International Journal of Science and Research Archive
eISSN: 2582-8185
Cross Ref DOI: 10.30574/ijsra
Journal homepage: https://ijsra.net/

(Research Article)

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Phytochemical screening, GC-MS analysis and larvicidal potentiality of *Lantana camara* red petals collected from wad Medani City, Gezira State, Sudan

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International Journal of Science and Research Archive, 2024, 11(02), 001–007

Publication history: Received on 16 January 2024; revised on 23 February 2024; accepted on 26 February 2024

Article DOI: https://doi.org/10.30574/ijsra.2024.11.2.0371

Abstract

Plants are a major source of medicine with a variety of biological deliberations, including antioxidant, antibacterial, antifungal and insecticidal activities. This study aimed to determine the phytochemical and GC-MS composition, in addition to the larvicidal potentiality of *Lantana camara* red petals collected from Wad Medani city, Gezira State, Sudan. The dried granules of red petals samples of *L. camara* were used to run the phytochemical screening tests, whereas the counterpart ethanol extract was used for the GC-MS analysis and the larvicidal potentiality and the morphological deformities using *Anopheles arabiensis* Patton and *Culex quinquefasciatus* Say larvae. The results of these tests showed that, flavonoids, steroids and terpenoids were presented in the red petals of *L. camara*, whereas, alkaloids, glycosides, saponnins and tannins were not detected. The GC-MS identified 42 compounds from the ethanol extract of the red petals, of which Benzyl beta d-glucoside (23.83%), 6-O-acetyl-1[[4-bromophenyl]thio]-beta- (10.12%), Galactopyranoside,1-octylthio-1-deoxy- (9.28%) and the terpenoids caryophyllene (1.40%), Beta-curcumene (1.66%) and Trans-sesquisabinene hydrate (1.72%) were the main components. At the concentration of 1120 mg/L of red ethanol extract, 50% of *Anopheles*, and 47.5% of *Culex* larvae were survived after 24 hour, but after 7 days, no one larva of *Anopheles* nor *Culex* were survived. The deformation monitored on mosquitoes larvae that submitted to Lantana red flowers were dark and swelled bodies, dark guts, loose bodies, dead and stretched body-pupae, in addition to dead emerged adults and emergence of short wings adults.

Keywords: Phytochemical; GC-MS; Larvicidal; *Lantana camara;* Wad Medani; Gezira State

1. Introduction

Lantana camara (common lantana) is a species of flowering plant within the verbena family (Verbenaceae), native to the American tropics [*1*]. The native range of *L. camara* is Central and South America; however, in around 60 tropical and sub-tropical countries worldwide [*2*]. *L. camara* is known to be toxic to livestock such as cattle, sheep, horses, dogs and goats. The active substances causing toxicity in grazing animals are pentacyclic triterpenoids, which result in liver damage and photosensitivity [*3*]. Studies conducted in India have found that *Lantana* leaves can display antimicrobial, fungicidal and insecticidal properties. *L. camara* has also been used in traditional herbal medicines for treating a variety of ailments, including cancer, skin itches, leprosy, chicken pox, measles, asthma and ulcers [4].

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Plants are a major source of medicine with a variety of biological deliberations, including antioxidant, antibacterial, and antifungal activities. Almost 25% of conventional drugs and primary health care of majority relies essentially on plants. Natural antioxidants are broad-spectrum, safe and effective in regulating destructive processes triggered by oxidative stress, induced by free radical's overproduction [5].

Mosquitoes are members of a group of small flies within the family Culicidae. Mosquitoes have a slender segmented body, one pair of wings, one pair of halteres, three pairs of long hair-like legs, and elongated mouthparts [6]. The mosquito life cycle consists of egg, larva, pupa, and adult stages. Eggs are laid on the water surface; they hatch into motile larvae that feed on aquatic algae and organic material. These larvae are important food sources for many freshwater animals, such as dragonfly nymphs, many fish, and some birds such as ducks. The adult females of most species have tube-like mouthparts (called a proboscis) that can pierce the skin of a host and feed on blood, which contains protein and iron needed to produce eggs. Thousands of mosquito species feed on the blood of various hosts vertebrates, including mammals, birds, reptiles, amphibians, and some fish; along with some invertebrates [7]. Mosquitoes are efficient at sucking blood from one individual and mainlining it into another, providing an ideal route for the spread of pathogenic microbes. The control of disease-carrying mosquitoes may in the future be possible using gene drives [8].

The plant based pesticides include several types of pest management intervention: through predatory, parasitic, or chemical relationships. They are obtained from organisms including plants, bacteria and other microbes, fungi, nematodes, *etc*. They are often important components of integrated pest management (IPM) programs, and have received much practical attention as substitutes to synthetic chemical plant protection products [9].

2. Material and method

2.1. The plant materials

The samples of *L. cammara* red petals were collected from the gardens of the main campus, around the Experimental Farm, University of Gezira, Wad Medani City, Gezira State, Sudan.

2.2. Preparation of extracts

The collected red petals were first cleaned manually and then let to dry at room temperature away from direct sunlight. When they became dried, they were crushed to fine granules, and extracted with 99% ethanol for 24 hours using cold extract (5 g plant powder dissolved in 25 ml ethanol), then filtered. The obtained extract was used for running GC-MS analysis and larvicidal potentiality using the mosquito larvae (*Anopheles* and *Culex*) as bioindicators.

2.3. GC–MS analysis

Five ml of Lantana red petals extract were analyzed using GC-MS technique at Central Laboratory, University of Gezira. The chemical constituents revealed from the GC-MS analysis along with their retention time, molecular formula and compound names were presented in the result sections. The library used to identify compounds was NIST 14S. The fragmentation pattern spectra of the unknown components were compared with those of known components stored in the NIST library.

2.4. Phytochemical screening tests

Phytochemical screening for the presence of the main classes in the selected samples were done according to Khalifa and Kehail [10]:

- **Test for alkaloids**: In a test tube, to 3 ml of each extract, 2 drops of Dragendoff's reagent was added. The precipitation of turbid orange red complex denoted the presence alkaloids.
- **Test for flavonoids and flavonones**: In a test tube, to about 4 mg/ml of each extract a piece of magnesium ribbon was added then a drop of concentrated HCl was wisely added. The presence of flavonones was confirmed when the orange colour changed to red; while the presence of flavonoids was indicated by the red to crimson changes.
- **Test for glycosides**: In a test tube, to about1 ml of the filtrate, 10 ml of 50% H_2SO_4 was added. The mixtures was heated for 15minutes then 10 ml of Fehling's solution was added and boiled. The presence of glycosides was indicated by brick red precipitate.

- **Test for saponnins**: in a test-tube, to about 0.5 g of each powdered plant materials, 5 ml of distilled water was added and the test-tube was shaken vigorously for 2 minutes. The presence of saponnins was indicated by the formation of froth that lasted for about 15 minutes.
- **Test for steroids**: In a test tube, 2 ml of each extracts were evaporated to dryness. The residues were dissolved in acetic anhydride then by addition of chloroform. Concentrated H_2SO_4 was gently added. The presence of steroids was confirmed by formation of brown ring at the mid-layer between the liquids and the violet supernatant.
- **Test for tannins**: In a test tube, to 2 ml of each extracts were diluted with distilled water, 3 drops of 5% ferric chloride ($FeCl_3$) solution was added. The appearance of green, black or blue coloration indicated the presence of tannins.
- **Test for terpenoids**: About 0.5 g of each plant powder was extracted in test tube with 2 ml of chloroform; 5 ml of concentrated sulfuric acid was carefully added. The formation of a reddish brown layer indicated the presence of terpenoids.

2.5. The biological potentiality test

Following the instructions of WHO [11], the susceptibility *An. arabiensis* and *C. quinquefasciatus* larvae were tested. The test period was 24 hours and the total number of each larvae used were 80. The dead larvae were counted in percentages and the survived larvae were separated in new rearing cups filled with tap water. The abnormalities in the morphology (deformities) of the dead and survived larvae, pupae and adults were monitored and photographed.

2.6. Statistical analysis

The data obtained from the result of each experiment was summarized as table and was analyzed using suitable statistical tool. The deformities noticed on the mosquitoes larvae were photographed using digital microscope provided with camera.

3. Results

3.1. The phytochemical analysis

The phytochemical analysis of Lantana red petals were represented in Table (1). It was cleared that, flavonoids, steroids, resins and terpenoids were presented, whereas, alkaloids, glycosides, saponnins and tannins were absent from the red petals.

Table 1 Phytochemical screening of *L. camara* red petals

Main class	**Result**
Alkaloids	-
Flavonoids	+
Glycosides	-
Saponnins	-
Steroids	+
Tannins	-
Terpenoids	+

(-) means absence of the main class; (+) means present of the main class

3.2. GC-MS analysis

The GC-MS identified 42 different compounds from the ethanol extract of the red petals of lantana, of which 11 compounds were the main constituents (Table 2), the rest were considered as traces since the concentration of each compound did not exceeded (1.0 %). The main component were Benzyl beta d-glucoside (23.83%), the sulfur containing compounds: 6-O-acetyl-1[[4-bromophenyl]thio]-beta- (10.12%), Galactopyranoside,1-octylthio-1-deoxy- (9.28%) and 2,2-dimethyl-3-[3-methyl-5-(phenylthio)penta- (6.07%), the terpenoids caryophyllene (1.40%), Beta-curcumene

(1.66%) and Trans-sesquisabinene hydrate (1.72%). The GC-MS also identified the nitrogen complex compound glutamine (1.07%).

Table 2 GC-MS main identified compounds from *L. camara* red petals

No.	Ret. time	Compound name	Mol. Form	Area %
1	12.599	Benzoic acid	$C_7H_6O_2$	3.28
2	13.977	Glutamine	$C_5H_{10}N_2O_3$	1.07
3	18.705	2-furanmethanol, 5-ethenyltetrahydr-alpha	$C_{10}H_{18}O_2$	3.42
4	20.122	Caryophyllene	$C_{15}H_{24}$	1.40
5	20.773	Trans-sesquisabinene hydrate	$C_{15}H_{26}O$	1.72
6	22.072	Beta-curcumene	$C_{15}H_{24}$	1.66
7	33.560	Benzyl beta d-glucoside	$C_{13}H_{18}O_6$	23.83
8	34.479	Galactopyranoside,1-octylthio-1-deoxy-	$C_{14}H_{28}O_5S$	9.28
9	35.118	2,2-dimethyl-3-[3-methyl-5-(phenylthio)-	$C_{16}H_{22}OS$	6.07
10	35.480	6-O-acetyl-1[[4-bromophenyl]thio]-beta-	$C_{14}H_{17}BrO_8S$	10.12
11	35.587	9,12,15-octadecatrienoic acid, 2(acetyloxy)-	$C_{25}H_{40}O_6$	4.50

3.3. The biocidal potentiality of Lantana extracts

The survived (No. and %) of mosquitoes 3rd instar larvae against ethanol extract of red petals of Lantana were presented in Table (3). At the concentration of 1120 mg/L, 50% of *Anopheles*, and 47.5% of *Culex* were survived after 24 hour. After 48 hour the survived larvae decreased to 38.8% of *Anopheles*, and 41.3% of *Culex*. The survived larvae decreased to 28.8% of *Anopheles*, and 33.8% of *Culex* after 72 hours, No single pupa of *Anopheles* or *Culex* were formed. After 7 days, no one larva of *Anopheles* nor *Culex* were survived, but 6.3% of *Culex* were completed to adults.

Table 3 Survived (No. and %) mosquitoes 3rd instar larvae against ethanol extract of red petals of Lantana at 1120 mg/L and its impact on late stages

Time	Species	Larvae		Pupae		Adults	
24 hours	*Anopheles*	40	50%	0	0%	0	0%
	Culex	38	47.5%	0	0%	0	0%
48 hours	*Anopheles*	31	38.8%	0	0%	0	0%
	Culex	33	41.3%	0	0%	0	0%
72 hours	*Anopheles*	23	28.8%	0	0%	0	0%
	Culex	27	33.8%	0	0%	0	0%
7 days	*Anopheles*	0	0%	0	0%	0	0%
	Culex	0	0%	4	5%	5	6.3%

3.4. Deformities of the survived mosquito's larvae, pupae and adults

The deformation monitored on mosquitoes larvae that submitted to Lantana red flowers were dark and swelled bodies, dark guts, loose bodies, dead and stretched body-pupae, in addition to dead emerged adults and emergence of short wings adults (Plate 1).

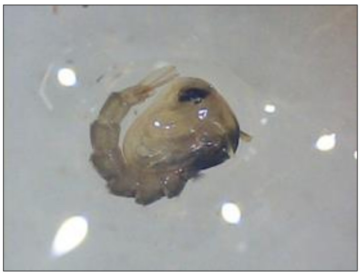
	
Dark body	Loose body
	
Swelled and dark gut	Stretched dead pupa
	
Dead pupa	Short wing male
	
Dead adult	Dead adult

Plate 1 Deformities on mosquitoes larvae caused by the ethanol extract of *L. camara* red petals (*Anopheles* at left, and *Culex* at middle)

4. Discussion

In similar study, the extracts of leaves and flowers from *L. camara* were tested for their polyphenol content (total phenol, total flavonoid, and total alkaloid) and antioxidant potential (total antioxidant activity, iron chelating activity and enzymatic activity (peroxidase and polyphenol oxidase)). Both extracts exhibited high antioxidant and free radical scavenging activities with relatively stronger antioxidant activity in the case of whole flower extracts [12].

In another study, it was cleared that, flavonoids, steroids, and terpenoids were presented in the red petals of Lantana, whereas, alkaloids, glycosides, saponnins and tannins were absent from the tested samples. Terpenoid, has been used as a protective agent against oxidative stress-induced diseases [13].

Plant-derived quinones have shown better antioxidant activity compared to synthetic antioxidants and impart superoxide scavenging activity [14].

The larvicidal potentiality of *L. camara* can be attributed to its chemical composition. This finding was agreed with the suggestion that: anthraquinones, coumarins, and anthocyanin are endowed with many biological activities like antimicrobial, antioxidant, anti-inflammatory and anticancer [15]. Steroids are electron donors, which act upon free radicals and alter them to a more stable compound, thus culmination the terminal chain reaction [16].

L. camara is known to be toxic to livestock such as cattle, sheep, horses, dogs and goats [17]. The active substances causing toxicity in grazing animals are pentacyclic triterpenoids, which result in liver damage and photosensitivity. Kehail [18] who conducted a study in Gezira State found that, *L. camara* red flowers is among 20 different local plant products that can be used to control *Anopheles arabiensis* and *Culex quinquefasciatus* larvae.

5. Conclusion

Flavonoids, steroids and terpenoids were presented in the red petal of Lantana. The GC-MS identified 42 compounds. At the concentration of 1120 mg/L of red petals ethanol extract, and after 7 days, no *Anopheles* nor *Culex* larva were survived. Some deformities were monitored on mosquito's larvae, pupae and adults.

Compliance with ethical standards

Acknowledgements

Thanks are extended to Blue Nile National Institute for Communicable Disease and Applied Chemistry and Central Laboratories, University of Gezira, for hosting the Laboratory work.

Disclosure of conflict of interest

No conflict of interest to be disclose.

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