MINOR RESEARCH PROJECT

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Title of the Project

Entomological Investigation of Ipomoea carnea Leaves

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SUMMARY OF THE FINDINGS

Natural products are used as traditional medicines from ancient times. They are having a great importance in Ayurveda. One of the medicinal plant species is Ipomoea belongs to family convolvulaceae. Ipomoea species have very high medicinal value as reported in Ayurveda. Ipomoea carnea (Mahananda in Marathi) is a native of South America and available in all states of India due to its adaptation to the Indian climatic conditions. *I. carnea* has been used as folk medicine. Its ash is used for the treatment of skin diseases. The milky juice of this plant is used for the treatment of leucoderma.

The material was analyzed qualitatively for carbohydrates. Total six carbohydrates namely L-Sorbose, L-Rhamnose, D-Glucose, Maltose, D-Fructose and D-Lactose were found to be present in the leaves of Ipomoea carnea.

The present study is an attempt to find new larvicidal products from the extracts of *Ipomea carnea* leaves to control the filarial vector *Cx. quinquefasciatus* and dengue vector *Ae. aegypti*. The attempts are made in the present study to analyze the larvicidal effect of the *Ipomoea carnea* leaf extract and the isolates whose activity has been mentioned in this project. This potent plant, *I. carnea* is vastly grown near water sources.

The present study investigated the order of activity for the total extracts is observed as Acetone > Ethanol > Ethyl acetate against both the species. From the above results it has been worked out after observing the results that acetone and ethanol extracts are more active. Fractionation of ethanol extract exhibited that fractions n-heaxne (A) and ethyl acetate (C) of ethanol extracts are found to be inactive. Both above extracts were tried at 1000 ppm to 100 pm concentrations. The exposure of 24, 48 and 72 hrs. were employed for testing the samples against *Ae. aegypti* and *Cx. quiquefasciatus*. Of all the fractions of ethanol, chloroform fraction **B** is found to be the most active than crude ethanol extract.

The attention for gaining the knowledge for leaves of the plant can be studied for further assay to evaluate effectiveness as insecticidal agents. The extracts can be evaluate against other insects,

larva, pupa etc. Further studies might be carried out to explore the lead compound responsible for aforesaid activity from this plant.

INTRODUCTION

In India, the use of different parts of several medicinal plants to cure specific ailments has been in vogue from ancient times. The indigenous system of medicine namely Ayurvedic, Siddha and Unani have been in existence for several centuries.² Isolation and characterization of active principles from medicinal plants are reported from 19th century.³ India has a rich traditional background in herbal medicines. Detailed accounts of remedies are given texts like the Ayurveda and Rigveda. In the "Atharva Veda", one finds the more varied use of drugs. Today there is a "Return to Nature" call in both advanced countries as well as countries like India⁴. In the convolvulaceae family, the Ipomoea species are cultivated and found in all regions of the world^{4,5}. The Ipomoea family is of approximately 1650 species in 55 genera consisting chiefly of tropical and subtropical climbers. In India there are 180 species belonging to approximately 20 genera. Ipomoea is represented by approximately 18 species in India⁶. Many Ipomoea species have very high medicinal value as those are used in Ayurvedic systems of medicine⁷. I. batata, Marginata, digitata etc. have very high medicinal value⁷. In the convolvulaceae family, the Ipomoea species occur throughout in the plains especially on the bank of rivers and streams in India. Most of the species of Ipomoea are abundantly distributed all over the world⁸. *Ipomoea carnea* is a non woody plant, cheap, representing an annually renewable source of high quality fibers that can be successfully grown in temperate and tropical climatic conditions, without requiring much attention⁹. It is frequently found in planes and low lands near water sources¹⁰. It is an ornamental plant due to its variety of flowers which appear pale rose, pink or light violet and whitish blue¹¹. It possesses two types of extra floral nectories, located on the petiole and on the pedicel. These secrete a complex nectar containing sugars and amino acids. The insects get attracted to the extrafloral nectories are predominantly ants and they are relatively abundant throughout the year. A number of incidents of plant defense as a result of the presence of the extrafloral nectory, visitors at the extrafloral nectories of I. carnea were observed and are consistent with the ant-guard theory of the function of extrafloral nectories¹². Botanical Identification¹¹

Kingdom	Phamerogamae	Family	Convolvulaceae
Division	Angiospermae	Sub family	Convolvulaceae
Class	Dicotyledous	Genus	Ipomoea
Sub class	Gamopetalae	Species	Carnea
Order	Polemeniales	Sub species	Fistulosa
Category	Shrub	Duration	Perrenial

Recent report shows that *I carnea* has a medicinal value. The species used for skin disease in rural area of Chattisghara, India¹³.

Present work is in continuation and advanced study of the work done for Ph. D. degree by principle investigator. The prepared n-hexane, ethyl acetate, acetone and ethanol extracts of I. carnea leaves were tested against Culex quinquefasciatus and Aedes aegypti mosquito larvae. The activity was tested at different concentrations ranging from 25 to 1000 ppm for definite exposure of time. Mortality was noticed and recorded at each level under same set of conditions. Ethyl acetate, acetone and ethanol extracts exhibited promising activity against both the species. The acetone extract was fractioned and fractions were tested against the both mosquito species. LC50 and LC90 values were reported. Fractions of acetone extracts also demonstrated the entomological activity against IVth instar larvae of both the mosquito species. The study was stucked to find out more potent fraction of acetone extract. Still potent molecule/s from ethyl acetate and ethanol extracts is to be carried out. Thus in the present study attempts will be made to find out active fractions from ethanol extracts. Screening of their bioactivity is necessary to get more active fraction as new larvicidal products from Ipomea carnea leaves to control the filarial vector *Culex quinquefasciatus* and dengue vector *Aedes aegypti*. So the efforts will be made to isolate potent molecule/s against the larvicidal activity. Here at each stage entomological activity will be performed to achieve more potent extract, thus molecule/s.

REVIEW OF RESEARCH AND DEVELOPMENT IN THE SUBJECT

Prevalence of Mosquito borne diseases is one of the world's most notable health hazards. Mosquitoes are the most important arthropods in medical entomology. Several mosquito species

belonging to genera Anopheles, Culex and Aedes are vectors for the pathogens of various diseases like malaria, filariasis, Japanese encephalitis, dengue, yellow fever and chickungunya¹⁴. Nearly 300-500 million people are infected worldwide with mosquito-born diseases and 1.5 to 2.0 millions die per year¹⁵. The most efficient approach to control the vector is to target the immature stages of their life cycles. The current mosquito control approach is based on synthetic insecticides of organophosphate compounds and insect growth regulators. Continuous use of synthetic insecticides has disrupted natural enemies and led to outbreak of some insect species, resulted in developing resistance, had undesirable effects on non-targeted organisms, pollution and toxic side effect on human being. There is a continuous and urgent need to discover new environmentally safe, biodegradable indigenous method for vector control. Therefore. researchers are increasingly turning their attention to herbal products to use as insecticides for controlling larval mosquitoes. The use of plants for medicinal and insecticidal purposes dates back to antiquity. Plant extracts of roots, leaves and flowers were found to have mosquito larvicidal activity¹⁴. Many researchers have been reported on the effectiveness of plant extracts or essential oils against mosquito larvae. Insecticidal activities of plant - derived compounds have been evaluated and few of these developed commercially¹⁶.

There were reports on synergistic effect of insecticides of *I. carnea* leaves extract against malarial vector *Anopheles stephensi*¹⁷. The steam distilled essential oil from the leaves of *I. cairica* was found highly toxic against *A. aegypti*, *A. stephensi* and *C. quinquefasciatus*¹⁸. Immunomodulatory activity of *I. carnea* is also reported¹⁹. Another species of Ipomoea, *Ipomoea cairica* Linn. is also reported as mosquito larvicide²⁰

Dengue fever/ yellow fever and Filariasis contribute major disease burden and their control has become more difficult. Sanitary problems are also responsible for controlling such diseases.

Mosquito resistance has been increased with synthetically produced compounds which are available in the market. So, in recent years attention has been made towards the use of ecofriendly and easily biodegradable natural insecticide of plant origin have gained importance, as they constitute a rich source of bioactive molecules. Studies on the efficacy of plant products as larvicides indicated that they could be possible alternatives to synthetic chemical insecticides²¹⁻²⁵. Much effort have been focused on plant extracts, as phytochemicals are potent sources of commercial mosquito control agents or as lead compounds²¹⁻²⁵.

However to the best of our knowledge, studies have not been conducted. So for to evaluate quantitavely the activity of *I. carnea* leaves extract against larvae of *Aedes aegypti* and *Culex quinquefasciatus*. In the present study I report the larvicidal activity of crude ethanol extract of *I. carnea* and its fractions against two mosquitoes *Aedes agypti* and *Culex quinquefasciatus*.

PRESENT WORK

Vector control is facing a threat due to the emergency of resistance in vector mosquitoes to conventional synthetic insecticides, warranting either countermeasures or development of newer insecticides. Therefore, it is necessary to look for and find a better insecticide or larvicide, which could provide a safer and long-lasting control against *Cx. quinquefasciatus* and *Aa. aegypti* mosquitoes. The deficiency of dissolved oxygen and active presence of the antioxidants-phenol, flavonoide molecules might be the reason for larval death. From the same extract antioxidants have been estimated. Such molecules interact with the cuticle membrane of the larvae, ultimately disarranging the membrane could be the most probable reason for the larval death.

RESULTS AND DDISCUSSION

The qualitative study is carried out on the extracts of *I. carnea* leaves using different polarity solvents. It reveals the presence of medicinally active constituents such as carbohydrates, saponins, phytosterols, phenols, flavonoids, tannins, etc. Such components are responsible for various bioactivities. The active components of the extracts may weakens the cuticle defence system of the larvae causing easy penetration of pathogenic molecules into insect bio systems. One of the approaches for control of these mosquito- Borne diseases is the interruption of disease transmission by either killing, preventing mosquitoes to bite human beings (by using repellents) or by causing larval mortality in a large scale at the breeding centers of the vectors.

Larvicidal Efficacy of Extracts

The results of this study leads to the various larvicidal activities of different extracts of *I. carnea.* Four extracts namely n-heaxne, ethyl acetate, acetone and ethanol are tested for their activity against 4th instar larvae of *Culex quinquefasciatus* and *Aedes aegypti* mosquito larvae. The results exhibit that n-hexane extract is found to be inactive, so further study is carried out with ethyl acetate, acetone and ethanol extracts.

The larvicidal activities of ethyl acetate, acetone and ethanol extracts are reported against *Ae*. *aegypti* and *Cx. quinquefasciatus* (**Table 1.1, 1.2, 1.3**) respectively.

Five different concentrations of ethyl acetate, acetone and ethanol extracts are tested against both mosquito species in various concentrations such as 1000, 750, 500, 250 and 100 ppm. Results of 100% mortality of various extracts at various exposure time are reported in the results and those are discussed, other details are incorporated in the respective tables.

Ethyl acetate extract

The results of this study present that EtOAc extract exhibits 100 % kill for both species after 24 hrs. exposure at 1000 ppm concentration. At 750 ppm. concentration 100 % kill is observed after 48 hrs. exposure for *Ae. aegypti* while it shows 100 % kill after 48 hrs. exposure for *Cx. Quinquefasciatus*. At 500 ppm. concentration 100 % mortality is illustrated after 48 hrs. exposure for *Ae. aegypti* while for *Cx. quinquefasciatus* 100 % kill is produced after 72 hrs. exposure. For *Ae. aegypti* at 250 ppm. concentration 100 % mortality is visible after 72 hrs. exposure. The details are reported (**Table 1.1**).

Acetone extract

The results produce that acetone extract shows 100 % kill after 42 hrs. exposure at concentration 500 ppm. for both species. At lower concentration of 250 ppm. acetone extract exhibits 100% mortality after 24 hrs. exposure for both species. So the experiments are performed for lower doses such as 50, 25 and 10 ppm. concentrations. The results exhibit 100% kill after 24 hrs. exposure for *Ae. aegypti* and 72 hrs. exposure for *Cx. quinquefasciatus* at 50 ppm concentration (**Table 1.2**). Lower doses of 25 ppm appeares 80% kill after 72 hrs. exposure in case of *Ae. aegypti* only. Lower doses have very poor activity for both species.

Ethanol Extract

EtOH extract shows 100% kill at 750 ppm. concentration after 24 hrs. exposure, while 100% mortality after 48 hrs. exposure at 500 ppm for both species. At 100 ppm. concentration after 48 hrs. exposure 100 % mortality is appeared for *Ae. aegypti* (**Table 1.3**). The comparable result is observed for *Cx. quinquefasciatus* species at 250 ppm. concentration. Results indicated that **Acetone Extract** is found to be more effective against both mosquito species.

Bioefficacy of extracts

Bioefficacy of ethyl acetate, acetone and ethanol extracts obtained by reflux in *I. carnea* leaves are tested against 4th instar larvae of *Ae. aegypti* and *Cx. quiquefascitus* species. The details of the experiments for the lethal concentrations for 50% and 90% kill using Probit analysis data are reported (**Table 2** and **3**).

The data for extracts of EtOAc, acetone and EtOH is analyzed for their bioefficacy. The lethal concentrations, LC_{50} values are 260.33, 24.52 and 163.37 ppm. while LC_{90} values are 1272.79, 101.68 and 750.938 ppm. for EtOAc, acetone and EtOH extracts respectively, when tested against *Ae. aegypti* mosquito species. The lethal concentrations, LC_{50} values are 336.60, 87.57 and 254.25 ppm while LC_{90} denote 802.68, 313.23 and 645.47 ppm. for EtOAc, acetone and EtOH extracts respectively, when tested against *Cx. quiquefasciatus* species.

The order of activity for the total extracts is observed as Acetone > Ethanol > Ethyl acetate against both the species. From the above results it has been worked out after observing the results that acetone and ethanol extracts are more active, so further fractionation of ethanol extract has been performed using different polarity solvents (Table 4) whose details are mentioned in the section, Materials and Methods. Larvicidal efficacies of fractions of acetone and ethanol extracts have been studied.

Larvicidal efficacy of Fractions of Ethanol Extract

Aedes aegypti

Larvicidal activity of Ethanol fractions are tested against *Ae. aegypti* species. The fractions, n-hexane (**A**), chloroform (**B**), EtOAc (**C**), acetone (**D**) and EtOH (**E**) are tested using different concentrations such as 1000, 750, 500, 250 and 100 ppm. Result of this experiment shows that fraction **A** and **C** are found to be inactive. Fraction **B** exhibits 100% mortality after 24 hrs. exposure till very low concentration upto 100 ppm(**Table 4.1**). Fraction **D** appears 100% kill after 24 hrs. exposure at 750 ppm. Concentration (**Table 4.2**). Fraction **E** leads 100% mortality till 500 ppm. concentration after 24 hrs. exposure. Fractions **E** exhibits 100% mortality till 250 ppm concentration after 48 hrs. exposure (**Table 4.3**).

Culex quiquefasciatus

Larvicidal activity of ethanol fractions (A-E) are tested against *Cx. quiquefasciatus* species. Fractions A and C are found to be inactive. Fraction B shows 100% kill after 24 hrs. exposure till 50 ppm. concentration. Fraction D demonstrates 100% kill after 24 hrs. exposure till 750 ppm. concentration. The same fraction exhibits 100% mortality after 48 hrs. at 500 ppm concentration. Fraction E shows 100% mortality till 500 ppm concentration after 24 hrs. exposure fraction E exhibits 100% kill for 250 ppm. concentration. (Table 4.1, 4.2, 4.3)

The results of this experiment show that fraction \mathbf{B} is very effective against both species.

CONCLUSION

The results of this study show that from the prepared extracts, acetone extract of *I. carnea* leaves is very effective than other extracts against *Cx. quinquefasciatus* and *Ae. aegypti* mosquito larvae. The order of activity for the extracts is observed as **Acetone** > **Ethanol** > **Ethyl acetate** against both the species. Ethanol extract has comparable activity as acetone. Fraction **B** obtained by fractionation of ethanol extract shows more activity against both the species.

Due to the problem of pollution and vector resistance, safe plant products are being tested around the world as pest control agents. Crude extracts or isolated bioactive phytochemicals from the plant could be used in stagnant water bodies which are known to be the breeding grounds for mosquitoes. However, further studies on the identification of the active principals involved and their mode of action and field trials are usually needed to recommend any of these plant materials as an anti-mosquito product used to combat and protect from mosquitoes in a control program.

MATERIALS AND METHODS

Plant Material

The plant of *Ipomoea carnea* was collected from river side of Pune, Maharashtra, India. It was authenticated by comparing herbarium voucher specimen deposited at Botanical Survey of India, India, Pune. Its authentication number is E LICAI BSI/WC/Tech/2009/96.

Entomological Material:

 4^{th} Instar larvae of *Ae. egypti* and *Cx. quinquefasciatus* were drawn from the laboratory culture of mosquitoes maintained at $27\pm2^{\circ}$ C temperature and $80\pm5\%$ Relative Humidity. The details of the experiment are mentioned in biological assay part.

Preparation of crude extracts

Air shaded dried, leaves powder of *I.Carnea* (200 gm) was extracted using soxhlet extractor. The continuous soxhlet extraction was performed with solvents n-hexane, ethyl acetate, acetone and ethanol. Each solvent was removed under reduced pressure. The hexane extract (2.4%), ethyl acetate extract (9.5%), acetone extract (8.2%) and ethanol extract (8.5%) were obtained. Among these extracts acetone and ethanol extracts were found to be most active. Column chromatography of acetone and ethanol extracts were carried out.

Column chromatography of ethanol extract

The crude ethanol extract (5.0 g) was chromatographed using silica gel (60-120,100 g). It was fractioned using polarity gradient solvents as: n-hexane (**A**), chloroform (**B**), ethyl acetate (**C**), acetone (**D**) and ethanol (**E**) which produced in five broad fractions (**Table 4**). The fractions of 300 ml volume were collected. The progress of the column chromatographic separation was monitored by performing thin layer chromatography of the fractions. Fractions depicting similar composition were combined together to get total five fractions (**A-E**, **Table 4**)

Fraction No.	Eluent	Volume (ml)	Weight (g)	Approximate composition
		5×300	0.341	
Α	n-Hexane			Mixture of unidentified compounds
		3×300	0.231	
В	Chloroform			Mixture of unidentified compounds
		4× 300	0.365	
E	Ethyl acetate			Mixture of unidentified compounds
		6× 300	0.750	
F	Acetone			Mixture of unidentified compounds
		5×300	0.870	
G	Ethanol			Mixture of unidentified compounds

 Table 4 column chromatography of Ethanol extract

Biological assay

For biological studies, the different extracts were carried out according to the solubility in organic solvents. These extracts were tested for larvicidal activity. All experiments were performed against 4th instar larvae (0-24 hr old) of *Aedes egypti* and *Culex quinquefasciatus* which were cultured and maintained during the experiment at $80\pm 5\%$ relative humidity at $27\pm 2^{\circ}$ C. The larvae were exposed to the desired concentration of given extract by keeping them in 100ml beaker containing 50 ml of water. Larva food ground dog biscuits/yeast tablets 1:1 was provided every alternate day. Five replicates were taken for each concentration and each experiment was repeated four times. The mortality was counted after every 24 h, until adult emergence. Dead larvae were removed and the total cumulative mortality was noted. Untreated controls were also taken in each test. The data was analyzed by the "log Probit conversion" to drive LD 50 values (in parts per million (ppm)) for each extract.⁷⁷

Table 1 Larvicidal Efficacy of Extracts

Conc	Aedes aegyptiCulex quiquefasciatus					
(ppm)	% Mortality	y after a perio	od of			
	24 hrs.	48 hrs.	72 hrs.			
1000	100			100		
750	82.5 ± 1.7	100		100		
500	55.0 ±1.80	100		61.5±1.83	80.5±1.82	100
250	40.5±1.62	90.5±2.15	100	20.0 ± 0	52.5±1.32	80.5±1.86
100	31.0± 1.30	50.0 ±0.83	90.0±2.0	10.5±0.85	30.0 ±1.04	52.5 ±1.32

 Table 1.1 Ethyl Acetate Extract

Conc	Aedes aegypt	i		Culex quiquefasciatus		
(ppm)) % Mortality after a period of					
	24 hrs.	48 hrs.	72 hrs.	24 hrs.	48 hrs.	72 hrs.
500	100			100		
250	100			81.5 ± 2.1	100	
100	84.5 ±1.83	100		52.5 ±1.82	72.5 ±1.62	100
50	80.3±1.63	100		32.0 ±1.02	66.5 ±1.76	100
25	50.0 ±1.52	70.5 ±1.84	80.5±1.60			
10	20.15±0.85	28.0 ± 0.5	30.0 ±0.80			

Table 1.2Acetone Extract

Table 1.3 Ethanol Extracts

Conc	Aedes aegypti			Culex quiqu	efasciatus			
(ppm)	% Mortality after a period of							
	24 hrs.	48 hrs.	72 hrs.	24 hrs.	48 hrs.	72 hrs.		
1000	100			100				
750	100			100				
500	70.5 ± 1.62	100		71.5 ± 1.76	100			
250	60.75 ±1.32	100		52.0 ± 1.32	90.0 ± 2.8			
100	40.0 ± 0.85	100		10.5 ± 1.02	70.0 ± 1.84			

Total	Regression equation	Lethal	Fiducial Limits	χ^2
extracts		concentration(ppm)		
Ethyl	Y = 0.507 + 1.859 x	Lc 50 = 260.33	(92.43 – 733.17)	41.52
Acetate	$SE \pm 0.625$	Lc 90 = 1272.79	(486.65–1679.32)	
Acetone	Y = 2.116 + 2.075 x	Lc 50 = 24.52	(20.90 – 28.76)	5.21
	$SE \pm 0.355$	Lc 90 = 101.68	(79.69 – 129.74)	
Ethanol	Y = 0717 + 1.935 x	Lc 50 = 163.37	(49.65 - 537.59)	39.04
	SE ± 0.691	Lc 90 = 750.938	(141 – 109.2)	

Table 2	Bioefficacy	of extracts	against Aedes	aegypti

 Table 3 Bioefficacy of extracts against Culex quinquefasciatus

Total	Regression equation	Lethal	Fudicial Limits	χ^2
extracts		concentration (ppm)	(lower-upper)	
Ethyl	Y = -3.582 + 3.39 x	Lc 50 = 336.60	(164.71 - 687.85)	51.95
Acetate	SE ± 0.897	Lc 90 = 802.68	(247.55 – 1602.65)	
Acetone	Y = 0.501 + 2.315 x	Lc 50 = 87.57	(76.27 – 100.57)	5.44
	SE ± 0.375	Lc 90 = 313.23	(245.69 - 402.61)	
Ethanol	Y = -2.619 + 3.167 x	Lc 50 = 254.25	(152.54 – 423.77)	21.77
	SE ± 0.850	Lc 90 = 645.47	(303.79 – 1071.47)	

Table 4 Larvicidal Efficacy of fractions of Ethanol Extract

Conc	Aedes aegyptiCulex quiquefascia							
(ppm)	% Mortality after a period of							
	24 hrs.	48 hrs.	72 hrs.	24 hrs.	48 hrs.	72 hrs.		
1000	100			100				
750	100			100				
500	100			100				
250	100			100				
100	100			100				
50	50			100				

Table 4.1 Fraction B [chloroform]

Table 4.2Fraction D[acetone]

Conc	Aedes aegypti			Culex quiq	Culex quiquefasciatus			
(ppm)	% Mortality after a period of							
	24 hrs.	48 hrs.	72 hrs.	24 hrs.	48 hrs.	72 hrs.		
1000	100			100				
750	100			100				
500	30	50	80	70.5± 1.8	100			
250	10	70		52.5 ± 1.2	70.0± 1.8			
100		50		$20.0\pm~0.8$	50.5 ± 1.3			

Conc	Aedes aeg	ypti		Culex quiquefasciatus				
(ppm)	% Mortal	ity after a pe	riod of	1				
	24 hrs.	48 hrs.	72 hrs.	24 hrs.	48 hrs.	72 hrs.		
1000	100			100				
750	100			100				
500	100			100				
250	90	100		20	50	100		
100	20	90		20	40			
50				10	60	70		

Table 4.3Fraction E[ethanol]

Fractions n-heaxne (**A**) and ethyl acetate (**C**) of ethanol extracts are found to be inactive. Both above extracts were tried at 1000 ppm to 100 pm concentrations. The exposure of 24, 48 and 72 hrs. were employed for testing the samples against *Ae. aegypti* and *Cx. quiquefasciatus*. Of all the fractions of ethanol, chloroform fraction **B** is found to be the most active than crude ethanol extract.

The attention for gaining the knowledge for leaves of the plant can be studied for further assay to evaluate effectiveness as insecticidal agents. The extracts can be evaluate against other insects, larva, pupa etc. Further studies might be carried out to explore the lead compound responsible for aforesaid activity from this plant.

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2.	Detection of Carbohydrates from	Asian Journal of	1.498
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No. of publications out of the project