



GCMS CHARACTERIZATION AND MOSQUITO REPELLING POTENTIAL EVALUATION OF COIL PRODUCED USING *OCIMUM BACILLICUM* OIL

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ABSTRACT

Mosquito a vector for many protozoa, bacterial and viral diseases facilitating their transmission to human, resulting to loss of millions of lives annually. Its vectors capacity is enhanced by development of its resistance to many insecticides. The present study aimed at characterizing using FTIR and GC-MS methods of essential oil from *Ocimum basilicum*, production of mosquito repellent coil from the oil and bio-efficacy evaluation of the coil on adult mosquito (*A. stephensis*). The FTIR Spectral revealed the presences of P-OH, N-O, C-O, NO₂, C=O, O-H, =C-H, O-H and C-X which correlate with the functional groups of the major phyto-chemical constituent compounds revealed by GC-MS spectral. 15 phyto-constituents were identified with 9, 12, 15-Octadecanoic acid as the major phyto-constituent with percentage of 35.61%. Bio-efficacy evaluation shows high potential of the coil at all concentration and with highest value at 300mg and 400mg compared to the result obtained for commercially made coil (Baygon) at laboratory condition. *Ocimum basilicum* can therefore be used as an alternative repellent ingredient to repel adult mosquito.

Keywords: Malaria, *Ocimum basilicum*, Mosquito replant, FTIR, GC-MS, and Bio-efficacy

INTRODUCTION

Mosquitos are among the most disturbing blood sucking insects affecting human. The behaviour of female mosquito feeding on human blood is responsible for the transmission of a number of the diseases. The most prevalent of these diseases include malaria, *bancrofti*, *filariasis*, yellow fever, dengue fever and some arbovirus infections (Curtis *et al.*, 1991; White, 1996). It is established that the transmission of these infectious diseases to human is via competent mosquito vector; hence its control initiatives remain top most to the prevention and control of the diseases (White, 1973; Curtis *et al.*, 1991; White, 1996).

In the recent the hopes in control of malaria was revitalized in seeking to reduce the mosquito bite by the introduction and use of Insecticide Treated Nets (ITNs), especially with pyrethroids as the active ingredient and the protective utility of the nets was improved (Curtis *et al.*, 1991; Chavasse *et al.*, 1999). The reduction in morbidity and mortality rate of malaria demonstrated the potency of ITNs method (Abdulla *et al.*, 2001). However, despite the common knowledge of the excite-repellent activity by pyrethroid based insecticide nets against indoor mosquito (Malima, 2008), they still bite people before retiring to bed or when out of the net at night. So the most viable malaria control measures are the mosquito control and personal protection from mosquito bites. Henceforth an additional mosquito control tool is therefore, urgently needed to complement ITNs, to curtail human-mosquito contact when not under the net.

About two decades ago, the discovery of insecticide activity of phyto-toxins present in *Asteracea* species had stimulated the interest in botanicals as part of the search for new plant derived insecticides (Rawls, 1986). Some plant extracts includes, Basil (*Ocimum basilicum* L, *O. grassiticum* L, *O. americanum*, *O. anvilforium* L). *Citronella* grass (*Cytopagan nardus randle*), *Alpinagalangl* L, *Polvos synzyanium aromaticum* L) and thyme *V. vlgaria*) were reported to have demonstrated good efficacy against some mosquito species (Bernard, 1999).

As such natural repellent of plant based, like rotenone and nicotine, among others have been extensively used for insects control (Balandrin, 1985). Also *Limanoids* such as *Azadirachtin* and *Gedunin* present in species from the *Leliaceae* and *Rutaceae* are recognized for their toxicity effects against insects, and that properties is exploited in several insecticide formulations in many parts of the world (Dua *et al.*, 1995; Nagpal *et al.*, 1996; Nkunya, 2002).

However, bio-efficacy evaluation and chemical characterization of the repelling activity and ingredients of some of these extracts were not exhaustive on experimental models (WHO, 1996). Therefore there is the need for such scenario. This work therefore, was designed to characterize using FTIR, GC-MS and evaluate the bio-efficacy of mosquito repelling coil produce using (*O. bacillicum*) basil oil and Compare the repelling potential of the coil with commercial produced (Baygon) mosquito coil in a named species of mosquito under laboratory condition.

MATERIALS AND METHODS

Collection and Identification of Plant Sample

Plant materials; fresh leave of *O. basilicum* (sweet basil) plant was collected in March from the premises of the main campus Kaduna Polytechnic and identified by botanist in the Department of Botany, Ahmadu Bello University Zaria.

Extraction of the Essential Oil from Basil using Steam Distillation Method

Fresh leaves of *O. basilicum* were shredded into pieces within 12 hours after collection and 100-200 g was weighed and transferred in to the Clevenger apparatus containing boiled 500 ml of distilled water for 5-6 hours until oil distillation ceased. The essential oil in the distillate were dried over anhydrous Na_2SO_4 and collected into a flask with air tight cover kept in the freezer for further testing. The percentage yield of the oil was calculated using the formula below.

$$\text{Percentage yield} = \frac{\text{mass of extract}}{\text{total mass of sample extracted}} \times 100\%$$

The extract obtained was stored to be used for characterization using FTIR and GC-MS and also for the production of mosquito repellent coil

GC-MS Analysis of the Oil

The dried solid extract was dissolved in methanol and analyzed using GC-MS model Qp2010 plus SHIMADZU equipped with detector and slit injection system the initial temperature was maintained at 60^oc for 3 minutes and was gradually increased to 250^oc. 1.6 μ l of solution was injected for analysis the sample injection temperature was maintained at 250^oc throughout the experimental period. The carrier gas (Helium) flow was 2ml per minute. The identification of components was based on comparison of their mass spectra with those present in the National Institute for Standard Technology computer data bank (NIST 2009 LIB).

FTIR Analysis of the Oil

FTIR analysis of the extract was carried out using infrared spectroscopy of the SHIMAZU FTIR-8400s. The extract was

scanned in accordance with ASTM 125z-98. Small quantify of the extract was applied on the sodium chloride cell to obtain a thin layer pallet. The cell was mounted on the FTIR and scanned through the FTIR region.

Mosquito Coil Production

The mosquito repellent coil was produced using the following materials, activated carbon (adsorbent), sawdust (combusting agent), dye (colouring agent), starch (binding agent) and the essential oil (active ingredient). These materials were mixed in a proportion and coil into a spiral shape. It was allowed to dry at room temperature then stored for bio-efficacy testing.

Bio-efficacy testing of the Mosquito Repellent Coil Produced Breeding of Mosquito

The mosquito's larva was obtained from breeding site at Congo Zaria and identified to be *Aides stephensi*. The Larvae were reared on a diet of floating catfish food. Adult Mosquitoes obtained from the larvae were reared and maintained at 27 \pm 3^oC and 80 \pm 10 relative humidity (RH) under a 12:12 (L:D) photoperiod. The adults were maintained in screen cages on 10% sucrose solution but 24 h before experiments the sucrose solution was removed from cages. Repellency was tested against 3 to 5 days old, blood starved mosquitoes, and for each test 25 mosquitoes were used (Norashiqin, 2008; Barat, 2012)

Results

The Percentage Yield of the Extract

The percentage yield of the extract was 1.3%

Characterization of the Essential Oil of *O. basilicum*

The FTIR characterization of the extracted essential oil of *O. basilicum* as shown in the Table 1 and Figure 1 below indicates the presences of P-OH Stretching, N-O Stretching, C-H bending, NO₂ symmetric stretching, NO₂ Asymmetric, C=O stretching, O-H and N-H Stretching, C-H Stretching, O-H Stretching C-X stretching. This indicates the presences of Aldehydes, Amines, Alcohols, and Alkyl-halides as the functional groups present in the oil extracted (Table 1).

Table 1 The FTIR Characterization of the Essential Oil of *Ocimum basilicum*.

Molecular motion	Peak(cm ⁻¹)	Functional group
P-OH Stretching	353.02	Phosphorous compound
N-O Stretching	963.48	Nitrogen compound
C-H Bending	1107.18	Aromatic compound
NO ₂ symmetric stretching	1377.22	Nitrogen compound
NO ₂ Asymmetric	1452.45	Nitrogen compound
C=O stretching	1733.1	Aldehydes
O-H and N-H Stretching	2030.15	Amines
C-H Stretching	2858.6	Alkanes
=C-H Stretching	3000.37	Alkanes
O-H Stretching	3443.05	Alcohol
C-X stretching (X=F,Cl,Br,I)	1251.84	Alkyl halide

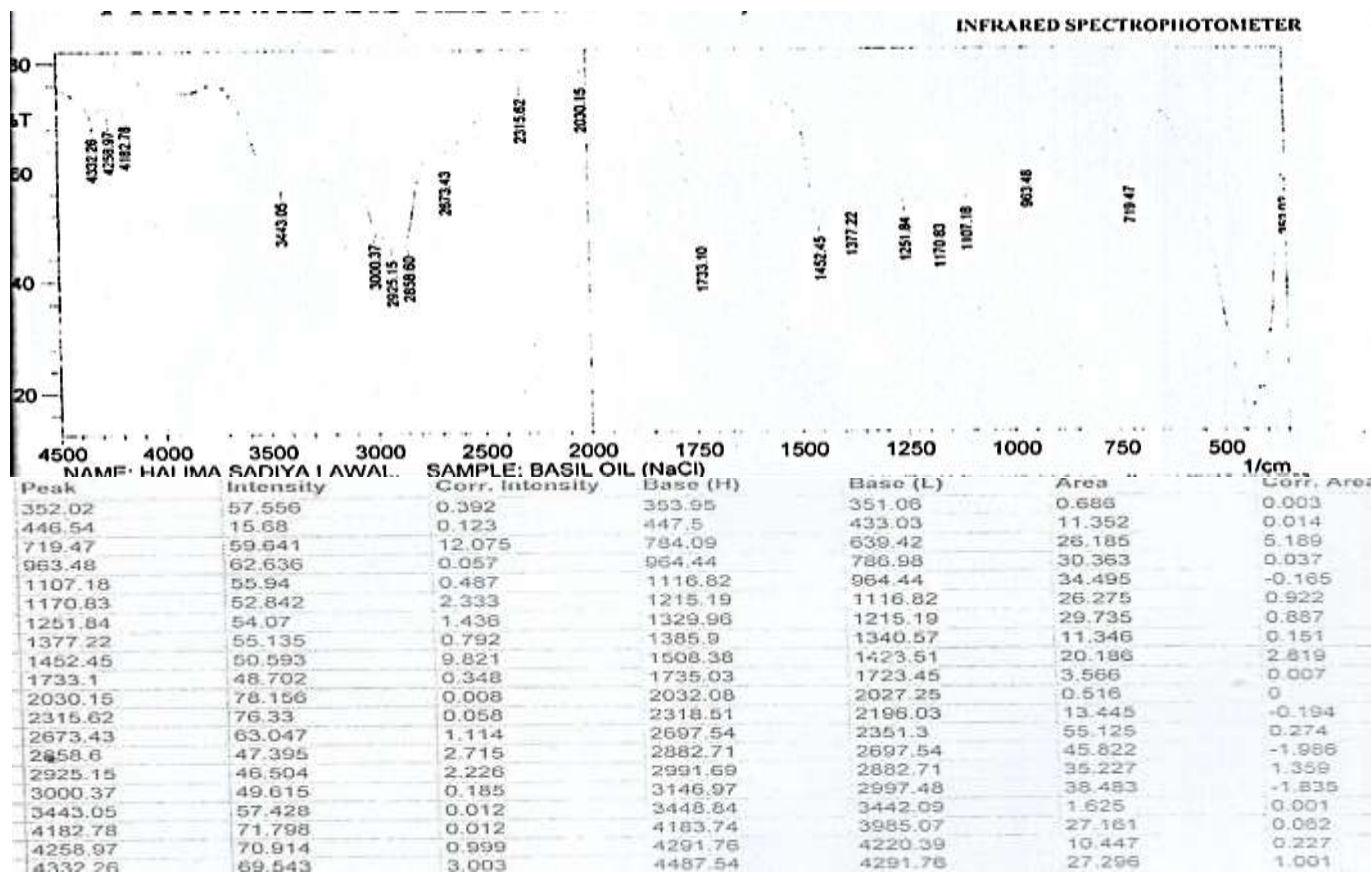


Figure 1: The FTIR Spectra of the Essential Oil of *Ocimum basilicum*

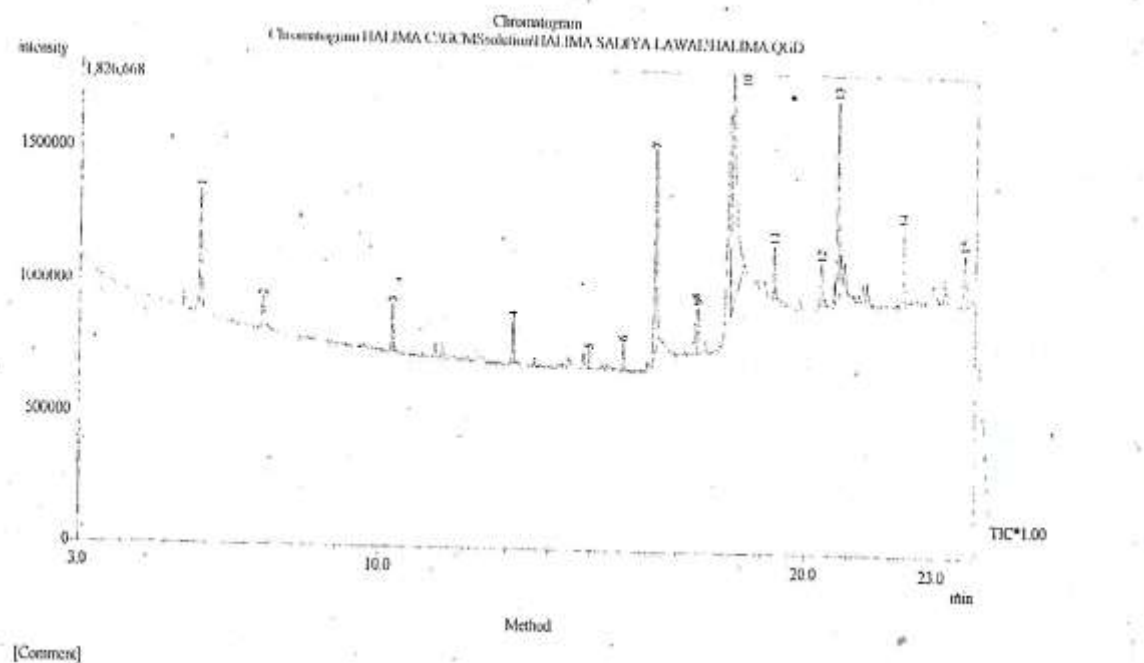



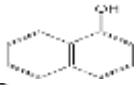


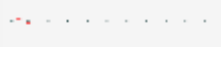




Figure 2: The GCMS Spectra of the Essential Oil of *Ocimum basilicum*

Table 2: The GC-MS analysis, showing, the Retention Time, Base Peak, Structure, Molecular Weight and Formular and the Percentage Composition of the Photo-constituents of the Essential Oil of *Ocimum basilicum*

Retention time	Base peak value	Pyto-constituent	Structure of phyto-constituent	Molecular weight	Molecular formular	% composition
5.825	42.95	1,6-Octadien-3-ol		154	C ₁₀ H ₁₈ O ₂	7.76
7.292	43.00	Butanoic Acid		170	C ₁₀ H ₁₈ O ₂	2.66
10.292	41.00	1,3,6,10-Dodecatetraene		204	C ₁₅ H ₂₄	2.33
13.117	42.95	1-Naphthalenol		222	C ₁₅ H ₂₆ O	2.70
14.925	43.00	2-heptanone		128	C ₈ H ₁₆ O	0.81
15.727	74.00	Tridecanoic acid		228	C ₁₄ H ₂₈ O ₂	1.33
16.475	43.00	Noanadecanoic Acid		29	C ₁₉ H ₃₈ O ₂	15.37
17.433	55.00	1-Octadecenoic		298	C ₁₉ H ₃₆ O ₂	4.07
17.5	79.05	9,12,15-Octadecatri-1-ol		264	C ₁₈ H ₃₂ O	2.23
18.275	79.05	9,12,15-Octadecatrienoic acid		278	C ₁₈ H ₃₂ O ₂	35.61
19.258	43.00	Octadecane		296	C ₂₀ H ₄₀ O	2.92
20.367	43.00	9-Tetradecenal		210	C ₁₄ H ₂₆ O	2.20
20.742	55.00	9-Octadecenal		266	C ₁₈ H ₃₄ O	12.15
22.292	57.05	2-Bromo-d0decane		245	C ₁₂ H ₂₅ br	4.31
23.742	69.05	Squalene		410	C ₃₀ H ₅₀	3.53

The Bio-efficacy Test of the Mosquito Coil Produced at Different Concentrations.

The bio-efficacy test result shows that Baygon a commercial coil had percentage mortality of 43.35% which is lower than the 63.50% of the coil produced with 100 mg concentration from basil oil. While that of 200, 300 and 400 mg give the percentage mortality of 85.00, 91.50 and 96.50% respectively. This showed the coil from basil has higher efficacy when compared with Baygon and the LC₅₀ and LC₉₅ are 1.225 and 3.136 respectively (Table 3).

Table 3: The Bio-efficacy Test of the Mosquito Coil Produced at Different Concentrations.

Concentration (mg)	Total number of mosquito	Response after 30 min (number of mortal mosquito)	% mortality after 30 min	LC ₅₀	LC ₉₅
Baygon	20	16	43.35	$y = 2.9719x + 0.1333$ $R^2 = 0.865$	
	20	0			
	20	10			
	60	8.67			
100 mg	20	20	63.50	1.225	3.136
	20	5			
	20	13			
	60	12.7			
200 mg	20	20	85.00		
	20	18			
	20	13			
	60	17			
300 mg	20	20	91.50		
	20	20			
	20	15			
	60	18.3			
400 mg	20	20	96.50		
	20	20			
	20	18			
	60	19.3			

DISCUSSION

Mosquitoes are among the most blood sucking insects which characteristic is responsible for the transmission of a number of diseases. Malaria been the most prevalent (Curtis *et al.*, 1991; White, 1996) and most devastating menace, a re-awaken searches for control of both causative agent and the host using the natural heal was necessitated. This work therefore characterized the oil of *O. basilicum*, used in mosquito repellent coil production, by FTIR and GC-MS methods. It further comparatively evaluates the bio-efficacy of the produced coil from *O. basilicum* on the adult *A. stephensi* with mosquito repelling coil (Baygon) commercial product. The 1.3% percentage yield of the oil was within the range obtained by Özcan and Chalchat (2002) that had the percentage yield of 1.28 and 1.73 even though the method and location of the research differs.

The identified functional groups corresponding to Aldehydes, carboxylic acid, Amines, Alcohols and Alkylhalides on the FTIR characterization of the essential oil of *O. basilicum* (Table

1) correlate with the chemical composition identified by the GC-MS analysis (Table 2). The chemical composition of the essential oils representing a total number of fifteen volatile compounds making 98% of the total composition identified to be fatty acids and aldehydes hydrocarbons to be the major group of compounds, Octadecatrienoic acid (35.61%) being the most prominent phyto-constituent followed by - Noanadecanoic Acid (15.37%) Octadecenal (12.15%). The most abundant components found in the oil was Octadecatrienoic acid also called linolenic fatty acid (Table 2). The essential oil chemical composition in this work however, differs with those identified by Özcan and Chalchat, (2002) whose compositions include Methyl eugenol (78.02%), α -cubebene (6.17%), nerol (0.83%), α -muurolene (0.74%), 3,7-dimethyloct-1,5-dien-3,7-diol (0.33%) and β -cubebene (0.30%). The variation could largely be influenced by the geography location and ecological factors that could possible determined the type of secondary metabolite that plant produce at particular point in time. The mosquito coil bio-efficacy increases with increase in concentration (Table 3)

as compared to commercially produced repellent (Baygon). The produced coil was observed to be more effective than the commercially produced coil (Baygon) which could be associated to the chemical composition and possible resistance of the mosquito to the commercial product.

CONCLUSION

This study revealed the major composition of the oil to be Octadecatrinoic acid having the highest percentage composition which is perfectly correlated with FTIR findings. While the result indicated high efficacy of the coil produced using basil, which repelling action against the adult mosquito is attributed to the chemical composition. It is pertinent to conclude that the coil of basil can competitively be a better product when employed for mosquito repelling activity.

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