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Original Research Article

In Vitro Antioxidant Properties of Aqueous and Methanol Extracts of the Stem Bark of *Anthocleista Vogelii* Planch (Loganiaceae)

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ABSTRACT

Anthocleista vogelii plant has applications in African traditional medicine against gastrointestinal diseases, fever, diabetes, constipation and inflammation. This work was been carried out with the objective of evaluating the in vitro antioxidant properties of aqueous and methanol extracts of the stem bark of *Anthocleista vogelii*. The antioxidant activities of the extracts have been evaluated by using in vitro assays and were compared to a standard antioxidant ascorbic acid. The evaluated parameters were the free radical scavenging activity, the ferric reducing power assay, the hydroxyl radical scavenging activity, the total phenolic content and the total flavonoids content. All the extracts showed effective H donor activity, reducing ferric power, free radical scavenging activity. The free radical scavenging and antioxidant activities may be attributed to the presence of phenolic and flavonoid compounds in the plant extracts. The results obtained in the present study indicate that the stem bark of *Anthocleista vogelii* a potential source of natural antioxidant.

Keyword: *Anthocleista vogelii*; antioxidant activities; aqueous and methanol extracts; stem bark.

INTRODUCTION

Reactive free radicals, such as superoxide anion (O2-), hydroxyl radical (OH), and peroxyl radical (ROO-), are particularly reactive and are known to be a biological product in reducing molecular oxygen. Reactive oxygen species (ROS) are an entire class of highly reactive molecules derived

from the metabolism of oxygen. ROS can cause extensive damage to cells and tissues, during infections and various degenerative disorders and neurodegenerative diseases [1,2,3,4]. Medicinal plants and natural products have become a great source of antioxidant and anti-ageing properties [5]. Many human diseases are caused by oxidative stress that results from imbalance between the formation and neutralization of pro-oxidants [6]. The role of medicinal plants in disease prevention or control has been attributed to antioxidant properties of their constituents, usually associated to a wide range of amphipathic molecules, broadly termed polyphenolic compounds [7]. The principle function of antioxidants is in delaying the oxidation of other molecules by inhibiting the initiation or propagation of oxidizing chain reactions by free radicals and they may reduce oxidative damage to the human body [9].

MATERIALS AND METHODS

Collection and authentication of the plant material

The stem bark of *Anthocleista vogelii* was collected in November 2011 in Bandjoun (West region, Cameroon) and authenticated in the National Herbarium of Yaoundé, Cameroon, where voucher specimen was deposited. The collected fresh stem bark was air dried and ground into fine powder in a high speed grinding mill.

Preparation of the extracts

Four types of extracts were prepared (decoction, maceration, infusion and methanol extract) were prepared in this study.

Decoction: 500 g of fine powder mixed with 2500 ml of distilled water were boiled for 15 min and then cooled for 20 min. After, the mixture was filtered using Whatman filter paper No.1 and the filtrate was evaporated to dryness in an air oven at 40°C and we obtained 40.17 g with a yield of 8.03 %.

Maceration: 500 g of powder were mixed with 2500 ml of distilled water and the mixture was left in a covered container for three days. The fourth day the mixture was filtered as previously. The filtrate was evaporated to dryness in an air oven at 40°C and we obtained 42.3 g with a yield of 8.46 %.

Infusion: 500 g of fine powder were mixed with 2500 ml of distilled water previously boiled for 15 min and then cooled for 15 min. After, the mixture was filtered using Whatman filter paper No.1 and the filtrate was evaporated to dryness in an air oven at 40°C and we obtained 28.48 g with a yield of 5.69 %.

Methanol extract: 500 g of extract powder were mixed in 2500 mL of methanol for 72 hours in cold condition. The whole mixture was filtered as previously and the filtrate obtained was evaporated at 60°C using rotary evaporator to obtain 42.3 g of the crude methanol extract, representing a yield of 8.46%.

DPPH radical scavenging assay

The free radical scavenging activity of crude extracts on 2, 2 diphenyl-2-picrylhydrazyl (DPPH) radical was measured according to the method described by Sanchez-Moreno and al. [10,11] with slight modification. One milliliter of different concentration (200, 100, 50 and 25 µg/ml) of extracts or standard (Vitamin C) in a test tube was added to1 ml of 0.3 mM of DPPH in the methanol. The mixture was vortexed and then incubated in a dark chamber for 30 minutes. Each measurement was made in triplicate. After 30 min, the optical density was read at 517 nm, methanol was used as a blank. Each measurement was made in triplicate. Ascorbic acid was used as standard. The percentage of DPPH free radical quenching activity was determined by the formula:

% inhibition DPPH = [(DO control – DO sample)/DO control] × 100%.

Ferric reducing power assay (FRAP)

The reducing power of the *Anthocleista vogelii* extracts was determined using the method of Oyaizu [12]. The extract solution (0.5 ml) at different doses was mixed with phosphate buffer (0.5 ml, 0.2 M, pH 6.6) and potassium ferricyanide [$K_3Fe(CN)_6$] (0.5 ml, 1%). The mixture was then incubated at 50 °C for 20 min. A portion of trichloroacetic acid (0.5 ml, 10%) was added to the mixture, which was then centrifuged for 10 min at 3000 rpm (1000 g). The upper layer of solution (0.5 ml) was mixed with distilled water (0.5 mL) and FeCl3 (0.1 ml, 0.1%) for 10 min, and then the absorbance was measured at 700 nm in a spectrophotometer, with higher absorbance indicating greater reducing power. Ascorbic acid was used as a standard.

Hydroxyl radical scavenging activity

The scavenging activity for hydroxyl radicals was determined using Fenton reaction with respect to the method describes by Selvakumar et al [13]. Briefly, 60 µl of 1.0 mM $FeCl_2$, 90 µl of 1 mM 1,10-phenanthroline, 2.4 ml of 0.2 M phosphate buffer (pH 7.8), 150 µl of 0.17 M H2O2and 1.5 m of extracts and fractions with various concentrations (ranging from 12.5 to 200 mg/mL) were mixed together. H_2O_2 was added to the reaction mixture in order to initiate the reaction and the mixture was kept for incubation at room temperature for 5 min. Butylated hydroxytoluene (BHT) was used as standard antioxidant. After incubation, the absorbance of the mixture was read at 560 nm using a spectrophotometer and the hydroxyl radicals scavenging (A) activity was calculated following this formula:

A (%) = [(Absorbance of control - Absorbance of sample) / Absorbance of control] x 100

Estimation of total phenolic content

The total phenolic compounds were determined by the method described by Ram de Tiendrebeogo et al [14] with slight modification. It involves the oxidation of phenols in alkaline solution by the yellow molybdotungstophosphoricheteropolyanion reagent and colorimetric measurement of the resultant molybdo-tungstophosphate blue. These blue pigments have a maximum absorption depending on the qualitative and/or quantitative composition of phenolic mixtures besides the pH of solutions, usually obtained by adding sodium carbonate. The reaction mixture consisted of 0.02 ml of extract and fraction (2 µg/ml), 0.02 ml of 2N FCR (Folin Ciocalteu Regent) and 0.4 ml of a 20% sodium carbonate solution. The mixture was left to stand at room temperature for 20 min and then the absorbance was measured at 760 nm. In the control tube, the extract volume was replaced by distilled water. A standard curve was plotted using gallic acid (0-0.2 µg/ml). Tests were performed in triplicate.

Determination of total flavonoids.

Total flavonoids content of the extracts and fractions of extracts were determined according to a modified colorimetric method of Padmaja et al [15] with slight modification. Methanolic solution of extracts and fractions (1 mL) were mixed with1 mL of distilled water and 75 µl of a 5% $NaNO_2$ solution. After 5 minutes, 0.03 ml of 10% $AlCl_3H_2O$ solution was added. After 5 minutes, 0.2 ml of 1 M Sodium hydroxide and 0.24 ml of distilled water were added. The solution was well mixed and kept for 15 minutes. The increase in absorbance was measured at 510 nm using a spectrophotometer. The total flavonoid content was calculated using standard catechin calibration curve. The results were expressed as milligrams of Catechin Equivalents (mgCE) per gram of extract/fraction.

STATISTICAL ANALYSIS

Results expressed as mean ± SEM for triplicate assays. Data were analyzed by one way ANOVA followed by Tukey's test using GraphPad Instant Biostatistic version 5.0. Values of $P < 0.05$ were the criteria for statistical significance.

RESULTS

IC50 of extracts of *Anthocleista vogelii*

Table 1 presents concentrations which inhibited 50% of DPPH (IC50). These results show that the concentration which inhibited 50% of DPPH from all the extracts of *Anthocleista vogelii* stem bark, show significant difference ($p < 0.05$) except the decoction. The extracts had a lowest IC50 (i.e. had the highest activity).

Table 1: IC_{50} values of extracts of *Anthocleista vogelii*

Extracts	IC_{50} (µg/ml)
Ascorbic acid	11,04 ± 0,03[a]
Infusion	12,18 ± 0,00[a]
Decoction	10,09 ± 0,01
Maceration	13,21 ± 0,01[a]
Methanol	10,03 ± 0,00[a]

Values given represent the mean ± SEM of three independent experiments carried out in triplicate.
[a] $P < 0.05$ statistically significant compared with the ascorbic acid.

DPPH radical scavenging assay

Figure 1 illustrates a significant ($p < 0.01$; $p < 0.001$) decrease in the concentration of DPPH radicals due to the scavenging ability of *Anthocleista vogelii* extracts. This activity was dose dependent. Maximum scavenging activity was observed at 200 µg/ml concentration in methanol extract.

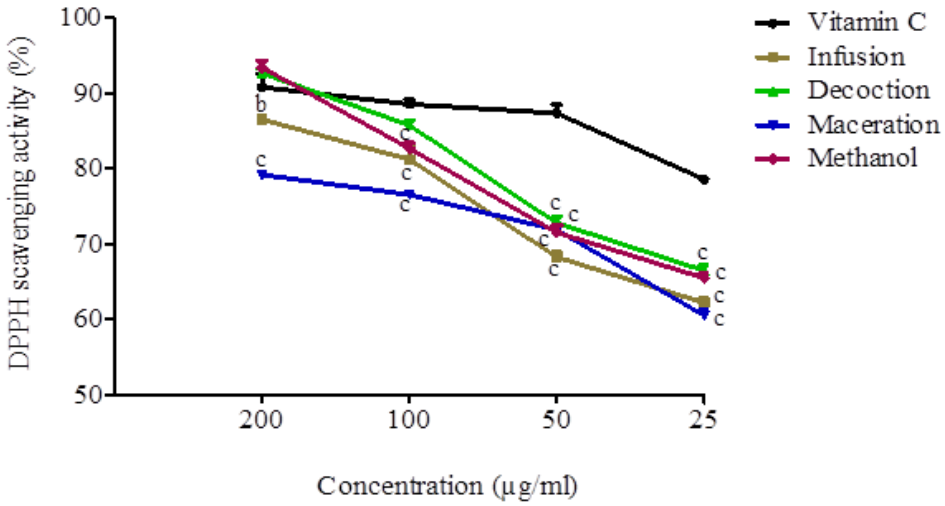

Fig.1: Effects of extracts on DPPH scavenging activity. Values given represent the mean ± SEM of three independent experiments carried out in triplicate. [b] $P < 0.01$; [c] $P < 0.001$ statistically significant compared with the vitamin C.

Ferric Reducing Power Assay (FRAP)

Figure 2 shows that the extracts showed reducing power with significant difference ($p < 0.001$) which increased with concentration of each sample. The highest reducing power was achieved with methanol extract at the concentration of 200 µg/ml. The reducing power assay correlated with the results obtained by the DPPH assay that the methanol extracts showed higher scavenging and reducing power activities than other extracts. Only maceration presented a dose dependent activity.

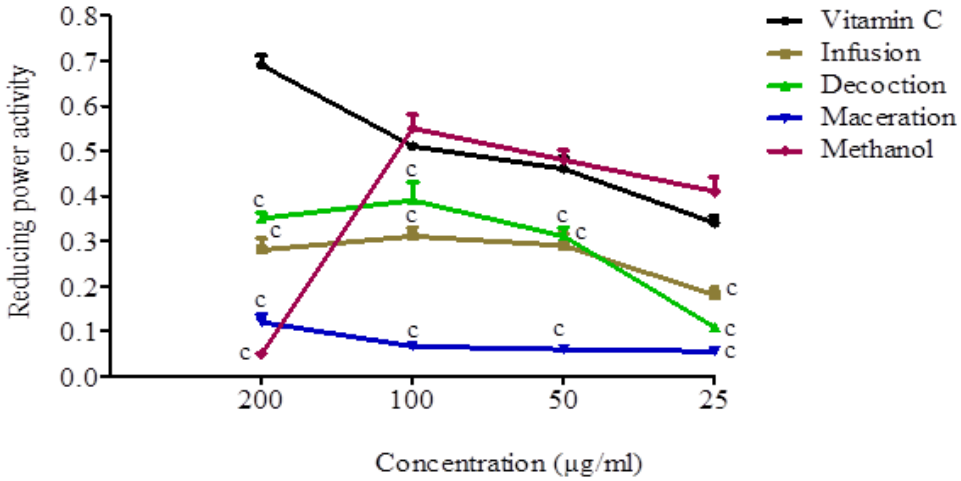

Fig.2. Effects of extracts on reducing power activity. Values given represent the mean ± ESM of three independent experiments carried out in triplicate. $^{c}P < 0.001$ statistically significant compared with the vitamin C.

Hydroxyl radical scavenging activity

The hydroxyl radical scavenging activity of the extracts is showed in figure 3. Extracts of *Anthocleista vogelii* stem bark are found to possess scavenging effect on hydroxyl radical with significant difference ($p < 0.05$; $p < 0.01$; $p<0.001$) and this inhibition effect is concentration dependent. Decoction, infusion and methanol extract at the concentration of 200 µg/ml each, shows the highest scavenging activity. However the activity of all concentrations of extracts was lesser than the butylated hydroxytoluene at the same concentration and presented a dose dependent relationship.

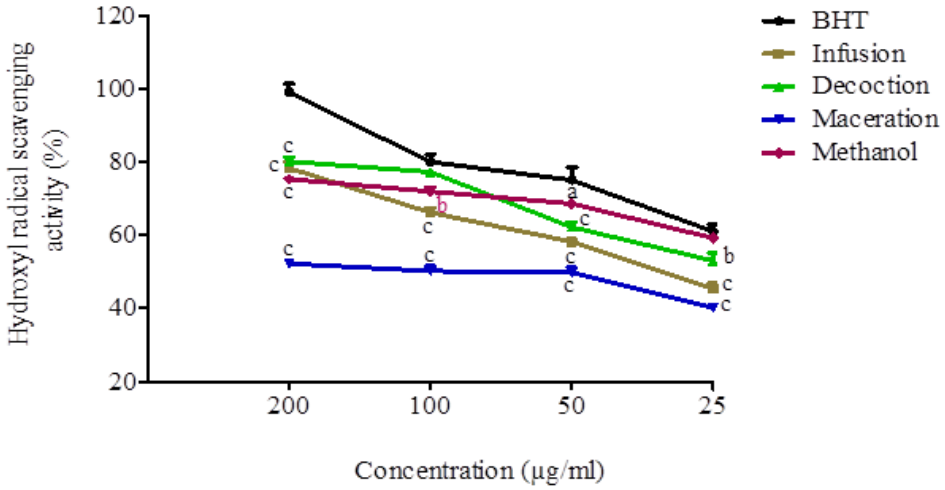

Fig.3. Effects of extracts on hydroxyl radical scavenging activity. Values given represent the mean ± ESM of three independent experiments carried out in triplicate. [a]P < 0.05; [b]P < 0.01; [c] P < 0.001 statistically significant compared with BHT. BHT: Butylated hydroxytoluene.

Estimation of total phenolic content

The estimation of total phenolic content was determined by using the Folin-Ciocalteu assay and was expressed as gallic acid equivalents (GAE). According to the results shown in figure 4, the total phenolic content was present in all extracts. However, decoction possesses a great amount of phenolic compound while maceration showed the lower amount of phenolic compound. The amount of total phenolic compound present in each extract was dose dependent.

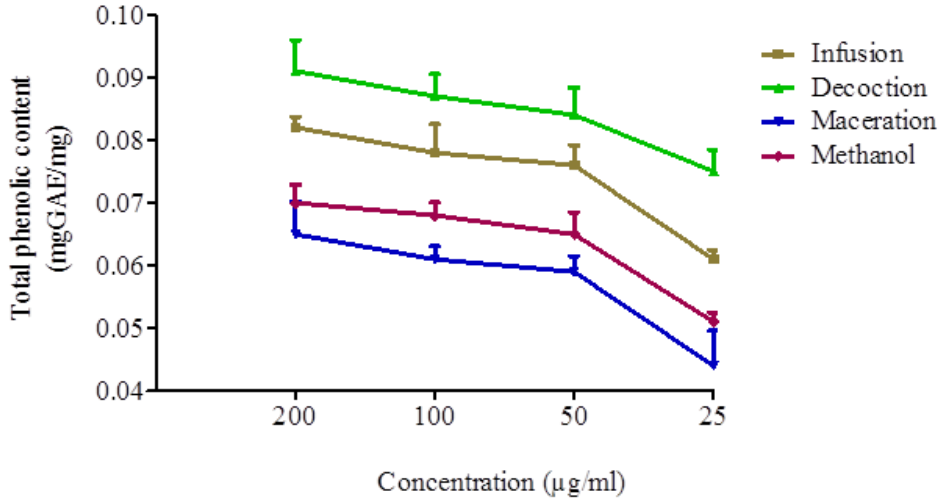

Fig.4. Effects of extracts on total phenolic content. Values given represent the mean ± ESM of three independent experiments carried out in triplicate. GAE: gallic acid equivalents.

Determination of total flavonoids

The results presented in Figure 5 shown that the extracts possess a great amount of flavonoids. However the greater amount of total flavonoids was presented in the decoction.

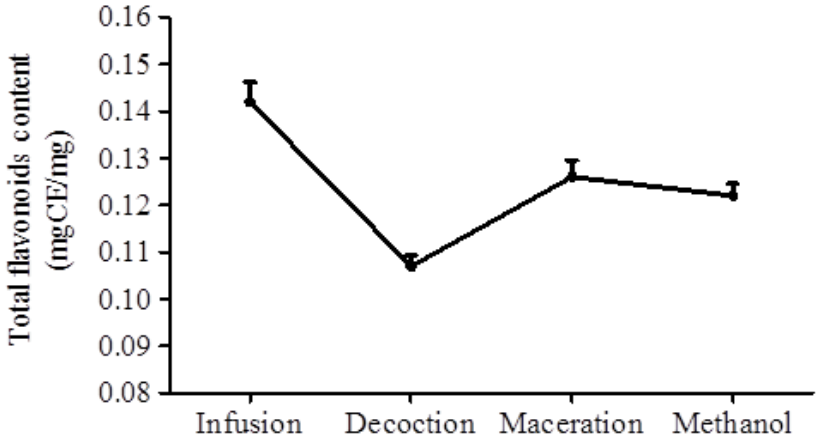

Fig.5. Effects of extracts on total flavonoids content. Values given represent the mean ± ESM of three independent experiments carried out in triplicate.

DISCUSSION

DPPH free radical scavenging assay is more indirect assay, because DPPH assay measures the inhibition of reactive species (free radicals) generated in the reaction mixture and these results depend on the type of reactive species used16. The model for scavenging the stable DPPH radical is widely used model to evaluate antioxidant activities in a relatively short time to compare with other methods. The effect of antioxidants on DPPH radical scavenging was thought to be due to their hydrogen donating ability. DPPH is a stable free radical and accept an electron or hydrogen radical to become a stable diamagnetic molecule and therefore inhibit the propagation phase of lipid peroxide [17, 18].

The reducing capacity of a compound may serve as an indicator of its potential antioxidant activity19. The presence of reducing compounds causes reduction of the Fe3+/ferricyanide complex to ferrous ion (Fe2+) [19]. Many reports have revealed that there is a direct correlation between antioxidant activities and reducing power of certain plant extracts [20, 21, 23]. The reducing properties are generally associated with the presence of reductions, which have been shown to exhibit antioxidant action by breaking the chain reactions by donating a hydrogen atom. Reductions are also reported to react with certain precursors of peroxide, thus preventing peroxide formation [24].

Scavenging activity of H_2O_2 by the extracts may be attributed to their phenolics, which can donate electrons to H_2O_2 thereby neutralizing it into water [25]. Hydrogen peroxide is a weak oxidizing agent and can inactivate a few enzymes directly, usually by oxidation of essential thiol (-SH) groups. It rapidly transverses cell membrane and once inside the cell interior, H_2O_2 can probably react with Fe^{2+}

and possibly Cu^{2+} ions to form hydroxyl radical and this may be the origin of many of its toxic effects [26]. Thus, the removal of H_2O_2 is very important for antioxidant defense in cell or food systems.

There is a highly positive relationship between total phenols and antioxidant activity of many plant species, because of the scavenging ability of their hydroxyl groups [27]. It was also reported that Phenolic compounds are effective hydrogen donors, making them very good antioxidants [28].

Flavonoids are a group of polyphenolic compounds, which exhibit several biological effects such as anti-inflammatory, anti-hepatotoxic, anti-ulcer, anti-allergic, anti-viral and anti-cancer activities [29]. The antioxidant activity of *Anthocleista vogelii* stem bark could be attributed to its flavonoids content. Flavonoids act as scavengers of various oxidizing species that is super oxide anion (O^{2-}), hydroxyl radical or peroxy radicals, they also act as quenchers of singlet oxygen [30]. They are capable of effectively scavenging the reactive O2 species because of their hydroxyl groups and so they are potent antioxidants also [30].

CONCLUSION

This study reveals that *Anthocleista vogelii* stem bark have a significant free radical scavenging activity. This benefit antioxidant effect of the plant extract might be attributing to the presence of flavonoids in this plant extract.

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CONFLICT OF INTEREST STATEMENT

The authors declare that they have no conflict of interests.

REFERENCES

1. Ames B. Micronutrients prevent cancer and delay aging. Toxicol Lett 1998; 102: 5-18.
2. Cox DA, Cohen ML. Effects of oxidized low density lipoprotein on vascular contraction and relaxation. Pharmacol Rev 1996; 48: 3-9.
3. Finkel T, Holbrook NJ. Oxidants, oxidative stress and the biology of ageing. Nature 2000; 408: 239-247.
4. Harman D. Free radical theory of aging, increasing the functional life span. Ann New York Acad Sci 1994; 717: 1-15.
5. Sumazian Y, Syahida A, Hakiman M, Maziah M. Antioxidant activities, flavonoids, ascorbic acid and phenolic contents of Malaysian vegetables. J Med Plant Res 2010; 4(10): 881-890.
6. Hazra B, Santana B, Nripendranath M. Antioxidant and free radicals scavenging activity of *Spondiaspinnata.* J BMC 2008; 8: 63-68.
7. Demiray S, Pintado ME, Castro PML. Evaluation of phenolic profiles and antioxidant activities of Turkish medicinal plants: *Tiliaargentea, Crataegifolium* leaves and *Polygonumbistorta* roots. World Acad Sci Eng Technol 2009; 54: 312-317.
8. Ismail A, Marjan ZM, Foong CW. Total antioxidant activity and phenolic content in selected vegetables. Food Chem 2004; 87: 581-586.
9. Sânchez-Moreno C, Larrauri J, Saura-Calixto F. Free radical scavenging capacity and inhibition of lipid oxidation of wines, grape juices and related polyphenolic constituents. Food Res Int 1999a; l32: 407-412.
10. Sànchez-Moreno C, Larrauri JA, Saura-Calixto F. Free radical scavenging capacity

of selected red, rose and white wines. J Sci Food Agri 1999b; 79(10): 1301-1304.
11. Oyaizu M. Studies on product of browning reaction prepared from glucose amine. Japanese J Nutri 1986; 44: 307-315.
12. Selvakumar K, Madhan R, Srinivasan G, Baskar V. Antioxidant Assays in Pharmacological Research. Asian J Pharmacol Tech 2011; 1 (4): 99-103.
13. Ramde-Tiendrebeogo A, Tibiri A, Hilou A, Lompo M, Millogo-Kone H, Nacoulma OG, Guissou IP. Antioxidative and antibacterial activities of phenolic compounds from Ficus Forssk. Int J Biol Chem Sci 2012; 6(1): 328-336.
14. Padmaja M, Sravanthi M, Hemalatha KPJ. Evaluation of Antioxidant Activity of Two Indian Medicinal Plants. J Phytol 2011; 3(3): 86-91.
15. Cao G, Sofic E, Prior RL. Antioxidant capacity of tea and common vegetables. J Agric Food Chem 1996; 4: 3426-3431.
16. Havsteen B. Flavonoids, a class of natural products of high pharmacological potency. Biochem Pharmacol 1983; 32: 1141-1148.
17. Soares JR, Dins TCP, Cunha AP, Almeida LM. Antioxidant activities of some extract of Thymus zygis. Free Rad Res 1997; 26: 469-478.
18. 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: 1813-1819.
19. Sousa A, Ferreira ICFR, Barros L, Bento A, Pereira JA. Antioxidant potential of traditional stoned table olives ‘‘Alcaparras”: influence of the solvent and temperature extraction conditions. LWT Food Sci Technol 2008; 41:739-745.
20. Duh PD, Du PC, Yen GC. Action of methanolic extract of mung beans hulls as inhibitors of lipid peroxidation and non-lipid oxidative damage. Food Chem Toxicol 1999; 37: 1055-1061.
21. Duh PD. Antioxidant activity of burdock (*Arctiumlappa linne*.): Its scavenging effect on free-radical and active oxygen. J Am Oil Chem Soc 1998; 75: 455-461.
22. Yildirim A, Mavi A, Oktay M, Kara AA, Algur OF, Bilaloglu V. Comparison of antioxidant and antimicrobial activities of tilia (*Tilia arentea* Desf. Ex. D.C.) sage (*Salvia triloba* L.) and black tea (*Camellia sinensis* L.) extracts. J Agr Food Chem 2000; 48(10): 5030-5034.
23. Matsushige K, Basnet P, Kadota S, Namba T. Potent free radical scavenging activity of dicaffeoyl -quinicacid derivatives from propolis. Trad Med 1996; 13(13): 217-228.
24. Mathew S, Abraham TE. In vitro antioxidant activities and scavenging effects of *Cinnamomum verum* leaf extract assayed by different methodologies. J Food Chem Toxicol 2006; 44: 198-206.
25. Contreras-Guzman ES, Strong FC. Determination of tocopherols (vitamin E) by reduction of cupric ion. J Assoc Anal Chem 1982; 65: 1215-1221.
26. Vinson JA, Yong H, Xuchui S, Zubik L. Phenol antioxidant quantity and quality in foods: vegetables. J Agric Food Chem 1998; 46: 3630-3634.
27. Yen GC, Duh PD, Tsai CL. The relationship between antioxidant activity and maturity of peanut hulls. J Agric Food Chem 1993; 41: 67-70.
28. Umamaheswari, Chatterjee TK. In vitro antioxidant activities of the fraction s of Cocciniagrandis L. leaf extract. Afr J Trad Complement Alt Med 2008; 5(1): 61-73.
29. Das NP, Ratty AK. Effect of flavonoids on induced non enzymatic lipid peroxidation. In, Cody V, Middleton E and Harborne J (eds.), Plant flavonoids in Biology and Medicine: Biochemical, Pharmacological and Structure-Activity Relationships. New York, Liss AR, 1986; p 243.

30. Cao G, Sofic E, Prior RL. Antioxidant and pro-oxidative behavior of flavonoids: Structure activity relationships. Free Radic Biol Med 1997; 22: 749-760.

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