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Evaluation of the Antioxidant Activity of *Ocimum* sp.

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Abstract: Extracts of leaves of *Ocimum* gratissimum and *O.* basilicum were investigated for antioxidant activities. The proximate composition determination revealed that extracts of *Ocimum* sp. had a high crude protein content (20.18 and 20.15), respectively. The crude fibre and ash content were high and the physicochemical parameters of the extracts were found to be similar. About 11-14% Vitamin E was obtained from the extracts as Vitamin E is said to be the most abundant lipid soluble antioxidants. An evaluation of the antioxidant effectiveness of the two extracts were carried out and compared with the synthetic antioxidant (BHT). Extracts of the spice were found to exhibit effective antioxidant activities as the synthetic antioxidants except for the green pigment of the extract. *O. basilicum* exhibited an appreciable antioxidant effectiveness which is significantly equal to the activity of BHT at THE 5th and 10th days during the 20 days of storage.

Key words: *Ocimum gratissimum*, *O. basilicum*, antioxidant, BHT, vitamin E

INTRODUCTION

Utilisation of spices as food additives has been an age long practice carried out by different people in many part of the world. Spices extractives, such as oleoresin of rosemary, can provide inhibition of oxidative rancidity and retard the development of warned-over flavour in some products. Thus, some spices not only provide flavour and aroma to food and retard microbial growth but are also beneficial in prevention of some off-flavour development[1].

Lipid peroxidation is a major cause of food quality deterioration during processing and storage Shahidi and Naizk[2]. Oxidation of lipid imitates other changes in food, which affects its nutritional quality, wholesomeness, safety, colour, flavour and texture[3].

Antioxidants are known to protect food quality by delaying or inhibiting free radical oxidation of fats and oils and the resulting off flavour and colour[4]. Although synthetic antioxidants like butylated hydroxytoluene, butylatedhydroxy anisole and propyl gallate are currently being used in food industries, concerns about the possible adduce effects of these and others have been documented[5].

Ocimum sp. belong to the plant family Labiates, which comprises of several species such as *O. gratissimum, O. basilicum O. americanus* and *O. tenuiflorum*[6].

The leaves from these various species have been put to various uses such as condiments, used for abdominal pains, as a tooth gargle, to regulate menstruation and cure prolapsed of the rectum[7]. Also the fragment leaves are also added to various dishes with a fishy or disagreeable smell. It occurs directly or indirectly for example in the development of ketch up, spices are ground, mixed and put in muslin bag which is dipped into the ketch up Sethip Aggarual[8].There is dearth of literature on the antioxidant effectiveness of *Ocimum gratissimum* and *Ocimum basilicum.* Thus, the objective of this study was to evaluate the antioxidant properties of these spices that have been put to various uses.

MATERIALS AND METHODS

Sample collection and preparation: *Ocimum gratissimum* and *O. basilicum* leaves were plucked from three different farms located around Federal University of Technology, Akure road in Akure. The freshly plucked lerics were identified at the Crop Soil and Pest Management Department FUTA. These leaves were sorted, washed under running tap water for 2 min, drained and dried at 45°C in the oven. The dried leaves were blended to obtain a fine powder and were sealed, respectively in high density polyethylene film and stored until required for use.

Proximate analysis determination: Proximate composition of the spices were determined using the method of AOAC[2]. The spices were analyzed for percentage fat, moisture, protein, ash and carbohydrate contents.The

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moisture content and ash content were determined using A.O.A.C.[9] procedure. The average of the triplicate readings gave the percentage moisture and ash contents, respectively.The fat content , crude fibre and crude protein were all determined using A.O.A.C[9]. All the analysis were carried out in triplicates.The carbohydrate content was determined by difference.

Determination of physicochemical properties of *O.basilicum* and *O.gratissimum*: Some physicochemical properties of the spice extracts were determined.This include; the specific gravity, refractive index, pH peroxide value and iodine value.The specific gravity was determined as the weight of equal volume of oil to to the same volume of water. Refractive index was carried out using Abbe refractometer as. The pH was determined using pH meter (Mettler 350). The peroxide value was determined using the method. The iodine value was determined by Wijs method.

Extraction of antioxidant: Antioxidant extraction were carried out using the method of Adegoke and Gopalakrishna[5] modified by extracting 25 g of finely ground spice with 250 mL Hexane in a Soxhlet extraction apparatus for 8 h. This consists of a high recovery unit and the extraction rate of spice to solvent was 5:50, that is for every 5 g of sample; 50 mL of solvent was used.To maximize antioxidant recovery, the defatted spices were subjected to further extraction using fresh solvents. In order to reduce the extraction solvent in the antioxidant to permissible level, the antioxidant was desolventized. This was done by drying the antioxidant in an air oven at 50°C for 10 min. The crude extracts were evaporated to dryness and the percentage yield of the extracts was determined.

Application of the antioxidant: Three antioxidants were incorporated by direct addition into the groundnut oil (groundnut oil bought from the market) and were then thoroughly mixed to achieve a uniform dispersion- U.S. Food and Drug Administration regulations governing the use of antioxidants limits usage levels to 0.02% w/w of the lipid content of the food. The antioxidants inoculated into the groundnut oil sample include; extracts of O.basilicum, O.gratissimum and BHT. Hence, the minimum concentration of the antioxidant employed was 100 ppm while the maximum was 300 ppm. All the experiments were done in triplicates.

Assessment of the antioxidant activities: In order to assess the efficacy and potency of the spice extracts as antioxidants, the inoculated oil samples and the control were subjected to accelerated stability test. This experiment was done at 63°C in an air light oven for a period of 20 days. The samples were shaken twice a day during storage and monitored for the peroxide value every 5 days. Decrease in the rate of formation of peroxides was taken as a measurement of the antioxidant activity of each extract[5]. The peroxide value, iodine value and refractive index were carried out using.

Determination of vitamin e content (tocopherol): About 0.5 g of sample (antioxidant extract) was weighed into a 100 mL beaker. 40 mL of petroleum ether was added and shaken to ensure a uniform mixing. The mixture was filtered using whatman No 42 filter paper and made up to mark with petroleum ether in a 100 mL volumetric flask. Standard Vitamin E (α-tocopherol) were prepared from range 25-200 μg mL^{-1} in petroleum ether and treated as sample. 2 mL of sample and standard petroleum ether extracted were each treated with 10 mL of the iron dipyridyl in glacial acetic acid and made up to 100 mL volume with glacial acetic in 100 mL volumetric flask. The absorbance of the standards were read on a spectronic 20 spectrophotometer at a wavelenght of 460 nm. Vitamin E in mg 100 g was calculated using the formular;

Absorbance sample×gradient factor×dilution factor/weight of sample source; association of vitamin chemist, methods of vitamin.

RESULTS AND DISCUSSION

Proximate composition: The two extracts have low moisture contents but high crude protein contents. The result of the proximate composition of the extracts, Table 1, shows that there is no much difference in the values of moisture, protein and carbohydrate of the two samples. However, *O. gratissimum* was found to be considerably higher in total ash and crude fibre than *O. basilicum.* This could give higher digestion aiding ability over *O. basilicum* Adejumo[10]. The low moisture content obtained is similar to that observed by Adegoke[5] where he obtained a moisture contents of 8.44% and 7.51% for *A. Melegueta* and *X. aethiopica,* respectively. Whereas, the protein content of *Ocimum* sp. is higher (20.18 and 20.15%) as against 6.44 and 8.53% for *A. melegueta* and *X. aethiopics*[5].

Physicochemical properties of the extracts: The result of the physicochemical properties of the two natural extracts which include, specific gravity, refractive index, pH, peroxide value and iodine value Table 2, were found to be similar except for the iodine value which was found to be higher in the extracts and O.gratissimum value was shown to be higher than that of the O.gratissimum. The

Table 1: The proximate composition of *O. gratissimum* and *O. basilicum*

Sample	Moisture (%)	Fat (%)	Protein (%)	Total Ash	Crude fibre	CHO (%)
Ocimum gratissimum	8.11±0.10	11.78±0.09	20.18±0.09	15.62±0.07	8.70±0.09	35.61±0.05
O. basilicum	9.35±0.02	14.38±0.03	20.15±0.01	13.25±0.01	6.73±0.01	36.14±0.01

Table 2: Chemical values of *O.* gratissimum and *O. basilicum*

Parameters	*O* gratissimum	*O.*basilicum
Specific gravity	0.947±0.01	0.955±0.01
Refractive Index	1.43±0.01	1.44±0.01
pH	6.90±0.01	7.10±0.08
Peroxide value	5.80±0.07	7.10±0.09
Iodine value meq.kg	48.48±0.13	41.12±0.01

Table 3: Vitamin E content and %yield of extract of the spices

Samples	Vit. E (mg 100 g)	Yield of Extracts (%)	Fat (%)
O. basilicum	10.29±0.01	14.69±0.01	14.38±0.01
O. gratissimum	13.34±0.01	11.26±0.01	11.78±0.01

implication of this high iodine value is that the extracts have high unsaturated linkages and they could be more susceptible to oxidation[11].

Vitamin E content and % yield of extract of the spices: The spices were found to contain 11-14% vitamin E Table 3, which shows that the spice extracts can act as strong antioxidant[11]. Vitamin E is reported to favour the absorption of iron and play a role in maintaining stability of biological membranes[11]. All tocopherols and tocotrienols when not esterified , have the ability to act as antioxidants; quench free radicals by donating the phenolic H and an electron. Naturally occuring tocopherols has been shown to contribute to the stability of highly unsaturated vegetable oils through their antioxidant activity[12]. Due to its antioxidant activity, Vitamin E is able to spare carotene and vitamin A from oxidative destruction[11]. The extracts were also found to contain 11-14% Vitamin E, which is the most abundant lipid soluble antioxidant and protects the lipid portions of the cell, especially cellular membranes[13].

The antioxidant yield obtained from 10g of each spice are also shown in Table 3. *O.basilicum* had a higher yield than *O. gratissimum* but the two spices antioxidant yield is lower when compared with *Xylopia aethiopica* which had a higher yield of 44%[5]. The higher percentage yield of *X. aethiopica* may be due to its higher fat content. From Table 3, the extraction yield showed a correlation with the fat contents, the more the fat content the more the extract yield.The fat content of any food may be considered to consist of the free lipid and the bound lipid constituent. The free lipid constituent is that which may be extracted by less polar solvent such as diethyl ether, hexane and petrolueum spirit whereas the bound constituents require more polar solvents such as alcohol for their extraction[14]. In this study hexane was used for the extraction.

Table 4: Antioxidant activity of *O.basilicum*, *O. gratissimum* and BHT (100 ppm)

No. of days	Control	BHT	O.basilicum	O.gratissimum
0	6.89±0.01	6.89±0.01	6.89±0.01	6.89±0.01
5	8.0 ± 0.2^{d}	2.86 ± 0.15^{a}	3.19 ± 0.01^{b}	4.29 ± 0.01^{c}
10	8.5 ± 0.24^{d}	3.06 ± 0.11^{a}	3.39 ± 0.01^{b}	4.49 ± 0.01^{c}
15	8.9 ± 0.2^{d}	3.10 ± 0.02^{a}	3.59 ± 0.02^{b}	4.79 ± 0.01^{c}
20	9.1 ± 0.1^{d}	3.13 ± 0.11^{a}	4.0 ± 0.2^{b}	5.10 ± 0.1^{c}

Each value represent the mean and standard deviation from three lots. a-c means within a column with a different letter are significantly different ($p<0.05$)

Table 5: Antioxidant activity of *O.basilicum*, *O. gratissimum* and BHT (300 ppm)

No. of days	Control	BHT	O.basilicum	O.gratissimum
0	6.89±0.01	6.89±0.01	6.89±0.01	6.89±0.01
5	8.0 ± 0.2^{d}	2.03 ± 0.05^{a}	2.08 ± 0.13^{a}	3.13 ± 0.15^{b}
10	8.5 ± 0.24^{d}	2.20 ± 0.1^{a}	2.60 ± 0.02^{a}	3.60 ± 0.05^{b}
15	8.9 ± 0.2^{d}	2.20 ± 0.1^{a}	2.96 ± 0.12^{b}	3.90 ± 0.1^{c}
20	9.1 ± 0.1^{d}	2.30 ± 0.1^{a}	3.16 ± 0.15^{b}	4.20 ± 0.1^{c}

Each value represent the mean and standard deviation from three lots. a-c means within a column with a different letter are significantly different ($p<0.05$)

Antioxidant activity of the spice: The antioxidant activities of the spice extracts were compared with the antioxidant activity of BHT at 100 and 300 ppm inclusions, respectively Table 4 and 5. The control (groundnut oil without antioxidant) showed that the peroxide value of the oil increases with storage days. This shows that as storage days increase there is increas in the rate of oxidation of the unsaturated bonds. However, when the natural antioxidants (spice extracts) and the synthetic antioxidant (BHT) were added there were reductions in the peroxide values which were different significantly.

At 100 ppm, the peroxide value of the control on the 0 day was (6.89±0.01) and it increased to 9.1±0.1) on the 20th day .The oil sample treated with BHT had a peroxide values (6.89±0.15 and 3.13±0.11) on the 0 and 20 days, respectively. However, the oil samples treated with spice extracts O.basilicum had values of (6.89±0.01 and 4.02±0.2) for 0-20 days, respectively, while O. gratissimum treated oil had peroxide values reduced from (6.89±0.01 to 5.10±0.1). Though the peroxide value of the BHT treated oil was significantly dirfferent from the oil sample treated with the natural antioxidants these natural extracts could as well act as antioxidant.

At 300 ppm inclusion however, there were no significant difference in the antioxidant activiyies of BHT and O.basilicum on the 5 and 10th days (Table 5). This shows that if the natural antioxidants are added in higher concentrations they would be as effective as the the synthetic antioxidants[5]. But as the storage days

increases there is tendency for the natural antioxidant to loose its antioxidant ability due to evaporation because on the 15th and 20th days the BHT activity was found to be significantly different again (Table 5). This study is in agreement with the research carried out by Wu[15] where they reported that naturally occuring compounds in rosemary extract exhibited antioxidant properties like BHA and equal or slightly less than BHT. The difference in antioxidant effectiveness of natural antioxidants couldbe as a result of plant species and the extraction method[16].

CONCLUSION

This study entails the determination of the antioxidant activities of the *Ocitmum* sp. The proximate composition revealed that the leaves are rich in protein and the Vitamin E content of the extracts are indications to their ability to serve as antioxidants. Thus, the oily, green aromatic extracts from *Ocimum* sp. possessed good antioxidant properties in oil except for the green colour of the extracts.

REFERENCES

1. Giese, J., 1994. Spices and seasoning blends. A taste for all season. Food Tech., 48: 87-98.
2. Shahidi, F. and M. Naizk, 1995. Food phenolic sources, chemistry, effects and application. Technomic, Lancaster, Basel, pp: 235-273.
3. Pomesanz, V., 1991. Functional Properties of Food Components. 2nd Edn. Academic Sci. Diego., pp: 16-20.
4. Buck, D.F., 1991. Antioxidants in food additives, users handbook. Academic, Glagow, pp: 5.
5. Adegoke, G.O., M.V. Kumar, A.G. Gopalakrishna, M.C. Varadaraj, K. Sambacah and B.R. Lokesh, 1998. J. Food Sci. Tech., 35: 283-298.
6. Tindall, H.D., 1983. Vegetables in the Tropics. Macmillan Educational Publisher Ltd. 3rd Edn., pp: 247-248.
7. Chogo, J.B. and G. Grank, 1981. Chemical composition and biological activity of the Tanzanian plant *O. suave*. J. National Prod., 44: 308-311.
8. Sethi, S.C. and J.S. Aggarual, 1950. Stabilization of edible fats by spices and Condiments. Nature, 166: 518-519.
9. AOAC, 1984. Official Methods of Analysis. 14th Edn. Association of Official Analytical Chemistry, Washington, D.C. USA.
10. Adejumo, M.A., 2000. Effect of varying dehydration temperature and processing on the organoleptic properties of local herbs use as flavour Efirin. ND Project Submitted to the Dept. of Food Tech., Osun State Poly. Iree. Osun State Nig.
11. Patter, N.N., 1978. Fats, Oils and their Product. In: Food Science, 3rd Edn., Avc Publishing Co-Inc. Westpart Connecticut, pp: 499-500.
12. Fenema, O.R., 1996. Food Chemistry. 3rd Edn. Library of Congress Cataloging- in-Publication Data.
13. Kaur Charanjit and Kapoor Harish, 2001. Antioxidants in fruits and vegetables-the millennium's health. Intl. J. Food Sci. Tech., 36: 711.
14. Kirk, R. and R. Sawyer, 1991. Pearson's Composition and Analysis of Foods. Longman Publisher London, pp: 1-650.
15. Wu, T.W., M.H. Lie, C.T. Ho and S.S. Chang, 1982. Elucidation of the chemical structure of natural antioxidants isolated from rosemary. J. Am. Oil Chem. Soc., 59: 339.
16. Fernandez-Lopex, J., N. Zhi, L. Aleson-Carbonell, J.A. Perez-Alvarez and V. Kuri, 2004. Antioxidant and antibacteria activities of natural extracts: Application in beef meatballs. Meat Sci., 69: 371-380.