

Effect of Drying Method on the *in vitro* Antioxidant Capacity of Ethanol Extracts of *Ocimum basilicum L.* Leaves.

*Lawrence Edemhanria, Israel Ebhohimen, Augustine Amama,
Bayowale Olubokun and Enthrono Okoh.

Department of Chemical Sciences, Biochemistry Unit, College of Basic and Applied Sciences, Samuel Adegboyega University, Ogwa, Edo State, Nigeria

ABSTRACT

This study investigated the effect of air and sun drying methods on the antioxidant capacity of *Ocimum basilicum L.* leaves. The total antioxidant capacity, nitric oxide scavenging activity, hydroxyl radical scavenging activity, metal chelating activity and ferric reducing power were evaluated using standard spectrophotometric methods against different concentrations (30 to 120µg/ml) of the plant extract and ascorbic acid as reference standard. There was no significant difference in the total antioxidant capacity estimated as ascorbic acid equivalent between the air and sun-dried extracts. A concentration dependent increase in antioxidant capacity was observed in the other assays. The air – dried extract had the highest percentage activity except in the metal chelating and ferric reducing power assays. Hydroxyl radical scavenging activity of the air-dried extract (86.21±1.19%) was significantly higher than ascorbic acid (85.69±0.90%) and the sun-dried extract (77.14±1.19%) at 120µg/ml. The result of this study suggests a better potential for air drying of basil leaves for use as spices, food additive and in traditional medicine.

Keywords: Antioxidant capacity, Drying method, *Ocimum basilicum L.*, Plant extract, Spices, Traditional medicine.

Date of Submission: 20-07-2017

Date of Publication: 19-08-2017

I. INTRODUCTION

Indigenous herbs are used as food, spices and traditional medicines [1],[2]. This is associated with nearly all cultures and civilizations from ancient times to the present day with immense contribution to the health of individuals and the communities [1],[2]. As a result, there is increasing interest in fruits, vegetables and herbs from consumers and researchers [3],[4]. Greater emphasis is also being placed on the use of traditional knowledge of ethnic people in bioprospecting of biological resources as a new source of drugs, food and other industrial and pharmaceutical raw materials [5]. Phytochemicals have antioxidant properties that are of great nutritional and therapeutic values [3],[4]. The most important of these bioactive constituents include alkaloids and phenolic compounds with phenolic compounds being the most abundant [6],[7].

Antioxidants are capable of delaying or preventing oxidative stress caused by free radicals [3],[8] as they have the ability to inhibit reactive oxygen species (ROS) formation, scavenge free radicals, or chelate metals [9]. Several evidences show that oxidative stress play important role in the development of several pathological conditions related to cardiovascular diseases, diabetes, inflammatory diseases, cancer, Alzheimer and Parkinson disease, mongolism, ageing [10],[11]. Dietary intake of natural antioxidants helps to support the body's natural endogenous antioxidant systems to deal with the production of free radicals from cellular and xenobiotic metabolism [6],[12]. Antioxidants can also prevent oxidative damage to food during processing and storage; they are useful in the development of food with low levels of lipid and protein oxidation products [13].

Ocimum basilicum L. (sweet basil) is an aromatic herb that is used as a spice, flavoring agent and preservative in various food products as well as effective drug for many applications in folk medicine especially in Africa and Asia [14]. Phytochemical evaluation of *Ocimum basilicum L.* shows that it is rich in alkaloids, glycosides, phenolic compounds and phytates [13],[15].

In Nigeria and other parts of Africa, sun – drying of the leaves of herbs/vegetables is a popular way of preservation before use [16]. Though there are increasing numbers of research aimed at validation of the acclaimed traditional uses of these herbs, many discrepancies are still present in the determination of the antioxidant potency of the plant extracts as affected by the drying method employed. This formed the basis of this study aimed at investigating the effect of drying method on antioxidant capacity of ethanol extract of the leaves of this herb using different *in vitro* antioxidant assays.

II. MATERIALS AND METHODS

2.1 Collection and Preparation of Sample

Whole *Ocimum basilicum* plants with the vegetative parts were collected from the premises of Samuel Adegboyega University, Ogwa, Edo State. They were authenticated in the Department of Biological Sciences, Samuel Adegboyega University, Ogwa, Edo State. The leaves were removed from the whole plant, rinsed and divided into two parts for drying. A part was dried by direct exposure to sun (sun – drying) at an average temperature of 33°C for five days and the other was exposed to ambient condition in the laboratory (air – drying) at an average temperature of 28°C for seven days. The dried samples were homogenized, macerated in 80% ethanol for 72 hours and filtered. The filtrate was concentrated using rotatory evaporator. Different concentrations of the plant extracts and ascorbic acid: 30, 60, 90, 100 and 120µg/ml were prepared respectively and used for the study.

2.2 Total Antioxidant Capacity (TAC) Assay

The total antioxidant capacity (TAC) of the *Ocimum basilicum* leaf extract was determined by the phosphomolybdate method [17] using ascorbic acid as standard. A portion of the leaf extract, 0.3ml (100µg/ml) was mixed with 3 ml of the reagent solution containing 0.6M sulphuric acid, 28mM sodium phosphate and 4mM ammonium molybdate in different test tubes respectively. The tubes were capped with aluminum foil and incubated in a water bath at 95°C for 90 min. The reaction mixture was allowed to cool to room temperature and the absorbance of the solution was measured at 695 nm against a blank containing 3ml of reagent solution and 0.3ml of distilled water incubated under the same conditions as the test samples. The total antioxidant capacity of the plant extracts were extrapolated from a standard curve prepared using ascorbic acid.

2.3 Hydroxyl Radical Scavenging Assay

Hydroxyl radical scavenging activity of *Ocimum basilicum L.* extract was assayed spectrophotometrically [18]. The reaction mixture containing 1.0ml of 1.5mM FeSO₄.7 (H₂O), 0.7ml of 6mM hydrogen peroxide, 0.3ml of 20mM sodium salicylate and 1ml each of the different concentrations of the extracts or ascorbic acid (standard) solution in different test tubes respectively were incubated at room temperature for one hour and the absorbance measured at 562 nm. Two other setups, one without the leaf extracts or ascorbic acid and the other without sodium salicylate served as control. The percentage hydroxyl radical scavenging activity was calculated using (1):

$$\text{Hydroxyl radical scavenging activity (\%)} = [1 - (A_1 - A_2) / A_0] \times 100 \quad (1)$$

Where:

A₀ is absorbance of the control (without extract or ascorbic acid)

A₁ is the absorbance in the presence of the extracts or ascorbic acid

A₂ is the absorbance without sodium salicylate.

2.4 Determination of Ferric Reducing Power

The ferric reducing power of *Ocimum basilicum L.* extract was determined spectrophotometrically [19]. One (1) ml each of the various concentrations of leaf extracts or ascorbic acid were mixed with 2.5ml of 0.2M phosphate buffer (pH 6.6) and 2.5ml of 1% potassium ferricyanide respectively. The mixtures were incubated at 50°C for 20 min. Thereafter, 2.5ml of 10% trichloroacetic acid, was added to the mixtures and then centrifuged at 3,000 rpm for 10 min. 2.5ml of the supernatant of each solution was mixed with 2.5 ml distilled water and 0.5 ml of 0.1% ferric chloride respectively. The absorbance was measured at 700 nm. Two experiments, one without the plant extracts or ascorbic acid and the other without ferric chloride served as control. The ferric reducing power activity was calculated using (2):

$$\text{Ferric reducing power activity (\%)} = [1 - (A_1 - A_2) / A_0] \times 100 \quad (2)$$

Where:

A₀ is absorbance of the control (without extract or ascorbic acid)

A₁ is the absorbance in the presence of the extract or ascorbic acid

A₂ is the absorbance without ferric chloride.

2.5 Determination of Nitric Oxide Scavenging Activity

The Nitric Oxide scavenging activity of the extracts was determined as described by [20]. Six (6) ml of 5mM sodium nitroprusside solution was mixed with 6ml of the different concentrations of the extracts or ascorbic acid respectively and incubated at 25°C for 120 minutes. Thereafter, 0.5ml of each reaction mixture was removed

and mixed with 0.5 ml of Griess reagent. The absorbance was read at 546 nm. A control experiment was done without the extracts or ascorbic acid. The percentage nitric oxide scavenging activity was calculated using (3):

$$\text{Nitric oxide scavenging activity (\%)} = [(A_0 - A_1) / A_0] \times 100 \quad (3)$$

Where:

A₀ is absorbance of the control (without extracts or ascorbic acid)

A₁ is the absorbance in the presence of the extracts or ascorbic acid

2.6 Ferrous Scavenging (Metal Chelating) Assay

The metal chelating activity of the extracts was measured following the method of [18]. The reaction mixture containing 0.5ml each of the various concentrations of the extracts or ascorbic acid in 1.6 ml of deionized water, 0.05 ml of ferrous chloride (2mM) and 0.1ml of 1,10-phenanthroline (5mM) respectively were incubated at 40°C for 10 min and the absorbance was measured at 562nm. Two other setups, one without the plant extracts and the other without ferric chloride served as control. The percentage metal chelating activity was calculated using (4):

$$\text{Metal chelating activity (\%)} = [1 - (A_1 - A_2)/A_0] \times 100 \quad (4)$$

Where:

A₀ is the absorbance of the control (without the extracts or ascorbic acid)

A₁ is the absorbance of extracts or ascorbic acid

A₂ is the absorbance without ferrous chloride

2.7 Statistical Analysis

All assays were performed in triplicates and results expressed as mean ± standard deviation (SD). Statistical difference was calculated for the total antioxidant capacity assay using Students T-Test and Analysis of variance (ANOVA) was used for determination of statistical differences for the other *in vitro* antioxidant parameters. *P* < 0.05 was taken as significant.

III. RESULTS

3.1 Total Antioxidant Capacity Assay

The total antioxidant capacity of ethanol extracts of the leaves of basil was extrapolated from the ascorbic acid standard curve prepared. The values obtained were 17.78 ± 0.83 µg/ml ascorbic acid equivalent (ambient) and 17.32 ± 1.34 µg/ml ascorbic acid equivalent (sun – dried) respectively. There was no significant difference between these values.

3.2 Nitric Oxide Scavenging Activity

The highest nitric oxide scavenging activity (57.90 ± 6.03%) at 120µg/ml was obtained for air – dried extract compared to 56.84 ± 1.38% for ascorbic acid and 51.25 ± 2.65 % for sun – dried extract respectively. The results obtained for all the concentrations of the extracts and ascorbic acid investigated are presented in Table 1.

Table 1: Percentage nitric oxide scavenging activity of *Ocimum basilicum L. leaf extracts*/ascorbic acid.

Concentration (µg/ml)	Ambient (%)	Sun – dried (%)	Ascorbic acid (%)
30	42.24 ± 5.82	40.62 ± 1.30	36.17 ± 5.29
60	43.60 ± 3.57	44.75 ± 0.64	44.53 ± 3.16
90	50.37 ± 3.89	47.64 ± 3.19	52.13 ± 0.94
120	57.90 ± 6.03	51.25 ± 2.65	56.84 ± 1.38

3.3 Hydroxyl Radical Scavenging Activity

The highest hydroxyl radical scavenging activity (86.21 ± 1.19%) was obtained for air – dried extract compared to 85.69 ± 0.90% for ascorbic acid and 77.14 ± 1.60% for sun – dried extract respectively. The results obtained for all the concentrations of the extracts and ascorbic acid investigated are presented in Table 2.

Table 2: Percentage hydroxyl radical scavenging activity of *Ocimum basilicum L. leaf extracts*/ascorbic acid.

Concentration (µg/ml)	Ambient (%)	Sun – dried (%)	Ascorbic acid (%)
30	60.82 ± 1.07	57.12 ± 1.14	60.64 ± 0.76
60	71.43 ± 1.41	62.28 ± 1.74	65.23 ± 2.14
90	79.20 ± 1.21	69.98 ± 1.70	77.30 ± 1.22
120	86.21 ± 1.19	77.14 ± 1.19	85.69 ± 0.90

3.4 Ferrous Scavenging (Metal Chelating) Activity

The highest ferrous scavenging activity ($88.72 \pm 0.71\%$) was obtained for ascorbic acid compared to $87.19 \pm 2.40\%$ for sun – dried and $86.44 \pm 1.90\%$ for air – dried (ambient) extract respectively. The results obtained for all the concentrations of the extracts and ascorbic acid evaluated are presented in Table 3.

Table 3: Percentage ferrous scavenging activity of *Ocimum basilicum L. leaf extracts*/ascorbic acid.

Concentration ($\mu\text{g/ml}$)	Ambient (%)	Sun – dried (%)	Ascorbic acid (%)
30	76.12 ± 3.11	65.03 ± 1.03	74.58 ± 1.10
60	79.80 ± 2.72	76.72 ± 1.50	80.81 ± 1.66
90	84.87 ± 2.20	80.32 ± 1.02	84.66 ± 0.79
120	86.74 ± 1.90	87.19 ± 2.40	88.72 ± 0.71

3.5 Ferric Reducing Power Activity

The highest ferric reducing power ($65.31 \pm 1.28\%$) was obtained for ascorbic acid compared to $62.57 \pm 0.64\%$ for air – dried and $61.52 \pm 1.76\%$ for sun – dried extract respectively. The results obtained for all the concentrations of the extracts and ascorbic acid investigated are presented in Table 4.

Table 4: Percentage ferric reducing power of *Ocimum basilicum L. leaf extracts*/ascorbic acid.

Concentration ($\mu\text{g/ml}$)	Ambient (%)	Sun – dried (%)	Ascorbic acid (%)
30	51.25 ± 3.43	50.68 ± 1.50	54.42 ± 3.35
60	55.08 ± 2.43	56.55 ± 1.11	58.37 ± 0.84
90	59.12 ± 1.29	59.74 ± 1.42	61.96 ± 0.95
120	62.57 ± 0.64	61.52 ± 1.76	65.31 ± 1.28

IV. DISCUSSION

The biochemical basis for the medicinal and food preservative properties of *Ocimum basilicum L.* leaves is of great importance to researchers as the antioxidant parameters frequently focused on is informed by the health benefits of the constituent phytochemicals [3],[4]. A number of drying techniques have been used to reduce water content, prevent microbial growth and hinder deteriorative biochemical changes as well as improving the quality of the herb [21],[22]. This study investigated air and sun – drying methods. The higher total antioxidant capacity observed in the extract of air-dried leaves is in agreement with the report [23] which showed that air – dried extract of *Cosmos caudatus* had higher total antioxidant capacity compared to oven and freeze dried ones used in the study. Subjecting fresh herbs to air – drying at ambient temperature leads to slow loss of moisture that can induce increased production of phenolic compounds as a stress response defense mechanism by metabolically active plants [22],[24].

4.1 Nitric Oxide Scavenging Activity

The results obtained for the nitric oxide scavenging activity assay showed a concentration – dependent increase for the extracts and the reference standard (ascorbic acid). The values were higher in the extracts compared to ascorbic acid. The results also showed the influence of drying method as the air – dried extracts had higher values compared to the sun – dried one except at the $60 \mu\text{g/ml}$. This is in agreement with other works reported in literature [22],[23].

4.2 Hydroxyl Scavenging Activity

There was a significant difference in the hydroxyl radical scavenging activity assay and also a concentration – dependent increase for the extracts and the reference standard – ascorbic acid. The values were higher in the air – dried extract compared to ascorbic acid. The air – dried extracts had higher values compared to the sun – dried. This is also in agreement with other works reported in literature [22],[23],[25].

4.3 Ferrous Scavenging Activity

Metal ion chelating capacity plays a significant role in antioxidant mechanism; it reduces the concentration of the catalyzing transition metal [25]. The chelating effects of the extract on ferrous ions increased with increasing concentrations of the extracts and ascorbic acid. The air – dried sample had higher values compared to sun – dried extract in agreement with reported literature [22],[23],[25]. However, in comparison with ascorbic acid, the air – dried extract had higher value at $30 \mu\text{g/ml}$, lesser value at $60 \mu\text{g/ml}$ and $120 \mu\text{g/ml}$ with almost the same value at $90 \mu\text{g/ml}$. The differences observed in the values obtained were however not significant.

4.4 Ferric Scavenging Activity

The results obtained for the ferric scavenging activity assay showed a concentration – dependent increase for the extracts and the reference standard – ascorbic acid. The values were higher in the ascorbic acid compared to the extracts. The results also showed the influence of drying method as the air – dried extract had higher values compared to the sun – dried extract except at 60µg/ml concentration (lower) and 90µg/ml (same) respectively. The higher values obtained for ascorbic acid compared to the extracts is in agreement with [25].

V. CONCLUSION

The antioxidant capacity of extracts of *Ocimum basilicum* L. leaves subjected to air - drying method compared well with ascorbic acid used as reference standard in this study. Although there was no significant difference in the total antioxidant capacities of the extracts obtained by both air and sun - drying methods, results of the other *in vitro* antioxidant assays investigated suggest that air – drying method should be preferred whenever drying is required in the preservation of the herb for use either as a spice, food preservation additive or in traditional medicine. However, this preference should be subjected to microbiological and toxicological evaluation.

REFERENCES

- [1]. D. Okwu, Evaluation of the chemical composition of indigenous spices and flavoring agents, *Global Journal of Pure and Applied Science*, 7(3), 2001, 455-459.
- [2]. A. Lino, and O. Deogracious, The *in vitro* antibacterial activity of *Annona senegalensis*, *Securidacca longipendiculata* and *Steanotaenia araliacea* - Ugandan medicinal plants, *African Health Sciences*, 6(1), 2006, 31-35.
- [3]. F. Danesi, and A. Bordoni, Effect of home freezing and Italian style of cooking on antioxidant activity of edible vegetables, *Journal of Food Science*. 73(6), 2008, H109 - H112.
- [4]. B. Halliwell, Are polyphenols antioxidants or pro-oxidants? What do we learn from cell culture and *in vivo* studies? *Archives of Biochemistry and Biophysics*, 476(2), 2008, 107-112.
- [5]. A. K. Dixit, K. Kadavul, S. Rajalakshmi, and M. S. Shekhawat, Ethno-medicobiological studies of South India, *Indian Journal of Traditional Knowledge*, 9(1), 2010, 116-118.
- [6]. G. Rimbach, J. Fuchs, and L. Packer, Application of nutrigenomics tools to analyze the role of oxidants and antioxidants in gene expression, in G. Rimbach, J. Fuchs, L. Packer (Eds.), *Nutrigenomics*, (FL, USA: Taylor and Francis Boca Raton Publishers, 2005), 1-12.
- [7]. A. Fiorentino, B. D'Abrosca, S. Pacifico, C. Mastellone, V. Piscopo, R. Caputo, and P. Monaco, Isolation and structure elucidation of antioxidant polyphenols from quince (*Cydonia vulgaris*) peels, *Journal of Agricultural and Food Chemistry*, 56(8), 2008, 2660-2667.
- [8]. B. Halliwell, and J. M. C. Gutteridge, The antioxidants of human extracellular fluids, *Archives of Biochemistry and Biophysics*, 280(1), 1990, 1-8.
- [9]. V. Panteleon, I. K. Kostakis, P. Marakos, N. Pouli, and I. Andreadou, Synthesis and free radical scavenging activity of some new spiropyranocoumarins, *Bioorganic & Medicinal Chemistry Letters*, 18(21), 2008, 5781-5784.
- [10]. E. Sahlin, G. P. Savage, and C. E. Lister, Investigation of the antioxidant properties of tomatoes after processing, *Journal of Food Composition and Analysis*, 17(5), 2004, 635-647.
- [11]. B. Halliwell, and J. M. C. Gutteridge, *Free radicals in biology and medicine* 4th ed. (New York: Oxford University Press Inc., 2007).
- [12]. M. C. Rojas, and M. S. Brewer, Effect of natural antioxidants on oxidative stability of frozen, vacuum-packaged beef and pork, *Journal of Food Quality*, 31(2), 2008, 173-188.
- [13]. F. Danesi, S. Elementi, R. Neri, M. Maranesi, L. D'Antuono, and A. Bordoni, Effect of Cultivar on the Protection of Cardiomyocytes from Oxidative Stress by Essential Oils and Aqueous Extracts of Basil (*Ocimum basilicum* L.), *Journal of Agricultural and Food Chemistry*, 56(21), 2008, 9911-9917.
- [14]. T. T. Adebolu, and S. A. Oladimeji, Antimicrobial activity of leaf extracts of *Ocimum gratissimum* on selected diarrhoea causing bacteria in south western Nigeria, *African Journal of Biotechnology*, 4(7), 2005, 682-684.
- [15]. I. I. Ijeh, O. U. Njoku, and E. C. Ekenza, Medicinal Evaluation of *Xylopiya aethiopica* and *Ocimum gratissimum*. *Journal of Medicinal Aromatis Science*, 26(1), 2004, 44-54.
- [16]. G. Oboh, and A.A. Akindahunsi, Change in the ascorbic acid, total phenol and antioxidant activity of sun-dried commonly consumed green leafy vegetables in Nigeria, *Nutrition and Health*, 18(1), 2004, 29-36.
- [17]. P. M. Prieto, M. Pineda, and M. Aguilar, Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: specific application to the determination of vitamin E, *Analytical Biochemistry*, 269(2), 1999, 337-341.
- [18]. T. C. P. Dinis, V. M. C. Madeira, and L. M. Almeida, Action of phenolic derivatives (acetaminophen, salicylate, and 5-amino salicylate) as inhibitors of membrane lipid peroxidation and as peroxy radical scavengers, *Archives of Biochemistry and Biophysics*, 315(1), 1994, 161-169.
- [19]. A. Kumaran and R. J. Karunakaran, Antioxidant and free radical scavenging activity of an aqueous extract of *Coleus aromaticus*, *Food Chemistry*, 97(1), 2006, 109-114.
- [20]. S. A. Daljit, and C. Priyanka, Assay of antioxidant potential of two *Aspergillus* isolates by different methods under various physiochemical conditions, *Brazilian Journal of Microbiology*, 41(3), 2010, 465-467.
- [21]. M. C. Diaz-Maroto, M. S. Perez Coello, and M. D. Cabezudo, Effect of different drying methods on the volatile components of parsley (*Petroselinum crispum* L.), *European Food Research Technology*, 215(3), 2002, 227-230.
- [22]. M. B. Hossain, C. Barry-Ryan, A. B. Martin-Diana, and N. P. Brunton, Effect of drying method on the antioxidant capacity of six *Lamiaceae* herbs, *Food Chemistry*, 123(1), 2010, 85-91.
- [23]. A. Mediani, F. Abas, C. P. Tan, and A. Khatib, Effects of different drying methods and storage time on free radical scavenging activity and total phenolic content of *Cosmos caudatus*, *Antioxidants* 3(2), 2014, 358-370.

- [24]. M. J. C. Rhodes, The physiological significance of plant phenolic compounds, *Annual proceedings of the Phytochemical Society of Europe*, Oxford, UK, Oxford University Press, 25, 1985, 99–117.
- [25]. E. A. Uyoh, P. N. Chukwurah, I. A. David, and A. C. Bassey, 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*, 4(2), 2013, 221-229.

Lawrence Edemhanria. "Effect of Drying Method on the in vitro Antioxidant Capacity of Ethanol Extracts of *Ocimum basilicum* L. Leaves." *The International Journal of Engineering and Science (IJES)*, vol. 6, no. 8, 2017, pp. 23–28.