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Insecticide phytocoil production from neem based materials and characterization of neem oil extract

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ABSTRACT

Neem based materials are abundant in almost every part of the world. It is allowed to litter the environment and eventually swept and disposed. In this study, neem oil was extracted from neem seeds kernel and its characterization was done by proximate analysis to determine the physico-chemical properties of the oil extracted. From the experiment, the moisture content, oil content, saponification (SAP) value, color and nature at room temperature of the grinded neem seed are 11.4%, 48.4%, 171 mg KOH, golden yellow and liquid, respectively. The primary objective of this work is to formulate an insecticide phytocoil from neem materials. The formulated insecticide phytocoil from both oil and neem leaves can be used as substitute to synthetic insecticides. Examining the repelling ability of the phytocoil confirms its ability to deter mosquitoes for up to 4 hrs in a small toilet room. Packaging as well as the improvement of the methodology of its mass production is suggested to enhance its looks and shape it to acceptable standard.

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1. INTRODUCTION

Azadirachta indica (neem) is a drought resistant tree originating from India and declared "Tree of the 21st Century" by the United Nations (UN) [1–3]. Parts of neem includes roots, leaves, seed, fruits, bark and flowers, with significant oil content in the kernel [4, 5]. [6], [7] and [8] highlight several benefits associated with neem including wound healing, antibacterial, antioxidant, anticancer, anti-inflammatory, antinephrotoxicity, blood and immune system regulation, skin moisture and softness, hair growth, hepaprotective, dentistry, antifungal, neuroprotective, potential contraceptive properties, AIDS, vitiligo, anticarcinogenic, psoriasis, arthritis, antihyperglycemic, immune stimulatory, immunomodulatory, cardiovascular (heart) disease, antiulcer, stress, antimutagenic, antimalarial and insect repellent. Neem-based materials especially neem oil contains Azadirachtin that repels insect and may be extracted by cold extrusion, mechanical pressing, supercritical fluid method and Soxhlet apparatus, which makes it an essential oil-based insecticide [5, 9–11]. Hence, it can be called a plant-derived insecticide as the neem oil protect humans from bites of mosquitoes of different species [12–15].

Production of insecticides with high repellency and low production cost is a great challenge that needs to be addressed. Insects in general, represent a great threat to people's health because of their ability to carry infectious diseases such as onchocerciasis, plague and malaria. Thus, the manufacture of materials that can mitigate or reduce these effects is deemed important. In view of that, the aim of this study is to produce and test the effectiveness of insecticide phytocoil from neem leaves and neem oil. Specific objectives of the study are to extract oil from neem seed, characterize the oil using proximate analysis and produce an insecticide phytocoil. The present study utilizes related research conducted previously by [7] and [3]. In those studies, methanol was used as solvent despite other available solvent. This work uses n-hexane as solvent and tries to compare analysis result obtained with those in the literature, where methanol is used. To characterize neem seed oil, properties such as water and sediment content, viscosity, specific gravity, density, color, pH, sulphur, acid and free fatty acid (FFA) values, flash, pour and cloud points, cetane number, saponification (SAP) value, iodine and peroxide values, molecular weight, total, free and bound glycerin contents needs to be determined [16–18]. In this study, only few properties of the oil that best describe the repelling ability of the phytocoil product produced would be determined. However, it is worthy of note that neem seed oil contains 59.4% moisture, 3.4% mineral, 7.1% proteins, 22.9% carbohydrates, 1% fat and 6.2% fiber [4].

2. RESEARCH METHOD

2.1. Materials

Equipment, tools and chemicals used in the course of executing the set objectives are as listed in Table 1.

Table 1: Materials Used and Model Information

S/No.	Materials	Model	Manufacturer	Function
1.	Neem leaves	Fresh	UNIMAID	Target Plant
2.	Neem oil	Extracted	UNIMAID LNG Lab	Target Plant
3.	Candle	Homesick	Kumos Laboratories and Company Kaduna	Oil Binder
4,	Distillation Apparatus	Still	Shakambari enterprise India	Separation
5.	Nitric acid (HNO_3)	Concentrated	MERU Chem PVT. Ltd.	Burning Aid
6.	n-Hexane	Concentrated	Gayatri Enterprises	Extracting Agent
7.	Plate	Stainless Steel	Mumbai Hangzhou Goldking Material Co. Ltd.	Basin
8.	Spatula	Stainless Steel	China Hangzhou Goldking Material Co. Ltd	Transferring Particles
9.	Scale	PCE-BS 6000	PCE Instruments, China	Measurements
10.	Thermometer	Liquid Thermometer	Angel Bliss Medical Technology	Temperature Measurement
11.	Water	Treated	UNIMAID Waters	Additive
12.	Potato Starch	Rotten	UNIMAID Commercials	Binder Extractor
13.	Soxhlet	KIMAX	PCE Instruments, China	Oil Extraction

These tools and chemicals were obtained from both the Chemical Engineering Analysis Laboratory and the LNG Laboratories of the Faculty of Engineering, University of Maiduguri (UNIMAID) and Kumos Laboratories and Company Kaduna, both in Nigeria.

2.2. Sample Collection and Preparation.

Neem leaves and seed samples were collected from UNIMAID, with the following coordinates: 11.8024° N and 13.1931° E. Using n-hexane as solvent in a solvent extraction setup, the neem seed oil was obtained at the LNG Lab, Faculty of Engineering, UNIMAID. Next, fresh leaves were washed and grinded to obtain the fresh leaves sample. To obtain the binder (potato starch), rotten potatoes were peeled off, washed and grated, sieved with water as sieving aid and sun dried after draining off the water. The use of potato starch as binder and sawdust as base material in this study, was based on similar work carried out by [7]. Lastly, the candles were pulverized into fine tiny particle size.

2.3. Determination of Seed Kernel Moisture Content

First, 120 mm diameter petri dish was dried in an oven at 100°C for 20 minutes to remove the moisture content of the petri dish before putting and allowing it to cool in a desiccator. The petri dish was placed on a balance machine and its weight was measured. Next, 10g of crushed kernel nuts sample was added to the petri dish and then placed inside a hot air oven to remove the moisture in the sample. The oven temperature was set to 135°C and left for 2 hours. After 2 hours, the sample was removed and cooled in a desiccator. After cooling the sample, its final weight was measured and the moisture content was determined using literature correlation [18].

2.4. Extraction of Oil from Neem Nuts (Kernel)

Ab initio, the extraction equipment was setup as follows: First, a round bottom flask was placed in a heating mantle with a Soxhlet extractor fixed on top of the round bottom flask. Second, a condenser was also placed on top of the Soxhlet extractor (the extractor has sight glass – double glass tube – the inner tube purge into the round bottom flask when the equipment is completely set while the other indicates the level of the solvent in the extractor). To initiate the extraction process, hexane (solvent) was introduced into the round bottom flask which was placed inside the heating mantle. The minimum amount of solvent required was determined by filling the Soxhlet extractor to the point where it purges. At this point, 200g crushed seed (kernel) was placed in the thimble, put inside the extractor and fixed on top of the round bottom flask. The condenser was placed on top of the extractor. The mantle was connected to power source (220V) before turning it ON.

The solvent was observed to heat up and vaporize. Its vapor passes through one tube of the extractor into the chamber where the crushed seeds (kernel) were placed. Some of the rising vapors were condensed back into the extractor by the condenser to ensure maximization of the solvent. Also observed is the hot solvent contact with the crushed seed (kernel) inside the thimble which extracts the oil from the kernel. This continues until the mixture of oil and solvent reached the purge point of the extractor. At this point, the mixture was purged into the round bottom flask. The process is then repeated continuously. When the color of the solvent in the glass was almost the same as its original color, it was assumed that the majority of the oil have been extracted and the process was halted by turning off the heating mantle. After the extraction process, the round bottom flask contained mixture of the seed (kernel) and the solvent. The oil was separated from the solvent by simple distillation. After distillation, again, 50g of crushed seed (kernel) was placed in the thimble repeating same procedure, making a total of 250g kernel used for the extraction. The forgone is in accordance with [16]. However, methanol is the most widely used solvent in Soxhlet extraction associated with neem materials reported in the literature [1, 17].

2.5. Oil Yield Computation

Oil percent recovered was determined by weighing the sample before extraction and the weight of the oil after extraction using Equation 1, given by [16], [18] and [17]:

$$Y_{oil} = \frac{Weight\ of\ Total\ Oil}{Weight\ of\ Sample} \times 100 \quad \text{.........} \quad (1)$$

where Y_{oil} = oil yield (%).

2.6. Extracted Oil Saponification Value Determination

After extracting the oil, 5g of the oil sample was measured and poured into a round bottom flask. A 4% ethanolic KOH (5 mL) was measured and poured into the 5g neem oil sample inside the round bottom flask. Automatically, two layers were formed within the round bottom flask. Another 50 mL of 4% KOH was measured and poured into a blank round bottom flask. A reflux condenser was attached to the round bottom flask containing the first sample before heating it to 78°C (boiling temperature of ethanol) using a heater. It is then observed that after 30-minute, saponification of the sample started as the condensed ethanolic sample began to fall drop-wise into the round bottom flask. It stopped after 38 minutes as the two layers within the round flask were undetectable, indicating an end of the saponification experiment/process. Similar procedure was repeated for the second sample containing only the KOH solution. After the saponification of the samples were concluded, the first sample was titrated by 0.5N HCl solution in a burette and the initial burette reading was recorded. Subsequently, 5 drops of phenolphthalein indicator were added, which turns the sample color, pink. As the titration continues, the round bottom flask was agitated gently for proper titration until the pink color disappeared. The final burette reading was recorded. The procedure was repeated for the blank solution and the initial and final burette readings were measured. SAP value equation given by [5] was then used to compute the SAP value of the oil.

2.7. Phytocoil Formulation

50 mL of water was measured in a beaker and boiled to a temperature within 85-90°C (above the gelling point of potato starch), before pouring into 20g of starch. Various trials were carried out using different compositions of the materials. The appropriate composition that was found suitable to create a perfect coil shape mold was 20g of grinded candle, 50g of grinded fresh leaves and 30g neem oil. A plate was loaded with a fine sand and a spiral shape was drawn around the surface of the sand. Groundnut oil was spread around the shape to prevent the sand from sticking to the coil. The kneaded mixtures were later poured all across the drawn shape and left for 2 hrs. Finally, the formulations were taken off and observed to be similar to a typical mosquito coil, in shape and size. Methodology associated with this section steps were drawn from [7].

2.8. Coil Effect on Mosquito Activity

Standard cage test method was used to capture the effectiveness of the phytocoil. The insecticidal characteristics were observed after igniting the formulations in a closed bathroom in which there are significant number of mosquitoes. After their complete combustion, the number of mosquitoes neutralized was counted.

3. RESULTS AND DISCUSSIONS

3.1. Product of Proximate Analysis

The results obtained from the work performed is shown in Table 2.

Table 2. Physico-Chemical Properties of Neem Oil from Laboratory Analysis

S/No.	Properties	Value
1.	Color	Golden Yellow
2.	Moisture Content	11.4%
3.	Oil Content	48.4%
4.	Saponification (SAP) Value	171 mg KOH
5.	Nature at Room Temperature	Liquid

From the Soxhlet extraction technique carried out, the 250g neem seed powdered sample used yielded 107g oil, enough to use in making insecticides. The golden yellowish color and the liquid nature of the extracted oil (Table 2) are just a physical property of the oil produced. [5] and [4] had described the color of neem oil as light to dark brown and brownish yellow in their respective work. Moisture content as obtained, was determined using the weight of the sample after drying (53.86 g) by plugging into equation in the cited literature source in the methodology. To compute the moisture content, it must be recalled that the weight of the measured petri dish weighed is 45g and the weight of sample oven-dried was 10g. Oil yield was the ratio of weight of oil after extraction (121g) and the sample weight before extraction (250g), multiplied by 100, as given in Equation 1. SAP value was calculated using 40 and 12 mL initial and final burette readings of the mixed sample and 78 and 20 mL initial and final burette readings of the blank solution,

3.2. Properties Analysis and Mosquito Kill Rate

SAP value of 182-193 mg KOH/g sample of canola oil, as obtained in the literature, can be used for making soap and detergent in a similar fashion as the neem plant used in this study, where a SAP value of 171 mg KOH was obtained. The oil content in the plant will also play significant role in selecting a material for phytocoil production. Groundnut (40-55%), neem powder (48.4% - this study), coconut seed (30-40%), orange peel (0.5-5.0% w/v) and many other plants have different oil content [19–23]. The 11.4% moisture content of the material, indicates the wettability of the seed samples which was used for the experiment. In a similar study conducted by [17] using methanol as solvent in a Soxhlet apparatus, the 41.5% yield of neem seed oil obtained, has 1.82% water content and 196.89 mg KOH/g SAP value.

After testing the formulated coil (Figure 1), it was seen to have great effect on mosquitoes. During the 4 hrs of complete combustion, 13 observable mosquitoes within the domain of the smoke were killed in the early period of its application. Apart from mosquitoes, [24] tested the effect of insecticidal activity of neem oil on bean weevil. The formulation of phytocoil using neem leaves was previously the work of [7], of which its method this research borrows. [3] also produced natural insecticide from neem leaves without formulating a phytocoil. However, their neem-based insecticide where tested based on the length of time it took before the user perceive the first mosquito bite. According to them, after an hour no mosquito bite occurred until in the second hour when few bites were perceived. In a study carried out by [25], neem oil offered 3 hrs of protection after application, closely in line with this study.

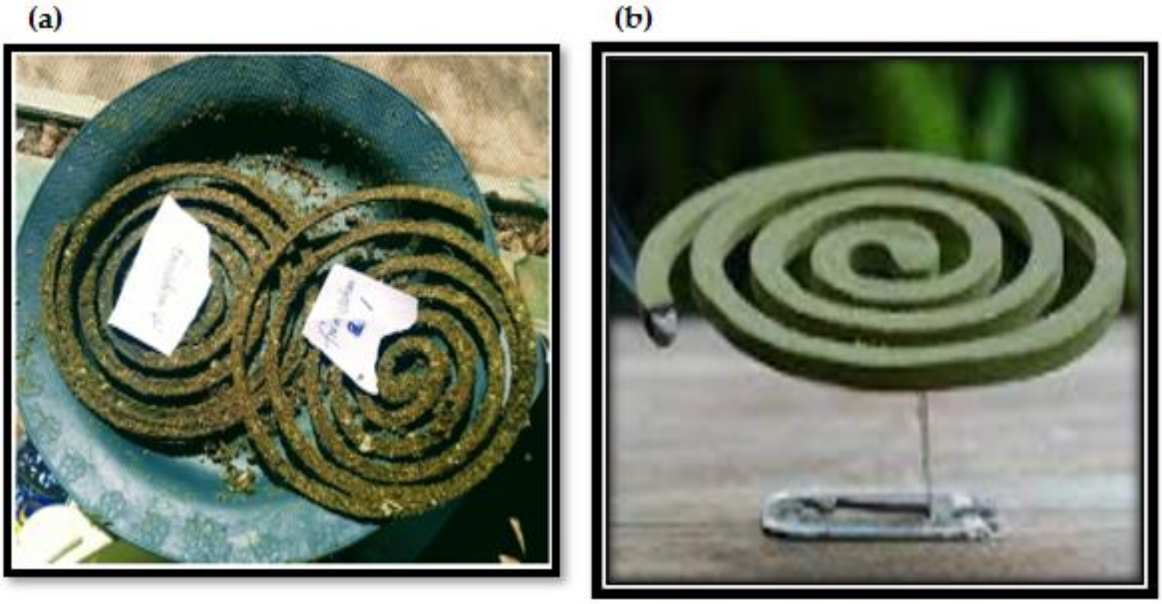

Figure 1. (a) Formulated Phytocoil and (b) Synthetic Phytocoil

The formulation needs additional touch or improvement to make its surface smooth and softer, in comparison with the synthetic type shown in Figure 1(b). Perfecting the shape will enable easy packaging for sale as well as prevent the escape of its scent which is its biggest property. Insecticide phytocoils' efficiency or ability to kill insects drops drastically when left outside its pack. Thus, the plant-base model in Figure 1(a) would be ineffective in killing insects (including mosquitoes) if not used immediately.

4. CONCLUSION

Production of a high insect repellant plant-base insecticide that can be used as a substitute to synthetic insecticide was achieved using *Azadirachta indicia* (neem). Neem plant can be use alone or combined with other plants for effective protection against insects, especially mosquitoes. A phytocoil with the right property or ability was obtained due to the following properties of the oil: 48.4% oil content, 11.4% moisture content and a 171 mg KOH SAP value. The phytocoil effectiveness is traced to the emission of scent from the leave and the oil when smoldered. It therefore offer a low cost and safer alternative to synthetic insecticides. Comparing the effectiveness of neem plant and bush mint and whether there is any serious health implication using the phytocoils generated from individual or combination of the two materials, remains to be researched. Furthermore, this work did not test the killing ability of other insects like bugs, bean weevil, crickets and cockroaches. Such test will facilitate the classification of the insecticide produced as either for mosquitoes alone or for a great number of (or diverse) insect type. Additional field test on the length of the phytocoil combustion after ignition using other essential oils and solvents, are ways that would help know or select the best combination of materials to produce a very effective insecticide.

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