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PHYTOCHEMICAL SCREENING AND LARVICIDAL ACTIVITY OF EXTRACTS OF *Ocimum basilicum* (Lamiaceae) LEAVES AGAINST *Aedes aegypti*

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ABSTRACT
The leaves of Ocimum basilicum were washed, air dried, ground and soaked in ethanol. The ethanol extract obtained was labeled (OB1) and macerated sequentially using petroleum ether, chloroform and water. The resulting fractions obtained were labeled OB2, OB3 and OB4 respectively. Each fraction was screened for the presence of secondary metabolites using standard procedures. Terpenoids, phenols, flavonoids, steroids, saponins, tannins and volatile oils were detected. All the fractions of O. basilicum were found to be active against fourth instars larvae of Aedes aegypti at 500, 1000, 2000 and 5000µg/ml. The activity was highest with chloroform fraction (OB3) which showed percentage mortality of 67, 77, 80, 97 (LC_{50} of 241.99µg/ml) and 83, 90, 97, 100 (LC_{50} of 75.15µg/ml) after 24hrs and 48hrs respectively, followed by ethanol (OB1), aqueous (OB4) and petroleum ether (OB2) with the least activity. The results could encourage the search for bioactive compounds that will provide an alternative to synthetic larvicides.
Keywords: Phytochemicals, Ocimum basilicum, larvicidal, Aedes aegypti, Activity.

INTRODUCTION

Mosquitoes are the vectors of several life threatening diseases in Human beings. *Aedes aegypti* is the vector of tropical and subtropical diseases such as yellow fever, dengue fever, zika fever and Chikungunya, WHO (2020). Vector control is the most successful method for preventing the problem of mosquito-borne diseases, the widespread of insecticide resistance and the environmental problems associated with some synthetic insecticides proved that the search for an alternative method to control the population of mosquitoes is necessary. Plant derived natural products used as larvicides have the advantage of being harmless to beneficial non-target organisms and to the environment, Kumar *et al*. (2017). The chemical compounds derived from plants have been projected as weapons in the future mosquito control programs because they function as general toxicant, growth and reproductive inhibitors, repellents, and oviposition-deterrent, Amerasan *et al.* (2012). The use of natural products is one of the best alternatives for mosquito control. Most of the mosquito control programmes target the larval stage in their breeding sites with larvicides, Knio *et al.* (2008).The search for herbal preparations that do not produce any adverse effects on the non-target organisms and are easily biodegradable remains a top research issue for scientists associated with alternative vector control, Navneet Kishore *et al*. (2011).

The genus *Ocimum* belongs to the family *Lamiaceae*. It is otherwise known as sweet basil. There are about 150 species, Simon *et al.* (1999), they are used to treat different types of ailments since ancient times especially the species of *Ocimum basilicum*, Siddiqui *et al.* (2012). It is used as a kitchen herb, culinary herb and ornamental herb, Nguyen and Niemeyer (2012). It has also been used as commercial fragrances, flavors and to improve the food products shelf life, Makinen *et al*. (1999); Nguyen and Niemeyer (2012); Suppakul (2003). It is mostly used to treat anxiety, cold, fevers, migraines, diabetes, menstrual cramps, sinusitis, cardiovascular diseases, nerve pain, insect bites, and headache, Sarita *et al*. (2017); Radulovic *et al*. (2013). It also acts as an anti-microbial, Suppakul *et al*. (2003), insecticidal, Freire *et al*. (2006) and cytotoxicity effect, Aarthi and Murugan (2010). It is mostly used to treat anxiety, cold, fevers, migraines, diabetes, menstrual cramps, sinusitis, cardiovascular diseases, nerve pain, insect bites, and headache, Nguyen and Niemeyer (2008); Radulovic *et al*. (2013).

Some basil essential oils have been asserted to possess larvicidal activity against mosquito larvae, Abo-Elseoud *et al.* (2005).

Ocimum basilicum majorly contains about 20 compounds such as linalol, estragole, methyl eugenol, 1, 8-cineole which have been identified by GC-MS, Radulovic *et al*. (2013). The essential oil extract from the leaves of *O. basilicum* has shown significant effect against against third stage larvae of *Culex tritaeniorhynchus*, *Aedes albopictus* and *Anopheles subpictus* with an LC_{50} values of 14.01 ppm, 11.97 ppm and 9.75 ppm and an LC_{90} values of 23.44 ppm, 21.17 ppm and 18.56 ppm respectively, Govindarajan *et al*. (2013).

Kumar *et al*. (2017) reported the exposure of early fourth instars of *Aedes aegypti* with various concentrations of *O. basilicum* essential oil for 24 h revealed a moderate LC_{50} and LC_{90} value of 141.95 ppm and 445.12 ppm, respectively.

The present study was aimed at exploring four different extracts from the leaves of *Ocimum basilicum* for phytochemical screening and larvicidal activity against *Aedes aegypti*.

MATERIALS AND METHODS

Collection of plant material

The fresh leaves of *O. basilicum* were collected from the Garden of Aminu Kano College of Islamic Studies, Kano State, Nigeria. The leaves were washed under running tap water, air dried and ground into fine powder.

Extraction and fractionation

The air dried sample (200 g) was percolated using ethanol (1 L) for a period of two weeks. The crude extract was drained and concentrated at 40^0C on a rotary evaporator. The ethanol extract was then labeled (OB1) and stored in a freezer until use. The crude ethanol extracts (13.7g) was macerated sequentially using petroleum ether, chloroform and water. The fractions were dried by exposing them to air at room temperature.

Phytochemical screening

All the fractions obtained from the leaves of *O. basilicum* were screened for the presence of secondary metabolites using standard procedure as described by Trease and Evans (2007).

Collection of larvae

Fourth instars of *Aedes aegypti* mosquito larvae were collected from Dan Agundi vicinity, Gwale Local Government Area, Kano State, Nigeria and transported to Chemistry Department laboratory of Yusuf Maitama Sule University, Kano in a white plastic bucket for larvicidal bioassay.

Larvicidal bioassay

The larvicidal activity of *Ocimum basilicum* leaves extracts was assessed by using the standard method as prescribed by World Health Organization (2005) with slight modification. From the stock solution, four different test concentrations (5000 µg/ml, 2000 µg/ml, 1000 µg/ml and 500 µg/ml) were prepared. Twenty fourth instars larvae were exposed to small disposable test cups each containing 100 ml of distilled water and 1 ml of prepared test concentrations or solvent (ethanol) as control. Each experiment was carried out in triplicate. The control experiments was also run parallel with each replicate. The larval mortality was calculated after 24 hours and 48 hours of the exposure period. The data was subjected to Probit analysis using SPSS to determine the lethal concentration (LC_{50}) at 95 % confidence intervals.

RESULTS AND DISCUSSION

The phytochemical screening result in Table 2 showed the distribution of secondary metabolites in the fractions. Terpenoids, phenols and saponins were found to be present in all the fractions, Volatile oils are present in all the fraction except OB3, tannins are also recorded in all except OB2. Flavonoids are absent in all the fractions except OB4. Steroids have been detected in OB2 and OB4 only.

The results of flavonoids and saponins from chloroform and ethanol extracts are in consistent with that of Nithya and Dhivya (2019), but there is contradiction in tannins. Lawrence *et al*. (2022) reported the phytochemical screening of ethanolic extracts of *ocimum basilicum* leaves with terpenoids, tannins, saponins and volatile oils similar to the results of this findings but differ in flavonoids. The differences in the distribution of secondary metabolites might be as a result of environmental factors.

The larvicidal analysis of fourth instars larvae of *Aedes aegypti* mosquito on the different fractions obtained from the leaves of *Ocimum basilicum* was carried out and the results are shown in Table 3. At the concentration of 2000 µg/ml and 5000 µg/ml, Chloroform fraction (OB3) exhibited a very high percentage mortality of 80 % and 97 % then 100 % and 97 % after 24 h and 48 h of exposure respectively. The ethanol fraction (OB1) also showed remarkable activity of 70 % and 83 % at 24 h then 87 % and 97 % at 48 h at the above mentioned concentrations respectively. While the petroleum ether fraction (OB2) proved to be less active than any other fraction as indicated in Table 3. The percentage mortality of all the extracts was higher after 48 h exposure period as shown in figure 2 than 24 h exposure period from figure 1.

However, the results of the larvicidal activity of *O. basilicum* leaves extracts are in good agreements with that of Asaad (2013), which reported highest percentage mortality with the ethylacetate leaves extracts at 96 % after 24 h with an LC_{50} at 0.390 mg/l, and 100 % mortality was observed after 48 h with LC_{50} at 0.284 mg/l against 3rd instar larvae of *Anopheles arabiensis*.

RESULTS

Table 1: Weight and appearances of extracts of *Ocimum basilicum* Leaves

Plant fraction	Fraction code	weight (g)	Texture	Colour
Ethanol	OB1	13.7	Sticky	Black green
Petroleum ether	OB2	1.5	Sticky	Black green
Chloroform	OB3	2.1	Sticky	Black green
Aqueous	OB4	4.3	Solid	Brown

Key: OB; Ocimum basilicum

Table 2: Phytochemical Screening of Extracts of *Ocimum basilicum* Leaves

Fraction	Phytochemicals
OB1	Terpenoids, Tannins, Phenols, Saponins, Volatile oils
OB2	Terpenoids, Steroids, Phenols, Saponins, Volatile oils
OB3	Terpenoids, Tannins, Phenols, Saponins.
OB4	Terpenoids, Tannins, Phenols, Flavonoids, Saponins, Steroids, Volatile oils

Key: OB1: Ethanol extract, OB2: Petroleum ether extract, OB3: Chloroform extract, OB4: Aqueous extract.

Table 3: Larvicidal Activity of Extracts of *Ocimum basilicum* Leaves.

Fraction	Conc. (µg/ml)	% Mortality After 24hr	LC_{50} (µg/ml) (LCL-UCL)	% Mortality After 48hr	LC_{50} (µg/ml) (LCL-UCL)
Ethanol (OB1)	5,000	83	234.66	97	178.55
	2,000	70	(3.74-551.97)	87	(25.47-
	1,000	67		83	356.93)
	500	60		70	
Petroleum ether (OB2)	5,000	53	> 1000	73	> 1000
	2,000	42		58	
	1,000	33		47	
	500	30		35	
Chloroform (OB3)	5,000	97	241.99	100	75.15
	2,000	80	(51.70-	97	(0.208-222.7)
	1,000	77	439.99)	92	
	500	67		88	
Aqueous (OB4)	5,000	83	845.51	87	406.05
	2,000	63	(491.56-	77	(118.08-
	1,000	50	1192.08)	60	676.61)
	500	33		57	

Key: LCL; lower confidence limit, UCL; upper confidence limit.

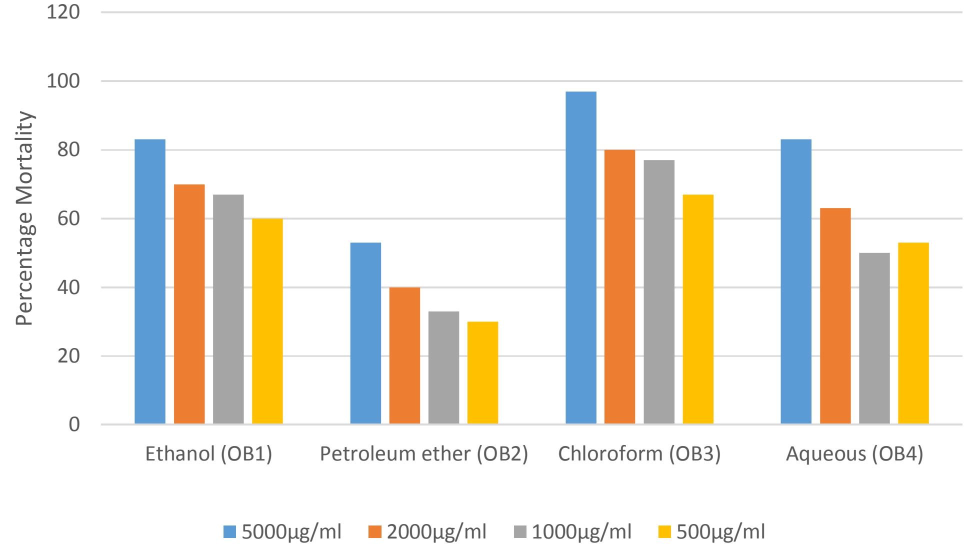

Fig. 1: Larvicidal activity of *O. basilicum* leaves extracts against *Aedes aegypti* after 24hrs exposure.

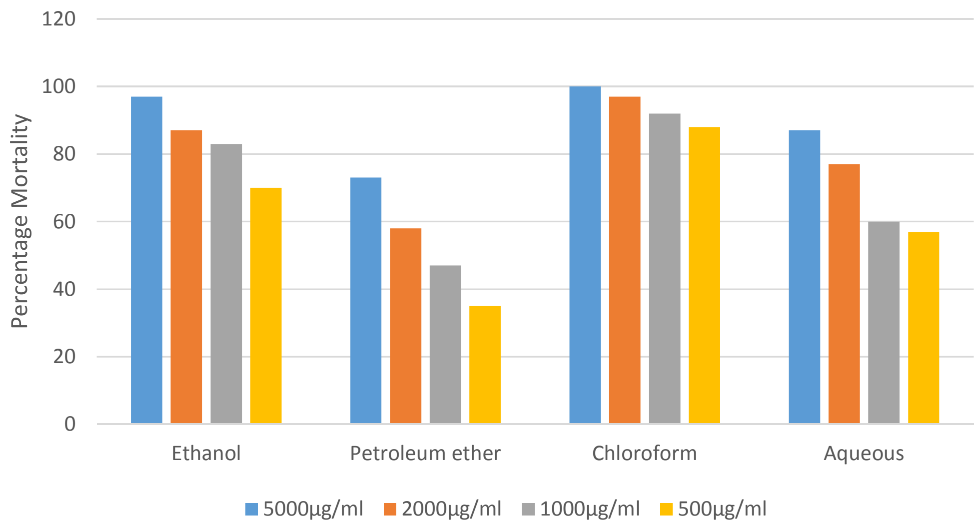

Fig. 2: Larvicidal activity of *O. basilicum* leaves extracts against *Aedes aegypti* after 48hrs of exposure

CONCLUSION

All the fractions obtained from the leaves extracts of *Ocimum basilicum* showed significant activity against fourth instars larvae of *Aedes aegypti*, the activity could be attributed to the presence of the secondary metabolites detected. These results would further encourage the search for a bioactive compound as larvicides from plants.

REFERENCES

Aarthi N. and Murugan K. (2010). Larvicidal and repellent activity of Vetiveri azizanioides L, Ocimum basilicum L and the microbial pesticide spinosad against malarial vector, Anopheles 76 stephensi Liston (Insecta: Diptera: Culicidae). Journal of Biopesticides. 3: 199–204.

Abo-Elseoud MA, Sorhan MM, Omar AE, Helal MM (2005). Biocides formulation of essential oils having antimicrobial activity. Arch Phytopathol Pflanzenschutz 38: 175-184.

Asaad G. M. Basheer (2013). Larvicidal activity of ocimum basilicum l. (basil) chemical extracts on anopheles arabiensis patton, Journal of Pharmaceutical and Biological Sciences 1(6): 66-73.

Duraisamy Amerasan, Kadarkarai Murugan, Kalimuthu Kovendan, Palanisamy Mahesh Kumar, Chellasamy Panneerselvam, Jayapal Subramaniam, Samuel John William, Jiang-Shiou Hwang, (2012). Adulticidal and repellent properties of Cassia tora Linn. (Family: Caesalpinaceae) against Culex quinquefasciatus, Aedes aegypti, and Anopheles stephensi. Parasitology Research DOI 10.1007/s00436-012-3042-3.

Freire MM, Marques OM, Costa M. (2006). Effects of seasonal variation on the central nervous system activity of Ocimum gratissimum L. essential oil. Journal of Ethnopharmacology. 105: 161–6. https://doi.org/10.1016/j.jep.2005.10.013 PMid:16303272

Gulcin I, Elmastas M, Aboul-Enein HY (2007). Determination of antioxidant and scavenging activity of Basil (Ocimum basilicum L. family lamiaceae) assayed by different methodologies. Phytotherapy Research. 21: 354–61. https://doi.org/10.1002/ptr.2069 PMid:17221941

Govindarajan M, Sivakumar R, Rajeswary K, Yogalakshimi (2013). Chemical composition and larvicidal activity of essential oil from *Ocimum basilicum* (L.) against *Culex tritaeniorhynchus*, *Aedes albopictus* and *Anopheles subpictus.* Experimental parasitology 134:7-11.

James E. Simon, Mario R. Morales, Winthrop B. Phippen, Roberto Fontes Vieira, Zhigang Hao. (1999). Basil: A source of aroma compounds and a popular culinary and ornamental herb. Perspectives on new crops and new uses. Alexandria: ASHS Press.

Knio, K.M., Usta, J., Dagher, S., Zournajian, H. and Kreydiyyeh, S. (2008). Larvicidal activity of essential oils extracted from commonly used herbs in Lebanon against the seaside mosquito, Ochlerotatus caspius. Bioresource Technology, 99:763–768.

Luka Lawrence, Attama Chika, Yahaya Muhammad Falalu, Njiddah Kwaji, Thomas Polar (2022). Phytochemical screening and bioassay of the ethanolic extracts of Ocimum basilicum and Commiphora kerstingii leaves. World Scientific News 167: 28-40.

Makinen S, Paakkonen K, Hiltunen R, Holm Y. (1999). Processing and use of basil in foodstuffs, beverages and in food preparation. Basil: the genus Ocimum. Netherlands: Harwood Academic Publishers.

Navneet Kishore, Bhuwan B. Mishra, Vinod K. Tiwari and Vyasji Tripathi (2011). A review on natural products with mosquitosidal potentials. Opportunity, Challenge and Scope of Natural Products in Medicinal Chemistry, 335-365 ISBN: 978-81-308-0448-4.

Nguyen PM, and Niemeyer ED (2008). Effects of nitrogen fertilization on the phenolic composition and antioxidant properties of basil (*Ocimum basilicum* L.). Journal of Agricultural and Food Chemistry. 56: 8685–91. https://doi.org/10.1021/jf801485u PMid: 18712879.

Nithya R. and Dhivya R. (2019). Phytochemical Screening and Repellent Activity of Leaf Extracts of *Ocimum basilicum* and Albizia amara against the Mosquito *Culex quinquefasciatus*. International Journal of Research & Review. Vol.6; Issue: 3.

Ozcan M, Chalchat JC (2005). Essential oil of Ocimum basilicum L. and Ocimum minimum L, in turkey. Journal of Food Science. 20: 223–8.

Radulovic NS, Blagojevic PD, Miltojevic AB (2013). α-Linalool marker compound of forged/synthetic sweet basil (Ocimum basilicum L.) essential oils. Journal of the Science of Food and Agriculture. 93: 3292–303. https://doi.org/10.1002/jsfa.6175 PMid:23584979

Ramalingam PS, Sagayaraj M, Ravichandiran P, Balakrishnanan P, Nagarasan S, Shanmugam K. (2017). Lipid peroxidation and anti-obesity activity of Nigella sativa seeds. World Journal of Pharmaceutical Research. 6(10): 882–92.

Sarita Kumar, Radhika Warikoo, Monika Mishra, Roopa R Samal, Shrankhla, Kungreiliu Panmei, Vinay S Dagar and Aarti Sharma (2017). Impact of Ocimum basilicum Leaf Essential Oil on The Survival and Behaviour of An Indian Strain of Dengue Vector, Aedes aegypti (L.) Vector Biol J 2017, 2:2

Siddiqui BS, Bhatti HA, Begum S, Perwaiz S (2012). Evaluation of the Anti-mycobacterium activity of the constituents from *Ocimum basilicum* against Mycobacterium tuberculosis. Journal of Ethnopharmacology. 144:220–2. https://doi.org/10.1016/j.jep.2012.08.003 PMid:22982011

Suppakul P, Miltz J, Sonneveld K, Bigger SW (2003). Antimicrobial properties of basil and its possible application in food packaging. Journal of Agricultural and Food Chemistry. 51: 3197–207.

Trease, G.E., Evans, W.C. (2002). Text Book of Pharmacognosy (16th edition) WB Saunders Harcourt Publishers Ltd. London, UK. 137-139, 230-240.

Wattal, B. L., Joshi, G. C. and Das, M. (1981). Role of agricultural insecticides in precipitation vector resistance. Journal of Communicable Disease; 13: 71-3

World Health Organization, (2005). Guidelines for laboratory and field testing of mosquito larvicides. Communicable disease control, prevention and eradication, WHO pesticide evaluation scheme. WHO, Geneva, WHO/CDS/WHOPES/GCDPP/1.3.

World Health Organization, (2020). Vector-borne disease fact sheet. https://www.who.int/news-room/fact-sheets/details/vector-borne-diseases.