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Investigating chemical composition and larvicidal potency of the essential oil of *Citrus sinensis* fruit rind

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Abstract
Malaria, filariasis and other related fever caused by insects constitute major health challenges in the world. Since synthetic insecticides used for the control of the vectors may not be environmentally friendly, essential oil of *Citrus sinensis* (sweet orange) rind was tested for larvicidal potency. The essential oil was obtained via hydrodistillation method and subjected to gas chromatography-mass spectrometry (GC-MS) analysis. (R)-(+)-limonene, a monoterpene was obtained as the major constituent of essential oil of *Citrus sinensis* rind. Mortality of the larvae of culex mosquitoes was observed after 24 hours of exposure to different concentrations of the essential oil. Result obtained showed that the mortality rate of the larvae increased with increase in concentration of the essential oil with LC_{90} value of 4.6 ppm. The essential oil of *Citrus sinensis* rind showed potent larvicidal efficacy and can be considered for further investigation.

Keywords: *Citrus sinensis*, esssential oil, hydrodistilation, larvicidal activity, limonene, Culex mosquitoes, sweet orange

Introduction

Mosquitoes are the most important insects in terms of public health importance. They transmit several diseases such as malaria, dengue fever, yellow fever, filariasis and encephalitis which continue to have devastating effect on human beings; and affect more than 700 million people worldwide annually [1]. Mosquito bites may also cause allergic responses including local skin reactions and systemic reactions such as urticarial. Mosquito control is essential to prevent proliferation of mosquito borne diseases and to improve quality of environment and public health [2]. During the past several decades, many synthetic insecticides have been developed and effectively used to eliminate mosquitoes. Unfortunately, the management of these disease vectors using synthetic insecticides has failed in part due to their efficiency in attaining physiological resistance. In addition, the application of such chemicals has resulted in long-term harmful effects on non-target organisms and other environmental components [3].

Larviciding is a successful way of reducing mosquito densities in their breeding places before they emerge into adults. Pesticides are indeed very effective in its use. However, the use of chemical insecticides is often toxic to both human and non-target animals. The intensive use of chemical insecticides led to the development of resistant insect populations, resulting in reduced control, environmental pollution resulting in bio-amplification in food chain and contamination [2].

Plants have the major advantage of still being the most effective and cheaper alternative green measure for the control of arthropods of public health importance. Therefore, natural products of plant origin are safe to use than the synthetic insecticides [2].

Citrus sinensis fruit (sweet orange) is of the family Rutacea. It is also called sweet orange, to distinguish it from the related *Citrus aurantium* referred to as bitter orange. Orange is a hybrid of pomelo (*Citrus maxima*) and mandarin (*Citrus reticulate*). The sweet orange reproduces asexually, and its trees are widely grown in tropical and subtropical climates. The fruit of the orange tree can be eaten fresh or processed for its juice or fragrant peel [4-6].

Essential oils from neem, camphor, lemon, cinnamon, pine, mint and several plants have been shown to possess mosquito larvicidal properties [1, 7].

In view of an increasing interest in developing plant-based insecticides as an alternative to chemical insecticides, hence, this study was carried out to investigate the effect of essential oil from *Citrus sinensis* fruit rind on Culex mosquito larva.

Materials and Methods

Extraction of Essential Oil

Mature ripe healthy fresh fruits of *Citrus sinensis* were bought from a local market in Port Harcourt metropolis, Nigeria. The fruits were washed thoroughly. Rinds (1.894 kg) from the washed *Citrus sinensis* fruits were removed, cut into tiny pieces and kept in a clean container. This was subjected to hydro-distillation in a 2.5L round bottom flask adapted to a Clevenger type apparatus to obtain the essential oil. Extracted oil was kept in an air tight glass sample bottles and refrigerated at 4℃ for further analysis [8-10].

GC-MS Analysis

GC-MS analysis was carried out using Agilent 6890N chromatograph with Agilent 5975 mass selective detector instrument employing the following condition: 30m x 0.25mm x 0.25µm Elite-5MS column (Perkin Elmer, USA). The column temperature was increased from 40 °C to 220 °C at a rate of 4 °C. MS parameters were as follows: EL mode, with ionization voltage. 70eV, ion source temperature 180 °C, scan range, 50-600 DA. The peaks were tentatively identified based on library search using NIST and Wiley registry 8 edition. The identities of some component were confirmed by both mass spectral and retention data of the authentic chemicals obtained under identical GC-MS conditions. International standards were applied, and concentration of selected compounds was determined based on standard curve.

Test organisms

Third instar larvae Culex specie was used in this study. All larvae of Culex species were obtained from a natural breeding site at Eagle Island of Port Harcourt, Nigeria and were transported to the Malaria Research Institute Laboratory, Rivers State University, where the larvae were introduced to clean non-chlorinated water and identified. Test organisms were identified at the Malaria Research Institute, Rivers State University by Dr. E. N. Ebere. The larvae were maintained under sterile condition at 27 ℃ and 70-80 % relative humidity under a photo period of 12-hour light. They were fed with a mixture of yeast powder and dog biscuits in the ratio of 1:3.

Larvicidal bioassay

Bioassay for the larvicidal activity of *Citrus sinensis* fruit rind essential oil was conducted with reference to the standard World Health Organization (WHO) procedure [11]. Essential oils do not dissolve in water therefore, tween 80 (JHD) was used as an emulsifier at the concentration of 0.05% in the test solutions. Different concentrations (2.5, 5, 10 and 20 ppm) of the essential oil in distilled water were prepared as test solutions. Twenty-five (25) larvae each were taken with a dropping pipette and transferred gently into 400ml of each test solutions and control experiments (without essential oil) were run in parallel. Control group consisted of larvae exposed to water and water/tween 80. The larvae were exposed to concentrations of 2.5 ppm, 5 ppm, 10 ppm and 20 ppm for over 24 hours at room temperature. During the test, no food was given to the larvae. Each treatment was done in three replicates. Larvae were considered dead if they settle and remain motionless in the bottom of the test beaker with no response to light or mechanical stimulus or not recovering life functions even after being transferred to their growth medium.

Results

Chemical composition of essential oil

The yield of *Citrus sinensis* fruit rind essential oil was 0.55% based on the fresh weight of the rind. Component of essential oil of *C. sinensis* as revealed in GC-MS result is shown on table 1. (R)-(+)-limonene, β-myrcene, camphene, carvacrol, tricyclene, β-pinene and trans-sabinol whose structures are given in figure 1 were the major components with percentage concentrations of 80.57, 5.59, 3.28, 2.57, 2.16, 1.14 and 1.03 respectively. The remaining 14 compounds were found to be minor; all of which made up a total of only 3.66% of the essential oil.

Table 1: Chemical Composition

S/N	Compound	Retention Time	Concentration (%)
1.	Camphene	6.235	3.28
2.	(R)-(+)-Limonene	6.826	80.57
3.	Pinocarvone	8.382	0.01
4.	Linarylacetate	8.610	0.03
5.	Ascaridole	10.315	0.18
6.	Carvacrol	10.581	2.57
7.	Thymol	10.835	0.36
8.	1,8-cineole	12.437	0.02
9.	Tricyclene	12.791	2.16
10.	trans-Sabinol	13.246	1.03
11.	α-Terpinene	13.843	0.01
12.	α-Pinene	15.453	0.19
13.	Bornyl acetate	15.719	0.16
14.	β-Pinene	18.235	1.14
15.	Farnesol	18.668	0.89
16.	α-Thujene	19.182	0.42
17.	Cymene	19.683	0.65
18.	Nerol	20.152	0.71
19.	Linalool	20.357	0.02
20.	β-Myrcene	21.583	5.59
21.	Spathulenol	21.812	0.01

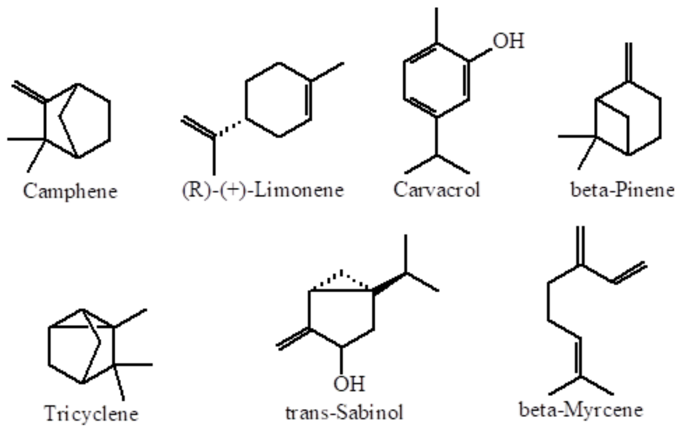


Fig 1: Structures of major components of *Citrus sinensis* fruit rind essential oil

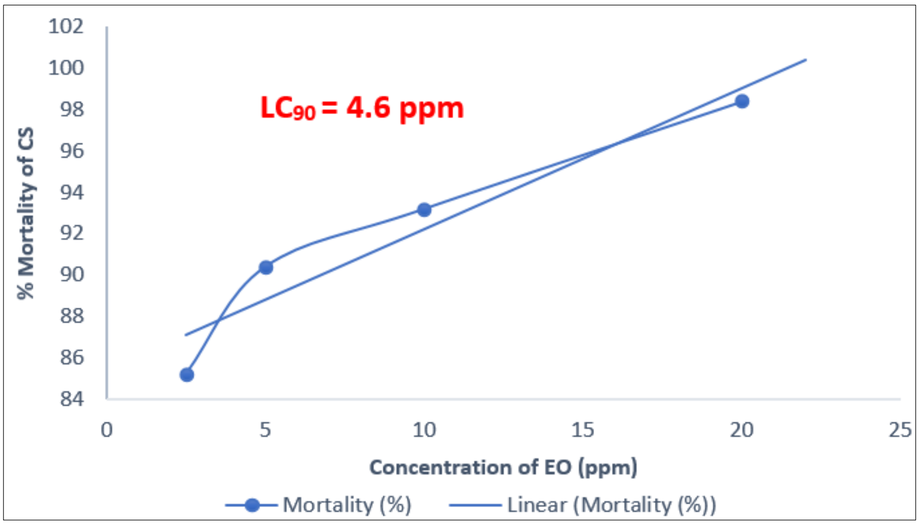
Larvicidal activity of essential oil

The result (table 2 and figure 2) shows mortality rates (85.2 - 98.4 %) of third instar larvae of Culex species by the action of *Citrus sinensis* fruit rind essential oil. The highest larval mortality of 24.6 was recorded for 20 ppm while the lowest larval mortality (21.3) was observed for 2.5 ppm. Larval mortality observed for the control was negligible. LC_{90} value of 4.6 ppm was derived by extrapolation.

Table 2: Larvicidal Activity

Concentration of essential oil (ppm)	Mortality of Culex species (Mean ± SEM)
2.5	21.3±3.7
5.0	22.6±2.4
10.0	23.3±1.4
20.0	24.6±0.2
Control	1

Key: SEM – Standard error of mean



Key: CS - Culex species; EO - Essential oil; LC_{90} – 90% Lethal concentration

Fig 2: Larvicidal activity of essential oil on Culex species

Discussion

(R)-(+)-Limonene, a monoterpene was the major constituent of the *Citrus sinensis* fruit rind essential oil (80.57%). This result corroborates the findings of Abbassy *et al.* and Ezeonu *et al.* [4, 5] (R)-(+)-Limonene has been reported to be a biodegradable insecticide, non-toxic petroleum-based grease cleaner; used in some medicine and cosmetic products, and of tremendous health benefits [12, 13].

β-Myrcene, a noncyclic monoterpene, which accounted for 5.59% of the essential oil of *C. sinensis* fruit rind is widely used in the perfume industry, but more commonly used as an industrial precursor to various other terpenes including geraniol, menthol, citral and nerol; it synergizes the antibiotic potential of other terpenes and is a potent analgesic [14].

Camphene (3.28%) is the third major constituent of *C. sinensis* fruit rind essential oil. It is a bicyclic monoterpene that occurs in one or both of its enantiomeric forms in a variety of essential oils, including turpentine, cypress oil, bergamot oil, and valerian. Camphene has bactericidal, antiviral, antifungal and anti-edematous properties as well as the pharmacological potential to protect the lungs against oxidative stress [15].

The fourth major constituent of the essential oil of *C. sinensis* fruit rind is carvacrol (2.57%). Carvacrol is a phenol that is a natural monoterpene derivative of cymene. It is an inhibitor of bacterial growth, a food additive, an antioxidant, a flavouring agent and a botanical anti-fungal agent [16-18].

Citrus sinensis fruit rind essential oil showed great larvicidal activity even at concentrations as low as 2.5 ppm. This is also in agreement with Ezeonu, *et al.* who reported that sweet orange has promising insecticidal activity against Aedes mosquitoes and cockroaches [5]. It was observed that the essential oil used in this study did not only affect the larval stage but also other developing stages. This is in accordance with the result findings of Shalaby *et al.* that the toxicity effect of citrus oils on larvae extends to other developing stages [6]. The mortality rate of Culex larvae increased with increase in concentration. Thus, high mortality rate recorded in this study could be due to the presence of (R)-(+)-limonene which was the major compound present in the essential oil of *C. sinensis* fruit rind.

Conclusion

Essential oil from *Citrus sinensis* fruit rind showed strong larvicidal activity and has potential as an ideal ecofriendly future larvicidal agent and biopesticide for use against Culex species of mosquito.

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