



Drying Methods Alter Angiotensin-I Converting Enzyme Inhibitory Activity, Antioxidant Properties, and Phenolic Constituents of African Mistletoe (*Loranthus bengwensis* L) Leaves

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Abstract

This study investigated the most appropriate drying method (sun drying, oven drying, or air drying) for mistletoe leaves obtained from almond tree. The phenolic constituents were characterized using high-performance liquid chromatography–diode array detector, while the inhibitory effect of the aqueous extracts of the leaves on angiotensin-I converting enzyme (ACE) was determined in vitro as also the antioxidant properties. Oven-dried extract (kidney [276.09 µg/mL] and lungs [303.41 µg/mL]) had the highest inhibitory effect on ACE, while air-dried mistletoe extract (kidney [304.47 µg/mL] and lungs [438.72 µg/mL]) had the least. Furthermore, the extracts dose-dependently inhibited Fe²⁺ and sodium nitroprusside-induced lipid peroxidation in rat's heart and kidney. Also, all extracts exhibited antioxidative properties as typified by their radical scavenging and Fe-chelating ability. Findings from this study revealed that oven drying is the best of the 3 drying methods used for mistletoe obtained from almond host tree, thus confirming that diversity in drying methods leads to variation in phenolic constituents and biological activity of plants.

Keywords

mistletoe, almond, drying, antioxidant, angiotensin-I converting enzyme (ACE)

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Drying is the unique method for producing powder forms of fruits and vegetables. The main benefits of powdering, as compared with fresh fruits and vegetables, are the potential for long storage at ambient temperature and a significant reduction in the costs of transportation and storage. This is particularly important for seasonal and scarce herbs^{1,2} such as mistletoe. Many factors, such as the characteristics of the food material to be dried, the quality of the desired final product, and processing costs, that is, energy and space requirements, are considered before drying.² Díaz-Maroto et al³ stated that drying of herbs inhibits microbial growth and forestalls certain biochemical changes, while Hossain et al⁴ observed that drying can alter the quality of herbs such as changes in appearance and aroma by loss of volatiles or formation of new volatiles. On the contrary, Yousif et al⁵ affirmed that certain compounds that are present in some fresh herbs have been observed to increase in these herbs after drying, while Hung and Tran⁶ reported that drying methods play important roles in the production of herbs, although the bioactive compounds and antioxidant capacities of the herbs might be lost during the process of drying. He stated further that the loss of these bioactive compounds may

affect the herbs' potency in the management of diseases such as hypertension.

Hypertension refers to an increase in arterial pressure that arises from peripheral resistance to blood flow due to increased vasoconstriction (ie, increase in the deposition of atherosclerotic plaque, mainly by cholesterol, around the arterial walls), thereby requiring excess pressure to circulate blood at the normal rate.⁷ One major cause of hypertensive condition is attributed to the action of angiotensin-I converting enzyme (ACE). ACE is involved in maintaining vascular tension as it converts angiotensin I to II, which is a potent vasoconstrictor and stimulator of aldosterone secretion by the adrenal gland.⁸ According to Lieberman,⁹ Inhibition of ACE is considered a

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useful therapeutic approach in the treatment of hypertension, and dietary phenolic phytochemicals have shown promising potential in this regard. Also, Ademiluyi and Oboh¹⁰ reported that plant phenolic compounds inhibit ACE and modulate the production of angiotensin II, hence eliciting an antihypertensive effect.

African mistletoe (*Loranthus bengwensis* L) is a parasitic plant normally found growing on a variety of trees such as cocoa, kola, citrus, almond, cashew, and so on, in the tropical rainforest area of Africa. The antihypertensive property of this plant is demonstrated in rats.¹¹ It was also deduced from the conclusions of Melzer et al¹² that the extract of the plant has both immunomodulatory and cytolytic properties. The leaves of this parasitic plant are usually made into concoctions, infusions, or teas, which are then taken to manage diseases including hypertension. Mistletoe teas have been used for the prevention and management of strokes in parts of Nigeria, as it is believed to improve the circulatory system and heart function in traditional medicine.¹³ Findings by Ademiluyi and Oboh¹⁴ revealed that mistletoe leaves are rich in phenolic compounds with potent antioxidant properties. These phenolic compounds might be responsible for the reported therapeutic properties of the plant in folklore for the treatment of many degenerative diseases. However, information on the effect of drying methods on the phenolic constituents and biological activity of mistletoe leaves is scarce. Hence, this study sought to investigate the influence of different drying methods (sun drying, oven drying, and air drying) on the phenolic constituents, antioxidant properties, and antihypertensive potentials of mistletoe leaves obtained from almond host tree using some in vitro analysis.

Materials and Methods

Sample Collection and Preparation

Mistletoe leaves growing on almond (*Terminalia catappa*) tree were harvested from a farm located at Ilara-mokin near Federal University of Technology, Akure, Ondo State, Nigeria. The authentication of the plants was done at the Department of Crop, Soil and Pest Management, Federal University of Technology, Akure, Nigeria. A voucher specimen was deposited at the Department of Crop, Soil and Pest Management Herbarium with Herbarium No. FUTA/HB/101013LB2. Subsequently, the leaves were rinsed under running tap and then divided into 3 portions. The first part was dried to constant weight using direct sunlight, the second part was dried to constant weight using a heat drying oven at a temperature of 50°C, and the third portion air dried to constant weight at room temperature and kept away from the intensity of the sun. The dried samples were then milled into fine powder. The aqueous extraction was carried out as described by Oboh et al.¹⁵

Chemicals and Reagents

All chemicals used were sourced from Sigma Co (St Louis, MO). Except where stated otherwise, all the chemicals and reagents used are of analytical grade, while the water used was glass distilled.

Assays

Angiotensin-I Converting Enzyme Assay. Appropriate dilution of the extracts (0-500 µL) and ACE solution (50 µL, 4 mU) was incubated at 37°C for 15 minutes. The enzymatic reaction was initiated by adding 150 µL of 8.33 mM of the substrate Bz-Gly-His-Leu in 125 mM Tris-HCl buffer (pH 8.3) to the mixture. After incubation for 30 minutes at 37°C, the reaction was arrested by adding 250 µL of 1 M HCl. The Bz-Gly produced by the enzymatic reaction was extracted with 1.5 mL ethyl acetate. Thereafter the mixture was centrifuged to separate the ethyl acetate layer; then 1 mL of the ethyl acetate layer was transferred to a clean test tube and evaporated. The residue was redissolved in distilled water and its absorbance was measured at 228 nm.¹⁶ The ACE inhibitory activity was expressed as percentage inhibition.

Lipid Peroxidation and Thiobarbituric Acid Reactions. Rats were decapitated under mild diethyl ether anesthesia and the heart and kidney were rapidly isolated and placed in ice and weighed. These tissues were subsequently homogenized differently in cold saline (1/10 w/v) with about 10 up-and-down strokes at approximately 1200 rev/min in a Teflon glass homogenizer. The homogenate was centrifuged for 10 minutes at 3000 × g to yield a pellet that was discarded, and a low-speed supernatant (SI) was kept for lipid peroxidation assay.¹⁷ The lipid peroxidation assay was carried out using the modified method of Ohkawa et al.¹⁸ Briefly 100 µL SI fraction was mixed with a reaction mixture containing 30 µL of 0.1 M, pH 7.4, Tris-HCl buffer, extracts (0-100 µL), and 30 µL of 250 µM freshly prepared FeSO₄ (the procedure was also carried out using 7 µM sodium nitroprusside). The volume was made up to 300 µL by water before incubation at 37°C for 1 hour. The reaction was developed by adding 300 µL 8.1% sodium dodecyl sulfate to the reaction mixture and this was subsequently followed by the addition of 600 µL of acetic acid/HCl (pH 3.4) and 600 µL 0.8% thiobarbituric acid. This mixture was incubated at 100°C for 1 hour and the thiobarbituric acid reactive species produced were measured at 532 nm. Subsequently, lipid peroxidation was calculated as malondialdehyde (MDA) produced (percentage of control) using MDA as the standard.

Inhibition of Fenton Reaction (Degradation of Deoxyribose). The method of Halliwell and Gutteridge¹⁹ was used to determine the ability of the extract to prevent Fe²⁺/H₂O₂-induced decomposition of deoxyribose. The extract (0-100 µL) was added to a reaction mixture containing 120 µL of 20 mM deoxyribose, 400 µL of 0.1 M phosphate buffer, 40 µL of 500 µM FeSO₄, and the volume was made up to 800 µL with distilled water. The reaction mixture was incubated at 37°C for 30 minutes and the reaction was then stopped by the addition of 0.5 mL of 2.8% trichloroacetic acid. This was followed by addition of 0.4 mL of 0.6% thiobarbituric acid solution. The tubes were subsequently incubated in boiling water for 20 minutes and the absorbance was measured at 532 nm in a spectrophotometer.

2,2'-Azino-bis(3-ethylbenzthiazoline-6-sulphonic acid) (ABTS⁺) Radical Scavenging Ability. The ABTS⁺ scavenging ability of the extracts was determined according to the method described by Re et al.²⁰ The ABTS⁺ was generated by reacting 7 mM ABTS aqueous solution with K₂S₂O₈ (2.45 mM, final concentration) in the dark for 16 hours and adjusting the absorbance 734 nm to 0.700 with ethanol. Thereafter, 200 µL of appropriate dilution of the extract was added to 2.0 mL ABTS⁺ solution and the absorbance was measured at 734 nm

after 15 minutes. The trolox equivalent antioxidant capacity (TEAC) was subsequently calculated using trolox as the standard.

Nitric Oxide Radical Scavenging Assay. The scavenging effect of the extract on nitric oxide (NO) radical was measured according to the method of Marcocci et al.²¹ Samples of the extract (100-400 μ L) were added in test tubes to 1 mL of sodium nitroprusside solution (25 mM) and tubes incubated at 37°C for 2 hours. An aliquot (0.5 mL) of the incubation was removed and diluted with 0.3 mL Griess reagent (1% sulfanilamide in 5% H₃PO₄ and 0.1% naphthylethylenediamine dihydrochloride). The absorbance of the chromophore formed was immediately read at 570 nm against distilled water as blank. Results were expressed as percentage radical scavenging activity.

1,1-Diphenyl-2-picrylhydrazyl (DPPH) Free Radical Scavenging Ability. The free radical scavenging ability of the extracts against DPPH free radical was evaluated as described by Gyamfi et al.²² Briefly, appropriate dilution of the extracts (0-500 μ L) was mixed with 1 mL, 0.4 mM methanolic solution containing DPPH radicals; then the mixture was left in the dark for 30 minutes and the absorbance was taken at 516 nm. The DPPH free radical scavenging ability was subsequently calculated.

Determination of Reducing Property. The reducing property of the extracts was determined by assessing the ability of the extract to reduce FeCl₃ solution as described by Oyaizu.²³ A total of 2.5 mL aliquot was mixed with 2.5 mL 200 mM sodium phosphate buffer (pH 6.6) and 2.5 mL 1% potassium ferricyanide. The mixture was incubated at 50°C for 20 minutes and then 2.5 mL 10% trichloroacetic acid was added. This mixture was centrifuged at 650 \times g for 10 minutes. Five milliliters of the supernatant was mixed with an equal volume of water and 1 mL 0.1% ferric chloride. The absorbance was measured at 700 nm. The ferric reducing antioxidant property (FRAP) was subsequently calculated.

Fe²⁺ Chelating Assay. The Fe²⁺ chelating ability of the extracts was determined using a modified method of Minnoti and Aust.²⁴ Freshly prepared 500 μ M FeSO₄ (150 μ L) was added to a reaction mixture containing 168 μ L 0.1 M Tris-HCl (pH 7.4), 218 μ L saline, and the extracts (0-25 μ L). The reaction mixture was incubated for 5 minutes, before the addition of 13 μ L 0.25% 1,10-orthophenanthroline (w/v). The absorbance was subsequently measured at 510 nm in a spectrophotometer. The Fe²⁺ chelating ability was subsequently calculated as a percentage.

Determination of Ascorbic Acid Content. Vitamin C (ascorbic acid) content of extracts of the samples was determined using the method of Benderitter et al.²⁵ Briefly, 75 μ L DNPH (2 g dinitrophenylhydrazine, 230 mg thiourea, and 270 mg CuSO₄·5H₂O in 100 mL of 5 M H₂SO₄) was added to 500 μ L of the reaction mixture (300 μ L of the test sample with 100 μ L of 13.3% trichloroacetic acid and water). The reaction mixture was subsequently incubated for 3 hours at 37°C, then 0.5 mL of 65% H₂SO₄ (v/v) was added to the medium and the absorbance was measured at 520 nm. The vitamin C content of the samples was subsequently calculated.

Determination of Total Phenol Content. The total phenol content was determined according to the method of Singleton et al.²⁶ Briefly,

appropriate dilutions of the extracts (200 μ L) was oxidized with 2.5 mL 10% Folin-Ciocalteu's reagent (v/v) and neutralized by the addition of 2.0 mL of 7.5% sodium carbonate. The reaction mixture was incubated for 40 minutes at 45°C and the absorbance was measured at 765 nm in a spectrophotometer. The total phenol content was subsequently calculated as gallic acid equivalent.

Determination of Total Flavonoid Content. The total flavonoid content was determined using a slightly modified method reported by Meda et al.²⁷ Briefly, 0.5 mL of appropriately diluted sample was mixed with 0.5 mL methanol, 50 μ L 10% AlCl₃, 50 μ L of 1 M potassium acetate, and 1.4 mL distilled water, and allowed to incubate at room temperature for 30 minutes. The absorbance of the reaction mixture was subsequently measured at 415 nm; the total flavonoid content was subsequently calculated. The non-flavonoid polyphenols were taken as the difference between the total phenol content and total flavonoid content.

High-Performance Liquid Chromatography-Diode Array Detector (HPLC-DAD) Characterization of the Phenolic Constituents. Two-stage extraction procedures were followed for the removal of the phenolic compounds as described by Kelley et al.²⁸ with slight modification by Provan et al.²⁹

Stage 1. The sample was centrifuged (5000 \times g), rinsed with water, centrifuged again, and the supernatants were combined and placed in a disposable glass test tube and heated at 90°C for 2 hours to release the conjugated phenolic compounds as described by Whitehead et al.³⁰ The heated extract was cooled, titrated with 4 M HCl to pH <2.0, diluted to 10 mL, with de-ionized water, and centrifuged to remove the precipitate. The supernatant was saved for subsequent purification and the residue was extracted further in stage 2.

Stage 2. The residue from stage 1 was extracted with 5 mL of 4 M NaOH and heated to 160°C. After cooling, the mixture was filtered. The supernatant was collected and the residue washed with water (de-ionized). The supernatants were combined and adjusted to pH < 2.0 with 4 M HCl. The filtrates were combined for further preparation.

Purification. An aliquot (5-15 mL) of the various supernatants was passed through a conditioned Varian (Varian Associates, Harbor City, CA) Bond Elut PPL (3 mL size with 200 mg packing) solid-phase extraction tube at ~5 mL/min attached to a Visiprep (Supelco, Bellefonte, PA). The tubes were then placed under vacuum (-60 kPa) until the resin was thoroughly dried after which the phenolic compounds were eluted with 1 mL of ethyl vials. The PPL tubes were conditioned by first passing 2 mL of ethyl acetate followed by 2 mL of water (pH < 2.0). Purified phenolic extracts (1 μ L: 10:1 split) were analyzed for composition by comparison with phenolic standards (Aldrich Chemical Co, Milwaukee, WI) and chromatography with standards on a Waters 600 High Performance Liquid Chromatograph LCD System equipped with Waters 515 HPLC pump, Waters 2487 UV/VIS detector, C18 column with dimensions 5 μ m, 4.6 \times 250 mm with Hamilton microliter syringe, and injection volume of 20 μ L. The following conditions were employed per separation: wavelength, 280 nm; flow rate, 1.0 mL/min; gradient elution total run time of 31 minutes, having Solvent A as acetonitrile, solvent B as 0.1% phosphoric acid in de-ionized water, which was started with 85% A and held at this for 13 minutes. This was followed by 75% eluent B for 10 minutes and then the concentration of B was increased to 85% for another 8 minutes.

Data Analysis

The results of the replicate experiments were pooled and expressed as mean \pm standard deviation (SD). A one-way analysis of variance (ANOVA) was used to analyze the mean. The post hoc treatment was performed using the Duncan multiple range test. Significance was accepted at $P < .05$.³¹ The EC₅₀ (extract concentration causing 50% enzyme inhibition/antioxidant activity) was performed using non-linear regression analysis.

Results and Discussion

ACE Activity

Table 1 presents the effect of some drying methods on the in vitro kidney and lungs ACE inhibitory activity of mistletoe leaves extract harvested from almond host tree. The result revealed that the extracts inhibited kidney ACE activity in a dose-dependent manner (0-357.14 $\mu\text{g/mL}$). Furthermore, as revealed by the EC₅₀ values (Table 1), the air-dried sample exhibited the least ACE inhibitory activity. However, oven-dried mistletoe (277.78 $\mu\text{g/mL}$) had the highest inhibitory effect on the kidney ACE activities. This same trend was observed in the lung ACE activity where air-dried mistletoe leaves extract had the least ACE inhibitory activity while the oven-dried mistletoe leaves extract had the highest ACE inhibitory activities (Table 1). Nevertheless, the ACE inhibitory activities of the samples were more pronounced in the kidney than in the lungs.

The use of African mistletoe (*Loranthus bengwensis* L) in the treatment/management of hypertension is established in folklore, and findings have corroborated the antihypertensive effect of mistletoe in experimental animals.¹¹ Also, previous studies have revealed that mistletoes are rich in phenolic compounds with strong antioxidant properties.¹⁴ Hence, the ACE inhibitory activity of the mistletoe leaves could be attributed to their phenolic content and this is evident in its agreement with the total phenol, total flavonoid, and the phenolic constituents as revealed by HPLC as well as antioxidative properties in this study. Furthermore, studies have reported that the ACE inhibitory property of plants and plant foods is a function of the type and amount of its constituent phenolic compounds.^{32,33} However, the ACE inhibitory activity of the differently dried mistletoe leaves extract is consistent with earlier studies on ACE inhibitory activity of plant phenolic extracts.^{10,34}

ACE is a known powerful vasoconstrictor that cleaves angiotensin-I to form angiotensin-II and has been identified as a major factor in hypertension.³⁵ As a result, ACE inhibitors have been widely developed to prevent angiotensin-II production in cardiovascular disease and utilized in clinical applications since the discovery of ACE inhibitor in snake venom.³⁶ Therefore, this ACE inhibitory activity of the mistletoe leaves indicates an antihypertensive property and could provide the biochemical basis for its use as antihypertensive agent in traditional medicine and provide useful information in the discovery of alternative/complementary therapy in the management of hypertension.

Table 1. EC₅₀ Values of the Effects of Drying Methods on Angiotensin-I Converting Enzyme (ACE) Inhibitory Activity of Mistletoe Leaves From Almond Tree in Rat Kidney and Lungs*[†].

Sample	EC ₅₀ for ACE inhibition ($\mu\text{g/mL}$)	
	Kidney	Lungs
Oven-dried mistletoe	277.78 \pm 2.3 ^a	344.83 \pm 5.2 ^a
Sun-dried mistletoe	297.62 \pm 1.8 ^b	359.71 \pm 6.9 ^b
Air-dried mistletoe	357.14 \pm 3.0 ^c	495.05 \pm 11.1 ^c

*Values represent mean \pm standard deviation (n = 3).

[†]Values with the same superscript letter on the same column are not significantly ($P < .05$) different.

Lipid Peroxidation and Thiobarbituric Acid Reactions

Incubation of rat's heart in the presence of 250 μM FeSO₄ caused a significant increase ($P < .05$) in the MDA content (Table 2). However, all the mistletoe extracts inhibited MDA production in heart in a dose-dependent (0-155.89 $\mu\text{g/mL}$) manner as represented by the EC₅₀ values in Table 2. Likewise, incubation of the rat's heart in the presence of 7 μM sodium nitroprusside also caused a significant increase ($P < .05$) in the heart MDA content with the mistletoe extracts also inhibiting the production of MDA in the tissue homogenates.

There was no significant ($P > .05$) difference in the inhibition of Fe²⁺-induced MDA production in the heart by the mistletoe extracts as affected by the air-dried and sun-dried samples. However, oven drying caused the highest inhibition among the 3 drying methods. This same trend was also observed when sodium nitroprusside was used as the pro-oxidant (Table 2). Furthermore, rat kidney homogenate was separately assaulted with 250 μM Fe²⁺ and 7 μM sodium nitroprusside and this led to significant increase ($P < .05$) in the MDA content (Table 2). However, incubating these challenged kidney homogenates in the presence of the mistletoe extracts resulted in the inhibition of MDA production dose-dependently (0-164.74 $\mu\text{g/mL}$).

The inhibition of lipid peroxidation induced by various pro-oxidants (Fe²⁺ and sodium nitroprusside) in both rat's heart and kidney homogenates by differently dried mistletoe extracts is an indication of potent antioxidant capacity. However, the effect of drying methods on this property suggests its dependence on the total phenol content and phenolic constituents.

This assertion is in line with Chu et al³⁷ that established a strong correlation between the antioxidant properties of plants and their phenolic constituents. Inhibition of lipid peroxidation has been identified as an antioxidant mechanism of action. Furthermore, the free cytosolic Fe has been implicated in the initiation of lipid peroxidation in biological systems.³⁸ Peroxidation of biomolecules and biological membranes has been linked to the etiology and progression of a number of diseases with hypertension being one of them.

OH⁻ Radical Scavenging Ability

The hydroxyl radical (OH[•]) scavenging ability of differently dried mistletoe leaves extract obtained from almond host tree

Table 2. EC₅₀ Values of the Effects of Some Drying Methods on the Inhibition of Sodium Nitroprusside (SNP) and Fe²⁺-Induced Lipid Peroxidation in Rat Kidney and Heart by Mistletoe Leaves From Almond Tree*†.

Tissue →	EC ₅₀ for Inhibition of SNP and Fe ²⁺ -Induced Lipid Peroxidation (μg/mL)			
	Kidney		Heart	
	SNP	Fe ²⁺	SNP	Fe ²⁺
Prooxidants →				
Sample ↓				
Oven-dried mistletoe	118.38 ± 1.8 ^a	141.84 ± 0.7 ^a	136.56 ± 2.6 ^a	145.76 ± 2.2 ^a
Sun-dried mistletoe	123.16 ± 1.0 ^b	156.56 ± 1.2 ^b	140.11 ± 2.4 ^b	152.55 ± 2.3 ^b
Air-dried mistletoe	129.34 ± 2.3 ^c	164.74 ± 3.6 ^c	147.07 ± 1.5 ^c	155.89 ± 1.7 ^b

*Values represent mean ± standard deviation (n = 3).

†Values with the same superscript letter on the same column are not significantly (P < .05) different.

Table 3. Effects of Drying Methods on the OH⁻, NO, DPPH Radical Scavenging and Fe²⁺ Chelating Ability of Mistletoe Leaves Harvested From Almond Host Tree*†.

Sample	EC ₅₀ for Scavenging and Chelating Abilities (μg/mL)			
	OH*	NO*	DPPH*	Fe ²⁺ Chelation
Oven-dried mistletoe	294.12 ± 8.8 ^a	403.23 ± 2.8 ^a	581.40 ± 11.0 ^a	340.14 ± 5.2 ^a
Sun-dried mistletoe	384.62 ± 11.8 ^b	427.35 ± 1.7 ^b	675.68 ± 16.5 ^b	362.32 ± 6.9 ^b
Air-dried mistletoe	537.63 ± 21.2 ^c	515.46 ± 4.2 ^c	1063.83 ± 19.2 ^c	406.50 ± 5.8 ^c

*Values represent mean ± standard deviation (n = 3).

†Values with the same superscript letter on the same column are not significantly (P < .05) different.

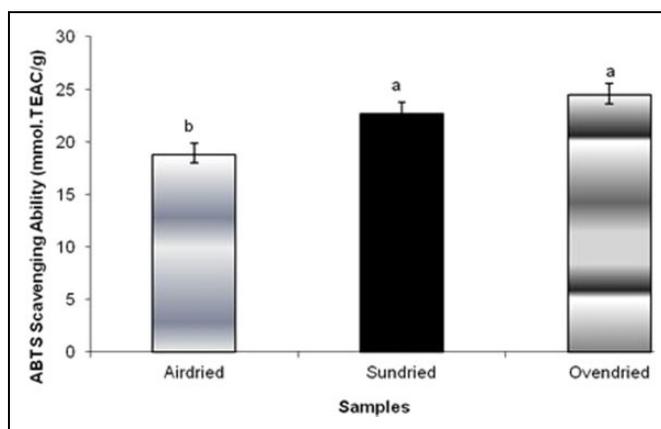
is presented in Table 3. This revealed that the extracts scavenged OH⁻-induced decomposition of deoxyribose in Fenton reaction in a dose-dependent manner (0-537.63 μg/mL). Furthermore, as revealed by the EC₅₀ values (Table 3), oven-dried mistletoe leaves extract (294.12 μg/mL) had the highest scavenging ability followed by the sun-dried extract (384.62 μg/mL), while the air-dried extract (537.63 μg/mL) had the least.

It is an indication that participation of Fe in the Fenton reaction will lead to generation of reactive oxygen species such as OH⁻ radical with potency to attack the polyunsaturated fatty acids of the cell membrane, thereby triggering a chain reaction of peroxidized molecules and eventually cell disruption and death. Hence, Fe²⁺ and OH radical scavenging ability of plant foods and herbs is accepted as effective antioxidant defense mechanisms.³⁸

ABTS⁺, NO, and DPPH Radical Scavenging Abilities

The ABTS⁺ scavenging ability of the mistletoe leaves extract presented as TEAC in Figure 1 revealed that all the extracts scavenged ABTS⁺. However, the air-dried sample (17.68 mmol TEAC/μg) had significantly (P < .05) lower ABTS⁺ scavenging ability compared to its corresponding sun-dried (22.21 mmol TEAC/μg) and oven-dried (24.67 mmol TEAC/μg) samples.

As revealed by the EC₅₀ values in Table 3, the extract of mistletoe leaves that are dried with an oven showed the highest NO free radical scavenging ability followed by the sun-dried sample while the air-dried sample showed the least. Furthermore, the DPPH free radical scavenging abilities of extracts

**Figure 1.** Effects of drying methods on the ABTS radical scavenging ability of mistletoe leaves from almond tree.

of mistletoe leaves subjected to oven drying, sun drying, and air drying revealed that all the extracts scavenged DPPH radicals in a dose-dependent pattern (0-1063.84 μg/mL) as presented in Table 3. The extract from oven-dried mistletoe carried (581.40 μg/mL) the highest DPPH free radical scavenging ability while air-dried sample (1063.84 μg/mL) showed the least scavenging ability as revealed by the EC₅₀ values in Table 3.

The mistletoe leaves demonstrated strong free radical scavenging abilities as exemplified by their scavenging activity of moderately stable ABTS⁺, NO, and DPPH in vitro. There was agreement between the ABTS⁺, NO, and DPPH free radical scavenging ability, with the extract from oven-dried

mistletoe leaves having the highest radical scavenging abilities. The radical scavenging ability of the mistletoe extracts also followed the trends for both the total phenol and flavonoid contents. These findings agreed with earlier discoveries of Chu et al³⁷ as well as Ademiluyi and Oboh¹⁴ that plant antioxidant properties (free radical scavenging ability) correlates with their phenolic content.

Free radicals may play an important role in the causation and complications of hypertension. Alterations in the endogenous free radical scavenging defense mechanisms (associated with hypertension) could lead to ineffective scavenging of reactive oxygen species, resulting in oxidative damage and tissue injury. Hence, steady supply of dietary antioxidants to augment the endogenous antioxidant defense mechanisms could be one practical approach through which free radical-mediated oxidative stress in hypertension may be curtailed. Simi et al³⁹ demonstrated that elevated consumption of plant antioxidants is accompanied by increased activity of extracellular antioxidant enzymes such as glutathione peroxidase and superoxide dismutase.

Ferric Reducing Antioxidant Property and Fe^{2+} Chelating Ability

The FRAP of the mistletoe leaves extract reported as ascorbic acid equivalent (AAE) is presented in Figure 2. The air-dried extracts (12.61 mg/AAE g) had significantly ($P < .05$) lower FRAP compared to the sun-dried (20.57 mg/AAE g) and oven-dried (31.58 mg/AAE g) samples. Also, as shown in Table 3, all the extracts chelate Fe^{2+} following the same trend with the extracts' free radical scavenging abilities oven dried (339.21 μ g/mL), sun dried (361.79 μ g/mL), and air dried (423.55 μ g/mL).

Likewise, the FRAP result from this study showed that mistletoe leaves extract demonstrated strong reducing properties; however, oven-dried sample had the highest reducing power while its corresponding air-dried sample had the least. This trend also agreed with the total phenol and flavonoid contents, in addition to the radical scavenging abilities, which is in line with earlier reports by Chu et al³⁷ as well as Yuan et al,⁴⁰ which stated that antioxidant activity of plant foods are a function of their phenolic content.

Reducing power is an antioxidation defense mechanism that deals with both electron and hydrogen atom transfer.⁴¹ This is determined in vitro by the ability of an antioxidant compounds to reduce Fe^{3+} to Fe^{2+} . This is because the ferric-to-ferrous ion reduction occurs rapidly with all reductants with half reaction reduction potentials above that of Fe^{3+}/Fe^{2+} ; the values in the FRAP assay will express the corresponding concentration of electron-donating antioxidants.⁴²

Furthermore, drying methods were also found to alter the Fe^{2+} chelating property of the differently dried mistletoe leaves extract. The high Fe^{2+} chelating property of the oven-dried mistletoe from almond tree also agreed with its phenolic content and radical scavenging abilities. The ability of antioxidants to chelate and deactivate transition metals prevent such metals from participating in the initiation of lipid peroxidation and

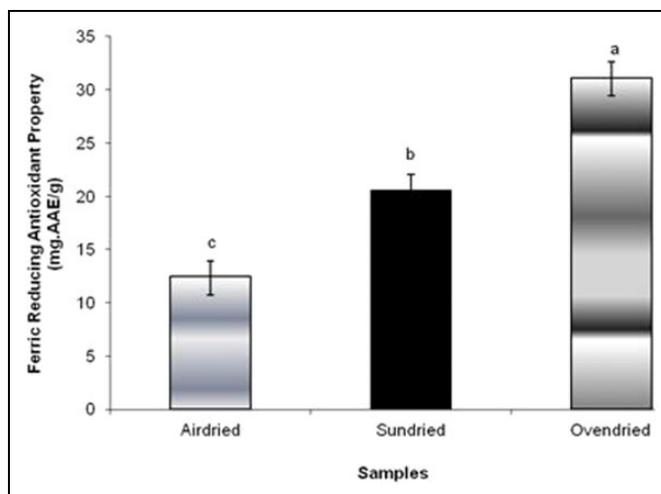


Figure 2. Effects of drying methods on the ferric reducing antioxidant properties of mistletoe leaves from almond tree.

Table 4. Total Phenol, Flavonoid, and Vitamin C Contents of Aqueous Extract of 3 Differently Dried Mistletoe Leaves From Almond Host Tree*[†].

Sample	Total Phenol (mg GAE/100 g)	Total Flavonoid (mg QUE/100 g)	Vitamin C (mg AAE/100 g)
Oven-dried mistletoe	10.26 ± 0.25 ^a	4.05 ± 0.21 ^a	17.33 ± 1.03 ^a
Sun-dried mistletoe	8.28 ± 0.13 ^b	3.23 ± 0.10 ^b	14.25 ± 0.45 ^b
Air-dried mistletoe	6.60 ± 0.22 ^c	2.09 ± 0.11 ^c	8.09 ± 0.19 ^c

*Values represent mean ± standard deviation (n = 3).

[†]Values with the same superscript letter on the same column are not significantly ($P < .05$) different.

oxidative stress through metal catalyzed reaction is an important antioxidant defense mechanism.¹⁵ The least Fe^{2+} chelating property of the extracts of air-dried mistletoe leaves also agreed with their phenolic content and radical scavenging ability. This is totally consistent with previous a study, which showed that the antioxidant properties of plant food are directly proportional to their phenolic contents.³⁷

Total Phenol, Flavonoid, and Vitamin C Contents

The results of the total phenol, flavonoid, and vitamin C contents of aqueous extracts of differently dried mistletoe leaves harvested from almond host tree were assessed and the results, as shown in Table 4, revealed that the oven-dried sample (10.26 mg GAE/100 g) had the highest total phenol content followed by the sun-dried sample (8.28 mg GAE/100 g) while the air-dried sample (6.60 mg GAE/100 g) had the least. Furthermore, total flavonoid content followed the same trend with the result of total phenol content as the oven-dried sample (4.05 mg QUE/100 g) had the highest flavonoid content followed by the sun-dried sample (3.23 mg QUE/100 g) while the air-dried sample (2.09 mg QUE/100 g) had the least. However, for the vitamin

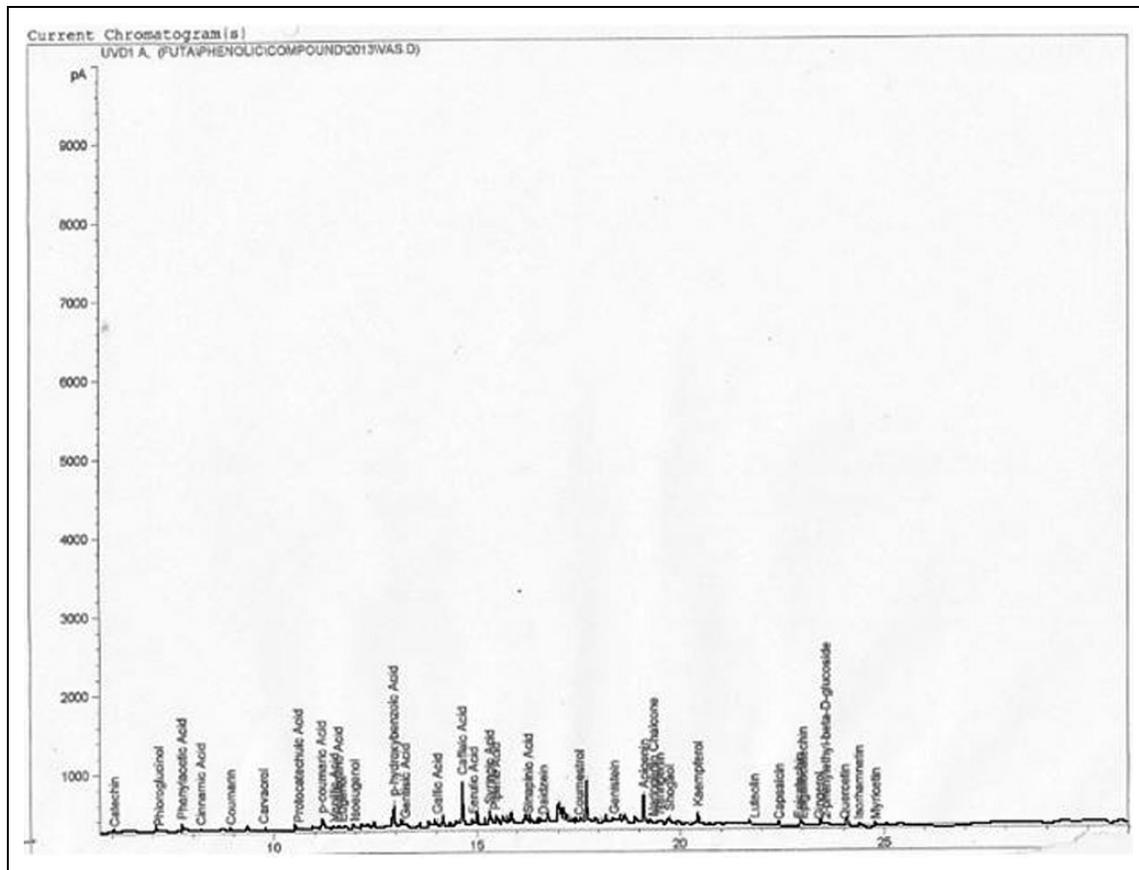


Figure 5. HPLC chromatogram of sun-dried mistletoe leaves harvested from almond host tree.

Table 5. Effect of Some Drying Methods on the Phenolic Constituents of Mistletoe (*Loranthus bengwensis* L) Leaves Harvested From Almond Host Tree*[†].

Drying Methods →	Amount of Phenolic Compounds in Mistletoe Leaves (mg/100 g)		
	Air-Dried	Sun-Dried	Oven-Dried
Phenolics ↓			
Protocatechuic acid	17.72 ± 0.60 ^c	23.43 ± 0.72 ^b	30.93 ± 1.06 ^a
p-Coumaric acid	38.19 ± 1.02 ^c	41.58 ± 1.61 ^b	49.07 ± 1.30 ^a
o-Coumaric acid	0.01 ± 0.01 ^c	0.30 ± 0.06 ^b	0.50 ± 0.10 ^a
Vanillic acid	0.02 ± 0.01 ^c	0.35 ± 0.05 ^b	0.55 ± 0.18 ^a
p-Hydroxybenzoic acid	0.01 ± 0.01 ^b	0.99 ± 0.02 ^a	0.95 ± 0.06 ^a
Gentisic acid	18.24 ± 0.62 ^c	20.74 ± 1.12 ^b	24.40 ± 1.00 ^a
Gallic acid	0.04 ± 0.01 ^b	0.16 ± 0.02 ^a	0.15 ± 0.06 ^a
Caffeic acid	297.42 ± 10.6 ^c	321.51 ± 12.6 ^b	399.93 ± 15.2 ^a
Ferrulic acid	0.01 ± 0.01 ^a	0.02 ± 0.01 ^a	0.02 ± 0.01 ^a
Syringic acid	0.01 ± 0.01 ^b	0.02 ± 0.01 ^b	0.14 ± 0.02 ^a
Apigenin	7.58 ± 0.72 ^c	10.16 ± 0.88 ^b	11.67 ± 0.42 ^a
Kaempferol	10.42 ± 0.56 ^c	14.76 ± 0.72 ^b	19.86 ± 1.02 ^a
Luteolin	26.26 ± 1.06 ^c	30.00 ± 2.02 ^b	38.48 ± 2.06 ^a
Gingerol	0.004 ± 0.002 ^b	0.001 ± 0.001 ^b	0.009 ± 0.002 ^a
Quercetin	37.80 ± 2.00 ^c	47.52 ± 3.06 ^b	54.00 ± 2.30 ^a
Isorhamnetin	0.064 ± 0.02 ^a	0.005 ± 0.002 ^b	0.008 ± 0.002 ^b
Myricetin	5.64 ± 0.50 ^c	8.03 ± 0.90 ^b	10.00 ± 0.62 ^a
Phenol	0.033 ± 0.002 ^a	—	0.026 ± 0.012 ^a
Phenylacetic acid	0.046 ± 0.02 ^c	0.147 ± 0.06 ^b	0.227 ± 0.08 ^a

*Values represent mean of triplicate analysis.

[†]Values with the same superscript letter on the same row are not significantly different.

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Author Contributions

GO participated actively in the design of the experiment and provided mentorship support. AOA participated in the design of the experiment, supervised the experiment, and carried out data analysis. OSO participated in the design of the research, conducted the experiment, and drafted the article. All authors read and approved the article for publication.

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Ethical Approval

The ethics regulation standards of the Helsinki Declaration of 1975 as revised in 2000 was followed strictly in accordance with Federal University of Technology, Akure guidelines for laboratory experiments and the protection of animals during experiments (Reference number FUTA/SOS/1410). The experiment was carried out at the Functional Food, Nutraceuticals and Phytomedicine Laboratory, Department of Biochemistry, Federal University of Technology, Akure, Ondo State, Nigeria.

References

1. Fellows PJ. *Food Processing Technology, Principles and Practice*. 2nd ed. Cambridge, England: Woodhead; 2000.
2. Tang J, Yang T. Dehydrated vegetables: principles and systems. In Hui YH, Chazala S, Graham DM, Murrell KD, Nip WK, eds. *Handbook of Vegetable Preservation and Processing*. New York, NY: Marcel Dekker; 2004:335-372.
3. Díaz-Maroto MC, Pérez-Coello MS, Cabezudo MD. Effect of different drying methods on the volatile components of parsley (*Petroselinum ispum* L). *Eur Food Res Technol*. 2002;215:227-230.
4. Hossain M, Barry-Ryan C, Martin-Diana A, Brunton N. Effect of drying methods on the antioxidant capacity of six Lamiaceae herbs. *Food Chem*. 2010;123:85-91.
5. Yousif AL, Scaman CH, Durance TD, Girard B. Flavor volatiles and physical properties of vacuum-microwave and air dried sweet basil (*Ocimum basilicum* L.). *J Agric Food Chem*. 1999;47:4777-4781.
6. Hung PV, Tran DL. Effects of drying methods on bioactive compounds of vegetables and correlation between bioactive compounds and their antioxidants. *Int Food Res J*. 2012;19:327-332.
7. Bullock J, Boyle J, Wang MB. Hypertension. In: *Physiology*. 3rd ed. London, England: Williams & Wilkins; 1995:189-190.
8. Skeggs LT, Khan JR, Shumway NP. The preparation and function of the hypertension-converting enzyme. *J Exp Med*. 1956;103:295-299.
9. Lieberman J. Elevation of serum angiotensin-converting-enzyme (ACE) level in sarcoidosis. *Am J Med*. 1975;59:365-372.
10. Ademiluyi AO, Oboh G. Phenolic-rich extract from selected tropical underutilized legumes inhibit α -amylase, α -glucosidase and angiotensin I converting enzyme in vitro. *J Basic Clin Physiol Pharmacol*. 2012;24:17-25
11. Obatomi DK, Bikomo EO, Temple VJ. Effect of African mistletoe extract on blood pressure in spontaneously hypertensive rat. *J Pharm Biol*. 1996;34:124-127.
12. Melzer J, Iten F, Hostanska K, Saller R. Efficacy and safety of mistletoe preparations (*Viscum album*) for patients with cancer diseases. *Forsch Komplementmed*. 2009;16:217-226.
13. Deeni YY, Sadiq NM. Antimicrobial properties and phytochemical constituents of the leaves of African mistletoe (*Tapinanthus dodoneifolius* (DC) Danser) (Loranthaceae): an ethnomedicinal plant of Hausaland, Northern Nigeria. *J Ethnopharmacol*. 2002;83:235-240.
14. Ademiluyi AO, Oboh G. Antioxidant properties of methanolic extracts of mistletoes (*Viscum album*) from cocoa and cashew trees in Nigeria. *Afr J Biotechnol*. 2008;7:3138-3142.
15. Oboh G, Puntel RL, Rocha JBT. Hot pepper (*Capsicum annum*, Tepin and *Capsicum chinese*, Habanero) Prevents Fe²⁺-induced lipid peroxidation in brain—in vitro. *Food Chem*. 2007;102:178-185.
16. Cushman DW, Cheung HS. Spectrophotometric assay and properties of the angiotensin-converting enzyme of rabbit lung. *Biochem Pharmacol*. 1971;20:1637-1648.
17. Bellé NA, Dalmolin GD, Fonini G, Rubin MA, Rocha JB. Polyamines reduces lipids peroxidation induced by different prooxidant agents. *Brain Res*. 2004;1008:245-251.
18. Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Anal Biochem*. 1979;95:351-358.
19. Halliwell B, Gutteridge JMC. Formation of a thiobarbituric-acid-reactive substance from deoxyribose in the presence of iron salts: the role of superoxide and hydroxyl radicals. *FEBS Lett*. 1981;128:347-352.
20. Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice-Evans C. Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radic Biol Med*. 1999;26:1231-1237.
21. Marcocci L, Maguire JJ, Droy-Lefaix MT, Packer L. The nitric oxide-scavenging properties of *Ginkgo biloba* extract EGb 761. *Biochem Biophys Res Commun*. 1994;201:748-755.
22. Gyamfi MA, Yonamine M, Aniya Y. Free-radical scavenging action of medicinal herbs from Ghana: *Thonningia sanguine* on experimentally induced liver injuries. *Gen Pharmacol*. 1999;32:661-667.
23. Oyaizu M. Studies on products of browning reaction: antioxidative activity of products of browning reaction prepared from glucosamine. *Jpn J Nutr*. 1986;44:307-315.
24. Minnoti G, Aust SD. An investigation into the mechanism of citrate Fe²⁺-dependent lipid peroxidation. *Free Radic Biol Med*. 1987;3:379-387.
25. Benderitter M, Maupoil V, Vergely C, Daloz F, Briot F, Rochette L. Studies by electron paramagnetic resonance of the importance

- of iron in the hydroxyl scavenging properties of ascorbic acid in plasma: effects of iron chelators. *Fundam Clin Pharmacol.* 1998;12:510-516.
26. Singleton VL, Orthofer R, Lamuela-Raventos RM. Analysis of total phenols and other oxidation substrate and antioxidants by means of Folin-Ciocalteu reagent. *Methods Enzymol.* 1999;299:152-178.
27. Meda A, Lamien CE, Romito M, Millogo J, Nacoulma OG. Determination of the total phenolic, flavonoid and proline contents in Burkina Faso honey, as well as their radical scavenging activity. *Food Chem.* 2005;91:571-577.
28. Kelley WTD, Coffey DL, Mueller TC. Lipid chromatographic determination of phenolic acids in soil. *J Assoc Anal Commun Int.* 1994;77:805-809.
29. Provan GL, Scobbie L, Chesson A. Determination of phenolic acids in plant cell walls by microwave digestion. *J Sci Food Agric.* 1994;64:63-65.
30. Whitehead D, Dibb D, Hartley R. Bound phenolic compounds in water extracts of soils, plant roots and leaf litter. *J Soil Biol Biochem.* 1983;15:133-136.
31. Zar JH. *Biostatistical Analysis.* Englewood Cliffs, NJ: Prentice-Hall; 1984.
32. Kwon YI, Apostolidis E, Shetty K. Evaluation of pepper (*Capsicum annuum*) for management of diabetes and hypertension. *J Food Biochem.* 2007;31:370-385.
33. Kwon YI, Apostolidis E, Shetty K. In vitro studies of eggplant (*Solanum melongena*) phenolics as inhibitors of key enzymes relevant for type 2 diabetes and hypertension. *Bioresour Technol.* 2008;99:2981-2988.
34. Saliu JA, Ademiluyi AO, Akinyemi AJ, Oboh G. In vitro antidiabetes and antihypertension properties of phenolic extracts from bitter leaf (*Vernonia amygdalina* Del.). *J Food Biochem.* 2012;36:569-576.
35. Ahnfelt-Ronne I. Enzyme inhibitors as drugs. In: Krogsgaard-Larsen P, Bundgaard H, eds. *A Textbook of Drug Design and Development.* Geneva, Switzerland: Harwood Academic; 1991:302-307.
36. Villar A, Paya M, Terencio MC. Plants with antihypertensive action. *Fitoterapia.* 1986;57:131-145.
37. Chu Y, Sun J, Wu X, Liu RH. Antioxidant and antiproliferative activity of common vegetables. *J Agric Food Chem.* 2002;50:6910-6916.
38. Oboh G, Rocha JBT. Antioxidant in foods: a new challenge for food processors. In: Panglossi HV, ed. *Leading Edge Antioxidant Research.* New York, NY: Nova Science; 2007:35-64.
39. Simi D, Simi T, Mimi-Oka J. Antioxidant status in patients with essential hypertension. Paper presented at: XIII World Congress of Cardiology; Monduzzi Editore Sp. A. Bologna, Italy; 1998.
40. Yuan YV, Bone DE, Carrington MF. Antioxidant activity of dulce (*Palmaria palmata*) extract evaluated in vitro. *Food Chem.* 2005;91:485-494.
41. Dastmalchi K, Dorman HJD, Kosar M, Hiltunen R. Chemical composition and in vitro antioxidant evaluation of a water soluble Moldavian balm (*Dracocephalum moldavica* L.) extract. *Leben Wiss Technol.* 2007;40:239-248.
42. Halvorsen BL, Holte K, Myhrstad MC, et al. A systematic screening of total antioxidants in dietary plants. *J Nutr.* 2002;132:461-471.
43. Kiremire BT, Musinguzi E, Kikafunda JK, Lukwago FB. Effects of vegetable drying techniques on nutrient content: a case study of South-Western Uganda. *Afr J Food Agric Nutr Dev.* 2010;10(5).
44. Oboh G, Akindahunsi AA. Change in the ascorbic acid, total phenol and antioxidant activity of some sun-dried green leafy vegetables in Nigeria. *Nutr Health.* 2004;18:29-36.
45. Oboh G. Effect of blanching on the antioxidant property of some tropical green leafy vegetables. *Leben Wiss Technol.* 2005;38:513-517.
46. Alia M, Horcajo C, Bravo L, Goya L. Effect of grape antioxidant dietary fiber on the total antioxidant capacity and the activity of liver antioxidant enzymes in rats. *Nutr Res.* 2003;23:1251-1267.
47. Amic D, Davidovic-Amic D, Beslo D, Trinajstic N. Structure-related scavenging activity relationship of flavonoids. *Croatia Chem Acta.* 2003;76:55-61.
48. Ogunmefun OT, Fasola TR, Saba AB, Oridupa OA. The ethnobotanical, phytochemical and mineral analyses of *Phragmanthera incana* (Klotzsch); species of mistletoe growing on three plant hosts in South-Western Nigeria. *Int J Biomed Sci.* 2013;9:33-40.
49. Moustapha B, Marina GA, Raúl FO, Raquel CM, Mahinda M. Chemical constituents of the Mexican mistletoe (*Psittacanthus calyculatus*). *Molecules.* 2011;16:9397-9403.
50. Hajimehdipoor H, Adib N, Khanavi M, Mobli M, Amin GR, Hamzeloo Moghadam M. Comparative study on the effect of different methods of drying on phenolics content and antioxidant activity of some edible plants. *Int J Pharm Sci Res.* 2012;3:3712-3716.