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

Ololade Z.S.[1*], Fakankun O.A.[1], Alao, F.O.[2], Udi O.U.[1]

[1]Department of Chemical Sciences, Bells University of Technology, P.M.B. 1015, Ota, Nigeria
[2]Department of Biological Sciences, Bells University of Technology, P.M.B. 1015, Ota, Nigeria
zacchsnatpdt@gmail.com; suntolgroup@yahoo.com

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 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 86-73% and the antioxidant power of the essential oil increased with concentration. The essential oil was found to be 90% more active than the synthetic antioxidant (ascorbic acid). The essential oil was also found to exert excellent antibacterial properties compared to standard antibiotics. The floral essential oil was significantly active against all tested species of Gram-positive and Gram-negative bacteria with high zones of inhibition between 15-30mm. The bacteria inhibition of the essential oil was found to be positively correlated with their terpenoid and phenolic contents. The results from this study indicate that the floral essential oil show potential as a good source of natural antioxidant and antimicrobial drugs and may impart health benefits by its pharmacological property.
[Ololade Z.S., Fakankun O.A., Alao, F.O., Udi O.U. ***Ocimum basilicum* var. *purpureum* Floral Essential Oil: Phytochemicals, Phenolic Content, Antioxidant, Free Radical Scavenging, Antimicrobial Potentials.** *Life Sci J* 2017;14(9):104-110]. ISSN: 1097-8135 (Print) / ISSN: 2372-613X (Online). http://www.lifesciencesite.com. 10. doi:10.7537/marslsj140917.10.

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

1. Introduction

Phytochemicals are huge variety of organic substances which accumulated in plants. Plant secondary metabolites, especially essential oils, are recognized as one of the most promising groups of natural compounds 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 as secondary metabolites. In addition to essential oils, odouriferous 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 food and toiletries, fine chemicals, and pharmaceutical industries, and 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 of diuretic, constipation, intestine ache, galactogogue, stomachic, headaches, coughs, diarrhoea, warts, worms, and kidney malfunction, anti-inflammatory and antispasmodic agent (Khelifa *et al*., 2012; Uyoh *et al*., 2013).

To the best 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 at looking into the composition and pharmacological properties in the floral essential oil of *O. basilicum* var. *purpureum* from Nigeria.

2. Material and Methods

Plant Materials and Isolation of the Essential Oil

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

GC and GC-MS Analyses

Analysis of the floral essential oil of *O. basilicum* var. *purpureum* was 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 analysis. 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.

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).

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

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 in methanol were incubated with a methanolic solution of DPPH. After 30 minutes of incubation at room temperature in the dark, the 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).

In vitro Antimicrobial assay

The antibacterial potentials of the floral essential oil were evaluated by agar-well diffusion method against representative multi-drug resistance Gram-positive organism (*Streptococcus agalactiae*, *Staphylococcus aureus* and *Streptococcus viridans*), Gram-negative organisms (*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 adsorption.

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 10µgml^{-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^{o}C for 24 hours before visual assessment of the inhibition zones. Antibacterial potential of the essential oil was evaluated by measuring the clear zones of growth inhibition against the test organisms. Gentamicin (GEN) and Cloxicillin (CXC) were used as control (Agu *et al*., 2013).

3. Results and Discussion

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 composition of this floral essential oil is 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%), *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
β-cis-caryophyllene	2.0	1494
2-phenyl-1-hexanol	14.0	1469
patchoulane	6.7	1968
Percentage Total	**99.7**	

RI = Retention Index

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 study on the related species such as methanolic seed extract 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 compound like methyleugenol and 2-phenyl-1-hexanol. This report is indicating that total phenolic content is directly proportional to antioxidant and pharmacological properties of the floral part of the plant. Therefore, the secondary metabolites contribute significantly to the total antioxidant and therapeutic potentials of the plant. 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 singlet oxygen, 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, were recently received attention for its 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, phenolic compounds have been shown to possess potential antioxidant ability, 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).

***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 estimated 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 54±0.002, 69±0.002 and 96±0.000 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* is 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 a result of their hydrogen donating ability. The reduction in the number of DPPH molecules can be correlated with the number of available hydroxyl groups (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 ($R^{\bullet}$) by interfere with the oxidation process by reacting with free radicals, chelating activity, catalytic activity and oxygen scavenging activity (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 Properties 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

Data are presented as triplicate of the mean

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. viridans* were shown in Table 3. The essential oil showed variable activities against tested bacteria. The essential oils were effective on all bacteria. The highest inhibitory effect of the floral essential oil of *O. basilicum* var. *purpureum* was observed against *E. coli* (30 mm), *S. aureus* (25 mm), *K. pneumoniae* (20 mm), *S. viridans* (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 terpenoids and phenolic compounds which are known to possess various medicinal activities in different organisms (Egharevba *et al*., 2010).

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 was reported that the antimicrobial actions of terpenoids are due to the fact that they diffuse into and damage the cell membrane structures. It is known that the antimicrobial properties of such molecules also depend on their presence in gaseous form which facilitates their solubilization in cell membranes. It has 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 and anticonvulsant properties, insecticidal, anthelminthic and nematicidal properties (Matasyoh *et al*., 2007 This study showed that the essential oils of floral of *O. basilicum* var. *purpureum* has greater potential as antibacterial compounds 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*

	Fruit 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. viridans	20	18	18	-	-

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

Conclusion

The results of the free radical scavenging, antioxidant and antimicrobial potentials of the part of the plant investigated in this study was thought to be 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 excellent 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 seed 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 potential of this species. Plant having antimicrobial

compounds have enormous therapeutic potential as they can act without any side effect as often found with synthetic antimicrobial products.

Conflict of interest: We have no conflict of interest.

Corresponding Author:
Ololade Z.S.
Department of Chemical Sciences,
Bells University of Technology,
P.M.B. 1015, Ota, Nigeria
Email: zacchsnatpdt@gmail.com;
suntolgroup@yahoo.com
Tel: +2348034836546

References

1. Agu KC, Igweoha CA, Umeh CN. Antimicrobial Activity of the Ethanolic and Petroleum Ether Extracts of Tangerine Seed on Selected Bacteria. *Inter. J. Agri. Biosci*. 2013; 2(1):22-24.
2. Angienda PO, Onyango DM, Hill DJ. Potential application of plant essential oils at sub-lethal concentrations under extrinsic conditions that enhance their antimicrobial effectiveness against pathogenic bacteria, *African Journal of Microbiology Research*. 2010; 4 (16): 1678-1684.
3. Burda S, Oleszek W, Antioxidant and antiradical activity of flavonoids. *J. Agric Food Chem*. 2001;49: 2774-2779.
4. Daferera DJ, Ziogas BN, Polissiou MG. GC-MS Analysis of Essential Oils from Some Greek Aromatic Plants and Their Fungitoxicity on *Penicillium digitatum J. Agric. Food Chem*. 2010; 2(10): 35-40.
5. Egharevba HO, Abdullahi MS, Okwute SK, Okogun JI. Phytochemical Analysis and Broad Spectrum Antimicrobial Activity of *Laggera pterodonta* (DC.) Sch. Bip. (Aerial Part), *Researcher*. 2005; 53: 1841-1856.
6. European Pharmacopoeia Commission. *European Pharmacopoeia 5th Ed.* Council of Europe: Strasbourg Cedex, France. 2004.
7. Georgiev V, Ananga A. Tsolova V. Recent Advances and Uses of Grape Flavonoids as Nutraceuticals, *Nutrients*. 2014; 6: 391-415.
8. Govindappa M, Naga SS, Poojashri, MN, Sadananda TS, Chandrappa CP. Antimicrobial, antioxidant and *in vitro* anti-inflammatory activity of ethanol extract and active phytochemical screening of *Wedelia trilobata* (L.) Hitchc., *Journal of Pharmacognosy and Phytotherapy*. 2011; 3(3): 43-51.
9. Hakkim FL, Arivazhagan G, Boopathy R. Antioxidant property of selected *Ocimum* species and their secondary metabolite content, *Journal of Medicinal Plants Research*. 2008; 2(9): 250-257.
10. Janick J, Simeon, J.E., Morales, M.R., Phippen, W.B., Vieira, R.F. and Hoa, Z. (1999): Basil: A Source of Aroma Compounds and a Popular Culinary and Ornamental Herb, reprinted from: *Perspectives on new crops and new uses*, ASHS Press, Alexandria VA. 1999; 499-505.
11. Joshi RK. Chemical Composition, *In vitro* Antimicrobial and Antioxidant Activities of the Essential Oils of *Ocimum gratissimum*, *O. sanctum* and their Major Constituents. *Indian J. Pharm. Sci*. 2013; 75: 457-462.
12. Khelifa LH, Brada M, Brahmi F, Achour D, Fauconnier ML, Lognay G. Chemical Composition and Antioxidant Activity of Essential Oil of *Ocimum basilicum* Leaves from the Northern Region of Algeria, *Top class Journal of Herbal Medicine*. 2012; 1(2): 25-30.
13. Matasyoh LG, Matasyoh, J.C., Wachira, F.N., Kinyua, MG, Muigai, AWT, Mukiama, TK. Chemical composition and antimicrobial activity of the essential oil of *Ocimum gratissimum* L. growing in Eastern Kenya, *African Journal of Biotechnology*..2007; 6(6): 760-765.
14. Mbagwu FN, Unamba, CIN, Nwosu, IC. Phytochemical Screening on the Seeds of *Treculia africana* and *Artocarpus atilis*, *New York Science Journal*. 2010; 3(12): 51-55.
15. Mediani A, Abas F, Khatib A, Tan CP. *Cosmos Caudatus* as a Potential Source of Polyphenolic Compounds: Optimisation of Oven Drying Conditions and Characterisation of Its Functional Properties. *Molecules*. 2013; 18: 10452-10464.
16. Mimica-Dukic N, Bozin B. Mentha L. species (*Lamiaceae*) as promising sources of bioactive secondary metabolites. *Curr. Pharm. Des*. 2008; 14 (29): 3141-3150.
17. Patil DD. Mhaske, D.K., Wadhawa, G.C. (2011): Antibacterial and Antioxidant study of *Ocimum basilicum Labiatae* (sweet basil), *Journal of Advanced Pharmacy Education and Research*. 2011; 2: 104-112.
18. Prior RL, Wu X, Schaich K. Standardized methods for the determination of antioxidant capacity and phenolics in food and dietary supplements. *J. Agric. Food Chem*. 2005; 53: 4290-4302.
19. Rajendran MP, Palliyan BB, Selvaraj N. Chemical composition, antibacterial and antioxidant profile of essential oil from *Murraya koenigii* (L.) leaves, *Avicenna J. Phytomed*. 2014; 4(3):200-214.
20. Sarma DSK, Babu, AVS. Antioxidant and Antimicrobial Activity of *Ocimum americanum*,

International Journal of Advances In Pharmaceutical Sciences. 2011; 2: 211-218.
21. Stefan M, Zamfirache, M.M., Padurariu, C., Truta, E., Gostin, I. (2013): The composition and antibacterial activity of essential oils in three *Ocimum* species growing in Romania, *Central European Journal of Biology*. 2013; 8(6):600-608.
22. Tyagi AK, Malik**,** A. Liquid and vapour-phase antifungal activities of selected essential oils against *candida albicans*: microscopic observations and chemical characterization of *cymbopogon citratus*, *BMC Complementary and Alternative Medicine*. 2010; 10:65.
23. Uyoh EA, Chukwurah PN, David IA, Bassey AC. Evaluation of Antioxidant Capacity of Two *Ocimum* Species Consumed Locally as Spices in Nigeria as a Justification for Increased Domestication, *American Journal of Plant Sciences*. 2013; 4, 221-229.
24. Varma J, Dubey NK. Efficacy of essential oils of *Caesulia axillaris* and *Mentha arvensis* against some storage pests causing biodeterioration of food commodities. *International Journal Food Microbiology*. 2001; 68: 207-210.
25. Wang YW, Zeng WC, Xu, PY, Lan YJ, Zhu RX, Zhong K, Huang YN, Gao H. Chemical Composition and Antimicrobial Activity of the Essential Oil of Kumquat (*Fortunella crassifolia* Swingle) Peel, *Int. J. Mol. Sci*. 2012; 13(3): 3382-3393.
26. Yadav RNS, Agarwala M. Phytochemical analysis of some medicinal plants, *Journal of Phytology*. 2011; 3(12): 10-14.

9/25/2017