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In vitro Medicinal Studies on *Ocimum gratissimum* Leaves

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Abstract: ***Background:*** *Ocimum gratissimum known as 'scent leaf' is commonly used as spice in food and it is used also in traditional medicine practice for treatment of epilepsy, diarrhoea, mental illness and fever etc. The study was carried out to screen for phytochemicals, determine mineral constituents as well as show in vitro antioxidant potentials of Ocimum gratissimum leaves.* ***Methods:*** *Phytochemical screening, and mineral determination were carried out using standard procedures. DPPH free radical scavenging activity and reducing power activity were evaluated for their antioxidant activities.* ***Results:*** *Phytochemical screening revealed the presence of tannins, flavonoids, alkaloids, saponins, steroids and the absence of anthraquinones and glycosides while mineral analysis revealed the presence of sodium, calcium, potassium, manganese, copper, zinc, iron etc. in various concentrations. The result of reducing power activity and DPPH radical scavenging showed that, the higher the concentration, the better the scavenging activity.* ***Conclusion:*** *Our findings give credence to the use of Ocimum gratissimum in traditional medicine in the management of microbial infection and free radical induced ailments.*

Keywords: DPPH, *in vitro*, Minerals, Phytochemicals, *Ocimum gratissimum*

1. INTRODUCTION

Ocimum gratissimum commonly known as 'scent leaf' is used In trado-medical practice, throughout West Africa as anti-malarial, antibacterial, antifugal, antiseptic, mosquito repellent and anti-convulsant [1]. *Ocimum gratissimum* is also used to treat different diseases including upper respiratory tract infections, diarrhea, headache, ophthalmic, skin diseases, pneumonia, and also as a treatment for cough, fever and conjunctivitis [2-3]. The aqueous leaf extract and seed oil showed anti-proliferative and chemo-preventive activity on HeLa cells [4-5] reported that, aqueous extract of *O. gratissimum* leaves inhibits tumor growth and angiogenesis by affecting tumor cell proliferation, migration, morphogenesis, stromal apoptosis and induction of inducible cyclooxygenase (COX-2). In this study, we intend to analyze the phytochemicals, investigate the *in vitro* antioxidant activities and analyze the mineral components of *Ocimum gratissimum*

2. MATERIALS AND METHODS

2.1 Collection, Identification and Preparation of Plant materials

Fresh *Ocimum gratissimum* leaves were collected in January 2016 from a local farm in South Eastern part of Nigeria. Identification and authentication were carried out after which the leaves were sorted, washed and air dried at room temperature in Benson Idahosa University, Nigeria for fourteen (14) days. They were grounded into fine powder using an electric blender and stored in a cool dry container until use for analysis.

Phytochemical analysis

Qualitative phytochemical screening using standard methods as described [6-10] were carried out.

Mineral analysis

Mineral analysis was carried out using Atomic Absorption Spectrophotometer (AAS) as previously done [11-12].

Determination of Reducing Power Ability

The reducing power activity of *Ocimum gratissimum* leaves was carried out using the reducing power method as described by Aiyegoro and Okoh [13]. A mixture containing 2.5 ml of 0.2 M phosphate buffer (pH 6.6) and 2.5 ml of $K_3Fe(CN)_6$ (1% w/v) was added to 1.0 ml of stock *Ocimum gratissimum* leaves filtrate (0.2–1.0 mg/ml) prepared in distilled water. The resulting mixture was incubated for 20 min at 50°C, followed by the addition of 2.5 ml of TCA (10% w/v), followed by centrifugation at 3000 rpm for 10 min. 2.5 ml of the supernatant was mixed with 2.5 ml of distilled water and 0.5 ml of $FeCl_3$ (0.1% w/v). The absorbance was measured at 700 nm against reagent blank sample. Increased absorbance of the reaction mixture indicates higher reducing power of *Ocimum gratissimum* leaves.

2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging ability

The DPPH method according to Liyana-Pathiana and Shahidi [14] was used for the determination of DPPH free radical scavenging activity of the *Ocimum gratissimum* leaves as follows: DPPH (1 ml, 0.135 mM) prepared in methanol was mixed with 1.0 ml of stock *Ocimum gratissimum* leaves filtrate ranging in concentration from 0.2 to 1.0 mg/ml. The reaction mixture was then vortexed thoroughly and left in the dark at room temperature for 30 min. The absorbance was measured at 517 nm. The scavenging ability was calculated using the equation: DPPH scavenging activity (%) = $[(Abs_{control} - Abs_{sample})]/(Abs_{control})] \times 100$,

Where: $Abs_{control}$ is the absorbance of DPPH + methanol and Abs_{sample} is the absorbance of DPPH radical + sample (sample or standard).

Statistical analysis

Data obtained from this study were expressed as mean value ± standard deviation.

3. RESULTS AND DISCUSSION

The phytochemical screening of *ocimum gratissimum* leaves showed the presence of various secondary metabolites including alkaloids, tannins, flavonoids, saponins etc as shown in Table 1.

Table 1. Phytochemical screening of *ocimum gratissimum* leaves

Phytochemicals	*Ocimum gratissimum*
Flavonoid	Present
Saponin	Present
Alkaloid	Present
Tannin	Present
Glycoside	Absent
Anthraquinone	Absent
Steroid	Present

The presence of tannins, saponins, flavonoids, alkaloids and steroids in *Ocimum gratissimum* leaves may contribute to antioxidant, anti inflammataory, analgesic, hypolipidaemic and antibacterial activity in *in vivo* studies. Flavonoids are polyphenolic compounds known to inhibit formation of plaques and streaks in arteries and so hinder hypertension, and other cardiovascular diseases [15-16]. They also are strong scavengers of reactive oxygen radicals known to be involved in many conditions that cause diabetes, inflammatory diseases, cancers and neurodegenerative diseases [17]. Tannins exerts many physiological effects, such as acceleration of blood clotting, reduction of blood pressure, decreasing the serum lipid level and modulating immunoresponses [18]. Saponins helps to lower cancer risks by lowering blood cholesterol levels. Alkaloids have a wide range of pharmacological activities including antimalarial (e.g. quinine), antiasthma (e.g. ephedrine), anticancer (e.g. homoharringtonine) [19], cholinomimetic (e.g. galantamine) [20], vasodilatory (e.g. vincamine), antiarrhythmic (e.g. quinidine), analgesic (e.g. morphine) [21], antibacterial (e.g. chelerythrine) [22], and antihyperglycemic activities (e.g. piperine) [23]. These phytochemicals may contribute analgesic, anti-pyretic, protective, antibacterial, anti-ulceration and ameliorating properties of *ocimum gratissimum* leaves.

The result of mineral composition of *Ocimum gratissimum* leaves revealed calcium to be highest in concentration and Chromium least in concentration as shown in Table 2. Calcium plays role in coagulation of blood, proper heart and nervous system functioning and normal contraction of muscles. Calcium also aid formation of strong bones and teeth. Sodium and Potassium regulate the acid-base balance. Sodium remains one of the major electrolytes in the blood. Without sodium the body cannot be hydrated, it would dry up [24]. Iron is important for the building up of red blood cells essential for formation of haemoglobin, the oxygen carrying pigment in red blood cells. Iron is used against anaemia, tuberculosis and disorder of growth [25]. Zinc is very important for nerve function and male fertility. It is important for normal sexual development especially for the development of testes and ovaries. Zinc stimulates the activity of vitamins, formation of red and white corpuscles, healthy functioning of the heart and normal growth [26]. According to Burkhard et al [27], manganese is necessary for the functioning of the pituitary gland, the pineal gland the brain, it promotes hepatorenal function, combats anaemia and also essential for growth. Calcium content of *Ocimum gratissimum* (436mg/100g) is high compared to 295mg/100g of *Celosia argentea* [28] but low compared to 1118mg/100g of *Annona muricata* and 1264mg/100g of *Vernonia amygdalina* [11-12]. Zinc content of *Ocimum gratissimum* (3.30mg/100g) compared favorably to 5.42mg/100g of *Celosia argentea* [28] but high compared to 0.83mg/100g of *Annona muricata* and 1.42mg/100g of *Vernonia amygdalina* [11-12]. Sodium content of *Ocimum gratissimum* (48.95mg/100mg) compared favorably with 48.31mg/100g of *Vernonia amygdalina* [12], but low compared to 69.49mg/100g of *Annona muricata* [11] and 71.32mg/100g of *Celosia argentea* [28]. Potassium content of *Ocimum gratissimum* (109.60mg/100g) is low compared to 128.33mg/100g of *Celosia argentea* [28] but high when compared to 36.31mg/100g of *Annona muricata* and 62.79mg/100g of *Vernonia amygdalina* [11-12]. Copper content of *Ocimum gratissimum* (1.35mg/100mg) compared favorably with 1.95mg/100g of *Vernonia amygdalina*, 1.42mg/100g of *Annona muricata* [11-12] and 2.18mg/100g of *Celosia argentea* [28]. Iron content of *Ocimum gratissimum* (9.48mg/100g) compared favorably with 13.90mg/100g of *Annona muricata* but low compared to 32.20mg/100g of *Vernonia amygdalina* [11-12] and 35.16mg/100g of *Celosia argentea* [28].

Table 2. Minerals present in *Ocimum gratissimum* leaves (mg/100g)

Minerals	*Ocimum gratissimum* (mg/100g)
Calcium	436±4.22
Magnesium	186.71±3.12
Potassium	109.60±3.09
Sodium	48.95±1.65
Phosphate	127.40±2.01
Iron	9.48±0.56
Zinc	3.30±0.26
Copper	1.35±0.20
Manganese	1.21±0.12
Chromium	0.89±0.10

Values are means ± SD for 2 determinations

The result of reducing power activity of *Ocimum gratissimum* leaves showed a concentration dependent increase in reducing power ability as concentration increases from 0.2 - 1.0mg/ml as revealed in Figure 1. The DPPH radical scavenging activity of *Ocimum gratissimum* is shown in Figure 2. The result revealed that the higher the concentration, the higher the percentage (%) inhibition of DPPH radical. DPPH assay is commonly used for the assessment of free radical-scavenging abilities of herbal medicines and health foods due to their simplicity, stability, accuracy and reproducibility [29]. DPPH is a stable radical which can accept hydrogen from an antioxidant to form reduced DPPH [30]. From our results using reducing power and DPPH assays, *Ocimum gratissimum* leaves have strong abilities to react with free radicals and could convert them into more stable nonreactive forms that eventually lead radical chain reactions to termination.

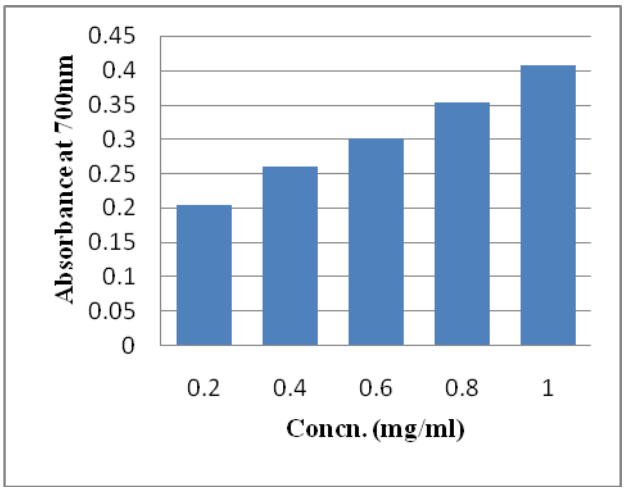

Figure 1. Reducing power ability of *Ocimum gratissimum* leaves

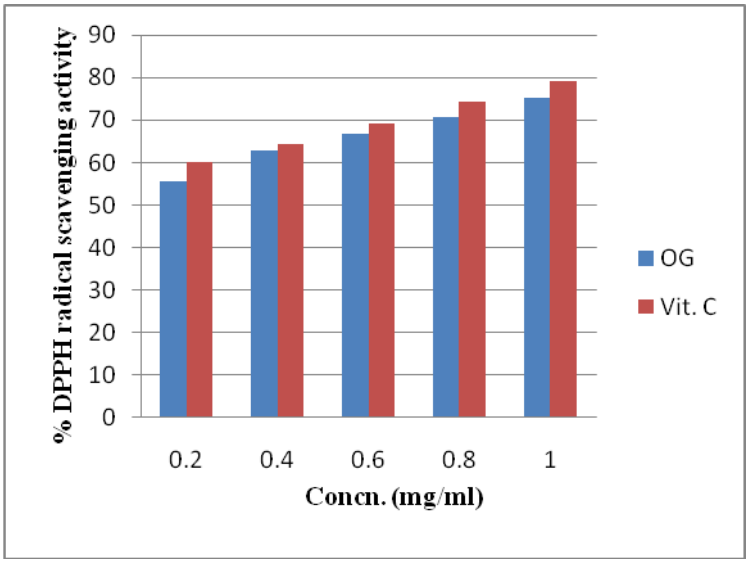

Figure 2. DPPH radical scavenging activity of *Ocimum gratissimum* (OG) leaves

4. Conclusion

In conclusion, this study supports the rationale for the use of the *Ocimum gratissimum* leaves in treating infections in traditional medicine. Research should continue to isolate and purify active components of this plant and its use in experimental animals.

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