ORIGINAL ARTICLE

Isolation and characterization of essential oils from *Jatropha curcas* and *Ricinus communis* and their larvicidal activity against mosquito vector *Aedes* and *Culex*

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ABSTRACT

The present study was undertaken for isolation and characterization of essential oil of Jatropha curcas and Ricinus communis and to evaluate their larvicidal activity against mosquito vector Aedes and Culex. The seed of Jatropha curcas and Ricinus communis was used for the study and oil was extracted from the seeds by hydrodistillation. The extracted oil was then subjected to GC-MS analysis and the larvicidal activity of the essential oil was tested against Culex and Aedes larvae (3rd instar). The GC-MS analysis of Jatropha curcas showed the presence of different compounds such as Oleic acid, n-Hexadecanoic acid, 9, 12-Octadecadienoic acid, 17-Octadecynoic acid. The GC-MS analysis of Ricinus communis showed the presence of eight compounds i.e. Benzaldehyde,3-methyl, 4-tert-Butoxystyrene, Oleic acid, 10-Undecenoyl chloride, 2-Piperidione, N-[4-bromo-n-butyl], 1,2-15,16-Diepoxyhexadecane, 11-Tetradecyn-1-ol and cis-Vaccenic acid. For larvicidal bioassay, different concentration of the oil (0.25, 0.5, 1 and 1.5 mg/ml) of both Jatropha curcas and Ricinus communis was tested against Aedes and Culex larvae. The percent mortality of the larvae was recorded after 24 and 48 hours and LD₅₀ and LD₉₀ values were calculated. The larvicidal activity of the essential oil from Jatropha curcas and Ricinus communis. From the present study it can be concluded that Jatropha curcas and Ricinus communis seed oils can be used as potential larvicides against mosquito vectors.

Keywords: Jatropha curcas, Ricinus communis, essential oil, Aedes, Culex, larvicidal bioassay, GC-MS analysis.

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INTRODUCTION

Mosquitoes are at the forefront of entomological research worldwide due to their primary role in the spread of major illnesses like malaria, dengue, encephalitis, lymphatic filariasis, yellow fever and viral diseases [1]. Mosquito- borne diseases are considered as one of the significant cause of human mortality and is estimated that more than 700 million individuals are affected globally every year. Such outbreak of infectious diseases and their high prevalence strains the economy as well as health services of a country [2].

There are more than three thousand mosquito species present worldwide, however Aedes aegypti and Culex quinquefasiatus are considered as the most significant targets for population management in India and other nations in South-East Asia [3]. They act as a primary vector and transmit various diseases. Aedes mosquitoes are the primary vector of dengue virus and are mostly common in tropical and sub-tropical regions [4]. Other diseases transmitted by this mosquito include Zika viruses, yellow fever, chikungunya. Dengue cases have increased more than 8 folds over the past two decades from 505,430 in 2000 to 24 million in 2010 and 5.2 million in 2019 and about 390 million dengue cases are recorded every year [5]. Also, no medications are known to be available for dengue [6]. Culex mosquitoes are universal in nature and are a vector for filariasis. These mosquitoes are commonly present in areas where people live and are frequently found in ditches, drains, septic tanks etc and breed in contaminated water

[7]. It infects about 120 million people worldwide and 44 million individuals develop chronic manifestation [4].

Different measures are undertaken to restrict the spread of diseases through insect vectors. This is accomplished by targeting the adult stage, larvicidal stage or any developmental stages of the vectors [8], use of repellents or inducing widespread larval mortality at their breeding grounds [9]. Use of synthetic insecticides like organophosphates (temephos, fenthion, etc.) is one such measure to control the disease causing vectors [6]. However, excessive use of synthetic pesticides results in a variety of environmental and ecological issues which includes the emergence of resistant strains of insects, contamination of air, water and land [10], ecological imbalance, adverse effect on human health [4] and harm to untargeted organisms [10].

Therefore, to control the disease causing vectors, interest in natural pesticides particularly those produced from plants, has recently increased immensely [4]. Many studies have shown that plants possess different active chemical components or phytochemicals that are effective against mosquitoes and possess mosquitocidal properties [3]. These studies suggest the significance of investigating and creating herbal insecticides from plants as they are effective, less harmful, less toxic, environment friendly and easily biodegradable [7].

The present study was undertaken for isolation and characterization of essential oils from Jatropha curcas and Ricinus communis and to explore their larvicidal activity against mosquito vector Aedes and *Culex. Jatropha curcas* is a shrub that belongs to the Euphorbiaceae family. The plant serves many purposes and has gained immense interest on a global scale as a raw material for biodiesel. The seeds of *Jatropha curcas* yields non-edible oil which is used as biofuel [11][7]. Different parts of this plant such as leaves, seeds, stem and bark are also used as antiseptic, purgative, diuretic, larvicides [8] and is also used as traditional medicine to cure fevers, dysentery, wounds [4], arthritis, jaundice, tumors, allergies, burns, smallpox, cuts [7], rheumatic and muscular pains, malaria [12]. The leaves and seeds of Jatropha curcas are known to possess a wide range of phytochemicals mainly phobol esters, stigmasterol, campesterol, β sitosterol etc and shows antileukemic, molluscicidal, fungicidal, nematicidal and insecticidal properties [4][8]. Ricinus communis also known as castor oil plant or castor bean belongs to the Euphorbiaceae family and is widely distributed around the world [6][13]. There are numerous health bernefits of *R*. *communis* which has been widely demonstrated in different literatures such as anti-inflammatory, hepatoprotective, diuretic, antibacterial, anticancer, free radial scavenging, insecticidal and hypoglycaemic. These therapeutic benefits are attributed due to the presence of abundant secondary metabolites like phenols, alkaloids, tannins etc which also helps in the protection against various diseases [6].

MATERIAL AND METHODS

1. Collection of plant samples: Mature seeds of *Jatropha curcas* and *Ricinus communis* were collected from Makum Railway Station of Tinsukia district and identified. The seeds were washed properly and dried at room temperature [7]. The seeds were then crushed into powder and stored for future use [14]

2. Extraction of essential oil: The seed oil is then extracted by using clevenger apparatus (hydrodistillation) following the method of Shah et al., 2017 and Sogan et al., 2018. The apparatus comprised of water condenser and round bottom flask (with 500 ml capacity). The crushed seeds were then plaed in the bottom flask along with distilled water and were heated. For *Jatropha curcas* 120 mg of crushed seeds and 300 ml of distilled water was taken and for *Ricinus communis* 250 gm of the crushed seeds and 200 ml of distilled water was used. On sufficient heating, the oil was released from the tissues (oil glands) along with water vapour and gradually condensed after passing through the condenser. On further condensation, the oil is separated forming a layer between the water and the oil. The oil was then collected using a TLC tip and stored at 4°C until use [14]. The extraction was carried out at the Department of Pharmaceutical Sciences, Dibrugarh University, Dibrugarh.

3. Collection of larvae: The eggs of *Aedes* and *Culex* were collected from Indian Council of Medical Research (ICMR), Dibrugarh. The eggs were kept in a bowl with water for seven days for hatching. After seven days, the eggs hatched into larvae and biscuit powder was introduced for their feeding. The larvae used were late 3rd or early 4th instars [7].

4. **Identification of larvae**: The larvae were identified for reconfirmation. The larva were taken on a clean slide with a drop of water and observed properly. The Aedes larvae have a single hair, and every air tube has a three branch hair tuft in bunch. The presence of the single hair tuft differs it from that of other species of mosquitoes. The air tube of other species has two or sometimes more air tufts and hair branch [16]. Culex larvae are semi-transparent [17]. A respiratory siphon is present on the 8th abdominal segment which is longer than that of Aedes larvae [18].

5. Larvicidal bioassay: The larvicidal bioassay wascarried out in different concentration of the extract. The extracts were dissolved in 0.5% Dimethyl Sulfoxide (DMSO) and different concentrations of the extract were prepared in distilled water following the method of Vinchurkar et al., 2017. For preparing 0.5% DMSO solution, 0.5 ml of DMSO was added to 99.5 ml of distilled water. Similarly, to make different concentrations of the extract, different volume of the oil was mixed with DMSO which gives the final concentrations of 0.24, 0.5, 1 and 1.5 mg/ml on dilution with distilled water to make the final volume of 100 ml as shown in Table 1.

Sl no.	Concentrations (mg/ml)	Volume of DMSO (ml)	Volume of oil (ml)	Volume of distilled water (ml)	Total volume (ml)
1.	0.25	1	0.25	98.75	100
2.	0.5	1	0.5	98.50	100
3.	1	1	1	98	100
4.	1.5	1	1.5	97.5	100

100 ml of different concentrations of the extracts were taken in a 250 ml beaker separately and 10 numbers of larvae of *Aedes* and *Culex* (3^{rd} instar) each were introduced in each beaker. Mortality rate of the larvae was then observed after 24 and 48 hours respectively and mortality rate was calculated. 100 ml of DMSO and distilled water was taken as control. The experiment was repeated three times and the results were expressed as ±SD.

Percentage mortality (%) = (Number of dead larvae / Number of larvae introduced) × 100

GC-MS analysis: The extracted oil was then subjected to GC-MS analysis. It was carried out at SAIF, IIT, BOMBAY. GC-MS is a chemical analysis that gives a spectral output of all the compounds in a sample that get separated during the analysis. It combines the features of gas chromatography and mass spectrometry to identify different substances within a sample. The gas chromatography (GC) device vapourises the sample, producing a spectral peak with retention time (time elapsed between elution and injection of the sample). The spectral peak is measured from peak to the base. GC-MS inherits the features of high resolution and accurate mass measurement with simple operation and high sensitivity. The GC-MS detection was carried with ionization energy of 70 eV. Helium was used as carrier gas. The flow rate of helium was 1ml/min (constant). The name of the compounds, their molecular weight, retention time and the structure of the sample was ascertained [15].

Statistical analysis: The mortality of the larvae was recorded after 24 and 48 hours respectively and LD₅₀ and LD₉₀ values were calculated by regression analysis using MS-EXCEL. For each concentration the experiment was repeated thrice and the result was expressed as ±SD.

RESULTS AND DISCUSSION

The results of the present study showed that the selected plants, *Jatropha curcas* and *Ricinus communis*, possess different bioactive compounds that have significant larvicidal property. Both the plants exhibited significant larvicidal activity, however, the essential oil from *Jatropha curcas* was found to be more effective than *Ricinus communis*.

(A) Larvicidal activity of *Jatropha curcas* oil against *Culex* larvae after 24 and 48 hours.

The larvicidal activity of *Jatropha curcas* against Culex larvae is shown in Table 2. The results showed that the mortality rate after 24 hours of exposure was highest in 1.5 mg/ml concentration i.e. 63.33% and least in 0.25 mg/ml i.e. 30%. The LD₅₀ and LD₉₀ after 24 hours were found to be 1.00 mg/ml and 2.5 mg/ml respectively. Similarly, after 48 hours of exposure, the mortality rate was highest in 1.5 mg/ml concentration i.e. 86.66% and least in 0.25 mg/ml concentration i.e. 53.33%. The LD₅₀ after 48 hours were found to be 0.028 mg/ml and 1.52 mg/ml respectively.

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Concentrations	% Mortality						Control		
(mg/ml)	24	Mean (%) ±	LD ₅₀	LD ₉₀	48	Mean (%) ±	LD ₅₀	LD ₉₀	(DMSO and distilled
	nours	50			nours	20			water)
	30	30 ± 0			50	53.33 ± 5.77			
0.25	30				50				
	30				60				
	30	36.66 ±5.77			60	66.66 ±5.77			
0.5	40				70				
	40		1.00	2.5	70		0.028	1.52	No mortality
	50	50 ± 0			80	80 ±0			
1	50				80				
	50				80				
	60	63.33 ± 5.77			90	86.66 ±5.77			
1.5	60				80				
	70				80				

Table 2: Table showing percentage mortality of Culex after 24 and 48 hours in Jatropha curcasplant extract.

Figure 1: Graph showing percentage mortality of *Culex* after 24 and 48 hours in *Jatropha curcas* plant extract.



Figure 2: Standard curve showing % mortality of *Culex* after 24 hours in *Jatropha curcas* plant extract.





Figure 3: Standard curve showing % mortality of *Culex* after 48 hours *Jatropha curcas* plant extract.



B) Larvicidal activity of *Jatrophs curcas* oil against *Aedes* after 24 and 48 hours.

The larvicidal activity of *Jatropha curcas* against *Aedes* larvae is shown in Table 3. The results showed that the mortality rate after 24 hours of exposure was highest in 1.5 mg/ml concentration i.e. 66.66% and least in 0.25 mg/ml i.e. 33.33%. The LD₅₀ and LD₉₀ after 24 hours were found to be 0.875 mg/ml and 2.375 mg/ml respectively. Similarly, after 48 hours of exposure, the mortality rate was highest in 1.5 mg/ml concentration i.e. 96.66% and least in 0.25 mg/ml concentration i.e. 46.66%. The LD₅₀ and LD₉₀ after 48 hours were found to be 0.299 mg/ml and 1.41 mg/ml respectively.

Table 3: Table showing percentage mortality of Aedes after 24 and 48 hours Jatropha curcas plantextract.

Concentrations	% Mortality								Control
(mg/ml)	24	Mean (%) ±	LD ₅₀	LD90	48	Mean (%) ±	LD ₅₀	LD90	(DMSO and distilled
	hours	SD			hours	SD			water)
	40	33.33 ± 5.77			40	46.66 ± 5.77			
0.25	30				50				
	30				50				
	40	40 ±0			60	60 ± 0			
0.5	40				60				
	40		0.875	2.375	60		0.299	1.41	No mortality
	50	53.33 ± 5.77			70	73.33 ± 5.77			
1	50				70				
	60				80				
	60	66.66 ± 5.77]		90	96.66 ±5.77]		
1.5	60				100				
	80				100				





Figure 5: Standard curve showing % mortality of *Aedes* after 24 hours *Jatropha curcas* plant extract.



Figure 6: Standard curve showing % mortality of *Aedes* after 48 hours *Jatropha curcas* plant extract.



(C) Chemical composition of *Jatropha curcas* oil (through GC-MS analysis).

The GC-MS analysis of the *Jatropha curcas* essential oil showed the presence of four compounds i.e. Oleic acid, n-Hexadecanoic acid, 9, 12-Octadecadienoic acid, 17-Octadecynoic acid. The gas spectrogram produced two major peaks which has been shown in Fig 7. The name of the compounds along with their chemical structure and molecular formula are given in the table 4.



Figure 7: Gas spectrogram of Jatropha curcas oil.

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Sl	Compounds	Molecular	Molecular	Retention	Chemical structure
no.		formula	weight	time	
			(g/mol)	(mins)	
1.	n-Hexadecanoic acid	C16H32O2	256	28.03	OH OH
2.	9,12- Octadecadienoic acid	C ₁₈ H ₃₂ O ₂	280	31.92	H ^O H ^H H
3.	17-Octadecynoic	C ₁₈ H ₃₂ O ₂	280	36.23	
4.	Oleic acid	C ₁₈ H ₃₄ O ₂	282	32.18	

Table 4: Chemical compositions of essential oil of Jatropha curcas.







(D) Larvicidal activity of *Ricinus communis* oil against *Culex* larvae after 24 and 48 hours.

The larvicidal activity of *Ricinus communis* against Culex larvae is shown in Table 5. The results showed that the mortality rate after 24 hours of exposure was highest in 1.5 mg/ml concentration i.e. 60% and least in 0.25 mg/ml i.e. 26.66%. The LD₅₀ and LD₉₀ after 24 hours were found to be 1.265 mg/ml and 2.817 mg/ml respectively. Similarly, after 48 hours of exposure, the mortality rate was highest in 1.5 mg/ml concentration i.e. 80% and least in 0.25 mg/ml concentration i.e. 40%. The LD₅₀ after 48 hours were found to be 0.446 mg/ml and 1.797 mg/ml respectively.

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Concentrations	% Mortality							Control	
(mg/ml)	24 hours	Mean (%) ± SD	LD ₅₀	LD ₉₀	48 hours	Mean (%) ± SD	LD ₅₀	LD90	(DMSO and distilled water)
	30	26.66 ± 5.7			40	40 ± 0			
0.25	20				40				
	30				40				
	30				60	56.66 ±5.7			
0.5	40	30 ±0			50				
	20		1.265	2.817	60		0.446	1.797	No mortality
	40	36.66 ± 5.77			70	66.66 ±5.7			
1	30				70				
	40				60				
	50	60 ± 0			80	80 ±0			
1.5	70				80				
	60				80				

Table 5: Table showing percentage mortality of Culex after 24 and 48 hours in Ricinus communis
plant extract.





Figure 13: Standard curve showing % mortality of *Culex* after 24 hours in *Ricinus communis* plant extract.



Figure 14: Standard curve showing % mortality of *Culex* after 48 hours in *Ricinus communis* plant extract.



(E) Larvicidal activity of *Ricinus communis* oil against *Aedes* after 24 and 48 hours.

The larvicidal activity of *Ricinus communis* against *Aedes* larvae is shown in Table 6. The results showed that the mortality rate after 24 hours of exposure was highest in 1.5 mg/ml concentration i.e. 63.33% and least in 0.25 mg/ml i.e. 30%. The LD₅₀ and LD₉₀ after 24 hours were found to be 1.037 mg/ml and 2.576

mg/ml respectively. Similarly, after 48 hours of exposure, the mortality rate was highest in 1.5 mg/ml concentration i.e. 86.66% and least in 0.25 mg/ml concentration i.e. 43.33%. The LD₅₀ and LD₉₀ after 48 hours were found to be 0.386 mg/ml and 1.590 mg/ml respectively.

Table 6: Table showing percentage mortality of Aedes after 24 and 48 hours in Ricinus communisplant extract.

plant extract.									
Concentrations	oncentrations % Mortality					Control			
(mg/ml)	24	Mean (%) ±	LD ₅₀	LD ₉₀	48	Mean (%) ±	LD ₅₀	LD ₉₀	(DMSO and
	hours	SD			hours	SD			distilled water)
	40	30 ± 10			40	43.33 ± 5.7			
0.25	20				40				
	30				50				
	30	36.66 ±5.7			60	56.66 ± 5.7			
0.5	40				50				
	40		1.037	2.576	60		0.386	1.590	No mortality
	50	46.66 ± 5.7			70	70 ± 10			
1	50				80				
	40				60				
	60	63.33 ± 15.2			80	86.66 ±5.7			
1.5	50				90				
	80				90				

Figure 15: Graph showing percentage mortality of *Aedes* after 24 and 48 hours in *Ricinus communis* plant extract.



Figure 16: Standard curve showing % mortality of *Aedes* after 24 hours in *Ricinus communis* plant extract.



Figure 17: Standard curve showing % mortality of *Aedes* after 48 hours in *Ricinus communis* plant extract.



(F) Chemical composition of *Ricinus communis* oil (through GC-MS analysis).

The GC-MS analysis of the *Ricinus communis* essential oil showed the presence of eight compounds i.e. Benzaldehyde,3-methyl, 4-tert-Butoxystyrene, Oleic acid, 10-Undecenoyl chloride, 2-Piperidione, N-[4-bromo-n-butyl], 1,2-15,16-Diepoxyhexadecane, 11-Tetradecyn-1-ol and cis-Vaccenic acid. The name of the compounds along with their chemical structure and molecular formula are given in the table 4.



Fig 18: Gas spectrogram of *Ricinus communis* Table 7: Chemical compositions of essential oil of *Ricinus communis.*

Sl no.	Compounds	Molecular formula	Molecular weight (g/mol)	Retention time (mins)	Chemical structure
1.	Benzaldehyde, 3-methyl	C ₈ H ₈ O	120.151	22.85	
2.	4-tert-Butoxystyrene	C ₁₂ H ₁₆ O	179.259	24.51	70

3.	Oleic acid	C ₁₈ H ₃₄ O ₂	282.47	25.53	*
4.	10-Undecenoyl chloride	C11H19ClO	202.722	27.13	O CI
5.	2-Piperidione, N-[4- bromo-n-butyl]	C9H16BrNO	234.137	27.65	Br
6.	1,2-15,16- Diepoxyhexadecane	C ₁₆ H ₃₀ O	254.414	30.16	~~~~~ <u>°</u>
7.	11-Tetradecyn-1-ol	C14H26O	212.377	31.11	ОН
8.	cis-Vaccenic acid	C ₁₈ H ₃₄ O ₂	282.468	34.11	HO

















Figure 26: Mass spectra of Benzaldehyde, 3-methyl.

Mosquitoes are the most significant vectors that cause different diseases such as malaria, lymphatic filariasis and several viral illnesses [19]. The spread of such mosquito-borne diseases can be prevented by controlling the growth of mosquito larvae using different larvicides [9]. A variety of synthetic pesticides or insecticides are known to control the disease causing insects. However, these pesticides come with a number of concerns related to the environment as well as human health [20], are also expensive [21] and affect many untargeted organisms [10]. Therefore, plants are viewed as an alternative to control the disease causing mosquitoes. Plants and plant-based products have been used since ancient times to control mosquitoes and other vectors as they are effective, less harmful, less toxic, environment friendly and easily biodegradable [7].

In the present study, essential oils from Jatropha curcas and Ricinus communis was isolated and larvicidal activity of Jatropha curcas and Ricinus communis was explored against mosquito vectors Aedes and Culex. The result showed that Jatropha curcas and Ricinus communis exhibited significant larvicidal activity and the mortality rate was highest in 1.5 mg/ml concentration after 24 and 48 hours of exposure for both Culex and Aedes mosquitoes respectively. The plant also showed the presence of different phytochemicals in their seed oil. The GC-MS analysis of *Jatropha curcas* essential oil showed the presence of six compounds such as Oleic acid, n-Hexadecanoic acid, 9,12-Octadecadienoic acid, 17-Octadecynoic acid. The GC-MS analysis of Ricinus communis essential oil showed the presence of Benzaldehyde,3methyl, 4-tert-Butoxystyrene, Oleic acid, 10-Undecenoyl chloride, 2-Piperidione, N-[4-bromo-n-butyl], 1,2-15,16-Diepoxyhexadecane, 11-Tetradecyn-1-ol and cis-Vaccenic acid. These compounds are known to have a wide range of additional properties besides insecticidal abilities. Hexadecanoic acid also known as palmitic acid is a type of saturated fatty acid, is known for its high economic importance and antimicrobial properties [22][23]. 9,12-Octadecadienoic acid, also known as linoleic acid, is reported to possess moisture-retention and anti-inflammatory properties. Similarly, Octadecanoic acid or steriac acid and oleic acid are also used for the production of shampoos, detergents, soaps, moisturizers, lotions etc [22]. Many reports suggested that the insecticidal properties of any essential oils are significantly influenced by the chemical composition of its fatty acids and the presence of fatty acids with high molecular weight along with some other active ingredients [24]. These chemical constituents detected from the seed oil of Jatropha curcas and Ricinus communis are thought to be responsible for its effectiveness and promising repellent and insecticidal activity against mosquito vectors [25]. The findings of the study also open door for future research for isolation of bioactive compounds that can be used as effective control against mosquito vectors.

CONCLUSION

From the present study it can be concluded that the essential oil obtained from *Jatropha curcas* and *Ricinus communis* possess different bioactive compounds or phytochemicals that has significant larvicidal property against mosquito vectors, *Aedes* and *Culex*. The larvicidal activity of the essential oil from *Jatropha curcas* was found to be more effective than *Ricinus communis*. This study, therefore, shows that *Jatropha curcas* and *Ricinus communis* plants can be used as potential alternative for synthetic larvicides. These findings motivate researchers to look for novel, biologically active plant-based natural compounds that can serve as an alternative to synthetic insecticides.

INTEREST CONFLICT

There is no interest conflict.

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