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An evaluation of phytochemical and biopesticidal composition of scent leaf

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Abstract

Phytochemicals are therapeutic while biopesticides are naturally occurring forms pesticides which are eco-friendly. The phytochemicals as well biopesticidal contents of scent leaf were investigated. The result indicated that glycosides and alkaloids not detectable, phenols and terpenoids least present, flavonoids and saponins moderately present while steroids and tannins were high in abundance. The result as investigated showed that scent leaf extract contains various secondary metabolites in the following concentrations, saponins (0.130 ± 0.361 mg/g), tannins (0.133 ± 0.365 mg/g), flavonoid (0.033 ± 182 mg/g) steroid (0.005 ± 0.071 mg/g), terpenoid (0.071 ± 0.266 mg/g) as well as oxalic acid which is the biopesticidal content. Owing to the phytochemicals and biopesticide in this leaf, scent leaf could be used both therapeutically and as a biopesticide.

Keywords: Phytochemicals; Biopesticidal; Glycosides; Alkaloids; Phenols; Terpenoids; Steroids; Tannins; Oxalic acid

1. Introduction

Biopesticides have immensely proven both economically and otherwise a great benefit to plants and animals as it can be applied both for curative (in animals) and treatment (in plants) measures. Biopesticides are certain types of pesticides derived natural materials such as animals, plants, and bacteria [1]. In recent years, some environmental problems have aroused the concern of the public on the use of pesticides. There have been studies on the subject of pesticide and human health, but there still remains deep controversy surrounding it [2]. Farmers were in dilemma to either sacrifice a significant of their crops to pest or use highly toxic pesticide that can harm human health and environment [3].

Biopesticides are key elements of Incorporated Insect Management (IIM) programs and are receiving much practical attention as a means to reduce the rate of artificial chemicals of pest control which are being used. Heavy use of synthetic chemicals of pest control started from 1940s and before then, farmers were using natural insecticides such as rotenone, essential oil, pyrethroids, and neem oil from roots of demis plants and pyrethrum from flower heads of a species of chrysanthemum [4].

Over the years, it was found that the level of synthetic pesticides were building and were not biodegradable and their harmful effect started coming out which led to a need to create biopesticides which are more effective, eco-friendly and do not leave any harmful effect on the environment [5]. Biopesticides are inherently less toxic than conventional

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pesticides, it generally affect only the target pest and closely related organism, in contrast to broad spectrum, conventional pesticides that effect organisms different from insects and pest [6]Har. They often are effective in every small quantity and often decompose quickly, thereby resulting in lower exposures and largely avoiding the pollution problems caused by conventional pesticides [7]. When used as a component of Integrated Pest Management (IPM) programs, biopesticides can greatly decrease the use of conventional pesticides, while crop yields remain high. To use biopesticides effectively, however, users need to know a great deal about management pest [6].

Scent leaf is a well know medicinal plant and has been used as a traditional agent for numerous human diseases since ages in many part of the world [8]. In rural areas of the developing countries, they are the primary source of medicine [9]. Scent leaf has been used as spice in food and medicinal purposes shown to have antibiotic, antiviral and antifungal qualities. Scent leaf exhibits a broad antibiotic spectrum against Gram positive and Gram negative bacteria. Other therapeutic effects of scent leaf include lowering of cholesterol levels, blood pressures, immune system boosting and treatment of infections such as athlete's foot, convulsion, antioxidant effects as well as anti-asthmatic effect [8].

Scent leaf aids digestion. It improves immunity and contributes to the general sense of wellbeing. It is also an important substance regarding the production of biopesticides which helps in controlling of pests such as mosquitoes. Scent leaf has a warming antiseptic qualities and ability to normalize cholesterol, blood pressure and help also to reduce the effects of cold, flu, cough and other bacterial infections [10]. The research aimed at assessing the phytochemical and biopesticide content of scent leaf.

2. Methods

2.1. Sample collection

The sample used *Ocimum gatissium* (Scent leaf) were obtained from Ogbete main market, Enugu State, Nigeria.

2.2. Sample preparation

The sample scent leaf was separated from the stem by hand picking and were air-dried at room temperature before grinding to form a powder using an electric grinder. The grinding was repeated severally to obtain the finest surface area and the ground leaf kept in an air-tight container.

3. Qualitative analysis

3.1. Tannins

A quantity, 0.1 g of extract was stirred with 10 ml of distilled water and filtered; few drops of 1% ferric chloride solution were added to 2 ml of each filtrate. The presence of a blue-black indicated the presence of tannins [11].

3.2. Alkaloids

A quantity, 0.1 g of the extract was dissolved individually in dilute hydrochloric acid and filtered. Filtrates were treated with Dragendorff's reagent, formation of red precipitate indicated the presence of alkaloids [11].

3.3. Saponins

A quantity, 0.1 g of the extract was boiled with 5 ml of distilled water and filtered. To each filtrate, about 3 ml of distilled water was further added and shaken vigorously for about 5 mins. Frothing which persisted on warming was taken as an evidence for the presence of saponins [12].

3.4. Glycosides

A quantity, 0.1 g of the extract was mixed with 30 ml of distilled water and heated on a water bath for 5 mins. To 5 ml of each of the filtrates, 0.2 ml of Fehling's solution A and B were added until it turned alkaline. The solutions were heated on a water bath for 7 mins. A brick -red precipitate indicated the presence of glycoside [12].

3.5. Terpenoids

A quantity of the extract, 0.1 g was dissolved in ethanol. Acetic anhydride 1 ml was added, followed by the addition of concentrated H₂SO₄. A change in colour from pink to violet showed the presence of terpenoids [12].

3.6. Flavonoids

A quantity of the extract, 0.1 g was dissolved in water and filtered. To 5 ml of each of the filtrates, 3 ml of lead ethanoate solution was added. Appearance of a buff-colour (pale yellow – brown) precipitate indicated the presence of flavonoids [11].

3.7. Steroids

To 0.1 g of extract, 2 ml of acetic acid was added. The solution was cooled well in ice followed by the addition of conc. tetraoxosulphate (vi) (H_2SO_4) carefully. Colour development from violet to blue or bluish green indicated the presence of a steroidal ring [12].

3.8. Phenols

A quantity of 0.1g extract was boiled with distilled water and then filtered. To the filtrate, few drops of 10% ferric chloride solution were then added. A green-blue or violet colouration indicated the presence of phenolic hydroxyl group [11].

4. Quantitative analysis

4.1. Saponins

Scent leaf powder was weighed (1.0 g) using an electric weighing balance into 250 ml conical flask and soaked with 100 ml of 20% ethanol for three (3) minutes and heated for three (3) hours at 55 °C for proper extraction and then filtered. The residue was re-extracted with another 100 ml of 20% ethanol. The two extracts were combined and heated to 40 ml at 90 °C on a water bath. The concentrate was transferred into a 500 ml separating funnel and 20 ml of diethylether was added and shaken vigorously, the upper layer was discarded. The purification process was repeated and 60ml of n-batanol was added, the lower layer was discarded while the upper layer was collected. The combined n-butanol extract was washed with 10 ml of 5 % aqueous NaCl and the lower layer was discarded while the upper layer was collected in a weighed beaker and heated to dryness. The beaker is allowed to cool in a desiccator and re-weighed. The saponin content was determined using the following formula.

$$\text{Concentration of saponin} = \frac{W_2 - W_1}{W_3}$$

Where

W_1 = weight of empty beaker

W_2 = weight of beaker + sample heating

W_3 = weight of sample used

4.2. Tannins

Scent leaf powder was weighed (1.0 g) into a plastic bottle and 50 ml of distilled water was added and shaken for 3 hours in a vibrator. The sample was filtered into a 50ml volumetric flask and made up to mark. A volume, 5ml of the filtrate was dispensed into a test tube and mixed with 2 ml of 0.1 M $FeCl_2$ in 0.1 N HCl and 0.008 M potassium ferrocyanide, the absorbance was measured at 720 nm for 10 mins. The tanning concentration was determined using the following relation.

$$\text{Concentration of tannin} = \frac{\text{Abs} \times D.F}{1000 \times \text{weight of sample used}}$$

Where

Abs = value of absorbance read

D.F = dilution factor

4.3. Flavonoids

Scent leaf powder (1.0 g) was repeatedly extracted with 100 ml of 80 % aqueous methanol at room temperature, the solution was shaken for 30 mins and filtrate was transferred into a weighed beaker and evaporated to dryness over a water bath and weighed again. The time for the first extraction was 1 hour, 45 mins for the second extraction and 30 mins for the third extraction. Flavonoid was determined using the following formula.

$$\text{Concentration of flavonoid} = \frac{W_2 - W_1}{W_3}$$

Where

W_1 = weight of empty beaker

W_2 = weight of beaker + sample after drying

W_3 = weight of sample used

4.4. Steroids

Scent leaf powder (1.0 g) was dispersed in 100 ml of distilled water into a conical flask; the mixture was shaken for 3 hours and allowed to stand overnight. Then it was filtered, the filtrate was eluted with 10 ml normal ammonium hydroxide solution, 2ml of the elute was put into a test tube and mixed with 2 ml of chloroform and also 3ml of acetic hydride was added to the mixture, followed by 2ml of concentrated H_2SO_4 drop wisely. The absorbance was measured in a spectrophotometer at 420 nm.

The steroid concentration was determined using the following relationship.

$$\text{Concentration of steroids} = \frac{\text{Abs} \times \text{Path length}}{100 \times \text{weigh of sample used}}$$

4.5. Terpenoid content

A quantity (0.1 g) of the extract was weighed out separately, macerated with 20 ml of ethanol and filtered through Whatman No.1 filter paper. The filtrates (1 ml) were pipetted out and 1 ml of 5% phosphomolybdic acid solution was added and shaken. Gradually 1 ml of concentrated H_2SO_4 was added to each. The mixtures were left to stand for 30 minutes. Ethanol (2 ml) was added and absorbance was measured at 700 nm.

$$\text{Concentration of terpenoid} = \frac{\text{Abs} \times \text{Path length}}{100 \times \text{weigh of sample used}}$$

5. Assay of the biopesticidal content of scent leaf

5.1. Determination of oxalic acids in scent leaf

A standard solution of oxalic acid was prepared by dissolving 100 mg of oxalic acid ($C_2H_2O_4 \cdot 2H_2O$ mol) in distilled water and diluted to 100 ml with distilled water. A quantity of the powdered sample 0.5 g was transferred to 50 ml- capacity volumetric flask, to which, 30 ml, 0.25 N HCl was added and kept in boiling water bath for about 15 min, cooled to room temperature and volume was made up with 0.25 N HCL. This solution was used as extract for determination of oxalic acid.

Assay mixture contained 2 ml standard oxalic acid solution at various concentrations, ranging from 0.100 to 1.00 mg per ml, prepared in 1 N H_2SO_4 . Blank was prepared with 2 ml in sulfuric acid instead of oxalic acid solution. Then 2 ml of indole reagent was added in each test tube including blank, allowing the reagent to run down the side of the tube to minimize heat development. All test tubes were placed in water bath at 80 to 90 °C for 45 minutes, cooled to room temperature and absorbance was measured at 525 nm.

6. Results

6.1. Phytochemical in scent leaf

Table 1 shows the phytochemicals in scent leaf. The result indicates high abundance of steroids and tannins, moderate presence of flavonoids and saponins, slight presence of terpenoids while alkaloids and glycosides were not detectable.

Table 1 Phytochemicals in scent leaf

Parameters	Abundance
Alkaloids	ND
Glycosides	ND
Flavonoids	++
Steroids	+++
Tannins	+++
Phenols	+
Saponins	++
Terpenoids	+

Key = Interpretation; +++= Highly present ;++= Moderately present; += Slightly present; ND= Not detected

6.2. Quantitative constituents of scent leaf

Table 2 shows the chemical constituents of scent leaf. The result indicated that terpenoids, tannins and saponins was the highest chemical constituents in scent leaf, flavonoids and steroids contained slightly chemical constituents while phenols, alkaloids and glycosides were not detectable.

Table 2 Chemical constituents of scent leaf

Parameters	Abundance
Saponins	0.130±0.361
Tannins	0.133±0.365
Flavonoids	0.033±0.182
Steroids	0.005±0.071
Terpenoids	0.071±0.266
Phenols	ND
Alkaloids	ND
Glycosides	ND

KEY WORDS; ND – Not detectable

6.3. Determination of oxalic acid in scent leaf

Table 3 shows the determination of oxalic acid in scent leaf. The results indicated that oxalic acid was detectable in scent leaf.

Table 3 Determination of oxalic acid in scent leaf

Parameter	Absorbance
A	0.198 ± 0.440
B	0.211 ± 0.450
B	0.219 ± 0.460
E + filtrate	0.031 ± 0.180

7. Discussion

Phytochemical analysis is of a paramount importance in identifying new sources of therapeutically and industrially valuable compounds having nutritional and medicinal properties [13]. Traditional herbs are generally cheaper, accessible or readily available and more culturally acceptable to many. Furthermore, some synthetic drugs have been associated with some side effects, thus many turn to traditional herbs as complementary therapies and for preventive medicines. Plants thus, are used as medicinal plant in many countries of the world to manage tumour, diseases associated with oxidative stress and in countries with improved health care [14, 15].

The results of the phytochemical analysis of this study confirmed the presence of saponin, tannins, flavonoids, steroids, terpenoids and phenols. These secondary metabolites like the steroids, flavonoids and alkaloids are all valuable medicinal plants which are widely used in many traditional cultures and are increasingly becoming popular in modern society as natural alternatives to synthetic medicines. According to [16] exploitation of plant using modern biotechnology in purification, separation of compounds and metabolic engineering have produced important compounds that may function as antioxidant, anti-inflammatory and antimicrobial compounds.

According to [8], steroids are useful in diabetes, arthritis, ulcer and stomach ache. People also use them in some sports settings to boost muscle mass, performance, endurance and to shorten recovery time between workouts. Steroids are artificially derived from the main male hormone testosterone which is important for promoting and maintaining muscle growth and developing secondary male sex characteristics, such as a deepening voice and facial hair.

Flavonoids have been reported to exhibit other multiple biological effects, antiviral, antibacterial, anti-inflammatory, vasodilatory, anticancer, anti-ischemic, etc. [17]. Since there has been a global upsurge in the incidence of cancer, hypertension and other diseases related to oxidative stress especially in developing countries, plant sources containing flavonoids will be very helpful.

Phenols present in plants have powerful medicinal effects as it can help to manage ulcer and cancer. More so, phenols and glycosides are both powerful secondary metabolites with enormous antioxidant activities, these metabolites also contains vitamins which plays a vital role in human health.

Oxalic acid also known as ethanedioic acid is a naturally occurring compound which in many different types of vegetable [18]. For human, oral and topical applications of this acid are highly toxic to the body due to its bleach like and corrosive properties [18]. These properties are however, useful for waste water applications and general cleaning and therefore this acid is commonly employed for this purpose today. The oxalic acid is an ideal chemical for cleaning purposes. Its bleach like quality makes it perfect for stylizing household items. It is also efficient in removing rust on various different surfaces [19]. Stains on counters, bath tubs and kitchen sinks can be removed through careful application of this chemical. Today, it can be found as a passive ingredient in various cleaning products, bleaches and detergents. It is used medically to purify chemicals. Oxalic can cause unwanted side effect to those who ingest a high amount of food containing the chemical or use drugs with concentrated amount of it present [19].

However, oxalic biopesticidal effect are seen or applied in form of fertilizer during plant cultivation, it helps to enhance the growth of plants when applied just like fertilizer. Any alternative to the use of chemical pesticide to the use of chemical pesticide will be a highly welcome approach. The oxalate content of neem plants have also been determined [20] and there is the need for more exploration of oxalate contents of range of plants.

8. Conclusion

The study shows that scent leaf extract has an enormous medicinal value as indicated by the metabolites present in the plant extract. Further, it can be exploited in biopesticidal application due to oxalic acid.

Compliance with ethical standards

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Disclosure of conflict of interest

There is no conflict of interest in this research article.

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