Research Journal of Chemical Sciences ____ ISSN 2231-606X
Vol. **5(10),** 7-12, October **(2015)** *Res. J. Chem. Sci.*

Compositional Analysis and Anti-Oxidant Assesment of Essential Oil of some Aromatic Plants Obtained from North-Eastern Nigeria

Runde M., Kubmarawa D. and Maina H.M.
[1]Department of Chemistry, Modibbo Adama University of Technology Yola Adamawa State, NIGERIA

Available online at: www.isca.in, www.isca.me
Received 12th August 2015, revised 15th September 2015, accepted 17th October 2015

Abstract

Fresh leaves of Ocimum americanus, Vossia cuspidata,Eucalyptus camaldulensis and the bark of Bosweillia dalzielii were collected and pretreated for essential oil analysis. DPPH scavenging capacity of the respective essential oils was used to determine the potential anti-oxidant activities of the oils. The results obtained from the analysis shows that the major compounds in essential oil of Ocimum americanus are; Terpinen-4-ol (14.507 %), Copaene (7.438 %) and Terpinen (6.178 %). The essential oil of Vossia cuspidata are predominated by 4-acetyl-7-hydroxybenzo-2,1,3-thiadiazole (15.037 %), α-Caryophyllene (11.397) and α-Pinene (10.285). The major compounds present in the essential oil of Eucalyptus camaldulensis being M-Cymene (19.74 %), α-Phellandrene (19.280 %), and Eucalyptol (13.101 %) whereas the major componds of the oil of Bosweillia dalzielii are α-Pinene (18.515 %), Isophthaldehyde (10.695 %) and β-Pinene (5.641 %). The samples have exhibited some degree of antioxidant activities with values above 80%. However, the lowest scavenging capacity for each of the sample is observed in the corresponding lowest concentrations of vitamins E (80.39 %) a water insoluble antioxidant which presented with the least scavenging capacity, whereas vitamins C (98.87%) has the highest scavenging property followed by vosia cuspidata (97.44 %) at the most increased concentration of 50 µL/ml.

Keywords: Antioxidant, essential oils, free radical.

Introduction

Free radicals are said to play a negative major role in the ageing process and in diseases progression. Antioxidants are the main defense agents against free radicals invasions, and are necessary for maintaining good health and wellbeing. The desire for antioxidant becomes even more important with increased risks for coming in contact with free radicals. Environmental pollutants such as cigarette smoke and other factors like drugs, illness, stress and even exercise can increase risk of free radical exposure[1].

Humans utilize oxygen in order to metabolize fats and carbohydrates for energy; however this does not come without a cost. Oxygen is a highly reacting atom that is capable of becoming part of potentially damaging molecules commonly called free radicals. Free radicals can attack the healthy cells of the body, causing them to lose their morphology[1]. Excessive production of oxygen-derive free radicals are involve in initiating many diseases such as cancer, rheumatoid arthritis, cirrhosis and arteriosclerosis[2]. Although oxidation reactions are crucial to life, antioxidants are therefore essential for life sustenance. They are substances that shield cells from the damage caused by free radicals[3]. Example of antioxidants includes: beta-carotene, lycopene, ascorbic acid, alpha-tocopherol, and other substances[4]. The use of these antioxidants in supplement form in the body may help in reducing oxidative damage[2]. Natural antioxidants are being thoroughly studied for their ability to protect human cells from damage due to oxidative stress[5]. The use of essential oil as active ingredients in various industries such as foods, drinks, toiletries and cosmetics is becoming more common[6]. Before now, essential oils have been studied mostly from view point of their flavor and fragrance chemistry for flavoring food, drinks and other goods.

There is an increasing interest in antioxidants, particularly in those intended to prevent the presumed deleterious effects of free radicals in the human body, and to prevent the deterioration of fats and other constituents of foodstuffs. In both cases, there is a preference for antioxidants from natural rather than from synthetic sources[7,8]. There is therefore a parallel increase in the use of methods for estimating the efficiency of such substances as antioxidants[9,10]. One such method that is currently popular is based upon the use of the stable free radical 2,2-Diphenylpicryl-1-hydrazyl (DPPH). The purpose of this paper is to examine, analyze the composition of various essential oils and to establish the possibility for their use as antioxidant.

Material and Methods

Essential oil extraction: The leaves of *Ocimum americanus, Vosia cuspidata, Eucalyptus camaldulensis* and the stem bark of *Bosweilia dalzielii* (each weighing 1 kg) obtained in the month of January 2015 from Girei Local Government of Adamawa state, North-eastern Nigeria and immediately subjected to extraction to avoid loss of some essential oils as a result of drying process, and using a modified type of steam distillation apparatus (in which the receiver end of the distiller has been pass through another vessel containing ice) for 2.5 h essential oils of the plants were collected over water and later kept at 4 ^{O}C until further required.

Gas chromatography Mass spectroscopy (GC-MS): GC-MS analysis was performed on a J and W Scientific gas chromatography directly coupled to the mass spectrometer system (model GC Agilent technologies 7890A, Agilent technologies MSD 5975C), 5 % phenyl methyl silox: 469.56 509 packed capillary column (30M x 250µm) was used under the following conditions: ovum temperature 50^0 C for 1 min, then raised within intervals of 10^0C/min to 200^0C for 1min, and 20^0 C/min to 300^0 C for 2 min. Injector temperature was 230^0C while the carrier gas was Helium flowing at the rate of 1ml/min, the volume of the injected sample was 0.2µL of diluted oil in hexane, splitless injection techniques was used and the ionization energy was 70ev in the electron ionization (EI) mode. Ion source temperature was 230^0C while the scan mass range of M/Z 60-335 was used.

The constituents of the essential oils were identified based on comparison of the retention indices and mass spectra of most of the compounds with data generated under identical experimental conditions by applying a two dimensional search algorithm considering the retention index as well as mass spectral similar with those of authentic compounds available in NBS75K and NIST08 Libraries.

The retention indices (RI) are in relation to a homologous series of n-alkanes on the GC column under the same chromatographic condition. Relative concentration will be obtained by peak area normalization as describe by Brand-Williams W et. al.[11].

DPPH free radical scavenging assay: The 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay was carried out for the evaluation of the antioxidant activity of the various essential oils. This assay measures the free radical scavenging strength of the tested essential oil. DPPH is a molecule containing a stable free radical however, in the presence of an antioxidant which can donate an electron to DPPH, the purple color, typical for free DPPH radical fades, and the change in absorbency at λ = 517 nm is measured spectrophotometrically. This test provides information on the ability of a compound to serve as antioxidant. The method will be carried out as described previously by Wei C.L. et. al.[12] and Kubmarawa D. et.al.[13]. The essential oil will be dissolved in methanol, and various concentrations (2, 6, 12, 24, and 50 µL/mL) will be used. The assay mixture contained in a total volume of 1 mL, 500 µL of the oil, 125 µL prepared DPPH (1 mM in methanol), and 375 µL solvent (methanol). After 30 min incubation at 25°C, the decrease in absorbance will be measured at λ = 517 nm. The radical scavenging activity will be calculated from the equation below:

% or radical scavenging = [(Abs control – Abs Sample) ÷ Abs control] x 100

Results and Discussion

Chemical composition of essential oils of various plants: The GC-MS analysis of the essential oil obtained from the stem bark of *Bosweilia dalzielii,* leaves of *Ocimum americanus, Vossia cuspidata* and *Eucalyptus camaldulensis* (table-1) reveals that α-Pinene (18.515 %), Isophthaladehyde (10.695 %) and β-Pinene (5.641 %) were the major component of the essential oil of *Boweillia dalzielii* stem bark whereas, the oil of *Ocimum americanus* was dominated by Terpinene-4-ol (14.507 %), Copaene (7.438 %), and Terpinene (6.178 %). Similar analysis carried on the essential oil of Vossia cuspidata shows that 4-Acetyl-7-hydroxybenzo-2,1,3-thiadiazole (15.037 %) was the most abundant compound, followed by α-Caryophyllen (11.397 %), α-Pinene (10.285 %). On the other hand the major compounds of the essential oil of *Eucalyptos camaldulensis* were M-Cymene (19.74%) α-Phellandrene (19.280 %) and Eucalyptol (13.101 %). The result also pointed at α-Pinene as the most common compound with appreciable concentration among the essential oils of all the plants subjected under this test. Similar work on composition of *Bosweillia dalzielii* in Nigeria reveals that α-Pinene (45.7 %) and Terpinene (11.5 %) were the major compounds[14]. In Iran, the analysis of essential oil of *Eucalyptus camaldulensis* obtained from two locations (Ghalegardan and seashore) indicate that the dominant compounds were Eucalyptol (29.2 %), α-phellandrene (17.43 %) and α-pinene (7.1 %) for Ghalegardan and for the seashore were Eucalyptol (46.74 %), Arommadenderene (12.1 %) and Terpinen-4-ol (7.6 %)[14]. These works by different authors from different locations have confirmed the result found in our work as being the composition of essential oils of various plants.

DPPH scavenging activity of essential oils of various plants: The antioxidant activities of the essential oils of 4 plants were investigated using 2, 2-Diphenyl-1-picryl hydrazyl (DPPH). The DPPH percentage Scavenging capacity for each essential oil and that of the two standards (vitamin C and vitamin E) were measured at varying concentrations and recorded for further discussion.

From the result all the samples have exhibited antioxidant activities with values above 80%. However, the lowest scavenging capacity for each of the sample is observed in the corresponding lowest concentrations vitamins E (80.39 %) a water insoluble antioxidant which is presented with the least scavenging capacity whereas vitamin C (98.87%) has the highest scavenging property followed by *vosia cuspidata* (97.44 %) at the most increased concentration of 50 µL/ml.

Table-1
Composition of essential oils of various plants

Compounds	**Bosweilia dalzielii**	**Ocimum americanus**	**Vossia cuspidata**	**Eucalyptus camaldulensis**
α-Pinene	18.515	2.664	10.285	8.073
3-Carene	1.317	00	00	3.729
β-pinen	5.641	2.277	00	00
Limonene	1.485	00	00	00
α-Cubebene	1.137	00	00	00
Furanacetic acid, 4-hexyl-2,5-dihydro-2,5-dioxo	1.226	00	00	00
Bicycle[5.2.0] nonane, 2-methylene-4,8,8-trimethyl-4-vinyl	2.169	00	00	00
Imidazo [4,5-e][1,4] diazepine-5,8-dione, 2-chloro-1,4,6,7-tetrahydro-1,4-dimethyl-	1.430	00	00	00
Isophthaldehyde	10.695	00	00	00
Acetonitril (3,5,5-trimethyl-2-cyclohexen-1-ylidene)-(z)-	3.051	00	00	00
3-penimidic acid, 3-methyl-N-phenyl, methyl ester	4.598	00	00	00
1,3,8-Menthatriene	2.108	00	00	00
1,2-Naphthalenediol,1,2,3,4-tetrahydro-1-methyl-cis	1.017	00	00	00
1,3-cyclopentadiene, 1,2,3,4-tetramethyl-5-methylene	4.439	00	00	00
Cinnamyl carbanilate	2.298	00	00	00
5-Isopropylidene-4,6-dimethylnona-3,6,8-triene-2-ol	2.554	00	00	00
Humulene	3.412	00	00	00
10,12-Tricoadiyoic acid, methyl ester	3.048	00	00	00
Cyclohexanone, 5-ethenyl-5-methyl-4-(1-methylethenyl)-2-(1-methylethylidene)-, cis	2.794	00	00	00
β-elemenone	1.009	00	00	00
α-Thujene	00	1.483	00	00
Phenol, 3-(1-methylethyl)-	00	2.467	00	00
P-Cymene	00	1.278	00	00
D-Limonene	00	3.149	1.332	00

Compounds	**Bosweilia dalzielii**	**Ocimum americanus**	**Vossia cuspidata**	**Eucalyptus camaldulensis**
Terpinene	00	6.178	00	00
Cis-β-Terpineol	00	3.914	00	00
Terpinolene	00	2.006	00	00
5-caranol, (1s,3R,5s,6R)-	00	2.123	00	00
Octan-1-ol.acetate	00	1.662	00	00
Terpinene-4-ol	00	14.507	00	00
Cyclone	00	1.377	00	00
Bornyl acetate	00	1.025	00	00
Myrtenyl acetate	00	1.139	00	00
Bicyclo [7.2.0] undec-4-ene,4,11,11-trimethyl-8-methylene-	00	4.285	00	00
Copaene	00	7.438	00	00
α-Caryophyllene	00	1.147	11.397	00
1H-Cyclopenta [1,3], cyclopropa [1,2]benzene, octahydro-7-methyl-3-methylene-4-(1-methylethyl)-	00	1.144	00	00
Isocaryophyllene	00	1.041	00	00
α-bergomotene	00	2.598	00	00
Cadinene	00	2.062	00	00
α-Gurjunene	00	1.698	00	00
1H-Indole-2-carboxylic acid, 3-amino-5-methoxy-ethyl ester	00	4.059	00	00
2,2,5,6-Tetramethyl-1,3-oxathiane	00	1.344	00	00
4-Piperidinecarboxamide,1-(2-hydroxybenzoyl)-	00	2.641	00	00
β-Myrcene	00	00	4.179	00
Benzene, 1,3,5-trimethyl-	00	00	1.459	00
β-Linalool	00	00	1.153	00
3-Cyclohexen-1-carboxylic acid, 4-methyl- ester	00	00	3.753	00
Benzyl cyclobutane	00	00	6.565	00
Thiophene	00	00	3.477	00
Aziridine, 1-phenyl-	00	00	3.487	00

Compounds	**Bosweilia dalzielii**	**Ocimum americanus**	**Vossia cuspidata**	**Eucalyptus camaldulensis**
1,5-Cyclooctadiene,1,5-dimethyl-	00	00	1.989	00
4-acetyl-7-hydroxybenzo-2,13-thiadiazole	00	00	15.037	00
Caryophyllene	00	00	6.079	00
Ylangene	00	00	4.390	00
Camphene	00	00	2.770	00
Octahydropyrolo [1,2-a]pyrazine perdeutero benzene	00	00	5.829	00
Sabinene	00	00	00	1.022
α-Phellandrene	00	00	00	19.740
(+)-2-carene	00	00	00	5.437
M-Cymene	00	00	00	14.981
Benzene, 1-methyl-3-(1-methylethyl)-	00	00	00	4.736
Eucalyptol	00	00	00	13.101
1,4-Cyclohexadiene, 1-methyl-4-(1-methylethyl)-	00	00	00	5.031
Isovaleric acid, butyl ester	00	00	00	2.114
3-Cyclohexan-1-ol, 4-methyl-1-(1-methyl)-	00	00	00	4.085
Piperitone	00	00	00	1.830
2-Hydroxy-4,5-dimethylacetophenone	00	00	3.862	00
TOTAL	**22**	**24**	**18**	**10**

Table-2
Evaluation of % scavenging activity of essential oils of various plants

Conc.(µL/ml)	**Bosweillia dalzielii**	**Ocimum americanus**	**Vossia cuspidata**	**Eucalyptus camaldulensis**	**Ascorbic acid (µg/ml)**	**α-tocopherol (µg/ml)**
2	90.67	94.47	89.66	88.74	98.77	80.39
6	90.79	94.49	90.17	88.86	98.77	81.20
12	90.89	94.89	90.77	90.37	98.85	86.16
24	91.19	95.35	92.73	94.88	98.87	87.17
50	91.79	95.49	97.44	95.15	98.87	88.53

Conclusion

The major components of Bosweillia dalzielii Hutch essential oil, included α-Pinene (18.515%) Isophthaldehyde (10.695 %) and β-pinen (5.641 %). Ocimum bassilicum essential oil mainly contained Terpinen-4-ol (14.507 %), Copaene (7.438 %) and Terpinene (6.178). The essential oil from Vossia cuspidata is characterized by a high content of 4-Acetyl-7-hydroxhbenzo-2,1,3-thiadiazole (15.037 %), α-caryophyllene (11.397 %) and α-pinene (10.285%). The predominant compounds in Eucalyptus camaldulensis essential oil M-cymene (19.74%), α-phellandrene (19.280) and α-Pinene (8.073 %). There is then, great variability and similarities in the chemical composition of essential oils obtained from these plants. The essential oil of these plants also exhibit great antioxidant activity as revealed by their DPPH scavenging activity.

References

1. Mark P, Antioxidant Clinical Nutrition Insight Advanced Nutrition, **(1998)**

2. Mahmood R., Gouthamchandra K. and Manjunata H, Free radical Scavenging, Antioxidant enzyme and Wound healing activities of leaves extracts from *Clerodendrum L., Environmental Toxicology and Pharmacology,* **30(1),** 11-18 **(2010)**

3. Hamid A.A., Ayelagbe O.O., Usman L.A., Ameen O.M. and Lawal A., Antioxidand: its Medicinal and Pharmacological Applications, *Afri. J. of pure and app. chem.,* **4(8)** 142-151 **(2010)**

4. Sie H, Oxidative Stress: Oxidants and Antioxidant, *Exp. Physiol;* **82(82),** 291-295 **(1997)**

5. Cazzi R., Richard R., Aglitti T., Petricone P. and De-Salvia P, Ascorbic acid and Beta-carotene as Modulators of Oxidative Damage, *Carcinogenesis,* **18(1),** 223-228 6 **(1997)**

6. Ormancey X., Sisalli S. and Contriere P, Formulation of Essential oils in Functional Perfumery, *Perfumes, Cosmetique, Actualities,* **157,** 30-40 **(2001)**

7. Abdalla A.E. and Roozen P.J., Effect of plant extract on the oxidative stability of Sunflower oil and emulsion, *Food chemistry*, **64(3)**, 323-329 **(1999)**

8. Sanchez-Moreno C., Methods used to Evaluate the Free Radical Scavenging Activity in Foods and Biological Systems, *Fd. Sci. and Technol. Int.*, **8(3),** 121-137 **(2002)**

9. Schwarz K., Bertelsen G., Nissen I.R., Gardner P.T., Heinonen M.I, Hopia A., Huynh-Ba T., Lamblet P., Mcphail D., Skibsted L.H. and Tijburg LInvestigation of Plants Extract for the Protection of Processed Food Against Lipid Oxidation, Comparison of Antioxidant Assays based on Radical Scavenging, Lipid Oxidation and Analysis of the Principal Antioxidant Compounds, *Eur. Fd. Res. Technol.*, **212,** 319-328 **(2001)**

10. Ramzi A.M., Mansour S.A., Mohammed A.A., Adnan J.A. and Jamal M.K., GC and GC/MS Analysis of Essential Oil Composition of the Endemic Soqotraen Leucas virgata Balf.f. and Its Antimicrobial and Antioxidant Activities*, Int. J. of Mol. Sci.*, **14(11)**, 23129-23139 **(2013)**

11. Brand-Williams W., Cuvelier M.E. and Berset C., Use of a Free Radical Method to Evaluate Antioxidant Activity, *Fd. Sci. Technol.*, **28,** 25–30 **(1995)**

12. Wei C.L., Roziahanim M., Rahmad N., Suthagar P.P., Shanmugapriya P. and Sabariah I., Free Radicals Scavenging Activity of Essential Oils of Pisidium Guajava L. Leaves against Toxoplama Gondii, *J. of Essenti. Oils Bear. Pla.*, **16(1),** 32-38 **(2013)**

13. Kubmarawa D., Ogunwande I.A., Okorie D.A., Olawore N.O and Kasali A.A. Constituent of the Essentials of *Bosweillia dalzielii* Hutch from Nigeria, *J. of Essenti. Oil Res.*, **2(18),** 119 **(2006)**

14. Lida M.L., Balak B., Seyed A.H.B., Mahmoud A. and Raziech M.B., Essential oils Composition and Antibacterial Activities of *Eucalyptus camaldulensis* Dehn, *Int. J. of Med. Arom. Pla.*, **3(2),** 214-219 **(2013)**