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Proximate, Phytochemical, and *In Vitro* Antimicrobial Properties of Dried Leaves from *Ocimum gratissimum*

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ABSTRACT: *Ocimum gratissimum* is a common plant in the tropics and has been used in food and medicine. Its usage in food and medicine could be attributed to its phtyochemical and antimicrobial properties. In this study we investigated the proximate, phytochemical, and antimicrobial attributes of air dried leaves of *O. gratissimum*. The aqueous extract was found to contain phtyochemicals with alkaloid and saponin present in appreciable amounts. The proximate analysis (crude protein and crude fibre content were 15.075% and 17.365%, respectively) showed that the leaf could be a good source of protein and fibre. The aqueous ethanolic extract of the leaf exhibited activity against a wider range of organisms when compared to the aqueous extract at the investigated concentrations. Aqueous ethanolic extracts of *O. gratissimum* leaf was active against *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Bacillus cereus* and the aqueous extract of the leaf was active against *P. aeruginosa*.

Keywords: *Ocimum gratissimum*, phytochemical, proximate analysis, antimicrobial activity

INTRODUCTION

The use of plants as medicine is an ancient practice common to all societies especially the African society (1). *Ocimum gratissimum* grows in the tropics and subtropics especially in tropical Africa and India (2). *O. gratissimum* has found usage in food and medicine. Its application in food includes the use as flavourings and nutraceuticals. In Nigeria, the leaf is used as a condiment in the preparation of dishes such as '*pepper soup*', '*jollof rice*', and vegetable soups. It was initially used in the preparation of these dishes to enhance their flavour. However, their usage in the preparation of these dishes is gaining increased acceptance due to the perceived nutraceutical benefit. The extract from the leaves of *O. gratissimum* possesses good antioxidant potential, which may be attributed to its phytochemical constituents (3). *O. gratissimum* is also used in traditional medicine for the treatment of several ailments such as urinary tract, wound, skin, and gastrointestinal infections, and this practice continues to exist in the developing nations (4). The steam distillation extract of the leaf has also been reported to have inhibitory effects on some selected bacteria that cause diarrhoea (5). The ethanolic extract of the leaf has been reported to inhibit the growth of *Proteus mirabilis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Candida albicans* (4). Traditionally, in Nigeria, fresh leaves are usually harvested, rinsed, and squeezed in cold water for 3 to 5 min. The squeezing in cold water is repeated three times, and the extracts are collected and served for drinking immediately. However, with increased urbanisation, access to freshly harvested leaves has decreased. Drying of the fresh leaves provides an option for preserving the leaves and making it available in the urban centers. Thus this study sought to investigate the qualitative and quantitative phytochemical, proximate and antimicrobial properties of dried leaves from *O. gratissimum*.

MATERIALS AND METHODS

Dry leaf preparation

The leaves were obtained from a garden in Ado Ekiti, Nigeria. Leaves were sorted and gently rinsed. The leaves were then spread on paper inside a room for 5 days to dry and then ground using a blender.

Phytochemical screening

Leave powder was soaked in water for 24 h at room temperature and then filtered. Chemicals tests were carried

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out on the extract using standard procedure to identify the constituents as described by Sofowora (6), Trease and Evans (7) and Harborne (8). Phytochemicals screened were: tannin, phlobatannin, saponin, flavonoid, steroid, terpenoid, glycoside, cardenolide, alkaloids, anthraquinone, chalcones, and phenols.

Proximate analysis

Proximate analysis was assayed as described in Association of Official Analytical Chemists (AOAC) (9). The leaf powder was analysed for crude protein, crude fat, crude fibre, ash, and moisture, and carbohydrate was calculated by difference.

Phytochemical quantification

Analyses were carried out in the aqueous extract. Alkaloids were measured as described in Soetan (10). Tannins were measured using the method of AOAC (11). Saponins were determined using the method of Brunner (12). Glycosides were determined as described by Sofowora (6). Phenols were measured using the method of Mako (13) and phlobatannins were assayed as described by Salau (14).

Microbial inhibition study

***Extract preparation*:** Fifty grams of the ground sample was soaked with 250 mL of sterile water for 24 h. The mixture was filtered with Whatman No 1 filter paper. The filtrate was concentrated to 1/10 of its original volume using a rotary evaporator. The influence of using aqueous ethanolic solvent as medium for extraction on the antimicrobial property of the leaf extract was also investigated. The same procedure as the aqueous extraction was used for the ethanolic extract, but 80% ethanol was used instead of water.

***Microbial assay*:** The antimicrobial activity of the ethanolic and aqueous extracts was evaluated by the agar well diffusion method (15). Inocula of test bacterial isolates were prepared by inoculating a loopful of test bacteria from a stock culture into freshly prepared nutrient both and incubated at 37°C for 24 h. Absorbance of the grown culture was read at 530 nm after adjustment with sterile distilled water to match that of 0.5 M McFarland standard solution which is equivalent to between $1.0\times10^6\sim 1.0\times10^7$ CFU/mL. One milliliter of the bacterial suspension was spread on Mueller-Hinton agar. The plates were allowed to stand for 1.5 h for the test bacterial isolates to be fully embedded and properly established in the seeded medium. With a sterile cork borer (No 4 Gallenkamp), wells of equal depth of 0.5 cm (Δ=5 mm diameter) were dug inside the agar. Each well was aseptically filled up with 0.5 mL of the respective extracts while avoiding splashes and overfilling. The sensitivity of the test organisms to the different extracts was indicated by clearing around each well. The halo's diameter as an index of the degree of sensitivity was measured with a transparent plastic ruler. Isolates tested were *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Bacillus cereus*.

Table 1. Phytochemical screening of *Ocimum gratissimum* leaf

Parameters	
Alkaloids	+++
Saponins	+++
Tannins	+
Phlobatannins	+
Glycosides	++
Phenols	++
Anthraquinones	+
Cardenolides	−
Steroids	−
Terpenes	−
Flavonoids	−
Chalcones	−

+++, appreciable amount; ++, moderate amount; +, a minute or trace amount; −, completely absent.

RESULTS

Alkaloids and saponins were present in appreciable amounts. Glycosides and phenols were present in moderate amounts while tannins, phlobatannins, and anthraquinones were present in minute quantities (Table 1). Cardenolides, steroids, terpenes, flavonoids, and chalcones were absent.

The proximate analysis indicated that the leaf powder had a high carbohydrate content 44.36% (Table 2). The crude protein and crude fibre content were 15.08% and

Table 2. Proximate and phytoquantitative analysis of *Ocimum gratissimum* leaf

Parameters	% Composition
Proximate analysis	
Crude protein	15.08±0.29
Crude fat	5.10±0.014
Crude fibre	17.37±0.021
Ash	9.79±0.035
Moisture	8.32±0.042
Carbohydrate	44.36±0.25
Phytoquantitative analysis	
Phytate	0.13±0.0014
Oxalate	0.11±0.0014
Tannin	0.012±0.0014
Saponin	0.23±0.0021
Alkaloid	0.29±0.0021
Phlobatannin	0.006±0.0014
Phenol	0.030±0.0021
Glycoside	0.11±0.0021

Table 3. Diameters of inhibition zones of aqueous and aqueous ethanolic extracts

Organisms	Inhibition zone (mm)				
	0.1 mg/mL	0.2 mg/mL	0.3 mg/mL	0.4 mg/mL	0.5 mg/mL
Aqueous extract					
Escherichia coli	0	0	0	0	0
Pseudomonas aeruginosa	9.43	9.93	10.9	12	13.1
Staphylococcus aureus	0.5	1.1	1.57	2.43	3.03
Bacillus cereus	0	0	0	0	0
Aqueous ethanolic extract					
Escherichia coli	7.03	8.01	9	10.03	10.1
Pseudomonas aeruginosa	11.03	11.83	11.97	12.9	14.97
Staphylococcus aureus	6.5	7.03	8.1	9.1	10.07
Bacillus cereus	9.47	10.07	10.97	11.97	12.97

17.37%, respectively. The crude fat, ash, and moisture contents had the lowest percentages of 5.10%, 9.79%, and 8.32%, respectively (Table 2).

As observed in the phytochemical screening, quantitative analysis also indicated that alkaloids (0.2875%) and saponins (0.225%) had the highest concentrations compared to the other phytochemicals that were quantified (Table 2). Phytates, oxalates, and glycosides were also present in moderate quantities with a concentration of 0.127%, 0.106%, and 0.106%, respectively. The phytochemical with the lowest concentrations was phlobatannin with a concentration of 0.006% (Table 2).

The aqueous extract exhibited antimicrobial activity against *P. aeroginosa*, and to a lesser extent against *S. aureus* (Table 3). The aqueous extract showed no microbial activity against *E. coli* and *B. cereus*. The aqueous ethanolic leaf powder extract showed antimicrobial activity against *E. coli*, *P. aeroginosa*, *S. aureus*, and *B. cereus* (Table 3). The aqueous and aqueous ethanolic extracts showed the highest inhibition against *P. aeruginosa* (13.10±0.17 mm and 14.97±0.06 mm, respectively) at the highest extract concentration of 0.5 mg/mL compared with the other organisms tested. The increase in diameter of the inhibition zones was found to have a linear relationship with the concentration of the extracts. The regression equations and coefficients of determination (r^2) are shown in Table 4.

Table 4. Coefficient of determination of the relationship between extract concentration and inhibition zone

Organisms	Extract	r^2	Regression equation
Escherichia coli	Aqueous ethanol	0.952	y=8.16x+6.39
Pseudomonas aeruginosa	Aqueous	0.983	y=9.41x+8.25
Pseudomonas aeruginosa	Aqueous ethanol	0.876	y=8.95x+9.86
Staphylococcus aureus	Aqueous	0.992	y=6.39x−0.19
Staphylococcus aureus	Aqueous ethanol	0.990	y=9.21x+5.40
Bacillus cereus	Aqueous ethanol	0.992	y=8.90x+8.42

r^2: coefficients of determination.
y, inhibition zone; x, extract concentration.

DISCUSSION

Akinmoladun et al. (3) and Nweze and Eze (4) carried out the phytochemical screening of *O. gratissimum*; however, quantitative analysis of the individual phytochemical components were not studied. Also, their study did not screen for phenols, cardenolides, and chalcones. Akinmoladun et al. (3) also reported the absence of alkaloids in the aqueous extracts of the leaf, while this study confirmed the presence of alkaloids in the aqueous extracts of the leaf. Akinmoladun et al. (3) confirmed the presence of steroids, terpenoids, and flavonoids in the aqueous and ethanolic leaf extracts; however, these phytochemicals were found to be absent in this current investigation. Flavonoids were also reported to be absent in the ethanolic extract of the leaf (4). Variation in the phytochemical content of the leaf extract could be due to planting location, seasonal variation, and extraction variables (temperature, time, concentration, and particle size).

This study showed that the *O. gratissimum* leaf has a crude fat content of 5.1%. Essential oils that have been extracted from the oil of the leaf include eugenol, thymol, citral, geraniol, and linalool (16). Adebolu and Oladimeji (5) suggested that only the oil from the leaves had antibacterial activity against some selected diarrhoea causing bacteria. Ethanol could improve the extraction of essential oils and this might have been responsible for the enhanced activity of the aqueous ethanolic extract against the tested bacteria compared to the aqueous extract. According to Lahlou (16), essential oils are poorly soluble in water, and the use of various solvents (such as acetone and ethanol) in the dilution of essential oils has been recommended.

Inhibition zones ≥10 mm can be considered active (18).

This suggests that the aqueous ethanolic leaf extract was active against *E. coli*, *P. aeruginosa*, *S. aureus*, and *B. cereus* while the aqueous extract was only active against *P. aeruginosa* at the concentrations studied. The antimicrobial activity of the leaf extract could be attributed to the phytochemical content of the leaf.

P. aeruginosa has become an important cause of Gram-negative infection, especially in patients with compromised host defense mechanisms. It is the most common pathogen isolated from patients who have been hospitalised longer than one week and a frequent cause of nosocomial infection (19). Also, three of the organisms in this investigation (*E. coli*, *S. aureus*, and *B. cereus*) have been implicated in food borne diseases (20,21). According to the FDA, there are 48 million cases of foodborne illness annually, and each year, these illnesses result in an estimated 128,000 hospitalizations and 3,000 deaths (22). This study suggests that the aqueous and aqueous ethanolic extracts of *O. gratissimum* could be potent therapeutic agents in treating some opportunistic infections and food borne illnesses caused by these bacteria.

While the leaf extract is useful in the inactivation of pathogenic microorganisms, its usage should be balanced with respect to its effect on beneficial microorganisms in the intestinal microflora. This brings to fore the importance of dosage in the use of the leaf extract of *O. gratissimum*.

Aqueous ethanolic extracts of the *O. gratissimum* leaf were active against *E. coli*, *P. aeruginosa*, *S. aureus,* and *B. cereus*, and the aqueous extract of the leaf was active against *P. aeruginosa* at the investigated concentrations. This brings to fore the role of solvent type in influencing the activity of *O. gratissimum* against microbes. Further investigation is required to understand how the phytochemical contents of *O. gratissimum* extracts could be affected by planting conditions and other processing variables such as variation in particle size, drying method, extraction temperature and extraction time.

AUTHOR DISCLOSURE STATEMENT

The authors declare no conflict of interest.

REFERENCES

1. Usman H, Osuji JC. 2007. Phytochemical and *in vitro* antimicrobial assay of the leaf extract of *Newbouldia laevis*. *Afr J Tradit Complement Altern Med* 4: 476-480.
2. Aruna K, Sivaramakrishnan VM. 1990. Plant products as protective agents against cancer. *Indian J Exp Biol* 28: 1008-1011.
3. Akinmoladun AC, Ibukun EO, Afor E, Obuotor EM, Farombi EO. 2007. Phytochemical constituent and antioxidant activity of extract from the leaves of *Ocimum gratissimum*. *Sci Res Essays* 2: 163-166.
4. Nweze EI, Eze EE. 2009. Justification for the use of *Ocimum gratissimum* L in herbal medicine and its interaction with disc antibiotics. *BMC Complementary Altern Med* 9: 37.
5. Adebolu TT, Oladimeji SA. 2005. Antimicrobial activity of leaf extracts of *Ocimum gratissimum* on selected diarrhoea causing bacteria in southwestern Nigeria. *Afr J Biotechnol* 4: 682-684.
6. Sofowora A. 1993. *Medicinal plants and traditional medicines in Africa*. John Wiley and Sons Ltd., New York, NY, USA. p 256.
7. Trease GE, Evans WC. 1989. *Trease and Evans' pharmacognosy*. 13th ed. Bailliere Tindall, London, UK. p 53.
8. Harborne JB. 1973. *Phytochemical methods: a guide to modern techniques of plant analysis*. Chapman and Hall, London, UK. p 279.
9. AOAC. 2005. Official methods 988.05, 2003.06, 958.06, 942.05, and 967.08. In *Official Methods of Analysis of the Association of Analytical Chemists International*. 18th ed. Gathersburg, MD, USA.
10. Soetan KO. 2012. Comparative evaluation of phytochemicals in the raw and aqueous crude extracts from seeds of three *Lablab purpureus* varieties. *Afr J Plant Sci* 6: 410-415.
11. Edeogu CO, Ezeonu FC, Okaka ANC, Ekuma CE, EIom SO. 2007. Antinutrients evaluation of staple food in Ebonyi State, South-Eastern, Nigeria. *J Appl Sci* 7: 2293-2229.
12. Brunner JH. 1984. Direct spectrophotometer determination of saponin. *Anal Chem* 34: 1314-1326.
13. Mako AA. 2013. Performance of West African Dwarf goats fed graded levels of sun-cured water hyacinth (*Eichhornia crassipes* Mart. Solms-Laubach) replacing Guinea grass. *Livestock Res Rural Dev* 25: 127.
14. Salau AK, Yakubu MT, Oladiji AT. 2013. Cytotoxic activity of aqueous extracts of *Anogeissus leiocarpus* and *Terminalia avicennioides* root barks against Ehrlich ascites carcinoma cells. *Indian J Pharmacol* 45: 381-385.
15. Nair R, Chanda SV. 2004. Antibacterial activity of some medicinal plants of Saurashtra region. *J Tissue Res* 4:117-120.
16. Sulistiarini D. 1999. *Ocimum gratissimum* L. In *Plant Resources of South East Asia. No.19: Essential-Oils Plants*. Oyen LPA, Dung NX, eds. ProSea, Bogor, Indonesia. p 140-142.
17. Lahlou M. 2004. Methods to study the phytochemistry and bioactivity of essential oils. *Phytother Res* 18: 435-448.
18. Usman H, Haruna AK, Akpulu IN, Ilyas M, Ahmadu AA, Musa YM. 2005. Phytochemical and antimicrobial screenings of the leaf extracts of *Celtis integrifolia* Lam. *J Trop Biosci* 5: 72-76.
19. Friedrich M, Cunha BA, Lessnau KD, Lazo KG. *Pseudomonas aeruginosa* Infections. http://emedicine.medscape.com/article/226748-overview (accessed Apr 2017).
20. Le Loir Y, Baron F, Gautier M. 2003. *Staphylococcus aureus* and food poisoning. *Genet Mol Res* 2: 63-76.
21. Biswas B, Rogers K, McLaughlin F, Daniels D, Yadav A. 2013. Antimicrobial activities of leaf extracts of guava (*Psidium guajava* L.) on two Gram-negative and Gram-positive bacteria. *Int J Microbiol* 2013: 746165.
22. FDA. Foodborne Illnesses: What You Need to Know. http://www.fda.gov/Food/FoodborneIllnessContaminants/FoodborneIllnessesNeedToKnow/default.htm (accessed Jun 2016).