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ORIGINAL ARTICLE

In vitro antioxidant activity of extracts from leaves of ten commonly used medicinal plants – a comparative study

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Abstract

Objective: Reactive oxygen has been implicated in degenerative diseases and medicinal activity of most herbs has been attributed to their ability to scavenge free radicals.

Methods: Free radical scavenging and antioxidant activities of ethanolic extracts from the leaves of varieties of medicinal plants, namely *Azadiratcha indica, Mangifera indica, Moringa oleifera, Psidium guajava, Terminalia catappa, Anacardiaceae occidentale, Cassia siamae, Chromolaena odorata, Telfaira occidentalis* and *Paraquetina nigresiens* were evaluated. Reducing power, DPPH scavenging, hydroxyl radical scavenging and ferrous ion chelating activities, phenolic, flavonoid and vitamin C contents were determined.

Results: *C.siamae* had excellent DPPH scavenging activity while *P.guajava* presented the lowest value. *T.catappa* had peak hydroxyl radical scavenging activity whereas *T.occidentalis* had the least capacity. *C.siamae* had the highest total phenolic and flavonoid contents while *P.guajava* and *P.nigresiens* showed the lowest phenol and flavonoid values. *C.siamae* also exhibited the highest reducing power activity whereas *P.nigresiens* had the least value. *P.nigresiens* had excellent ferrous iron chelating capacity while *C.odorata* had only poor activity. The total phenolic and flavonoid contents were highly correlated with the DPPH scavenging and reducing power activities, respectively. *M.oleifera* had the highest vitamin C content while *P.guajava* was the least..

Conclusion: Different values were obtained for each parameter for the medicinal leaves and free radical scavenging activity could be attributed to total phenolic and total flavonoid content. Among the plants tested, *Cassia siamae* leaf extract consistently exhibited the highest antioxidant activity and seems to be a promising source of natural antioxidants.

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INTRODUCTION

Free radicals generation now appears to be the fundamental mechanism underlying a number of human patho-physiological diseases and over a hundred diseases including malaria, diabetes, heart diseases, cancer, inflammation, viral infections, autoimmune pathologies, digestive system disorders, ulcer and neurodegenerative disorders (*e.g.* Alzheimer disease, Parkinson disease, multiple sclerosis, Down's syndrome)[1, 2]. In recent times, antioxidants have become really important in cancer prevention, therapy and longevity [3]. Epidemiological and experimental evidence have demonstrated that phenolics exert chemopreventive and therapeutic effects on diseases such as cancers, diabetes, cardiovascular, renal disorder and several other human ailments [4-7]. Over-production of reactive oxygen species (ROS) can oxidize oxygen, polyunsaturated fatty acids, phospholipids, cholesterol and DNA [8]. ROS includes non-radical oxidants such as ozone and hydrogen peroxide as well as radicals such as superoxide and hydroxyl. Overproduction of ROS could overwhelm the antioxidant defense mechanism of the organism and thus the need for supplementation from dietary source, especially neutraceuticals [9, 10]. These have been associated with the antioxidative and free radical scavenging properties of these plants, thus preventing, delaying or ameliorating many of disorders associated with oxidative stress [11-13].

The antioxidative and free radical scavenging properties of plants commonly used in traditional medicine have been attributed to the presence of polyphenols, most especially flavonoids and phenolic acids which are secondary metabolites accumulated through evolution as a natural means of surviving in a hostile environment [14]. They may also be influenced by other organic and inorganic compounds such as coumarins and antioxidant micronutrients (*e.g.*, cupper, manganese and zinc) [15]. Some of these higher plants used in traditional medicine are widely distributed in the tropics and since Nigeria is located within the heart of the tropics with enormous biodiversity of these natural resources. Apart from this, the recommended dietary supplements for antioxidants from epidemiological studies are fruits, vegetables and minimally processed staple foods.

Indigenous vegetables eaten in Nigeria are cooked, fruits are not readily available due to cost and we have considered commonly used medicinal herbs. We do not have complete data base of antioxidant content in food supplements; the level of any single antioxidant may not reflect the total antioxidant potential of foods and environmental factors could influence data from different regions. Earlier studies have been done on some of the plants in our study on cultivars, leaves at various stages of maturity and some on the matured leaves extract [16-19]. The leaves chosen in this study those frequently used in traditional medicine in tropical Africa such as 'village dispensary' (*Azadiratcha indica*), 'miracle tree' (*Moringa* spp), heamatonic (*Telfaira ocidentalis*), others for diarrhoea, nephritis, cough, febrile ailments, stomach aches. This informed our decision to evaluate the potential antioxidative and anti-radical properties of ten commonly used tropical medicinal plants (*Azadiratcha indica, Mangifera indica, Moringa oleifera, Psidium guajava, Terminalia catappa, Anacardiaceae occidentale, Cassia siamae, Chromolaena odorata, Telfaira occidentalis* and *Paraquetina nigresiens*) using seven different assay methods to quantify the antioxidants present and correlation that exist between the assayed parameters.

MATERIALS AND METHODS

Chemicals

Gallic acid, 1,1-diphenyl-2-picrylhydrazyl (DPPH), 3-(2-pyridyl)-5,6-diphenyl-1,2,4-triazine-4',4"-disulfonic acid sodium salt (Ferrozine), (+)-cyanidol-3(2R,3S)-2-(3,4-dihydroxylphenyl-3,4-dihydro-1(2H)-benzopyran-3,5,7-triol (+)-catechin hydrate and Folin-Ciocalteau reagent were from Sigma-Aldrich (St. Louis, MO, USA). Ferrous chloride ($FeCl_2$), sodium nitrite, sodium hydroxide, potassium ferricyanide, sodium carbonate and aluminum chloride were from Labtech (Uckfield, East Sussex, England, UK). All other reagents used were of the highest purity grade commercially available.

Plant extraction

Fresh leaves of the plants were collected from Botanical Garden University of Ibadan were authenticated at the Herbarium of Botany Department, University of Ibadan, Ibadan. The leaves were air-dried at room temperature in the Nutritional and Industrial Biochemistry Laboratory for 8 weeks and were milled manually into a uniform powder, packed in air tight cellophane bags and stored. The leaf ethanol extracts were prepared by cold maceration using 100 g each of the dried powdered plant materials in 300 ml of 80% ethanol at room temperature for 72 h. The extracts were filtered, concentrated to dryness on a rotary evaporator (Buchi Rotavapor R114) at 60ºC and the weight of dried crude extracts ranged from 10.6-25 g.

Antioxidant assays

The total phenol content (TPC) of the extracts was determined using Folin-Ciocalteu reagent method of Singleton *et al* [20]. Total flavonoid content (TFC) was measured using the aluminum chloride colorimetric assay [21]. The capacity of leave extracts to scavenge the 'stable' free radical DPPH were monitored according to the method of Ardestani and Yazdanparast [22]. The hydroxyl radical averting capacity assay (HROAC) was done using Fenton reaction as recommended by Yu *et al* [23] and the potassium ferricyanide reducing power (FRAP) of the prepared extracts were assayed according to the method of Oyaizu [24] using the formation of potassium ferrocyanide in the presence of antioxidants. The ferric ion chelating antioxidant power of the extracts was done using ferric ion TPTZ (2,4,6-tri(2-pyridyl)-1,3,5 triazine) method of Dinis *et al* [25] and vitamin C content was determined using the titrimetric method based on the redox properties of vitamin C [26]. Stock solutions of extracts were made and aliquots of 200 µg were used for the assays.

Statistical analysis

All measurements were carried out in triplicate and reported as means ± standard error of the mean (SEM). Linear correlations between the content of total flavonoid, total phenolic content and data for some of the antioxidant assays were also assessed. All tests were performed using the SPSS software version 17 (SPSS Inc, Chicago, IL, USA). $P < 0.05$ was accepted as significant.

RESULTS

Scavenging activity of different plant leaf extracts on FRAP, HROAC and DPPH radical are shown in Table 1. Extracts showed noticeable variation in the antioxidant assays. *C.siamae*, *P.nigresiens*, *A.occidentale*, *C.odorata* and *A.indica* had good DPPH radical scavenging activity. *T.catappa*, *M.oleifera*, *P.nigresiens* and *P.guagava* were found to be good hydroxyl radical scavengers. Reducing power activity of the plant extracts was highest with *C.siamae*, *M.indica*, *A.occidentale*, *T.catappa* and *C.odorata*.

Data for TPC, TFC, vitamin C content and ferrous ion chelating capacity of different plant leaves extracts are shown on Table 2. TPC of plant extracts were found to be high in *C.siamae*, *C.odorata*, *P.nigresiens*, *A.occidentale* and *A.indica*. TFC was high in *C.siamae*, *M.indica*, *A.occidentale*, *T.catappa*, *T.occidentalis* and *C.odorata*. Out of ten plants screened, *P.nigresiens*, *A.occidentale*, *T.occidentalis*, *P.guajava*, *M. indica* and *C.siamae* were shown to have high ferrous ion chelating activity. Vitamin C contents of extracts were highest in *M.oleifera*, followed by *A.occidentale*, while *P.guajava* had the least.

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Table 1. Reducing power, Hydroxyl and DPPH radical scavenging activity of the different plant leaf extracts

Plant extract	Hydroxyl radical scavenging (%)	DPPH radical scavenging (%)	Reducing power assay (AA mg/100g)
Azadiratcha indica	74.16 ± 0.217	84.1 ± 0.207	296 ± 0.577
Mangifera indica	-	63.87 ± 0.127	310 ± 1.155
Moringa oleifera	93.73 ± 0.106	63.59 ± 0.243	288.67 ± 1.453
Psidium guajava	80.48 ± 0.282	62.84 ± 0.049	288.33 ± 0.882
Terminalia catappa	96.99 ± 0.362	66.8 ± 0.121	306 ± 0.577
Anacardiaceae occidentale	62.22 ± 0.248	87.73 ± 0.056	306.67 ± 0.667
Cassia siamae	78.73 ± 0.112	95.78 ± 0.358	327.67 ± 0.882
Chromolaena odorata	63.74 ± 0.17	86 ± 0.055	300.67 ± 0.667
Telfaira occidentalis	49.26 ± 0.316	77.37 ± 0.155	299 ± 1.155
Paraquetina nigresiens	84.48 ± 0.247	91.06 ± 0.088	212.67 ± 0.667

Each value is mean ± SD (n = 3)

Table 2. Ferrous ion chelating capacity, total phenol, total flavonoid and vitamin C content of the different plant leaf extracts

Plant extract	Ferrous ion chelation (%)	Total phenol (GAE mg/100 g)	Total flavonoids (CE mg/100 g)	Vitamin C (100 mg/100 g)
Azadiratcha indica	24.98 ± 0.196	109.77 ± 0.385	437.47 ± 9.950	28 ± 1
Mangifera indica	64.71 ± 0.619	74.973 ± 0.018	468.2 ± 0.584	25.33 ± 1.528
Moringa oleifera	30.76 ± 0.39	74.58 ± 0.12	423.62 ± 9.369	203.67 ± 1.5
Psidium guajava	67.61 ± 0.428	71.75 ± 0.086	435.13 ± 1.715	25 ± 4.35
Terminalia catappa	68.74 ± 0.228	80.23 ± 0.026	461.84 ± 0.868	33.33 ± 1.528
Anacardiaceae occidentale	80.25 ± 0.459	117.69 ± 0.075	462.12 ± 1.21	139.67 ± 1.5
Cassia siamae	62.59 ± 0.283	131.92 ± 0.06	483.28 ± 13.19	125.67 ± 2.517
Chromolaena odorata	14.4 ± 0.219	132.05 ± 0.057	452.38 ± 0.89	66.67 ± 1.528
Telfaira occidentalis	74.48 ± 0.237	99.07 ± 0.11	452.84 ± 0.905	100.33 ± 2.517
Paraquetina nigresiens	85.12 ± 0.072	124.84 ± 0.673	318.38 ± 3.554	44 ± 2

Each value is mean ± SD (n = 3)

DISCUSSION

Antioxidant intake has received increased awareness because of the health benefits derived from the consumption. The recommended dietary supplements for antioxidants from epidemiological studies are fruits, vegetables and minimally processed staple foods. Leaves screened in this study are used commonly as decoction for febrile ailments, tonic for anemia, wound dressing, herbal tea for general wellness, diabetes and culinary purposes. Antioxidant capacity and free radical scavenging activity could be due to wide variety of antioxidant constituents such as phenolics, ascorbate and carotenoids and antioxidants could be inhibitors of free radicals which initiate oxidation and/or inhibitors of free radical chain propagation reactions. Phenolics, being the most wide spread secondary metabolite in plant kingdom, have received much attention as potential natural antioxidant due to their ability to act as both free radical scavengers and metal chelator [27]. The reducing capacity of a compound may as well serve as a significant indicator of its potential antioxidant activity [28]. The activity of antioxidants has been assigned to various mechanisms such as prevention of chain initiation, binding of transition metal ion catalysts, decomposition of peroxides, prevention of continued hydrogen abstraction, reductive capacity and radical scavenging.

Antioxidant capacity gives information about health benefits and functions of foods and supplements and can be measured by DPPH radical and HROAC radical scavenging relatively within a short time. The order in the decrease of DPPH radical scavenging activities from the leaves extract decreased from *C.siamae* (having the highest value) to *P.guajava* (having the least value). *C.siamae*, *P.nigresiens*, *A.occidentale*, *C.odorata* and *A.indica* had high DPPH radical scavenging values and we observed a strong linear correlation coefficient ($R^2 = 0.981$, $P < 0.05$) between DPPH radical scavenging activity and the TPC, suggesting the

positive influence of phenolics on antioxidant properties of the medicinal leaves. Similar relationships between TPC and DPPH assays have been found by other researchers [29-32]; so, phenolics could be the main antioxidant phytochemicals in leaves.

Out of ten plants screened, *T.catappa*, *M.oleifera*, *P.nigresiens* and *P.guajava* were found to have good hydroxyl radical scavenging activity, wheras *A.occidentalis* and *T.occidentalis* had the lowest. Similar quantification could not be done for *M.indica*; serial dilutions up to 10-fold did not help due to gel formation because of high mangiferin content, the natural C-glucoside xanthone. The high HROAC of *M.oleifera* and *P.nigresiens* could give credence to the use of plants as anti-inflammatory agents [33].

The reducing property is a measure of the ability of the leaves extract to transfer electron and could serve as measure of potential antioxidant activity [34]. Reducing power of extracts is. the presence of reductants in the herbal extracts causing the reduction of the Fe^{3+}/ferricyanide complex to the ferrous form. Flavonoids, the most common group of polyphenols found in plants [35], appear to function as electron and hydrogen-atom donors and could terminate radical chain reactions by converting free radicals to more stable products. Interestingly, we observed a strong linear correlation ($R^2 = 0.989$, $P < 0.05$) between the reducing power and TFC of plants. Thus, the elevated reducing power activities of *C.siamae*, *M.indica*, *A.occidentale*, *T.catappa*, *T.occidentalis* and *C.odorata* could directly be linked to the high TFC content of the plant extracts. The leaves of *C.odorata* are marched and applied on wounds for pain relief and wound healing traditionally. In addition, high content of flavonoids in *C.siamae* and *A.occidentale* could account for the use of the leaves in the treatment of diarrhea, since flavonoids have been shown to inhibit the development of fluids [36].

With regard to TPC and TFC, *C.siamae* had the highest values among the ten plants tested. The phenolic compounds are free radical terminators and flavonoids function as scavengers or chelators. Although correlation was reported by other researchers using different plant extracts [37, 38], there was no correlation between the TPC and TFC in this study.

Transition metals play important roles in initiation and propagation steps of lipid oxidation. The presence of metal ions can accelerate the initiation step of lipid oxidation, decompose hydrogen peroxide to form both peroxyl and alkoxyl radicals, and thus accelerate lipid oxidation at an exponential rate. Hydrogen peroxide can react with transition metal ions to form hydroxyl radical. Metal chelators could convert metal ions into insoluble metal complexes or generate steric hindrance, thus hindering lipid peroxidation. In our study, *P.nigresiens*, *A.occidentale*, *T.occidentalis*, *P.guajava*, *M.indica* and *C.siamae* leaves showed high ferrous ion chelating activity; so, they could be used to hinder lipid peroxidation on intake but also in foods.

In conclusion, the collected results of the present work suggest that TPC and TFC contents of herbs can account for their medicinal. The antioxidant activity levels of the plants tested differ with assays method.

REFERENCES

1. Demir H, Leyla A, Burcu BE, Yasemin KL, Gonul K. Antioxidant and antimicrobial activities of *Solidago virgaurea* extracts Afr J Biotechn 2009; 8:274-9.

2. Aruoma OI. Methodological considerations for characterizing potential antioxidant actions of bioactive components in food plants. Mutat Res 2003; 523-524:9-20.

3. Kalcher K, Svancara I, Buzuk M, Vytras K, Walcarius A. Electrochemical sensors and biosensors based on heterogenous carbon materials. Monatsh Chem 2009; 140:861-89.

4. Surh YZ, Ferguson LR. Dietary and medicinal antimutagens and anticarcinogens: molecular mechanisms and chemopreventive potential-highlight of a symposium. Mutat Res 2003; 523-524:1-8.

5. Park EJ, Pezzuto JM. Botanicals in cancer chemoprevention. Cancer Metastasis Rev 2002; 21:231-55.

 DOI 10.5455/oams.171114.or.077

6. Wattenberg LW. Chemoprevention of cancer. Prev Med 1996; 25:44-5.

7. Greenwald P. Science, medicine and the future of cancer chemoprevention. Brit Med J 2002; 324:714-8.

8. Yang MH, Schaich KM. Factors affecting DNA damage caused by lipid hydroperoxides and aldehydes. Free Radic Biol Med 1996; 20:225-36.

9. Espin JC, Soler-Rivas C, Wichers HJ. Characterization of the total free radical scavenging capacity of vegetable oils and oil fractions using 2, 2-diphenyl-1-picrylhydrazyl radical. J Agric Food Chem 2000; 48: 648-656.

10. Halliwell B. Free radicals, antioxidants and human disease: curiosity, cause or consequence. Lancet 1994; 344:721-4.

11. Youdim KA, Joseph JA. A possible emerging role of phytochemicals in improving age-related dysfunction: A multiplicity of effects. Free Radic Biol Med 2001; 30:583-94.

12. Delanty N, Dichter MA. Antioxidant therapy in neurologic diseases. Arch Neurol 2000; 57:1265-70.

13. Mantle D, Lennard TW, Pickering AT. Therapeutic applications of medicinal plants in the treatment of breast cancer: A review of their pharmacology, efficacy and tolerability. Adverse Drug React Toxicol Rev 2000; 19:223-40.

14. Robards K, Prenzler PD, Tucker G, Swatsitang P, Glover W. Phenolic Compounds and their role in oxidative processes in fruits. Food Chem 1999; 66:401-36.

15. Repetto MG, Llesuy SF. Antioxidant properties of natural compounds used in popular medicine for gastric ulcers. Braz J Med Biol Res 2002; 35:523-34.

16. Streelatha S, Padma PR. Antioxidant activity and total phenolic of *Moringa oleifera* leaves in two stages of maturity. Plant Food Hum Nutri 2009; 64(4): 303-311.

17. Charng-Cherng C, Pei-Tzu K, Jeng LM. Antioxidant properties of aqueous extracts from *Terminalia catappa* leaves. LWT Food Sci Technol 2006; 39:1099-108.

18. Charan AA, Gupta P. Comparative analysis of antibacterial, antioxidant and photosynthetic activity of *Azardirachta indica*, *Rosa indica* and *Moringa oliefera* cultivars. Int J Curr Res 2013; 15:556-61.

19. Qian H, Nihorimbere V. Antioxidant power and phytochemicals from *Psidium guajava* leaf. J Zhejiang Univ Sci 2004; 5:676-83

20. Singleton VL, Orthofer R, Lamuela-Raventos RM. Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. Methods Enzymol 1999; 299:152-78.

21. Jagadish LK, Krishnan VV, Shenbhagaraman R, Kaviyarasan V. Comparative study on the antioxidant, anticancer and antimicrobial property of *Agaricus bisporus* imbach before and after boiling. Afr J Biotechnol 2009; 8:654-61.

22. Ardestani A, Yazdanparast R. Antioxidant and free radical scavenging potential of *Achillea santolina* extracts. Food Chem 2007; 104:21-9.

23. Yu W, Zhao Y, Shu B. The radical scavenging activities of *Radix puerariae* isoflavonoids: A chemiluminescence study. Food Chem 2004; 86:525-9.

24. Oyaizu M. Studies on products of browning reaction: antioxidative activity of products of browning reaction prepared from glucosamine. Jpn J Nutr 1986; 44:307-15.

25. Dinis TCP, Madeira VMC, Almeida LM. Action of phenolic derivatives (Acetaminophen, salicylate, and 5-aminosalicylate) as inhibitors of membrane lipid peroxidation and as peroxyl radical scavengers. Arch Biochem Biophys 1994; 315:161-9.

26. VanderJagt DJ, Garry PJ, Hunt WC. Ascorbate in plasma as measured by liquid chromatography and by dichlorophenolindophenol colorimetry. Clin Chem 1986; 32:1004-6.

27. Cao G, Sofic E, Prior RL. Antioxidant and prooxidant behavior of flavonoids: structure-activity relationships. Free Radic Biol Med 1997; 22:749-60.

28. Apak R, Guclu K, Demirata B, Ozyurek M, Celik SE, Bektasoglu B, Berker KI, Ozyurt D. Comparative evaluation of various total antioxidant capacity assays applied to phenolic compounds with the CUPRAC assay. Molecules 2007; 12:1496-547.

29. Roy MK, Takenaka M, Isobe S, Tsushida T. Antioxidant potential, antiproliferative activities and phenolic content in water soluble fractions of some commonly consumed vegetables: effects of thermal treatment. Food Chem 2007; 103:106-14.

30. Chew YL, Lim YY, Omar M, Khoo KS. Antioxidant activity of three edible seaweeds from two areas in South East Asia. LWT Food Sci Technol 2008; 41:1067-72.

31. Sikora E, Cieslik E, Leszczynska T, Filipiak-Florkiewicz A, Pisulewski PM. The antioxidant activity of selected cruciferous vegetables subjected to aquathermal processing. Food Chem 2008; 107:55-9.

32. Xu B, Chang SKC. Effect of soaking, boiling and steaming on total phenolic content and antioxidant activities of cool season food legumes. Food Chem 2008; 110:1-13.

33. Surh YJ, Chun KS, Cha HH, Han SS, Keum YS, Park KK, Lee SS. Molecular mechanisms underlying chemopreventive activities of anti-inflammatory phytochemicals: down-regulation of COX-2 and iNOS through suppression of NF-kappa B activation. Mutat Res 2001; 480-481:243-68.

34. Meir S, Kanner J, Akiri B, Philosoph-Hadas S. Determination and involvement of aqueous reducing compounds in oxidative defense systems of various senescing leaves. J Agric Food Chem 1995; 43:1831-9.

35. Upadhay RK. Plant natural products: Their pharmaceutical potential against disease and drug resistant microbial pathogens. J Pharm Res 2011; 4:1179-85.

36. Schuier MH, Sies B, Billek G, Fischer H. Cocoa-related flavonoids inhibit CFTR-Mediated chloride transport across T84 human colon epithelia. J Nutr 2005; 135:2320-5.

37. Turkmen N, Sari F, Velioglu S. The effect of cooking methods on total phenolics and antioxidant activity of selected green vegetables. Food Chem 2005; 93:713-8.

38. Zhang D, Hamauzu Y. Phenolics, ascorbic acid, carotenoids and antioxidant activity of broccoli and their changes during conventional and microwave cooking. Food Chem 2004; 88:503-9.

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