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Phytochemical Composition and Larvicidal Activity of *Ocimum canum* (L.) Essential Oil against *Anopheles gambiae* (Diptera: Culicidae)

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Authors' contributions

This work was carried out in collaboration between all authors. Author SKB planned, designed the research and wrote the first draft. Authors RMA, EAA and IBA designed the methodology, performed the Lab. experiment and author SO performed the statistical analysis and edit final draft. All authors read and approved the final manuscript.

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ABSTRACT

Background: Malaria is a serious health problem in many countries of the world especially sub-Saharan African. *Anopheles mosquito* which is the vector of this disease has developed resistance against synthetic pyrethroids which are the main stay of insecticide treated bed nets. The development of insecticide resistance and side effects associated with synthetic pesticides has triggered intense research efforts towards natural products.
Aim: This study aimed at investigating quality and quantity of phytochemical constituents of *Ocimum canum* leaf extract and determined larvicidal effect of essential oil component of this plant extract on 3rd instar larvae stage of *Anopheles gambiae*.
Methods: The essential oil was extracted by hydro-distillation using Clevenger type apparatus.

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Standard methods previously described by other authors were used to determine the quality and quantity of phytochemical components of the plant and larvicidal bioassay method was used to determine the effect of essential oil of *O. canum* on the mosquito larva.
Results: The analysis of the phytochemical revealed the presence of the following compounds: Tannin, phenol, saponin, trepenoid, alkaloid, steroid, and flavonoid, with the following quantities 22.14, 19.83, 14.08, 10.97, 8.16, 3.40 and 5.29 mg/g respectively. The mosquito larvicidal bioassay showed LC_{50} and LC_{90} values of 49.51 and 103.54×10^{-3} mg/ml respectively after 24 hours exposure to essential oil extract.
Conclusion: The results obtained showed that the essential oil of *O. canum* is a promising mosquito larvicidal compound.

Keywords: Anopheles mosquito; Ocimum canum; essential oil; phytochemicals; larvae.

1. INTRODUCTION

Malaria is one of the world's most common and severe tropical diseases transmitted mainly by the female *Anopheles gambiae* mosquito. According to Breman et al. [1] and Snow et al. [2], more than 300 millions of people are infected with malaria annually. This infection is estimated to cause approximately 1.5 to 3 million deaths globally each year, with about 90% of all infections mainly affecting pregnant women and children under 5 years of age [3], occurring in sub-Sahara Africa. Despite considerable efforts to eradicate or control malaria, no effective malaria vaccine is yet available [4]. The parasites develop quick resistance to many effective drugs.

However, vector control has been touted as primordial and an essential means of controlling transmission of not only malaria; but also several other mosquito-borne diseases such as yellow fever, dengue fever, filariasis and Japanese encephalitis [5,6]. Due to the concern over the quality and safety of life and the environment, the emphasis on controlling mosquito vectors has shifted steadily from the use of conventional chemical insecticides towards alternative insecticides that are target-specific, biodegradable, and environmentally safe; and these are generally botanicals in origin.

A lot of phytochemicals extracted from various plant species have been tested for their larvicidal and repellent actions against mosquitoes [7]. This has propelled the search for and use of eco-friendly plant based products for the control of insects such as mosquitoes [8]. Hence, there is a renewed interest in the exploration and use of plant products with insecticidal properties for mosquito control.

The essential oil of aromatic plants is very important sources of many compounds that are useful for medicine and other applications [9]. Essential oils are simply volatile fractions obtained by steam or water distillation of aromatic plants [10]. Essential oils have received considerable renewed attention as potent bioactive compounds against various species of mosquitoes. They are potentially suitable for application in larval control management because they constitute a rich source of bioactive compounds that are effective and naturally biodegradable into non-toxic products [11-13].

Plants are the richest resource of drugs of traditional systems of medicine. In modern medicines, plants are particularly useful as sources of food supplements and pharmaceutical intermediates for synthetic drugs [14]. Traditional medical system has great value for plants and many medicinal plants have been identified from indigenous pharmacopoeias, because plants are still making imperative contribution to healthcare regardless of modern medicines; and they are known to possess many advantages [15].

The medicinal values of plants lie in their phytochemicals, which produce definite physiological actions on the human body. Some phytochemicals have antioxidant property, while others have hormone-like effect which helps in fighting against many diseases including cancer, heart disease, diabetes, high blood pressure and preventing the formation of carcinogens on their target tissues [16].

The genus *Ocimum* belongs to lamiaceae family and is found in many parts of the world especially in tropics and sub-tropical regions of Asia, Africa and Central and South America. It is a source of essential oils and aromatic compounds, a culinary herb and an attractive, fragrant ornamental plant [17]. *O. canum* is considered as a sacred plant and its various medicinal properties have been mentioned in ancient

medicinal test, *Ayurveda* [13]. Different parts of this plant are used for treatment of various ailments.

The distribution of *Ocimum canum* depends on the climatic conditions [17]. The plant has been identified as a medicinal plant in Nigeria and other parts of Africa to treat conjunctivitis, malaria and headache [17]. It has also been used to manage diabetes mellitus in Ghana [18].

Most of previous researches on larvicidal activity of essential oils of aromatic plants were done against *Aedes aegypti* and *Culex quinquefasciatus* larvae that are not vector of malaria [19], while others worked on other species of Ocimum such as *Ocimum basilicum* and *Ocimum sanctum* [20]. The prevention of mosquito breeding through the use of larvicidal compounds is the more effective way to fight mosquito imposition than against the adult stage. In addition, recent increased interest in developing plant insecticides as an alternative to chemical insecticides has motivated this research work. This research was carried out to elucidate the phytochemical components of extracts of *Ocimum canum* and investigate the larvicidal activity of extracted essential oil of the plant against larvae *Anopheles gambiae*.

2. MATERIALS AND METHODS

2.1 Collection, Identification and Maintenance of Plant Materials

Ocimum canum leaves were collected from Okoru village of Moro Local Government area of Kwara State, Nigeria. It was identified and authenticated by a plant taxonomist from the Department of Botany, Obafemi Awolowo University, Ile-Ife, Nigeria with a voucher number A20695.

All the plant leaves needed for the test were rinsed with distilled water and air dried to crispiness at room temperature on the laboratory work bench within a week. The dried leaves were grinded to powdered form using an electric blender (Binatone, Japan) and stored in an enclosed container in the refrigerator.

2.2 Breeding, Collection, Identification and Maintenance of Mosquito Larvae

Using the dipping technique WHO [21] 30 litres of 10 plastic rubber containing water was set in stagnant pool of water bodies in Malete community to breed the mosquito larvae. Large numbers of mosquito larvae were harvested within a period of 5 to 7 weeks. By gently dipping a clean container with a fairly long handle into the stagnant water containing the mosquito larvae, the larvae were collected, ensuring that human shadow was kept away from the water body, this is because larvae are sensitive to darkness and this tends to make them dive away from the surface to the bottom of the water body. The larvae collected were transported to the laboratory in small basins. The larvae were identified by an entomologist in the Department of Zoology, Kwara State University, to be third instar larvae of *Anopheles gambiae*. It was then kept and properly maintained in order to determine its susceptibility to the essential oil extracts of *O. canum* leaves.

2.3 Preparation of the Leaf Extract

Extraction of the leaves was prepared following the method of [11], by adding 25 ml of methanol, ethanol and aqueous solutions to 0.5 gram of powder leaves each contained in a covered 50 ml centrifuge tube; this was shaken continuously for 1 hour at room temperature. The mixture was centrifuged at 3,000 rpm for 10 minutes, and then the supernatant was collected and stored at 4ºC for further analysis.

2.4 Phytochemical Screening of the Leaf Extracts of *Ocimum canum*

Ethanolic, methanolic and aqueous extract of *Ocimum canum* leaves were subjected to phytochemical analysis using standard techniques described by Odebiyi and Sofowora [22]. The detection of steroids, saponins, phenolics, tannins, flavonoids, terpenoid and alkaloid were carried out as previously described by Trease and Evan [23], Hiai et al. [24], Kale et al. [25], Padmaja, [26], Kale et al. [25], Harbourne, [27] and Singh et al. [28] respectively. Methanol extract of *O. canum* was used for quantitative of phytochemicals. The quantitative analyses were for steroids, saponins, phenolics, tannins, flavonoids, terpenoid and alkaloid were carried out with methods of El-Olemy et al. [29], Makkar [30] Chan et al. [31], Padmaja [26], Kale et al. [25], Doneva-Sapceska et al. [32] and Singh et al. [28] respectively.

2.5 Extraction and Isolation of Essential Oil

Two hundred and fifty grams (250 grams) of the air-dried leaves of *Ocimum canum* was subjected

to hydro distillation for 3 hours using a Clevenger type apparatus (Soham Scientific, UK). Sodium chloride (1 gram) and 20 mL of dichloromethane was added with the aqueous distillate in a separating funnel and shaking was continued for 40 minutes and this was allowed to stand for 15 minutes. The organic layer was separated and concentrated under reduced pressure. The oils dissolved in the organic layer was dried over anhydrous sodium sulphate and preserved in a sealed vial at refrigerated temperature until further analysis.

2.6 Mosquito Larvicidal Bioassay

The larval toxicity test was carried out following the standard World Health Organization [32] larval bioassay method [32], with slight modifications. Since oil does not dissolve in water, it was first solubilized in dimethyl-sulphoxide (DMSO), and diluted with spring river water to a stock solution of 250 mg/mL. Serial dilution of the stock solution were done at different concentrations which include: 250, 200, 150, 125, 100, 75, 45, 35, 17.50, 7.95, 3.72× 10^{-3} mg/mL. The concentration of DMSO was kept below 1% since at this level it does not affect larval mortality. The bioassays were performed with third instar larvae of *Anopheles gambiae.* Three replicates were set up for each concentration, and equal numbers of controls were set up simultaneously using local spring water in 1% DMSO. The larvae were collected by direct pipetting from the plastic trays and transferred to 25 ml disposable Petri-dishes containing 10 ml of test solution and fed on cray fish feed during all testing. Ten larvae were placed in each Petri-dish. The mortality and survival in each plastic tray was counted after 24 hours. Larvae were considered dead if they were unmovable within a period of time, even when gently prodded with a micropipette. The dead larvae in the three replicates were combined and expressed as the percentage mortality for each concentration.

2.7 Statistical Analysis

The average larval mortality data were subjected to probity regression methods for calculating LC_{50} and LC_{90} at 95% fiducial limits of upper confidence limit and lower confidence limit using IBM SPSS software version 16.

3. RESULTS

The phytochemical screening of the ethanol, methanol and aqueous extracts of *O. canum* leaf revealed the presence of tannin, saponin, alkaloid, flavonoid, trepenoid, steroid and phenol. The larvicidal bioassay of the extracted essential oil revealed mortality rate. Qualitative analysis of the ethanol and methanol extracts showed the presence of all the tested phytochemicals in *O. canum* leaves, compared to the aqueous extracts that showed that saponin, alkaloid and trepenoid were absents. In methanol extracts, all the phytochemicals were most present except steroids; in ethanol extracts only tannins and phenol were most present (Table 1). The quantitative analysis of *Ocimum canum* leaves showed that tannin and phenol had the highest phytochemicals content that account for 22.14 and 19.83 mg/g respectively; while steroids had a lower composition of all the phytochemicals with the value of 3.40 mg/g (Table 2).

Essential oil of *O. canum* leaves showed good larvicidal activity against the third instar larvae of *A. gambiae* after 24 hours of exposure. Larvicidal bioassay concentration at 250 ×10^{-3} mg/ml which was the highest concentration had the highest lethal effect followed by the subsequent concentrations, but least concentration which were 17.50, 7.95 and 3.72 ×10^{-3} mg/ml had no effect, as well as on the controls which was the spring water and DMSO. The percentage mortality, mean and standard deviation of the three replicates and the average larval mortality data were subjected to probity regression methods for calculating LC_{50} and LC_{90} at 95% fiducial limits of upper confidence limit and lower confidence limit which accounted for the value of 49.50 and 103.54× 10^{-3} mg/ml respectively (Table 3).

4. DISCUSSION

Many plant extracts produce lethal effects on various stages of mosquitoes such as oviposition, ovicidal, larvicidal, pupicidal and adulticidal. The effects of phytochemical components of *O. canum* on larvae of *Anopheles gambiae* were influenced by extrinsic and intrinsic factors, that can only be found in phytochemical components of the leaf [33]. Our result also showed that the higher the concentration of essential oil of *O. canum* applied the higher the number of larvae killed. At the concentration of 250 mg/ml all the larvae of the mosquitoes were killed. This is similar to the result obtained by Pugazhvendon and Elumali, [33] that showed highest larval mortality at high concentration of 400 ppm, though they used a different plants and mosquito species.

Table 1. Qualitative analysis of phytochemicals in *O. canum*

Extraction solvent	Tannin	Saponin	Alkaloid	Flavonoid	Trepenoid	Steroids	Phenol
Ethanol	+++	+	+	++	+	++	+++
Methanol	+++	+++	+++	+++	++	+	+++
Aqueous	+	-	-	+	-	+	+

Key: + = present, ++ = more present, +++ = most present and - = absent

Table 2. Quantitative analysis of phytochemicals in *O. canum* leaves methanolic extract

Quantities of phytochemicals (mg/g)						
Phenol	steroids	Flavonoids	Tannin	Saponins	Alkaloids	Terpenoids
19.83	5.29	22.14	14.08	8.16	10.97	3.40

Table 3. Larvicidal activity of essential oil of *O. canum* leaves against third instar larvae of *A. gambiae* after 24 hours of exposure

Concentration ($x10^3$ mg/mL)	Mean mortality	% Mortality ±SD	LC_{50} ($x10^{-3}$ mg/mL)	LC_{90} ($x10^{-3}$ mg/mL)
3.72	0.0000	0.00±0.00	49.50 (41.64-58.28)	103.54 (87.50-123-54)
7.95	0.0000	0.00±0.00		
17.50	0.6667	3.33±1.00		
35.00	3.0000	15.00±1.00		
45.00	5.3333	26.67±0.58		
75. 00	7.3333	36.67±0.78		
100.00	10.0000	50.00±1.00		
125.00	12.3333	63.33±2.08		
150.00	15.3333	81.67±2.08		
200.00	18.6667	95.00±1.00		
250.00	20.0000	100.00±0.00		
Control	0.0000	0.00±0.00		

From the mosquito bioassay analysis result obtained, the essential oil of *O. canum* plant exhibited mortality at LC_{50} and LC_{90} at 49.50 mg/L and 103.54 mg/L respectively against *A. gambiae*. This is similar to reports from earlier studies on activity of essential oil from *Fagaropsis angolensis* (Rutaceae) plant against the third instar larvae of *A. gambiae* exhibited LC_{50} and LC_{90} value of 83.7 mg/L and 324.0 mg/L, respectively by Mudalungu et al. [34]. Kweka and colleagues [35] also reported larvicidal activity of *Plectranthrus. amboinicus* essential oil against late third instar larvae of *A. gambiae* and observed LC_{50} and LC_{90} values of 67.53 and 107.60 ppm, respectively.

The present study has shown that the essential oil of *O. canum* has larvicidal activity against *A. gambiae.* It could be used selectively in places where water is stagnant and may affect the fertility of adults emerged from larvae exposed to oil [36]. However, further study is still needed to determine which of the seven phytochemical components detected in essential oil of *Ocimum canum* produce larvicidal effect. Study by Omibito and his colleagues [7] was able to further break down the components of essential oil present in *Zanthoxylum gilleti* into over 50 compounds, however the research did not determine which of these compounds is responsible for larvicidal activity.

5. CONCLUSION

In conclusion, this study and others [7,37,38] on essential oil of aromatic plants have revealed that products based on these plants can be used to control population density of this important vector (*A. gambiae*) of sub-Saharan killer disease-Malaria.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Breman JG, Alilio MS, Mills A. Conquering the intolerable burden of malaria: What's new, what's needed: A summary. Am. J. Trop. Med. Hyg. 2004;71:1–15.
2. Snow RW, Guerra CA, Noor AM, Myint HY, Hay SI. The global distribution of clinical episodes of *Plasmodium falciparum* malaria. Nature. 2005;434:214–217.
3. Steketee RW, Nahlen BL, Parise ME, Menendez C. The burden of malaria in pregnancy in malaria-endemic areas. Am. J. Trop. Med. Hyg. 2001;64(1-2Suppl): 28-35.
4. Matasyoh JC, Wathuta EM, Kariuki ST, Chepkorir R, Kavulani J. Aloe plant extracts as alternative larvicides for mosquito control. Afr. J Biotechnol. 2008;7: 912-915.
5. Vatandoost H, Vaziri M. Larvicidal activity of neem extract (*Azadirachta indica*) against mosquito larvae in Iran. Pestol. 2001;25:69-72.
6. Radhika D, Ramathilaga A, Prabu CS. Evaluation of larvicidal activity of soil microbial isolates (*Bacillus* and *Acinetobacter* Sp.) against *Aedes aegypti* (Diptera: Culicidae) - The vector of chikungunya and dengue. Proceed Inter Acad. Ecol. Environ. Sci. 2011;1:169-178.
7. Ombito OJ, Matasyoh C, Josphat M, Vulule MJ. Chemical composition and larvicidal activity of *Zanthoxylum gilletii* essential oil against *Anopheles gambiae*. African Journal of Biotechnology. 2014; 13(21):2175-2180.
8. Navneet K, Bhuwan B, Mishra V, Tiwari K, Tripathi V. A review on natural products with mosquitocidal potentials. Mosq Nat. Prod. 2011;201:335-365.
9. Abduelrahman A, Elhussein S, Osman N, Nour A. Morphological variability and chemical composition of essential oils from nineteen accession of basil (*Ocimum basilicum* L) growing in Sudan. Int. Jo. Chem. Techn. 2009;1(1):1-10.
10. Rabha B, Gopalakrishnan R, Baruah I, Singh L. Larvicidal activity of some essential oil hudrolates against dengue and filariasis vectors. E3 J. Med. Res. 2012;1(1):014-016.
11. Lucia A, Audino GA, Seccacini E, Licastro S, Zerba E, Masuh H. Larvicidal effect of *Eucalyptus grandis* essential oil and turpentine and their major components against *Aedes aegypti* larvae. J. Am. Mosq. Contr. Assoc. 2007;3:299-303.
12. Cheng SS, Huang CG, Chen, WJ, Kuo YH, Chang ST. Larvicidal activity of tectoquinone isolation from red heartwood-type *Cryptomeria japonica* against two mosquito species. Bioresour. Technol. 2008;99:3617-3622.
13. Cheng SS, Chua MT, Chang EH, Huang CG, Chen WJ, Chang ST. Variation in insecticidal activity and chemical composition from leaf essential oils of *Cryptoemeria japonica* at different ages. Bioresour Technol. 2009;100(4):65-470.
14. Hammer KA, Carson CF, Riley TV. Antimicrobial activity of essential oils and other plant extracts. Journal of Applied Microbiology. 1999;86:985-990
15. Adebolu TT, Salau AO. African Journal of Biotechnology. 2005;4(7):682-684.
16. Daniel VN, Daniang IE, Nimyel ND. IJET-IJENS. 2011;11(6):161-165
17. Ngassoum MB, Ousmaila H, Ngamo LT, Maponmetsem PM, Jirovetz L, Buchbauer G. Aroma compounds of essential oils of two varieties of the specie plant; *Ocimum canum* Sims from northern Cameroon. J. Food Compost Anal. 2004;17(2):197-204.
18. Nyarko AK, Asare-Anane H, Ofosuhene M, Addy M. Extract of *Ocimum canum* lowers blood glucose and facilitates insulin release by isolated pancreatic β-islet cells. Journal of Phytomedicine. 2002;9(4):346-351.
19. Manzoor FF, Beena W, Naz N, Naz S. Preliminary evaluation of Ocium santum as toxicant and repellent against termites *Heterotermes indicola* (Wasmann) (Ispoptera) (Rhinotermitidae). Paskistan J. Sci. 2011;63(1):59-62
20. Bhatnagar M, Kapur KK, Jalees S, Sharma SK. Laboratory evaluation of insecticidal properties of *Ocimum basilicum* Linnaeus and O. sanctum Linnaeus plant's essential oils their constituents against vector mosquito species. J. Entomol Res. 1993; 17(1):111-119.
21. World Health Organization. Guidelines for laboratory and field testing of mosquito larvicides. Communicable disease control, prevention and eradication, WHO pesticide evaluation scheme. WHO, Geneva; 2005: WHO/CDS/WHOPES/GCDPP/1.3.

22. Odebiyi O, Sofowora A. Medicinal plants and traditional medicine in Africa. Journal of Phytomedicine. 1993;8:107-119.
23. Trease GE, Evans W. Pharmacognsy. 11th edn. Brailliar Tiridel Can. Macmillian Publishers. 1989;127-198.
24. Hiai S, Oura H, Nakajaima H. Colour reaction of some sapogennins and saponnins with vanillin and sulphuric acid. Plant Med. 1976;29:116-122.
25. Kale A, Gaikwad S, Mundhe K, Deshpande N, Salvekar J. Quantification of phenolics and flavonoids by spectrophotometer from *Juglan regia*. International Journal of Pharma and Biosciences. 2010;1:1-4.
26. Padmaja G. Evaluation of techniques to reduce assayable tannin and cyanide in cassava leaves. J. Agric Food Chem. 1989;37:712-716
27. Harbourne JB. Phytochemical methods, London. Chapman and Hall, Ltd. 1983;49-188.
28. Signh DK, Srivastava B, Sahu A. Spetrophotometric determination of Rauwolfia alkalnoids, estimation of reserpine in pharmaceuticals. Analytical Sc. 2004;20:571-573.
29. El-Olemy MM, Al-Muhtadi FJ, Afifi AFA. Experimental phytochemistry: A laboratory Manual. King Saud University Press, Saudi Arabia. 1994;21-27.
30. Makkar HS, Sidhuraju P, Becker K. Plant secondary metabolites. Humana Press Inc. Totowa, NJ, USA. 2007;34-45.
31. Chan EC, Lim Y, Chew YL. Antioxidant activity of *Camellia sinensis* leaves and tea from a lowland plantation in Malaysia. J Food Chem. 2007;102:1214-1222.
32. Doneva-Sapceska D, Dimitrovski A, Bojadziev T, Milaov G, Vojnovski B. Free and potentially volatile monoterpenes in grape varieties from the republic of Macedonia. Maced. J. Chem. 2006;25(1): 51-56.
33. Pugazhvendan SR, Elumali K. Larvicidal activity of selected plant essensial oil against vector mosquitoes: Dengue vector, *Aedes aegypti* (L), Malarial vector, *Anopheles stephensi* (Liston) and Filaria vector, *Culex quinquefasciatus* (Say) (Diptera: Culicidae). Middle-East Journal of Scientific Research. 2013;18(1):91-95.
34. Mudalungu CM, Matasyoh JC, Vulule JM, Chepkorir R. Larvacidal compounds from *Fagaropsis angolensis* leaves against malaria vector (*Anopheles gambiae)*. Int. J. Malaria. Res. Rev. 2013;1(1):1-7.
35. Kweka JE, Senthikumar A, Venkatesalu V. Toxicity of essential oil from Indian borage on the larvae of African malaria vector mosquito, *Anopheles gambiae*. Parasites Vectors. 2012;5:277.
36. Cavalcanti ES, Morais SM, Lima MA, Santana EW. Larvicidal activity of essential oils from Brazilian plants against *Aedes aegypti.* L. Mem Inst Oswaldo Cruz. 2004; 99(5):541-544.
37. Santhoshkumar T, Rahuman AA, Rajakumar G, Marimuthu S, Bagavan A, Jayaseelan C, Zahir AA, Elango G, Kamaraj C. Synthesis of silver nanoparticle using *Nelumbo nucifera* leaf extract and its larvicidal activity against malaria and filariasis vectors. Parasitol. Res. 2010;2: 2115-2117.
38. Pushpanathan T, Jebanesan A, Govindarajan M. The essential oil of *Zingiber offinalis* Linn (*Zingiberaceae)* as mosquito larvicidal and repellent agent against the filarial vector *Culex quinquefasciatus* Say (Diptera: Culicidae). Parasitol. Res. 2008;102:1289-1291.

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