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Full Length Research Paper

Antioxidant activity of some African medicinal and dietary leafy African vegetables

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Seven medicinal and dietary plant species from Southern Africa were analysed for their antioxidant and total phenolic content. These were *Lippia javanica, Tagetes minuta, Bidens pilosa, Vigna unguiculata, Amaranthus spinosus, Telfairia occidentalis* and *Corchorus olitarius*. Aqueous methanol extracts were tested for free radical scavenging and anti-oxidant activity using three standard assays including 2,2'azinobis-(3-ethylbenzthiazoline-6-sulphonic acid) (ABTS), 1,1-diphenyl-2-picrylhydrazyl (DPPH) and ferric reducing antioxidant potential (FRAP). The Folin-Ciocalteu assay was used to determine the total phenolic content with gallic acid as a standard. The antioxidant activity of the plants ranged from 0.76 to 5.77 mmol TEAC/100 g (ABTS), 16.29 to 1711.22 mmol TEAC/100 g (DPPH) and 0.58 to 6.12 mmol TEAC/100 g (FRAP). *B. pilosa* and *C. olitarius* had the best activity in all assays, while *V. unguiculata* and *A. spinosus* were the least active. The total phenolic content ranged from 19.79 to 333.56 mg gallic acid equivalent (GAE)/100 g. In general, there was a good correlation between antioxidant activity and total phenolic content. These results imply that these plant species may possess health promoting effects and might be potential sources of potent natural antioxidants.

Key words: Dietary plants, metabolic stress, antioxidant activity, total phenolic content.

INTRODUCTION

Free radicals are generally unstable reactive molecules that are produced in animals and humans under physiological and pathological conditions (Fang et al., 2002). There is increased scientific evidence that oxidative stress which results in the generation of free radicals contributes to many common ailments including cancer, cardiovascular disease, cataract formation, as well as accelerating the ageing process (Bagchi et al., 2000; Fang et al., 2002; Bugg et al., 2006; Dasgupta et al., 2007). Epidemiological studies have shown a strong and consistent protective effect of dietary antioxidants against the risk of such illnesses (Block et al., 1992; Varma et al., 1995; Steinmetz and Potter, 1996; Hunter and Fletcher, 2002). This protective effect is often attributed to different antioxidant components, such as vitamin C, vitamin E, carotenoids, polyphenolic compounds and other phytochemicals (Amin et al., 2006). A high intake of food rich in natural antioxidants has been shown to increase the antioxidant capacity of the plasma and reduce the risk of some, but not all, cancers, heart diseases and stroke (Kris-Etherton et al., 2002). Recent research has also shown that through overlapping or complementary effects, the complex mixture of phytochemical compounds in fruits and

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Abbreviations: ABTS, 2,2'Azinobis-3-ethylbenzthiazoline-6-sulphonic acid; **DPPH,** 1,1-diphenyl-2-picrylhydrazyl; **FRAP,** ferric reducing antioxidant potential; **GAE,** gallic acid equivalent.

vegetables provides a protective effect on health than single phytochemicals (Eberhardt et al., 2000).

The present study investigated the total phenolic content and related total antioxidant potential of extracts of *Amaranthus spinosus* (Amaranthaceae), *Vigna unguiculata* (Fabaceae), *Lippia javanica* (Verbenaceae), *Tagetes minuta* (Asteraceae), *Bidens pilosa* (Asteraceae), *Telfairia occidentalis* (Cucurbitaceae) and *Corchorus olitarius* (Tiliaceae). The plant species were selected because of their dietary use in southern Africa. In addition, all of them are important in the traditional medical armamentarium. *L. javanica*, for example, has been reported to have antimicrobial activity and is used in the treatment of colds, coughs and bronchial problems (Muchuweti et al., 2006). The species has also been reported to be effective against fever caused by malaria, influenza and measles (Viljoen et al., 2005). For example, *T. minuta* has been reported to have antibacterial activity against *Staphylococcus aureus* and *Staphylococcus epidermidis* (Vasudevan et al., 1997). It is used in the treatment of colds, diarrhoea and suspected liver ailments (Rios and Recio, 2005). *A. spinosus* is used in the treatment of menorrhagia, gonorrhoea, eczema and colic (Azhar et al., 2004) and is also cited in the treatment of diabetes (Katerere and Eloff, 2005). The root infusion of *V. unguiculata* is used to treat dysmenorrhoea and epilepsy, while a soup made from the seeds is reported for the treatment of bilharziasis (Gelfand et al., 1993). *B. pilosa* has been reported to have antibacterial, antifungal and anticancer activities (Khan et al. 2001), and *T. occidentalis* is used to treat anaemia (Aiyeloja and Bello, 2006; Mensah et al., 2008).

Furthermore, the leaves of *C. olitarius* have been reported to possess diuretic, antipyretic, analgesic and antimicrobial activity, to contain antitumor compounds (Furumoto et al., 2002) and antioxidant carotenoids and flavonoids (Azuma et al., 1999; Khan et al., 2001; Zeid, 2002). *A. spinosus, V. unguiculata, B. pilosa, T. occidentalis* and *C. olitarius* are eaten as a relish with maize staple (pap or sadza). These plant species play an important role as famish foods in rural Southern Africa and they may be cooked (fresh or dry) with tomato or peanut sauce and served alone or with meat. Aerial parts of *T. minuta* and *L. javanica* are consumed as herbal teas and commercially available in some African countries, for example, in Ghana and Kenya.

However, there are few studies on the healthful properties of these dietary plant species. In this study, we screened them for both antioxidant activity and total phenolic content, considering the increasing importance of both parameters in human health and nutrition.

MATERIALS AND METHODS

Chemicals

All chemicals used were of analytical grade. 2,4,6-Tri(2-pyridyl)-s-triazine (TPTZ), 2,2′azinobis-(3-ethylbenzthiazoline-6-sulphonic acid) (ABTS), gallic acid and Trolox were obtained from Sigma Aldrich Co. (St. Louis, MO). Anhydrous sodium carbonate (Na_2CO_3), ferric chloride hexahydrate ($FeCl_3 \cdot 6H_2O$), Folin–Ciocalteu phenol reagent, hydrochloric acid (HCl), glacial acetic acid, potassium persulphate, methanol and sodium acetate trihydrate were obtained from Merck (Darmstadt, Germany).

Plant materials

T. minuta, L. javanica and *B. pilosa* were collected from Onderstepoort, Pretoria, South Africa, while *A. spinosus, V. unguiculata, T. occidentalis* and *C. olitarius* were collected from Moruleng village of Rustenburg, North West Province, South Africa. Voucher specimens were collected and deposited at the UWC herbarium in Cape Town. The plants were collected between March and April 2007, oven-dried at 50°C and separately ground into a fine powder using a Romer Labs Series II Grinding/Sub-sampling mill (Romer Labs, Tulln, Austria). They were then shipped to the University of Naples, Federico II in Naples, Italy, where the analysis was done.

Extract preparation

Three grams (3 g) of each plant material were extracted with 30 ml of 70% aqueous methanol by sonication for 30 min and centrifuged (Jouan CR3i of BICASA Spa, Italy) for 5 min at 4000 rpm. The extracts were filtered using Whatman no. 1 filter paper and the aliquots were analyzed for their antioxidant capacity and total phenol content using the methods subsequently described. Each sample was prepared and analyzed for each assay in triplicate.

ABTS radical scavenging assay

The free radical scavenging activity of the plant extracts was determined using 2,2′azinobis-(3-ethylbenzthiazoline-6-sulphonic acid) (ABTS) radical cation decolorization assay and 6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox) as a standard (Re et al., 1999). ABTS was dissolved in water to a 7 mM concentration. ABTS radical cation ($ABTS^{*+}$) was produced by reacting ABTS stock solution with 2.45 mM potassium persulphate (final concentration) and allowing the mixture to stand in the dark at room temperature for 12 to 16 h before use. The free radical was stable in this form for more than two days when stored in the dark at room temperature. For this study $ABTS^{*+}$ solution was diluted with ethanol to an absorbance of 0.70 (±0.02) at 734 nm. Reagent blank reading was also taken (A_0). After the addition of 1.0 ml of diluted $ABTS^{*+}$ solution to 100 µL of each plant extract, the absorbance reading (A_e) was taken exactly 2.5 min after initial mixing. The plant extracts were first adequately diluted (where necessary) to fit within the linearity range. The percentage inhibition of absorbance at 734 nm was calculated using the formula: % inhibition = $(1 - A_e / (A_o) \times 100$. The antioxidant capacity based on the ABTS free radical scavenging ability of the extract was expressed as mmol Trolox equivalence antioxidant capacity (TEAC) per 100 g of plant material.

Ferric reducing antioxidant potential assay (FRAP)

The FRAP assay was carried out according to the method of Benzie and Strain (1996) with few modifications. The FRAP reagent was prepared from sodium acetate buffer (300 mmol/L, pH 3.6), 10 mmol/L 2,4,6-tri(2-pyridyl)-s-triazine (TPTZ) solution in 40 mmol/L HCl and 20 mmol/L $FeCl_3$ solution in proportions of 10:1:1 (v/v),

Table 1. Total antioxidant activity and phenolic content of the plant extracts.

Plant species	TEAC (mmol/100 g)			Phenolic Content (mg GAE/100 g)
	ABTS	DPPH	FRAP	
Vigna unguiculata	0.76	95.93	0.58	109.14
Amaranthus spinosus	1.02	16.29	0.75	79.79
Tagetes minuta	2.3	1399.42	4.22	216.84
Lippia javanica	1.5	1462.54	2.38	221.31
Bidens pilosa	5.77	1210.05	6.12	333.56
Telfairia occidentalis	3.37	293.29	2.68	222.94
Corchorus olitarius	4.42	1711.22	5.04	316.34

ABTS, 2,2'Azinobis-3-ethylbenzthiazoline-6-sulphonic acid; DPPH, 1,1-diphenyl-2-picrylhydrazyl; FRAP, ferric reducing antioxidant potential; GAE, gallic acid equivalent

respectively. The FRAP reagent was prepared fresh daily. Antioxidant potential was determined by adding 651 µL sodium acetate buffer solution, 279 µL of FRAP reagent and 70 µL of each plant extract. The absorbance reading was taken at 593 nm exactly 4 min after initial mixing. The plant extracts were first adequately diluted to fit within the linear dynamic range. Solvent blanks were also prepared and the absorbance reading taken. The antioxidant capacity based on the ability to reduce ferric ions of the extract was expressed as mmol Trolox equivalents (TEAC) per 100 g of plant material.

DPPH radical scavenging assay

The 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging activity of plant extracts was determined using the method by Yen and Chen (1995). The DPPH radical ($DPPH^+$) solution was prepared in MeOH to make a 1 mM $DPPH^+$ solution. DPPH solution was further diluted with MeOH at the ratio of 1:25. The scavenging activity of the plant extracts was determined by adding 600 µl of diluted DPPH solution and 300 µl of each plant extract. The absorbance reading of 0.900 ± 0.020 at 517 nm was taken exactly 4 min after initial mixing. The plant extracts were first adequately diluted to fit within the linearity range. The absorbance of the DPPH radical without antioxidant (the control) was also measured. All determinations were carried out in triplicate. The percentage inhibition of the DPPH radical by the samples was calculated using the same formula as in ABTS assay. The antioxidant capacity based on the DPPH free radical scavenging ability of the extract was expressed as mmol Trolox equivalents (TEAC) per 100 g of plant material.

Determination of total phenolic content (TPC)

Total phenolic concentration in plant extracts was determined spectrophotometrically by the Folin–Ciocalteu assay (Singleton and Rossi, 1965) using gallic acid as a standard. An aliquot of 125 µL of each plant extract was mixed with 125 µL of Folin–Ciocalteu phenol reagent and allowed to react for 6 min. Afterward, 1.25 ml of saturated Na_2CO_3 solution (7.5%) was added and allowed to stand for 90 min before the absorbance of the reaction mixture was measured at 760 nm. The total phenolic content of the plant extracts was expressed as mg gallic acid equivalents (GAE) per 100 g of plant material.

Statistical analysis

All determinations of the antioxidant activity by the assays were conducted in triplicate. The reported values of each sample and the correlation coefficients (R^2) were calculated as the mean of three measurements using Microsoft Excel 2000.

RESULTS AND DISCUSSION

Antioxidant activity

The ABTS, FRAP and DPPH assays were used to measure the antioxidant activity of the plant extracts. Because different antioxidant compounds may act *in vivo* through different mechanisms, no single method can fully evaluate the total antioxidant capacity of foods. For this purpose, in this study three different assays were applied to obtain robust data on antioxidant activity of the selected plants (Table 1). The ABTS and DPPH assays measure the ability of antioxidants to quench a radical cation, while the FRAP assay evaluate the reducing potential of the samples.

The TEAC values of ABTS assay ranged from 0.76 - 5.77 mmol Trolox/100 g, FRAP from 0.58 - 6.12 mmol Trolox/100 g and for DPPH from 16.29 - 1711.22 mmol Trolox/100 g. *B. pilosa* was found to have the highest antioxidant activity in both ABTS (5.77 mmol/100 g) and FRAP (6.12 mmol/100 g) assays, while *C. olitarius* had the highest antioxidant activity in DPPH (1711.22 mmol/100 g) assay. *V. unguiculata* had the lowest antioxidant activity in both ABTS (0.79 mmol/100 g) and FRAP (0.58 mmol/100 g) assays. Moreover, the antioxidant activity obtained from ABTS and FRAP were better correlated (R^2 = 0.8296) compared to those obtained from ABTS-DPPH (R^2= 0.2585) and DPPH-FRAP (R^2 = 0.5943). This implies that these plants contain compounds that are capable of scavenging free cation radicals ($ABTS^{*+}$) as well as reducing oxidants (ferric ions). Activity against DPPH radical was not potent on the other hand. According to Surveswaran et al. (2007), it is not surprising to find differences in antioxidant activity among the assays, as each has a different mechanism of action and different reaction conditions. ABTS*+ is soluble in both aqueous and organic solvents and so can determine both hydrophilic

and lipophilic antioxidant capacities (Arnao, 2000; Thaipong et al. 2006).

V. unguiculata and *A. spinosus* showed the lowest antioxidant activity in all the assays, but this activity was similar to that previously reported for other commonly consumed African green leafy vegetables (Akindahunsi and Salawu, 2005; Oboh, 2006). Antioxidant activity of *V. unguiculata* seeds has been previously reported and it was demonstrated that the DPPH radical and ABTS cation radical scavenging activities were well correlated with the ferric reducing antioxidant capacity (Oboh and Akindahunsi, 2004; Siddhuraju and Becker, 2007). Oboh (2005) reported the antioxidant activity of the aqueous and ethanolic extracts of *T. occidentalis* leaf showing the hepatoprotective properties after garlic-induced oxidative stress in rats; in particular the aqueous extract is more effective than the ethanolic extract and this could be attributed to the higher antioxidant activity of the aqueous extract. To date, the reported antioxidant activity of *L. javanica* and *T. minuta* are mainly from essential oils (Muyima et al., 2004). A comparative study of the antioxidant properties of hydrophilic and lipophilic extract constituents of the *C. olitorius* leaves by Oboh et al.,(2009) reported that hydrophilic extract showed a higher DPPH radical-scavenging ability, reducing power and trolox equivalent antioxidant capacity (TEAC), while lipophilic extract showed a higher OH scavenging ability. Zeashan et al. (2009) also reported that ethanolic extracts of *A. spinosus* possess significant hepatoprotective activity which might be due to antioxidant capacity, and they attributed this to its high phenolic content.

In general, limited information is available on the antioxidant potential of extracts of these plants which are important dietary and medicinal plants in rural sub-Saharan Africa. Our results show that the free radical scavenging ability of the plants analyzed was generally lower than that of fruits and vegetables like spinach and red pepper which were previously studied by Chu et al. (2002). It is also complicated to compare results from other studies because of immense variations in environment, climate and time of harvest, among numerous other parameters which affect any wild growing plant (Howard et al., 2003; Zhou and Yu, 2004).

Total phenolic content

Phenolics constitute one of the major groups of compounds which act as primary antioxidants (Muchuweti et al., 2006). They inhibit autoxidation of unsaturated lipids, thus preventing the formation of oxidized low-density lipoprotein (LDL), which is considered to induce cardiovascular diseases. The assay used for total phenols determination detects phenolic acids, flavonoids, tannins, anthocyanins, lignans and coumarins. The content of phenolic compounds is expressed as milligrams gallic acid equivalence (mg GAE) per 100 g plant sample. The amounts of total phenolics of the plant extracts are shown in Table 1. The total phenolic content of the plants decreased in the order: *B. pilosa* (333.56 mg GAE/100 g) > *C. olitarius* (316.34 mg GAE/100 g) > *T. occidentalis* (222.94 mg GAE/100 g) > *L. javanica* (221.31 mg GAE/100g) > *T. minuta* (216.84 mg GAE/100g) > *V. unguiculata* (109.14 mg GAE/100 g) > *A. spinosus* (79.79 mg GAE/100 g).

While some authors found a strong correlation between phenolic content and the antioxidant activity (Velioglu et al., 1998), others found no such relationship (Kaehkoenen et al., 1999) showing that the antioxidant activity of an extract cannot be predicted on the basis of its total phenolic content because the activity of phenolic compounds depends on their chemical structure (Statue-Gracia et al., 1997). This is probably because there are structure-activity relationships governing anti-oxidant activity. Flavones and catechins are, in general, superior to other polyphenols (for example, lignans and coumarins) in this regard (Nijveldt et al., 2001). In addition, the extent of hydroxylation and glycosylation also plays an important role in determining anti-oxidant potency. The antioxidant activity of flavonoids has been shown to reside in the aromatic hydroxyl groups (Rezk et al., 2002) with maximum radical scavenging activity displayed by flavonoids with 3-OH groups attached to the 2,3–double bond neighboring the carbonyl group in the C-ring (Cotelle, 2001). Resorcinol and phloroglucinol substituents also show substantially higher antioxidant activity compared to phenol (Rezk et al., 2002). In this regard, two anti-oxidant pharmacores are now recognized in flavonoids - the catechol structure in ring B and in rings A and C (Rezk et al., 2002).

In this study, the findings show that phenolic content was better correlated with both FRAP (R^2= 0.8923) and ABTS (R^2= 0.8266) assays than with that of DPPH (R^2= 0.622) (Table 2). These results suggest that in the plants analyzed in the current study, 89% of the ferric reducing power and 82.6% of the ability to scavenge the $ABTS^{*+}$ radical cations, is probably due to phenolic compounds. Also, it can be concluded that antioxidant activity of plant extracts is not limited to phenolics. Activity may also be due to the presence of other secondary metabolites such as volatile oils, carotenoids, and vitamins, among others, that in this case contributed to 11% (for FRAP assay) and 17.4% (for ABTS assay), respectively to the antioxidant capacity. The non-polyphenolic compounds appear to be more important in contributing to activity in the DPPH assay. The antioxidant activity of phenolics is mainly due to the ease with which they can be involved in redox reactions, which implies that they can act as reducing agents, hydrogen donors and singlet oxygen quenchers.

The extract with the lowest total polyphenolic content was that of *A. spinosus*, which also showed the lowest antioxidant activity in both assays. Our results confirm

Table 2. Correlation between the different assays used in this study.

Correlation	R^2
ABTS - DPPH	0.8296
ABTS - FRAP	0.2585
DPPH - FRAP	0.5943
ABTS - TPC	0.8266
DPPH - TPC	0.622
FRAP - TPC	0.8923

previous observations. Muchuweti et al. (2006) reported that the measured antioxidant activity of plant extracts may be due to the synergistic effect of polyphenolics with one another and/or with other components present in an extract. However, there is a need to identify and to characterize active components within each plant extract since it is reported in the literature that different classes of phenolics have varying antioxidative strengths and that synergy of polyphenolics (condensed tannins, gallotannins and flavonoids) with one another or with other components present in an extract may contribute to the overall observed antioxidant activity (Shahidi et al., 1994).

Conclusion

African leafy vegetables have long been known and reported to have health protecting properties and uses. They have a long history of being consumed by humans as both food and medicine. However, due to westernization and urbanization, the consumption of these vegetables appears to be declining and yet they may be an important resource in promoting good nutrition in sub-Saharan Africa (Odav et al., 2007; Smith and Eyzaguirre, 2007). The current study shows the potential of some common African vegetables as functional foods whose uses should be further investigated and encouraged. The antioxidant activity and total phenolic content of the extracts analyzed were appreciable. Results highlighted a good correlation between antioxidant activity and total phenolic content. This may indicate that phenolic compounds play a role in the antioxidant activity of plant materials. Meanwhile, further work is necessary to elucidate the identity the compounds responsible for the antioxidant activity of these plant species.

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REFERENCES

Aiyeloja A, Bello O (2006). Ethnobotanical potentials of common herbs in Nigeria: A case study of Enugu state. Edu. Res. Review 1: 16-22.

Akindahunsi A, Salawu S (2005). Antioxidant indices of some green leafy vegetables. *Trop. Sci.* 45: 33-35.

Amin I, Norazaidah Y, Hainida K (2006). Antioxidant activity and phenolic content of raw and blanched Amaranthus species Food Chemistry, 94:47-52.

Arnao MB (2000). Some Methodological Problems in the Determination of Antioxidant Activity Using Chromogen Radicals: A Practical Case. Trends Food Sci. Tech. 11, 419-421.

Azhar ul H, Malik A, Khan A, Shah M, Muhammad P (2004). Spinoside, new coumaroyl flavone glycoside from Amaranthus spinosus. Archiv.pharmaceut. Res. 27(12): 1216-1219.

Azuma K, Nakayama M, Koshioka M, et al (1999). Phenolic Antioxidants from the Leaves of Corchorus olitorius L. J Agric Food Chem. 47(10): 3963-3966.

Bagchi D, Bagchi M, Stohs SJ, Das DK, Ray SD, Kuszynski CA, Joshi SS, Pruess HG (2000). Free radicals and grape seed proanthocyanidin extract: importance in human health and disease prevention. Toxicology, 148(2-3): 187-197.

Benzie IFF, Strain JJ (1996). The Ferric Reducing Ability of Plasma (FRAP) as a Measure of "Antioxidant Power": The FRAP Assay. Analytical biochemistry, 239: 70-76

Block G, Patterson B, Subar A (1992). Fruit, vegetables, and cancer prevention: a review of the epidemiological evidence. Nutr Cancer.18(1):1-29.

Bugg GJ, Crocker I, Baker PN, Johnston TA, Taggart MJ (2006). The effect of superoxide free radicals on human myometrial contractility in vitro. Eur J Obstet Gynecol Reprod Biol.125(1): 140-141.

Chu YF, Sun J, Wu X, Liu RH (2002). Antioxidant and antiproliferative activities of common vegetables. J. Agric. Food Chem. 50(23): 6910-6916.

Cotelle N (2001). Role of flavonoids in oxidative stress. Curr. Top. Med. Chem. 1(6): 569-590.

Dasgupta N, De B (2007). Antioxidant activity of some leafy vegetables of India: A comparative study. Food Chemistry, 101: 471-474.

Eberhardt MV, Lee CY, Liu RH (2000). Antioxidant activity of fresh apples. Nature,405(6789): 903-904.

Fang YZ, Yang S, Wu G (2002). Free radicals, antioxidants, and nutrition. Nutrition, 18(10): 872-879.

Furumoto T, Wang R, Okazaki K, AFMF H, Ali M, Kondo A, Fukui H, (2002). Antitumor promoters in leaves of jute (Corchorus capsularis and Corchorus olitorius). Food Sci Technol Res 8 239-243.

Gelfand M, Mavi S, Drummond R, Ndemera B (1993) *The traditional medical practitioner in Zimbabwe* 2nd ed. Gweru: Mambo Press.

Howard LR, Clark JR, Brownmiller C (2003). Antioxidant capacity and

phenolic content in blueberries as affected by genotype and growing season. J. Sci. Food Agric. 83: 1238-1247
Hunter K, Fletcher J (2002). The antioxidant activity and composition of fresh, frozen, jarred and canned vegetables. Innovative Food Science & Emerging Technologies, 3: 399-406.
Kaehkoenen M, Hopia A, Vuorela H, Rauha J (1999). Antioxidant Activity of Plant Extracts Containing Phenolic Compounds. J. Agric. Food Chem. 47: 3954-3962.
Katerere D, Eloff J (2005). Management of diabetes in African traditional medicine. In: Soumyanath A, ed. Tradi. Med. modern times: Antidiabetic plants: Taylor and Francis Group, CRC Press. pp. 157-158.
Khan MR, Kihara M, Omoloso AD (2001). Anti-microbial activity of Bidens pilosa, Bischofia javanica, Elmerillia papuana and Sigesbekia orientalis. Fitoterapia, 72(6): 662-665.
Kris-Etherton PM, Hecker KD, Bonanome A, Coval SM, Binkoski AE, Hilpert KF, Griel AE, Etherton TD (2002). Bioactive compounds in foods: their role in the prevention of cardiovascular disease and cancer. Am. J. Med. 113(9): 71-88.
Mensah J, Okoli R, Ohaju-Obodo J, Eifediyi K (2008). Phytochemical, nutritional and medical properties of some leafy vegetables consumed by Edo people of Nigeria. . Afr. J. Biotechnol. 7: 2304-2309.
Muchuweti M, Nyamukonda L, Chagonda L, Ndhlala A, Mupure C, Benhura M (2006). Total phenolic content and antioxidant activity in selected medicinal plants of Zimbabwe. Int. J. Food Sci. Technol. 41: 33-38.
Muyima N, Nziweni S, Mabinya L (2004). Antimicrobial and antioxidative activities of Tagetes minuta, Lippia javanica and Foeniculum vulgare essential oils from the Eastern Cape Province of South Africa. J. Essential Oil-Bearing Plants, 7: 68-78.
Nijveldt RJ, van Nood E, van Hoorn DE, Boelens PG, van Norren K, van Leeuwen PA (2001). Flavonoids: a review of probable mechanisms of action and potential applications. Am. J. Clin. Nutr. 74(4): 418-425.
Odav B, Beekrum U, Baıjnath H (2007). Preliminary assessment of nutritional value of traditional leafy vegetables in KwaZulu-Natal. J. Food Compos. Anal. 20:430-435.
Oboh G (2006). Antioxidant properties of some commonly consumed and underutilized tropical legumes. Eur. Food Res. Technol. 224 61-65.
Oboh G, Akindahunsi A (2004). Change in the ascorbic acid, total phenol and antioxidant activity of sun-dried commonly consumed green leafy vegetables in Nigeria. Nutrition and Health, 18: 29-36.
Oboh G (2005). Effect of blanching on the antioxidant properties of some tropical green leafy vegetables. Food Sci. Technol. 38: 513-517.
Oboh G, Raddatz H, Henle T (2009). Characterization of the antioxidant properties of hydrophilic and lipophilic extracts of Jute (Corchorus olitorius) leaf. Int. J. Food Sci. Nutr. 60: 124-134.
Rezk BM, Haenen GR, van der Vijgh WJ, Bast A (2002). The antioxidant activity of phloretin: the disclosure of a new antioxidant pharmacophore in flavonoids. Biochem. Biophy. Res. Commun. 295(1): 9-13.
Rios JL, Recio MC (2005). Medicinal plants and antimicrobial activity. J Ethnopharmacol. 100 (1-2): 80-84.
Steinmetz K, Potter J (1996). Vegetables, fruits and cancer prevention: a review. J. Am. Diet Assoc. 96: 1027-1039.
Statue-Gracia M, Heionen M, Frankel E (1997). Antioxidant activity of anthocyanin in LDL and lecithin liposome systems. J. Agric. Food Chem. 5: 3362-3367.
Shahidi F, Wanasundara UN, Amarowicz R (1994). Natural antioxidants from low-pungency mustard flour. Food Res. Int. 27(5): 489-493.
Siddhuraju P, Becker K (2007). The antioxidant and free radical scavenging activities of processed cowpea (Vigna unguiculata (L.) Food Chemistry, 101: 10-19.
Singleton VL, Rossi JA (1965). Colorimetry of Total Phenolics with Phosphomolybdic-Phosphotungstic Acid Reagents. Am. J. Enol. Vitic. 16(3): 144-158.
Smith F, Eyzaguirre P (2007). African leafy vegetables: Their role in the World Health Organisation's global fruit and vegetables initiative. Afr. J. Food, Agric, Nutr. Develop.7(3).
Surveswaran S, Yi-Zhong C, Corke H, Sun M (2007). Systematic evaluation of natural phenolic antioxidants from 133 Indian medicinal plants. Food Chemistry, 102: 938-953.
Thaipong K, Boonprakob U, Crosby K, Cisneros-Zevallos L, Byrne D (2006). Comparison of ABTS, DPPH, FRAP, and ORAC assays for estimating antioxidant activity from guava fruit extracts. J. Food Compos. Anal. 19: 669-675.
Varma S, Devamanoharan P, Morris S (1995). Prevention of cataracts by nutritional and metabolic antioxidants. . Crit. Rev. Food Sci. Nutr. 35: 111-129.
Vasudevan P, Kashyap S, Sharma S (1997). Tagetes: A multipurpose plant. Bioresour Technol. 62 (1-2): 29-35.
Velioglu Y, Mazza G, Gao L, Oomah B (1998). Antioxidant activity and total phenolics in selected fruits, vegetable, and grain products, . J. Agric.Food Chem. 46: 4113-4117.
Viljoen AM, Subramoney S, van Vuuren SF, Baser KH, Demirci B (2005). The composition, geographical variation and antimicrobial activity of Lippia javanica (Verbenaceae) leaf essential oils. J Ethnopharmacol. 96 (1-2): 271-277.
Yen G-C, Chen H-Y (1995). Antioxidant Activity of Various Tea Extracts in Relation to Their Antimutagenicity. J Agric Food Chem. 43(1): 27-32.
Zeashan H, Amresh G, Singh S, Rao CV (2009). Hepatoprotective and antioxidant activity of Amaranthus spinosus against CCl4 induced toxicity. J Ethnopharmacol. 125(2): 364-366.
Zeid A (2002). Stress metabolites from Corchorus olitorius L. leaves in response to certain stress agents. Food Chemistry, 76: 187-195.
Zhou K, Yu L (2004). Antioxidant Properties of Bran Extracts from Trego Wheat Grown at Different Locations. J. Agric .Food Chem. 52(5): 1112-1117.