

Potentials of *Ocimum Gratissimum* Extracts as Mosquito Repellents and Mosquitocidal Candles

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Abstract: Ethanol and distilled water extracts of the *Ocimum gratissimum* leaves were obtained using standard methods. The preliminary phytochemical analysis carried out showed the presence of alkaloids, tannins, glycosides, saponins, flavonoids and absence of steroids in both extracts. Three extractives were used in compounding candle samples. The produced candle samples were tested for their mosquitoes' repellent ability by knockdown effect. The knockdown effect result showed that the number of mosquitoes still flying for hot water extract after 5 minutes was 8(20%), then at 10 minutes was 6(20%). However, the highest mosquito's knockdown effect was at 15 minutes which had 3 mosquitoes knockdown. Cold water extract result showed the lowest knockdown effect at 10 minutes 9(10%) while for ethanol extract, there was highest knockdown effect at 10 minutes which have 4(40%). Studies revealed that *ocimum gratissimum* has the tendency and ability of eliminating mosquitoes in less than 10 minutes of application with ethanolic extracts being the most efficient. However, the health implication of this research was not studied to ascertain possible implication on the person inhaling the product.

Keywords: Candles, Knockdown, Malaria, Mosquito, Phytochemical, Repellants

1. INTRODUCTION

The leading cause of morbidity in Nigeria and in Africa as a whole is vector borne diseases transmitted by mosquitoes. The diseases transmitted include malaria, filariasis, yellow fever, Japanese encephalitis and dengue fever while seeking for a human blood meal. Malaria, in particular causes about 1.2 million deaths annually [1,2]. In south eastern Nigeria, *Anopheles gambiae* is the major vector transmitting malaria [3]. There has been exploration of various methods over the centuries to combat threats from mosquito borne diseases such discovery of insecticidal properties of DDT in 1939. However its deleterious impact on non-target population and the development of resistance prompted for the search of alternative, simple and sustainable methods of mosquito control [4].

In recent transmitting times, the use of environment friendly and biodegradable natural insecticides of plant origin to control insect vectors of diseases is gaining ground [5]. These botanical insecticides have been found to be effective, user-friendly and inexpensive [6]. *Ocimum gratissimum* commonly called "nchuanwu" in South-Eastern Nigeria, meaning mosquito repellent is cultivated around houses. It is an herb, which grows up to six feet high with an erect stem. Ethnomedicinally, it is used as remedy for cold and catarrh, stomach pain, diarrhoea and piles [7-9]. The plant has numerous uses both medicinal and non-medicinal in the modern scientific world same of these uses include insecticidal, pharmacological, flavoring agent, raw materials and also serve as vetegetables. Other documented ethnopharmacological activities of the herb include; antipyretic [10], divietic, laxative and hepatoprotective [11] and treatment for mental illness [12]. It is also used as food spices by the people [13].

There are various research reported on the insect repellent and insecticidal activities of *ocimum gratissimum* in the field and laboratory. [14], [15] and [16] have tried the activity on mosquitoes and

black files respectively using human baits. [17], did a preliminary study on mosquito repellent and mosquitocidal activities of *Ocimum gratissimum* (L.) grown in eastern Nigeria and concluded that the plant grown in eastern Nigeria has mosquito-repellent and mosquitocidal potentials and the formulations could be used to reduce human-mosquito contacts and hence mosquito-borne diseases and irritations caused by their bites. [18], formulated creams with the *Ocimum gratissimum* extract to test for its mosquito repellents potentials against *Aedes aegypti* L. The author [18] observed that the repellent activity was dependent on the strength of the extracts and fractions. Among the tested formulations, the maximum protection time was observed in methanol extract and ethyl acetate fractions.

A study done by the American College of Physicians showed that persons who lit citronella candles had 42% fewer bites than controls, which had no protection [19]. However, for *ocimum gratissimum* there is paucity of published work regarding its use in producing mosquito repellent candles. Therefore, the purpose of this work is to produce mosquito repellents candles using different extractives of *Ocimum gratissimum*. This will increase its availability especially repellents using local herbs will provide source of employment with its attendant reduction of unemployment. The specific objectives include; (a) Extraction of *ocimum gratissimum* leaves with different solvent (b) Qualitative test of extracts of *ocimum gratissimum* (c) Prepare candles with extract (d) Test for repellent properties of candles by using knockdown effect and calculate efficiency of candles to repel mosquitoes under laboratory conditions.

2. EXPERIMENTAL SECTION

2.1. Plant Collection and Identification

Fresh leaves of *Ocimum gratissimum* were collected from Ekeonuwa market in Owerri Municipal LGA in Imo state. They were identified at the Imo State University, Owerri, Chemistry laboratory.

2.2. Preparation and Extraction of Leaves of *Ocimum Gratissimum*

The leaves of *ocimum gratissimum* were separated manually. The materials were cleaned with distilled water, sun dried for five days and were ground using a grinder mill. Log of the fine powder from *ocimum gratissimum* leaves were placed in 250ml of solvent (100ml distilled H₂O), placed in a conical flask and refluxed for 1 hour. The powder of the medicinal plants was extracted similarly with ethanol and hot water.

2.3. Determination of Electrical Conductivity (EC)

The electrical conductivity was measured using HANNA H18733 electrical conductivity meter in $\mu\text{S}/\text{cm}$. It was calibrated using KCL. 50g of *Ocimum gratissimum* was weighed into a beaker and in 100ml of water was added. Then it was stirred gently and allows standing for 30minutes. The E.C probe is then introduced into the water-extract suspension for 60 seconds and readings is taken.

2.4. Qualitative:- Phyto-Chemical Analysis

The extracts were evaluated for the presence of Alkaloid (Al), Tannins (TA) Glycosides (GL) Saponins (Sap) Steroids (ST) and Flavonoids (FL). The identifications of the phytochemicals under study were carried out in the various extracts. The various methods applied in the analysis are outlined below.

2.4.1. Alkaloids

1cm³ of 1% Hcl was added to 3cm³ of the extracts in a test tube. The mixture was heated for 20 minutes it was cooled and filtered. The filtrate was used for the following tests.

- 2 drops of Mayer's reagent (potassium mercuric iodide solution) was added to 1cm³ of the extract.
- 2 drops of Wagner's reagents (iodine-potassium iodide solution) was added to 1cm³ of the extracts.

2.4.2. Tannins

- 1cm³ of freshly prepared 10% KOH was added to 1cm³
- 2 drops of 5% FeCl₃ was added to 1cm³ of the extracts

2.4.3. Glycosides

10 cm³ of 50% H₂SO₄ was added to 1cm³ of the extract in a test tube. The mixture was heated in boiling water for 15 minutes 10cm³ of Fehling's solution was added and the mixture was boiled

2.4.4. *Flavonoids*

1cm³ of 10% NaOH was added to 3cm³ of extracts

2.4.5. *Steroids*

Salkowski test, 5 drops of concentrated H₂O₄ was added to 1cm³ of the extracts.

2.4.6. *Saponins*

Frothing test 2cm³ of the extracts in a test tube was bigorously shaken for 2 minutes.

2.5. Preparation of Candle of *Ocimum Gratissimum*

10g of fine powdered *Ocimum gratissimum* was put in two different beakers and ethanol was added in one of the beakers and in the other and was both heated. Evaporation took place while the main extract remained 30g of paraffin wax was transferred to an aluminum pot and heated gently until it melt to a colourless liquid and the main extract of *Ocimum gratissimum* was poured into the melt and stirred thoroughly using a stirrer. The mixture was carefully decanted into the candle mould and allowed to solidify for one hour. At the expiration of the time, the candles were extruded from the candle mould and then the wicks were finally trimmed.

2.6. Determiration of Knockdown Effect

Thirty healthy mosquitoes captured from a breeding site prepared by allowing rain water in a nearby bush during the month of October 2015. The mosquitoes were then released in a transparent glass chamber where the candles produced were lit in turns for a period of 20 minutes. The number of mosquitoes still flying after for 5,10,15,20,25 and 30 minutes was counted and the difference expressed as percent of mosquitoes knockdown by the smoke (Eq. 1) from the burning candles because paraffin wax doesn't go well with solvent. Any little solvent content makes the candle not to burn for long.

$$Efficiency = \frac{\text{No.of Mosquitoes knockdown}}{\text{Total No.of Mosquitoes}} \times 100 \dots\dots (1)$$

3. RESULT AND DISCUSSION

The characteristics of *ocimum gratissimum* leaves showed brownish colouration in hot water and cold water extracts while the presence of greenish colouration in Ethanol. The hot water and cold water extracts shows pleasant odour while ethanol has a choking odour. The conductivity value in hot water and cold water was 1826 and 1824 respectively and ethanol was 229. The conductivity relates to the ability of the material to conduct electrical current through it [20].

Table2. *Physiochemical Properties of Ocimum Gratissimum Leave Extract*

| Parameter | Hot water H ₂ O | Ethanol | Cool water H ₂ O |
|--------------|----------------------------|----------------|-----------------------------|
| Colour | Brownish | Greenish black | Brownish |
| Odour | Spicy | Choking | Spicy |
| Conductivity | 1826 | 229 | 1824 |

The results for the phytochemical screening of six different bioactive compounds in three extracts of *Ocimum Gratissimum* are presented in Table 3. In these screening, results show that *ocimum gratissimum* leaves does not contain steroids but showed variations of alkaloids, tannins, saponins, flavonoids, glycosides for each solvents and method used.

The medicinal properties of the plants are determined by the components presents in them. The presence of alkaloids, tannins, saponins, flavonoids, and glycosides, contributes to the medicinal and aesthetic quality of the leave.

Flavonoid has various pharmacological effects like anti-oxidant activity, free-radical scavenging, anti-cancer and anti-aging [21] etc. Tannins are anti-cancerous in nature [22]. Flavonoids and tannins were present in cold and hot water extracts but absent in ethanol extracts.

Alkaloids could be toxic but at the right dose they act as life saving drugs in some serious disorders like heart-failure, cancer, blood pressure etc. Saponin is being used as mild detergent and in intracellular histochemistry staining to allow antibody access to intracellular protein. It is of great importance in medicine because it is used in anti-inflammatory and body loss [23]. Alkaloids and saponins were present only in the ethanol extract. This could be due to the amphipathic property of alcohol that ensures dissolution of both polar and non-polar constituents of plants.

Table3. Phytochemical Screening of the Extract

| Test | Reagent | Observation | Inference | Cold H ₂ O | Ethanol | Hot H ₂ O |
|------------|--|--------------------------|---------------------|-----------------------|---------|----------------------|
| Alkaloids | Wagner’s reagent | Redish brown precipitate | Alkaloid present | - | + | - |
| | Mayer’s reagent | Creamy PPT | Alkaloid present | - | + | - |
| Tannins | KOH, | A dirty while PPT | Tannins present | - | - | + |
| | FeCL ₃ | Greenish ppt | Tannins present | + | - | + |
| Glycosides | H ₂ SO ₄ Fehling solu | A brick-red ppt | Glycosides present | - | ++ | - |
| Saponins | Frothing test | Frothing observed | Saponins present | + | - | + |
| Steroids | Salkouski test, H ₂ SO ₄ | No red colour | Steroids absent | - | - | - |
| Flavonoids | NaOH | Yellow colour | Flavonoids presents | + | - | + |

“+” Indicates presence , “-” Indicates absence

Glycosides are basically elements bound to a sugar and have several important functions across it various categories in all living organisms. It was strongly present in the ethanolic extract of the plant, while absent in cold and hot water.

Table 4, 5, and 6 shows the knockdown effect results of the three extractives (hot water, cold water and ethanol) produced candle samples. Varying knockdown result was obtained for the different extracts used in the candle production.

Table4. Knockdown Effect of Candles with Hot Water Extract.

| Time (minutes) | Initial No of mosquitoes | Final No of mosquitoes | No of mosquitoes knocked down | Efficiency |
|----------------|--------------------------|------------------------|-------------------------------|------------|
| 5 | 10 | 8 | 2 | 20% |
| 10 | 8 | 6 | 2 | 20% |
| 15 | 6 | 3 | 3 | 30% |
| 20 | 3 | 1 | 2 | 20% |
| 25 | 1 | - | 1 | 10% |
| 30 | - | - | - | 0% |

In Table 4, the knockdown result shows that the number of mosquitoes still flying after 5minutes was 8 and 6 at 10minutes. However, the highest mosquito’s knockdown was at 15minutes which have 3 mosquito knockdowns.

Table5. Efficiency of Candles Produced with Cold H₂O

| Time (mins) | Initial No of mosquitoes | Final No of mosquitoes | No of mosquitoes knocked down | Efficiency |
|-------------|--------------------------|------------------------|-------------------------------|------------|
| 5 | 10 | 9 | 1 | 10% |
| 10 | 9 | 7 | 2 | 20% |
| 15 | 7 | 5 | 2 | 20% |
| 20 | 5 | 3 | 2 | 20% |
| 25 | 3 | - | 3 | 30% |
| 30 | - | - | - | 0% |

In table 5, the number of mosquitoes knocked down after 5 mins was 1 while 2 mosquitoes were knocked down repeatedly between 10 and 20 mins of exposure. However, the highest knockdown was obtained after 25 mins of exposure to the candle made with cold water extract.

Table6. Knockdown effect of Candle Produced with Ethanol extract

| Time (minutes) | Initial No of mosquitoes | Final No of mosquitoes | No of mosquitoes knocked down | Efficiency |
|----------------|--------------------------|------------------------|-------------------------------|------------|
| 5 | 10 | 10 | 0 | 0% |
| 10 | 10 | 6 | 4 | 40% |
| 15 | 6 | 4 | 2 | 20% |
| 20 | 4 | 2 | 2 | 20% |
| 25 | 2 | - | 2 | 20% |
| 30 | - | - | - | 0% |

Table 6 shows that there was no knockdown of mosquito after 5minutes and the highest knockdown effect were at 10minutes which have 4 mosquitoes knocked down.

Figures 1-3 is showing a plot of efficiency of the candles in knocking down mosquito at various time intervals.

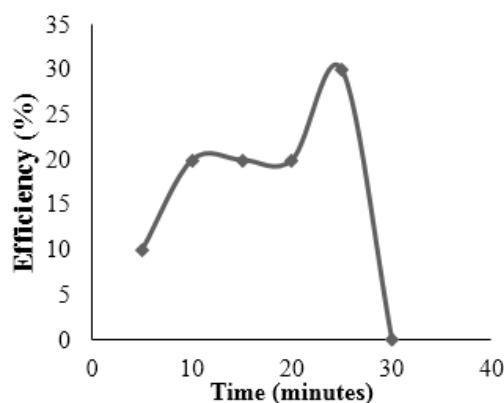


Fig1. Knockdown effect of candles produced with hot water extract

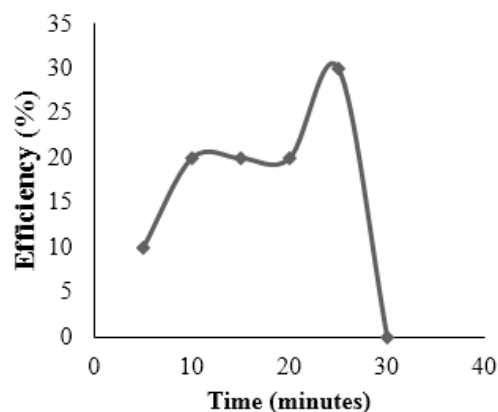


Fig2. Knockdown effect of candles produced with cold water extract.

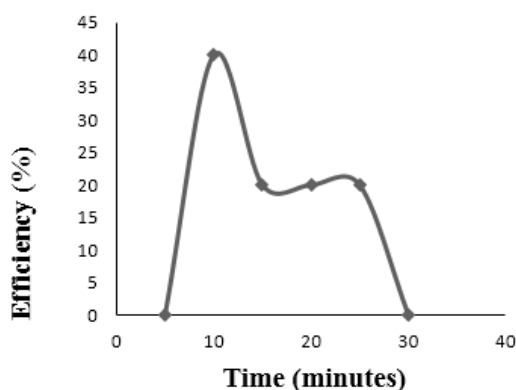


Fig3. Knockdown effect of candles produced with ethanolic extract

The efficiency in relation to time were in the order; Ethanolic extract 40 %: 10 mins > Hot water extract 30 %: 15 mins > Cold water 30 %: 25 mins. The highest efficiency obtained for the candle made with the ethanolic extract is in agreement with observation made by [18], for alcoholic extracts of *ocimum gratissimum* used in cream production. Therefore, ethanolic extract is most effective in repelling and knocking down mosquitoes.

4. CONCLUSION

From the results obtained, it is obvious that *ocimum gratissimum* can be incorporated into candles without losing their repelling properties. The mosquito repelling candle repels mosquitoes and also gives illumination at the same time, thereby serving two purposes. If this candle is further produced and used in the country, the money that would have been spent on lighting and repellents would be reduced to nothing compared to what would have been spent. Finally, result also revealed that *ocimum gratissimum* has the tendency and ability of eliminating mosquitoes in less down 10minutes of application with ethanolic extract proving more efficient. However, the health implication of this research was not noted to ascertain possible implication on the person inhaling the product.

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