
Qualitative and Quantitative Phytochemical Screening of Nigerian Indigenous *Ocimum gratissimum* Linn (Clove Scented Basil Leaves)

By

Jumare, Fatima. M

Department of Integrated Science,
Federal College of Education Zaria, Kaduna State, Nigeria.

Email: fatimajumare@gmail.com

ABSTRACT

*Phytochemical compounds are secondary metabolites produced by all plants in which some has medicinal uses. The phytochemical analysis of aqueous leaf extract of indigenous *Ocimum gratissimum* (Scent leaf) was investigated in the present study. Based on qualitative analysis, the results revealed the presence of alkaloids, saponins, tannins, flavonoids, terpenoids, coumarins, quinones, glycosides, and steroids while phenols and phlobatannins were absent. The quantitative analysis on saponins, alkaloids and flavonoids recorded mean results of 3.67 mg/100g, 2.03 mg/100g and 4.17 mg/100g respectively. This investigation suggested that Nigerian indigenous *Ocimum gratissimum* leaves are rich in phytochemical constituents which contributed to its medicinal uses. Therefore, the study justifies the use of scent leaf in Nigeria as a traditional medicine for treatment of various diseases.*

Keywords: Phytochemical Screening, *Ocimum gratissimum* Linn

INTRODUCTION

Ocimum gratissimum (Scent leaf) is a popular Nigerian herb that is utilized for both medicinal and culinary purposes. According to Abdullahi, 2012 it is an herbaceous plant which belongs to the Lamiaceae family. The plant is indigenous to tropical areas especially India and West Africa. In Nigeria, it is found in the Savannah and coastal areas. It is known by various names in different parts of the world. In India it is known by its several vernacular names, the most commonly used ones being Vriddhutulsi (Sanskrit), Ram tulsi (Hindi), Nimma tulasi (Kannada). In the southern part of Nigeria, the plant is called “effirin” by the Yoruba speaking tribe. It is called “Nichonwu” by the Igbos, while in the Northern part of Nigeria; the Hausas call it “Daidoya” (Abdullahi *et al.*, 2003; Idris *et al.*, 2011 and Alexander, 2016).

The plant is commonly used in folk medicine to treat different diseases such as upper respiratory tract infections, diarrhoea, headache, diseases of the eye, skin diseases, pneumonia, cough, fever and conjunctivitis (Gupta *et al.*, 2011). The flowers and the leaves of *O. gratissimum* are rich in essential oils so it is used in preparation of teas and infusion (Prabhu *et al.*, 2009). In the Southeastern areas of Nigeria, the plant is used in the treatment of epilepsy, high fever, diarrhoea as well as in the management of the baby’s cord, to keep the wound surfaces sterile (Alexander, 2016). The Kenyan and sub Saharan African communities’ use this plant for various

purposes like viz., the leaves are rubbed between the palms and sniffed as a treatment for blocked nostrils, they are also used for abdominal pains, sore eyes, ear infections, coughs, barrenness, fever, convulsions, and tooth gargle, regulation of menstruation and as a cure for prolapse of the rectum (Abdullahi, 2012). In India, the whole plant has been used for the treatment of sunstroke, headache, and influenza, as a diaphoretic, antipyretic and for its anti-inflammatory activity (Venuprasad *et al.*, 2014).

Hitherto, there is an increasing interest worldwide in herbal medicines accompanied by increased laboratory investigation into the pharmacological properties of the bioactive ingredients and their ability to treat various diseases (Justina and Solomon, 2017; Omodamiro and Jimoh, 2015; Gupta *et al.*, 2011). Furthermore, numerous drugs have entered the international market through exploration of ethnopharmacology and traditional medicine. However, Prabhu *et al.*, (2009) has lamented that scientific studies have been done on a large number of herbs but only a considerably smaller number of marketable drugs or phytochemical entities have entered the evidence-based therapeutics. Thus, this study aims to reveal the qualitative and quantitative phytochemical constituents of *Ocimum gratissimum* being a commonly used herb and condiment in most Nigerian homes.

MATERIALS AND METHOD

Plant collection (Source of plant material)

Fresh leaves samples of *Ocimum gratissimum* were collected from naturally growing populations located in Samaru district in Zaria local government area of Kaduna state, Nigeria. The samples were identified at the herbarium section of the Department of Biological Sciences, Faculty of Science Ahmadu Bello University Zaria, Kaduna state, Nigeria. The fresh leaves plant samples were washed by tap water, air dried in shadow at room temperature milled well into a fine powder in a grinder and then stored for further use.

Preparation of leave extracts

The aqueous extract of fine pulverized leaves were prepared according to Salem *et al.*, (2016) by taking the weighed amount of fine powdered leaves sample separately in proper volume of distilled water at a 40% (w/v) concentration (200g leaves powder in 500 ml water) for 72h with alternating shaking, and then extract was filtered using Whatman filter paper No. 1. The dried yield was stored in refrigerator at 4 °C until further use.

Qualitative Phytochemical Analysis

Chemical investigations were carried out on the aqueous extract using standard procedures to identify the constituents (Roopashr *et al.*, 2008, Suman Kumar *et al.*, 2013 and Salem *et al.*, 2016).

Test for Tannins

About 0.5 g of the extract (dry extract) was dissolved in distilled water and about 10 ml of bromine water added. Decolouration of bromine water indicated the presence of tannins.

Test for saponins

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

Test of Flavonoids (Alkaline Reagent Test)

10 ml of each extracts (separately) were treated with few drops of sodium hydroxide solution. Formation of intense yellow colour, which becomes colorless on addition of dilute acid, indicates the presence of flavonoids.

Test of Terpenoids (Salkowski's test)

10 ml of each extracts (separately) were mixed in 4 ml of chloroform, and concentrated H₂SO₄ (6 ml) was carefully added to form a layer. A reddish brown coloration of the interface was formed to show the presence of terpenoids.

Test of Cardiac glycosides (Keller-Killani test)

10 ml of each extracts (separately) were treated with 4 ml of glacial acetic acid containing one drop of ferric chloride solution. This was under layer with 1 ml of concentrated Sulphuric acid. A brown ring of the interface indicates a deoxysugar characteristic of cardenolides. A violet ring may appear below the brown ring, while in the acetic acid layer, a greenish ring may form just gradually throughout thin layer.

Test for alkaloids

10 ml each extracts (separately) were treated with 8 ml of picric acid solution in a test tube. The formation of orange coloration indicated the presence of alkaloids in the extract.

Test for steroids

0.5 g of the various solvent extract fraction of each plant was mixed with 2 ml of acetic anhydride followed by 2 ml of sulphuric acid. The colour changed from violet to blue or green in some samples indicated the presence of steroids.

Test for Phenols

To 1ml of various solvent extracts of sample, 2ml of distilled water followed by a few drops of 10% aqueous ferric chloride solution were added. Formation of blue or green colour indicated the presence of phenols.

Test for Coumarins

0.5 g of the moistened various extracts was taken in a test tube. The mouth of the tube was covered with filter paper treated with 1 N NaOH solution. Test tube was placed for few minutes in boiling water and then the filter paper was removed and examined under the UV light for yellow fluorescence indicated the presence of coumarins.

Test for Quinones

One ml of each of the various extracts was treated separately with alcoholic potassium hydroxide solution. Quinines give coloration ranging from red to blue.

QUANTITATIVE PHYTOCHEMICAL ANALYSIS

Extraction of Plant Materials

The quantities of the phytochemicals present were determined using the methods of Obadoni, and Ochuko, 2001; Krishnaiah *et al.*, 2009 and Salem *et al.*, 2016 as shown below:

Alkaloid Determination

6g of the sample were weighed into a 500 ml beaker and 25% acetic acid in ethanol was added and covered to stand for 4hrs. This was filtered and the extract was concentrated using a water-bath to one quarter of the original volume. Concentrated ammonium hydroxide was added drop wise to the extract until the precipitation was complete. The whole solution was allowed to settle and the precipitate was collected by filtration, dried and weighed.

Saponin Determination

25g of fine powdered leaves were poured in 250ml of 25% ethanol. The suspension was heated over a hot water bath for 4hrs with continuous stirring at about 60 °C. The mixture was filtered and the residue re-extracted with another 200ml of 20% ethanol. The combined extracts were reduced to 40ml over water bath at about 90 °C. The concentrate was transferred into a 250ml separator funnel and 20ml of diethyl ether was added and shaken vigorously. The aqueous fraction was recovered while the ether layer was discarded. The purification process was repeated thrice. 60ml of n-butanol extract was washed twice with 10ml of 5% aqueous sodium chloride. The remaining solution was heated in a water bath. After evaporation, the sample was dried in the oven to a constant weight. The Saponin content was calculated in percentage.

Determination of flavonoids

10 g of fine powdered leaves were frequently extracted with 100ml of 80% aqueous methanol at room temperature. The mixture was then filtered through a filter paper into a pre-weighed 250ml beaker. The filtrate was transferred into a water bath and allowed to evaporate to dryness and weighed. The percentage flavonoid was calculated by difference.

RESULTS AND DISCUSSION

Qualitative Phytochemical Screening

Preliminary phytochemical analysis of aqueous leaves extracts of *Ocimum gratissimum* revealed the presence of various phytochemicals as shown in Table 1. Phlobatannins, Tannins, Saponins, Flavonoids Steroids, Terpenoids, Cardiac Glycosides, Alkaloids, Quinone, Phenol and Coumarin were all analyzed in the plant leaves. In accordance with the table, Tannins, Saponins, Flavonoids Steroids, Terpenoids, Cardiac Glycosides, Alkaloids, Quinone, and Coumarin were found present in the extract while Phlobatannins and Phenol were found absent. The presence of these constituents gives more authentication to the findings of Afolabi *et al.*, (2007) where alkaloids, flavonoids and tannins were detected in aqueous extract of *Ocimum gratissimum*. Moreso, several studies have proven that these metabolites have varied pharmacological actions in man and animals, the presence of these metabolites suggest great potentials of the plants as a source of useful phytomedicine. The phytochemicals are naturally occurring chemicals in plants which serve as medicine for the protection of human disease; the phytochemical are also

nonnutritive plants chemical that have protection or disease preventive properties (Cheng and Linn, 2002; Venuprasad *et al.*, 2014; Alexander, 2016; Justina and Solomon, 2017).

Table 1: Qualitative Phytochemical analysis of the leaves extracted

Chemical Constituent	Extract
Phlobatannins	-
Tannins	+
Saponins	+
Flavonoids (Alkaline Reagent Test)	+
Steroids	+
Terpenoids (Salkowski test)	+
Cardiac glycosides (Keller-Killani test)	+
Alkaloids	+
Quinone	+
Coumarin	+
Phenol	-

Key: + = Present, - = absent

Quantitative Phytochemical Screening

Quantification of Flavonoids, Alkaloids, and Saponins content of aqueous extract *Ocimum gratissimum* was measured in mg/100g as presented in Table 2. The results showed the mean content of saponins, alkaloids and flavonoids as 3.67 mg/100g, 2.03 mg/100g and 4.17 mg/100g respectively after three replications. Obviously the highest levels were the flavonoids content and saponins while moderate content of alkaloids was revealed.

Table 2: Quantitative Phytochemical analysis of the leaves extracted

Leaves Extract	Saponin (mg/100g)	Flavonoids (mg/100g)	Alkaloids (mg/100g)
Replication 1	3.68	4.15	2.05
Replication 2	3.68	4.16	2.02
Replication 3	3.66	4.20	2.02
Mean	3.67	4.17	2.03

Flavonoids are polyphenolic compounds that contribute to many other colours found in nature particularly the yellow and orange of petal, they have been reported to have antiviral and anti-allergic activities. Presence of flavonoids might be responsible for its use as anti-inflammatory effects on both acute and chronic inflammation (Gupta *et al.*, 2011). 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 (Gupta *et al.*, 2011). Thus, the overall results suggested that aqueous extract of *Ocimum gratissimum* has the potential for curing various human diseases due to the presence of these

phytochemicals. However, further work is necessary to ascertain the safety of plants extracts and to determine appropriate concentration for therapy so as to safeguard the health of the traditional users whom do not take these factors into consideration.

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 (Stanley, 2007; 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 (Alexander, 2016).

CONCLUSION

In conclusion, aqueous leaves extract from *Ocimum gratissimum* plant possessed a reasonable amount of phytochemical constituents that qualifies it as a medicinal plant. This gives support to its traditional use against infectious diseases. Also, the presence of most general phytochemicals especially flavonoids with highest content may possibly be responsible for its therapeutic effect. Thus, the present study further initiates optimism for the advancement of research on *Ocimum gratissimum* and many more novel chemotherapeutic agents with a good potential of being synthesized as therapeutic agents in the future.

ACKNOWLEDGMENTS

The author expresses gratitude to the authorities and analysts of National Research Institute for Chemical Technology Basawa, Zaria Kaduna State for providing facilities and constant reassurance for this research work.

REFERENCES

- Abdullahi, M (2012). Phytochemical Constituents and Antimicrobial and Grain Protectant Activities of Clove Basil (*Ocimum gratissimum* L.) Grown in Nigeria, *International Journal of Plant Research*, 2(1), pp. 51-58.
- Abdullahi, M., Muhammed, G. and Abdulkadir, N.U. (2003). Medicinal and Economic plants of Nupeland, 1st edition, *Jube Evans publisher, Bida, Niger state, Nigeria: 139*.
- Afolabi, C, Akinmoladun, E. O, Ibukun, E. A, Obuotor, E. M. and E.O. Farombi. (2007). Phytochemical constituent and antioxidant activity of extract from the leaves of *Ocimum gratissimum*. *Scientific Research and Essay*. 2(5), pp. 163-166.
- Alexander, P. (2016). Phytochemical Screening and Mineral Composition Of The
- Cheng, H. and Linn, T. (2003). Antihrebs simplex Virus of Terminalia Arjuana Linn. *Antiviral* 55 (3) 47-55.
- Gupta, V. K, Singh, J, Kumar, R and Bhanot, A. (2011). Pharmacognostic and Preliminary Phytochemical Study of *Ocimum gratissimum* Linn. (Family: Lamiaceae). *Asian Journal of Plant Sciences*, 10 (7): 365-369.

- Idris, S., Iyaka, Y. A., Ndamitso, M. M and Paiko, Y. B. (2011). Nutritional Composition of the Leaves and Stems of *Ocimum gratissimum*. *Journal of Emerging Trends in Engineering and Applied Sciences (JETEAS)*. 2 (5): 801-805.
- Krishnaiah, D, Devi, T, Bono, A, Sarbatly, R. (2009). Phytochemical analysis of some selected plants. *J Medicin Plant Res*. 3(2):067-072.
- Leaves of *Ocimum Gratissimum* (Scent Leaf). *Int J Appl Sci Biotechnol*, 4(2):161- 165.
- Obadoni, B. O., and Ochuko, P. O. (2001). Phytochemical Methods. *Global J Pure and Applied Sciences*. 8: 203-208.
- Omodamiro O. D. and Jimoh. M. A. (2017). Antioxidant and Antibacterial Activities of *Ocimum gratissimum*. *American Journal of Phytomedicine and Clinical Therapeutics*. 3 (1)
- Prabhu, K. S., Lobo, R., Shirwaikar, A. A. and Shirwaikar, A. (2009). *Ocimum gratissimum*: A Review of its Chemical, Pharmacological and Ethnomedicinal Properties. *The Open Complementary Medicine Journal*, 1(1-15).
- Roopashree, T. S, Dang, R, Rani, R. H. S, Narendra, C. (2008). Antibacterial activity of antipsoriatic herbs: *Cassia tora*, *Momordica charantia* and *Calendula officinalis*. *International J App Res Nat l Prod*; 1:20-28.
- Salem, M. E, Atega, A, Afifa, O, Fouzy, A. (2016). Qualitative and quantities analysis of phytochemicals of various extract for *Ephedra altissimo* from Libya. *Journal of Medicinal Plants Studies*; 4(3): 119-121
- Stanley, D. (2007). *Anogeissus Leiocarpus* (DC). Grill pub. pp119.
- Suman, R.K, Venkateshwar, C.G, Samuel, S, Gangadhar, R. (2013) Phytochemical Screening of some compounds from plant leaf extracts of *Holoptelea integrifolia* (Planch.) and *Celestruse marginata* (Grah.) used by Gondu tribes at Adilabad District, Andhrapradesh, India. *International Journal of Engineering Science Invention*. 2(8): 65-70.
- Venuprasad, M. P., Hemanth, K. KuSakina, R., Khanum, K. (2014). Phytochemical analysis of *Ocimum gratissimum* by LC-ESI-MS/MS and its antioxidant and anxiolytic effects, *South African Journal of Botany*, 92, 151-158