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Chemical Composition and Free Radical Scavenging Activities of 10 Elite Accessions of Ginger (*Zingiber officinale Roscoe*)

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Abstract

The polyphenolic composition, oleoresin contents and antioxidant activities of 10 elite accessions of ginger (*Zingiber officinale* Roscoe) were investigated using standard procedures. The accessions of ginger contained significant quantities of polyphenols and oleoresin which suggested their therapeutic potentials. The antioxidant activities of extracts of the ginger accessions as determined by reducing power tests and their scavenging activities on 2, 2 diphenyl-1-picylhydrazyl radical, indicated that most of them possessed very strong antioxidant activities with higher reductive capacity than standard quercetin at higher concentrations. There was a negative correlation between percentage oleoresin versus phenol (r^2=-0.328) but a positive correlation between oleoresin versus percentage inhibition of DPPH radical (r^2=0.251) and reducing power (r^2=0.494). We obtained a positive correlation between the percentage inhibition of DPPH radical versus phenol (r^2=0.827) and reducing power (r^2=0.736) suggesting that any of the three assays could be used to determine the antioxidant activity of ginger, but a negative correlation between the percentage inhibition of DPPH radical versus flavonoids (r^2=-0.754). Finally, we obtained a negative correlation between flavonoids versus reducing power (r^2=-0.926). Results show these accessions of ginger will possess high economic potentials and could be utilized in the treatment of diseases that implicate free radicals. In addition, the oleoresins in ginger could contribute significantly to the reductive capacity of ginger. Finally, the negative correlation we obtained between the percentage inhibition of DPPH radical versus flavonoids and reducing power suggest to us that the flavonoids in ginger may have no contribution to their reductive capacity or hydrogen donating ability.

Keywords: Free radicals; Antioxidants; Ginger; Oleoresin; Nutrition

Introduction

A free radical is an atom or molecule with one or more unpaired electrons. Its tendency to acquire electrons from other sources makes it reactive. Free radicals are generated either through normal body metabolism or exposure to various carcinogens e.g. Tobacco smoke, radiation, etc. The human body has a natural antioxidant defensive mechanism both enzymatic (catalase, proxidase) and non enzymatic (Ubiquinone, NADPH, Vitamins A, E, etc) that scavenges free radicals. However, in disease conditions, the body's natural antioxidant defensive mechanism is compromised. In addition, these free radicals can oxidize nucleic acid, proteins, lipids or DNA causing diseases such as: cancer, hypertension, heart attack, diabetes, AIDS and malaria [1,2]. Free radicals act by creating an imbalance between the pro-oxidants and antioxidants homeostasis [3] which balance plays critical roles in maintaining a healthy biological system [4-6]. In malaria, the parasite stimulates certain cells to generate free radicals resulting in hemoglobin degradation [7]. The use of synthetic antioxidants such as Butylated Hydroxytoluene (BHT), tannic acid and propyl gallate has been reported to be harmful to human health [8]. Hence, strong restrictions have been placed on their application and the trend now is to substitute them with naturally occurring antioxidants.

The antioxidant activity (total antioxidant capacity) of plants and plant extracts can be determined by several in vitro methods. However, there are two general types of methods that are widely employed for antioxidant studies. The first set of assays involve electron or radical scavenging and they include: the 2,2 diphenyl-1-picrylhydrazyl (DPPH) radical, Trolox Equivalent Antioxidant Capacity (TEAC), and FRAP assays. They are based on reduction reaction. The second set is associated with lipid peroxidation which includes: the thiobarbituric acid and β-carotene bleaching assays [9].

Ginger (*Zingiber officinale* Roscoe) is a rhizome with a sharp pungent odour. It is used in most Nigerian homes as an ingredient in preparing soups, stews as well as ginger drinks. It can also be made into candy or used as flavouring for cookies, crackers and cake. In the Northern parts of Nigeria, ginger is used in making an indigenous drink known as 'Kunu'. In addition to its food value, ginger also has some considerable medicinal values such as: allay of motion sickness, protection of the DNA and other molecules from cell damage induced by oxidation, improvement of sperm quality and reproductive efficiency of men as well as hypoglycemic actions which were reported in animal models [10-12]. These biological functions of ginger have been attributed to its antioxidant potentials that arise from its contents of oleoresin and essential oils.

These oleoresins and essential oils also determine the quality of ginger rhizome in the world trade. In addition, the oleoresins have been credited for various pharmacological effects such as: anti-nauseant, antimicrobial, anti-inflammatory [13], anticoagulant, anti-hypercholesterolemic, anti-hypertensive, anti-hyperglycemic, antispasmodic [14], and vasodilatory properties. The chemical constituents of the oleoresins that are responsible for the pungent and pharmacological properties of ginger are 1-(3'-methoxy-4'-hydroxyphenyl)-5-hydroxyalkan-3-ones, also known as {3,6}-,{8-10} and {12}-gingerols [15].

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In most countries in Africa especially in Nigeria, several diseases such as cardiovascular, cancer, diabetes, hepatitis and prostatitis are the leading causes of mortality. This situation is further worsened by the development of different strains of disease resistant micro-organisms. This has therefore increased the search for new therapeutic agents and spices are increasingly being considered as one of the most promising agents [16].

Although the oleoresin, polyphenolic contents and antioxidant activities of ginger have been reported [17-19], these parameters tend to vary with different species of ginger.

In 2009, a breeding activity to identify and select some ginger genotypes with high yield and oleoresin content commenced at the National Root Crops Research Institute, Umudike, Nigeria. Preliminary investigation of six selected accessions in 2010 as published in 2012 [19] showed that they contained lower oleoresin contents than reported values for ginger. As a measure of selecting the most elite accessions, further breeding as well as screening studies were carried out and the most promising accessions in terms of oleoresin were selected and analyzed for polyphenolic contents and antioxidant activities and the results are reported in this paper.

Materials and Methods

Gallic acid, Folin-ciocalteu reagent, 2,2 diphenyl-1-picylhydrazyl radical (DPPH) and quercetin standards were obtained from Sigma and Aldrich Chemical Company, Germany and were of analytical grade.

Preparation of plant extracts

Ginger rhizomes, freshly harvested at maturity from the experimental farm of National Root Crops Research Institute, Umudike in 2012, were properly washed, sliced into chips and dried in an oven at a temperature of 65°C for 24 hours for the determination of moisture contents. Six grams of each flour of the different accessions of ginger was dissolved in 2 ml of methanol, made up to 60 ml with water, and left overnight. The mixtures were filtered using Whatman No 1 filter paper and stored in a refrigerator for the analysis of phenols, flavonoids and reducing power.

Determination of percentage oleoresin

The method of AOAC [20] was used with modifications. Ten grams of the fresh ginger rhizomes of each accession was washed properly with distilled water, sliced into chips of approximately 0.5 cm in diameter and dried in an oven at a temperature of 65°C for 8 hours to obtain a constant weight. Five grams of the dried samples (W_1) were mashed with 100 ml of acetone and the mixture was left overnight. It was later filtered (Whatman No 1 filter paper) into a pre-weighed empty beaker (W_2) and the acetone was evaporated on a water bath at 65°C, cooled and the whole setup (beaker + oleoresin) was re-weighed (W_3). The percentage oleoresin contents of the samples was determined by weight difference as:

$$\text{Percentageoleoresin}\left(\text{dry weight}\right) = \frac{W_3 - W_2}{W_1} \text{ X } 100$$

W_3=Weight of beaker + oleoresin

W_2=Weight of empty beaker

W_1=Weight of sample taken

Phenolic assay

The method of Singleton and Rossi [21] was used with modifications. Briefly, 0.1 ml of each extract of the flours of the ginger accessions and 50 µl of Folin-ciocalteau reagent were thoroughly mixed together. After 3 minutes, 0.3 ml of 20% Na_2CO_3 was added to the reaction mixture and the whole setup was shaken and incubated for 15 minutes at room temperature. One ml of distilled water was added to the reaction mixture and the absorbance was read at 725 nm using a UV spectrophotometer (Genesys 10 VIS Thermo Electron Corporation) against the reagent blank. Gallic acid was used as the standard for this assay.

Assay of total flavonoids

The method of Meda et al. [22] was used with modifications. To 0.5 ml of the extract were added, 0.5 ml of methanol, 50 µl of 10% $AlCl_3$ (in ethanol), 50 µl of 1 mol/L of potassium acetate and 1.4 ml of water. The mixture was incubated at room temperature for 30 minutes and the absorbance read using a UV spectrophotometer (Genesys 10 VIS Thermo Electron Corporation) at 420 nm instead of 415 nm against the reagent blank. Quercetin was used as the standard for this assay.

Assay of flavonols

The method of Kumaran and Karunakaran [23] was used with modifications. One gram of each flour was dissolved with 1 ml of ethanol, made up to100 ml with water and left overnight. It was filtered, centrifuged and the supernatant was collected. One ml of each extract + 1ml of 2% $AlCl_3$ (in ethanol) + 1 ml of sodium acetate solution (2 g in 40 ml of water) were thoroughly mixed together and left in a water bath at 20°C for 10 minutes. The absorbance was read at 440 nm against the reagent blank that contained 1 ml of ethanol. Quercetin was used as the standard for this assay.

2,2-Diphenyl-1- picrylhydracyl (DPPH) radical scavenging assay

The method of Blois [24] was used with modifications. A measured amount (0.5 g) of each sample was dissolved in 200 ml of methanol to give a concentration of 2.5 mg/ml and the mixture was filtered with Whatman No 1 Filter Paper. Then, 0.1, 0.2, 0.3, 0.4 and 0.5 ml of each filtrate was diluted with methanol to give final concentrations of 125, 250,375, 500 and 625 µg/ml respectively. Finally, 0.1 ml of 0.3mM DPPH in methanol was added to each of the reaction mixtures and the whole setup was well shaken and left in the dark for 30mins before the absorbance was read spectrophotometrically at 517 nm against the reagent blank that contained 1 ml of methanol. The same procedure was followed for standard quercetin (2.5 mg/ml in methanol) which was diluted to the concentrations 125, 250, 375, 500 and 625 µg/ml. The radical scavenging activity was calculated as: % Scavenging activity=[(Absorbance of control – Absorbance of sample)/Absorbance of control] × 100

Reducing power assay

The method of Pulido et al. [25] was used with modifications. A measured amount: 1.25, 2.5, 3.75, 5.0 and 6.25 ml of each extract was mixed with 2.5 ml of sodium phosphate buffer (0.2 m pH 6.6) and 2.5 ml of potassium ferri-cyanide (1% in water) in a test tube and reacted for 20 minutes at 50°C.The mixture was cooled using crushed ice and 0.5 ml of trichloroacetic acid (10% in water) was added and the set up was centrifuged for 10 minutes. One ml of the supernatant was collected and an equal volume of water was added + 0.2 ml of (0.1% in water) ferric chloride. The absorbance was read at 700 nm against the reagent blank. Quercetin was used as the control.

Statistical analysis

Results are reported as the means ± standard deviations of triplicate

experiments. Data was subjected to analysis of variance and differences between means were separated using the Duncan Multiple Range Test and results were considered to be significant at P<0.05.

Result

The analysis of the oleoresin contents of all the 10 accessions of ginger as shown in Figure 1, indicated that they contained high quantities of oleoresin which did not differ significantly from each other (P>0.05), suggesting their economic and antioxidant potentials. In addition, the large amounts of percentage oleoresin that were observed in the acetone extracts of the ginger accessions indicate possibilities of biological properties such as: anti-microbial, anti-nauseant, anti-inflammatory, anticoagulant, anti-hypercholesterolemic, anti-hypertensive, and vasodilatory properties.

Flavonoids are the largest group of polyphenolic compounds found in higher plants and are synthesized from the shikimic acid and malonic acid pathways [26,27]. Based on the structural differences, flavonoids are further subdivided into six sub-groups namely flavanones, flavones, flavonols, flavan-3-ols, anthocyanins and isoflavones [28]. Flavonoids possess free radical scavenging activities which prevent oxidative cell damage, have anti-inflammatory, anticancer activities as well as protection against the different levels of carcinogenesis. The total flavonoid contents of the accessions of ginger that were investigated fell within the range that was reported for ginger on dry basis [17] but higher than the previous reports of Ali and Hawa [18]. Thus the large amount of flavonoids that were observed in these lines of ginger as shown in Table 1, infer that they could possess some considerable levels of biological functions.

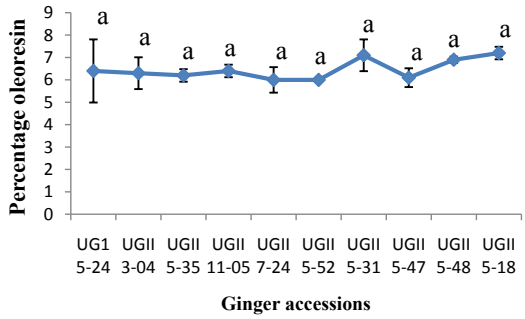

Figure 1: Percentage oleoresin contents of 10 accessions of ginger.

Sample	Phenol (gGAE/100g)	Flavonoids (gQE/100g)	Flavonols (gQE/100g)
UGII 11-05	0.63 ± 0.13^{ab}	1.061 ± 0.008^{cd}	0.69 ± 0.00^{c}
UGII 7-24	0.81 ± 0.00^{b}	1.027 ± 0.004^{a}	0.33 ± 0.03^{a}
UGI 5-24	0.56 ± 0.12^{a}	1.076 ± 0.00^{d}	0.83 ± 0.00^{d}
UGII 5-18	0.60 ± 0.04^{a}	1.062 ± 0.013^{cd}	0.74 ± 0.00^{cd}
UGII 5-35	0.75 ± 0.12^{ab}	1.058 ± 0.011^{bcd}	0.68 ± 0.00^{c}
UGII 5-48	0.76 ± 0.05^{ab}	1.035 ± 0.002^{ab}	0.36 ± 0.01^{a}
UGII 5-52	0.76 ± 0.09^{ab}	1.027 ± 0.004^{a}	0.28 ± 0.07^{a}
UGII 5-47	0.75 ± 0.09^{ab}	1.035 ± 0.005^{ab}	0.33 ± 0.04^{a}
UGII 5-31	0.74 ± 0.07^{ab}	1.040 ± 0.009^{abc}	0.38 ± 0.01^{a}
UGII 3-04	0.65 ± 0.01^{ab}	1.053 ± 0.023^{bcd}	0.53 ± 0.11^{b}

Values with the same superscripts in each vertical column are not significantly different from each other (P > .05). GAE = Gallic Acid Equivalent; QE = Quercetin Equivalent

Table 1: Polyphenolic composition of 10 elite accessions of ginger.

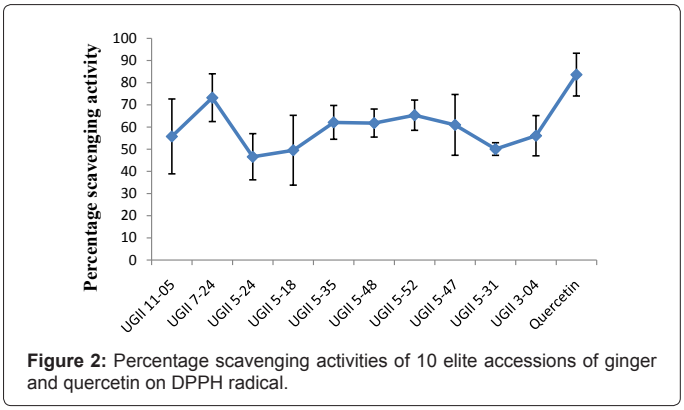

Figure 2: Percentage scavenging activities of 10 elite accessions of ginger and quercetin on DPPH radical.

Flavonols are the most ubiquitous flavonoid sub-group present in plant-based foods and beverages. Flavonols act as prominent antioxidants in biological systems and they have a saturated C-ring with a hydroxyl group at the C-3 position. They do not exist in glycosylated form as the other flavonoids but can be found in both monomer (catechins) and polymer forms (procyanidins). The most common flavonols found in the diet include: quercetin, kaempferol, and myricetin [28]. Results shown in Table 1 indicate that all the accessions contained low quantities of flavonols. In addition, UGII 5-24 was observed to have the highest quantities of flavonols (0.83 ± 0.00 g Quercetin Equivalence (QE)/100 g) while UGII 5-52 had the least (0.28 ± 0.07 g QE/100 g).

The presence of phenolic compounds with antioxidant activities is believed to be responsible for the free radical scavenging activities of many medicinal plants [8]. Results presented in Table 1 indicated that all accessions studied, contained significant quantities of phenols. In addition, UGII 7-24 was observed to have the highest phenolic content (0.81 ± 0.00 g Gallic Acid Equivallent (GAE)/100g) while UGI 5-24 had the least (0.56 ± 0.12 g GAE/100 g).

The DPPH assay is used to predict antioxidant activities of various food substances by mechanisms in which antioxidants in a substance act to inhibit lipid oxidation by scavenging DPPH radical and this therefore gives an idea of the antioxidant capacity of the substance being investigated. The method is widely used due to relatively short time required for the analysis. DPPH is a stable free radical in methanol or aqueous solution and accepts an electron or hydrogen radical to turn into stable diamagnetic molecule [29-31]. The tendencies of electron or hydrogen donation are critical factors in characterizing antioxidant activity. As observed in Figure 1, the methanolic extracts of the flours of most of the ginger accessions studied contained considerable scavenging activities on DPPH free radical compared with standard quercetin. The scavenging activities of the methanolic extracts of the ginger accessions and standard quercetin decreased in the following order: Quercetin > UGII 7-24 > UGII 5-52 > UGII 5-35 > UGII 5-48 > UGII 5-47 > UGII 3-04 > UGII 11-05 > UGII 5-31 > UGII 5-18 > UGII 5-24 (Figure 2). The high scavenging activities of most of these accessions of ginger on DPPH free radical could be attributed to the presence of phenols in them which are polyphenolic compounds with high antioxidant activities. The hydrogen donating abilities of the phenolic compounds in the extracts of the ginger accessions is thought to be responsible for the inhibition of the extracts on DPPH free radical.

The DPPH assay has the limitation of color interference and sample solubility [32] (Dorman and Hiltunen, 2004) and this informed the assay of the reducing power of all the ginger accessions.

Reducing power assay is a novel method that is used in the assay

of the antioxidant activities of various medicinal plants and it employs the reduction of Fe^{3+} to Fe^{2+}. This is so because antioxidants are strong reducing agents. Moreover many researchers have reported that the reducing power of bioactive compounds is associated with antioxidant activity [33,34]. Hence, it is essential to determine the reducing power of phenolic constituents to explain the relationship between their antioxidant effects. The reducing power of the ginger accessions as shown in Figure 3 indicated that they possessed strong antioxidant activities. In addition, the reducing power of some of the extracts of the ginger accessions (0.4 to 1.9 nm for 0.1 g of sample) showed higher activity at higher concentrations compared with standard quercetin (0.592 to 1.2 nm for 0.1 g of quercetin) and previous studies of Chen et al. [35] who obtained a range of 0.34 to 1.6 nm (for 100 mg of sample) in 18 different species of ginger and this is a significant finding in this present study.

Correlation analysis that was carried out, revealed that there was a negative correlation between percentage oleoresin versus phenol (r^2=-0.328) but a positive correlation between oleoresin versus percentage inhibition of DPPH radical (r=0.251) and reducing power (r=0.494) (Table 2). Similarly, we obtained positive correlation between the percentage inhibition of DPPH free radical versus phenol (r^2=0.827) and reducing power (r^2=0.736), but a negative correlation between percentage inhibition of DPPH radical versus flavonoids (r^2=-0.754) (Table 2). Finally, we also obtained a negative correlation between flavonoids versus reducing power (r^2=-0.926). It does appear from the study that the oleoresins in ginger could contribute significantly to the reductive capacity of ginger. The significant correlation we obtained between the percentage inhibition of DPPH radical versus phenols and reducing power suggest to us that any of the three assays could be utilized to determine the antioxidant activity of ginger. Finally, the negative correlation we obtained between the percentage inhibition of DPPH radical versus flavonoids and reducing power suggest to us that the flavonoids in these accessions of ginger may have no contribution to their reductive capacity or hydrogen donating ability. Similar reports of negative correlation between percentage inhibition of DPPH radical versus flavonoids have been previously reported [21,36].

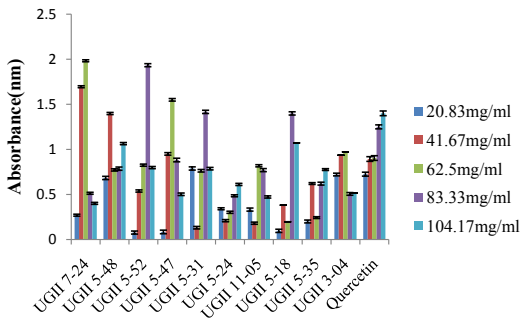

Figure 3: Reducing power of 10 elite accessions of ginger and quercetin.

Parameter	Phenol	Flavonoids	Inhibition	Reducing power	Flavonols
Oleoresin	-0.328 (NS)		0.251 (NS)	0.494*	
Percentage inhibition	0.827**	-0.754**		0.736**	
Flavonoids				-0.926**	

**Correlation is significant at 0.01 levels; *Correlation is significant at 0.05 level. NS- Not-significant

Table 2: Pearson correlation between percentage oleoresin versus antioxidant parameters; DPPH inhibition versus antioxidant parameters; flavonoids versus antioxidant parameters.

Conclusion

The high quantities of oleoresin in all the accessions of ginger that were investigated as observed in this study, confer on them very high economic potentials and biological properties. Finally, the high antioxidant activities of most of the ginger accessions that were studied indicate that they could be useful in the treatment of diseases that could arise from free radicals and oxidative stress.

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References

1. Halliwell B (2007) Dietary polyphenols: good, bad, or indifferent for your health? Cardiovasc Res 73: 341-347.
2. Eleazu CO, Okafor PN (2012) Antioxidant effect of unripe plantain (Musa paradisiacae) on oxidative stress in alloxan induced diabetic rabbits. International Journal of Medicine and Biomedical Research 1.
3. Halliwell B, Whiteman M (2004) Measuring reactive species and oxidative damage in vivo and in cell culture: how should you do it and what do the results mean? Br J Pharmacol 142: 231-255.
4. Davies KJ (2000) Oxidative stress, antioxidant defenses, and damage removal, repair, and replacement systems. IUBMB Life 50: 279-289.
5. Tiwari AK (2001) Imbalance in antioxidant defense and human diseases: Multiple approach of natural antioxidant therapy. Curr Sci 8: 1179-1187.
6. Katalinic V, Milos M, Kulisic T, Jukic M (2006) Screening of 70 medicinal plant extracts for antioxidant capacity and total phenols. Food Chem 94: 550-557.
7. Omale J, Omajali JB (2010) Evaluation of bio-safety and antioxidant activity of the fruit and leaf of Saba florida (Benth.) from Ibaji forest. International Journal of Medicine and Medical Sciences 2: 100-105.
8. Pourmorad F, Hosseinimehr SJ, Shahabimajd N (2006) Antioxidant activity, phenols, flavanoid contents of selected Iranian medicinal plants. S Afr J Biotechnol 5: 1142-1145.
9. Moon JK, Shibamoto T (2009) Antioxidant assays for plant and food components. J Agric Food Chem 57: 1655-1666.
10. Yang HS, Han DK, Kim JR, Sim JC (2006) Effects of alpha-tocopherol on cadmium-induced toxicity in rat testis and spermatogenesis. J Korean Med Sci 21: 445-451.
11. Khaki A, Fatemek F, Mohammad N, Amir AK, Chelar CO, et al. (2009) The effects of ginger on spermatogenesis and sperm parameters. Iranian J Reproduc Med 7: 7-12.
12. Al-Amin ZM, Thomson M, Al-Qattan KK, Peltonen-Shalaby R, Ali M (2006) Anti-diabetic and hypolipidaemic properties of ginger (Zingiber officinale) in streptozotocin-induced diabetic rats. Br J Nutr 96: 660-666.
13. Verma SK, Singh J, Khamesra R, Bordia A (1993) Effect of ginger on platelet aggregation in man. Indian J Med Res 98: 240-242.
14. Bradley PR (1992) British Herbal Compendium. British Herbal Medicine Association, Bournemouth, Dorset, UK.
15. Bruneton J (1995) Pharmacognosy, Phytochemistry, Medicinal Plants: Phytochemistry, Medicinal Plants. Intercept Limited.
16. Ríos JL, Recio MC (2005) Medicinal plants and antimicrobial activity. J Ethnopharmacol 100: 80-84.
17. Shirin Adel PR, Jamuna P (2010) Chemical composition and antioxidant properties of ginger root (Zingiber officinale). Journal of Medicinal Plants Research 4: 2674-2679.
18. Ghasemzadeh A, Jaafar HZE (2012) Effect of salicylic acid application on biochemical changes in ginger (*Zingiber officinale* Roscoe). J Med Plant Res 6: 790-795.
19. Eleazu CO, Eleazu KC (2012) Physico-chemical properties and antioxidant properties of 6 new varieties of ginger. American Journal of Food Technology 7: 214-221.

20. AOAC (1975) Official Methods of Analysis. (12thedn), Association of Analytical Chemists, Washington DC.

21. Singleton VL, Rossi JA (1965) Colorimetry of total phenolics with phosphomolybdic–phosphotungstic acid reagents. Am J Enol Vitic16: 144-158.

22. Meda A, Lamien CE, Romito M, Millogo J, Nacoulma OG (2005) Determination of the total phenolic, flavonoid and proline contents in Burkina Fasan honey, as well as their radical scavenging activity. Food Chem 91: 571-577.

23. Kumaran A, Karunakaran RJ (2007) In vitro antioxidant activities of methanol extracts of Phyllanthus species from India. Lebens-Wiss Technol 40: 344-352.

24. Blois MS (1985) Antioxidant determination by use of stable free radicals. Nature 29: 1199-1200.

25. Pulido R, Bravo L, Saura-Calixto F (2000) Antioxidant activity of dietary polyphenols as determined by a modified ferric reducing/antioxidant power assay. J Agric Food Chem 48: 3396-3402.

26. Croft KD (1998) The chemistry and biological effects of flavonoids and phenolic acids. Ann N Y Acad Sci 854: 435-442.

27. Rupasinghe HPV (2008) The role of polyphenols in quality, post harvest handling and processing of fruits. In: Paliyath G, Lurrie S, Murr D, Handa A (Eds) Post Harvest Biology and Technology of Fruits, Vegetables and Flowers. Wiley-Blackwell Publishers.

28. D'Archivio M, Filesi C, Di Benedetto R, Gargiulo R, Giovannini C, et al. (2007) Polyphenols, dietary sources and bioavailability. Ann Ist Super Sanita 43: 348-361.

29. Vinson JA, Dabbagh YA, Serry MM, Jang J (1995) Plant flavonoids especially tea flavonols are powerful antioxidant using in vitro oxidation model for heart disease. J Agric and Food Chem 43: 2800-2802.

30. Je JY, Park PJ, Kim EK, Ahn CB (2009) Antioxidant and angiotensin-1-converting enzyme inhibitory activity of Bambusae caulis in Liquamen. Food Chem 113: 932-935.

31. Duh P, Tu Y, Yen G (1999) Antioxidant activity of aqueous extract of harn jyur (*Chyrsanthemum morifolium* Ramat). LWT J Food Sci Tech 32: 269-277.

32. Dorman HJD, Hiltunen R (2004) Fe (II) reductive and free radical scavenging properties of summer savory (Satureja hortensis L.) extract and sub-fractions. Food Chem 88: 193-199.

33. Yen GC, Duh PD, Tsai CL (1993) Relationship between antioxidant activity and maturity of peanut hulls. J Agric Food Chem 41: 67-70.

34. Shiddhuraju P, Mohan PS, Becker K (2002) Studies on antioxidant activity of Indian Laburnum (Cassia fistula L.): a preliminary assessment of crude extracts from stem back leaves, flower and fruit pulp. Food Chem 79: 61-67.

35. Chen IN, Chang CC, Ng CC, Wang CY, Shyu YT, et al. (2008) Antioxidant and antimicrobial activity of Zingiberaceae plants in Taiwan. Plant Foods Hum Nutr 63: 15-20.

36. Mohammed FA, Nagendra K, Prasad KW, Amin I (2010) Flavonoid, hesperidine, total phenolic contents and antioxidant activities from Citrus species Afric J Biotechnol 9: 326-330.