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EVALUATION OF INSECTICIDAL POTENCY AND DOSE-RESPONSE RELATIONSHIP OF NEPETA CATARIA EXTRACT AGAINST ANOPHELES MOSQUITOES

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ABSTRACT
This study investigated the repellent and insecticidal effectiveness of catnip (Nepeta cataria L.) leaf extract against mosquitoes with a view to formulating an effective repellent and an alternative to the gold-standard synthetic DEET (N, N-diethyl-3-methylbenzamide) repellent. The leaves were extracted using ethyl acetate, and the extract was prepared at five different (5, 10, 20, 50, and 100 %) concentrations. Mosquito repellency tests were conducted using arm-in-cage trials, in which a control arm and an arm treated with the extract were placed in a cage containing a predetermined number of unfed mosquitoes. A similar setup was used, but with a synthetic DEET-based product (Odomos) acting as a positive control. The result of the repellency study showed that at concentrations of ≥10%, the extract effectively repels mosquitos, according to efficacy studies. There was no substantial difference in the extract's repellent properties vs Odomos. Mortality test results show that the extract lacks acute toxicity at low dosages (5%), with mortality rates increasing significantly as concentrations scaled. Lethality rose from 8.67% at a 10% concentration to 27.78% at 20%, eventually peaking at 39.62% at the 100% dosage. A critical efficacy threshold was identified between the 10% and 20% concentrations, suggesting that bioactive compounds—potentially nepetalactones—reach levels sufficient to disrupt insect physiological activity within this range. While the extract's performance remained lower than the positive control (which peaked at 49.22%), the narrow margin at high dosages underscores its potency. These findings support the potential of ethyl acetate extract of N. cataria as a viable, eco-friendly alternative to conventional synthetic insecticides. It also indicated that the plant extract could be utilized in mosquito control management in two ways: it could be utilized at low concentrations to formulate mosquito repellents and at high concentrations to produce insecticides.

Keywords: Nepeta cataria, Anopheles mosquitoes, repellent, insecticides, bioactive compounds, DEET-based products.

1. INTRODUCTION
Insects cause enormous damage and loss of storage grains, crop waste, and even injure plants and trees, resulting in major economic losses (Padin *et al.*, 2002). Some of these insects also transmits parasites and microbes causing diseases ranging from Lyme, malaria, dengue fever, bubonic plague and Chagas disease (Niroumand *et al.*, 2016). Mosquito-borne illnesses and diseases has caused the deaths of many people throughout the world. Dengue fever and Dengue

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Hemorrhagic fever, are life-threatening diseases caused by the bite of an infected Aedes mosquito. They are the most common mosquito-borne viral diseases affecting a wide range of people in the world. There is no proper vaccine or treatment for these diseases and the only way to prevent infection is by avoid being bitten by an infected mosquito (Elisa *et al.,* 2004). Furthermore, other insect vectors, such as Anopheles and Culex mosquitoes, are responsible for spreading serious human diseases like malaria, Japanese encephalitis, yellow fever, and filariasis (Karunamoorthi and Hailu, 2014). Synthetic compounds (pesticides) and agents are frequently implemented as remedial measures to either repel or eliminate these insects (Nicolopoulou-Stamati *et al.,* 2016). Insect repellents are an alternative to the use of insecticides because they may be applied topically to the skin to protect an individual from the bites of insects such as mosquitoes, mites, ticks and lice or less commonly, may be used to exclude insects from an area, such as a packaging, to prevent infestation of stored products (Peterson and Coats, 2001). Prevention of insect bites, and exclusion of insects from residential areas and where agricultural crops are kept is very essential due to their economic and health implications. Agricultural crops, stored cereals, even human health and the environment are all negatively impacted by these chemical compounds (Nicolopoulou-Stamati *et al.*, 2016). For instance, long term exposure of new born babies and children to parathyroid based mosquito repellents is known to cause clinical, biochemical, and neurological changes (Sinha, 2004). In addition, these synthetic repellents also produce undesirable effects such as rashes, eye and skin irritation, and dizziness in humans (Patel *et al*., 2012). Furthermore, insects are increasingly becoming resistant to some of the commonly used pesticides thereby making the control of insect pests to much more difficult (Khan *et al.,* 2017). Hence, there is a need to find new, effective, non-toxic, safe and environmentally friendly mosquito repelling agents. It has already been publicised those certain phytochemicals, produced by plant species traditionally used in treating numerous diseases, exhibit a significant mosquito repellent activity against variety of Anopheles and culex species (Sharma *et al.*, 2024). Some of these botanical plants such as *Azadirachta indica*, *Citrus medica, Murraya koenigii, Ocimum tenuifloreum*, *Ricinus communis,* and *Plectranthus incanus* that have been used successful to control mosquitoes had been documented (Maia and Moore, 2011; Pal *et al*., 2011; Sai Shankar *et al*., 2013; Rani *et al.*, 2013; Obico and Ragragio, 2014). This biological control methods, involving the use plants and herbal materials to ward off, repel or kill insect pests, have become viable alternatives because these plants are very rich in phytochemicals. Many of these phytochemicals have been reported to possess strong biological activity and can work as larvicides, insect growth regulators, repellents, and attractants for laying eggs (Govindarajan & Sivakumar, 2012). Furthermore, the utilization of plant extracts in the control of insect pests is an environmentally beneficial and cost-effective approach (Khan *et al.*, 2017).

Nepeta cataria (Figure 1) is a perennial herbaceous plant belonging to the genus Nepeta and family Lamiaceae (Acimovic *et al.*, 2021). It is native to southern Europe, the middle East, central Asia, and parts of China. Although, it has also been widely naturalized in northern Europe, New Zealand, and North America (Suntar *et al.*, 2018). It is commonly referred to as catnip, a name probably derived from its pseudo-narcotic effects on cats (Small, 2012). *N. cataria* is an aromatic plant with medicinal or pharmaceutic properties, and it is also cultivated for bee keeping, ornamental, or aesthetic purpose (Ibrahim *et al.*, 2017; Manju, *et al*. 2019). It has been reported to possess antimicrobial, anti-inflammatory, antioxidant, anticancer, insecticidal, and insect repellent activities (Edewol and Usman, 2011; Khan *et al.*, 2011; Acimovic *et al.,* 2021). *N. cataria* is rich in bio-active phytochemicals and essential oils, flavonoids, phenolic acid, steroids, terpenoids, and terpenoid hydrocarbons (Sharma *et al.*, 2019). It also contains a bioactive chemical called nepetalactone (Figure 2), isolated in the plant's essential oil (Peterson and Coats, 2001; Saharkhiz *et al.*, 2016). This chemical has been reported to have the potential to repel mosquitoes.

Figure 1. Habit Image of *Nepeta cataria*

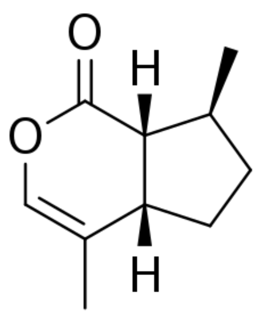

Figure 2. Structure of Nepetalactone.

2. MATERIALS AND METHODS

Sample Collection and Processing

Fresh leaves of *Nepeta cataria* L., were collected from Mgbom village, Afikpo North, Ebonyi State, Nigeria and duly authenticated. The leaves were washed, to remove extraneous materials, and air-dried. The dried leaves were then pulverized and kept for extraction.

Preparation of Extract and Impregnated Filter paper

Approximately 1000 g of powdered leaves of *N. cataria* was subjected to solvent extraction by macerating in 700 mL of ethyl acetate (purity > 99.5%). The mixture was properly mixed and left to stand for 48 hours to aid extraction. The resultant liquid extract was filtered through Whatman No. 1 filter paper to separate the marc from the filtrate. The filtrate was concentrated using a rotary evaporator under decreased pressure at 40 ^{o}C, yielding a crude extract and was stored at 4 ^{o}C until needed. Stock solutions of the crude extract were later prepared by serial dilution to achieve five concentrations 5, 10, 20, 50, and 100% (v/v) using Tween 80 as a solubilizer. DEET-based Odomos, a positive control solution, was also prepared using the same concentration regimes as for the test extract. The extract solubilizer, Tween 80, was used as a negative control.

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Circular filter paper discs (4 cm in diameter) were prepared and impregnated in 0.5 mL of each test solution and the controls. The solvent was allowed to evaporate completely by placing the discs separately on glass plates in a fume hood for 24 hours.

Phytochemical Screening

The crude extract was subjected to a variety of qualitative phytochemical tests to determine the presence of secondary metabolites. The screening procedures used were previously reported by Harborne (1973) and Trease and Evans (1989). The extract was screened for the presence of alkaloids, flavonoids, tannins, saponins, phlobatannins, steroids, phenols, terpenoids, volatile oils, resins, and cardiac glycosides.

Mosquito Rearing

Anopheles mosquitoes used in the experiment were obtained from a laboratory colony raised at an Insectary in Akanu Ibiam Federal Polytechnic, Unwana. The mosquitoes were raised under typical laboratory conditions, including a photoperiod of 12:12 (light: dark), a temperature of 27±2 ◦C, and a relative humidity of 80±10%. To encourage egg laying, water-filled Petri dishes were put in a dark region of the insectary. Larvae were then raised in water and fed brewer's yeast and dog biscuits. After eclosion, the adult mosquitoes were transported to a cage and fed a 10% glucose solution. Using a mechanical aspirator, adult mosquitoes were transported into a transparent bioassay cage of 24×24×24 cm.

Repellent Efficacy Bioassay

The laboratory bioassay to determine the repellant efficacy of the produced plant extract was carried out using a modified arm-in-cage method, which was derived from Schreck and McGovern (1989). Prior to testing, individuals were screened for adverse skin reactions by applying a tiny patch of the test extract and the positive control, a commercial DEET-based ® repellent, to the forearm. Twenty-five unfed adult mosquitoes were utilized at each of the five extract concentrations. The test involved both a control arm and a treatment arm.

The participant's forearm was washed with unscented soap, rinsed, and allowed to air dry about 12-24 hours before testing, and the use of perfumes/cosmetics was avoided. The control arm was then placed in the mosquito cage for three minutes, and the number of mosquitoes that landed on the hand was counted and recorded. The control arm was withdrawn from the cage, and the test extract was evenly applied to the forearm from elbow to fingers.

Treatment: The repellent efficacy of each concentration of the test extract and the commercial DEET-based repellent (positive control) was measured on three volunteers who served as biological replicates. The extract/ control was applied to the arm, allowed to dry for 5 minutes, and returned to the cage for 2 minutes. The number of mosquitoes landing on the hand was counted and recorded. This process was repeated on the participant's opposite forearm. To ensure the validity of the bioassay, a fresh set of mosquitos, that had not previously been exposed to either the test extract or the control repellant, were utilized in each test trial. To avoid potential desensitization of the mosquitos' antennal chemoreceptors, which could lead to a decrease in perching activity, testing was done with a timed rest interval between insertions.

The repellent activity of the extract (RA), evaluated as reduction in numbers of mosquitoes landing on the exposed forearm compared to the negative control, was calculated using Equation 1.

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$$RA\ (\%) = C - TC\ X\ 100 \quad (1)$$

T is the average number of mosquitoes landing on the surface of treated forearm; C is the average number of landings (or bites) on the untreated forearm (negative control).

Mortality Bioassay

The insecticidal activity of the plant extract was evaluated, using a modified WHO (1998) susceptibility test method, at five concentrations (5, 10, 20, 50, and 100%). Each extract concentration, DEET-based Odomos (Positive control), and Tween 80 (negative control) included four replicates, each containing 25 adult female Anopheles mosquitoes (3-5 days old). The mosquitoes were transported from the rearing cages to cylindrical air-tight container lined with the impregnated filter paper discs. The tubes were closed at both ends with mosquito netting. The mosquitoes were exposed to the impregnated papers for 24 hours. To prevent direct contact between the mosquitoes and the impregnated filter paper, a thin layer of cotton was placed between them. After the exposure time, the number of dead and live mosquitoes in each tube was counted. Mortality was defined as a mosquito's incapacity to stand or fly when the tube was gently nudged. Mosquitoes that remained active were considered alive. Abbott's formula (Equation 2) was used to adjust the observed mortality for each concentration using the control mortality.

$$\text{Corrected Mortality (\%)} = \frac{\text{Sample Mortality (\%)} - \text{Control Mortality (\%)}}{100 - \text{Control Mortality (\%)}}\ X\ \frac{100}{1} \quad (2)$$

Statistical Analyses

The repellency and insecticidal activity of *N. cataria* extract was evaluated by subjecting the dose-response data to descriptive statistics. All statistical calculations and regression modeling were performed using SPSS software (Version 16.0), and the results were expressed in percentage.

3. RESULTS AND DISCUSSION

The preliminary phytochemical screening of *N. cataria* extract revealed the presence of numerous secondary metabolites, as shown in Table 1. The screening selectively detected the presence of flavonoids and glycosides.

Table 1. Preliminary Phytochemical Screening of *N. cataria* leaf extract

Phytochemical	Presence/Absence	Proportion
Alkaloids	+++	Fully present
Flavonoids	+	Trace
Tannins	++	Moderately present
Glycosides	++	Moderately present
Resins	+	Trace
Steroids	-	Absent
Saponins	++	Moderately present
Terpenoids	+++	Fully present
Phenols	+++	Fully present
Volatile oils	+++	Fully present
Phlobatannins	+	Trace

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These findings are consistent with prior research on *N. cataria* by Nadeem *et al.*, (2022) and Khan *et al.* (2011), who detected the presence of bioactive compounds such as saponins, phenols, cardiac glycosides, flavonoids, triterpenoids, anthraquinones, alkaloids, and tannins in *N. cataria* stem and leaf extracts. However, the result is in variance with the finding of Edewor and Usman (2011), who observed the presence of only flavonoids, coumarins and glycosides in the methanol and dichloromethane extracts of *N. cataria*. The variation could be as a result of geological location, phonological age of the plant, climatic condition, percentage humidity of the harvested plant, soil composition, time of harvest, solvent used for extraction and methods of extraction (Khan *et al.*, 2011).

Repellency Bioassay

The results of repellency assay displayed in Figure 3 showed that the extract displayed considerable dose-dependence repellent activity. It was also observed that the extract outperformed the commercial DEET-based repellent, with the highest significant repellency observed at concentrations of 50%. At these doses, the extract was just as effective as the positive control repellant. However, lowering the concentration to 50% resulted in a significant reduction in repellency for both the test extract and the control. Overall, there was no significant difference in repellant efficacy between the test extract and the control.

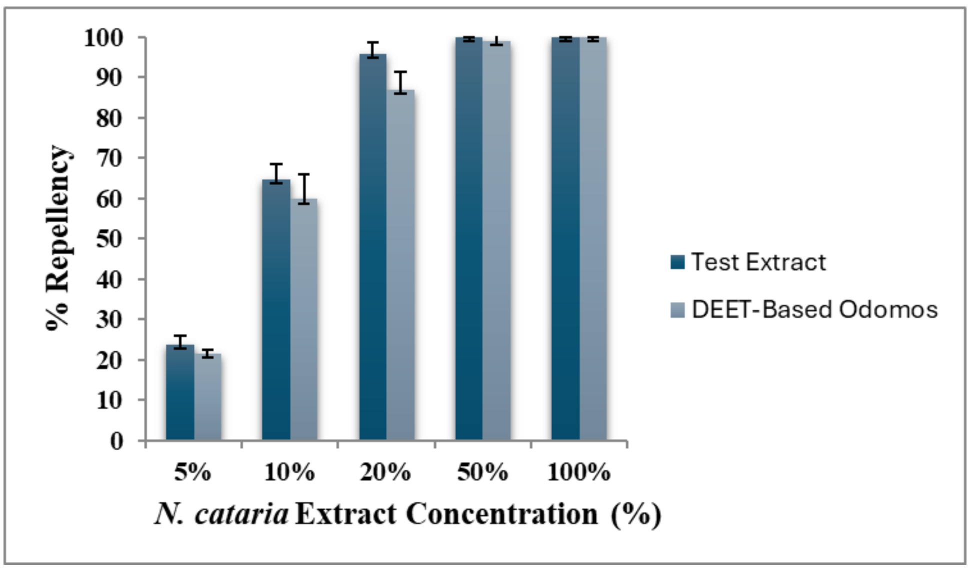

Figure 3. Repellent activity of *N. cataria* extract compared with DEET-based Odomos.

A notable observation from this study was the inverse correlation between the extract dosage and the number of mosquitoes perching on the treated arm, thereby indicating that the insect repellent was effective at increasing extract concentrations. It was also observed that increasing the concentration of the extract from 10% to 20% tripled the repellency activity of the test extract. Similarly, a four-fold increase in extract dosage resulted in an 80% repellency rate. The result showed that the extract protected the individuals from mosquito bites without triggering any allergic symptoms. The results showed that the extract exhibits a substantial repellent effect on the test insects. This finding is consistent with the result obtained by Patel *et al.*, (2023) who observed that 2.55 *N. cataria* essential oil exhibit 80% repellency against bed bug. The findings is also consistent with the 48-100% repellency exhibited by *Cassia mimosoides* petroleum ether extract (Alayo *et al.*, 2015).

Mortality Test

The insecticidal activity of the plant extract and the positive control (Odomos) was evaluated at five concentrations (5, 10, 20, 50, and 100%). The results, presented in figure 4, showed that the extract exhibited a clear dose-dependent increase in percentage mortality. The result showed that at the lowest dose of 5%, the plant extract had no insecticidal activity (0%), while positive control had a mortality rate of 13.64%. This implies that the minimum effective concentration of the plant extract is greater than 5%. At greater concentrations, the plant extract showed a significant increase in insecticidal activity, with a peak mortality of 39.62% at 100 % extract concentration. The findings of this study indicate that *N. cataria* leaf extract has strong insect repellant and insecticidal effects. The data show a clear association between extract concentration and repellent efficacy; higher concentrations resulted in a much lower number of mosquitoes perching on participants treated arms.

The insecticidal efficacy of the plant extract can be attributed to the bioactive phytochemicals observed during the preliminary phytochemical investigation. Specifically, the high concentration of volatile oils containing nepetalactone may be responsible for the mosquitocidal or larvicidal activity of the plant extract. The insecticidal action seen in this study could be attributed to the presence of myriads of bioactive phytochemicals with mosquitocidal and larvicidal activities present in the extract (Senthil-Nathan, 2020). This finding is consistent with previous studies that have investigated the phytotoxicity of *N. cataria* essential oil (Saharkhiz *et al.*, 2016).

The extract's insecticidal/ toxicological properties were evaluated against a separate set of female Anopheles mosquitoes as depicted in Table 3.

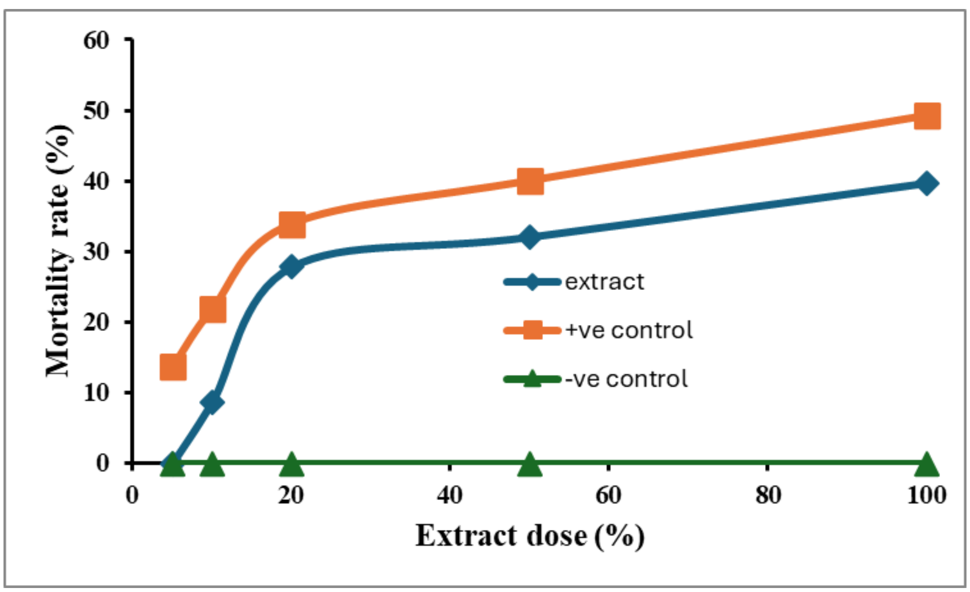

Figure 4. Insecticidal Activity of *N. cataria* leaf extract and ®Repel against female *Anopheles quadrimaculatus* mosquito.

The result showed the presence of a clear concentration-dependent relationship between the extract dosage and insect mortality rate. No insect mortality was observed at the lowest extract dosage (5%), whereas the positive control demonstrated 13.64% mortality at this concentration. This showed that the test extract lacked acute toxicity at low dosages. As the concentration increased, the extract's efficacy increased sharply, demonstrating 8.67% lethality at 10% concentration, which increased significantly to 27.78% at 20% dosage. The extract reached it's peak mortality rate (39.62%) at the highest dosage tested (100%) compared to the positive control's 49.22%. While the test extract's insecticidal activity remains consistently lower than that of the positive control, the narrow margin indicates that the plant extract indeed possessed potent bioactive compounds that become increasingly effective as the dosage scales. The consistent 0.0% mortality exhibited by the negative control validates the experimental setup, confirming that the observed deaths are attributable solely to the treatments. In contrast, the positive control demonstrated higher efficacy at all dosages, achieving nearly half-kill (49.22%) at the highest dosage. The substantial increase in mortality observed between 10 and 20% indicates a critical threshold where the bioactive compounds, probably nepetalactones, reach a dosage that is sufficient enough to disrupt the physiological activity of the insects. The result obtained in this study is consistent with findings of Boussaada *et al.*, (2008) who observed insecticidal activity in ethyl acetate extracts of some Asteraceae species against *Tribolium confusum*. The lower acute toxicity observed in the plant extract compared to the industry standard is consistent with findings by Zhu et al., (2020). However, the strong dose-dependence increase in percentage mortality observed during the study highlights the potential of ethyl acetate extract of this plant as a viable, eco-friendly alternative to conventional insecticide.

4. CONCLUSION

N. cataria ethyl acetate extract has significant insecticidal efficacy against female Anopheles mosquito, showing a distinct dose-dependent relationship with insect mortality. Despite its reduced efficacy compared to the commercial insecticide DEET, the results affirm its promise as a bio-pesticides. The findings also indicate that topical application of *N. cataria* extract may be a promising intervention for reducing human-vector contact, particularly in isolated populations with high malaria transmission rates and limited access to conventional healthcare.

While this study demonstrated the extract's repellent and insecticidal activity, it did not involve a thorough characterization of the essential oils or an in-depth assessment of the extract's biosafety. As a result, thorough biosafety and toxicological studies are required to determine whether the extract has any negative effects on humans or the environment before it can be fully approved for personal mosquito protection.

Subsequent research should concentrate on isolating the active chemicals and evaluating their insecticidal effects both separately and in combination. The effective formulation of a plant-derived pesticide may offer a more ecologically sustainable option for pest control.

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