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PHYSICOCHEMICAL PROPERTIES OF THE LEAF AND FLOWER EXTRACT OF CITRONELLA PLANT (*CYMBOPOGON NARDUS*), PIGNUT (*HYPTIS SUAVEOLENS*), AFRICAN BASIL (*OCIMUM GRATISSIMUM*), AND FORMULATED MOSQUITO COILS

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Abstract

This research aims to identify the physical and chemical characteristics of the leaf and flower extracts from Citronella plant (*Cymbopogon nardus*), Pignut (*Hyptis suaveolens*), and African basil (*Ocimum gratissimum*), as well as mosquito coils formulated with different plant concentrations. We evaluated nine mosquito coil formulations with varying amounts of *Cymbopogon Nardus*, *Ocimum gratissimum*, and *Hyptis suaveolens* (10g, 15g, and 20g, respectively), along with extracts from the three plant samples. The leaves and flowers of these plants were collected, dried, and ground into fine powder. The mosquito coil samples were prepared by crushing

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Keywords; *Occimum gratissimum, Hyptis suaveolens, Cymbopogon nardus,* formulated mosquito coils, physico chemical.

the coils. We then tested the finely powdered plant samples and the crushed mosquito coil samples for various physicochemical parameters, including appearance, color, pH, density, specific gravity, ash content, moisture content, acid value, and primary, secondary, and tertiary alcohols. The results showed that the pH of *Ocimum gratissimum* plant sample was highly acidic (pH=3), while *Cymbopogon nardus* was slightly acidic (pH=6), and *Hyptis suaveolens* was neutral (pH=7). The formulated mosquito coils had slightly acidic pH values ranging between 5 and 6. The plant samples of *Ocimum gratissimum* appeared brownish-green, *Hyptis suaveolens* appeared dark green, and *Cymbopogon nardus* appeared green. The formulated coils had colors of dark brown, black, and grey for *Ocimum gratissimum*, *Hyptis suaveolens*, and *Cymbopogon nardus*, respectively.
Cymbopogon nardus and *Hyptis suaveolens* showed similar values for ash content and acid value. These properties are consistent with those reported in other studies and are known to contribute to the repellent and medicinal properties of these plants.

INTRODUCTION

In previous times, synthetic chemicals have been utilized in agriculture, medicine, industry, and households. However, the use of synthetic insecticide is limited due to their carcinogenic and liver-toxic effects (Lourenço *et al*., 2019). The application of synthetic insecticides has been documented to have adverse effects on non-target organisms and to contribute to the development of insecticide resistance in mosquitoes (Rajkumar, 2004). Plant products have traditionally been utilized by human communities. (Dharmagadda *et al.,* 2005). In response to this issue, the priority for scientists is to find natural insecticide compounds in agriculture, medicine, industry, and households. As a result, numerous research studies have been conducted, and the findings have demonstrated that natural plant substances such as essential oils (EOs) are highly effective in preventing radical chain reactions and inhibiting the oxidation process caused by synthetic chemicals (Diniz do Nascimento *et al*., 2020; Šojić *et al*., 2023 & Odeh *et al*., 2022).

The Cymbopogon nardus plant, also known as the citronella plant, mosquito plant geranium, Citrosa geranium, and Pelargonium citrosum, is a type of scented geranium that emits a citronella-like scent when its leaves are crushed. *Hyptis suaveolens*, also called pignut or chan (Palmer *et al*., 1891), is a branching pseudo cereal plant that is native to tropical regions of Mexico, Central, and South America, the West Indies and is also found in tropical parts of Africa, Asia, and Australia (Gentry *et al*., 1990). It typically grows to a height of 1–1.5 m (3.3–4.9 ft) but can occasionally reach up to 3 m (9.8 ft) (Yoganarasimhan, 2000). When crushed, the leaves emit a strong minty odor.

Ocimum gratissimum, also referred to as clove basil, African basil, and wild basil in Hawaii, belongs to the Ocimum species (USDA, 2009). It is indigenous to Africa, Madagascar, southern Asia, and the Bismarck Archipelago. Additionally, it has been able to adapt and thrive in Polynesia, Hawaii, Mexico, Panama, the West Indies, Brazil, and Bolivia. This fragrant, perennial herb has the potential to reach a height of 1-3 meters. Its stem is upright, with a round-quadrangular shape, extensively branched, and may have a smooth or downy surface, along with a woody base, often accompanied by peeling epidermis (Dharsono *et al*., 2022).


Figure 1: Citronela grass


Figure 2: Ocimum gratisimum


Figure3: Hyptis suaveolens

Source: Sodangi *et al*., 2022; en.m.wikipedia.org.

These plants are highly prized for their aromatic properties and the volatile oils they produce. They are commonly used in the food industry as condiments and also have medicinal properties, including anti-inflammatory, pain-relieving, liver-protecting, anti-mutagenic, antihypertensive, and anticarcinogenic effects. Previous research has also shown their pain-relieving, intestinal motility-inhibiting, antibacterial, and antifungal properties (Coulibaly *et al*., 2023).

In addition to their use in the food and medicinal industries, these plants are commercially cultivated for the extraction of their essential oils. Like other chemicals, insecticides have properties that can be accurately measured. The behavior of insecticides is influenced by their chemical and physical properties as well as environmental factors, which in turn determine the fate and effectiveness of the insecticide. Understanding the chemical and physical characteristics of an insecticide can help applicators make better decisions regarding pesticide selection for specific situations (UNL Water, 2019).

MATERIALS AND METHODS

The leaves of *Cymbopogon nardus, Hyptis suaveolens*, and *Ocimum gratissimum* plants, weighing 100 kilograms each, were gathered from a field in Suleja, Niger state. These plant samples were then taken to the National Institute for Pharmaceutical Research & Development (NIPRD) in Abuja for identification.

Following the collection, the leaves were spread out to dry. Once dried, the plants were finely ground into powder using a mortar and an electric blender (Philips, HR 1707), and then stored in a black polyethylene bag. Charcoal was purchased at Garki market in Abuja. Talcum powder, starch, and sodium benzoate were acquired at the National Institute for Pharmaceutical Research & Development (NIPRD) in Abuja.

Charcoal Preparation

Charcoal (hardwood lump, 500 g) was sieved using a 4.75 mm sieve and then blended into fine particles with a Phillips HR 1707 electric blender.

Starch Preparation

The starch (40 g) was mixed with 70 cm3 of cold water (cold water starch) and stirred thoroughly.

Mosquito coil formulation

The charcoal samples weighing 20 g, 15 g, and 10 g were sifted and placed into plastic bowls. They were then combined with 10 g, 15 g, and 20 g of ground plants, respectively. Subsequently, 15 g of talcum powder was added to each mixture and thoroughly blended. Then, 1 g of sodium benzoate was incorporated into the mixtures and stirred well using a porcelain pestle. Cold starch was then added to the mixture and thoroughly mixed to form dough. The dough was spread onto a carton board with an aluminium sheet to create a smooth, thin paste (Kinball, 1999).

An already fabricated metal coil was placed on top of the smooth paste and gently pressed to outline the shape of the coil. The coils were then dried in an oven at 70°C for 6 hours, removed from the oven, and allowed to stand for 30 minutes (Phal *et al*., 2012).

Physicochemical Properties of the leaf and flower extract of the plant samples and the formulated coils.

The physical properties analyzed were pH, appearance, specific gravity/density, and moisture content. While the chemical properties analyzed were acid value, primary, secondary, and tertiary alcohols.

pH

The ground and a sieved sample of the plant (1.0 g) were weighed and dissolved in 10 cm^3 of distilled water, a universal indicator was dipped into the sample solution, and the colour that appeared on the paper was compared with the colours that corresponded to the pH range of 1-14 on the universal indicator and were recorded.

Appearance

The appearance of the plants and mosquito coil samples was observed physically with the naked eye.

Specific gravity/density

Empty density bottles were weighed and used to weigh out 10g each of crushed mosquito coils and sieved plant samples, the weight of the bottles containing the samples was then subtracted from the weight of the empty crucibles. The specific gravity of the samples was then calculated using Equation 1.

$$\text{Specific gravity} = \frac{\text{volume of sample}}{\text{equal volume of water}} \quad (1)$$

Essential Oil Extraction

The leaf sample (50 g) was immersed in 80 cm^3 of water in a 1 L extraction flask. The set was placed in a heater and connected to a condenser and the oils were condensed for about 3 hours. Two phases where observed (an aqueous and organic phase) at the end of the distillation. The oil was separated from the aqueous layer using a 100 cm^3 capacity separatory funnel. The collected oil was dried over anhydrous sodium sulphate and filtered using a Whatman filter paper no. 40. The extracted oil was stored at 4 °C in dark brown 5 cm^3 capacity sample vial until analysis (Baizabal, 2010).

The yield of oil was calculated using equation 4:

$$\text{Yield (\%)} = \frac{\text{oil (cm}^3\text{)}}{\text{plant (g)}} \times \frac{100}{1} \quad (2)$$

Acid value

The oil samples (2 cm^3) were accurately measured into a 250 cm^3 flask, and 25 cm^3 of a mixture of equal volumes of ethanol and ether reagent were added, which were neutralized with potassium hydroxide (0.1 mol/dm^3) after the addition of 1 cm^3 phenolphthalein. The mixtures were then heated using a steam bath until samples were completely dissolved, then cooled at room temperature and titrated with potassium hydroxide (0.1 mol/dm^3) volume solution with constant shaking until a pink colour was obtained (WHO, 2006). The volume (a) of the titrant was recorded. The acid value was calculated using Equation 2:

$$\text{Acid value} = \frac{a \times 0.00561 \times 1000}{\text{weight (in g) of sample}} \quad (3)$$

Moisture content

An evaporating dish was heated to a constant weight using an oven (N30C). The ground plant and coil samples (3.0 g) were weighed into the dish and then placed in an oven at 105°C and dried to a constant weight by checking the weight at 30 minutes intervals after drying for one hour (two consecutive weights confirm a constant weight). The loss in weight (the weight of the moisture) was found by subtracting the final weight of the dish and the ground sample from the initial weight of the dish and the ground sample. The percentage of the moisture content was calculated regarding the initial weight of the sample using Equation 3.

$$\text{Moisture content} = \frac{\text{weight loss}}{\text{weight of sample}} \times 100 \quad (4)$$

Test for alcohols (Lucas test)

Each sample (2 cm^3) was placed into separate clean, dry test tubes (100 × 13 mm) and 1 $cm^3$of Lucas reagent ($ZnCl_2$ + HCl) was added, then mixed properly by stoppering with a cork tapping the test tubes sharply with the fingers for a few seconds to mix. The cork was then removed and the test tubes were allowed to stand for 5 minutes to observe for any cloudiness that may have developed. The test tubes that showed no cloudiness after 10 minutes were warmed for 15 minutes in a 60 ^{o}C water bath. The observations were recorded.

Upon addition of Lucas reagent, a tertiary alcohol reacted rapidly and immediately to give a white layer, while a secondary alcohol reacted slowly and after heating slightly gave the white layer within 10 minutes. Primary alcohols do not react. Any formation of a heterogeneous phase or appearance of an emulsion was a positive test.

Results and Discussion

The physicochemical properties of the plant extracts and the formulated mosquito coils are presented in Tables 1 and 2 respectively; Tests for alcohols conducted on the plant samples and the formulated coils are presented in Tables 3 and 4 respectively.

Figure 4: Dried formulated Mosquito coils (Sodangi *et al*., 2022).

Table 1: Physical properties of the ground plant samples

Sample	pH	Appearance	Density (g/cm^3)	Ash content (%)	Moisture content (%)	Acid value (mgKOH/g)
Occimum gratissimum	3	Brownish green	1.0063	8.72	11.21	2.244
Hyptis suaveolens	7	Dark green	1.0046	6.62	17.11	1.683
Cymbopogon nardus	6	Green	1.0085	6.62	9.26	1.683

Table 2: Physical properties of the formulated coils

Sample	Ph	Appearance	Density (g/cm³)	Moisture content (%)
Occimum gratissimum	5	Dark brown	1.00097	7.96
Hyptis suaveolens	6	Black	1.00065	8.36
Cymbopogon nardus	6	Grey	1.00081	7.86

Table 3: Test for alcohols in the ground plant samples

Sample	Primary alcohol (1°)	Secondary alcohol (2°)	Tertiary alcohol (3°)
Cymbopogon nardus	-	+	-
Hyptis suaveolens	+	-	-
Ocimum gratissimum	+	-	-

Table 4: Test for alcohols in the produced coil samples

Coil sample	Primary alcohol (1°)	Secondary alcohol (2°)	Tertiary alcohol (3°)
Cymbopogon nardus (10 g)	-	+	-
Cymbopogon nardus (15 g)	-	+	-
Cymbopogon nardus (20 g)	-	+	-
Hyptis suaveolens (10 g)	-	-	-
Hyptis suaveolens (15 g)	-	-	-
Hyptis suaveolens (20g)	-	-	-
Ocimum gratissimum (10 g)	-	+	-
Ocimum gratissimum (15 g)	-	+	-
Ocimum gratissimum (20 g)	-	+	-

Positive (+) = present

Negative (-) = absent

Table 5: Essential oil yield of the plant samples

sample	% Yield
Cymbopogon Nardus	0.13
Hyptis Suaveolens	0.20
Occimum Gratissimum	0.27

The physicochemical properties of the plant samples and the formulated coils are presented in Tables 1 and 2, respectively. From the results, it was observed that the *Occimum gratissimum* plant sample (Table 1) was highly acidic (pH=3), while *Hyptis suaveolens* was neutral (pH=7) and *Cymbopogon nardus* was slightly acidic (pH=6). The pH values of the formulated mosquito coils (Table 2) were 5 for *Occimum gratissimum*, 6 for *Cymbopogon nardus*, and 6 for *Hyptis suaveolens*. The oils varied in color, with some appearing darker, reflecting the acidity of the samples. It was reported that one of the problems with repellent/insecticide products made of plant essential oils is that they can be irritating to the skin of humans and animals alike (Baube, 2013), which might be attributed to the degree of pH.

The acid value (2.244 mgKOH/g) and ash content (8.72 %) of *Occimum gratissimum* were observed to be higher than those of *Cymbopogon nardus* (1.683 mgKOH/g, 6.62 %) and *Hyptis suaveolens* (1.683 mgKOH/g, 6.62 %) as shown in Table 1. *Cymbopogon nardus* and *Hyptis suaveolens* showed similar values for ash content as well as acid value. However, there were not many differences between the three plant species with respect to their density/specific gravity. *Hyptis suaveolens* had the highest value for moisture content (17.11 %, 8.36 %). The ash, moisture, and acidic contents were similar to those reported by Gupta *et al.*, 2011) in Pharmacognostic and Preliminary Phytochemical Study of *Ocimum gratissimum* Linn. (Family: Lamiaceae) and Rai et al. (2013) in the study of the Physicochemical properties and elemental analysis of some non-cultivated seed oils collected from Garhwal region, Uttarkland (India). The density of *Cymbopogon nardus*, *Occimum gratissimum*, and *Hyptis suaveolens* reported were comparable to those reported by Elhassan *et al*. (2016) in the physicochemical investigation of essential oils from three *Cymbopogon* species cultivated in Sudan. The densities reported were also similar to the study of Ngozi et al. (2014) in a review on the efficacy, nutritional, and medicinal applications of *Hyptis Suaveolens*; and also similar to the research of Monteiro *et al*. (2011) in the Chemical evaluation and thermal analysis of the essential oil from the fruits of the vegetable species; where the densities reported were not more than 1 g/cm3.

The acid value shown for all three samples is normal for all volatile oils. The values are within the range of 0.6 and 10 mgKOH/g for virgin and non-virgin edible oil and fats (Dawodu, 2009); a value of ≤0.60 was given by the codex standard (2001). The acid value provides insight into the free acid content of essential oils. It is a crucial parameter of the physical and chemical properties that reflects the age, quality, edibility, and appropriateness of essential oils (Islam *et al*., 2022). A lower acid value indicates higher quality of the essential oil (Coulibaly *et al*., 2023), suggesting that the essential oils are of high quality. Solubility in ethanol is an important physical characteristic that distinguishes essential oils. The moisture content of the plant samples indicated that *Hyptis suaveolens* had the highest value (17.11 %) while *Cymbopogon nardus* and *Occimum gratissimum* showed lower values of 9.26 % and 11.21 % respectively for the ground plant samples. The formulated coils had values of 7.96 %, 8.36 %, and 7.86 % for *Occimum gratissimum, Hyptis suaveolens*, and *Cymbopogon nardus* respectively.

The samples of *Cymbopogon nardus* and *Ocimum gratissimum* plants showed traces of secondary alcohol on the addition of the Lucas reagent ($ZnCl_2$ + HCl) while *Hyptis suaveolens* showed the presence of primary alcohol when heated, but none showed the presence of tertiary alcohol (Table 3). The three different formulated coil samples of *Hyptis suaveolens* did not show any trace of primary, secondary, or tertiary alcohols. For the coil samples of

Cymbopogon nardus (Table 4), the 15 g and the 20 g coil samples indicated the presence of secondary alcohol before and after heating, while the 10 g coil sample was positive for secondary alcohol after heating. For the coil samples of *Occimum gratissimum*, all three compositions (10 g, 15 g, and 20 g) indicated the presence of secondary alcohol before and after heating.

CONCLUSION

Physico-chemical properties provide a favorable evaluation of the plant extracts and their essential oils and highlight their applications, particularly in therapeutic, culinary, industrial, and cosmetics fields. These characteristics of the physical and chemical properties reflect the quality, edibility, medicinal, ethnobotanical, and insecticidal properties of the plants and their essential oils. Understanding the chemical and physical characteristics of an insecticide can help applicators make better decisions regarding pesticide selection for specific situations (UNL Water, 2019).

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