
**PHYTOCHEMICAL SCREENING OF THE LEAVES OF CITRONELLA
(*Cymbopogon nardus*), PIGNUT (*Hyptis suaveolens*) AND AFRICAN BASIL
(*Ocimum gratissimum*)**

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ABSTRACT

The leaves of Citronella (*Cymbopogon nardus*), Pignut (*Hyptis suaveolens*), and African basil (*Ocimum gratissimum*) were investigated. The phytochemical screening of the leaves carried out in the laboratory using standard methods revealed the presence of tannins, saponins, phenols, flavonoids, alkaloids, terpenes, resins, anthraquinones, terpenoids, steroids, and glycosides. This investigation shows that *Hyptis suaveolens* has the most components as it contains alkaloids, flavonoids, phenols, saponins, and tannins, which are major components of plants. The phytochemical screening shows that these plants are volatile oils that possess medicinal and insecticidal properties due to the presence of these phytochemical constituents.

Keywords: phytochemical, *Cymbopogon nardus*, *Hyptis suaveolens*, *Ocimum gratissimum*, medicinal, insecticidal.

INTRODUCTION

Plant extracts consist of a wealth of naturally synthesized chemical compounds. (Muthukrishnan *et al*, 2014).

Plants serve as valuable sources of drugs in traditional and modern medicine, nutraceuticals, food supplements, pharmaceutical intermediates, and chemical compounds used in synthetic drugs. (Hammer *et al.*, 1999). Since the earliest days of human civilization, people have used plants and plant products as medicines (Alexander, 2016).

"Medicinal plants, an essential local legacy with worldwide significance, possess healing properties attributed to diverse complex chemical compounds present as secondary metabolites in one or more parts of these plants (Praveen *et al.*, 2010). Important bioactive components such as alkaloids, tannins, flavonoids, and phenolic compounds are found in several indigenous medicinal plants, which are also used as spices and food sources (Okwu, 1999 & 2001). The World Health Organization (WHO) and several developing nations are making more efforts to document the ethnomedical knowledge associated with the medicinal properties of African plants (Alexander, 2016).

Traditional African communities have traditionally used plants with insecticidal properties for personal protection against disease-causing parasites and pathogens, based on long-term observation and use of these plants, passed down through generations (Kidane *et al.*, 2013).

The *Cymbopogon* genus belongs to the *Andropoganeae* family of the family *Poaceae*, known for its high essential oil concentration, and is valued for its applications in cosmetics, pharmaceuticals, and phytotherapy. For instance, the citronella plant (*Cymbopogon nardus*) is recognized for its mosquito-repellent qualities due to its flavonoids and phenolic compounds (Tibenda *et al.*, 2022). Research has verified its mosquito-repellent qualities (Kim *et al.*, 2005). Research has indicated that the *Cymbopogon* genus primarily consists of flavonoids and phenolic compounds, which are the crucial pharmacologically active constituents. When paired with the intricate β cyclodextrin, phytochemicals like citronellal have been demonstrated to inhibit the descending pain pathway through a mechanism of action (Tibenda *et al.*, 2022).

Hyptis suaveolens, a member of the *Lamiaceae* or *Labiatae* family is a common weed that is utilized as a medicinal and food plant for treating various microbial infections (Akharaiyi *et al.*, 2023), with almost all parts of the plant used in traditional medicine for various purposes (Praveen *et al.*, 2010). *Hyptis suaveolens*, also referred to as pignut or chan (Palmer *et al.*, 1891), is a branching pseudo-cereal plant. It is indigenous to tropical areas of Mexico, Central and South America, and has also become established in tropical regions of Africa, Asia, and Australia (Gentry *et al.*, 1990). The leaves of *Hyptis suaveolens* have been used for stimulant, carminative, and anticancer properties (Praveen *et al.*, 2010).

Ocimum gratissimum, also called clove basil, is a species that is indigenous to Africa, Madagascar, and southern Asia. It is a perennial plant belonging to the Labiatae Family and is the most abundant in the *Ocimum* genus (Alexander, 2016). *Ocimum gratissimum* is a 1-3 m tall aromatic, perennial herb with an erect, round-quadrangular stem. The stem is much branched, glabrous or pubescent, and woody at the base, often with peeling epidermis in strips (Orwa *et al.*, 2009). It is extensively utilized for both medicinal and nutritional purposes. This understanding is founded on careful observation and the longstanding use of these plants within local communities and has been transmitted across generations (Karunamorthi and Hailu, 2014). It is essential to maintain comprehensive research documentation to preserve knowledge and transfer sustained insecticidal plant cultivation practices. This documentation also facilitates the discovery of bioactive components in plants that effectively combat blood-sucking insects (Adelaja *et al.*, 2021). Other names are Ebe-amwonkho in Edo, Tchayo in Fon [Benin], Efinrin in Yoruba, Daidoya in Hausa, Nchuanwu in Igbo, Ntonng in Ibibio, Fobazen in Haiti, Kunudiri in Okrika, Yerba di holé in Papiamento, Nunum in Akan, Nunu Bush in Jamaica (from the Akan language)

The aim of this work therefore is to carry out a phytochemical screening of the natural plant extracts (*Cymbopogon Nardus*, *Hyptis Suaveolens* and *Occimum Gratissimum*).

MATERIALS AND METHODS

Sample collection and preparation

One hundred kilograms (100 g) each of *Cymbopogon nardus*, *Hyptis suaveolens*, and *Occimum gratissimum* plants/leaves were collected at a field in Suleja, Niger state. The plant samples were identified at the National Institute for Pharmaceutical Research & Development (NIPRD), Abuja.

The leaves (100 kg each) of *Cymbopogon nardus*, *Hyptis suaveolens*, and *Ocimum gratissimum* plants that were collected were spread out to dry. The dried plants were ground into a fine powder using a mortar and an electric blender (Phillips, HR 1707) and stored in a black polyethene bag.

Preparation of Reagents

Mayer's reagent

Mercuric chloride (1 g) was dissolved in 60 cm³ of distilled water and potassium iodide (5 g) was dissolved in 20 cm³ of distilled water, the two solutions were mixed and made up to 100 cm³ with distilled water.

Dragendroff's reagent

Solution A: basic bismuth nitrate (1.7 g) and tartaric acid (20 g) were weighed and 80 cm³ of distilled water was added to make a solution.

Solution B: potassium iodide (16 g) was weighed and dissolved in 40 cm³ of distilled water.

Solution A and B were mixed to form the Dragendroff's reagent.

Wagner's reagent

Iodine (2 g) and potassium iodide (3 g) were dissolved in 100 cm³ of distilled water.

Hager's reagent

Picric acid (1 g) was dissolved in 100 cm³ of distilled water.

10% Tannic acid solution

Tannic acid (10 g) was dissolved in 100 cm³ of distilled water.

The presence of precipitate in at least 3 or all of the above reagents indicated the presence of alkaloids.

Extraction of the plants/leaves

Alkaloids test extraction

The sieved sample (10 g) was placed in a small beaker (25 cm³), and 3 drops of a concentrated ammonia solution were added until just moistened. The solution was allowed to stand for 10 minutes after a thorough mixing of the contents. A mixture of chloroform (50 cm³) and ethanol (50 cm³) was then added to the suspended powder and allowed to stand for 20 minutes with occasional stirring with a glass rod. The mixture was filtered through a plug of cotton wool. The mixture was washed twice with 2 cm³ of chloroform and the wash was combined with the filtrate. The bulked filtrate was concentrated to dryness with a steam bath. The residue was allowed to cool at room temperature and dissolved in 5 cm³ of chloroform and then transferred into a 100 cm³ separatory funnel to which 3 cm³ of dilute sulphuric acid was added and shaken to form two layers. The chloroform lower layer was drained off until the upper acid layer was colourless. The acid layer was made completely alkaline with a strong ammonium solution (tested with indicator paper). The chloroform extract was evaporated to dryness using a steam bath, and then dissolved in 3cm³ of ethanol and neutralized with dilute sulphuric acid.

Phenols test extraction

Each of the powdered samples (1.0 g) was extracted in a test tube with 5 cm³ of ethanol. Each mixture was filtered with filter paper.

Terpenes and Sterols test extraction

The powdered samples (5 g) were extracted by maceration with 50 cm³ of ethyl acetate and then filtered using filter paper.

Tannins test extraction

The powdered plant material (1g) was weighed into a test tube, boiled in 15 cm³ of distilled water for 3 minutes in a boiling water bath then filtered with filter paper.

Flavonoids test extraction

The sieved plant material (5 g) was extracted with acetone. The residue was extracted in warm water after evaporating the acetone in a water bath. The mixture was then filtered with a filter paper.

Anthraquinones test extraction

The sieved plant material (0.5 g) was weighed into two separate test tubes. Chloroform (10 cm³) was added and shaken for 5 minutes. The extract was filtered with a filter paper.

Alkaloids

Test for alkaloids

A set of three test tubes were used for each of the plant samples. The following reagents: Mayer's reagent, dragendroff's reagent, Wagner's reagent, Hager's reagent, and 10% tannic acid solution were added dropwise to each little amount of the ethanolic solution of the samples to check for precipitation.

Phenols

To 2 cm³ of the extract, 2 drops of 10 % ferric chloride were added. The formation of greenish colouration indicated the presence of phenols.

Terpenes and Sterols

The filtrate from the extraction was evaporated to dryness using a steam bath. The residues were dissolved in 10 cm³ of anhydrous chloroform and then filtered. The filtrate was divided into two equal portions and the following tests were carried out:

Lieberman-Burchard Test: Acetic anhydride (1 cm^3) was added to the first portion of the chloroform solution from the filtrate and was shaken to mix. Concentrated sulphuric acid (1 cm^3) was added down the wall of the test tube to form a lower layer. The formation of a reddish-violet colour in the lower layer indicated the presence of terpenes.

Salkowski's Test: Secondly, 2 cm^3 of concentrated sulphuric acid was carefully mixed with the second portion of the solution, allowing the sulphuric acid to form a lower layer. The formation of a reddish-brown colour at the interface indicated the presence of a steroidal ring.

Tannins

The filtrate from the extraction was used to carry out the following tests for Tannins:

Ferric chloride test

The filtrate (1 cm^3) was diluted with 4 cm^3 of distilled water and 2 drops of 10% ferric chloride was added. The formation of a blue or green colour is an indication of the presence of tannins (Evans, 1989).

Ferric ammonium citrate test

To 1 cm^3 of each of the filtrate, 0.25% ferric ammonium citrate solution was added to adjust the pH to 7. The mixture was then boiled in a water bath and filtered using filter paper. The formation of a coloured precipitate indicated the presence of tannins.

Flavonoids test

The filtrate from the extraction was used for the following tests:

Lead acetate test

The filtrate (5 cm^3) and 2 drops of 10 % lead acetate solution were added to the extract into a test tube. The formation of coloured precipitate indicated the presence of flavonoids.

Amyl alcohol test

The sieved plant material (3 cm³) was macerated with 50 cm³ of 1% HCl and filtered with filter paper. The filtrate (4 cm³) was shaken with 5 cm³ of amyl alcohol for colour change. The formation of yellow colour indicated the presence of flavonoid aglycones.

The filtrate (10 cm³) was shaken with 7 cm³ of amyl alcohol and transferred into a separatory funnel. The amyl alcohol layer was discarded and the aqueous layer boiled with 10 cm³ of 10% HCl for 2 minutes. The acidic solution was cooled and divided into two portions and the first portion was shaken with amyl alcohol. The production of a yellow colour indicated the presence of combined flavonoids.

Magnesium turnings were added to the second portion of the extracts and the solution was observed. The formation of a red coloration indicated the presence of flavonone and flavonol glycosides.

Anthraquinones test

Test for free Anthraquinones:

The filtrate from the extraction was shaken with a 10 % potassium hydroxide solution. The formation of bright pink-red colour in the upper aqueous layer indicated the presence of free anthraquinones (Evans, 1989).

Test for combined Anthraquinones:

The sieved plant material (0.5 g) was weighed and boiled with 10 cm³ of 10 % hydrochloric acid for 15 minutes. The extract while hot was filtered into a test tube and 5 cm³ of chloroform was added to the filtrate. The test tube was inverted a couple of times to avoid vigorous shaking. The solution was transferred into a separatory funnel and the two layers were allowed to separate. The lower chloroform layer was poured into a clean test tube. 2 drops of 10 % potassium hydroxide solution was added and shaken. Again, the two layers were allowed to separate. A pink, red, or violet coloration in the

upper aqueous layer indicated the presence of free and/or combined anthraquinones (Evans, 1989).

Results and Discussion

Table 1: Phytochemical screening of the plant samples

Parameter	<i>Occimum gratissimum</i>	<i>Cymbopogon nardus</i>	<i>Hyptis suaveolens</i>
Alkaloids	-	+	-
Tannins	+	+	+
Saponins	-	+	-
Flavonoids	+	+	+
Phenols	+	+	+
Resins	-	+	+
Terpenes	+	-	-
Sterols	+	-	+
Anthraquinones	-	-	-
Glycosides	-	-	-

Positive (+) = present

Negative (-) = absent

The phytochemical screening findings presented in Table 1 suggest that all plant samples contain volatile oils. "*Hyptis suaveolens*" stands out for having the most diverse array of oils, including alkaloids, flavonoids, phenols, saponins, and tannins as its major components. The screening indicates that these plants possess medicinal and insecticidal properties. Phytochemicals are believed to be responsible for the ethno-pharmacological activities of most medicinal plants (Omoyeni *et al*, 2012). The presence of phytochemical constituents implies that the plants have repellent activity (Shapiro, 2012). Compounds such as saponins have antifungal properties, and certain non-toxic glycosides can be hydrolyzed into phenolic compounds, which can act as insect repellents (Datti *et al*, 2014).

Alkaloids are nitrogenous bases found in plants and have significant physiological effects on humans. The presence of alkaloids suggests potential antimicrobial activity on microorganisms. Some plants containing alkaloids

are known for reducing blood pressure and balancing the nervous system in cases of mental illness. Alkaloids are also known for their anti-malarial properties; therefore, these plants may serve as good sources of traditional anti-malarial treatments (Stanley *et al.*, 2007).

Flavonoids are polyphenolic compounds that contribute to the yellow and orange colors found in nature. They have been reported to have antiviral and antiallergic activities.

Saponins belong to a class of natural products that can enhance the penetration of small molecules, such as proteins, through cell membranes. The presence of saponins indicates potential antimicrobial and antibacterial activity.

According to a report by Logita (2015), phytochemical analysis of gaggassa plant leaves extracted with water, acetone, and ethanol revealed several bioactive secondary metabolites such as alkaloids, polyphenols, terpenoids, flavonoids, steroids, tannins, and cardiac glycosides. These compounds, whether individually or in combination, may be responsible for the plant's insecticidal and antioxidant activity.

CONCLUSION

The leaves of plants such as *Ocimum gratissimum*, *Cymbopogon nardus*, and *Hyptis suaveolens* contain bioactive compounds that can inhibit the growth of microorganisms, making them potential sources of effective antibiotics. These compounds, which have scents reminiscent of rose and lemon, can be used in products such as soaps and cosmetics. Additionally, these phytochemicals have been found to have various pharmacological effects, including anti-protozoal, anti-bacterial, anti-inflammatory, and anti-cancer properties. The plants have proven to be valuable and have numerous applications. Their phytotherapeutic benefits are attributed to their rich phytochemical content, obtained from different parts of the plant, including leaves, roots, aerial parts, rhizomes, and essential oils.

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