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Chemical Composition and Repellent Activity of Methyl Cinnamate-Rich Basil (*Ocimum basilicum*) Essential Oil

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Abstract: Basil (*Ocimum basilicum* L.) is an important culinary herb and essential oil source widely recognized worldwide. The oil of the plant is beneficial for medicinal uses, and it has many biological activities such as insect repellent, larvicidal, and bactericidal. This study aimed to investigate basil's essential oil for its chemical composition and repellent activity. The essential oil of basil was extracted from fresh leaves by steam distillation method, and the chemical composition of the oil was determined by using GC/MS. Also, the repellent activity of the oil was tested against American cockroaches. Ebeling Choice-Box test with a little modification used in repellence test. The obtained results of chemical composition revealed that the amount of the oil contained forty-one chemical constituents (~97.1%); the major constituents were methyl cinnamate (25.3%), linalool (19.1%) and estragole (12.3%) as the major oxygenated monoterpenes. While α-bergamoten (5.3%), germacrene (4.6%), γ-cadinene (2.8%), and β-elemene (2.4%) were the main compounds in sesquiterpene hydrocarbons. Whereas, in oxygenated sesquiterpenes, Tau-cadinol (4.3%) was an important compounds and ocimene the highest compound in monoterpene hydrocarbons. The obtained results also indicated that the essential oil had good activity against the P. American, at a 100% concentration of oil; the repellence reaches 100% after 1 h. The IC50 and IC90 values of basil essential oil against P. Americans were 53.0 and 83.0%, respectively. The major compound methyl cinnamate which exists in basil essential oil is a very important compound and could be used in a wide area of industrial applications as repellent products, medicinal products, and cosmetics.

Keywords: *Ocimum basilicum*, Basil, Essential oil, Repellent activity, American cockroaches.

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INTRODUCTION

Natural products, especially plants, have served humanity as an important source for many uses. Humans use plants or their derivatives for many benefits, such as foods and medicines (1), antioxidant (2), rhizoremediation (3), fertilizers and pesticides (4), lipid-lowering potential, anti-malarial, anti-ulcer, antipyretic, anti-cancer, and anti-proliferation (5). The genus *Ocimum*, of *Ocimum basilicum* (Family: Lamiaceae formerly Labiatae), called basil has long been known worldwide as a diverse, rich source of essential oils and a significant culinary herb. Basils exhibit great variation in both morphology and diversity, such as inflorescence, leaf, and essential oil components (6). The taxonomy of *O. basilicum* more complicated due to the numerous varieties, cultivars and chemotypes within the species that do not vary significantly in morphology. The essential oil composition was utilized to characterize the diversity among the most economically important *Ocimum* species (7). The essential oils of basils are used as a food flavoring, medicines, and in the perfumery industry. In addition, previous studies have reported very interesting biological activities of these oils, such as being bactericidal (8), mosquito repellent (9) and larvicidal (10), etc. Besides, it was used in traditional medicine to sooth pain, treat vomiting and stress, and as an insect repellent (11). The leaves and flower of basil are utilized in folk medicine as a tonic and vermifuge, also basil tea is good for treating dysentery, nausea, and flatulence, its oil is useful for the mitigation of spasm, rhinitis, mental fatigue, cure of wasp stings and snake bites (12,13). It has used as a folk medicine for boredom and convulsion. Basil heals headache, improves digestion, and as well helpful for toothache, earache, and for curing epistaxis when used with camphor. The plant infusion is effective in cephalic, gouty joints, fever, otitis, and snakebite(12,14). In addition, it is efficient in remediation of stomach problems, fever, cough, gout, and given internally to cure cystitis, nephritis and interior piles. The plant is also used to keep away insects and snakes (12). The use of chemically synthetic repellents of insect control may result in disturbing natural ecosystems and resulted in the development of resistance to insecticides, and even adverse impact on non-target organisms. Hence, the idea of using natural repellent products as an alternative to develop new eco-friendly repellents could be a friendly solution for the reduction of adverse effects on the environment and human health. Therefore, this study aimed to investigate the chemical composition and repellent activity of *O. basilicum* essential oil.

MATERIALS AND METHODS

Plant Material

The fresh leaves of *O. basilicum* were collected in October 2017 from the Ministry of Agriculture and Forestry, General Directorate of Horticultural Production, Department of Medicinal and Aromatic Plants, Khartoum, Sudan.

Extraction of Essential oils

The fresh leaves of *O. basilicum* are used to obtain their essential oils. The extraction was conducted on a laboratory scale by Steam Distillation (SD) unit as the method described by Mesomo et al. (15) with slight modification. In brief, 100 g of fresh *O. basilicum* leaves were steam distilled for 4 h. Then, the extracted essential oils were dried over anhydrous sodium sulfate, filtered, stored in hermetically closed dark bottles, and kept at -4 °C for further studies. The percentage of extracted oils (v/w %) from the SD method was calculated according to the following formula (Equation 1):

$$\text{The Essential oil}\left(\%\right) = \frac{\text{volume of oil}}{\text{weight of sample}} \text{ X } 100 \quad \text{(Eq. 1)}$$

Gas chromatography-mass Spectrometry Analysis

The chemical composition of extracted *O. basilicum* essential oils was determined using an Agilent 7890A GC–MS instrument equipped with column nonpolar capillary DB-1 of 100% dimethyl-polysiloxane (30 m, 0.25 mm i.d, film thickness 0.25 μm) and mass spectrophotometric detector. The carrier gas was helium with a flow rate of 1 mL/min, and the injector mode was splitless with an injection volume of 1 μL/L and an injection temperature of 250 °C. The temperature program was 60 °C for 3 min, 240 °C at the rate of 3 °C/min, and held for 10 min. The run time was 93 min, and the lab data system was NIST Library Chem Station software.

Cockroaches Collection and Repellent Test

About 600 adults (male and female) of American cockroaches (*P. Americana*) were collected from the University of Khartoum, Sudan. They were kept in boxes and reared in the laboratory by feeding on water and biscuits. The healthy nymphs and adults (male & female) cockroaches were used in this repellence test. The temperature was maintained at 28±5 °C. The Ebeling choice box test, which describe by Ebeling (16) with some modification used in this experiment. The *O. basilicum* leaves essential oil was prepared in various concentrations (5, 25, 50, 75, and 100 v/v %) by dissolving in 1% DMSO. Ten adult and nymph cockroaches (male and female) were then released into the central choice box (untreated zone). Then, the choice boxes (treated and untreated location) were exposed to a photoperiod of 27 ºC for 72 hrs. 1% DMSO and naphthalene were used as negative and positive controls, respectively. The cockroaches at the treated and untreated zone were carefully observed and counted for 0, 3, 6, 9, 12, 24, 48, and 72 hrs. of treatment. Each treatment with a different concentration was conducted in three replicates. The percentage of repellency is calculated as follows (Equation 2):

$$\text{Repellency \%} = 100 - \left(\frac{T}{N} \text{ X } 100\ \%\right) \quad \text{(Eq. 2)}$$

Where T is the number of cockroaches located at the treated zone, and N stands for the total number (ten heads) of cockroaches been used in the repellency test. The mean percentage of the repellence was then calculated from the values obtained in three replicates.

Statistical Analysis

Statistical analysis of the obtained results conducted using MS Excel (2007), version 12.0.4518.1014. The results performed in three repetitions and expressed as mean ± standard deviation.

RESULTS AND DISCUSSION

Yield of essential oil

The essential oil of the collected *O. basilicum* leaves was extracted using the steam distillation method, and the percentage yield of the oil was expressed on a fresh leaf weight basis (v/w %). The oil content was found to be 0.78%, and the color is light yellow with a camphor-like smell. Previously, reported *O. basilicum* had a yield of 1.56% essential oil with yellowish green color (17) and also a yield of 1.98± 0.01% with a pale yellow oil (18). In addition, a yield of 0.65 to 1.90% (19) and 0.9–1.7% essential oil (20), where these results were higher than the obtained results in this study. Whereas, the yields were found to be 0.05 to 0.55% (19), 0.6% (20) and 0.28% essential oil too (21); where these results were lower than the result obtained in this study. The variation in the results may be due to climate and soil conditions, but the oil content is still in the range obtained in the previous studies.

Chemical Composition of Essential Oil

The chemical composition of the extracted *O. basilicum* essential oils was determined using GC-MS instrument and the obtained results were shown in Table 1. The obtained results were indicated forty-one chemical constituents (~97.1%); the major constituents were methyl cinnamate (25.3%), linalool (19.1%) and estragole (12.3%) as the major oxygenated monoterpenes. While sesquiterpenes hydrocarbons, ɑ-bergamoten (5.3%), germacrene (4.6%), *γ*-cadinene (2.8%) and *β*-elemene (2.4%) were major constituents. Whereas, in oxygenated sesquiterpenes, Tau-cadinol (4.3%) was major constituent and ocimene the major constituent in monoterpene hydrocarbons. In previous studies reported basil essential oils consisted of linalool were the most abundant component (56.7-60.6%), followed by epi-ɑ-cadinol (8.6-11.4%), *α*-bergamotene (7.4-9.2%) and *γ*-cadinene (3.2-5.4%) (21,22). In addition, a total of 17 compounds were identified with linalool (70.44%) as the major compound, followed by an estragole (14.4%), tau-cadinol (4.1%) and *α*-bergamoten (3.7%) (23). Also reported the dominant components were methyl chavicol (81.8%), *β*-(*E*)-ocimene (2.9%), *α*-(*E*)-bergamotene (2.5 %), *α*-epi-cadinol (2.1%), 1,8-cineole (1.6%), methyl eugenol (1.1%) and camphor (1.1%) (24).

Table 1: Chemical composition of essential oil.

Compound	Formula	Area %
Monoterpene hydrocarbons		
alpha-Pinene	$C_{10}H_{16}$	0.2
Camphene	$C_{10}H_{16}$	0.0
Sabinene	$C_{10}H_{16}$	0.3
Pseudopinen	$C_{10}H_{16}$	0.5
Myrcene	$C_{10}H_{16}$	0.9
Limonene	$C_{10}H_{16}$	0.5
Ocimene	$C_{10}H_{16}$	1.5
Total monoterpene hydrocarbons		**3.9**
Oxygenated monoterpenes		
Cineole	$C_{10}H_{18}O$	5.6
Sabinene hydrate	$C_{10}H_{18}O$	0.3
Fenchone	$C_{10}H_{16}O$	0.9
Linalool	$C_{10}H_{18}O$	19.1
Fenchol	$C_{10}H_{18}O$	0.6
(a)	$C_{10}H_{14}O_2$	0.2
Camphor	$C_{10}H_{16}O$	0.4
Isoborneol	$C_{10}H_{18}O$	0.3
Terpinenol-4	$C_{10}H_{18}O$	0.1
Terpineol schlethin	$C_{10}H_{18}O$	0.9
Estragole	$C_{10}H_{12}O$	12.3
Beta-Citral	$C_{10}H_{16}O$	0.1
Geraniol	$C_{10}H_{18}O$	1.2
Alpha-Citral	$C_{10}H_{16}O$	0.2
Methyl cinnamate	$C_{10}H_{10}O_2$	25.3
3-Allylguaiacol	$C_{10}H_{12}O_2$	1.0
8-Hydroxylinalool	$C_{10}H_{18}O_2$	0.04
Total oxygenated monoterpenes		**68.5**
Sesquiterpene hydrocarbons		
Elixene	$C_{15}H_{24}$	0.3
Alpha-Ylangene	$C_{15}H_{24}$	0.1
Copaene	$C_{15}H_{24}$	0.2
b-Elemene	$C_{15}H_{24}$	2.4
Caryophyllene	$C_{15}H_{24}$	1.0
Alpha-Bergamoten	$C_{15}H_{24}$	5.3
(b)	$C_{15}H_{24}$	0.1

Beta-Farnesene	$C_{15}H_{24}$	0.2
Humulene	$C_{15}H_{24}$	0.6
Beta-Cubebene	$C_{15}H_{24}$	0.6
Germacrene	$C_{15}H_{24}$	3.6
Alpha-Bulnesene	$C_{15}H_{24}$	1.4
Gamma-Cadinene	$C_{15}H_{24}$	2.8
Total sesquiterpene hydrocarbons		**19.1**
Oxygenated sesquiterpenes		
Beta-Elemol	$C_{15}H_{26}O$	0.5
Cubedol	$C_{15}H_{26}O$	0.8
Tau-Cadinol	$C_{15}H_{26}O$	4.3
Total oxygenated sesquiterpenes		**5.5**
Others		**2.9**

(a): Oxirane,2-(hexyn-1-yl)-3-methoxymethylene, **(b):** Cis-Muurola-3,5-diene

The qualitative and quantitative composition of essential oils was quite different: *O. basilicum* var. purpureum essential oil contained 57.3% methyl-chavicol (estragol); *O. basilicum* var. thyrsiflora oil had 68.0% linalool; the main constituents of *O. citriodorum* oil were nerol (23.0%) and citral (20.7%) (25). Moreover, claimed the major compounds were: linalool (32.8%), linalyl acetate (16.0%), elemol (7.4%), geranyl acetate (6.2%), myrcene (6.1%), allo-ocimene (5.0%), *α*-terpineol (4.9%), (*E*)-*β*-ocimene (3.7%) and neryl acetate (3.5%) (18). Thus, the most of previously mentioned compounds were present in the obtained results.

The obtained results in Table 1 also showed that the total monoterpenes represents 72.4% of the oil, the ratio is hydrocarbon 3.9% and oxygenated monoterpene was 68.5%. While the total sesquiterpenes were 24.6%, representing 19.1% sesquiterpene hydrocarbon and 5.5% are oxygenated sesquiterpenes. The distribution of mono and sesquiterpenes in *O. basilicum* essential oil was shown in Figure 1. Previously, reported samples collected in winter were found to be richer in oxygenated monoterpenes (68.9%), compared to those collected in summer where were higher in sesquiterpene hydrocarbons (24.3%) (21,22).

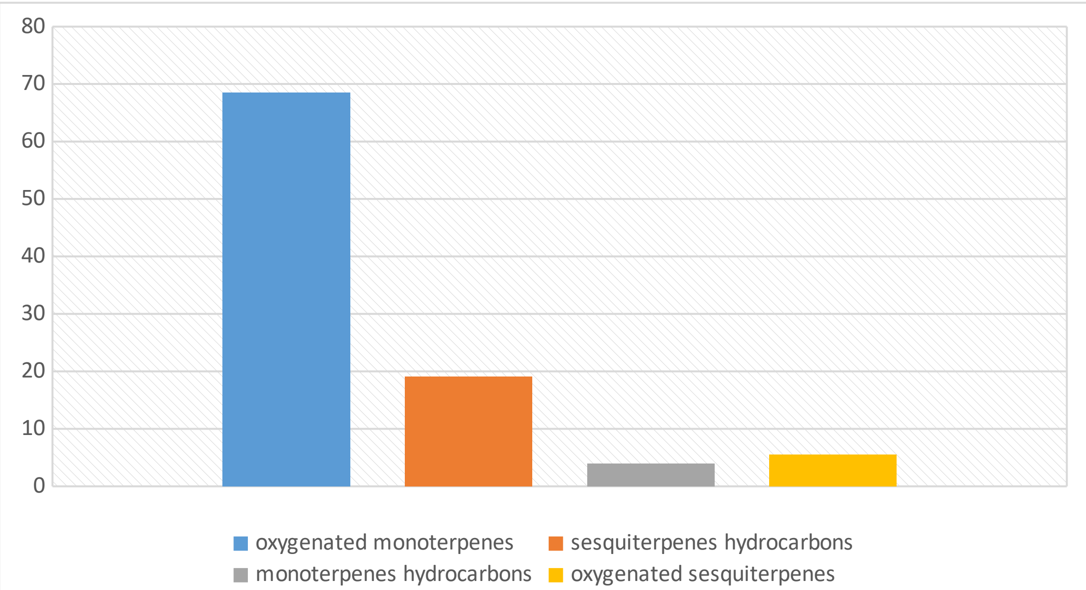

Figure 1: Distribution of mono and sesquiterpenes in *O. basilicum* essential oil.

Repellent Activity of Essential Oil

Repellency activity of *O. basilicum* essential oil (100%) against A. cockroaches after 72 hrs. of treatment and the IC50 and IC90 values against A. cockroaches after 24 hrs. of treatment was tested in this study and the obtained result showed in Figure 2 and Figure 3. The obtained results indicated that the essential oil had a good activity against the cockroaches, at a 100% concentration of oil; the repellence reaches 100% up to 1 h of exposure; whereas the repellency reaches 80.0% for more than 4 h and more than 70.0% after 10 h. The IC50 and IC90 values of *O. basilicum* essential oil against cockroaches were 53.0% and 83.0%, respectively. Yoon (26) was tested the repellent efficacies of certain components and their obtained results indicated that the efficacies were varied with different doses and the cockroach species, and the major components responsible for the repellent activity of the essential oils were limonene, β-pinene and γ-terpinene.

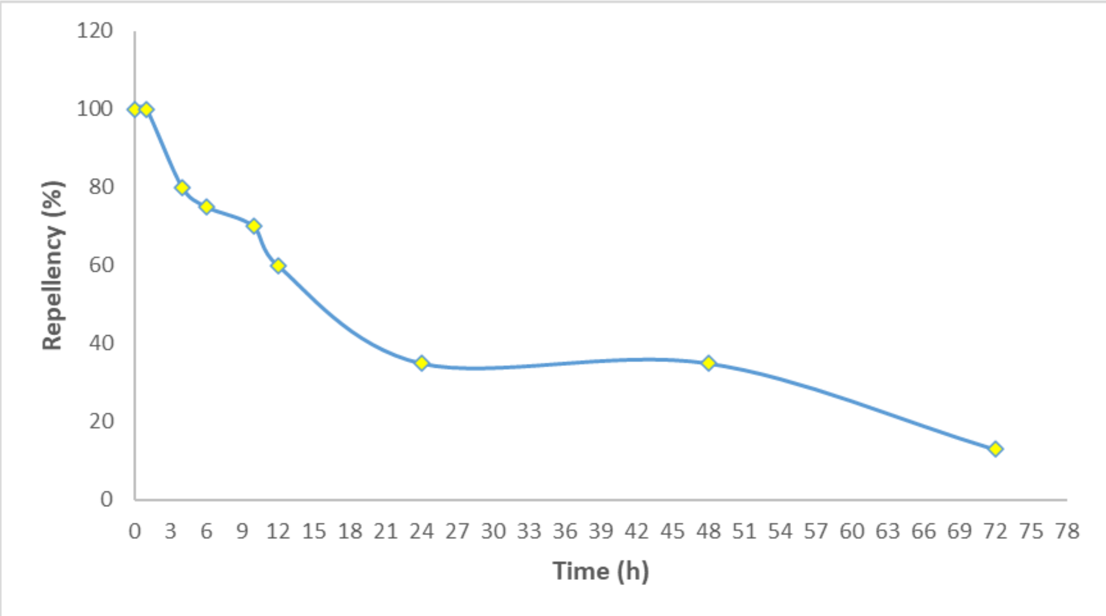

Figure 2: Repellency activity of *O. basilicum* essential oil (100%) against cockroaches after 72 h of treatment.

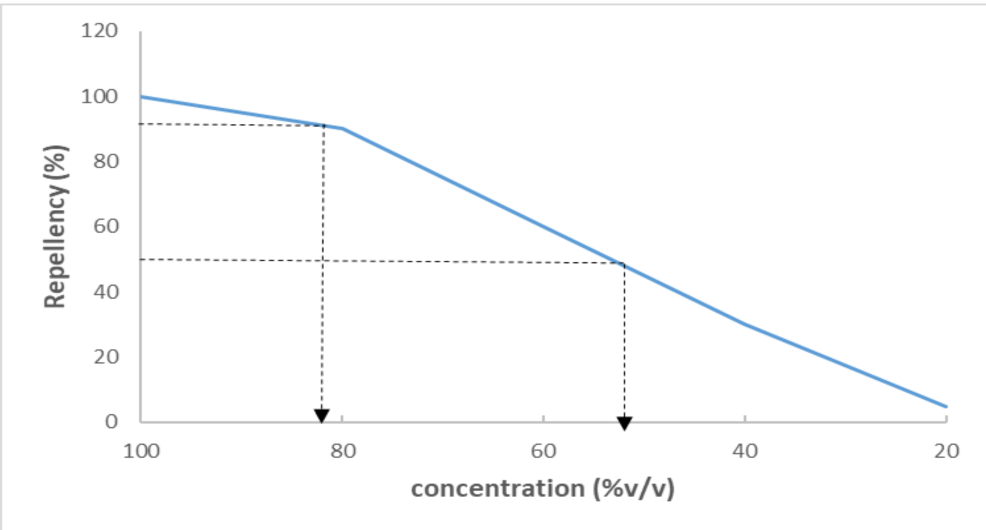

Figure 3: The IC50 and IC90 values of *O. basilicum* essential oil against cockroaches after 24 h of treatment.

El-Seedi et al. (27) claimed that the *O. basilicum* oil with major compounds of 1,8-cineole, camphor, linalool, 4-terpineol, borneol, and carvone was the most repellent oil among many oils tested against cockroach. In addition a number of compounds were showed repellent activity against insects such as: linalool (28), Estragole (29), ocimene (30), cineole (31), α-pinene (32), camphene (100%) against

German cockroaches (33), camphor (34), limonene (35), sabinene (36), terpinene 4-ol (35), myrcene (34), geraniol was toxic to the cockroaches by contact or injection and repellency (32). Moreover, caryophyllene (34), fenchone (29), *β*-citral and *α*-citral (37), *β*-elemene (38), *β*-cubebene, fenchol, *α*-bergamoten, *α*-guaiene and *β*-farnesene (32), *α*-ylangene, *β*-elemol and *γ*-cadinene (35), germacrene (39), humulene (35), *β*-bulnesene (38), *β*-cubebene (32), verbenone (33) and α-copaene (32). Nour et al. investigated the repellent activity of *Cyperus rotundus* rhizomes essential oil against American cockroaches; his obtained results indicated that the IC50 and IC90 values of *C. rotundus* rhizomes essential oil against American cockroaches were 57 and 88%, respectively (40). Sittichok et al. evaluated the repellent activity of the essential oils derived from Cymbopogon citratus (lemon grass), Cymbopogon nardus (citronella grass) and Syzygium aromaticum (clove) against adult American cockroach; in his results all of the essential oils in ethyl alcohol showed higher percent repellency (81-100%) against P. americana than all of the essential oils in soybean oil (66-84% repellency), The essential oil from C. citratus in ethyl alcohol exhibited the highest repellency (100%) among the tested repellents and naphthalene (83% repellency)(41). The repellent activity of essential oil in this study could be due to the presence of compounds that had already demonstrated repellent activity against certain insects, including cockroaches.

CONCLUSION

The obtained results in this study indicated that the *O. basilicum* essential oil contained various chemical constituents include monoterpenes, oxygenated monoterpenes, sesquiterpenes, and oxygenated sesquiterpenes. Also indicated that oil had a repellent activity, and it could be attributed to the presence of compounds that have already demonstrated their repellent activity towards certain insects, including cockroaches.

CONFLICTS OF INTEREST

The authors declare no conflicts of interest exist.

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