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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 Fe^{2+} 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 al[3] stated that drying of herbs inhibits microbial growth and forestalls certain biochemical changes, while Hossain et al[4] 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 al[5] affirmed that certain compounds that are present in some fresh herbs have been observed to increase in these herbs after drying, while Hung and Tran[6] 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 plague, 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 Oboh[10] 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 al[12] 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 Oboh[14] 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 decapacitated 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 (Sl) 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 Sl 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 $FeSO_4$ (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 Gutteridge[19] was used to determine the ability of the extract to prevent Fe^{2+}/H_2O_2-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 $FeSO_4$, 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) ($ABTS^{\cdot+}$) Radical Scavenging Ability. The $ABTS^{\cdot+}$ scavenging ability of the extracts was determined according to the method described by Re et al.[20] The $ABTS^{\cdot+}$ was generated by reacting 7 mM ABTS aqueous solution with $K_2S_2O_8$ (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 $ABTS^{\cdot+}$ 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 μL) 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% H_3PO_4 and 0.1% naphthlyethylenediamine 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 μL) 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 $FeCl_3$ 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% trichoroacetic acid was added. This mixture was centrifuged at 650 × *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.

Fe^{2+} Chelating Assay. The Fe^{2+} chelating ability of the extracts was determined using a modified method of Minnoti and Aust.[24] Freshly prepared 500 μM $FeSO_4$ (150 μL) was added to a reaction mixture containing 168 μL 0.1 M Tris–HCl (pH 7.4), 218 μL saline, and the extracts (0-25 μL). The reaction mixture was incubated for 5 minutes, before the addition of 13 μL 0.25% 1,10-orthophenanthroline (w/v). The absorbance was subsequently measured at 510 nm in a spectrophotometer. The Fe^{2+} 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 μL DNPH (2 g dinitrophenylhydrazine, 230 mg thiourea, and 270 mg $CuSO_4{\cdot}5H_2O$ in 100 mL of 5 M H_2SO_4) was added to 500 μL of the reaction mixture (300 μL of the test sample with 100 μL 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% H_2SO_4 (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 μL) was oxidized with 2.5 mL 10% Folin-Ciocalteau'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 μL 10% $A1C1_3$, 50 μL 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 × *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 μL: 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 μm, 4.6 × 250 mm with Hamilton microliter syringe, and injection volume of 20 μL. 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 ± 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 EC_{50} (extract concentration causing 50% enzyme inhibition/antioxidant activity) was performed using nonlinear 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 µg/mL). Furthermore, as revealed by the EC_{50} values (Table 1), the air-dried sample exhibited the least ACE inhibitory activity. However, oven-dried mistletoe (277.78 µg/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. EC_{50} 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*†.

	EC_{50} for ACE inhibition (µg/mL)	
Sample	Kidney	Lungs
Oven-dried mistletoe	277.78 ± 2.3[a]	344.83 ± 5.2[a]
Sun-dried mistletoe	297.62 ± 1.8[b]	359.71 ± 6.9[b]
Air-dried mistletoe	357.14 ± 3.0[c]	495.05 ± 11.1[c]

*Values represent mean ± 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 $FeSO_4$ 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 µg/mL) manner as represented by the EC_{50} 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 Fe^{2+}-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 Fe^{2+} and 7 µM sodium nitroprusside and this lead 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 µg/mL).

The inhibition of lipid peroxidation induced by various prooxidants (Fe^{2+} 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 al[37] 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. EC_{50} Values of the Effects of Some Drying Methods on the Inhibition of Sodium Nitroprusside (SNP) and Fe^{2+}-Induced Lipid Peroxidation in Rat Kidney and Heart by Mistletoe Leaves From Almond Tree*,†.

	EC_{50} for Inhibition of SNP and Fe^{2+}-Induced Lipid Peroxidation (μg/mL)			
Tissue →	Kidney		Heart	
Prooxidants →	SNP	Fe^{2+}	SNP	Fe^{2+}
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 Fe^{2+} Chelating Ability of Mistletoe Leaves Harvested From Almond Host Tree*,†.

	EC_{50} for Scavenging and Chelating Abilities (μg/mL)			
Sample	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 EC_{50} 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, Fe^{2+} and OH radical scavenging ability of plant foods and herbs is accepted as effective antioxidant defense mechanisms.[38]

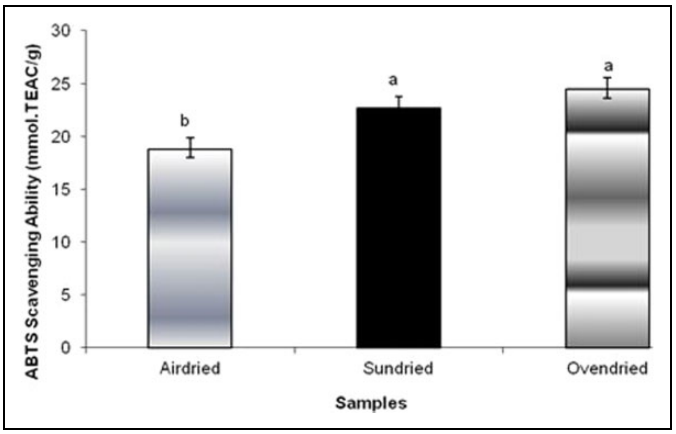

Figure 1. Effects of drying methods on the ABTS radical scavenging ability of mistletoe leaves from almond tree.

$ABTS^{\cdot+}$, NO, and DPPH Radical Scavenging Abilities

The $ABTS^{\cdot+}$ scavenging ability of the mistletoe leaves extract presented as TEAC in Figure 1 revealed that all the extracts scavenged $ABTS^{\cdot+}$. However, the air-dried sample (17.68 mmol TEAC/μg) had significantly ($P < .05$) lower $ABTS^{\cdot+}$ 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 EC_{50} 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 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 EC_{50} values in Table 3.

The mistletoe leaves demonstrated strong free radical scavenging abilities as exemplified by their scavenging activity of moderately stable $ABTS^{\cdot+}$, NO, and DPPH in vitro. There was agreement between the $ABTS^{\cdot+}$, 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 al[37] as well as Ademiluyi and Oboh[14] 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 al[39] 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 μg/mL), sun dried (361.79 μg/mL), and air dried (423.55 μg/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 al[37] 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 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]

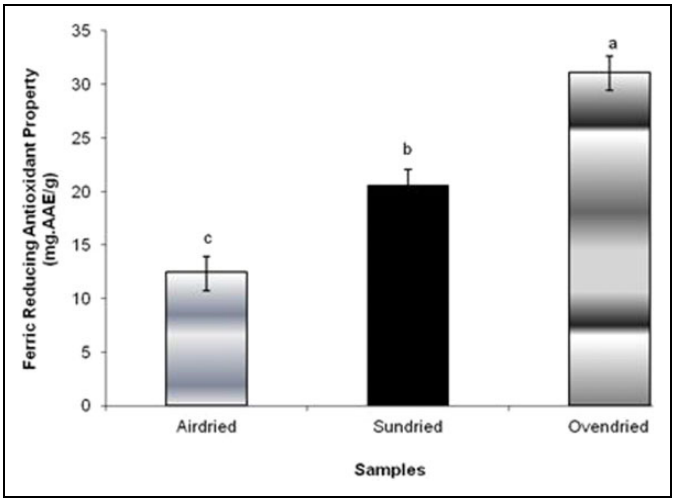

Figure 2. Effects of drying methods on the ferric reducing antioxidant properties of mistletoe leaves from almond tree.

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.

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

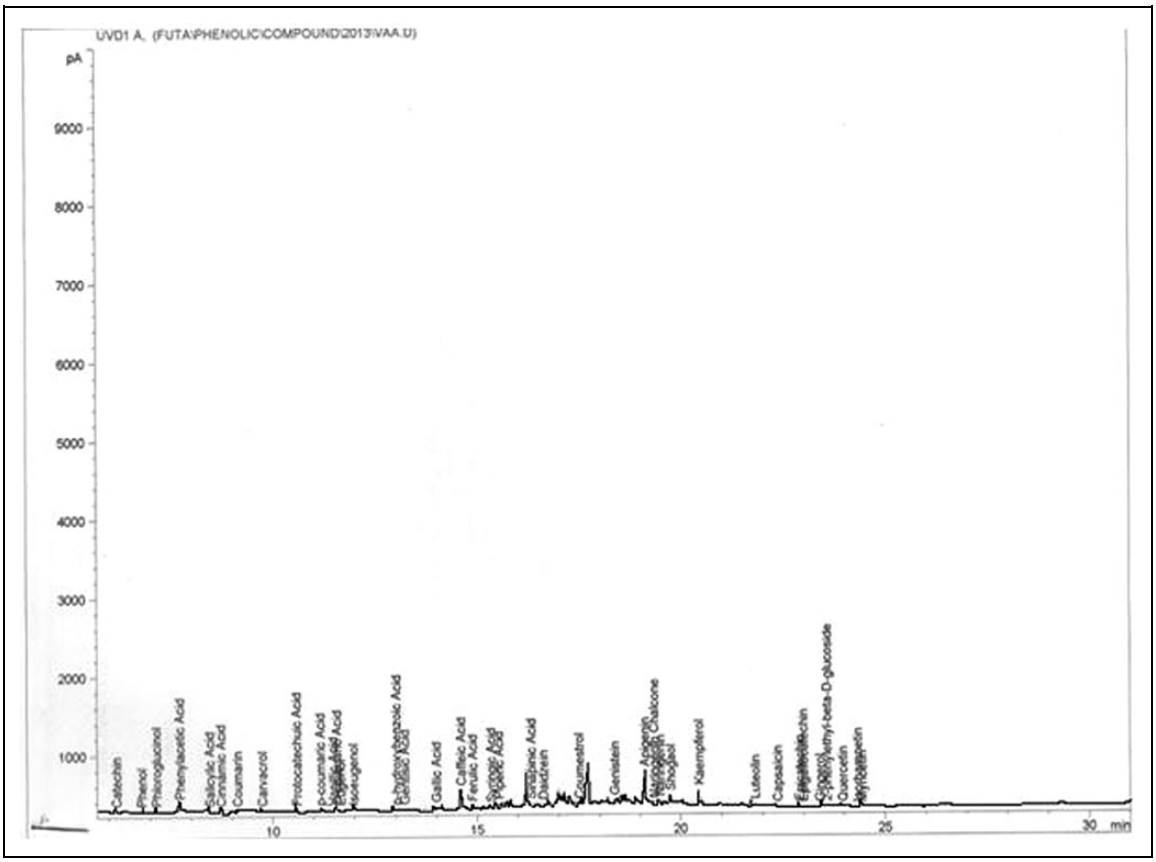

Figure 3. HPLC chromatogram of air-dried mistletoe leaves harvested from almond host tree.

C content, oven-dried mistletoe leaves (17.33 mg AAE/100 g) had the highest content, followed by the sun-dried sample (14.25 mg AAE/100 g) while the air-dried sample (8.09 mg AAE/100 g) had the least among the 3 drying methods.

The vitamin C content of the differently dried mistletoe leaves harvested from almond host plant as presented showed that the oven-dried sample had the highest vitamin C content than the sun-dried and air-dried samples. Sun drying result in great loss of vitamin C and β-carotene, as this occurs when ultraviolet light oxidizes vitamin C to a less stable dehydroascorbic acid, which is in turn oxidized to other compounds.[43] The lower vitamin C content in the sun-dried samples confirm the effect of ultraviolet light from direct sunlight in the reduction of this content in fruit and vegetables, while the very low vitamin C content of air-dried samples may be as a result of oxidation reactions that took place over the prolonged drying period. Vitamin C has been reported to contribute to the antioxidant activities of plant food as ascorbic acid is known to be a good reducing agent and exhibits its antioxidant activities by electron donation.[44,45]

HPLC-DAD Characterization of Constituent Phenolic Compounds

Figures 3, 4, and 5 show the HPLC chromatograms of constituent phenolic compounds of air-dried, oven-dried, and sun-dried mistletoe leaves, respectively. As shown in the figures, protocatechuic acid, *p*-coumaric acid, *o*-coumaric acid, vanillic acid, *p*-hydroxybenzoic acid, caffeic acid, ferulic acid, gentisic acid, gallic acid, syringic acid, quercetin, apigenin, gingerol, kaempferol, luteolin, isorhamnetin, myricetin, phenol, and phenylacetic acid are the predominant phenolic compounds in all the dried mistletoe leaves. Table 5 shows the quantity of identified phenolic compounds in each of the differently dried mistletoe leaves harvested from almond host tree. The oven-dried sample had the highest values followed by the sun-dried sample while the air-dried sample had the least for all the phenolic compounds. Also, phenol is not present in the sun-dried sample, signifying the high scavenging effect of ultraviolet radiation from the sun on this particular phenolic compound.

Plant foods are rich sources of phytochemicals, and intake of these plant chemicals has protective potential against degenerative diseases.[37] The HPLC-DAD characterization of mistletoe leaves revealed that this plant is rich in phytochemicals such as flavonoids and phenolic acids. These different phytochemicals have various protective and therapeutic effects essential to prevent diseases and maintain a state of well-being as phenolics are capable of scavenging free radicals, chelate metal catalysts, activate antioxidant enzymes, reduce α-tocopherol radicals, and inhibit oxidases. Their potent antioxidant activity is due to the redox properties of their hydroxyl groups.[46,47]

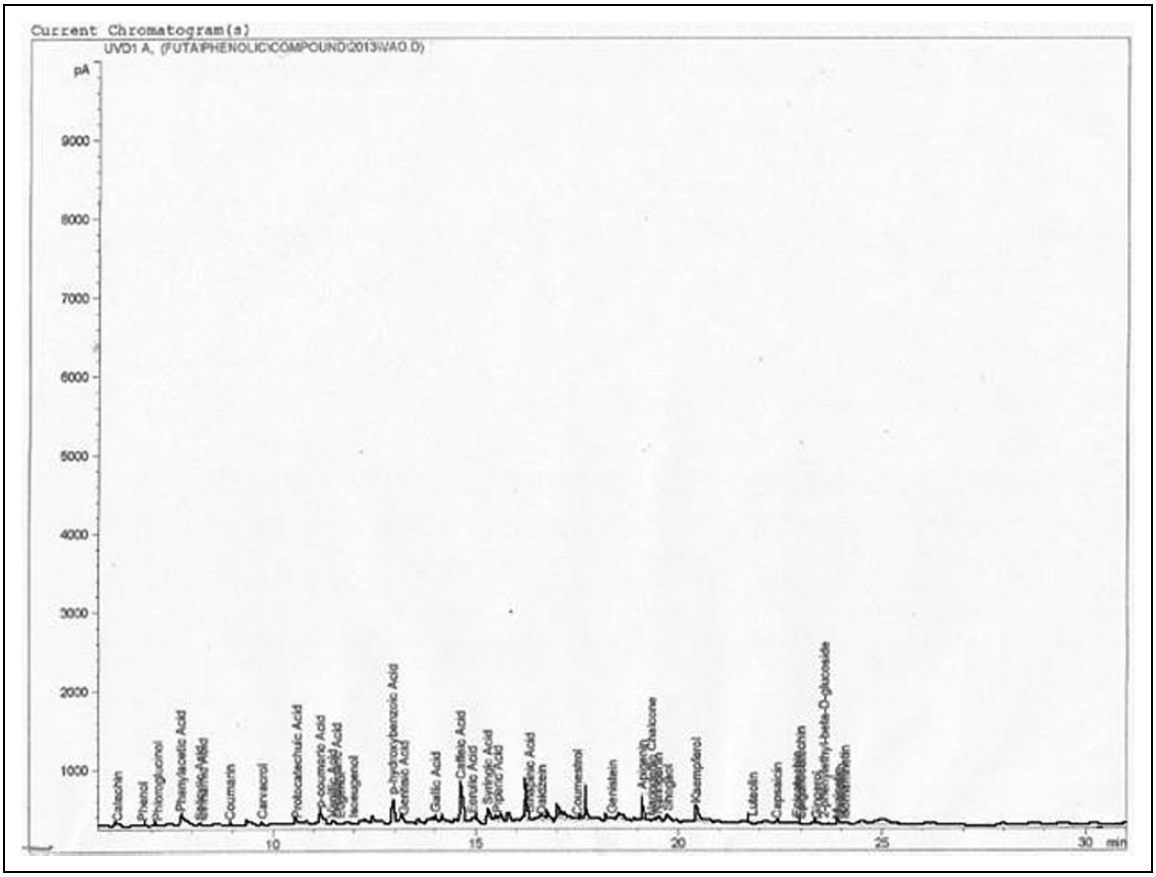

Figure 4. HPLC chromatogram of oven-dried mistletoe leaves harvested from almond host tree.

However, the observed higher phenolic content/constituents of the oven-dried extract relative to sun-dried and air-dried extracts may be as a result of rapid loss of water molecules to the atmosphere, which prevented cooking (oven dried) or deterioration resulting from slow dehydration observed in air drying under ambient temperature. There is longer retention of moisture content in air-dried samples compared to the 2 other drying methods, which may cause deterioration to the leaves and encourage some microbial growth, which in turn could induce rapid degradation of the phenolic compounds. This is tenable for the least phenolic content/constituents and bioactivity observed for the air-dried samples. Recently, Ogunmefun et al[48] reported that mistletoe being a parasitic plant would share some similarities to the host plant in terms of their phytoconstituents and this may influence their biological activities. Also, findings by Moustapha et al[49] have established that the phytoconstituents of mistletoes is a direct function of the host plant.

In this study, various drying methods significantly altered the phenolic constituents as well as the antihypertensive property of the various mistletoes leaves; as the methods with highest phenolic content exhibited the best biological activities. Hajimehdipoor et al[50] suggested that each plant needs a special drying method to retain its total phenolic contents and antioxidant activity. They further hypothesized that because of similarities in the secondary metabolites of a typical plant family, a particular drying method should be used for all plants in that family. Findings from this research work affirms the hypothesis of Hajimehdipoor et al[50] that diversity in drying methods leads to different losses of phenolic compounds and antioxidant property. It is suggested that each plant family needs a special drying method but further work needs to be done using mistletoe leaves harvested from a host tree that belongs to a different plant family to establish and fully substantiate this claim and hypothesis.

Conclusion

The inhibition of enzyme linked with hypertension (angiotensin-1 converting enzyme) by extracts of dried mistletoe leaves from almond host tree could be part of the mechanism through which mistletoe leaves exert its antihypertensive effect, thus giving the biochemical basis for its folkloric use in the management of hypertension. However, mode of drying significantly altered the phenolic phytoconstituents and bioactivity of these mistletoe leaves. Therefore, to obtain high phenolic content, antioxidant ability, and potent ACE inhibitory activity from mistletoe leaves harvested from almond host tree, oven drying is the most effective drying method followed by sun drying.

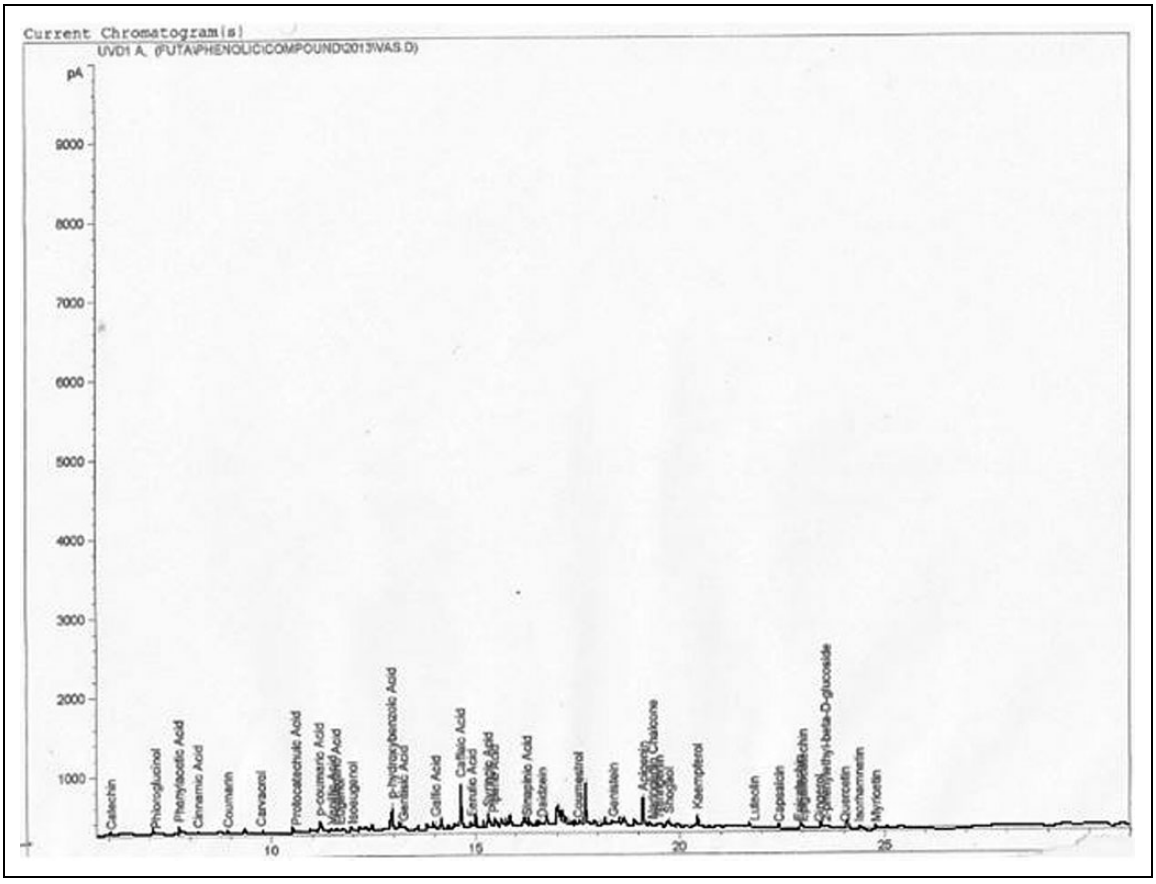

Figure 5. HPLC chromatogram of sun-dried mistletoe leaves harvested from almond host tree.

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

	Amount of Phenolic Compounds in Mistletoe Leaves (mg/100 g)		
Drying Methods →	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}
Kaemferol	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.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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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.

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