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Ocimum basilicum var. *purpureum* Floral Essential Oil: Phytochemicals, Phenolic Content, Antioxidant, Free Radical Scavenging and Antimicrobial Potentials

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Abstract- This study examined the phytochemicals and medicinal properties of the floral essential oil of *O. basilicum* var. *purpureum* from Nigeria. The GC and GC-MS analyses revealed the presence of twenty-five organic compounds making up 99.7% of the total percentage composition of the essential oil. The most abundant components was phenolic compound called methyleugenol (15.5%), followed by 2-phenyl-1-hexanol (14.0%), 1-(4,5-dimethyl-2-nitrophenyl)-1H-tetraazole (14.0%), 2-methyl-3,5-dodecadiyne (14.0%), *o*-nitrocumene (14.0%) and patchoulane (6.7%). The total phenolic content was quantitatively determined as 459 μgmg^{-1} gallic acid equivalent (GAE) confirming the presence of high amount of phenolic compounds in the floral essential oil. The DPPH IC_{50} value was 1.0 μgml^{-1}, the essential oil was capable of scavenging free radicals in a range of 73-86% and the antioxidant power of the essential oil increased with concentration.

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Keywords: Ocimum basilicum var. purpureum, floral essential oil, phytochemical, phenolic content, pharmacological potentials.

I. Introduction

Phytochemicals are huge varieties of organic substances which accumulated in plants. Plant essential oils are recognized as one of the most promising secondary metabolites for the development of cheap and safer drugs (Varma and Dubey, 2001). Essential oils are volatile, natural and complex compounds characterized by a strong odour and are produced from odoriferous medicinal plants. In addition to essential oils, odoriferous plants are also characterized by the presence of phenolic compounds that have been shown to possess multiple pharmacological activities. Essential oils, their fractions and isolated aroma chemicals are valuable ingredients of flavour foods, toiletries, fine chemicals and pharmaceutical industries, they are utilized as such or in diluted forms in therapy or by the aromatherapy sector (Daferera *et al.,* 2000; Mimica-Dukic and Bozin, 2008). According to world health organization (WHO), greater than 80% of the total world's population depends on natural products in order to satisfy their primary health care needs. Investigations of these secondary metabolites intensified when some commercial synthetic antioxidants were found to exhibit toxic, mutagenic and carcinogenic effects and other problems associated with their usage (Rajendran *et al.,* 2014). Knowledge of the chemical composition of medicinal plants is desirable because such information will be of value for the synthesis of complex chemical substances (Yadav and Agarwala, 2011).

The genus *Ocimum* comprises more than 150 species and is considered as one of the largest genera of the *Lamiaceae* family. *Ocimum basilicum* var. *purpureum* is an annual plant which grows well in Nigeria. The purple colour of the plant is due to the presence of anthocyanins mainly cyanidin-3-(di-*p*coumarylglucoside)-5-glucoside and small amount of peonidin compounds, therefore, this plant is considered a potential source of red pigments for the food industry (Janick *et al.,* 1999). The plant is widely used in food and oral care products. The plant is a good source of magnesium, which promotes cardiovascular health, also helps muscles and blood vessels to relax, thus improving blood flow and lessening the risk of irregular heart rhythms or a spasming of the heart muscle or a blood vessel. It is also an excellent source of vitamin K and manganese; a very good source of copper, vitamin A and vitamin C; a good source of calcium, iron, folate, and omega-3 fatty acids (Patil *et al*., 2011). The plant is also used as condiment, calmative and flavouring agents. Traditionally, it is commonly used in treatments

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of diuretic, constipation, intestine ache, galactogogue, headaches, coughs, diarrhoea, warts, worms, kidney, anti-inflammatory and antispasmodic agent (Khelifa *et al.,* 2012; Uyoh *et al.,* 2013).

To the best of our knowledge, there is paucity of information on the phytochemical, total phenolic content, free radical scavenging, antioxidant and antimicrobial potentials of this plant so far. Therefore, the present research was undertaken for the first time with the aim of looking into the composition and pharmacological properties in the floral essential oil of *O. basilicum* var. *purpureum* from Nigeria.

II. Material and Methods

a) Plant Materials and Isolation of the Essential Oil

The floral parts of the plant were collected from their natural habitat in Ota, Nigeria and were authenticated as *O. basilicum* var. *purpureum*. The floral parts of the plant were extracted by hydrodistillation using clevenger-type apparatus to give a neat essential oil which was preserved in a vial at low temperature to prevent evaporation (European pharmacopoeia, 2004).

b) GC and GC-MS Analyses

Analyses of the floral essential oil of *O. basilicum* var. *purpureum* were performed using multi-dimensional gas chromatograph coupled with Gas Chromatography-Mass Spectrophotometer (Shimadzu, Japan) equipped with non-polar and polar double capillary columns (25.0 m x 0.25 µm i.d., 0.25 µm df). High purity helium was used as the carrier gas at a constant flow rate of 0.99 ml/min. A total of 1 µl sample was injected (split ratio 100:1) into GC and GCMS using AOC20i auto injector for analyses. The initial temperature was set at 60°C, heated at a rate of 3°C/minutes to 280°C and held isothermally for 6 minutes. Ion source temperature for these analyses was set at 200°C, while the interface temperature was set at 250°C, solvent cut time was 3.0 minutes and the mass spectrometer was set to operate in electron ionization mode with an ionizing energy of 70 eV as acquisition mass range from 40-700 a.m.u. at 0.50 scan/s. The constituents were identified by comparison of their retention indices with those of the literature. The retention indices were determined in relation to a homologous series of n-alkanes under the same operating conditions. Further identification was made by comparison of their mass spectra with those stored in National Institute for Standards and Technology (NIST) and with mass spectra from literature.

c) Determination of Total Phenolic Content

Total phenolic content of the floral essential oil of *O. basilicum* var. *purpureum* was determined using the Folin-Ciocalteau method. 1 ml aliquot solution of the essential oil was mixed with 46 ml distilled water and 1 ml of Folin Ciocalteau reagent, then 3 ml of (2% w/v) Na_2CO_3 solution was added after 3 minutes and the mixture was allowed to stand for 2 hours for incubation in dark with intermittent shaking, the absorbance of the reaction mixture was measured on a UV-Visible spectrophotometer at 760 nm against a blank (containing all reagents except the test sample). The total phenolic content was expressed as gallic acid equivalents (Govindappa *et al.,* 2011).

d) In vitro 2,2´-Diphenyl-1-picrylhydrazyl (DPPH) Free Radical Scavenging and Antioxidant Activities

The free radical scavenging and antioxidant activities of the floral essential oil against the stable free radical DPPH were measured. Briefly, three different concentrations (1000, 100 and 10 µgml^{-1}) of the essential oil were incubated with a methanolic solution of DPPH for 30 minutes of incubation at room temperature in the dark, then absorbance at 517 nm was measured spectrophotometrically. Ascorbic acid was used as reference compound. The assay was carried out in triplicate. The percentage inhibition (I%) for each concentration was calculated by using the absorbance values according to the following formula:

$$I\% = [(A_{blank} - A_{eo})/A_{blank}] \times 100$$

Where: A_{blank} is the absorbance of blank solution and A_{eo} is the absorbance of the essential oil. The dose-response curve was plotted and IC_{50} value for the essential oil and the standard were calculated (Adeniran *et al.,* 2013).

e) In vitro Antimicrobial Activities

The antibacterial potentials of the floral essential oil were evaluated by agar-well diffusion method against representative multi-drug resistance Gram-positive organisms (*Streptococcus agalactiae*, *Staphylococcus aureus* and *Streptococcus* species) and Gram-negative bacteria (*Escherichia coli*, *Klebsiella pneumoniae*, *Proteus mirabilis*, *Pseudomonas aeruginosa* and *Salmonella typhimurium*). The bacteria isolates were first sub-cultured in Nutrient agar and incubated at 37°C for 24 hours. All the bacteria cultures were adjusted to 0.5 McFarland standards, 20 ml of sterilized Nutrient agar medium was poured into each Petri dish aseptically and plates were then swabbed with inocula of the test organisms, and kept for 15 minutes for absorption. Using sterile cork borer of 6 mm diameter wells were bored into the seeded agar plates, and these were loaded with 10 µl of different concentrations (1000, 100 and 10 µgml^{-1}) of the essential oil in dimethylsulfoxide (DMSO). The plates were allowed to stand in the refrigerator for 1 hour to allow proper diffusion of the essential oil into the medium and incubated at 37°C for 24 hours before visual assessment of the inhibition zones. The Antibacterial potential of the essential oil were evaluated by measuring the clear zones of growth inhibition against the test organisms. Gentamicin (GEN)

and Cloxicillin (CXC) were used as control (Agu *etal.,* 2013).

III. Results and Discussion

a) Chemical Constituents of the Essential Oil

In this study, the floral essential oil of *O. basilicum* var. *purpureum* was investigated for its chemical constituents. The essential oil imparted pleasant aromatic odour. The GC and GC-MS analyses of the floral essential oil of *O. basilicum* var. *purpureum* showed the presence of 25 compounds making up 99.7% of the total percentage composition (Table 1). Compounds were listed in order of their retention indexes. The most abundant component was phenolic compound called methyleugenol (15.5%), the other major compounds present in the essential oil were 2-phenyl-1-hexanol (14.0%), 1-(4,5-dimethyl-2-nitrophenyl)-1H-tetraazole (14.0%), 2-methyl-3,5-dodecadiyne (14.0%), o-nitrocumene (14.0%) and patchoulane (6.7%). The principal classes of organic compounds in the floral essential oil were phenolic compounds (29.5%), sesquiterpenes (16.9%) and monoterpenes (1.4%). Comparatively, the chemical constituents of the investigated floral essential oil were different from those reported in other studies. The main constituents in the leaf essential oil of *O. gratissimum* were eugenol (68.8%), methyl eugenol (13.21%) and *cis*-ocimene (7.47%) (Matasyoh *et al.,* 2007) while linalool (65.38%, 74.22%, 38.60%), eugenol (5.26%, 3.47%, 10.20%) and *tau*-cadinol (8.18%, 3.47%, 10.20%) were the main components in *O. basilicum* var. *genovese*, *O. gratissimum* and *O. tenuiflorum* from Romania (Stefan *et al.,* 2013). Joshi (2013) also reported that the main composition of *O. gratissimum* and *O. sanctum* were eugenol (75.1%) and methyl eugenol (92.4%) respectively.

Table 1 : Chemical Composition of the Floral Essential Oil of *O. basilicum* var. *purpureum*

Compounds	% Composition	RI
2,3,4-trimethyl-1,4-pentadiene	0.4	687
2,3,3-trimethyl-1,4-pentadiene	0.7	689
1,3-dimethyl-1-cyclohexene	0.4	852
1,9-decadiyne	1.0	1011
3-[(1Z)-1-butenyl]-4-vinyl-1-cyclopentene	1.0	1100
1-(1-Ethylvinyl)-1-(2-methylene-3-butenyl)cyclopropane	1.0	1115
iso-borneol	0.4	1138
8-methylenedispiro[2.1.2.4]undecane	1.0	1215
copaene	0.3	1221
1-(4,5-dimethyl-2-nitrophenyl)-1H-tetraazole	14.0	1250
megastigma-7(*E*),9,13-triene	0.3	1278
2-methyl-3,5-dodecadiyne	14.0	1284
nopol	0.3	1290
trans-7-hydroxymethyl-3-cyclopropylbicyclo[4.1.0]heptane	1.8	1307
o-nitrocumene	14.0	1324
1-(2-nitro-2-propenyl)-1-cyclohexene	1.0	1339
α-cubebene	0.5	1344
methyleugenol	15.5	1361
aromadendrene	2.0	1386
2,4-diisopropenyl-1-methyl-1-vinylcyclohexane	1.8	1398
β-elemene	3.6	1403

(5E,9E)-12-methyl-1,5,9,11-tridecatetraene	2.0	1404
2-phenyl-1-hexanol	14.0	1469
β-cis-caryophyllene	2.0	1494
patchoulane	6.7	1968
Percentage Total	99.7	

RI = Retention Index

b) Total Phenolic Content (TPC)

Total phenolic content analysis revealed the presence of high quantity phenolic compounds in the floral essential oil. This was found to be 459 μgmg^{-1} gallic acid equivalents. The essential oil gave a higher TPC when compared with the previous studies on the related species such as methanolic seed extracts of *O. gratissimum* (168 mgg^{-1}), *O. americanum* (123 mgg^{-1}), *O. minimum* (110 mgg^{-1}), *O. citriodorum* (96 mgg^{-1}), *O. kilimandscharicum* (82 mgg^{-1}), *O. grandiflorum* (61 mgg^{-1}), *O. lamiifolium* (54 mgg^{-1}), *and O. selloi* (42 mgg^{-1}) (Hakkim *et al*., 2008). The floral essential oil of *O. basilicum* var. *purpureum* exhibited the high TPC due to the presence of low molecular mass phenolic compounds like methyleugenol and 2-phenyl-1-hexanol. Phenolic compounds in the floral essential oil were oxidized by Folin-Ciocalteu reagent which reduced to a mixture of blue oxides of tungsten, W_8O_{23}, and molybdenum, Mo_8O_{23} after oxidation of the phenolic compounds (Walch *et al.,* 2011). Phytophenolic compounds are very important because their hydroxyl groups which are highly effective scavengers of most oxidizing molecules, including reactive oxygen species, and various free radicals implicated in several diseases. Plant phenolic compounds have been widely consumed for many years as dietary components with no side effect, they play important beneficial roles in mammalian systems, they are especially important in prevention of cancers, cardiovascular diseases, and other degenerative diseases. Methyleugenol and 2-phenyl-1-hexanol are natural phenolic compounds that recently received attention for their extensive pharmacological properties, including anti-tumor, antibacterial, cardio-protective and gastroprotective effects (Georgiev *et al*., 2014). Phenolic compounds play a key role in scavenging free radicals that cause oxidative stress because they have substantial antioxidant capacity against peroxyl radicals. In addition, they have been shown to possess potential antioxidant abilities, which helps them to scavenge electrophiles and active oxygen species, slow down nitrosation and chelate metal ions to limit auto-oxidation and increase the ability to adjust some enzyme actions (Mediani *et al.,* 2013).

c) In vitro Free Radical Scavenging and Antioxidant Potentials

The free radicals scavenging and antioxidant potentials of the floral essential oil of *O. basilicum* var. *purpureum* were evaluated by DPPH assay. The essential oil was able to inhibit the formation of DPPH radicals in a concentration dependent manner. The percentage inhibitions of the essential oil at various concentrations (1000, 100 and 10 μgml^{-1}) were 86±0.001, 78±0.001 and 73±0.000% respectively; while the IC_{50} value was found to be 1.0 μgml^{-1} in comparison to ascorbic acid which gave 96±0.000, 69±0.002 and 54±0.002% as the percentage inhibitions with IC_{50} value of 9.0 μgml^{-1}. The DPPH radical scavenging capacity of the floral essential oil of *O. basilicum* var. *purpureum* was higher than that of ascorbic acid. The free radical scavenging and antioxidant properties of the essential oil were found to be nine times more active than the synthetic antioxidant (ascorbic acid) as shown in Table 2 below. Moreover, the floral essential oil of *O. basilicum* var. *purpureum* inhibited the DPPH free radicals than extracts of other related species such as *O. americanum* which has lower percentage inhibitions ranging from 32.9-67.4% (IC_{50}: 290 μgml^{-1}) in ethanolic extract, 20.9-63.2% (IC_{50}: 350 μgml^{-1}) in chloroform extract, 37.2-59.8% (IC_{50}: 430 μgml^{-1}) in petroleum ether extract and 26.5-56.2% (IC_{50}: 510 μgml^{-1}) in aqueous extract at different concentrations between 100-500 μgml^{-1} (Sarma and Babu, 2011). The antioxidant activity has been related to the number and position of free hydroxyl groups in terpenoids and phenolic compounds, which could be as a result of their hydrogen donating abilities (Burda and Oleszek, 2001). The essential oil showed significantly higher inhibition percentage and positively correlated with the content of the secondary metabolites in the essential oil. As shown in the equation below DPPH involved hydrogen atom transfer reactions (HAT) and single electron transfer (SET). Natural antioxidants (AH) neutralize the free radicals (R•) by interfere with the oxidation process by reacting with free radicals, chelating, catalytic and reactive oxygen scavenging activities (Prior *et al.,* 2005).

Hydrogen Atom Transfer

$$R^{\bullet} + AH \rightarrow RH + A^{\bullet}$$

Single Electron Transfer

$$R^{\bullet} + AH \rightarrow R^{-} + AH^{\bullet+}$$

$$AH^{\bullet+} \xleftrightarrow{OH_2} A^{\bullet} + H_3O^{+}$$

$$R^{-} + H_3O^{+} \rightarrow RH + H_2O$$

Table 2 : IC_{50} of the Antioxidant Property of the Floral Essential Oil of *O. basilicum* var. *purpureum* and Reference drug

Essential Oil and Reference Compound	DPPH IC_{50} μgml^{-1}
O. basilicum var. *purpureum*	1.0
Ascorbic acid	9.0

d) Antibacterial Potentials

The antimicrobial activities of the floral essential oil of *O. basilicum* var. *purpureum* against *E. coli, K. pneumoniae*, *P. mirabilis*, *P. aeruginosa*, *S. typhimurium*, *S. aureus, S. agalactiae* and *S. species* were shown in Table 3. The essential oil showed variable activities against tested bacteria. The essential oil was highly effective on all the tested bacteria. The highest inhibitory effect of the floral essential oil of *O. basilicum* var. *purpureum* was observed against *E. coli* (30 mm) followed by *S. aureus* (25 mm), *K. pneumoniae* (20 mm), *S. species (20 mm), P. aeruginosa (20 mm), P. mirabilis (20 mm), S. agalactiae (18 mm) and S. typhimurium (18 mm).* The tested bacteria were found to be resistant to Cloxicillin (CXC) but some were sensitive to Gentamicin (GEN) synthetic antibiotics. The antibacterial properties of this essential oil were comparable to that of leaf essential oil of *Ocimum gratissimum* which gave zones of inhibition between 7.0-26.6 mm for the following Gram positive (*S. aureus*, *Bacillus* spp.*)* and Gram negative (*E. coli, P. aeruginosa*, *S. typhi, K. pneumoniae, P. mirabilis*) bacteria (Matasyoh *et al.,* 2007). The observed antibacterial effects of the plant correlate its folk uses. In this study the essential oil of the plant inhibited the growth of Gram positive and Gram negative bacteria to a high degree. The observed activities may be due to the presence of some secondary metabolites such as terpenoids and phenolic compounds which are known to possess various medicinal activities in different organisms (Egharevba *et al., 2010). The antimicrobial activities* may also be due to numerous free hydroxyls that have the capability to combine with the carbohydrates and proteins in the bacteria cell wall. It has also been reported that the antimicrobial properties of essential oil results from the combined effect of direct vapour absorption on organisms and indirect effect through the medium that absorbed the vapour (Wang *et al.,* 2012). The vapour absorption on microorganisms is determined by their membrane permeability. Gram negative bacteria are less susceptible to essential oils than Gram positive bacteria because they possess outer membrane surrounding the cell membrane which restricts diffusion of hydrophobic compounds through its lipopolysaccharide covering (Angienda *et al*., 2010). Therefore, higher cell damage is expected to occur from the floral essential oil on the tested bacteria (Tyagi and Malik, 2010). Methyl eugenol is a phenolic compound that has been reported to have antimicrobial, central nervous system depressant, anaesthetic, hypothermic, myorelaxant, anticonvulsant, insecticidal, anthelminthic and nematocidal properties (Matasyoh *et al*., 2007). This study showed that the floral essential oil of *O. basilicum* var. *purpureum* has greater potential as antibiotic against bacteria and that they can be used in the treatment of infectious diseases caused by resistant pathogenic organisms in human beings.

Table 3 : Zones of Inhibition (mm) showing the Antimicrobial Properties of the Floral Essential oil of *O. basilicum* var. *purpureum*

	Floral Essential Oil			GEN	CXC
Conc. / Organism	1000	100	10	10µg	5µg
E. coli	30	30	30	22	-
K. pneumoniae	20	18	15	21	-
P. aeruginosa	18	18	18	20	-
P. mirabilis	20	20	20	20	-
S. agalactiae	18	18	18	-	-
S. aureus	25	25	25	-	-
S. typhimurium	18	18	18	21	-
S. species	20	18	18	-	-

Keynote:--- = Resistant, 6-9 mm = low inhibition, 10-14 mm = moderate inhibition and ≥ 15 mm = high inhibition.

IV. Conclusion

The results of the free radical scavenging, antioxidant and antimicrobial potentials of the part of the plant investigated in this study were basically due to the synergic effects of the phytochemical constituents in the floral essential oil. Natural antioxidants are helpful in assisting the body to neutralize free radicals in healthy individuals. Therefore, phytochemicals in the floral essential oil of this plant which are good antioxidants would help to reduce the harmful effects of oxidative stress and could be used to handle health problems caused by reactive oxygen species. Moreover, the ability of the floral essential oil to inhibit the growth of the bacteria in this study at low concentrations is an indication of its broad spectrum antimicrobial and great therapeutic potentials of this species. Plant having antimicrobial compounds have enormous therapeutic potentials as they can act without any side effect as often found with synthetic antimicrobial products.

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