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Efficacy Evaluation of *Ocimum gratissimum* Stem Extract as Mosquito Repellent

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Abstract

Malaria is a mosquito-borne disease which poses a significant threat to human health globally. This research was designed to find out the efficacy evaluation of O*cimum gratissimum* stem extract as mosquito repellent. *Ocimum gratissimum* stems were harvested from different locations within Abuja. Solutions of ethanol (20%), acetone (20%) and aqeous(20%) extracts were prepared and tested on mosquito larvae and adult mosquitoes for repellency and knockdown. Results from this study indicate that 80% of mosquito larvae were killed by ethanol extract, with no mortality recorded for acetone and aqeous extracts. Further more, the ethanol extract repelled 37.5% of mosquitoes while both acetone and aqeous extracts repelled 25% each. All extracts exhibited 0% knockdown on the mosquitoes. Phytochemical investigation revealed that all extract contains flavonoid, phenol and tannin. Higher larvicidal effect was observed in ethanol extractas a result of its ability to extract some potent phytochemicals in higher concentration due to its polarity compared to other solvents. In conclusion, stem extract of O*cimum gratissimum* only controlled mosquitoes at the larva stage. It is however recommended that ethanol stem extract of O*cimum gratissimum* should be employed in the formulation of larvicide.

Keywords*: Ocimum gratissimum*, Extracts, Mosquito repellent, Phytochemicals

1. Introduction

Mosquitoes, insects of considerable relevance to public health, are a huge nuisance, in addition to serving as carriers of dangerous and deadly disease infections (Baraack *et al.*, 2017, CDC, 2018, WHO, 2019). The enormous human and financial costs of many of these mosquito-borne illnesses, particularly malaria, provide a barrier to national development amongst other things. According to Ali *et al.*, (2014), malaria is a deadly mosquito-borne illness that kills millions of people every year, mostly pregnant women and children under the age of five. It imposes significant expenses on both people and governments. According to Fang (2010), low productivity at work, reduced physical growth, and increased economic turnover are some other significant negative impacts caused by mosquitoes. According to Islam *et al.*, (2017), as a result of these negative impacts, several solutions have been developed over the years to effectively control mosquitoes. It would seem that controlling the mosquito population would be a quicker, better, and more cost-effective approach for controlling disease-causing mosquito-borne illnesses. However, the synthetic substances that are frequently utilized for this purpose have detrimental impacts on both the

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environment and human health. Unfortunately, using mosquito repellents repeatedly for extended periods of time can have negative effects on human health, including poisoning of the spleen and kidneys (Burfield and Reekie, 2005; Arnason *et al*., 2010).

Due to their hazardous and quick-acting side effects, frequently used chemical insecticides and repellents must presently be replaced or supplemented (chemically or biologically) in order to solve these difficulties (Oliver *et al.*, 2010). Use of extracts derived from plants appears to be a promising strategy. Wu *et al.*, (2019) stated that the poisonous properties of plants can be employed for insecticidal purposes. Due to their great abundance, their use can also be considered to be a cost-effective strategy. According to Younoussa *et al.*, (2016), plant extracts are efficient against a wide range of insect pests, easy to remove, environmentally safe, biodegradable, and has been proven to have very little toxicity to non-target species. Plant-based repellents have the added bonus of being aromatic and being beneficial for the environment. Mosquito antennae equipped with chemoreceptors can detect the scent of perspiration (Patel *et al.*, 2012). Most essential volatile oils derived from various plants have proven effective as topical insect repellents (Nerio, *et al.*, 2010).

In the literature that is currently available, common weeds like *Lantana Camara* Linn (L. camara) belonging to the family Verbenaceae and *Ocimum gratissimum* Linn belonging to the family Lamiaceae have been found to have larvicidal and mosquito-repelling capabilities (Keziah *et al.*, 2015; Maia and Moore, 2020; Sumitha and Thoppil, 2016; Mi, 2018). Based on this and the facts earlier presented, it appears that plant-based natural remedies must be created in order to combat mosquitoes and diseases spread by them, especially in light of the environmental problems and health hazards associated with current synthetic pesticides (Katz *et al*., 2019). Therefore, this paper seeks to present the effectiveness of *Ocimum gratissimum* stem extract as a mosquito repellent.

Ocimum gratissimum essential oil is also recognized for its insecticidal properties. This plant is grown in home gardens in Nigeria under the name "nchu anwu," which translates to "repellent against mosquitoes" (Oparaocha *et al.*, 2010). *Aedes aegypti L*. and *Aedes albopictus* (Skuse) mosquitoes had larvicidal and repellent effects in response to the essential oil (Cavalcanti *et al.*, 2004; Oparaocha *et al.*, 2010). Cruz *et al.* (2017) found *that Ocimum gratissimum* essential oil was poisonous to *Spodoptera frugiperda* larvae in their third instar as well as to the Asian blue tick *Rhipicephalus microplus* (Lima *et al.*, 2018).

2. Materials and Methods

2.1 Sample Collection and Preparation

Stem of Ocimum *gratissimum* plant was collected fresh from different locations along Airport Road, Abuja in the month of July, 2022. The samples were rinsed with distilled water and dried under shade for three weeks. The dried stems were milled with electric blender and the resulting powdered sample was stored in an air tight container and kept in a refrigerator.

2.2 Preparation of Sample Extracts

2.2.1 Aqueous Extract

The powdered sample (100 g) was soaked in 1000 ml of distilled water. The extraction was carried out by placing the sample in water bath at 80 ^{0}C for 30 min . This was allowed to cool and filtered. The extract was concentrated on steam bath at 40 ^{0}C, transferred into a sterilized beaker and stored inside a refrigerator until ready to use.

2.2.2 Ethanol extract

The powdered sample (100 g) was soaked in 1000 ml of 99.5% ethanol for 72 h with intermittent shaking. The extract was concentrated on rotary evaporator at 50 ^{0}C transferred into sterilized beaker and stored inside a refrigerator until ready to use.

2.2.3 Acetone extract

The powdered sample (100 g) was soaked in 1000 ml of 99.5% acetone for 72 h with intermittent shaking. The extract was concentrated on rotary evaporator at 50 ^{0}C, transferred into sterilized beaker and stored inside a refrigerator until it's ready to use.

2.3 Phytochemical Analysis of the Extracts

2.3.1 Test for glycosides

Fehling's reagent (1 ml) was added to the solution of the three extracts in different test tubes and each of the mixtures boiled for 2 min. A brick red color indicates presence of glycosides.

2.3.2 Test for tannins

Each of the plant extract was stirred with 1ml of distilled water in separate test tubes,1 ml of 10% Iron (III) chloride solution was then added to each test tubes. A blue black green precipitate indicates presence of tannins.

2.3.3 Test for flavonoids

To 1 ml of the extract solutions in separate test tubes, 2 drops of dilute sodium hydroxide (2 M) was added to each test tubes. An intense yellow colour that becomes colourless when 2 drops of dilute hydrochloric acid (2M) was added indicates presence of flavonoid.

2.3.4 Test for phenolic compounds

To 2 ml of each of the plant extracts in separate test tubes, 2ml of distilled water was added and then 10% $FeCl_3$ solution. Bluish black colour indicates the presence of phenolic compounds.

2.4 Larva Collection and Mosquito Rearing

Mosquito Larvae were collected from stagnant pool of water in the month of October along airport road, Abuja. The water containing the larvae was poured into transparent containers. The larvae were identified as mosquito larvae with the aid of microscope. The mosquito larvae were reared in the laboratory in a cage covered with mosquito net to prevent the escape of the adult mosquitoes during emergence.

2.5 Larvicidal Bioassay

To 2 g of each of the concentrated extracts, 5 ml of distilled water was added to form a solution. The extracts were soluble in water. Each of the extract solution was poured into three different beakers. With the aid of scoop spoon, 10 mosquito larvae were gently put into each of the extracts and observed for 48 h.

2.6 Mosquitocidal Bioassay

To 2 g of each of the extracts, 5 ml of distilled water was added to make a solution. Mosquitoes (n = 10) were collected in a cage. Ethanol extract solution was rubbed on the hand of a

volunteer and placed in the mosquito cage for 3 h testing for repellancy. This was repeated using acetone and aqeous extracts.

Rate of knockdown of the mosquitoes was carried out by collecting 10 mosquitoes in 3 different cages. Solution of each extract was sprayed at mosquitoes in each cage. One extract per cage and then observed for 24 h.

3. Results and Discussion

3.1 Results of the Phytochemical Screening

The Phytochemicals found in the extracts of *Ocimum gratissimum* stem are presented in Table 1

Table 1: Phytochemicals Found in *Ocimum gratissimum* Stem Extracts

S/No	Chemical Species	Inference
1	Glycoside	Absent
2	Tannins	Present
3	Flavonoids	Present
4	Phenol	Present

3.2 Larvicidal Potential of *Ocimum gratissimum* Stem Extract

The larvicidal potentials found in the extracts of *Ocimum gratissium* stem are presented in Table 2.

Table 2: Larvicidal Potential of *Ocimum gratissimum* Extract against Mosquito Larva

Extract	No. of Larvae	Mortality (12h)	Mortality (24h)	% Mortality (24h)
Acetone	10	0	0	0
Water	10	0	0	0
Ethanol	10	0	8	80

3.3 Mosquitocidal Potential of *Ocimum gratissimum* Stem Extract

The mosquitocidal ability found in the extracts of *Ocimum gratissimum* stem is presented in Table 3

Table 3: Mosquitocidal Activity of *Ocimum gratissimum* Stem Extract against Mosquito

Extract	No. of Mosquito	Repellence (3h)	Knockdown (24h)
Acetone	8	2	0
Water	8	2	0
Ethanol	8	3	0

4. Discussion

The phytochemicals present in *Ocimum gratissimum* stem extracts according to this study includes: flavonoids, tannins, balsams and phenols. The presence of flavonoids could be responsible for the larvicidal activity of the stem extract (Prapanthadara, *et al.*, 2008). The detection of tannins in this study is an indication that the extracts of *the* plant can be used to formulate cosmetics that could be able to protect the skin from being bitten by mosquitoes as it has insecticidal properties (Odeyemi et al., 2020).

The larvicidal results obtained revealed that ethanol extract had the highest mortality rate (80%) on mosquito larva over 24 h period. In contrast, acetone and aqueous extracts had no larvicidal impact on mosquito larva. This could be because ethanol extract contains certain chemical compounds that have larvicidal properties, which are absent or present in lower concentrations

in aqueous and acetone extracts. This is in agreement with study by Ileke *et al.*, (2016), which found out that ethanol extract of *Ocimum gratissimum* leaf had significant larvicidal activity against *Aedes aegypti* and *Culex quinquefasciatus* mosquito larvae while the aqueous extract had no significant effect on both species of mosquito larva. Thus, in comparison to water and acetone, the results presented indicate that ethanol is the most ideal solvent for the extraction of biolarvicidal active compound.

The result of mosquitocidal activity on repellency demonstrated that the *Ocimum gratissimum* stem extracts had mild effect to keep mosquitoes away for 180 minutes. This could be due to the presence of certain volatile compounds in low concentration that possess mosquito repellent properties such as eugenol (Muller, *et al*., 2009). Presence of tannin is another factor as it has been shown to have insect repelling activity against mosquitoes (Trongtokit, *et al.*, 2005). Tannins work by interfering with the mosquitoes ability to detect and locate host. Furthermore, no mortality was recorded following spraying of the extracts on the mosquitoes. This could be because the extracts do not have strong mosquito-killing properties or may not contain a high concentration of active compounds to effectively kill mosquitoes.

In addition, the yields (%) of the extracts obtained from the plant material followed a polarity pattern; the yield decreases with increase in polarity. Acetone, the least polar solvent extracted more of the constituents (5.3 %) than ethanol (4.8 %) and water (4.5 %). This is pointing to the richness of *Ocimum gratissimum* stem in mid-polar to non-polar constituents. This may account for the potency of the ethanol extract as the active phytochemical may be mid polar in nature.

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