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GCMS analysis and mosquitocidal effects of petroleum ether extract of *Datura stramonium* and *Morus alba* against *Aedes aegypti*

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Aedes aegypti (A. aegypti) causes millions of deaths every year being the vector of many human diseases like dengue virus, yellow fever, chikungunya, and Zika. Phytochemicals area blend of secondary metabolites which are eco-friendly as well as controlling agents against *A. aegypti* and therefore diseases spread by them. Chromatographs of the GC-MS analysis of the petroleum ether extract of *Datura stramonium* and *Morus alba* leaves showed the presence of various compounds. Phytol, tetratetraacontane, pentanoic acid in *D. stramonium* and mibemycin b,13-chloro-5-o-demethyl-28-deoxy-6,28-epoxy-25-1{1-mwthyl propyl}-(6r-13s,25A(S), acetic acid, 17-{4-chloro-5-methylhexyl}-4,4,10,13,14-pentamethyl-2,3,4,5,6,7,10, 11,12,13,14,15,16,17-tetradecahydro-1-phenanthyl, lup-20{29}-en-3-one in *M. alba* were the main constituents. The study screened for larvicidal, ovicidal, ovipositional deterrent, and adulticidal activity. The leaves of both plants were collected, washed in tap water, air dried in shade, powdered, and subjected to Soxhlet extractions with petroleum ether solvent. Seven different concentrations of *D. Stramonium* and *M. alba* extracts were performed to get LC_{50}.*D. stramonium* and *M. alba*, recorded LC_{50} at 80.42 and 118.627 mg/mL respectively after 24 h. Whereas LC_{50} after 48 h is recorded at 73.04 mg/mL in *D. stramonium* and 111.68 mg/mL in *M. alba*. The results indicated that *D. stramonium* is a better mosquitocidal agent than *M. alba*.

Keywords: *Aedes aegypti*, *Datura stramonium*, GCMS, Larvicidal, *Morus alba*, Ovicidal

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Introduction

Mosquitoes are one of the major vectors all over the world causing life-threatening diseases. They are major blood-sucking vectors as they carry and transmit deadly parasites and pathogens which cause a devastating impact on human beings. Every year, at least 500 million people in the world suffer from one or other tropical diseases including malaria, lymphatic filariasis, schistosomiasis, dengue, trypanosomiasis, and leishmaniasis[1].

Among all, *A. aegypti* is one well-known vector for transmitting dengue, yellow fever, and chikungunya. Dengue alone had 96 million instances all over the world, out of which, India alone contributed 34% (22-24 million) of the sicknesses in 2010. A total of 99,913 dengue cases and 220 deaths were reported in 2016 among 35 states and UTs in India. Therefore dengue is considered an endemic disease in India[2]. In a report by the European Centre for Disease Prevention and Control, India reported 110473 cases of dengue and 86 deaths in the span of just 10 months i.e., from January to October 2022. Along with high levels of morbidity and mortality, these diseases also inflict great economic loss and social disruption on developing countries like India. *A. aegypti* is the main vector for spreading dengue fever which is caused by an arbovirus. Therefore, mosquito control is the most important aspect to reduce disease cases as no vaccine has as yet been developed for dengue[3].

The constant use of synthetic insecticides for mosquito control leads to some major environmental hazards like persistence and accumulation of non-biodegradable chemicals, biological magnification through the food chains, development of insecticide resistance among vector species and toxic effects on human health and other non-target organisms[4].

Due to such reasons, there was a demand for eco-friendly alternative insecticidal agents along with high mosquito control potentiality. One such alternative could be bioactive products from phytochemicals by systematically exploring the global floral biodiversity.

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These secondary metabolites are produced by the plants for their own protection against herbivory and other infections, but can also be used for mosquito control programme[5].

Phytochemicals are superior to insecticides as they comprise blends of secondary metabolites that act concertedly on behavioural and physiological processes, not just single active ingredients like insecticidal agents. Therefore phytochemicals are nontoxic, easily available at affordable prices, biodegradable, and show broad-spectrum target-specific activities which act concertedly on both behavioural and physiological processes[6].

Although these phytochemicals are potent mosquitocidal agents their exact mechanism of action is not fully known. Due to this reason, investigation of the changes of basic biochemicals in the target specimens treated by phytochemicals is very essential. This biochemical profiling is very valuable in assessing and predicting the toxic effect of these extracts on the test organism. Further, the potential of phytochemicals in the alteration of the biochemicals of insects is of great interest in biological control applications as it explains that these phytochemicals can affect the biochemistry and physiology of insects. This makes biochemical quantification a good tool to understand about reasons for the toxicity of the extracts[7].

Therefore, *D. stramonium* and *M. alba* being easily available, fast-growing, and available throughout the year can be a good alternative. *D. stramonium* (F.) is a species of flowering plant belonging to the Solanaceae family and commonly known as thorn apple, or jimson weed. It is well known in many parts of the world as an aggressive invasive weed[8]. It shows toxicity and hallucinogenic effects on herbivores. It is also known for its medicinal importance to treat epilepsy, burns, and rheumatism[9]. Whereas *M. alba* L. commonly known as white mulberry belongs to the *Moraceae* family. Phenolic acids, flavonoids, flavonols, anthocyanins, macronutrients, vitamins, minerals, and volatile aromatic compounds are some of the bioactive compounds which are abundant in *M. alba*[10]. Many pharmacological effects like antioxidative, diuretic, antiobesity, hypoglycemic, hypotensive, anticholesterol, antidiabetic, and antimicrobial were shown by natural bioactive compounds[11].

A previous report on phytochemicals of *D. stramonium* shows the presence of alkaloids, tannins, flavonoids, terpenoids, and sterols which are known to specifically inhibit growth, morphogenesis, metamorphosis and reproduction in insect[12]. The higher larvicidal activities of ethanolic extract of *D. stramonium* leaves against mosquitoes are supported by earlier studies as well which state that LD50 values for larvicidal activity were found to be 86.25, 16.07, and 6.25 ppm against *Aedes aegypti*, Anopheles stephensi and *Culex quinquefasciatus* respectively[13-14]. Little work is done on, ovicidal, ovipositional and adulticidal activity of *D. stramonium*[15]. *M. alba* contains many bioactive compounds but mosquitocidal activity is not much explored.

Realising the eco-friendly, insecticidal potential of *D. stramonium* and *M. alba*, both were tested as a mosquitocidal agents at different development stages of the mosquito's life cycle e.g., egg, larvae, pupa and adult. Therefore larvicidal, ovicidal, ovipositional deterrent and adulticidal assay were conducted against *A. aegypti*. The development of a mosquitocidal agent that affects all stages of the mosquito life cycle will be beneficial and cost-effective also. Hence, the present study was conducted to investigate the potential mosquitocidal activity of *D. stramonium* and *M. alba* petroleum ether extract against *A. aegypti*. In order to know the underlying cause of the effect of both extracts, GCMS analysis was conducted to identify the phytoconstituents. The extract that shows better results in all mosquitocidal assays screened for the estimation of carbohydrates, proteins and enzyme activities to determine any change in the metabolic activity of treated larvae.

Materials and Methods

Collection and identification of plant samples

During the month of August – September, healthy leaves of *D. stramonium* and *M. alba* were collected from the natural surroundings of Pune (18.5204°N, 73.8567° E), Maharashtra (India) in 2018 and 2021. The authenticity of the sample was verified by the Botanical Survey of India, Pune. No. BSI/WRC/ IDEN.CER./2019/H3 128 and No.BSI/WRC/100-1/Tech./2019/128.

Preparation of plant extract

Leaves were washed thoroughly and kept for shade drying at room temperature (27±2°C). The dried leaves were first powered and later subjected to Soxhlet extractions with petroleum ether solvent for 1 day. Petroleum ether is a nonpolar solvent with a boiling point range of 35 to 60°C and can dissolve many organics, this makes it a good solvent for

extraction. The filtrates were concentrated to dryness by rotary evaporation at 50°C and kept in the freezer for further use[16].

Gas chromatography-mass spectrometry analysis

Analysis of effective components

The samples were subjected to gas chromatography (Agilent 7890A) and mass spectrometry (Accu TOF GCv, Jeol) to identify the bioactive constituents of the selected plants. Helium was used as carrier gas at a constant column flow rate of 1 mL/min. The GC programme was set for *D. stramonium* as 10; 60-1M-8-200-1M-8-275-15M-5-280 ET and for *M.alba* as 10; 60-1M-8-200-1M-8-200-15M-5-280 ET[17]. Here ET is Ethyl acetate in which the sample is dissolved before loading in GC column. M is representing the time duration in minutes for a specific temperature to vaporise the samples. The programme represents the pre-set conditions for the rise in temperature for a particular duration of time

Identification of major compounds of different plants

Compounds of each plant extract were identified on the basis of their area percentage calculated from the GC- chromatogram and mass spectrometry results in reference to the NIST standard database (NIST 2008).

Rearing of mosquito

A. aegypti mosquitoes were reared as per WHO guidelines. The mosquitoes were obtained from Ross life sciences, Pune. Larvae and eggs population were maintained according to the guidelines in different sets up. Temperature and relative humidity were maintained at 27±2°C and 75±5% respectively under 12:12 light and dark photoperiod cycle[18].

Larvicidal assay

Larvicidal activity of *D. stramonium* and *M. alba* were carried out in the laboratory on IVth instar larvae of *A. aegypti*. The IVth instar larvae were visually detected by relatively bigger size with prominent 10 abdominal segments, the last segment having prominent siphon and anal papillae, and a large head with enormous setae. WHO's (2005) bioassay protocol, with slight modifications, is used to calculate larvicidal activity (percentage of mortality) and LC_{50} values. Stock solution (1000 mg/mL), was prepared with 100 mg of test material dissolved in 1 mL of acetone and later diluted in 100 mL of distilled water in the conical flask. Series of five concentrations of 20, 40, 60, 80,100, 120, and 140 mg/mL was prepared for *D. stramonium* from the stock solutions. On the other hand 40, 60, 80, 100, 120, 160, and 180 mg/mL concentrations were prepared for *M. alba* from the stock solutions. Twenty-five larvae were taken and no. of dead larvae was counted every 24 and 48 h. Also, 25 IVth instar larvae in 1 mL of acetone mixed with water to make 100 mL were taken as control. Three replicates were set to check the mortality for each concentration. The percentage of larval mortality was recorded after 24 and 48 h and corrected using Abbott's formula[19-20].

Statistical analysis

The LC_{50} and LC_{90} values were calculated by SPSS software and the average mortality data was subjected to Probit analysis calculations. 95% fiducial limits of upper confidence limit and lower confidence limit and regression equation were calculated using the SPSS software[21].

Ovicidal assay

The ovicidal activity of *D. stramonium* and *M. alba*, was assessed by treating the eggs at LC_{50} value obtained from larvicidal assay as well as two concentrations above and below it, to determine the range. For *D. stramonium* 40, 60, 80, 100, 120 mg/mL and for *M. alba* 80, 100, 120, 140, 160 mg/mL concentrations were prepared and the eggs of *A. aegypti* were separately exposed to each concentration. Five replicates of each concentration was set to check the activity. The eggs were exposed for five days. A separate set for the control group was exposed to acetone. The per cent ovicidal activity was calculated by the following formula[22-23].

$$\text{Per cent ovicidal activity: } \frac{\text{Number of unhatched eggs}}{\text{Total number of eggs introduced}} \times 100$$

Oviposition deterrent assay

The deterrent activity of *D. stramonium* and *M. alba* was tested against *A. aegypti* mosquitoes by using the method of Elango[24]. Fifteen three-day-old gravid females were taken and after blood feeding, they were kept in the mosquito cage (45*38*38*cm^3) for the testing. The cage was fully covered with a plastic screen with a glass top and a muslin sleeve for access so as to prevent the escape of any mosquito. To prevent the starvation of mosquitoes, 10% sucrose solution was kept in the cage for feeding at all times. For testing the ovipositional deterrent, one bowl treated with the test material was kept in one corner whereas, the other one with control solvent i.e. acetone was kept in the opposite corner of the cage.

The position of both the bowls was kept interchanging at different intervals to nullify the effect of position on oviposition. Serial dilutions of the extract were made in acetone. The desired concentration of test solutions was obtained by treating 100 mL of water and acetone with the extract. The temperature was fixed at 27±2ºC and relative humidity at 70-80%. The number of eggs laid in treated and control bowls was counted and recorded after 24 h of experiment[25].

The following formula is used to get the ovipositional deterrence percentage.

$$ER\% = \frac{NC - NT}{NC} \times 100$$

where, ER= Per cent effective repellency, NC= Number of eggs in control, and NT= Number of eggs in treatment.

Adulticidal assay

The adulticidal activity was conducted by following the WHO standard method. Briefly, plant extracts were dissolved in acetone to prepare a testing concentration of 10 mg/mL. Whatman No 1. Filter papers (12 × 15 cm) were impregnated with 2.5 mL of testing concentration. The impregnated papers were air-dried for 5 min and then inserted into an exposure tube (10 x 8 cm)in the WHO testing kit. Negative control was set with acetone. Twenty, 2–5 day old, blood-starved female mosquitoes were used for the adulticidal experiment. These mosquitoes were introduced into the holding tube and held for 1 h to acclimatize and later were transferred to the exposure tube by gentle blowing for 1 h and then transferred back to the holding tube to recover. The recovering mosquitoes feed on a pad of cotton soaked with a 10% glucose solution. The number of dead mosquitoes was recorded and the per cent mortality was calculated after 24 h recovery period. Each extract was tested in duplicate and the assay was repeated three times[25].

Estimation of carbohydrates

Carbohydrates (glucose) content was determined by anthrone method[26].

Estimation of protein

Protein content was determined by folin-Lowry method[27].

Enzyme activity

The amylase and invertase enzyme content was determined by DNSA method[28-29].

Results

Major compounds

The GC-MS analysis of the Petroleum ether extracts of *D. stramonium* and *M. alba* accounted for 23 phytocompounds in *D. stramonium* and 6 phytocompounds in *M. alba* as represented in Tables 1 and 2 with their respective retention time, area, molecular weight and molecular formula. Molecular structures of active compounds of *D. stramonium* are shown in Fig. 1 whereas molecular structures of active compounds of *M. alba* is shown in Fig. 2. The chromatogram showed the peaks with their retention time are presented in Fig. 3 and 4. The three major compounds of *D. Stramonium* are phytol ($C_{20}H_{40}O$), tetratetraacontane ($C_{44}H_{30}$), pentanoic acid ($C_5H_{10}O_2$) whereas three major compounds of *M. alba* are mibemycin b,13-chloro-5-o-demethyl-28-deoxy-6, 28-epoxy-25-1{1-mwthyl propyl}-(6r-13s,25A(S) ($C_{34}H_{49}CO_7$), acetic acid, 17-{4-chloro-5-methylhexyl}-4,4,10,13,14-pentamethyl-2,3,4,5,6,7,10,11,12,13,14,15,16,17-tetradecahydro-1-phenanthyl ($C_3H_{55}CIO_3$), and lup-20{29}-en-3-one ($C_{30}H_{48}O$).

Larvicidal assay

The results of larvicidal activity obtained after exposing IVth instar larvae of *A. aegypti* to various concentrations of *D stramonium* and *M. alba* exhibits potent lethality against the mosquito species tested. Extract of *D. stramonium* shows 20-40% mortality against IV instar larvae of *A. aegypti* at 40-80 mg/mL when exposed for 24 h, while *M. alba* kills 20-40% IV instar larvae *A. aegypti* at 80- 100 mg/mL when exposed for 24 h. Mortality percentage is depicted in Table 3.

The data obtained from probit analysis of plant extract against fourth instar larvae of *A. aegypti* at different concentrations are represented in Table 4. The LC_{50} and LC_{90} of *D. stramonium* against IV instar larvae of *A. aegypti* after 24 h post treatment was 80.42 and 129.5 mg/mL respectively where as LC_{50} and LC_{90} after 48 h of exposure was 73.04 and 123.40 mg/mL respectively. The LC_{50} and LC_{90} of *M. alba* against IV instar larvae of *A. aegypti* after 24 h post treatment was 118.627 and 191.46 mg/mL respectively where as LC_{50} and LC_{90} after 48 h of exposure was 111.68 and 186.19 mg/mL (Table 4).

Ovicidal assay

D. stramonium recorded its highest ovicidal activity of 66.45% against *A. aegypti* at 80 mg/mL and whereas *M. alba* recorded 47.15% ovicidal activity against *A. aegypti* at 120 mg/mL (Table 5). In contrast, the ovicidal activity in control I and IIis 5.18 and 8.88% respectively

Table 1 — Major compounds of petroleum ether extract of *D. stramonium*

S. No	Retention time	Name of the compound	Molecular formula	Molecular weight	Area {Intens. *sec]
1	4.21	n-3-Triphosphacyclopropane molybdenum, dicarbonyl-(n-5,1, butylcyclopentadienyl	$C_{11}H_{13}MoO_2P_3$	368	19143.34
2	6.32	Pentanoic acid	$C_5H_{10}O_2$	105	624741.14
3	7.63	Butanoic acid	$C_4H_8O_2$	88	46765.25
4	8.32	Hexanoic acid, anhydride	$C_{12}H_{22}O_3$	214	18665.52
5	9.19	3-pyridinecarboxylic acid,2,7,10-tris(acetyloxy)-1,1a,2,3,4,6,7,10,11,11a-decahydro-1,1,3,6,9-pentamethyl-4-oxo-4a,7a-epoxy-5H-cyclopenta(a)cyclopropa(f)cy	C32H39NO10	597	7471.62
6	10.89	4a Phorbol12, 13 didecanoate	$C_{40}H_{64}O_8$	672	19905.72
7	22.96	Phytol	$C_{20}H_{40}O$	296	99480.34.
8	27.29	D-Glucopyranoside,[3β, 22α,25S]-22,25-epoxy-3methoxyurosl-5-en-26-yl,2,3,4,6-tetra-D-methyl	$C_{38}H_{62}O_9$	662	3563.52
9	28.13	3-Phorbinepropanoic acid,9-acetyl-14 ethyl-13,14-dihydro-21-[methoxycarbonyl]-4,8,13,18-tetramethyl-20-oxo-3,7,11,15-tetramethyl-2-hexadecenyl ester	$C_{55}H_{76}N_4O_6$	888	7486.92
10	29.27	Tetratetracontane	$C_{44}H_{90}$	618	83710.65
11	30.73	1,3,5, Trimethyl-2,4,6-tris(3,5 di-tert-butyl-4-hydroxybenzyl) benzene.	$C_{54}H_{78}0_3$	774	5895.94
12	31.11	10-Acetoxy-2-hydroxy-1,2,6a,6b,9,9,12a-heptamethyl-1,3,4,5,6,6a,6b,7,8,9,10,11,12,12a,12b,13, 14b-octadecahydro-2H-picene-4a-carboxylic acid,methyl	C33H52O5	528	3479.7
13	31.62	Tetratetracontane	$C_{44}H_{90}$	618	131265.87
14	32.73	Tetratetracontane	$C_{44}H_{90}$	618	89471.06
15	33.11	Octadecanoic acid,1-[[1-oxohexadecyl)oxy}methyl}-1,2-ethanediyl ester	C55H106O6	862	1949.72
16	34.3	Tetratetracontane	$C_{44}H_{90}$	618	111905.39
17	34.43	MibemycinB,%-demethoxy-5-one-6,28-anhydro-25-ethyl-4-methyl-13-chloro-oxime	C32H44ClNO7	589	13714.62
18	35.04	Tetratetracontane	$C_{44}H_{90}$	618	136187.76
19	36.11	Vitamin E	C29H50O2	430	142004.06
20	36.78	Vitamin E	C29H50O2	430	246961.7
21	37.31	Olean-12-ene-3,28-diol,[3β]	C30H50O2	442	4391.05
22	39.07	Tetratetracontane	$C_{44}H_{90}$	618	19457.13
23	40.18	1,3-dioxane,5-(hexadecyloxy)-2-pentadecyl-trans-	C35H70O3	538	7601.02

Table 2 — Major compounds of petroleum ether extract of *M. alba*

S. No	RT	Name of the Compound	Molecular formula	Molecular weight	Area {Intens.*sec]
1	32.6	1,1'.3',1'-Tetphenyl,3,3',5,5"-tetrabromo-5-{3,5-dibromophenyl)	C24H12Br6	774	34269.93
2	34.14	Milbemycinb,13-chloro-5-O-demethyl-28-deoxy-6,28-epoxy-25-{1-methylpropyl}-[6R,13S,25R(S)]-	C34H49ClO7	604	20465.35
3	34.44	ϒ-Tocopherol	C28H48O2	416	222316.98
4	36.09	α-Tocopherol-B-D-mannoside	C35H60O7	592	43445.44
5	41.22	Acetic acid,17-(4-chloro-5-methoxy-1,5-dimethylhexyl)-4,4,10,13,14-pentamethyl-2,3,4,5,6,7,10,11,12,13,14,15,16,17-tetradecahydro-1-phenanthryl-	C33H55ClO3	534	88936.39
6	42.98	Lup-20[29]-en-3-one	C30H48O	424	88187.67

Table 3 — Mortality % of fourth instar larvae of *A. aegypti* exposed for 24 and 48 h (in mg/mL)

S. No	Plant extract	Exposure (h)	Control	20 mg/mL	40 mg/mL	60 mg/mL	80 mg/mL	100 mg/mL	120 mg/mL	140 mg/mL
1	*D. stramonium*	24	0.3	3.3	19	33.5	47.7	70.5	83	93.7
		48	2.8	7.2	22.8	37.3	54.7	77.5	90	93.7
			Control	40 mg/mL	60 mg/mL	80 mg/mL	100 mg/mL	120 mg/mL	160 mg/mL	180 mg/mL
2	*M. alba*	24	2.0	2.5	14.8	29.3	46.0	51.5	67.0	89.0
		48	0.8	7.2	19.5	34.0	50.7	56.2	71.7	100.0

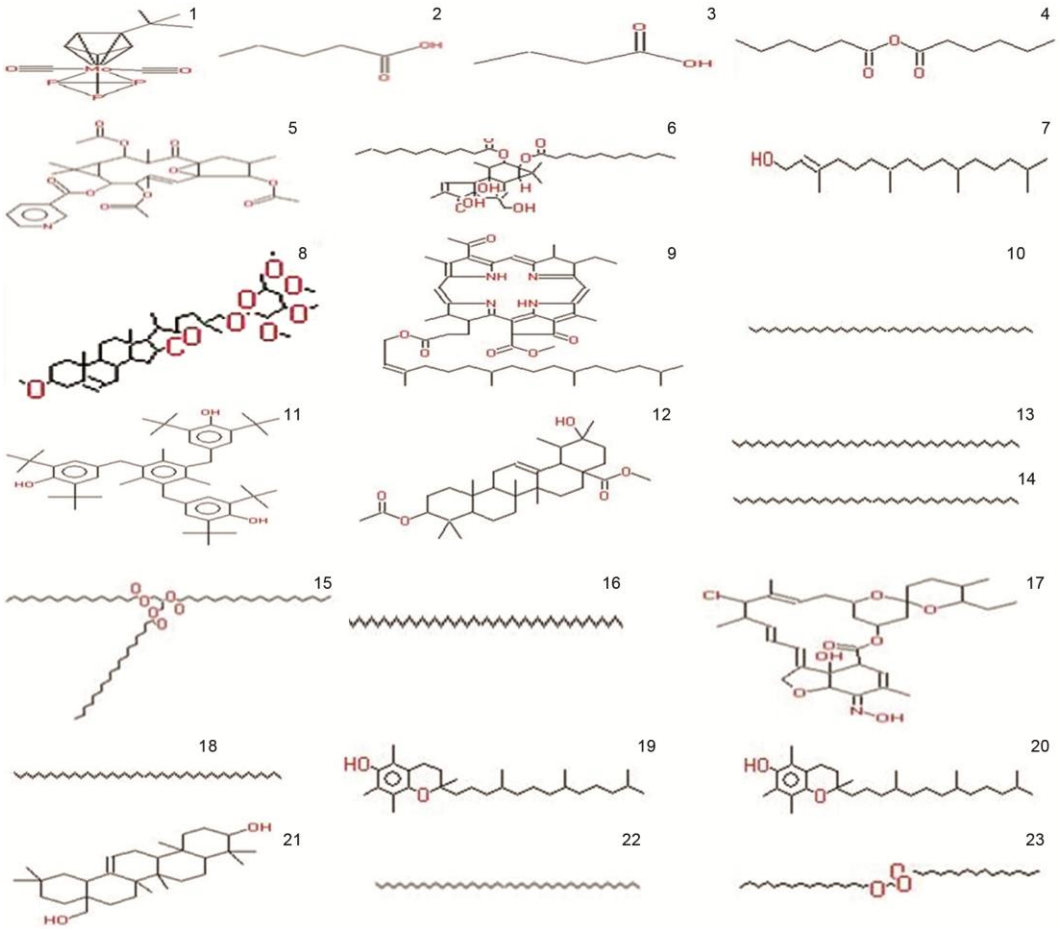

Fig. 1 — Structures of active compounds of *Datura stramonium*. (Names of corresponding s. no in Table 1)

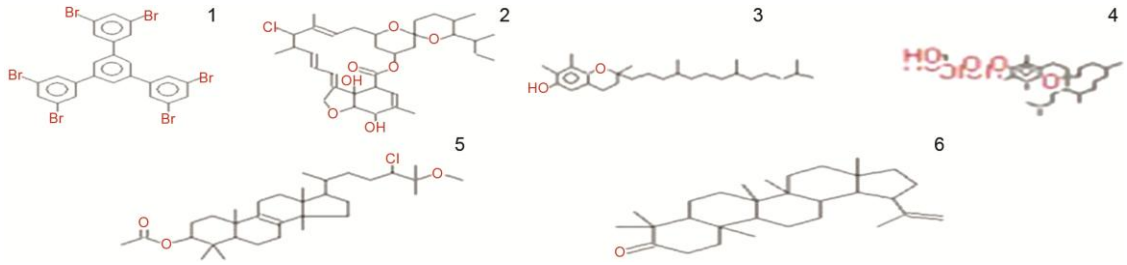

Fig. 2 — Structures of active compounds of *Morus alba*. (Names of corresponding s. no in Table 2)

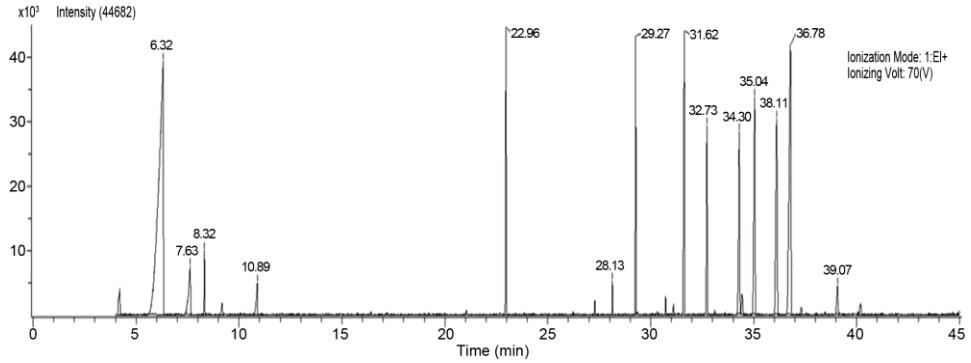

Fig. 3 — GC-MS chromatogram of petroleum ether extract of *D. stramonium*.

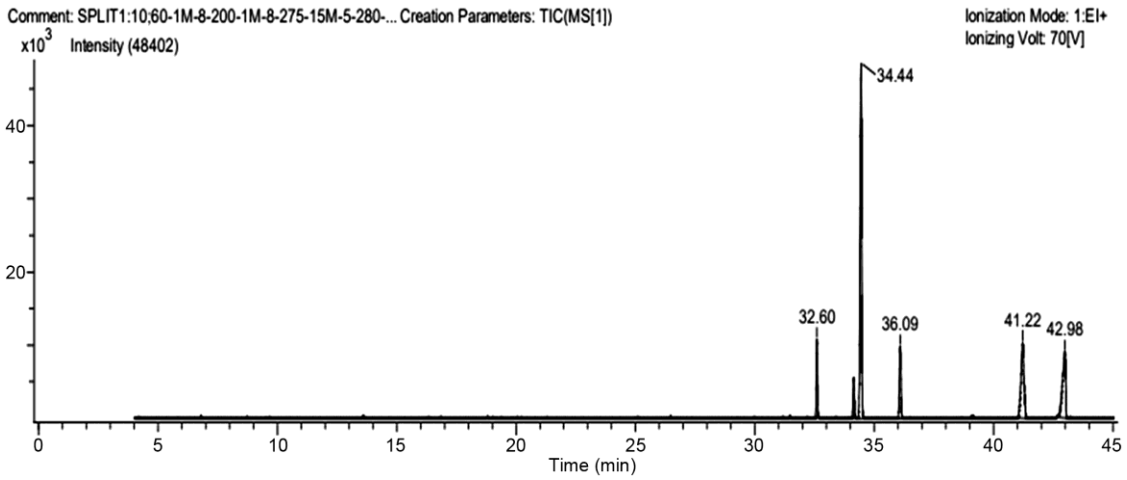

Fig. 4 — GC-MS chromatogram of petroleum ether extract of *M. alba*.

Table 4 — Lethal concentration of *D. stramonium* and *M. alba* against IVth instar larvae of *A. aegypti*

S. No	Plant extract	Exposure (h)	LC_{50} (mg/mL)	LC_{90} (mg/mL)	Regression equation	95% Confidence limits			
						LC_{50}		LC_{90}	
						LCL	UCL	LCL	UCL
1	*D. stramonium*	24	80.42	129.5	y=-2.100+0.026x	76.26	84.66	122.85	137.559
		48	73.04	123.4	y=-1.859+0.025x	68.08	77.3	116.81	131.33
2	*M. alba*	24	118.627	191.46	y=-2.087+.018x	112.67	124.98	180.34	205.37
		48	111.68	186.19	y=-1.921+0.017x	105.74	117.92	175.23	199.89

Note: SSPSS 16 software is used for statistical analysis.

Table 5 — Effect of plant extracts on egg hatchability of *A. aegypti*

S. No	Plant extract	Dose (mg/mL)	Parameters	Average	% Ovicidal activity
1	*D. stramonium*	80	Eggs exposed	152.60	66.45
			Unhatched eggs	101.40	
2	*M. alba*	120	Eggs exposed	126.40	47.15
			Unhatched eggs	59.60	
3	Control	Set- 1	Eggs exposed	180.20	5.88
			Unhatched eggs	10.60	
4	Control	Set- 2	Eggs exposed	165.20	6.42
			Unhatched eggs	10.60	

Oviposition deterrent assay

The ovipositional deterrent activity of petroleum ether extract of *D. stramonium* against *A. aegypti* was higher in contrast to *M. alba*. *D. stramonium* showed its highest ovipositional repellency activity of 92.67 at 80 mg/mL whereas *M. alba* showed 62.08% ovipositional repellency activity at 120 mg/mL against *A. aegypti*. On the other hand, the control group exhibited minimal activity as shown in Table 6.

Adulticidal activity

The no. of dead adult mosquitoes was recorded after treatment with extracts of *D.stramonium* and *M. alba* (Table 7). Analysis of petroleum extracts revealed the fact that both extracts show promising adulticidal activity against *A. aegypti*. *D. stramonium* showed 55.20% whereas *M. alba* showed 49.60% adulticidal activity against *A. aegypti*. On the other hand, adulticidal activity is negligible in control.

Estimation of carbohydrates, proteins, and enzymes

Generally, the carbohydrate concentration was increased in treated larvae in comparison with the control. The larvae treated with *D. stramonium* extract do not show any major difference in the concentration of carbohydrates among the control and treated samples (Table 8).

Table 6 — Effect of Petroleum ether extracts of *D. stramonium* and *M. alba* on oviposition deterrence of *A. aegypti*

S. No	Plant extract	Dose (mg/mL)	Parameters	Average	% Repellency	Ovipositional activity index
1	*D. stramonium* (leaves)	80	Control	507.20	92.67	0.86
			Treated	37.20		
2	*M. alba* (leaves)	120	Control	462.00	62.08	0.45
			Treated	175.20		

Where R= replication set

Table 7 — Effect of plant extracts for adulticidal activity against *A. aegypti*

S. No	Plant extract	Average in % after 1 h of exposure	Average in % after 24 h of exposure
1	*D. stramonium*	71.20	55.20
2	*M. alba*	65.60	49.60
3	Control I	2.8	5.6
4	Control II	2.0	2.4
5	Control III	1.8	4.0

Table 8 — Estimation of carbohydrates

S. No	Sample	Absorbance mean (nm)	Concentration of sample (ug/mL)
1	Standard(glucose)	1.680	400
2	*A. aegypti* treated with Control	0.723	172.14
3	*A. aegypti* treated with *D. stramonium*	1.513	160.23

Table 9 — Estimation of protein

S. No	Sample	Absorbance mean (nm)	Concentration of sample (ug/mL)
1	Standard (BSA)	1.518	1500
2	*A. aegypti* treated with Control	1.013	1000
3	*A. aegypti* treated with *D. stramonium*	0.787	777.6

Table 10 — Estimation of enzyme

S. No	Name of the sample	Absorbance mean (nm)	Product form	Enzyme activity (ug/mL/min)
I	Amylase enzyme			
2	*A. aegypti* treated with Control	0.105	139	0.0386
3	*A. aegypti* treated with *D. stramonium*	0.027	36	0.01
II	Invertase enzyme			
2	*A. aegypti* treated with Control	0.105	124	0.0181
3	*A. aegypti* treated with *D. stramonium*	0.035	40	0.0058

The result revealed that in *A. aegypti*, the concentration protein level of the treated decreased as compared to the control. The *A. aegypti* larvae when treated with *D. stramonium* extract it was seen that the 22% of protein level decreased (Table 9).

Enzyme activity was also decreased in treated larvae. Amylase activity in *A. aegypti* when treated with *D. stramonium* enzyme activity gets lowered by 73% as compared with control (Table 10).

Discussion

Mosquitoes are the vector of some of the world's most deadly diseases. In the present study, the focus is on the control of *A. aegypti*. The extensive use of synthetic organic chemical insecticides leads to many environmental hazards like resistance in major vector species, pollution, and bioaccumulation. This has necessitated the need to develop a more potent and environmentally safe insecticide. Phytochemicals serve as a suitable alternative to synthetic insecticides which are relatively safe, inexpensive and safe for non-target organisms which are readily available in several areas of the world[30]. Several studies have focused on natural products for controlling mosquitoes as larvicides and insecticides with varied results[31-33].

*D. stramonium*is a plant with both poisonous and medicinal properties and has been proven to have great pharmacological potential with great utility and usage in folklore medicine and is traditionally being used as a medicinal plant for the treatments of malarial fevers, and liver problems[34]. *M. alba* is an immune-suppression, antioxidant, antibacterial, anti cancer agent and very effective against cardiovascular diseases[35]. The toxicity level in petroleum ether extract could be attributed to the presence of phytol, tetratetraacontane, pentanoic acid in *D. stramonium* and mibemycin b,13-chloro-5-o-demethyl-28-deoxy-6,28-epoxy-25-1{1-mwthyl propyl}-(6r-13s,25A(S), acetic acid, 17-{4-chloro-5-methylhexyl}-4,4,10,13, 14-pentamethyl-2,3,4,5,6,7,10,11,12,13,14,15,16,17-tetradecahydro-1-phenanthyl, lup-20{29}-en-3-one in *M. alba*. These phytochemicals have been shown to have insecticidal properties against eggs, larvae, pupae, nymphs and adult insects. *D. stramonium* and *M. alba* both show good larvicidal, ovicidal,

ovipositional deterrent, and adulticidal activity and this high mosquitocidal activity of the active fractions was attributed to the ability of the non-polar solvent to extract toxic mosquitocidal agent. The study has revealed the potential of *D. stramonium* as a source of mosquito larvicidal agents with low LC_{50} values. *D. stramonium* shows larvicidal activity with LC_{50} at 80 and 73.04 mg/mL whereas *M. alba* shows larvicidal activity with LC_{50} at 118.62 and 111.68 mg/mL after 24 and 48 h respectively. *D. stramonium* shows 66.45% ovicidal activity, 92.67% ovipositonal deterrency and 55.2% adulticidal activity at 80.42 mg/mL in comparision to *M. alba* with 49.6% ovicidal activity, 62.08% ovipositonal deterrency and 49.6% adulticidal activity at 120 mg/mL (Fig. 5) A similar observation was reported by Olofintoye *et al.*[36]. The better activity of *D.stramonium* can be attributed to the phytol derivatives from a plant extract of *D. stramonium* proven by previous studies as well, which can bring the changes in the biochemical composition in the treated larvae proven by biochemical quantification done in the present study. The petroleum ether extracts of *D. stramonium* (leaves) show a 22% decrease in protein and a 73% decrease in enzyme level and not much change in carbohydrate level in treated larvae which causes high mortality among IVth instar larvae[37] Earlier studies has reavealed that extreme increase or decrease in the protein content during larval stages could be highly detrimental for the larval growth and sometimes pupation completely halts due to extreme changes in protein content[38,39]. Amylase enzyme is responsible for breaking down polysaccharides, so any decrease in its content will also halts larval growth. The petroleum ether extracts of *D. stramonium* could be employed in the control of mosquitoes as vectors. In summary, these reports revealed phytol in *D. stramonium* leaves being responsible for the mosquitocidal activity for the control of mosquitoes and subsequently in the prevention of, yellow fever virus, chikungunya virus, and Zika virus.

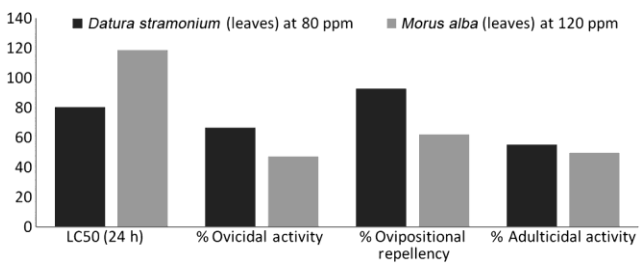

Fig. 5 — Mosquitocidal activities of *D. stramonium* and *M. alba.*

Conclusion

The present study showed that the petroleum ether extract of *D. stramonium* and *M. alba* exhibits good mosquitocidal effects against *A. aegypti*. While comparing the two, *D. stramonium* proves to be better and more effective mosquitocidal agent. These results suggest that the extract can be exploited as a potential natural product for mosquito management. However, further their effects on non target organisms needs to be tested.

Conflict of interest

The authors declare no conflict of interest.

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