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 phytochemical 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 phytochemicals 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 sub tropics 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
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 1.0×10^6 to 1.0×10^7 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 (A=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.

**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 1. Phytochemical screening of Ocimum gratissimum leaf

| Parameters       | % Composition |
|------------------|---------------|
| Alkaloids        | +++           |
| Saponins         | +++           |
| Tannins          | +             |
| Phlobatannins    | +             |
| Glycosides       | ++            |
| Phenols          | ++            |
| Anthraquinones   | +             |
| Cardenolides     | –             |
| Steroids         | –             |
| Terpenes         | –             |
| Flavonoids       | –             |
| Chalcones        | –             |

+++ appreciable amount; ++, moderate amount; +, a minute or trace amount; −, completely absent.
Properties of Dried Leaves from Ocimum gratissimum

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      |                      |           |           |           |           |           |
| Pseudomonas aeruginosa|                      |           |           |           |           |           |
| Staphylococcus aureus |                      |           |           |           |           |           |
| Bacillus cereus       |                      |           |           |           |           |           |
| Aqueous ethanolic extract |                  |           |           |           |           |           |
| Escherichia coli      |                      |           |           |           |           |           |
| Pseudomonas aeruginosa|                      |           |           |           |           |           |
| Staphylococcus aureus |                      |           |           |           |           |           |
| Bacillus cereus       |                      |           |           |           |           |           |

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 ethanol       | 0.983  | $y=9.41x + 8.25$    |
| Pseudomonas aeruginosa| Aqueous ethanol       | 0.876  | $y=8.95x + 9.86$    |
| Staphylococcus aureus | Aqueous ethanol       | 0.992  | $y=6.39x - 0.19$    |
| Staphylococcus aureus | Aqueous ethanol       | 0.992  | $y=9.21x + 5.40$    |
| Bacillus cereus       | Aqueous ethanol       | 0.992  | $y=8.90x + 8.42$    |

$r^2$: coefficients of determination.

Inhibition zones $\geq 10$ 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 therapeutically 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.

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