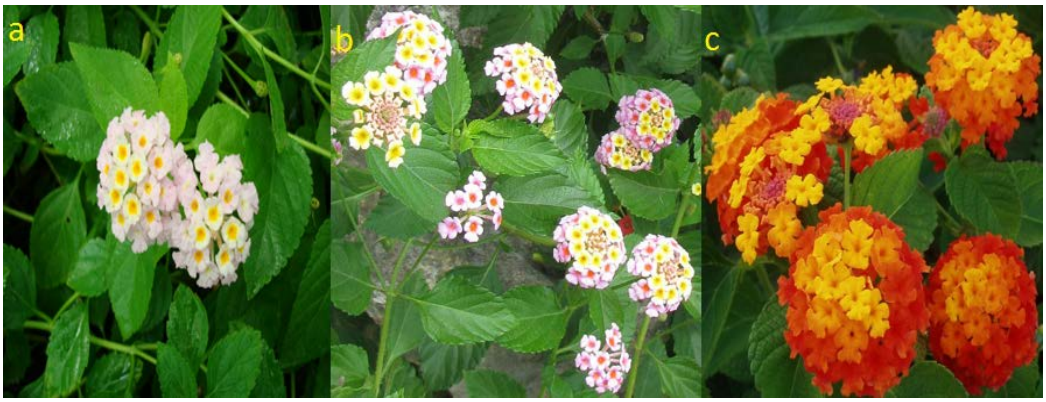


Figure 1. Different varieties of Lantana camara based on flower color. (A. White trailing. B. Purple trailing, C. Dallas Red) (0-no damage portion, 1-distinct damage portion, 2-massive damage portion)

Fresh leaves of the Dallas Red variety of *L. camara* were collected and air dried at room temperature before grinding them to powdered form with the help of mechanical grinder. The plant powder was soaked in 95% ethanol for 3 days at room temperature and the ethanolic extract filtered using Whatman filter paper to obtain clear extract.

Phytochemicals such as alkaloids, flavonoids, saponins, tannins, antraquinons, steroid, cyanogenic-glycosides and fatty acids were carried out and done in Department of Chemistry, College of Science and Mathematics, MSU- Iligan Institute of Technology.

The Antimicrobial assay was performed at the biology laboratory of the University of Philippines (Diliman). The following bacterial strains were used as test organisms: *Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Salmonella typhimurium, Staphylococcus aureus* and *Bacilius subtilis*.

The MTT assay [18] was performed in University of Philippines (Diliman) in January 2016. The MTT assay is a delicate, quantitative and reliable colorimetric assay that measures viability, proliferation and activation of cells. Splenocytes were used in this study for the evaluation of the extracts.

Results

Of the three *L. camara* varieties that were evaluated for resistance, the Dallas red variety was observed to be resistant to the damage of *U. giradi*. (Fig. 2). Based on the severity of the feeding marks, the purple and white trailing varieties were found to be susceptible especially on the feeding damage on the leaf. The damage based on the feeding marks includes missing portion (pierced, trimmed, entire leaf cropped) of the plants. The (DR) Dallas red variety of *L. camara* exhibited the least susceptibility to insect feeding especially on the leaf; thus, it can be argued to be containing an antifeedant or toxic components. The damage observed in the flowers were not used to select the variety to be used since the damage may be caused by how pollination process among insects and birds were done [19, 20].

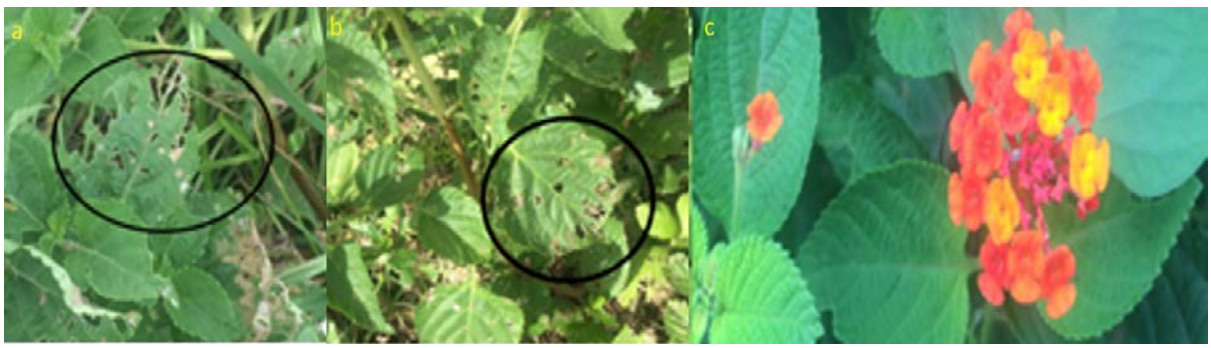


Fig. 2. Damage made on the leaves resulting from leaf feeding by *U. Girardi* (a) purple (b) white and (c) Dallas red

Antimicrobial screening

Table 2 shows *L. camara* Dallas red variety leaf extract showed antimicrobial activity against *K. pneumonia, P. aeruginosa, S. aureus* and *B. subtilis* with AI (antimicrobial index) of 0.3, 0.2, 1.6 and 1.0 respectively. This result is supported by several literatures stated *L. camara*'s significant antibacterial activity, including antibacterial activity against *S. aureus* [21].

Table 2. Antimicrobial screening of Dallas red *L. camara* leaf extract against the different bacterial strains with chloramphenicol as negative control

Test organism	*Sample*	Clearing zone			AI
		1	2	3	
E. coli	*L. camara* leaf extract	-[a]	-	-	0
	Chloramphenicol disc[b]	27			3.5
K. pneumoniae	*L. camara* leaf extract	14	13	12	0.3
	Chloramphenicol disc	38			5.3
P. aeruginosa	*L. camara* leaf extract	12	12	13	0.2
	Chloramphenicol disc	15			1.5
S. typhimurium	*L. camara* leaf extract	-	-	-	0
	Chloramphenicol disc	30			4.0
S. aureus	*L. camara* leaf extract	25	25	38	1.6
	Chloramphenicol disc	33			4.5
B. subtilis	*L. camara* leaf extract	19[a]	20[a]	20[a]	1.0
	Chloramphenicol disc[a]	20			2.3

([a] – no clearing zone; no inhibition of growth of test organism, [b] – 6mm diameter disc, contains 30 ug chloramphenicol)

The resistance of the Dallas red variety against feeding of the insect herbivore was hypothesized to have the presence of compounds that my not only deter feeding but may have antimicrobial and cytotoxic functions such as the phytochemicals observed in the ethanol extract such as Saponins, Flavonoids, Steroids > alkaloids and tannins. Cyanogenic glycosides, anthraquinones and fatty acids were absent.

Table 3. Phytochemicals identified in the Dallas red variety of *L. camara* [-=None, + = present in small quantity, +++ = present in large quantity

Extract	***Alkaloids***	***Saponins***	***Flavonoids***	***Steroids***	***Tannis***	***Cyanogenic-glycosides***	***Anthr-aquinones***	***Fatty Acids***
L. camara	+	+++	+++	+++	+	-	-	-

Stimulation/Suppression of Splenocyte Proliferation (MTT) assay

Results of the evaluation of the effects of different concentrations of the ethanolic extracts of *L. camara* Red Dallas variety against mouse splenocytes show no significant toxicity effects (Fig. 3). Cellular metabolic activity was observed in the lowest concentration but not significant enough to make a conclusive remark that it has toxicity to the cells. It can be observed in the results that at the lower concentration, the compounds in the extract may have slowed down the activity of the splenocytes to metabolize the tetrazolium salt (Table 4).

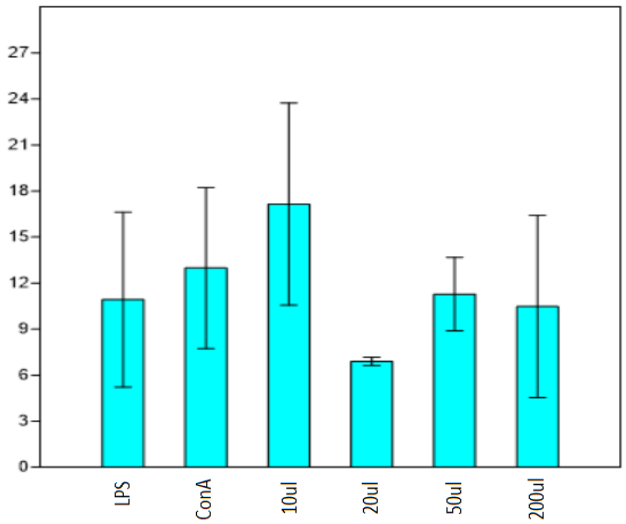


Fig. 3. Effects of the different concentrations of the ethanolic extract of the Dallas red variety of *L. camara* on mouse splenocytes.

Table 4. Statistical results of the comparison of the different concentrations of the ethanol extracts of the Dallas red variety of *L. camara* on mouse splenocytes.

	SS	df	Mean Square	F	P(same)
Between groups	118.046	5	23.6091	3.892	0.03216
Within Groups	60.6659	10	6.0669		
Total	178.712	15			
Omega^2	0.4747				
Levene's Test				Based on means	0.2036
				Based on Medians	0.3356
Welch F test in the case of unequal variances		3.14		9.387	0.04305

GC-MS analysis of the ethanolic extract

Result of the GC-MS spectrum is shown in Fig. 4. It can be seen from the figure that the different compounds that were isolated were of several types and of different concentrations (Fig. 4 and Table 5). These compounds were reported in many studies to have various functions ranging from being an antioxidant to antimicrobials.

Gas Chromatography Mass Spectrum

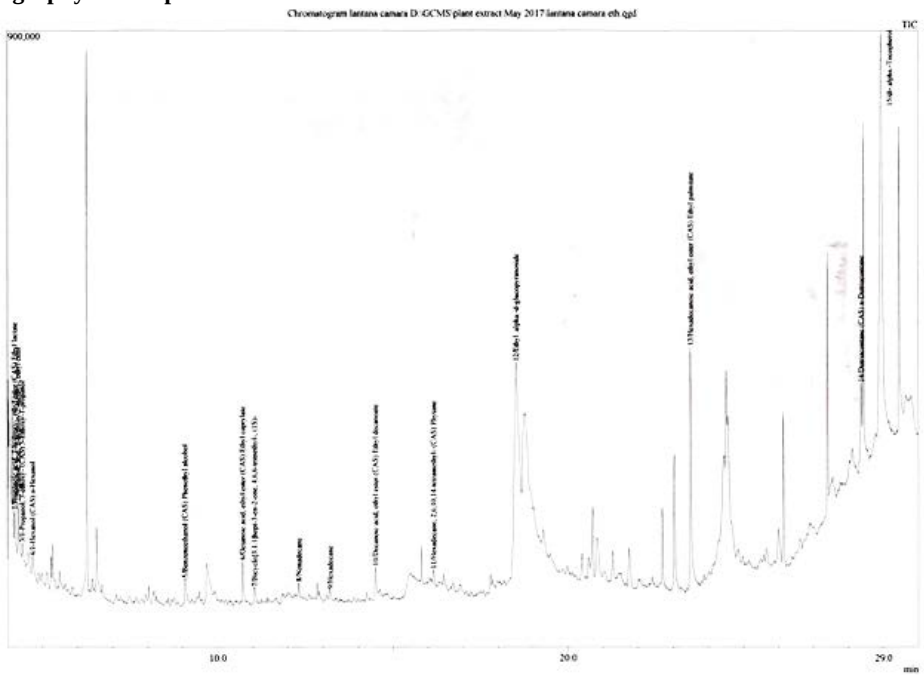
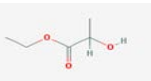
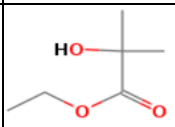
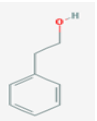
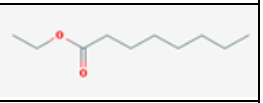
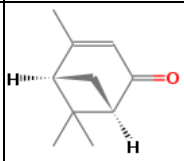
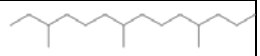
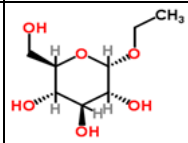
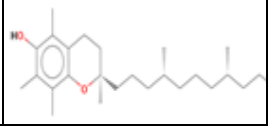


Fig. 4. GC-MS chromatograph of ethanolic leaf extract of *L. camara*

Table 5. The major phytocompounds detected in ethanolic leaf extract of the Dallas red variety of *L. camara* by GC-MS analysis

Sample No.	Name of Compound	Retention Time	Molecular Weight	Mass Peak	Biological Properties	Chemical Stucture
1	Propanoic acid, 2-hydroxy-, ethyl ester	4.205	118	209	Antimicrobial, Antifungal	
2	Propanoic acid, 2-hydroxy-2-methyl-, ethyl ester	4.305	132	217	Antimicrobial, Antifungal	
3	1-propanol, 3-ethoxy-	4.420	104	255	No activity reported	
4	1-Hexanol	4.715	102	241	Anesthetic, Nicotinic antagonists	
5	Benzeneethanol	9.070	122	244	Antimicrobacterial, Anti-infective agent, disinfectant, preservative,	
6	Octanoic acid, ethyl ester	10.705	172	284	No activity reported	
7	Bicyclo(3,1,1) hept-3-en-2-one, 4,6,6-trimethyl-	11.045	150	293	Fragrance agents	
8	n-Nonadecane	12.315	268	238	No activity reported	
9	n-Hexadecane	13.195	226	250	Toxic Substance	
10	Decanoic acid, ethyl ester	14.490	200	233	No activity reported	
11	Hexadecane,2,6,10,14-tetramethyl	16.155	282	239	No activity reported	
12	Ethyl alpha-d-glucopyranoside	18.515	208	281	No activity reported	
13	Hexadecanoic acid, ethyl ester	23.490	284	309	Antioxidant, Hypocholesterolemic, Nematicide, Pesticide, Antiandrogenic, Hemolytic, 5-alpha reductase inhibitor	
14	Dotriacontane	28.365	451	267	Toxic Substance	
15	dl-alpha, -tocopherol	28.925	430	332	Essential vitamin (E)	

The antimicrobial, phytochemical and GC-MS results of the current study confirm the results of several studies which reported that the genus *Lantana* exhibited a variety of biological activities relating these activities in its chemical structure [21-26]. Leaves, stem and root fixed extract analysis of *L. camara* indicated the presence of acid glycosides, alkaloids, flavonoids and triterpines [14, 15, 16, 27-32]. Studies also show the essential oil of *L. camara* showed antibacterial and antifungal activity against microorganisms that are actively working in respiratory and intestinal infections [33-35]. Several species are considered toxic, but the most important toxic species is *L. camara*, to cause photosensitization in ruminants when they ingest large quantities of leaves [36]. Other studies also show the secondary metabolites showing several biological activities such as antibacterial [24, 37-38], fungicidal [26, 27], inflammatory [39], nematicidal [40], inhibitors of α-human thrombin [29, 30], inhibitors of protein kinase C [16] and inhibitors of protein Bcl-xL, an anti-apoptotic protein of the family of Bcl-X [32,41].

Conclusion

The current study has shown the antimicrobial activity and mild toxicity responses of the mouse splenocytes to the ethanolic extracts of *L. camara* Dallas red variety. These responses can be attributed to the various identified compounds isolated from the plant extracts.

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