



Research Article

PHYTOCHEMICAL DETERMINATION OF *OCIMUM GRATISSIMUM* (SCENT LEAF) EXTRACT AGAINST *PYTHIUM APHANIDERMATUM* IN COWPEA IN UMUAGWO, IMO STATE, NIGERIA

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ABSTRACT

This study was designed to investigate the effects of *Ocimum gratissimum* in the control of *Pythium aphanidermatum* in cowpea. The experiment was conducted in the green house and agricultural laboratory of Imo State Polytechnic Umuagwo, Nigeria from August to October, 2007 using Completely Randomized Design. This experiment was replicated four times. The results of the experiment on cum appearance at different time intervals of 24, 36, 48, 60 and 72 hours (3days) revealed that the extracts (liquid) (L₁0g 0.0 – L₁0g 0.4) changed the colour from curtney brown to whitish. This was more pronounced in L₁0g 0.4mls > 0.3 > 0.2 > 0.1mls and 0.0mls which was the control. It was also noticed that after the medium was inoculated, there existed different forms of *Pythium aphanidermatum*, ring, circular and dotted forms which never appeared in the control. On application of different concentrations of the liquid extract, the different forms of growths were cleared. The extent of control was more on 0.4mls > 0.3mls > 0.2mls > 0.0mls. On colony diameter after test of significance at 5% level of probability, it was noticed that 0.4mls reduced the colony diameter more than other levels (0.3, 0.2 and 0.0mls respectively). The ability of the extracts to exert these effects may be likened to the presence of phytochemicals which were extracted from the plant materials.

KEYWORDS: *Ocimum gratissimum*, *Pythium aphanidermatum*, Scent Leaf, Cowpea, *Vigna unguiculata*.

INTRODUCTION

Plants are richest resources of drugs in the traditional and modern systems of medicine, nutraceuticals, food supplements, pharmaceutical intermediates and chemical entities for synthetic drugs (Alexander, 2016 and Hammer *et al.*, 1999). The use of plants and plants products as medicines could be traced back to the beginning of human civilization. Medicinal plants are of great importance to the health of individual and the communities. The medicinal values of some plants lie in some chemical substances that produce definite physiological actions in the human body. The most important of these bioactive constituents are alkaloids, tannis, flavonoids and phenolic compounds. Many of these indigenous medicinal plants are used as spices and food plants (Okwu, 2001). An Ethno botanical and ubiquitous plant serves as rich resources of natural drugs for research and development (Kong *et al.*, 2008).

The phytochemical evaluation of *Ocimum gratissimum* shows that it is rich in alkaloid, tannins, phytates, flavonoids and Oligosaccharides (Ijeh, *et al.*, 2004). In the coastal area of Nigeria, the plant *Ocimum gratissimum* is used in the treatment of epilepsy, high fever and diarrhea (Ladipo *et al.*, 2010). The plant *Ocimum gratissimum* is one of those plants widely known and used for both medicinal and nutritional purposes. It is a perennial plant that is widely distributed in the tropics of Africa and Asia. It belongs to the Family *Labiatae* and it is the most abundant of the genus *Ocimum*. The common names of the plant are Basil Fever plant or Tea bush and vernacular names include *Daidoya tagida* (Hausa), *Nichonwu* (Igbo), *Tanmotswangiwawagi*

(Nupe) and *Efinrin* (Yoruba) (Abdullahi *et al.*, 2003 and Idris *et al.*, 2011).

It is woody at the base and has an average height of 1-3 meters. The leaves are broad and narrowly ovate, usually 5-13cm long and 3-9cm wide. It is a scented shrub with lime-green leaves (Sofowara, 1984). The plant is consumed by the Igbos as a leafy vegetables and the nutritional importance of this plant center on its usefulness as a seasoning because of its aromatic flavor. It is also used by the Igbos in the management of the baby's cord. It is believed to keep the baby's cord and wound surface sterile. It is used in the treatment of fungal infections, fever, cold and catarrh. *Ocimum gratissimum* is used through West Africa as antimalarial and anti-convulsant. The crushed leaf juice is used in the treatment of convulsion, stomach pain and catarrh. Oil from the leaves have been found to possess antiseptics, antibacterial and antifungal activities (Edeoga and Eriata, 2001; Sofowara 1984). Alexander, (2016) stated that the extracts of *Ocimum gratissimum* are active in vitro against human pathogenic dematophyte.

Cowpea (*Vigna unguiculata*) (L.) Walp is an important crop for many subsistence farmers in tropical areas especially in Africa. The green plant parts of cowpea can be used as a vegetable or as fodder for cattle whereas the cowpea contain a high level of proteins and are used as human food (Bosah, 2013). The crop is well adapted to stress and has excellent nutritional qualities (El-Ameen, 2008). It is a very important food source in developing countries where animal protein is limited (Philip and McWatters, 1991 and Tenebe *et al.*, 1995) thereby, supplementing the low protein menus due to high cost of animal source of protein (Fawole *et al.*, 2006, Miko and Mohammed, 2007).

Almost all the parts of the cowpea are used as food. They contain proteins, vitamins and minerals (Fatokun, 2002). Its high protein level makes it extremely valuable in communities where many people cannot afford protein food derived from meat, fish and egg (IITA, 2007; Emeribe and Chukwuezi, 2018). The amino acid of cowpea compliments those of cereals (Fashakin and Ojo, 1988). Cowpea also contains minerals such as potassium, iron, calcium, phosphorus and magnesium. Cowpea has received a particular attention from the onset of research on grain legumes at the International Institute of Tropical

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Agriculture (IITA), Ibadan. Many African countries depend on its seeds and leaves for food. Adams (1984) reported that Nigeria is known to be the greatest consumer of cowpea in the World.

The value of cowpea lies in its high protein content and ability to tolerate drought. As a legume cowpea fixes atmospheric nitrogen, allowing it to grow on and improve poor soils (Amadi and Oso, 1996). Cowpea grain contains about 25% protein making it extremely valuable when many people cannot afford protein foods such as meat and fish. Despite the many uses and its relevance to the tropics particularly the Nigerian agriculture; cowpea crop losses due to insect pests were estimated at 55-62%. Several fungi attack the legume plants during growth, harvest and storage. While more than 25 different species of fungi are known to invade stored grains and legumes (Duan *et al.*, 2007), species of *Aspergillus*, *Penicillium* and *Fusarium* are responsible for most spoilage and germ damage before and during storage. They cause reduction in cooking or baking quality, and nutritive values, produce undesirable odors and color and change appearance of stored food grade grains and decrease germinability and total decay (Quenton *et al.*, 2003 and Castillo *et al.*, 2004). In Nigeria, there is a need for more information on the identification and pathogenicity of fungi associated with cowpea. This present study aimed at determining the fungicidal activity of *Ocimum gratissimum* in the control of *Pythium aphanidermatum*.

MATERIALS AND METHODS

The study was designed to investigate the efficacy of liquid extract of the leaves of *Ocimum gratissimum* in the control of *Pythium aphanidermatum* in cowpea. Leaves of *Ocimum gratissimum* were procured from a local market in Imo State, Nigeria.

Field Experiments:

The experiment was carried out at the Imo State Polytechnic green house and agricultural laboratory (longitudes 7° 01' 06¹¹E and 7° 03' 00¹¹ and latitudes 5° 28' 00¹¹N and 5° 30' 00¹¹N) (IMLS, 2009) from August through October, 2007

Experimental materials:

Experimental materials used are: petri-dishes, electron microscope, polythene bags, slides, cover slips, ethanol, mortal, petroleum spirit, potato dextrose, agar-agar, inoculating loop, syringes, streptomycin powder, dilution water, benzoyl and Soxhlet extractor respectively.

Soil mixture was made in the ratio of 3:2:1 of humus mature poultry droppings, top soil and sharp sand. The top soil provided the needed water retention capacity in the mixture and mineral content while the poultry droppings provided the nutrients and also helped in moisture conservation. The sharp sand aided in sufficient aeration.

The polythene bags served as source of (inoculum) *Pythium aphanidermatum*. After 3 months of growth of cowpea, the affected leaves (suffering from leaf rot) were collected from each replicate, crushed and used to inoculate the PDA medium.

Preparation of Liquid Extracts of *Ocimum gratissimum*:

After drying at room temperature till the leaves became crispy, the leaves were ground and the products was extracted using Soxhlet extractor with 2.25 liters of the petroleum spirit 40 – 60% thus yielding 150ml of the crude liquid extract or 0.43 g/ml (as calculated 2250/975 g/ml = 0.43 g/ml).

RESULTS AND DISCUSSION

The result of phytochemical screening Table 1 of *Ocimum gratissimum* showed that the plant leaves contains tannins, flavonoids, terpenoids alkaloids Phlobatannins, tannins saponins, steroids and glycosides. Further analysis of the phytochemicals constituents with

HPLC ascertains that *Ocimum gratissimum* contains all the necessary phytochemical constituents. These metabolites are known to have varied pharmacological actions in man and animals, the presence of these metabolites suggest great potentials of the plants as a source of useful phyto-medicines. The phytochemicals are naturally occurring chemicals in plants which serve as medicinal for the protection of human disease; the phytochemical are non-nutritive plants chemical that have protection or disease preventive properties (Cheng and Linn, 2002).

Phytochemical Determination:

Phytochemical Analysis:

Phytochemical screening was carried out using standard procedures to identify the constituents as describe by Okwu and Okwu, (2005) and Ladipo *et al.*, (2010).

Qualitative Analysis of the Constituents:

Test for Tannins:

0.5g of the dried powdered sample was boiled in 20cm³ of water in a test tube and was filtered. A few drops of 0.1% ferric chloride was added for observation of brownish green or a blue black colouration.

Test for Phlobatannins:

Aqueous extract of the plant sample was boiled with 1% aqueous hydrochloric acid and deposition of a red precipitate was seen as an evidence for the presence of Phlobatannins.

Test for Saponins:

2g of the powdered sample was boiled in 20cm³ of distilled water in a water bath and filtered. 10cm³ of the filtrate was mixed with 5cm³ of distilled water and was shaken vigorously for a stable persistent froth to be formed. The frothing was mixed with 3 drops of olive oil, and was shaken vigorously and then observed for the formation of emulsion.

Test for Flavonoids:

5cm³ of 10% diluted ammonia solution was added to a portion of the aqueous filtrate of the plant extract, and then followed by addition of concentrated H₂SO₄. The observation of a yellow colouration in the extract indicated the presence of flavonoids.

Test for Cardiac Glycosides:

5cm³ of the extract was treated with 2cm³ of glacial acetic acid containing 1 drop of ferric chloride solution (0.1%) was underlaid with 1cm³ of concentrated H₂SO₄. A brown ring of the interface was indicated by a de-oxy-sugar characteristic of cardenolides. The violet ring did not appear below the brown ring, while in the acetic layer, a greenish ring was not formed throughout thin layer.

Test for Terpenoids:

5cm³ of the extract was mixed in 2cm³ chloroform and 3cm³ conc. H₂SO₄ was added, to form a layer. A reddish brown colouration of the interface was formed to show the positive result for the presence of terpenoids.

Test for Steroids:

2cm³ of acetic anhydride was added to 0.5g ethanolic extract of the sample with 2cm³ of H₂SO₄. The colour does not change from violet to green to indicate the presence of steroids.

Sample preparation for (HPLC):

5g of prepared sample was placed into a 25cm³ standard volumetric flask and make up to mark with buffer diluents. The solution was reflux, shaken, centrifuged and decanted. The filtrate was filtered using the HPLC grade filter paper.

Table No. 1: Phytochemical constituents (Field work, 2007; Alexander, 2016)

Constituents	Result	HPLC
Tannins	+	+
Saponins	+	+
Flavonoids	+	+
Terpenoids	+	+
Alkaloids	+	+
Steroid	+	+
Glycosides	+	+
Phlobatannins	+	+

- =Absent, + = present

Alkaloids are also considered as nitrogenous bases that occur in plants, many of them have marked physiological effects on humans. Some alkaloids used as medicine are morphine, caffeine and coffee; in which caffeine in tea and coffee is alkaloids that stimulate the nervous system (Alexander, 2016). The presence of alkaloids suggests that it has potential antimicrobial activity on microorganisms. Some plants that posse alkaloids are known for decreasing blood pressure and balancing the nervous system in case of mental illness. Alkaloids are known to posses' anti-malaria property; hence the plants may be a good source of anti-malaria for which it is traditionally used (Edeoga and Eriata, 2001).

Flavonoids are polyphenolic compound that contribute to many other colours found in nature particularly the yellow and orange of petal, they have been reported to have antiviral and antalgic activities. Presence of flavonoids might be responsible for its use as anti-inflammatory effects on both acute and chronic inflammation (Boham and Kocipai, 1994). The presence of saponins serves as potential activity of an antimicrobial agent. The presence serves as an indicator towards

possible antibacterial activity. Saponins are a class of natural products involves and can be used to enhance penetration of micro molecules such as protein through cell membrane.

Mineral content:

The result of the mineral composition of the leaves was presented in Table 2. The result revealed the presence of the essential elements such as Mg, K, Cu, Zn, N, Fe, Na and Ca; which indicates the medicinal values of the plant. Different combination of these elements in the medicinal plant helps to cure the ailments. From the results of the investigation carried out, magnesium was the most abundant elements with the concentration of 1.712 ± 0.537 mg/kg. Magnesium helps in maintaining a normal heart rhythm and is sometimes given intravenously to reduce the chance of atrial fibrillation and cardiac arrhythmia (Holleman *et al.*, 1988).

Table No. 2: Mineral composition (Mg/Kg) (Field work, 2007; Alexander, 2016)

Elements	Concentration	WHO (mg/kg)
Magnesium	1.71 ± 0.537	0.62-2.64
Potassium	0.26 ± 0.077	0.07-0.34
Manganese	0.46 ± 0.107	0.23-0.67
Copper	0.80 ± 0.818	0.75-0.89
Lead	0.005 ± 0.002	0.01- 0.03
Zinc	0.20 ± 0.06	0.16-0.34
Nitrogen	0.29 ± 0.052	0.45- 0.67
Iron	0.31 ± 0.067	0.00012-0.46
Sodium	0.31 ± 0.049	0.048-0.56
Calcium	0.14 ± 0.111	0.24-0.28
Cadmium	ND	0.00012-0.00016
Chromium	ND	0.0024-0.0036

(\pm) mean and standard deviation of three determinations; ND – Not Detected; WHO – World Health Organisation

The concentration of sodium in the plant is 0.311 ± 0.04 mg/kg. Sodium has an important role in maintaining the water balance within cells and in the function of both nerve impulse and muscles. The sodium also helps in maintenance of normal acid-base balance. An adult need about 3g per day of sodium but modern dietary habits take in 5-20 per day (Milbury *et al.*, 2008). The sodium content of the plant is within the recommended level by (WHO, 1995) (70.048 - 0.56mg/kg). Calcium (Ca) was present at the concentration (0.138 ± 0.111 mg/kg), which plays an important role in building and maintaining strong bones and teeth, large part of human blood and extracellular fluids. Approximately 99 percent of the body's calcium is stored in the bones and teeth (Holleman *et al.*, 1988).

The studied plant of *O. gratissimum* is essential in building up the level of calcium in the body. The concentration of iron in the studied plan leaf of *O. gratissimum* is 90.312 ± 0.067 mg/kg. The presence of iron shows that the plant is essential for red blood cell production and oxygen transport in the body as supported by the work of (Bahl and Bahl, 2006).

Lead (Pb) was present at a very low concentration of (0.005 ± 0.0016 mg/kg). Lead occurs naturally in the environment. Everyone may be exposed to trace amounts of leads through air, soil, household dust, food, drinking water and various consumer products (Shivery and Sofora, 2009).

The presence of Cu, Mn, and Zn indicates that the plant is essential for: immune function, protein synthesis, blood clothing, Hormones, formation of hemoglobin and for secretion and potentiating insulin action, this has been also reported by (Bahl and Bahl, 2006), and their concentrations in the plant leaf range as Cu (0.80 ± 0.818 mg/kg), Mn (0.46 ± 0.107 mg/kg) and Zn (0.20 ± 0.006 mg/kg) respectively. Copper is an essential element in the human body and exist as an integral part of copper proteins ceruloplasmin, which is concern with the release of iron from the cells into the plasma and is involved in energy metabolism (Bahl and Bahl, 2006).

Chromium in trivalent state is an essential trace element that potentiates insulin action and thus influences carbohydrate, lipid and protein (Shivery and Sofora, 2009). Chromium was not detected in plant leaf of *O. gratissimum*. Cadmium was also not detected in the sample, cadmium causes kidney and liver problem including heart, brain and eyes problem on longer time of its accumulation (WHO, 1995). *Ocimum gratissimum* leaf is safe for consumption since these toxic elements were noted detected.

CONCLUSION

The phytochemical and elemental component of the leaves of *Ocimum gratissimum* was analysed. The result indicated that the leaf of the plant contains some major bioactive compounds that can inhibit the

growth of microorganism thereby proving it as an effective potentials source of antibiotic. However, the result further revealed that the plant leaf contains *saponins*, *tannins* and *alkaloids* which help to inhibits bacterial growth and control of *Pythium aphanidermatum* infestation in cowpea. The plant extract might also be a potential source for drugs formulation as the plant leaves are used traditionally for curing of many infectious diseases.

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