Chemical Profiling, Larvicidal Activity and Antihemolytic Property of *Allium sativum* L. and *Allium cepa* L. Essential Oil

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Abstract

Essential oils are plant derived concentrates of the secondary metabolites responsible for the aromatic flavor attributing to its various medicinal properties. Fresh *Allium sativum* (*A. sativum*) and *Allium cepa* (*A. cepa*) were subjected to steam distillation for isolation of essential oil characterized by performing Gas Chromatography – Mass Spectroscopy (GC-MS). Chromatogram of the essential oil depicted the presence diallyl sulfide (5.35%), 2-(2'-carbamoylphenoxy)-butanoic acid (2.64%), 2-ethyl-5-methylthiophene (0.42%), diallyl disulphide (18.76%), 3-(2-thia-4-pentenyl)-1-thia-cyclohex-5-ene (1.09%) and dimethyl tetrasulphide (0.15%), 2,4-dimethylpyrido[2,3-d]pyrimidin-5-one (47.91%), 2,4-Thiazolidinedione (0.01%), 5-chloro-2-hydroxy-1,3-dinitrobenzene (5.93%), 6-Methoxy-1-methyl-3,4-dihydroisoquinoline (47.91%) in *A. sativum* and *A.cepa* respectively. Larvicidal activity against third instar larvae of *Anopheles stephensi* (*A. Stephensi*) was assessed by following the standard protocol of World Health Organization. The 50% lethality (LC_{50}) of *A. stephensi* larvae was observed at 265.96 ± 1.88 ppm and 357.14 ± 2.36 ppm of *A. sativum* and *A. cepa* essential oil correspondingly. The mortality rate of the larvae was both time and dose dependent. Besides, the *in vitro* antihemolytic activity of the essential oil was also assessed using Sheep erythrocytes. The erythrocyte lysis was inhibited by the essential oils of both *A. sativum* and *A. cepa* in a concentration dependent manner with an IC_{50} of 427.35 ± 1.23 µl and 549.45 ± 1.38 µl respectively. On a comparative assessment between the essential oils of *A. sativum* and *A. cepa*, the former exhibited better larvicidal activity against the disease-causing vector, *A. stephensi*. Still, both could serve as potent insecticidal agents after further identification of the responsible chemical compound and its mode of action.

Keywords

Allium sativum, Allium cepa, Essential oil, GC-MS, Anopheles stephensi, Larvicidal activity, Antihemolytic activity

Introduction

Mosquitoes are the foremost important single group of insects in terms of public health importance. Blood feeding female mosquitoes are answerable for the transmission of immense number of diseases, like malaria, filariasis, dengue, Japanese encephalitis, etc. causing ample number of deaths once a year [1]. Within the last 20 years, the employment of chemical insecticides in mosquito control programme has resulted in the instability of the environment, mosquito resistance, mosquito resurgences and are toxic to non-target organisms including natural enemies within the agriculture ecosystem [2].

Mosquito control has become difficult of the indiscriminate uses of synthetic chemical insecticides which has an adverse impact on the environment and disturb ecological balance. Majority of the chemical pesticides are harmful to man and animals, a number of which aren't easily degradable and causes toxic effects. The increased use of those insecticides may enter into the organic phenomenon and thereby the liver, kidney, etc. is also irreversibly damaged. They even lead to mutation of genes and these changes become prominent after some generations. Besides, a significant drawback with the employment of chemical insecticides is that they're non-selective and will be harmful to other useful organisms within the environment.

This necessitates the event of another method which involves the utilization of potential biocontrol agents or compounds isolated from the biological world. Plants may be a replacement for mosquito larvicides because they constitute a possible source of bioactive chemicals and usually free from harmful effects. The look for herbal preparations that don't produce any adverse effects within the non-target organisms and are easily biodegradable remains a top research issue for scientists related to alternative vector control [3]. Phytochemicals derived from plant sources can act as larvicides, insect growth regulators, repellents, and oviposition attractants. They'll also play a vital role in the interruption of the transmission of mosquito-borne diseases at individual along with the community level [4 & 5].

Allium serves as a large genus containing about 400 species. The current study focused on a couple of species from this family namely *Allium sativum* and *Allium cepa*. *Allium sativum* (Garlic) belonging to the liliid monocot, Alliaceae family is a plant containing 1-2% volatile oil on a dry basis with wide variation of chemical composition as a function of generation diversity, habitat and agronomic treatment culture. Garlic incorporates a long folklore history as a treatment for cold, cough and asthma and is reported to strengthen immunity. It has sufficient medicinal effects like lowering of blood cholesterol level, antiplatelet aggregation, anti-inflammatory activity and inhibition of cholesterol synthesis. It is also known to possess antibacterial, antifungal, anticancer, antioxidant and antiviral activities [6]. *Allium cepa* (Onion) is involved in treatment of cold, allergies, toothaches, laryngitis and coughing. Homeopaths make a tincture of onion to treat heterogenous conditions including diarrhea, paralysis, hay fever, hernia, laryngitis, pneumonia and trauma. Onion has been recommended to treat bronchitis, coughing, asthma and other respiratory problems. It holds a belief to assist loosen congestion in the lungs and expand the airways [7].

Essential oils are volatile, natural, complex compounds characterized by a robust odor and are formed by aromatic plants as secondary metabolites. They're usually obtained by steam or hydro-distillation first developed during the Middle Ages by Arabs. Known for its antiseptic, i.e. bactericidal, virucidal and fungicidal, and medicinal properties and their fragrance, they're applied in embalmment, preservation of foods and as antimicrobial, analgesic, sedative, anti-inflammatory, spasmolytic and locally anesthetic remedies. Up to this day, these characteristics haven't changed much except that more is now known about few of their mechanisms of action, particularly at the antimicrobial level. They are also competent in attracting some insects which may well be applicable for its use as a larvicidal compound.

In accordance with all this literary support, the current study evaluated the chemical profiling of garlic and onion essential oil after which its larvicidal activity against *Anopheles stephensi* and antihemolytic property was assessed.

Materials and Methods

Collection and Processing of Sample

A. sativum and *A. cepa* samples were purchased from the local market. Contamination free garlic and onion samples were chosen for the study. The papery skin of both garlic and onion was peeled off and the bulb was chopped into small pieces.

Extraction of Essential Oil (EO)

The finely chopped sample was taken in a round bottomed flask and distilled water was added to it until it gets soaked completely. This was then kept in heating mantle set at 50°C and fit to a Clevenger apparatus. Essential oils were extracted from the samples by the process of steam distillation following the method of Surburg and Panten [8].

GC-MS Analysis of Essential Oil

GC-MS analyses were carried out with a Thermo GC – Trace Ultra Version 5.0, equipped with mass selective detector, Thermo MS DSQ II. The electron ionization energy was 70 eV, ion-source temperature 200°C and the interface temperature 280°C. A fused silica column 5% phenyl-poly-dimethyl-siloxane (DB- 5MS 30m x 0.25 mm in diameter. and 0.25µm film thickness, J&W Scientific) was used. The oven temperature was programmed as follows: from 70°C (3 min hold) raised at 6°C/min to 260°C (20 min hold). A sample of 1 µl was injected. Data acquisition was performed with Mass Lab software for the mass ranges 30 - 600 u with a scan speed of 1 scan/sec. The carrier gas used was Helium with a flow rate of 1 mL/ min. The identification of compounds was performed by comparing their mass spectra with data from Adams, US National Institute of Standards and Technology (NIST, USA), WILEY 1996 Ed. mass spectra library and a personal library of 600 spectra. The identification of compounds was also based on the Kovats retention indices. The Kovats retention indices were calculated using n-alkanes C8-C20 and the experimental values were compared with those reported in literature [9].

In vitro Antihemolytic Activity of Essential Oil

Antihemolytic activity of the five different concentrations (100-500 µl) of the essential oil was assessed as described by Naim *et al.,* [10]. The erythrocytes from sheep blood were separated by centrifugation and washed with 0.2M phosphate buffer (pH 7.4). The erythrocytes were then diluted with phosphate buffered saline to give 4% suspension. Different concentrations of essential oil were added to 2 ml of the erythrocyte suspension and the volume was made up to 5 ml with saline buffer. The mixture was incubated for 5 min at room temperature and then 0.5 ml of H_2O_2 solution in saline buffer was added to induce the oxidative degradation of the membrane lipids. The concentration of H_2O_2 in reaction mixture was adjusted to bring about 90% hemolysis of blood cells after 120 min. After incubation the reaction mixture was centrifuged at 1500 rpm for 10 min and the extent of hemolysis was determined by measuring the absorbance at 540 nm corresponding to hemoglobin liberation. The analysis was performed in triplicates and results were expressed in terms of percentage activity and IC_{50}.

Larvicidal Activity of Essential Oil

The egg rafts of *A. stephensi* were procured from Centre for Research in Medical Entomology, Indian Council for Medical Research, Madurai, India. Filter paper with attached eggs was dipped into a plastic tray containing 500 mL dechlorinated water for 30-40 min, and this time was enough to allow eggs to hatch into larvae. They were reared indoors at 28±2ºC temperature and 14:10 h light and dark period cycle. The larvae were fed with powdered mixture of dog biscuits and yeast powder 3:1 ratio. These larvae were used for assessing the larvicidal activity of *A. sativum* and *A. cepa* essential oils.

Experimental Design for Larvicidal Activity [11]

Group I: Control (100 Larvae)
Group II: 100 Larvae in 249 ml water + 1 ml of 100 ppm of *Allium sativum* EO
Group III: 100Larvae in 249 ml water + 1 ml of 200 ppm of *Allium sativum* EO
Group IV: 100 Larvae in 249 ml water + 1 ml of 300 ppm of *Allium sativum* EO
Group V: 100 Larvae in 249 ml water + 1 ml of 400 ppm of *Allium sativum* EO
Group VI: 100 Larvae in 249 ml water + 1 ml of 500 ppm of *Allium sativum* EO
Group VII: 100 Larvae in 249 ml water + 1 ml of 100 ppm of *Allium cepa* EO
Group VIII: 100 Larvae in 249 ml water + 1 ml of 200 ppm of *Allium cepa* EO
Group IX: 100 Larvae in 249 ml water + 1 ml of 300 ppm of *Allium cepa* EO
Group X: 100 Larvae in 249 ml water + 1 ml of 400 ppm of *Allium cepa* EO
Group XI: 100 Larvae in 249 ml water + 1 ml of 500 ppm of *Allium cepa* EO

Calculation of Lethal Dose

Mortality data was recorded and the LD_{50} values (the dose at which 50% of the Larvae was immobilized) was calculated.

Statistical Analysis

Data were analyzed for statistical significance using one way analysis of variance (ANOVA) followed by Dunnet's multiple comparison test and results were expressed as mean ± standard deviation (SD) using SPSS version 16.0.

Results

GC-MS Analysis of *Allium sativum* EO

The chemical profiling of *Allium sativum* essential oil performed by GC-MS resulted in hundreds of compounds out of which compounds greater than 10% probability were around thirty. Compounds with more than 95% probability of occurrence were Disulfide, di-2-propenyl, Diallyl disulphide and Trisulfide, di-2-propenyl whereas that around 80% probability were Dimethyl tetrasulphide, 3,6-Dimethylpiperazine-2,5-dione, 2,3-Dicarboxythiophene, (2-Trimethylsilyl)thioacetic acid S-methyl ester, 1-thia-2-(2,3-dithia-5-hexenyl)-5-cyclohexene and so on. The compounds with probability of occurrence more than 10% are given in **Table 1** and the Chromatogram is depicted in **Figure 1**.

Table 1: GC-MS Analysis of *Allium sativum* EO

S.No.	Compound name	Retention Time (min)	Molecular formula	Molecular weight	Peak area (%)
1	Diallyl sulfide	6.50	$C_6H_{10}S$	114	5.35
2	1,3-Benzenediol, 2-chloro	6.75	$C_6H_5ClO_2$	144	19.45
3	Disulfide, di-2-propenyl	11.45	$C_6H_{10}S_2$	146	18.76
4	Diallyl disulphide	12.24	$C_6H_{10}S_2$	146	18.76
5	Trisulfide, methyl 2-propenyl (CAS)	13.64	$C_4H_8S_3$	152	13.51
6	Dimethyl tetrasulphide	14.12	$C_2H_6S_4$	158	0.78
7	2-Phenyl-3-fluoropropene-1	14.63	C_9H_9F	136	1.11
8	Trisulfide, di-2-propenyl	15.74	$C_6H_{10}S_3$	178	7.93
9	3,6-Dimethylpiperazine-2,5-dione	16.76	$C_6H_{10}N_2O_2$	142	1.01
10	4,5-Dihydro-1H,3H-thieno[3,4-c]thiophene)	18.93	$C_6H_8S_2$	144	0.48
11	2,3-Dicarboxythiophene	22.17	$C_6H_4O_4S$	172	9.26
12	Peracetyl ketone oxime derivative of1,4,6-tri-O-Methyl-2-hexulose	22.88	$C_{15}H_{25}NO_9$	363	1.12
13	3-Ethoxycarbonylmethyl-2-imino-2,3-dihydrothiazole	23.65	$C_7H_{10}N_2O_2S$	186	2.17
14	3-(2-thia-4-pentenyl)-1-thia-cyclohex-5-ene	24.68	$C_9H_{14}S_2$	186	1.09
15	(2-Trimethylsilyl)thioacetic acid S-methyl ester	26.60	$C_5H_{11}OS$	147	0.82
16	(E)-1-(4-methoxyphenyl)-2-nonene	27.55	$C_{16}H_{24}O$	232	0.67
17	3-n-Propyl-2-thiabicyclo[4.4.0]decane (trans,cis)	30.35	$C_{12}H_{22}S$	198	0.42
18	4,6-Dimethyl-1,3-dihydro-1H-2.lamada.6-thieno[3,4-c]thiophene-2,2-dione	32.55	$C_8H_{10}O_2S_2$	202	0.81
19	1-thia-2-(2,3-dithia-5-hexenyl)-5-cyclohexene	35.26	$C_9H_{14}S_3$	218	2.14
20	Dibromoschizandrin	37.48	$C_{24}H_{30}Br_2O_7$	588	0.51
21	4-(4-Chlorobenzoyl)-1-cyclohexyl-5-tosylamino-1H-1,2,3-triazole	37.50	$C_{22}H_{23}ClN_4O_3S$	458	0.51

GC-MS Analysis of *Allium cepa* EO

GC-MS analysis of *Allium cepa* essential oil resulted in compounds with more than 80% probability like Trisulfide, dimethyl (CAS), 5-chloro-2-hydroxy-1,3-dinitrobenzene. The other important compounds found in *Allium cepa* essential oil were Disulfide, dipropyl (CAS), Dimethyl tetrasulphide, 6-Methoxy-1-methyl-3,4-dihydroisoquinoline, 2,4-

Dimethylpyrido[2,3-d]pyrimidin-5-one, Methyl 3-oxo-2,6,6-trimethylcyclohexen-1-carboxylate, (Z)-4-Methylphenylazo tert-butyl sulfide, 3-Chloro-2H-1-benzopyran-2-ol, 1,5-bis[(6-methoxyphenyl)methyl-1,3-benzodioxol-5-yl]-2-methylidene-1,5-pentadione and so on. (**Table 2** & **Figure 2**).

Figure 1: Chromatogram of *Allium sativum* EO

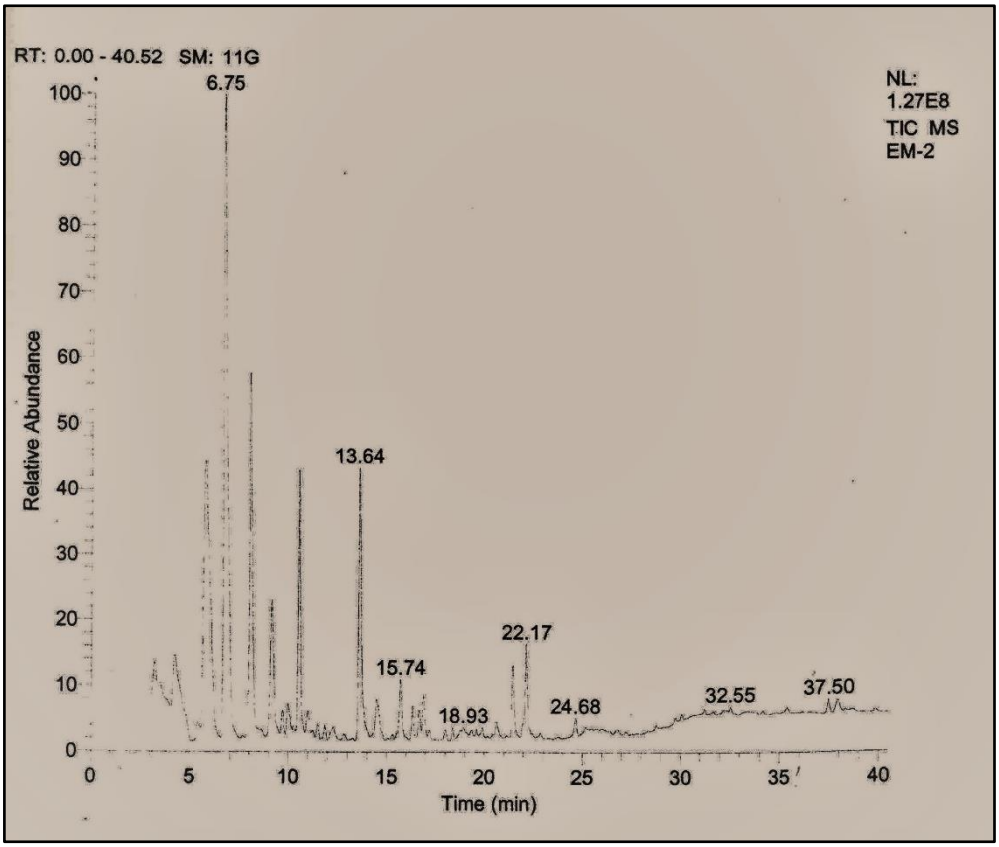
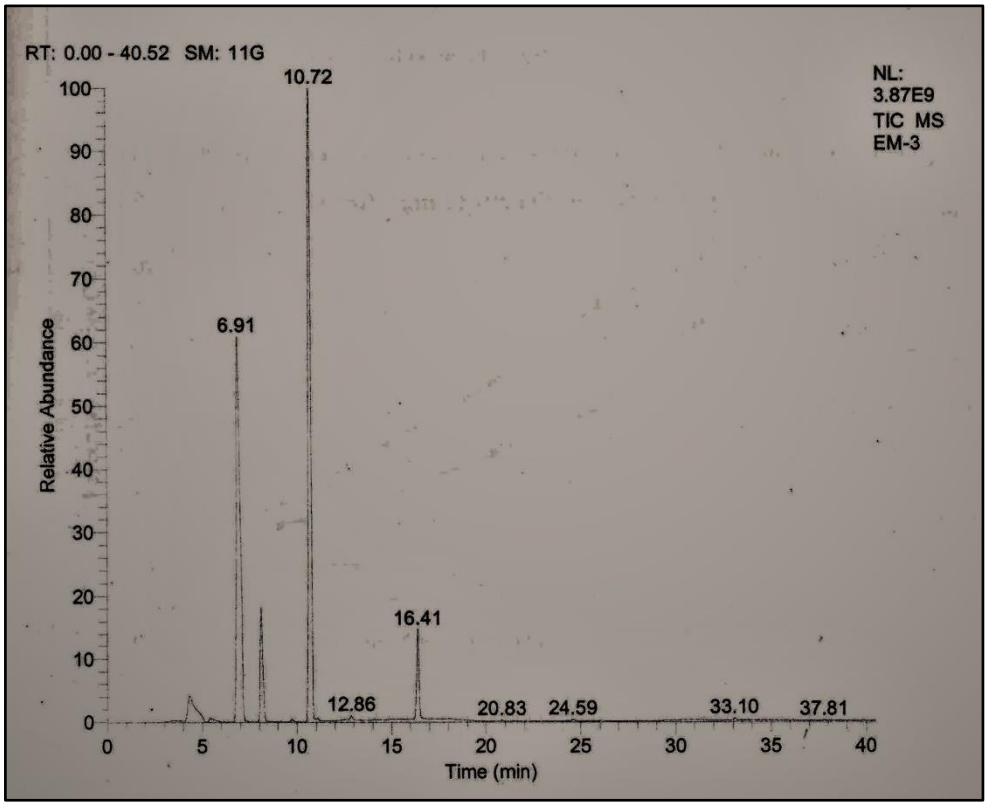


Table 2: GC-MS Analysis of *Allium cepa* EO

S.No.	Compound name	Retention Time (min)	Molecular formula	Molecular weight	Peak area (%)
1	Disulfide, dipropyl (CAS)	6.91	$C_6H_{14}S_2$	150	35.16
2	Trifluoromethyl-leucine	7.23	$C_7H_{12}F_3NO_2$	199	6.22
3	1-Methyl-3-(methylamino)-4-pyrazolecarboxamide	7.57	$C_6H_{10}N_4O$	154	6.22
4	Dimethyl tetrasulphide	8.42	$C_2H_6S_4$	158	0.15
5	2-Bromo[(15)N]pyrodine	9.51	C_5H_4BrN	157	0.15
6	6-Methoxy-1-methyl-3,4-dihydroisoquinoline	10.72	$C_{11}H_{13}NO$	175	47.91
7	2,4-Dimethylpyrido[2,3-d]pyrimidin-5-one	10.72	$C_9H_9N_3O$	175	47.91
8	Testosterone-M1	11.23	$C_{19}H_{24}O_4$	316	0.10

9	Methyl 3-oxo-2,6,6-trimethylcyclohexen-1-carboxylate	11.88	$C_{11}H_{16}O_3$	196	0.03
10	4,5-Dihydroindeno[1,2-b]thiopyran-4,5-dione	12.10	$C_{12}H_6O_2S$	214	0.28
11	(Z)-4-Methylphenylazo tert-butyl sulfide	12.13	$C_{11}H_{16}N_2S$	208	0.03
12	5,6-Dimethylidene-2-exo-bicyclo[2.2.1]heptylp-bromobenzoate	12.66	$C_{16}H_{15}BrO_2$	318	0.05
13	7,8-Dihydro-6(9H)-(methylene)pyrrolo[1,2-a]indole	13.54	$C_{13}H_{13}N$	183	0.05
14	2,4-Thiazolidinedione (CAS)	14.36	$C_3H_3NO_2S$	117	0.01
15	2,3-Dihydro-3,3-dimethyl-[1,2]benzoisothiazole 1,1-dioxide	15.45	$C_9H_{11}NO_2S$	197	0.01
16	5-chloro-2-hydroxy-1,3-dinitrobenzene	16.41	$C_6H_3CIN_2O_5$	218	5.93
17	9-Phenyl-4,4a,9,10-tetrahydro-2(3H)-phenanthrenone	18.33	$C_{20}H_{18}O$	274	0.01
18	Indoleazepinone	20.83	$C_{13}H_{14}N_2O$	214	0.11
19	Methyl 8-methoxy-3-methyl-2-hydroxy-1-naphthoate	21.51	$C_{14}H_{14}O_4$	246	0.01
20	Methyl 7-methoxy-5-methyl-2-hydroxy-1-naphthoate	22.46	$C_{14}H_{14}O_4$	246	0.01
21	3-O-dimethylethylsilyl-5,7,3',4'-tetra-O-methylquercetin	23.55	$C_{23}H_{28}O_7S$	444	0.01
22	N-Isopropylfuro[2,3-c]pyrrole	24.59	$C_9H_{11}NO$	149	0.06
23	3-Chlorobenzofuran	26.75	C_8H_5ClO	152	0.01
24	3-Chloro-2H-1-benzopyran-2-ol	30.23	$C_9H_7ClO_2$	182	0.12
25	Dihydro-1,4-benzothiazine-6,7-dione	33.10	$C_8H_7NO_2S$	181	0.07
26	1,5-bis[(6-methoxyphenyl)methyl-1,3-benzodioxol-5-yl]-2-methylidene-1,5-pentadione	34.87	$C_{36}H_{32}O_8$	592	0.07

Figure 2: Chromatogram of *Allium cepa* EO



***In vitro* Antihemolytic Activity of Essential Oil**

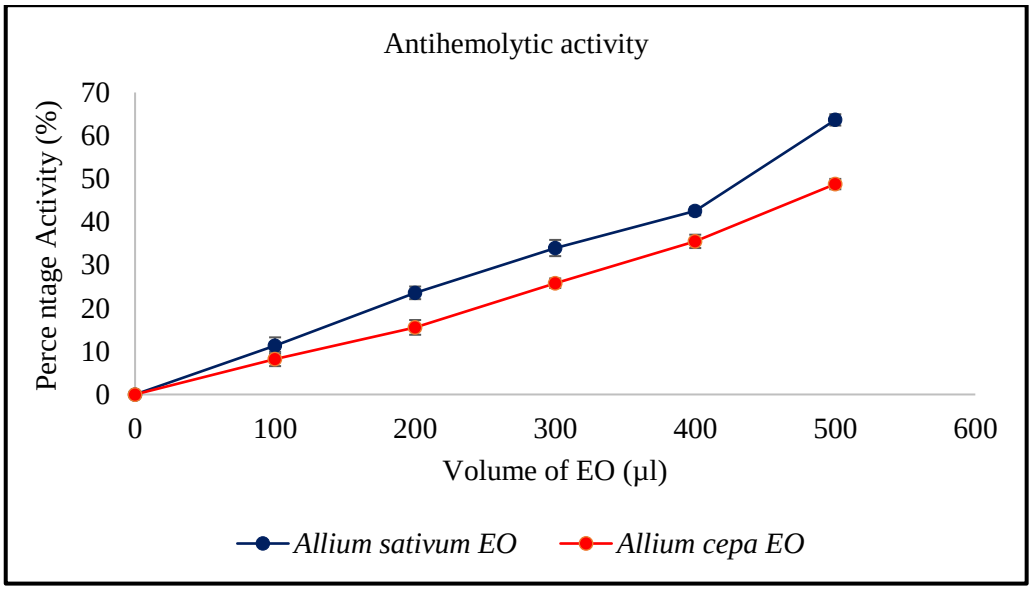
The potential of *Allium sativum* and *Allium cepa* essential oil to inhibit lysis of erythrocytes were assessed at five different concentrations namely 100 -500 µl. The percentage activity increased in a concentration dependent manner. 500 µl concentration of both garlic and onion essential oil extended a percentage activity of 63.65 ± 0.31% and 48.77 ± 0.20% respectively. The IC_{50} values of *Allium sativum* and *Allium cepa* essential oil were found to be 427.35 ± 1.23 µl and 549.45 ± 1.38 µl as given in **Table 3** and **Figure 3**.

Table 3: Antihemolytic Activity of Essential Oil

Sample	Concentration (µl)	Percentage activity (%)	IC_{50} (µl)
Allium sativum essential oil	100	11.35 ± 1.89	427.35 ± 1.23
	200	23.56 ± 1.47	
	300	33.95 ± 1.88	
	400	42.51 ± 0.97	
	500	63.65 ± 1.31	
Allium cepa essential oil	100	8.23 ± 1.66	549.45 ± 1.38
	200	15.55 ± 1.71	
	300	25.79 ± 1.11	
	400	35.49 ± 1.55	
	500	48.77 ± 1.20	

Values are means of three independent analyses ± standard deviation (n = 3).

Figure 3: Antihemolytic Activity of Essential Oil



Values are means of three independent analyses ± standard deviation (n = 3).

Larvicidal Activity of Essential Oil against *Anopheles stephensi*

The larvicidal activity was assessed at five different concentrations of essential oils namely 100 – 500 ppm. As the concentration of the essential oil increased, the mortality percentage of *Anopheles stephensi* larvae increased. The maximum mortality rate was found to be 95.67 ± 3.11% and 75.00 ± 2.67% for *A. sativum* and *A. cepa* essential oil respectively. **Table 4** and **Figure 5** indicates the 50% lethality of *Anopheles stephensi* larvae at 265.96 ± 1.88 ppm and 357.14 ± 2.36 ppm of *Allium sativum* and *Allium cepa* essential oil sequentially.

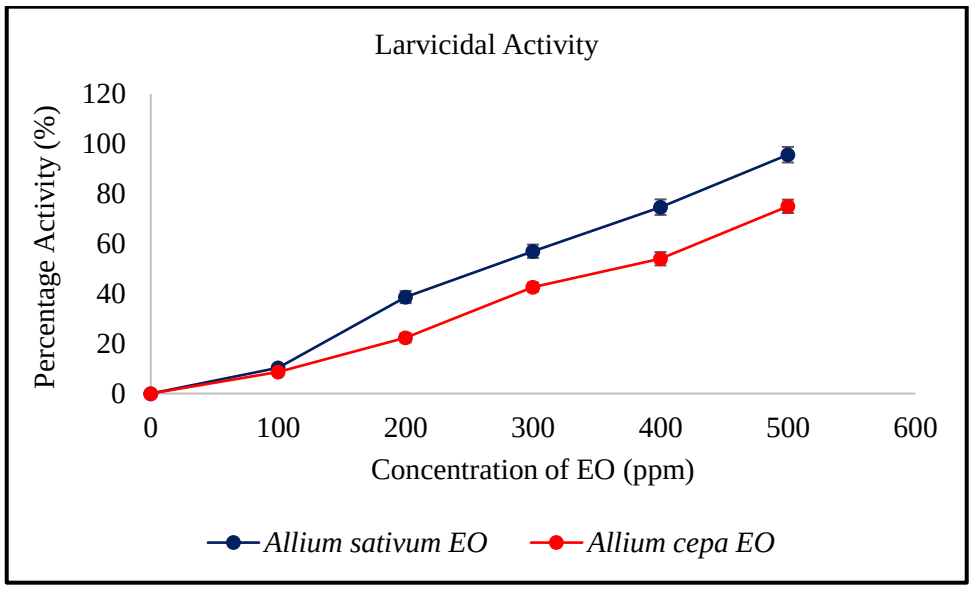
Figure 4: Third Instar *Anopheles stephensi* Larvae

Table 4: Larvicidal Activity of Essential Oil

Sample	Groups	Concentration (ppm)	Percentage activity (%)	LD_{50} (µl)
Control	I	0	0	
Allium sativum essential oil	II	100	10.33 ± 1.11*	265.96 ± 1.88
	III	200	38.67 ± 2.44**	
	IV	300	57.00 ± 2.67**	
	V	400	74.67 ± 3.11***	
	VI	500	95.67 ± 3.11***	
Allium cepa essential oil	VII	100	8.67 ± 1.11$^{\#}$	357.14 ± 2.36
	VIII	200	22.33 ± 1.78$^{\#\#}$	
	IX	300	42.67 ± 1.78$^{\#\#}$	
	X	400	54.00 ± 2.67$^{\#\#}$	
	XI	500	75.00 ± 2.67$^{\#\#\#}$	

*Change in activities at $p<0.05$ when groups II - VI compared to group I, **$p<0.01$, ***$p<0.001$; $^{\#}$Change in activities at $p<0.05$ when groups VII - XI compared to group I, $^{\#\#}p<0.01$, $^{\#\#\#}p<0.001$;Values are means of three independent analyses ± standard deviation (n = 3).

Figure 5: Larvicidal Activity of Essential Oil



Values are means of three independent analyses ± standard deviation (n = 3).

Discussion

The WHO reports convey that around 44% of the global population is susceptible to Malaria which is caused predominantly by the vector, *Anopheles* mosquito constituting about 537 species [12 & 13]. Around 116 districts in India were reported with Zero malaria cases in 2020 and marches with the theme of "Reaching the Zero Malaria Target" throughout the nation. Amidst this, there were also statements from WHO, [14] which leaves information that 92% of African countries are at the risk of developing Malaria. WHO has declared Srilanka and China to be Malaria Free Countries which is primarily because of their effective vector control

techniques. Potential pesticides used against mosquitoes namely Resmethrin, Sumithrin, Permethrin and Malathion are possible carcinogens and neurotoxins which causes birth defects in human beings and toxic effects in aquatic invertebrates and amphibians [15]. The toxicity free, reliable and effective alternative to these chemical compounds could only be a plant derivative.

On a thorough survey of literature, it was initiated that essential oil derivatives from plants could be employed in vector control as they possess many volatile organic compounds. Generally, these volatile organic compounds and more specifically organosulphur compounds are very well known for their mosquito repelling properties [16]. A deep study about the volatile organosulphur compounds revealed *Allium sativum* and *Allium cepa* to be their vital sources which paved us the way to choose both these *Allium sp.* for the present study. Among all the extraction procedures of essential oil, steam distillation process seems to be a traditional one but was found be the best suitable method. Owing to these data, the fresh samples of *Allium sativum* and *Allium cepa* were steam distilled which yielded a maximum of 0.9% and 0.8% essential oil correspondingly.

The quality of the herbal products used for therapeutic as well as commercial purposes cannot be compromised and an ideal technique for its characterization studies can only be GC-MS. Based on this knowledge, GC-MS was employed to gather information about the chemical constituents present in *Allium sativum* and *Allium cepa* which could be the better attributes for the current study as well as for the other pharmacological importance of these species. The extracted essential oil of both the *Allium sp.* depicted the presence of many organosulphur compounds as given in the table: 1 and table 2. The presence of these organosulphur compounds were on par with the earlier reports given by Satyal *et al.,* [17] & D'Auria and Racioppi [18].

In common, the pesticides used against mosquito control are mostly organophosphates, carbamates and pyrethroids which are toxic to the nervous system, reproductive system and endocrine function. They interfere the normal hormonal activities and influence cellular pathways which results in reproductive dysfunction, developmental impairment and cancer [15]. The herbal based essential oil derived from *Allium sativum* and *Allium cepa* were the key sources of organosulphur compounds which were known to possess repelling properties against female *Anopheles aegypti* [16]. In accordance with this the current study also generated reports supporting the presence of organosulphur compounds in both *Allium sativum* and *Allium cepa* (Table: 1 & Table 2). Compounds like Diallyl sulphide, Diallyl disulphide, Diallyl trisulphide, Methyl Propyl disulphide and Dimethyl tetrasulphide present in both the *Allium sp.* could contribute to the design of analogues which would more effectively repel *Anopheles stephensi* than their chemical counterparts.

In support of this mosquito repelling property of the *Allium sativum* EO and *Allium cepa* EO, the present study also focused on assessing its larvicidal effect against the third instar larvae of *Anopheles stephensi*. The results obtained were on a concentration dependent manner (Table: 4 & Figure: 5) providing 50% lethality at 265.96 ± 1.88 ppm and 357.14 ± 2.36 ppm of *Allium sativum* and *Allium cepa* essential oil respectively. Reports of Milugo *et al.,* [19] states that 81% of the plant derived compounds identified with larvicidal activities were essential oil and the prevailing study also justifies the larvicidal activity of essential oil acquired from *Allium sativum* and *Allium cepa*.

Besides using the EO from *Allium sativum* and *Allium cepa* externally as a repellent and larvicide in vector control, the idea of using it as a therapeutic compound in Malaria is also brought about in this study by assessing their Antihemolytic activity. The reports of Dey *et* al., [20] justify the acceleration of intravascular hemolysis during malarial infection eventually degrading the total RBC hemogobin and damaging the vital organs. The outcome from the Antihemolytic activity observed in the erythrocytes with H_2O_2 induced oxidative stress (Table: 3 & Figure: 3) suggests the prevention of erythrocyte lysis in a concentration dependent

manner. This property of the *Allium sativum* EO and *Allium cepa* EO could accredit their usage in the treatment of Malaria after profound pharmacological studies.

Conclusion

The persuading results from this ongoing research has opened up ideas to widen and deepen the search of plant derived analogues for mosquito repellents and antimalarial drugs present in the essential oil of *Allium sativum* and *Allium cepa*. The findings obtained from this work has ignited the minds to initiate rigorous research in deriving a better solution to make the humankind live in a Malaria free Globe.

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