Phytology

Evaluation of bio-insecticidal capacity of datura (*Datura stramonium* L.) leaves and flowers using GC-MS and phytochemical techniques

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Abstract

The widespread use of synthetic insecticides has led to many negative consequences, resulting in increasing attention to natural products. The aim of this study was to evaluate the bio-insecticidal capacity of *Datura stramonium* leaves and flowers using GC-MS and phytochemical techniques and also mosquito's larvae as bioindicators. The phytochemical screening and the biological effect tests were run at Basic Sciences Laboratories, whereas GC-MS applications were run at the Central Laboratory, University of Gezira. The results showed that, Datura leaves contained saponnins, alkaloids, tannins but the flowers contained also flavonoids and steroids. 14 compounds were detected from Datura leaves ethanol extracts of which Butanol, 3-methyl is the main component (79.76%) followed by Toluene (6.14%) and Phytol (3.9%), while that of the flowers detected also 14 compounds of which formic acid, 3-methylbut-2-yl ester is the main component (82.22%). The ethanol extract of Datura leaves reflected LC50 of: 844.43 mg/L, against *Cx. quiquefasciatus* larvae, while that of the flowers was 636.62 mg/L. The ethanol extract of Datura leaves, after one week, left no survived *Anopheles* or *Culex* larvae, whereas, 10% of *Aedes* larvae were survived. The ethanol extract of Datura flowers, after one week, left no survived *Anopheles* or *Culex* or *Aedes* larvae, but some were developed to pupae. Field assessment should be run to evaluate the impact on the aquatic predators.

Keywords: bio-insecticidal, datura, GC-MS, phytochemical techniques

Introduction

Datura stramonium (family Solanaceae), known by the common names thorn apple, jimsonweed, is a species of flowering plant. It's likely origin was in Central America (efloras.org, 2017) [3]. It is an aggressive invasive weed in temperate climates across the world. *D. stramonium* has frequently been employed in traditional medicine to treat a variety of ailments. It contains tropane alkaloids which are responsible for the deliriant effects, and may be severely toxic (Glatstein *et al.*, 2016) [7].

All parts of *Datura* plants contain dangerous levels of the tropane alkaloids atropine, hyoscyamine, and scopolamine, and the risk of fatal overdose is high among uninformed users, and many hospitalizations occur among recreational users who ingest the plant. Deliberate or inadvertent poisoning resulting from smoking jimsonweed and other related species has been reported (Pennachio, 2010) [9].

The Zuni people used Datura as an analgesic to render patients unconscious while broken bones were set. The Chinese also used it as a form of anesthesia during surgery (Turner, 2009) [12].

The term biological pesticides has been associated historically with biological pest control and it has been defined as a form of pesticide based on micro-organisms or natural products (European Commission, 2008) [4]. Plant produces naturally occurring substances to defend itself against disease, pathogens and some are known to have pesticidal properties (Benhamou *et al*., 2012; European Commission, 2020) [1, 5]. The needs for selective plant product have been rised because of the adverse effects of the broad products on environmental quality and on non-target organisms including human health.

Natural insecticides from plant origin against mosquito vectors have been the main concern for many researches due to their high level of eco-safety. No doubt, the chemical composition of any pant product determines to great extent their mechanism of action. The impact of plant extract on any subjected organisms depended on polarity of solvents used during extraction, level of maturity, nature of active ingredient and promising advances made by plant derived secondary metabolites (Ghosh *et al*., 2012) [6]. The objective of this work was to study the phytochemical composition of *Datura stramonium* (leave and flower parts) ethanol extract and to evaluate their larvicidal activity on some mosquitoes species.

Materials and Methods

Study materials

The samples of Datura (*D. stramonium*) leaves and flowers were brought from within Wad Medani City, Gezira State, Sudan. The larvae of mosquitoes (*Anopheles arabiensis*, *Culex quinquefasciatus* and *Aedes aegypti*) were brought from the insectary of the Blue Nile National Institute for Communicable Diseases, University of Gezira.

Preparation of extracts

The selected plant parts were cleaned manually and then let too dry at room temperature away from direct sunlight, and then crushed to fine granules. Each plant powder was extracted with 99% ethanol for 24 hours using cooled extract (5 g plant powder dissolved in 25 ml ethanol). Each extract was filtered to separate solid parts from ethanol-soluble extract (EE) under reduced pressure. The EE of each product was used to run the GC-MS tests and to estimate the biocidal potentialities of these products using the mosquito larvae as bioindicators.

GC-MS analysis

The ethanol extract of the selected plant parts was analyzed through GC-MS techniques using (GCMS-QP2010 Ultra, Shimadzu Europa GmbH) which was carried out at Central Laboratories, University of Gezira. The library used to identify compounds was NIST 11s.

Phytochemical screening tests

Phytochemical screening for the presence of the main classes (alkaloids, flavonoids, glycosides, saponnins, steroids, trepenoids and tannins) in the selected samples was done according to Khalifa and Kehail (2019) [8].

The biocidal potentiality

Following the instructions of WHO (2012) [13], the susceptibility *An. arabiensis*, *C. quinquefasciatus* and *Ae. aegypti* larvae were tested at different concentrations of Datura leaves and flowers. Two different tests were run: the first was to test the biocidal activity of the ethanol extract of the selected plant parts using only *C. quinquefasciatus* larvae, whereas in the second test the larvae of the three species were used to monitor the survived larvae against one diagnostic concentration for one week.

Statistical analysis

The data obtained from the result of each experiment was summarized as table and was analyzed using Probit analysis to detect the biocidal potentiality through calculating LC's for each product used. The cumulative mortality was calculated for the survival tests.

Results and Discussion

The phytochemical screening

The phytochemical analysis of *D. stramonium* leaves and flowers revealed that, leaves contained more amounts of saponnins than flowers and both contained also alkaloids, but leaves contained tannins and lack flavonoids and steroids which are component of flowers (Table, 1).

Table 1: Phytochemical analysis of *D. stramonium* leaves and flowers

No.	Datura leaf	Datura flower
Saponnins	++	+
Flavonoids	-	+
Tannins	+	-
Glycosides	-	-
Alkaloids	+	+
Steroids	-	+

(-) means absence; (+) means present; (++) means present of the main class in relatively more amount

GC-MS tests

The GC-MS tests of Datura leaves ethanol extracts (Table, 2) revealed the detection of 14 compounds at different retention time (R. time) and concentrations (Area %), of which 1-Butanol, 3-methyl is the main component (79.76%) followed by Toluene (the paint thinner; 6.14%), Phytol (the cyclic diterpine; 3.9%) and also 3-azabicyclo[3.2.2]nonane (0.99%) that have a distinct in vivo activity against *Plasmodium berghei* as stated by (Seebacher *et al*., 2015) [10]. The test also showed the presence of D-Alanine (the non-proteinogenic amino acid, 0.47%) and Cystine (the sulfur containing amino acid; 0.29%). The GC-MS tests of Datura flowers ethanol extracts revealed the detection of 14 compounds at different retention time and concentrations, of which formic acid, 3-methylbut-2-yl ester is the main component (82.22%) followed by Dodecanoic acid, ethyl ester (3.3%), Toluene (2.86%), 3-Methyl-oxiran-2-yl-methanol (2.1%) and Carbamic acid,2-(2-tolyloxycarbonylamino) (2.04%) (Table, 3).

Table 2: GC-MS detected compounds of Datura leaves ethanol extract

Peak	Compound Name	Formula	Mol Wt.	R. time	Area %
1	1-Butanol, 3-methyl	$C_5H_{12}O$	88	2.275	79.76
2	Toluene	C_7H_8	92	2.504	6.14
3	Propna-1-(1-Methylethoxy)-	$C_3H_7NO_2$	89	3.519	2.27
4	1-Propaol, 2-amine-	C_3H_8NO	75	6.539	0.27
5	1-(5-Bicyclo{2.2.1}heptyl)ethylamine	$C_9H_{17}N$	139	7.130	0.63
6	D-Alanine	$C_3H_7NO_2$	89	16.396	0.47
7	Cystine	$C_6H_{12}N_2O_4S_2$	240	13.131	0.29
8	(S)-(+)-1-Cyclohexylethylamine	$C_8H_{17}N$	127	14.394	1.22
9	n-Hexylmethlamine	$C_7H_{17}N$	115	16.366	0.43
10	2-Pentanamine	$C_5H_{13}N$	87	16.570	0.46
11	Undecanoic acid, ethyl ester	$C_{13}H_{26}O_2$	214	17.606	2.89
12	Phytol	$C_{20}H_{40}O$	296	18.835	3.90
13	3-Azabicyclo{3.2.2}nonane	$C_8H_{15}N$	125	19.289	0.99
14	1,2-Propanediamine	$C_3H_{10}N_2$	74	20.785	0.19

Table 3: GC-MS detected compounds of Datura flowers ethanol extract

Peak	Compound Name	Formula	Mol wt	R. time	Area %
1	Formic acid, 3-methylbut-2-yl ester	$C_6H_{12}O_2$	116	2.269	82.22

2	Toluene	$C_6H_5CH_3$	92	2.507	2.86
3	3-Methyl-oxiran-2-yl-methanol	$C_4H_8O_2$	88	3.516	2.10
4	1,2,3-Butanetriol	$C_4H_{10}O_3$	106	3.629	0.35
5	Benzyl alcohol, p-hydroxy-alpha-methylamine	$C_9H_{14}ClNO_2$	203	7.109	0.71
6	Cystine	$C_6H_{12}N_2O_4S_2$	240	7.678	0.47
7	Benzeneethanamine,2,5-dimethoxy-alpha-	$C_{11}H_{17}NO_2$	195	8.807	1.19
8	Carbamic acid,2-(2-tolyloxycarbonylamino)-	$C_{11}H_{14}N_2O_4$	238	10.529	2.04
9	(S)-(+)-1-Cyclohexylethylamine	$C_8H_{17}N$	127	17.689	1.06
10	D-Alanine	$C_3H_7NO_2$	89	13.132	0.78
11	Diisopropylamine	$C_6H_{15}N$	101	16.539	1.23
12	Dodecanoic acid, ethyl ester	$C_{14}H_{28}O_2$	228	17.607	3.30
13	Imidazole,2-amino-5{(2-carboxy)vinyl}-	$C_6H_7N_3O_2$	153	19.291	1.19
14	1-Methyldecylamine	$C_{11}H_{25}N$	171	22.479	0.50

Biocidal tests

1. for the ethanol extracts on *Culex* larvae

The ethanol extract of *D. stramonium* leaves was tested at concentrations of 444 - 1332 mg/L on *Culex* larvae. The tested mortalities ranged between 10 - 82.5% after 24 hrs. The calculated LC50's was 844.43 mg/L (Table, 4).

The ethanol extract of *D. stramonium* flowers was tested at concentrations of 393 - 1173 mg/L on *Culex* larvae. The mortalities ranged between 20 - 95%. The calculated LC50's was 636.62 mg/L, after 24 hrs (Table, 5). The LC_{50} of Datura leaves was lower than that of Datura flowers, i.e. Datura leaves was more potent against *Culex* larvae than Datura flowers.

Table 4: % mortality of *Culex* larvae on ethanol extract of *D. stramonium* leaves

Concentration		%Tested mortality	Probit
mg/L	Log		
444	2.65	10	3.72
666	2.82	30	4.48
888	2.95	57.5	5.20
1110	3.04	65	5.39
1332	3.12	82.5	5.95
Probit analysis			
R^2			0.98
Slope			4.63
LC_{50} (mg/L)			844.43
LC_{95} (mg/L)			1908.87

Control mortality= 0

Table 5: % mortality of *Culex* larvae on ethanol extract of *D. stramonium* flowers

Concentration		Tested mortality (%)	Probit
mg/L	Log		
393	2.59	20	4.16
589.5	2.77	30	4.48
786	2.89	65	5.39
982.5	2.99	80	5.84
1179	3.07	95	6.64
Probit analysis			
R^2			0.93
Slope			5.15
LC_{50} (mg/L)			636.62
LC_{95} (mg/L)			1325.35

Control mortality= 0

2. For the survived larvae

The ethanol extract of *D. stramonium* leaves (at concentration of 401.77 mg/L) was tested on *Anopheles*, *Culex* and *Aedes* larvae for one week to monitor the survived larvae (Table, 6). From the original number (80 individuals) of each mosquito's larvae, 75 larvae (93.8%) of *Anopheles* were killed after 24 hours, while only 15 larvae (18.8%) of *Culex* and 25 larvae (31.3%) of *Aedes* were killed under the same concentration and period. The number survived were 5 (6.2%), 65 (81.2%), and 55 (68.7%), respectively of *Anopheles, Culex* and *Aedes* larvae. After 48 hours the cumulative dead larvae increased to 77 (96.3%) in *Anopheles* with only 3 (3.7%) survived, 22 (27.5%) in *Culex* with 58 (72.5%) survived, and 38 (47.5%) in *Aedes* with 42 (52.5%) survived. After one week, the cumulative dead larvae reached 77 (96.3%) with 3 (3.7%) developed to pupae and no survived individuals of *Anopheles* larvae, while a total of 22 (27.5%) of the *Culex* larvae were died and 58 (72.5) developed to the next instars, whereas, 55 (68.7%) of *Aedes* larvae were killed and 17 (21.3%) developed and 8 (10%) survived. It is of important to mentioned that, not all the survived larvae turned to healthy pupae and adults; some died while they were pupae and some died during emergence from the pupal case, also some developed to week adults that cannot able to flight, but few were developed to complete healthy adults need to be monitored in their lifespan and fecundity. The same product has an LC_{50} of 844.43 mg/L on *Culex* larvae after 24 hours (Table, 4).

Table 6: Survived mosquito larvae on ethanol extract (at 401.77 mg/L) of *D. stramonium* leaves during one week

Time	Species	Ref. No. Larvae	No. Dead larvae	No. Survived	Cumulative Developed	Cumulative dead
24 hrs	*Anopheles*	80	75	5	0	75
	Culex	80	15	65	0	15
	Aedes	80	25	55	0	25
48 hrs	*Anopheles*	5	2	3	0	77
	Culex	65	7	58	0	22
	Aedes	55	13	42	0	38
72 hrs	*Anopheles*	3	0	2	1	77
	Culex	58	0	37	21	22
	Aedes	42	15	27	0	53
One week	*Anopheles*	*2*	0	0	3	77
	Culex	*37*	0	0	58	22
	Aedes	27	2	8	17	55

Ref. No. larvae: the number of larvae survived at the end of the previous day

The ethanol extract of *D. stramonium* flowers (at concentration of 333 mg/L) was tested on *Anopheles*, *Culex* and *Aedes* larvae for one week to monitor the survived larvae (Table, 7). From the original number (60 individuals) of each mosquito's species, 53 larvae (88.3%) of *Anopheles* were killed after 24 hours, while 30 larvae (50%) of *Culex* and 27 larvae (45%) of

Aedes were killed at the same concentration and period. The number survived were 7 (11.7%), 30 (50%), and 33 (55%), respectively of *Anopheles*, *Culex* and *Aedes* larvae. After 48 hours the cumulative dead larvae increased to 53 (88.3%) in *Anopheles* with only 6 (10%) survived, 53 (88.3%) in *Culex* with 7 (11.7%) survived, and 35 (58.3%) in *Aedes* with 25 (41.7%) survived. After one week, the cumulative dead larvae reached 54 (90%) with 6 (10%) developed to pupae and no survived individuals of *Anopheles* larvae, while a total of 59 (98.3%) of the *Culex* larvae were died and 1 (1.7%) developed to the next instars, whereas, 48 (80%) of *Aedes* larvae were killed and 12 (20%) developed and no survived. The same product has an LC_{50} of 636.62 mg/L on *Culex* larvae after 24 hours (Table, 5). It was noticed that, *Culex* larvae were more susceptible to Datura flowers more than *Anopheles* and *Aedes* larvae.

Table 7: Survived mosquito larvae on ethanol extract (at 333 mg/L) of *D. stramonium* flowers during one week

Time	Species	Ref. No. Larvae	No. Dead larvae	No. Survived	Cumulative Developed	Cumulative dead
24 hrs	*Anopheles*	60	53	7	0	53
	Culex	60	30	30	0	30
	Aedes	60	27	33	0	27
48 hrs	*Anopheles*	7	0	6	1	53
	Culex	30	23	7	0	53
	Aedes	33	8	25	0	35
72 hrs	*Anopheles*	6	0	0	6	54
	Culex	7	6	0	1	59
	Aedes	25	7	13	5	42
One week	*Anopheles*	*0*	0	0	6	54
	Culex	0	0	0	1	59
	Aedes	13	6	0	12	48

Ref. No. larvae: the number of larvae survived at the end of the previous day

It was noticed that, most of larvae died after 24 hours, and this may be due to the sustainability of the toxic ingredients of *D. stramonium* leaves and flowers, and the rest of mortality can be attributed to the impact of the toxic ingredients on the internal and external structures of mosquito's larvae. It was also noticed that, *Anopheles* larvae were more susceptible to Datura leaves more than *Aedes* and *Culex* larvae.

Chemical control is an effective strategy used extensively in daily life. However, the widespread use of synthetic insecticides has led to many negative consequences, resulting in increasing attention to natural products. Among bio-pesticides, botanical ones are experiencing a revival due to their eco-toxicological properties. Plants may provide potential alternatives to currently used insect-control agents because they constitute a rich source of bioactive chemicals. Many secondary plant metabolites are known for their insecticidal properties (Zoubiri and Baaliouamer, 2014) [14]. In this context, screening and evaluation of potentiality of Datura leaves and flowers as bio-pesticides were the main concern of this research.

The chromatogram of the leave of *D. stramonium* revealed the presence of six compounds using GC-MS analysis which include Glycerin (22.19%), 6-Pentyl-5,6-dihydro-2H-pyran-2-one (26.49%) and 2,2-Dimethylpropanoic acid, tridec2-ynyl ester (19.16%), Hexadecanoic acid (12.21%), Heneicosane (7.59%) and Di-n-octyl phthalate (12.36%) (Chintem *et al*., 2014) [2], but more compounds were identified in this study, and this may be due to several factors including geographical, seasonal and genetic differences between the two samples.

The ethanol extracts of leaves of *D. stramonium* was evaluated for larvicidal activity against *Ae. aegypti*, *A. stephensi* and *Cx. quinquefasciatus*. The LD50 values were 86.25, 16.07 and 6.25 ppm against *Ae. aegypti*, *A. stephensi* and *Cx. quinquefasciatus*, respectively (Swathi *et al*., 2012) [11].

Conclusions

It can be concluded that, Datura leaves contained saponnins, alkaloids, tannins but the flowers contained also flavonoids and steroids. The GC-MS tests of Datura leaves ethanol extracts detected 14 compounds of which 1-Butanol, 3-methyl is the main component (79.76%) followed by Toluene (6.14%) and Phytol (3.9%), while that of the flowers detected 14 compounds of which formic acid, 3-methylbut-2-yl ester is the main component (82.22%) followed by Dodecanoic acid, ethyl ester (3.3%), Toluene (2.86%). Methyl-oxiran-2-yl-methanol (2.1%) and Carbamic acid,2-(2-tolyloxycarbonylamino) (2.04%). The ethanol extract Datura leaves reflected LC50 of: 844.43 mg/L, against *Cx. quiquefasciatus* larvae, while that of the flowers was 636.62 mg/L. The ethanol extract of Datura leaves (at concentration of 401.77 mg/L) after one week, produced cumulative mortality of 96.3% and 3.7% developed to pupae and no survived individuals of *Anopheles* larvae, while 27.5% of *Culex* larvae were died and 72.5% developed to the next instars, whereas, 68.7% of *Aedes* larvae were killed and 21.3% developed and 10% survived. *Anopheles* larvae were susceptible to Datura leaves more than *Aedes* and *Culex* larvae. The ethanol extract of Datura flowers (at concentration of 333 mg/L), after one week, produced cumulative mortality of 90% but 10% developed to pupae and no survived individuals of *Anopheles* larvae, while the mortality was 98.3% of the *Culex* larvae and 1.7% developed to the next instars, whereas, mortality was 80% of *Aedes* larvae and 20% developed and no survived.

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