Fjrs.futa.edu.ng
FUTA Journal of Research in Sciences
ISSN: 2315 – 8239 (Print); E-ISSN: 2489 - 0413

FUTA Journal of Research in Sciences, Vol. 12 (2) 2016: 188-194

EFFECTS OF DRYING METHODS ON THE ANTIOXIDANT PROPERTIES OF PHENOLIC-RICH EXTRACTS OF AFRICAN MISTLETOE (*LORANTHUS BEGWENSIS L.*) LEAVES FROM ALMOND AND KOLANUT HOST TREES.

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ABSTRACT

African mistletoe (*Loranthus begwensis* L.) has been employed in sub-Sahara African folklore for the treatment of many degenerative diseases owing to its strong antioxidant properties. It has been reported that drying methods play an important role in herbs production. This study sought to investigate the most appropriate drying method by assessing the influence of these drying methods on the antioxidant properties of the leaves. Fresh leaves obtained from almond and kolanut host trees were subjected to oven drying, sun drying and air drying respectively after which the phenolic extraction was carried out. The influence of these drying methods on the total phenol and total flavonoid content as well as antioxidant properties (NO*, OH*, DPPH* scavenging and Fe^{2+} Chelating abilities) were assessed using standard methods. The total phenol ranged from 19.38 mg.GAE/100 g (Kolanut air-dried) to 39.47 mg.GAE/100 g (Almond oven-dried), while the flavonoid content ranged from 8.70 mg.QUE/100 g (Almond air-dried) to 21.81 mg.QUE/100 g (Almond oven-dried). In addition, all extracts scavenged DPPH [786.16 µg/ml (Almond oven-dried) to 1179.25 µg/ml (Kolanut air-dried)], NO [584.11 µg/ml (Almond oven-dried) to 1054.85 µg/ml (Kolanut air-dried)] and OH [404.86 µg/ml (Almond oven-dried) to 784.93 µg/ml (Kolanut air-dried)] radicals in dose-dependent manner as well as chelate Fe^{2+}[377.64 µg/ml (Almond oven-dried) to 593.82 µg/ml (Kolanut air-dried)]. This study revealed that oven drying is the best method for mistletoe leaves obtained from almond host tree while sun drying is the best for mistletoe leaves obtained from Kolanut host tree as exemplified by their radical scavenging abilities and total phenolic compounds. Thus, diversity in drying methods leads to different loss of phenolic compounds and antioxidant property, suggesting that each plant family needs a special drying method.

Keywords – Mistletoe, Kolanut, Almond, Drying, Phenolic-rich, Antioxidant.

INTRODUCTION

African Mistletoes (*Loranthus begwensis* L.) are highly specialized angiosperms of the family Loranthaceae, which are well known as broad range hemi-parasites of a variety of different gymnosperms and angiosperms (Deeni and Sadiq, 2002). They are of great economic importance due to the major damages they cause to their host (e.g kolanut, almond etc) upon heavy infestation which leads to economic losses (Zuber and Widmer, 2009). African mistletoe (*Loranthus bengwensis* L.) has been employed in sub-Sahara African folklore for the treatment of many degenerative diseases such as diabetes and hypertension (Obatomi *et al.*, 1994). Studies revealed that plant foods are rich sources of phytochemicals such as phenolics; with strong antioxidant properties (Kwon *et al.*, 2008; Hung and Tran, 2012). Many researchers have attributed the health promoting effects of plant foods to be a function of its phenolic constituents (Oboh *et al.*, 2014). A previous study demonstrated that mistletoe leaves are rich in phenolic compounds with potent antioxidant properties (Ademiluyi and Oboh, 2008).

Drying is by far the most widely used method of preserving medicinal herbs and plant materials in Nigeria. Drying of herbs inhibits microbial

growth and forestalls certain biochemical changes but, at the same time, it can give rise to other alterations that affect herb quality, such as changes in appearance and alterations in aroma caused by losses in volatiles (Hossain *et al.*, 2010) or the formation of new volatiles as a result of oxidation reactions or esterification reactions (Diaz-Maroto *et al.*, 2002). Studies by Di Cesare *et al.*, (2003) have reported change in colour and volatile compounds of the aromatic herbs after drying. Similarly, Hung and Tran (2012) reported that drying methods play an important role in production of the dried herbs and that the bioactive compounds and their antioxidant capacity might be lost during processing. However, there is dearth of information on the effects of conventional drying methods like sun drying, oven drying and air drying on the phenolic compounds and antioxidant properties of mistletoe leaves. Hence, this study sought to investigate the influence of these drying methods on the phenolic compounds and antioxidant properties of phenolic-rich extract of African mistletoe leaves from kolanut and almond host trees.

MATERIALS AND METHODS

Sample Collection and Preparation

African mistletoe leaves from Almond (*Terminalia catappa Linn.*) and Kolanut (*Kola notida*) host trees, were harvested from a farm location at Ibule-soro 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. Subsequently, the leaves were rinsed under running tap water and then divided into three portions. The first part was dried for about five days to constant weight using direct sunlight at temperature of approximately 27^{o}C, the second part was dried for about 48 h to constant weight using a heat drying oven at a temperature of 50^{o}C and the third portion air dried for about 15 days to constant weight at room temperature (approximately 25^{o}C) and away from direct effects of the sun. The dried samples were then milled into fine powder, put in air-tight polyethylene bag and kept in a refrigerator at 4^{o}C prior to analysis.

Phenolic Extraction

For the extraction of phenolics, 10 g of the powdered mistletoe leaves was soaked in an extraction medium of a mixture of 1M HCl and methanol (1:1) for 24 h and thereafter filtered through Whatman No. 2 filter paper. The filtrate was evaporated to dryness with rotary evaporator to remove the solvent and then stored at 4°C for further analysis.

Chemicals and Reagents

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

Assays

Determination of total phenol content

The total phenol content was determined according to the method of Singleton *et al.*, (1999). Briefly, appropriate dilutions of the extracts (200 µl) were 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 min at 45^{o}C and the absorbance was measured at 765 nm in the 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*., (2005). Briefly 0.5 ml of appropriately diluted sample was mixed with 0.5 ml methanol, 50 µl of a 10% solution of $AlC1_3$, 50 µ1 of 1 M Potassium acetate and 1.4 ml distilled water, and allowed to incubate at room temperature for 30 min. 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 and total flavonoid content.

OH^- Radical Scavenging Ability (Degradation of deoxyribose)

The method of Halliwell and Gutteridge (1981) 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^{o}C for 30 min and the reaction was then stopped by the addition of 0.5 ml of 2.8% trichloroacetic acid (TCA). This was followed by addition of 0.4 ml of 0.6% thiobarbituric acid (TBA) solution. The tubes were subsequently incubated in boiling water for 20 min and the absorbance was measured at 532 nm in a spectrophotometer.

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,* (1994). Samples of 100 – 400 μL of the extract were added to the test tubes containing 1 ml of Sodium nitroprusside solution (5mM) and the tubes were incubated at 37^0C for 2 h. An aliquot (0.5 ml) of the incubated solution was removed and diluted with 0.3 ml Griess reagent (1% sulphanilamide in 5% H_3PO_4 and 0.1% naphthlyethylenediaminedihydrochloride). 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 (RSA).

1,1-diphenyl-2 picrylhydrazyl (DPPH) free radical scavenging ability

The free radical scavenging ability of the extracts against DPPH (1,1-diphenyl-2 picrylhdrazyl) free radical was evaluated as described by Gyamfi *et al*. (1999). Briefly, appropriate volumes of the extracts (0 – 500 µl) were mixed with 1 ml of 0.4 mM methanolic solution containing DPPH radicals. The mixture was left in the dark for 30min and the absorbance was taken at 516 nm. The DPPH free radical scavenging ability was subsequently calculated.

Fe^{2+} chelating assay

The Fe^{2+} chelating ability of the extracts were determined using a modified method of Minotti and Aust (1987). 150 µl of freshly prepared 500 µM $FeSO_4$ was added to a reaction mixture containing 168 µl of 0.1 M Tris-HCl (pH 7.4), 218 µl saline and the extracts (0 – 25 µl). The reaction mixture was incubated for 5 min, before the addition of 13 µl of 0.25% 1,10-phenanthroline (w/v). The absorbance was subsequently measured at 510 nm in a spectrophotometer. The Fe^{2+} chelating ability was subsequently calculated as percentage (%).

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 Duncan multiple range test. Significance was accepted at $p<0.05$ (Zar, 1984). The EC_{50} (extract concentration causing 50% antioxidant activity) was performed using non-linear regression analysis.

RESULTS

Table 1 presents the results of the total phenol and flavonoid contents of polyphenol-rich extracts of differently dried African mistletoe leaves harvested from almond and kolanut host trees. For African mistletoe leaves harvested from almond host tree, oven dried (39.47 mg.GAE/100 g) had the highest total phenol content followed by the sun dried (28.85 mg.GAE/100 g) while air dried (19.78 mg.GAE/100 g) had the least. Likewise, for African mistletoe leaves harvested from kolanut host tree and sun dried (23.44 mg.GAE/100 g) had the highest total phenol content followed by the oven dried (21.34 mg.GAE/100 g) while air dried had the least(19.38 mg.GAE/100 g). Similarly, total flavonoid content of African mistletoe leaves harvested from almond host tree followed the same trend with the result of total phenol content with oven dried (21.81 mg.QUE/100 g) having the highest followed by the sun dried (12.17 mg.QUE/100 g) while air dried (8.70 mg.QUE/100 g) had the least. Also, total flavonoid content of African mistletoe leaves harvested from kolanut host tree followed the same trend as was observed in the results of its total phenol assay.

Table 1: Total Phenol and Total Flavonoid contents of methanolic extract of three differently dried African Mistletoe leaves from Almond and Kolanut trees.

Sample	Total Phenol (mg.GAE/100 g)	Total Flavonoid (mg.QUE/100 g)
Air dried Kolanut Mistletoe	19.38^{d}±0.80	9.09^{e}±0.21
Sun dried Kolanut Mistletoe	23.44^{c}±1.08	11.30^{c}±0.28
Oven dried Kolanut Mistletoe	21.34^{c}±0.78	10.43^{d}±0.42
Air dried Almond Mistletoe	19.78^{d}±0.11	8.70^{f}±0.22
Sun dried Almond Mistletoe	28.85^{b}±0.10	12.17^{b}±0.30
Oven dried Almond Mistletoe	39.47^{a}±0.21	21.81^{a}±1.35

Values represent mean of three readings

Values with the same superscript letter on the same column are not significantly ($p<0.05$) different.

Table 2 presents the EC_{50} values of hydroxyl (˙OH), nitric oxide (NO) and DPPH radical scavenging abilities as well as iron chelating ability of African mistletoe leaves obtained from kolanut and almond host trees. This revealed that the extracts scavenged ˙OH - induced decomposition of deoxyribose in Fenton reaction in dose-dependent manner (0 – 784 µg/ml). Furthermore, oven dried African mistletoe from almond host tree (404.86 µg/ml) had the highest scavenging ability followed by the sun dried sample (538.21 µg/ml) while air dried sample (701.26 µg/ml) had the least. Also, sun dried African mistletoe leaves from kolanut host tree (641.03 µg/ml) had the highest scavenging ability followed by the oven dried (681.20 µg/ml) while air dried (784.93 µg/ml) had the least. Similarly, the dried African mistletoe leaves extract scavenged NO radical as was observed for its OH* scavenging ability.

Furthermore, the DPPH free radical scavenging abilities of polyphenol-rich extracts of oven dried, sun dried and air dried mistletoe leaves harvested from almond and kolanut host plant as shown in Table 2 revealed that all the extracts scavenged DPPH radicals dose-dependently with oven dried mistletoe from almond host tree (786.16 µg/ml) showing the highest DPPH free radical scavenging ability while air dried mistletoe from kolanut host tree (1179.25 µg/ml) showed the least. Also, the chelating ability of the samples followed the same trend with the DPPH* scavenging ability.

Table 2. Effects of drying methods on the OH^{-}, NO, DPPH radical scavenging and Fe^{2+} chelating ability of African Mistletoe leaves harvested from Almond and Kolanut host tree.

	EC_{50} values for Radical Scavenging and Chelating abilities (µg/ml)			
Sample	OH*	NO*	DPPH*	Fe^{2+} Chelating
Air dried Kolanut Mistletoe	784.93^{f}±8.8	1054.85^{f}±2.3	1179.25^{f}±4.0	593.82^{f}±2.2
Sun dried Kolanut Mistletoe	641.03^{c}±6.8	771.60^{c}±1.8	996.02^{d}±4.5	443.26^{d}±2.9
Oven dried Kolanut Mistletoe	681.20^{d}±7.2	813.01^{e}±2.2	1033.06^{e}±5.2	471.70^{e}±3.8
Air dried Almond Mistletoe	701.26^{e}±8.8	805.15^{d}±2.3	888.10^{c}±4.0	438.30^{c}±1.2
Sun dried Almond Mistletoe	538.21^{b}±6.8	672.04^{b}±1.8	819.67^{b}±4.5	407.17^{b}±2.9
Oven dried Almond Mistletoe	404.86^{a}±7.2	584.11^{a}±2.2	786.16^{a}±5.2	377.64^{a}±3.8

Values represent mean of three readings. Values with the same superscript letter on the same column are not significantly ($p<0.05$) different.

DISCUSSION

The use of African mistletoes (*Loranthus begwensis L.*) in the treatment/management of hypertension is established in folklore and findings have corroborated its use in combating degenerative diseases like hypertension and diabetes in experimental animals (Obatomi *et al.,* 1994; Obatomi *et al.,* 1996). Plant foods are rich sources of phytochemicals, and intake of these plant chemicals have protective potential against degenerative diseases (Saliu *et al.,* 2012). Findings from this study is consistent with the one reported by Ademiluyi and Oboh, (2008) which revealed that mistletoes from cocoa and cashew host tree are rich in phenolic compounds with strong antioxidant properties. This study thus potentiates that phenolic compounds present and antioxidant properties elicited by mistletoe leaves could have accounted for its forkloric use in the management of degenerative diseases.

The radical scavenging and chelating ability of the mistletoe extracts followed the trends observed for both the total phenol and flavonoid content. This finding agreed with earlier findings where plant antioxidant properties (free radical scavenging ability) correlates with their phenolic content (Chu *et al.,* 2002; Ademiluyi and Oboh, 2008). It has been demonstrated that elevated consumption of plant antioxidants is accompanied by increased activity of extracellular antioxidant enzymes like glutathione peroxidase, catalase and superoxide dismutase (Ragavendran *et al.,* 2012).

The high Fe^{2+} chelating ability of the oven dried mistletoe from almond tree also agreed with its phenolic content and radical scavenging abilities. Oboh *et al.* (2007) stated that the ability of antioxidants to chelate and deactivate transition metals, prevent such metals from participating in the initiation of lipid peroxidation and oxidative stress as metal catalysed reaction is an important antioxidant defence mechanism in plants. These scavenging abilities could be assumed to have accounted for the strong antioxidant activity and other biological activities elicited by mistletoe leaves.

The least phenolic content and antioxidant property observed for the air dried samples maybe as a result of longer retention of moisture content in air dried samples over a prolonged drying period compared to the two other drying methods (sun dried and oven dried), this may cause deterioration to the leaves which in turn could induce rapid degradation of the phenolic compounds. Findings by Moustapha *et al.,* (2011) established that the phytoconstituents of mistletoes is a direct function of the host plant. Further studies by Hajimehdipoor *et al.,* (2012) suggested that each plant needs a special drying method to retain its total phenolic contents and biological activity. He 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. The difference in the phenolic constituents in relation to the host tree may have accounted for the distinctive variation in biological activity elicited by African mistletoe leaves from different host trees (Omojokun, 2014).

It is interesting to note that this study is consistent with the hypothesis of Hajimehdipoor *et al.,* (2012) in that, kolanut which belong to *Sterculiaceae* family (Martin, 2002) produced African mistletoes with the high phenolic content and antioxidative property after sun drying. Likewise, almond which belongs to the Rosaceae family (Potter, 2007) has oven drying as its best drying method. Hence, findings from this work affirms Hajimehdipoor *et al.,* (2012) hypothesis that diversity in drying methods leads to differences in loss of phenolic compounds and biological activity. We thus confirm that diversity in drying methods leads to differences in loss of phenolic compounds and antioxidant property. Hence, suggesting that each plant family needs a special drying method that best soothe it so as to retain appreciable quantity of its vital phytoconstituents.

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