Pharmacognostic Evaluation and Larvicidal Activity of Selected Three Ocimum Species.

Rajamma A.J, Sonal Dubey and Sateesha S. B

K. L. E. Society's College of Pharmacy,
II Block, Rajajinagar, Bangalore-560 010, Karnataka, INDIA.

Summary

This study was aimed to evaluate the efficacy of crude petroleum ether, chloroform, ethanol and aqueous extracts of *ocimum* species aerial parts against *Culex quinquefasciatus*. Initially the plants were subjected for successive extraction using various solvents ranging from non-polar to polar and secondary metabolites were extracted. The extracts were analyzed for the presence of various secondary metabolites and a thin layer chromatography study confirms the presence of terpenoids and sterols. Larvicidal activities of the extracts in the concentration range of 100 to1000 ppm were performed by WHO method on *Culex quinquefasciatus* at National institute of malaria research center (ICMR), Bangalore. The effect of extracts on the larval mortality rate, survival number were studied. Petroleum ether extracts of all the three species have shown better pesticidal activity. Further Pet. ether extract of *Ocimum basilicum* has shown the best result in comparison to other two species with the less LD_{50} value 46.67.

Key words: Ocimum species, pharmacognostic evaluation, WHO method, modified WHO method, Eliot's method

***Corresponding author: Sonal Dubey**
Associate Professor,
K. L. E. Society's College of Pharmacy,
II Block, Rajajinagar, Bangalore-560 010, Karnataka, INDIA.
E-mail: drsonaldubey@gmail.com

Introduction

The plant world comprises a rich storehouse of phytochemicals which are widely used in the place of synthetic insecticides. The continuous use of synthetic insecticides causes side effects to non-target organisms and insecticide resistance against mosquitoes (1) Researchers are now looking for natural insecticides which do not have any ill effects on non-target population and are easily degradable. The search is underway to find out newer insecticides which will be effective and safe, and also easily available at low cost. It has been pointed out that there is an urgent need to control vector population because the incidence of malaria (2), dengue fever and filariasis is increasing.

Due to spiralling costs of insecticides and labour, paucity of funds and due to resistance developed by plasmodia, *culex* or anophelines to chemicals, diseases carried by mosquitoes are back since 1980. So far only a few compounds of plant origin are used as insecticides. The genus *Ocimum* contains about 30 species native to the tropics and subtropics, some of which are grown in temperate regions as well (3). Several species from genus *Ocimum* have been popularly used to repel or kill insects. *Ocimum americanum* (syn. *Ocimum canum*) and *Ocimum basilicum*, for instance, have been widely employed against mosquitoes in East as well as in West Africa (4). The use of *Ocimum* spp. as insecticides and mosquito repellents seems to be supported by some experimental studies. The essential oils of *Ocimum basilicum* and *Ocimum americanum* were found to kill insects and to protect stored cereals from damage by cowpea weevils (*Callosobruchus maculatus*) (5,6) reported that thermal expulsion and or direct burning of *Ocimum americanum*, *Ocimum kilimandscharicum* and *Ocimum suave* were effective in repelling *Anopheles gambiae* in experimental huts within a screen-walled greenhouse. It has been demonstrated that potted *Ocimum americanum* repelled *Anopheles gambiae* in experimental huts under semi-field conditions (7). Furthermore, *Ocimum americanum* volatile oil was shown to repel *Aedesaegypti*, *Anopheles dirus* and *Culex quinquefasciatus*, under cage conditions, for up to 8 h (8). In addition to their use as insect repellents, *Ocimum* spp. and their essential oils have also been employed to flavor foods and oral hygiene products, in fragrances, in traditional medicines, and as a condiment in culinary. In India, *Ocimum* species have been widely used for Indian religious rituals and, in folk medicine, to treat fevers, stomachaches, cough, bronchitis, sore throat, and also as analgesics, stimulants, emmenagogues and emetics (3).

Materials and Methods

Plants material: The aerial parts of the plants of *Ocimum sanctum, Ocimum basilicum* and *Ocimum gratissimum* were obtained from Ghandi Krishi Vignana Kendra (G.K.V.K), Bangalore. The plants were cleaned, dried in shade and identified by G.K.V.K., Bangalore.

Collection of larvae: *C. quinquefasciatus* larvae were obtained and reared in National Institute of Malaria Research (ICMR), Bangalore, India, at 28.2˚c with a photoperiod of 12 h light and dark and 80±105 RH. A brewer's yeast powder mixed with an equal quantity (w/w) of ground dog biscuits is used in the laboratory as a food for larvae. The late third or early fourth instar larvae were collected.

Preparation of extracts: Ocimum leaves were visually inspected and only those, which were, clean and without any sign of insects or fungi attack were selected for extraction. Fresh leaves were dried at room temperature and then powdered by using a blender. Powdered leaves were subsequently stored in tightly closed containers, kept protected from light at room temperature and under 40–50% relative humidity. About 500 g of the powdered drug was packed in to the thimble of soxhlet extractor and was extracted with petroleum ether (40 - 60º). The extraction was carried out till the solvent in the thimble was clear, indicating the completion of extraction. The extract was filtered and the marc was dried.

The dried marc was repacked and successively extracted with chloroform and alcohol till the solvent in the thimble was clear. Extract was filtered and marc was dried and marc was macerated with water and chloroform for a week and marc was discarded.

The petroleum ether, chloroform, alcohol and water extract so obtained were concentrated under reduced pressure and dried to constant weights. The concentrated extracts were stored in airtight containers.

Preliminary phytochemical tests of the various extracts

The various extracts viz. pet ether, chloroform, alcohol and water extracts and the essential oils of the selected plant species were analyzed for the presence of secondary cell constituents by chemical tests. The main objective of this study was to establish any difference in the chemical composition of the different species.

Preparation of the sample for identification of active ingredients

All the oils and the dried petroleum ether extract which exhibited highest mortality amongst three species were subjected to TLC using pre coated plates (Silica GF_{254}). Toluene: ethyl acetate (93:7) and Petroleum ether: Acetone (9:1) were used as mobile phase for triterpenoids and sterols respectively.

The TLC plates were then sprayed with vanillin sulphuric acid reagent and antimony trichloride (20% solution in chloroform) spraying reagents and the R_f value of each active ingredient was calculated.

Bioassay: For the study of activity all the above extracts were made into suspensions. One gram of the extract was weighed and dissolved in acetone and to this 0.1 ml of Tween 80 was added and small amount of water. Shaken well and made up to 100 ml with water. This solution contains 10000 ppm of the extract. Further dilutions were made from this primary suspension according to the requirement of the study.

Method I (WHO method): Larvicidal activity was evaluated in accordance to World Health Organisation (WHO). All experiments were carried out in 500 ml beaker in triplicate. Twenty five larvae were collected with a Pasteur pipette, placed on a filter to remove excess amount of water and wiped with Whatmann filter paper to absorb remaining water. These are added to 250 ml of dechlorinated tap water containing various concentrations of the crude extract and volatile oil. Two controls in triplicate were set up, one with acetone, Tween 80 in dechlorinated tap water (250 ml) and positive control with Econeem containing 0.5% of azadirachtin was prepared in a concentration of 1 ppm as standard. The beakers are covered with net to avoid the entry of any foreign material. Mortality was recorded at the interval of 1h, 2h, 3h, 4h, 6h and 24 h by counting dead and moribund larvae. Dead larvae were those, which could not be induced to move when they were probed with a needle in the siphon or cervical region.

Moribund larvae were those incapable of rising to the surface or showing characteristic diving reaction when water was disturbed.

Method II (Modified WHO method): The test solutions were taken in 250 ml capacity and larvae released into each of the containers. The containers were covered with a muslin cloth and the mortality recorded every 60 mins for 5 hours. The mortality at five hours was considered as maximum for that test solution.

Method III (Eliot method) : The test suspensions were poured into the Petri dishes. The tests were transferred to the test dishes by nylon net, blotted dry between each operation. After one hour exposure, the larvae were transferred to clean distilled water and examined for mortality after 5 hrs.

Results

The thin layer chromatography of petroleum ether extract found contains triterpenoids and sterols. The chemical analysis of petroleum ether extract showed the presence of triterpenoids, sterols, eugenol, flavonoids and lactones. The pharmacognostic evaluation of ocimum species were tabulated in the table no.2

Amongst the all the extracts tested, the petroleum ether extracts shown the best activity; chloroform shown moderate activity; alcoholic extract shown very poor activity and water did not show any activity. Based on preliminary studies, petroleum ether extracts of *O. sanctum, O. basilicum* and *O. gratissimum* were selected for further studies table no 3.

According to WHO, modified WHO and Eliot's method toxicity evaluation on lab reared larvae showed following order of 24 hrs toxicity against *C. quinquefasciatus* larvae was *O. basilicum* pet ether extract > *O. sanctum* pet ether extract > *O. gratissimum* pet ether extract table no 4,5 and 6.

Table No.1 Phytochemical tests of the various extracts of ocimum species

S. No	Test for	*O.sanctum*				*O.basilicum*				*O.gratisimum*			
		P	C	A	W	P	C	A	W	P	C	A	W
1	Sterols	+	+	-	-	+	+	-	-	+	+	-	-
2	Triterpenoids	+	+	-	-	+	+	-	-	+	+	-	-
3	Eugenol	+	+	-	-	+	+	-	-	+	+	-	-
4	Flavoniods	+	-	+	+	+	-	+	+	+	-	+	+
5	Lactones	+	+	+	+	+	+	+	+	+	+	+	+
6	Saponins	-	-	-	+	-	-	-	+	-	-	-	+
7	Alkaloids	-	-	-		-	-	-		-	-	-	-
8	Carbohydrates	-	-	+	+	+	-	+	+	-	-	+	+
9	Tannins	-	-	-	+	-	-	-	+	-	-	-	+
10	Proteins	-	-	-	-	-	-	-	-	-	-	-	-

P-petroleum ether extract, C-chloroform extract, A- alcoholic extract, W- water extract

Table No.2 Pharmacognostic evaluation of ocimum species

Parameters		*O. sanctum*	*O. basilicum*	*O. gratissimum*
Extractive values	Water	0.92	1	0.76
	Alcohol	0.6	0.56	0.08
	Petroleum Ether	0.16	0.12	0.08
Ash values	Total ash	17.5	15.5	15.5
	Acid insoluble ash	3.2	11.1	1.16
	Water soluble ash	12	11.1	14.5
Loss on drying		10	8.5	10.5
Successive method of extraction	Petroleum ether extract	1.34	12.79	2.95
	Chloroform extract	9.8	8.05	13.42
	Alcohol extract	4.61	19.46	4.77
	Water extract			
Powder microscopy	Covering trichomes	Numerous, unicellular head, multicellular stalk Collapsed trichomes can be seen	Muticellular, warty and long	Collapsed trichomes, multicellular but uniseriate
	Glandular trichomes	-	Small, short with multicellular stalk.	unicellular head and multicellular stalk
	Stomata	Diacytic	Diacytic	
	Other characteristics	Vessels, Oil cells or glands can be seen which are brown in color	Oil cells which are ellipsoid are present many in number	Oil glands can be seen

Table No. 3 Preliminary screening for Ocimum species larvicidal activity against *C. quinquefasciatus*

Sample	LD_{50} values	LD_{90} values	χ^2	Limits for LC_{50}	
				LCL	UCL
O. sanctum pet ether extract	33.33 ppm	99.92	0.0036	26.91	41.21
O.sanctum chloroform extract	3469.66	-	10.276	315.91	41981.05
O. sanctum alcohol extract	467689.94	-	2.010	-	-
O. basilicum pet ether extract	12.28	45.44	0.0008	9.48	15.05
O. basilicum chloroform extract	1992.49	35122.29	10.56	303.45	13609.72
O .basilicum alcoholic extract	457735.24	-	0.45		
O. gratissimum pet ether extract	38.1	633.73641	2.21	25.3481	54.62
O. gratissimum chloroform extract	2746.18	-	5.90	1878.81	4257.08
O. gratissimum alcoholic extract	457735.24	-	0.454		
O. sanctum oil	31.6127	99.87	0.006	25.45	39.23
O. basilicum oil	28.53	89.49	0.0033	23.01	35.44
O.gratissimum oil	40.21	142.28	0.06	32.08	50.27

ppm: parts per million, LD_{50} : Lethal concentration required to kill 50% of the population exposed, LC_{90} : Lethal concentration required to kill 90% of the population exposed, χ^2 : Chi-square for heterogeneity

Table No.4 Larvicidal activity of Ocimum species against larvae of *C. quinquefasciatus* by WHO method

Sample	LD_{50} values	LD_{90} values	't' value	Slope	'χ^2'	Limits for LC_{50}	
						LCL	UCL
O. sanctum pet ether extract	50.62	107.08	6.69	3.93	33.61	40.67	62.56
O. basilicum pet ether extract	46.67	117.66	8.35	3.19	29.08	37.95	56.93
O. gratissimum pet ether extract	55.91	138.58	5.22	3.25	5.22	41.95	73.99
O. sanctum oil	39.31	106.66	5.07	2.95	46.00	27.08	56.66
O .basilicum oil	40.02	108.64	5.31	2.95	41.55	28.13	56.55
O. gratisimum oil	42.87	115.0	2.98	8.17	17.07	34.35	53.16

Table No.5: Larvicidal activity of Ocimum species against larvae of *C. quinquefasciatus* by Modified WHO method

Sample	LD_{50} values	LD_{90} values	't' value	Slope	'χ^2'	Limits for LC_{50}	
						LCL	UCL
O.sanctum pet ether extract	57.13	184.82	6.52	2.51	37.46	44.45	72.99
O.basilicum pet ether extract	50.68	125.1	8.27	3.27	28.36	41.66	61.18
O.gratissimum pet ether extract	76.01	196.59	7.08	3.11	26.82	64.08	89.99
O. sanctum oil	35.14	105.98	6.92	2.67	23.87	26.11	46.88
O.basilicum oil	19.81	77.05	13.36	2.17	9.97	16.82	22.74
O.gratisimum oil	37.34	115.35	7.1	2.62	21.83	28.13	49.14

Table No.6: Larvicidal activity of Ocimum species against *C. quinquefasciatus* by Eliot method

Sample	LD_{50} values	LD_{90} values	't' value	Slope	'χ^2'	Limits for LC_{50}	
						LCL	UCL
O.sanctum pet ether extract	85.41	196.1	13.06	3.55	12.84	80.1	91.06
O.basilicum pet ether extract	78.03	209.52	7.8	2.99	21.41	66.73	91.17
O.gratissimum pet ether extract	88.66	189.09	12.84	3.9	5.91	83.56	94.09
O. sanctum oil	52.37	103.63	8.4	4.32	14.55	44.69	61.00
O.basilicum oil	26.92	90.36	14.62	2.44	10.34	23.72	30.12
O.gratisimum oil	56.69	110.62	13.95	4.41	10.03	52.89	60.44

Discussion

Vector control is facing a threat due to the emergence of resistance in vector mosquitoes to conventional synthetic insecticides, warranting either counter measures or the development of newer insecticides. The results show that this botanical natural product could be used in mosquito control instead of synthetic larvicides. The use of botanicals in mosquito control is an alternative pest control method for minimizing the noxious effects of some pesticidal compounds on the environment.

Present study revealed that pet ether extracts from plant *Ocimum* could induce 50% of mortality in the larvae of *Culex quinquefasciatus* at very low concentration of 39.31 and 46.67 ppm.

Differences in the repellent activity of products, which were extracted by distinct processes from the same part of a particular plant, stated that the extraction procedure and nature of solvents used were the important factors affecting the bioactive principles within the same plant species. Petroleum ether extracts have shown the maximum activity compared to the other solvent s used for the extraction.

When all the three species *O. basilicum*, *O. sanctum, O. gratissimum* were compared *O. basilicum* has more activity, which responded with lower LD_{50} value. *O. sanctum, O. gratissimum* have shown all most same LD_{50} values.

References

1. Kelm MA, Nair MG, Schutzki RA. Mosquitocidal compounds from *Magnolia salicifolia*. International Journal of Pharmacognosy 1997; 35: 84–90.
2. WHO, Manual on larval control operations in Malaria programmes. WHO Offset Publications, No. 1, Geneva, 1973.
3. Vieira RF, Simon JE. Chemical characterization of basil (*Ocimum* spp.) found in the markets and used in traditional medicine in Brazil. Economic Botany 2000; 54: 207–216.
4. Pålsson K, Jaenson TGT. Plant products used as mosquito repellents in Guinea Bissau, West Africa. Acta Tropica. 1999; 72:39–52.
5. Keita SM, Vincent C, Schmit J, Ramaswamy S, Belanger A. Effect of various essential oils on *Callosobruchus maculates* (F.) (Coleoptera: Bruchidae). Journal of Stored Products Research. 2000; 15:355–364.
6. Seyoum A, Pålsson K, King'a S, Kabiru EW, Lwande W, Killeeen GF, Hassanali A, Knols BG. Traditional use of mosquito-repellent plants in western Kenya and their evaluation in semi-field experimental huts against *Anopheles gambiae*: ethnobotanical studies and application by thermal expulsion and direct burning. Transactions of the Royal Society of Tropical Medicine and Hygiene. 2002; 96: 225–231.
7. Seyoum A, Kabiru EW, Lwande W, Killeen GF, Hassanali A, Knols BG. Repellency of live potted plants against *Anopheles gambiae* from human baits in semi-field experimental huts. Am J Trop Med Hyg 2002 ;(67) 191–195.
8. Tawatsin A, Wratten SD, Scott RR, Thavara U, Techadamrongsin Y. Repellency of volatile oils from plants against three mosquito vectors. J Vector Ecol 2001; (26) 76–82.