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The *In Vitro* antioxidant and free radical scavenging Activities of suran (*Amorphophallus campanulatus* (Araceae)) tubers extracts

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Abstract

Total Phenolics and the antioxidative properties of *Amorphophallus campanulatus* were studied. The dried Powder of tubers was extracted successively with methanol, Hydroalcohol, ethanol and 70% acetone separately. After removing the solvents, the extracts were freeze-dried. The *Amorphophallus campanulatus* tubers contained relatively high levels of total Phenolics and tannins. The extracts were subjected to assess their potential antioxidant activities using systems such as diphenyl- β-picrylhydrazyl (DPPH˙), $ABTS^{+}$, FRAP, linoleic acid emulsion, $O_2^{-}$and OH´. The superoxide anion radical-scavenging activity was found to be higher in methanolic extract in comparison to other extracts' at the concentration of 600 µg in the reaction mixture. The DPPH radical and ABTS action radical-scavenging activities were well proved and related with the ferric-reducing/antioxidant capacity of the extracts. Interestingly, among the various extracts, methanolic extracts' shows significantly ($P < 0.05$) higher hydroxyl radical- scavenging activity. In general, all extracts exhibited good antioxidant activity (53.3–73.1%) against the linoleic acid emulsion system but were significantly ($P < 0.05$) lower than the synthetic antioxidant, BHA (93.3%).

Key-Words: Amorphophallus campanulatus, Flavonoid, Phenolics; Tannins; Antioxidant activity; $ABTS^{+}$

Introduction

There is an increasing interest in the protective biochemical function of naturally occurring antioxidants in biological systems and on the mechanism of their action. Several plant constituents have proven to show free radical scavenging or antioxidant activity (Mantle et al., 2000; Choy et al., 2002). There are some synthetic antioxidants compounds like butylated hydroxyl toulene, butylated hydroxyl anisole and tetrbutylhydroquinone have commonly used. However, it has been suggested that these compounds are carcinogens5. This led to an increased interest in natural antioxidants from plant sources..Flavonoids and other phenolic compounds of plant origin have been reported as scavengers and inhibitors of lipid peroxidation (Formica and Regelson, 1995; Rice-Evans et al., 1997). Flavonoids and Phenolics are the bioactive phytoconstituents having an important role in control & prevention of tissue damage by activated oxygen species(Rice Evans et al.,1998)

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And hence herbal drugs /herbal preparations containing such phytoconstituents are gaining importance in the prevention and treatment of various organ toxicities due to xenobiotic / environmental challenges (Lorson et al.,1988).Flavonoids is a large family consisting of more than 4000 ubiquitous secondary plant metabolites, which are further divided into five subclasses namely flavonols, flavones, anthocyanins, catechins and flavonones(Merker 2000). Flavonols such as Catechin mainly reduces atherosclerotic plaques (Chyu K.Y. et al., 2004) and cancer (Mittal et al., 2004) and also posse's antibiotic properties by disrupting bacterial DNA replication process (Gradisar et al., 2007). Quercetin acts as antioxidant, antiinflammatory, antiulcerative, antihepatotoxic and antiangiogenesis (Da Silva E.L, 1998: Igura K. et al., 1997).Other polyphenols like, Caffeic acid act as inhibitor for lipooxygenase enzyme, suppress acute immune and inflammatory response (Huang M.T.et al., 1996) and known to have antimitogenic, anticarcinogenic and immunomodulatory properties (Huang M.T.et al., 1998). pCoumaric acid has antioxidant properties and is believed to reduce the risk of stomach cancer(Fergusion L.R et al.,2005)by

reducing the formation of carcinogenic nitrosamines(Kikugawara K.et al., 1983).The antioxidant properties of polyphenols depend mainly upon factors such as metal reducing potential, chelating Behavior, pH and solubility characteristics (Decker EA.et al., 1997). *Amorphophallus campanulatus* (AC) (Araceae) a tuberous, stout indigenous herb commonly known as elephant foot yam, Suran, grown as vegetable is widely available (Nandkarni K.M.et al., 2000; Goshi S.G. et al., 2000) the tuber of *Amorphophallus campanulatus* (AC) (Araceae) is used for tumor, rheumatoid arthritis, carminative and liver tonic. It is also used in piles and given as the restorative in dyspepsia debility, Anti-inflammatory, anti-haemorrhoidal, haemostatic, expectorant, and anthelmintic. Previously studies have indicated that analgesic activity6, Antibacterial, Antifungal and Cytotoxic Activities(Khan et al., 2007)..The aim of present study was to antioxidant and free radical scavenging activities of Suran (Amorphophallus campanulatus(Ara.)) tubers extracts.

Material and methods

Chemicals

Butylated hydroxyanisole (BHA), potassium ferricyanide, a,a-diphenyl-b-picrylhydrazyl (DPPH), nitro blue tetrazolium (NBT), thiobarbituric acid (TBA), trichloroacetic acid (TCA), 2-deoxy-D-ribose, methionine, linoleic acid, ethylenediamine tetraacetic acid (EDTA), ammonium thiocyanate, potassium persulfate, ferrous chloride, ascorbic acid, Tween 20, 2,4,6-tripyridyl-s-triazine, ferric chloride, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid, 2,20-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt were obtained from Himedia, Merck, Sigma or S.D. Fine-Chem Ltd. All other reagents were of analytical grade.

Collection of Plant material and preparation of Extracts

The tubers of *Amorphophallus campanulatus* (AC) were collected from Sagar district of Madhya Pradesh and authenticated as *Amorphophallus campanulatus* family- Araceae by Zea ul Hasan Ast.Proffesar, Head Dept.of Botany, Safia Science collage, Bhopal and a specimen voucher no.192/BOT/Safia/19.

Extraction of Plant Material

The air dried powder of tubers was subjected to exhaustive soxhlation with solvents Methanol(ME), ethanol(EA), acetone (70%)(AE) and Hydroalcohol (70%)(HA) separately. The solvents of respective extracts were evaporated under reduced pressure using a rotatory vaccum evaporator at 40˚C and the remaining water was removed by lyophilization and weighed to determine the yield of soluble components. The freeze-dried extracts thus obtained were used directly for estimation of total Phenolics and tannins.

Total Flavonoidal content (FC)

To determine the total flavonoidal content, stock solutions of the respective extracts (ME: 1mg/ml; AE: 1mg/ml: HA 1mg/ml :EA 1mg/ml) were prepared with methanol to a suitable concentration for analysis. Total flavonoid content was measured according to the method previously reported by (Helmija et al.,1889) with slightly modifications using standard curve generated with Quercertin. Aliquots of each extract (ME, AE,HA and EA) were pipette out in series of test tubes and volume was made upto 0.5ml with distilled water; Sodium nitrate (5% : 0.3ml) was added to each tube & incubated for 5 min. at room temperature; Aluminium chloride solution (10%; 0.06ml) was added and incubated for 5 min, at room temperature; Sodium hydroxide (1M; 0.25ml) was added and total volume was made to 1ml with distilled water. Absorbance was measured at 510nm against a reagent blank using Schimadzu model 150 – 02 double beam spectrophotometer and concentration of flavonoids in the test sample was determined and expressed as mg of Quercertin equivalent per gram of sample.

Determination of total phenolic and tannins

The total phenolic content of the freeze-dried methanol and hydroalcoholic extract of raw and processed tubers was determined according to the method described by (Makkar et al., 2003). Known aliquots of the extract (each 10 mg of extracts dissolved in 100 mL of respective solvent) were taken in a test tube and made up to a volume of 1 ml with distilled water. Then 0.5 ml of Folin-Ciocalteu reagent (1:1 with water) and 2.5 ml of sodium carbonate solution (20%; w/v) were added sequentially in each tube. Soon after vortexing the reaction mixture, the tubes were placed in the dark for 40 min and the absorbance was recorded at 725 nm against the reagent blank. The amount of total phenolics was calculated as pyrogallol equivalents (even though pyrogallol was used as a standard in this work, it is not a natural constituent of Suran or tubers in general) from the calibration curve. Using the same methanol(ME), aqueous acetone(AE),Hydralcoholic(HA) and Ethanol (EA) solutions, which contained known amount of freeze-dried extract, the tannins (Makkar et al., 2003) were estimated after treatment with polyvinyl polypyrrolidone (PVPP). Hundred millgrams of PVPP were weighed in a 100 x12 mm test tube and to this 1.0 mL distilled water and then 1.0 mL of the tannin-containing phenolic extract were added. The content was vortexed and kept in the tube at 4˚C for 15 min.

Then the sample was centrifuged (3000g for 10 min at room temperature) and the supernatant was collected. This supernatant has only simple phenolics other than tannins (the tannins would have been precipitated along with the PVPP). The phenolic content of the supernatant was measured as mentioned above and expressed as the content of non-tannin phenolics on a dry matter basis. From the above results, the tannin content of the sample was calculated as follows:

Total phenolics (%) - non-tannin phenolics (%) = tannin (%)

Antioxidant activity assays

Superoxide radical-scavenging activity by NBT method

Superoxide radicals were generated by a modified method of Beauchamp and Fridovich (1971), described by Zhishen, Mengcheng, and Jianming (1999). All solutions were prepared in 0.05 M phosphate buffer (pH 7.8). The photo-induced reactions were performed using 20 W fluorescent ´lamps. The mass of extract in the reaction mixture was 200–600 µg. The total volume of the reactant mixture was 5 ml and the concentrations of the riboflavin, methionine and nitro blue tetrazolium (NBT) was $3x10^{-6}$, $1x10^{-2}$ and $1x\ 10^{-4}$ mol/L, respectively. The reactant was illuminated at 25 ˚C for 25 min. The photochemically reduced riboflavins generated ($O_2^{\cdot-}$) which reduced NBT to form blue formazan. The unilluminated reaction mixture was used as a blank. The absorbance was measured at 560 nm. The sample extracts were added to the reaction mixture, in which ($O_2^{\cdot-}$) radicals were scavenged, thereby inhibiting the NBT reduction. The net value of absorbance was calculated as the decrease in ($O_2^{\cdot-}$) level which was represented by A (illum) - A (unillum), where A (illum) was the absorbance value of illuminated reaction mixture and A (unillum) was the absorbance value of unilluminated reaction mixture for control or test sample. The degree of the scavenging was calculated as:

scavenging (%) = [A (control) -A (test sample)]/A (control) x100,

Where A (control) and A (test sample) were the net values of absorbance calculated for control and test sample, respectively.

Hydroxyl radical-scavenging activity

The scavenging activity of the powdered tubers of suran extracts on the hydroxyl radical (OH´) was measured by the deoxyribose method (Aruoma et al.,1994) modified by (Hagerman et al. 1998). The reactions were performed in 10 mM phosphate buffer, pH 7.4, containing 2.8 mM deoxyribose, 2.8 mM H2O2, 25 lM FeCl3, 100 lM EDTA, and the test sample (200 µg). The reaction was started by adding ascorbic acid to a final concentration of 100 µM and the reaction mixture was incubated for 1 h at 37 ˚C in a water bath. After incubation, the colour was developed by addition of 1% thiobarbituric acid followed by ice-cold 2.8% trichloroacetic acid and heating in a boiling water bath (95–100 ˚C) for 20 min. The sample was cooled, and the chromophore was extracted into n-butanol and the absorbance was measured at 532 nm against n-butanol (as blank). The reaction mixture not containing test sample was used as control. The scavenging activity on hydroxyl radicals (HRSA) was expressed as:

HRSA(%)= [1 - (OD at 532 nm in presence of sample) /OD at 532 nm in absence of sample)x100

The β-carotene/linoleic acid antioxidant activity

One millilitre of a β-carotene solution in chloroform (1 mg/100 ml) was pipetted into a flask containing 20 mg of linoleic acid and 200 mg of Tween 40. The chloroform was removed by rotary vacuum evaporator at 45˚C for 4 min and, 50 ml of oxygenated distilled water was added slowly to the semi-solid residue with vigorous agitation, to form an emulsion. A 5 ml aliquot of the emulsion was added to a tube containing 0.2 ml of the antioxidant (AC extracts, BHA or Trolox) solution at 1000 mg/liter and the absorbance was measured at 470 nm, immediately, against a blank, consisting of the emulsion without b-carotene (Taga, Miller, & Pratt, et al.,1984). The tubes were placed in a water bath at 50˚C and the absorbance was monitored at 15 min intervals until 180 min. All determinations were carried out in triplicate. The antioxidant activity of the AC extracts and pure compounds was evaluated in terms of bleaching of b-carotene using the following formula:

$$AA = [1 - (A_a - A_o)/ (A'_a - A'_o)] \times 100$$

Where A_a and A_o are the absorbance measured at zero time of incubation for the test sample and control, respectively, and A'_a and A'_o are the absorbances measured in the test sample and control, respectively, after incubation for 180 min.

Free radical-scavenging activity on α, α,-diphenyl-β picrylhydrazyl (DPPH).

The antioxidant activity of tubers extracts and BHA was measured in terms of electron transfer/hydrogen donating ability, using the stable radical, DPPH˙ method (Brand-Williams, Cuvelier, & Berset, 1995) modified by Sa´nchez-Moreno, Larrauri, and Saura-Calixto (1998). A methanol solution (0.1 ml) of the sample extracts at various concentrations was added to a 3.9 ml (0.025 g/L) of DPPH˙solution. The decrease in absorbance at 515 nm was determined continuously at every 1 min with a Hitachi UV–Visible model U-2000 Spectrophotometer until the reaction reached a plateau.

The remaining concentration of DPPH in the reaction medium was calculated from a calibration curve obtained with DPPH˙ at 515 nm. The percentage of remaining DPPH, (DPPH R̄) was calculated as follows:

$$DPPH^{\cdot}_{\varphi} = [DPPH_{\chi}/DPPH_{\chi} = 0] \times 100$$

where $DPPH_{\varphi}$ was the concentration of DPPH at the time of steady state and $DPPH_{\chi}$= 0 was the concentration of DPPH at the time of zero (initial concentration).The percentage of remaining DPPH˙ against the sample/ standard concentration was plotted to obtain the amount of antioxidant necessary to decrease the initial concentration of DPPH˙ by 50% (EC50). Based on the parameter EC50, the result was expressed in terms of mg dry matter of sample/standard equivalent g/l DPPH˙in the reaction medium.

Antioxidant activity by the ABTS⁺ assay

The total antioxidant activity of tubers extracts was measured by the $ABTS^{+}$ radical cation decolorization assay involving preformed $ABTS^{+}$ radical cation (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 persulfate (final concentration) and allowing the mixture to stand in the dark at room temperature for 12–16 h before use. Oxidation of the ABTS commenced immediately, but the absorbance was not maximal and stable until more than 6 h had elapsed. The radical cation was stable in this form for more than 2 days in storage in the dark at room temperature. Prior to assay, the solution was diluted in ethanol (about 1:88 v/v) to give an absorbance at 734 nm of 0.700 ± 0.02 in a 1 cm cuvette and equilibrated to 30 ˚C, the temperature at which all the assays were performed. The stock solution of AC extracts, AA and BHA in ethanol were diluted such that, after introduction of a 10 µL aliquot of each dilution into the assay, they produced between 20% and 80% inhibition of the blank absorbance. After the addition of 1.0 mL of diluted $ABTS^{+}$ solution to 10 µL of antioxidant compounds or Trolox standards (final concentration 0–15 µM) prepared in ethanol was incubated at 30 ˚C exactly for 30 min after the initial mixing. Appropriate solvent blanks were also run in each assay. Triplicate determinations were made at each dilution of the standard, and the percentage inhibition was calculated of the blank absorbance at 734 nm and then was plotted as a function of Trolox concentration. The activity of AC extracts BHA and ascorbic acid was estimated at a minimum of three different concentrations within the range of dose– response curve, and the mean value was derived as the TEAC (Trolox equivalent antioxidant capacity) value. The unit of total antioxidant activity (TAA) was defined as the concentration of Trolox having the equivalent antioxidant activity expressed as mmol/kg tubers extracts on dry matter basis.

Ferric-reducing/antioxidant power (FRAP) assay

The antioxidant capacity of *Amorphophallus campanulatus* extracts was estimated according to the procedure described by (Benzie and Strain et al.,1996) with slight modifications made by (Pulido, Bravo, and Saura-Calixto et al., 2000). FRAP reagent (900 µL), prepared freshly and incubated at 37 ˚C, was mixed with 90 µL of distilled water and 30 µL of test sample, BHA and Trolox or methanol (for the reagent blank). The test samples and reagent blank were incubated at 37 ˚C for 30 min in water bath. The final dilution of the test sample in the reaction mixture was 1/34. The FRAP reagent contained 2.5 mL of 20 mmol/L TPTZ solution in 40 mmol/ L HCl plus 2.5 mL of 20 mmol/L FeCl3. 6H2O and 25 mL of 0.3 mol/L acetate buffer, pH 3.6 (Benzie & Strain et al., 1996). At the end of incubation, the absorbance readings were taken immediately at 593 nm using a Spectrophotometer. Methanolic solutions of known Fe(II) concentration ranging from 100 to 2000µmol/L (FeSO4.7H2O) were used for the preparation of the calibration curve. The parameter Equivalent Concentration (EC1) was defined as the concentration of antioxidant having a ferric-TPTZ reducing ability equivalent to that of µ mmol/L (FeSO4.7H2O). (EC1) was calculated as the concentration of antioxidant giving an absorbance increase in the FRAP assay equivalent to the theoretical absorbance value of a µmmol/L concentration of Fe(II) solution determined using the corresponding regression equation.

Statistical analysis

The data were subjected to a one-way analysis of variance (ANOVA) and the significance of the difference between means was determined by Duncan's multiple range test ($P < 0.05$) using the Statistica (Statsoft Inc., Tulsa, USA). Values expressed are mean of three replicate determinations ± standard deviation

Results and Discussion

Recovery percent and phenolic content of extracts

The yield percent of flavonoid, total phenols and tannins of extracts obtained from tubers using methanol, Hydroalcohol, Ethanol and aqueous acetone (70%) solvent are shown in Table 1. Among all extracts, maximum yield was obtained for methanol extracts of tubers samples. The extractable total phenolics and tannins of the methanol extracted samples were found to be higher than in other samples for each solvent that could be due to the solubility of phenolics and other aroma compounds. However, the methanol and 70% acetone was found to be more

efficient solvent for extracting the phenolic constituents in tubers than the respective solvents.

Superoxide anion radical and hydroxyl radical scavenging activities

The effects of phenolic extracts of *Amorphophallus campanulatus* on superoxide anion radical scavenging activity were Estimated by the nitro blue tetrazolium (NBT) assay method and the results are compared to tannic acid and Quercetin (Siddhuraju & Becker, 2007) (Table 3). All of The extracts had a scavenging activity on the superoxide radicals in a dose dependent manner (200–600 µg in the reaction mixture). Nonetheless, when compared to Quercetin and tannic acid, the superoxide radical-scavenging activity of all the above-mentioned extracts was found to be low. This could be due to the presence of relative concentration of bioactive constituents and mixture of impurities/ other nutrients in the extracts. All the tubers extracts showed hydroxyl radical-scavenging activities (12.25–60.24%) at a level of 200 µg in the reaction mixture. Methanol extracts of *Amorphophallus campanulatus* showed relatively lower hydroxyl radical-scavenging activity. However, the hydroalcoholic extract of same had similar hydroxyl radical-scavenging activity to the methanol extracts and their values were significantly ($P < 0.05$) higher than all other sample extracts. Yen and Hsieh (1995) reported that xylose and lysine Maillard reaction products had scavenging activity. On hydroxyl radical that depends on dose response manner And which might have been attributed to the combined effects of reducing power, donate on of hydrogen atoms and scavenging of active oxygen. These results show that the potential scavenging abilities of phenolic substances might be due to the active hydrogen donor ability of hydroxyl substitution. Hagerman et al. (1998) have also explained that high molecular weight and the proximity of many aromatic rings and hydroxyl groups are more important for the free radical-scavenging activity by tannins than their specific functional groups.

Antioxidant activity in linoleic acid emulsion system

The antioxidant effects of the extracts Amorphophallus campanulatus, ascorbic acid and BHA on the peroxidation of linoleic acid were investigated and the results are presented in Fig. 1. At a level of 250 µg in the final reaction mixture, the methanol and hydroalcoholic extract samples inhibited 53.3–72.7% peroxidation of linoleic acid after incubation for 72 h (3 days). However, those values were significantly lower and higher than those of the positive controls such as BHA (93.3%) and AA (43.0%), respectively. In summary, the results show that the inhibitory potential follows the order BHA > ME > HA> EA>AA . The hydrolysable tannins from Osbeckia chinensis were found to have potential antioxidative efficiency in the linoleic acid-thiocyanate system (Su, Osawa, Kawakishi, & Namiki, 1998). The stability of antioxidant potential of dryheated samples may be due to the formation of products from the Maillard reaction. (Nicoli, Anese, Manzocco, and Lerici et al.,1997) reported that medium dark roasted coffee brews had the highest antioxidant properties due to the development of products as a result of Maillard reaction. Similarly, extract of roasted followed by defatted legume, peanut kernels, displayed most remarkable antioxidative activity on linoleic acid emulsions system (Hwang, Shue, & Chang, et al., 2001).

Antioxidant activity in the b-carotene bleaching assay

The antioxidant activity of tubers extracts as measured by bleaching of b-carotene is presented in Fig. 3. All extracts showed significantly higher antioxidant activity (ME and EA, 10.5% and 13.3%, respectively) than both the acetone and hydroalcohol treated extracts samples at 200 µg in the final reaction mixture. The antioxidant activity was related to phenolic concentration dependency, including high molecular phenolics, tannins and condensed tannins. However, the inhibition of β-carotene bleaching by AC extracts was lower than those of standards BHA and Trolox. Different solvent extracts of various fractions of common beans have also been reported to have potential inhibiting activity against the oxidation of b-carotene molecules (Cardador-Martı`nez et al., 2002).

Radical-scavenging activities on a,a-diphenyl-bpicrylhydrazyl (DPPH˚) and ABTS.

The DPPH˚ radical-scavenging activities of ME, AE, HA and EA extracts of tubers along with the reference standards ascorbic acid (AA) and BHA. A lower value of EC50 indicates a higher antioxidant activity. ME Extracts obtained from the tubers showed the highest DPPH radical-scavenging activity (2RA, black variety, raw, 70% acetone extracted; 0.26, 2HA, brown variety, heat treated, 70% acetone extracted; 0.44, 1HA brown variety, heat treated, 70% acetone extracted; 1.02 and 1RA, brown variety, raw, 70% acetone extracted; 1.44) and the values were significantly different ($P < 0.05$) when compared to the values of methanol extracts of the respective extracts samples. However, the synthetic antioxidants, BHA and AA were found to be more potent for providing the hydrogen donating/electron transfer ability than all other extracts samples. In a recent study (Siddhuraju, Mohan, and Becker et al., 2002) reported that a high concentration of tannins (proanthocyanidins) extracted from stem bark of Cassia

fistula was found to have elevated DPPH radical quenching capacity. Similarly, (Amarowicz, Naczk, and Shahidi et al., 2000) reported that the tannins extracted from canola and rape seed hulls exhibited a high scavenging efficiency toward DPPH radicals. The order of scavenging activity of AC extracts: 2HA > 2RA > 1HA > 1RA > 2RM > 2HM > 1HM > 1RM was found in the study. This antiradical-scavenging activity of untreated and treated extracts would be related to the nature of phenolics, thus contributing to their electron transfer/ hydrogen donating ability (Brand-Williams et al., 1995).

Ferric reducing antioxidant power

Antioxidants can be explained as reductants, and inactivation of oxidants by reductants can be described as redox reactions in which one reaction species (oxidant) is reduced at the expense of the oxidation of another antioxidant. The FRAP assay measures the antioxidant effect of any substance in the reaction medium as reducing ability. Antioxidant potential of the AC extract was estimated from their ability to reduce TPTZ-Fe(III) complex to TPTZ-Fe(II) complex. The AC extracts of ME and EA showed highest FRAP antioxidant activity, as has been recorded in DPPH and ABTS+ methods. Nonetheless, the order of FRAP activity of respective tubers samples extract is as follows: ME > EA > HA, followed by autoclaving, as in the case of DPPH_ and ABTS_+. Moreover, there was a noticeable correlation between extractable total phenolics. Similarly, high correlation has also been reported in guava fruit extracts (Jime`nez-Escrig, Rinco`n, Pulido, & Saura-Calixto, 2001). Yen and Duh (1993) and Siddhuraju et al. (2002) have reported that the reducing power of bioactive compounds (mainly low and high molecular phenolics), extracted from peanut hulls and stem bark of Indian laburnum, was associated with antioxidant activity, specifically scavenging of free radicals. This study suggests that not only the phenolic (tannins) substances from AC extracts. Nonetheless, evaluation of the occurrence of tannin–protein interactions in the above said processed samples by testing both the in vitro protein digestibility, together with the assessment of antioxidant properties might be a fruitful approach for advocating them as nutraceuticals in addition to them being potential protein and carbohydrate suppliers. Furthermore, such studies may also provide the information for the people who claim that the consumption of post cooking liquor of tubers is a potential health promoter. On the other hand, whether the presence of tannins–protein complexes and phenolics associated with dietary fibre in legume-based food protects against the risk of oxidative injury during gastrointestinal digestion in vivo remains to be demonstrated. After establishing a balance between the antinutrient and the biological antioxidant effects of phenolics, the consumption of a processed legume food would not only improve nutrient utilization but also provide potential nutraceuticals for human health. In addition, the isolation and preparation of bioactive compounds (benzoic acid and cinnamic acid derivatives) from the coloured tubers of AC, which is considered to be a waste material, could serve as potent natural antioxidants from an industrial perspective.

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References

1. Amarowicz, R., Naczk, M., & Shahidi, F. (2000). Antioxidant activity of crude tannins of Canola and Rapeseed hulls. Journal of the American Oil Chemists' Society, 77, 957–961.
2. Aruoma, O. I. (1994): "Deoxyribose assay for detecting hydroxyl radicals". Methods in Enzymology, 233, 57–66.
3. Ault, J.G., Lawrence, D.A. (2003): "Glutathione distribution in normal and oxidatively stressed cells". Experimental Cell Research, 285, 9–14.
4. Augustin S, Claudine M, Christine M, Christian R. (2005) Dietary polyphenols and the prevention of diseases. Crit. Rev. Food Sci; 45: 287-306.
5. Branien AL. (1975) "Toxicoly and biochemistry of butylated hydroxyanisole and butylated hydroxytoluene". JAOCS; 52: 59-63.
6. Beauchamp, C. & Fridovich, I. ,(1971) "Superoxide dismutase: Improved assays and an assay applicable to acrylamide gels". Analytical Biochemistry: 44, 276–287
7. Benzie, I. F. F. & Strain, J. J. (1996) "The ferric reducing ability of plasma (FRAP) as a measure of ''antioxidant power": The FRAP assay. Analytical Biochemistry: 239, 70–76.
8. Brand-Williams, W., Cuvelier, M. E., & Berset, C., (1995) "Use of a free radical method to evaluate antioxidant activity".Lebensmittel Wissenschaft-und Technologie:28, 25–30.
9. Bauer, J.D., Ackermann, P.G., Toro, G. (1974) "Clinical Laboratory Methods". C.V. Mosby Company, London, p. 97
10. Chiu, D., Kuypers, F., Lubin, B. (1989): "Lipid peroxidation in human red cells. Seminars in Hematology" 26, 257–276.

11. Cutts, J.H., (1970) "Cell Separation, Methods in Hematology". Academic Press, New York and London, p. 43.
12. Chyu KY, Babbidge SM, Zhao X, Dandillaya R, Rietveld AG, Yano J, et al. (2004) "Differential effects of green tea-derived catechin on developing versus established atherosclerosis in apolipoprotein E-null mice". Circulation; 109 (20): 2448 53.
13. Carbonaro, M., Virgili, F., & Carnovale, E. (1996). "Evidence for protein– tannin interaction in legumes: Implications in the antioxidant properties of faba bean tannins". Lebensmittel Wissenschaft-und Technologie, 29, 743–750.
14. Decker, E. A. (1998). Antioxidant mechanisms. In C. C. Akoh & D. B. Min (Eds.), Food lipids, chemistry, nutrition, and biotechnology (pp. 397–401). New York: Marcel Dekker.
15. Da Silva EL, Tsushida T and Terao J. (1998) "Inhibition of mammalian 15-lipoxygenase-dependent lipid peroxidation in low-density lipoprotein by quercetin and quercetin monoglucosides". Arch. Biochem. Biophys; 349: 313-320.
16. Ferguson LR, Shu otun, Z, Harris PJ. (2005) "Antioxidant and antigenotoxic effects of plant cell wall hydroxycinnamic acids in cultured HT-29". Molecular Nutrition & Food Research; 49 (6): 585–693.
17. Formica, J.V., Regelson, W. (1995)"Review of the biology of quercetin and related bioflavonoids". Food and Chemical Toxicology 33, 1061–1080.
18. Ghani A. (1998) "Medicinal Plants of Bangladesh, Dhaka, Bangladesh", Asiatic Society of Bangladesh. 12(1), 77-82.
19. Goshi SG. (2000) Medicinal plants. 1st ed., 53-54.
20. Gradisar H, Pristovsek P, Plaper A, Jerala R. (2007) "Green tea catechins inhibit bacterial DNA gyrase by interaction with its ATP binding site". J. Med. Chem; 50 (2): 264–71.
21. Huang MT, Ma W, Yen P et al. (1996)"Inhibitory effects of caffeic acid phenethyl ester (CAPE) on 12-O-tetradecanoylphorbol-13- acetate-induced tumor promotion in mouse skin and the synthesis of DNA, RNA and protein in HeLa cells".Carcinogenesis; 17 (4): 761–5.
22. Huang MT, Smart RC, Wong, CQ, Conney AH. (1998) "Inhibitory effect of curcumin, chlorogenic acid, caffeic acid, and ferulic acid on tumor promotion in mouse skin by 12-Otetradecanoylphorbol- 13-acetate". Cancer Res; 48 (21): 5941–5946.
23. Hagerman, A. E., Riedl, K. M., Jones, G. A., Sovik, K. N., Ritchard, N. T., Hartzfeld, P. W. (1998) " High molecular weight plant polyphenolics (tannins) as biological antioxidants". Journal of Agricultural and Food Chemistry,: 46, 1887–1892.
24. Hwang, J. Y., Shue, Y. S., & Chang, H. M. (2001) "Antioxidant activity of roasted and defatted peanut kernels". Food Research International,.34, 639–647.
25. Igura K, Ohta T, Kuroda Y and Kaji K. (1997) "Resveratrol and quercetin inhibit angiogenesis in-vitro". Cancer Let; 114: 11-16.
26. Kikugawa K, Hakamada T, Hasunuma M, Kurechi T (1983)"Reaction of p-hydroxycinnamic acid derivatives with nitrite and its elevance to nitrosamine formation". Journal of Agricultural and Food Chemistry; 1 (4): p.780–785.
27. Khan A, Rahman M, Islam S. Antibacterial, "Antifungal and Cytotoxic Activities of Tuberous Roots of Amorphophallus campanulatus". Turk J Biol. 2007; 31(3): 167-72.
28. Lowry, O.H., Rosebrough, N.J., Farr, A.L., Randall, R.J. (1951) " Protein measurement with the folin phenol reagent". Journal of Biological Chemistry 193, 265–275.
29. Konyalioglu, S., Karamenderes, C. (2004) "Screening of total flavonoid, phenol contents and antioxidant capacities of Achillea L. species growing in Turkey". Acta Pharmaceutica Turcica 46 (3), 163–170.
30. Merken HM, Beecher GR. (2000) "Liquid chromatographic method for the separation and quantification of prominent flavonoid aglycones". J Chromato A; 897:177 – 184.
31. Mittal A, Pate MS, Wylie RC, Tollefsbol TO, Katiyar SK. (2004) "EGCG down-regulates telomerase in human breast carcinoma MCF-7 cells, leading to suppression of cell viability and induction of apoptosis". Int. J. Oncol; 24 (3): 703–10.
32. Mantle, D., Eddeb, F., Pickering, A.T., (2000) "Comparison of relative antioxidant activities of British medicinal plant species in vitro". Journal of Ethnopharmacology. 72, 47–51.
33. Makkar, H. P. S.,(2003). "Quantification of tannins in tree and shrub foliage: A laboratory

manual". Dordrecht, The Netherlands: Kluwer Academic Publishers. 19:123-129.
34. Nicoli, M. C., Anese, M., Manzocco, L., & Lerici, C. R. (1997) " Antioxidant properties of coffee brews in relation to the roasting degree ". Lebensmittel Wisenschaft – und Technologie,:30, 292–297.
35. Nadkarni.KM, (2000) "Indian Materia Medica."Vol.I,3rd ed.popular prakashan limited, Mumbai., 94-95.
36. Pulido, R., Bravo, L., & Saura-Calixto, F. (2000) "Antioxidant activity of dietary polyphenols as determined by a modified ferric reducing antioxidant power assay". Journal of Agricultural and Food Chemistry,: 48, 3396–3402
37. Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., & Rice- Evans, C.. (1999) "Antioxidant activity applying an improved ABTS radical cation decolorization assay". Free Radical Biology in Medicine,: 26, 1231–1237.
38. Rice-Evans, C., Miller, N.J., Paganga, G. (1997) "Antioxidant properties of phenolic compounds". Trends in Plant Science 2:, 154–159.
39. Rice-Evans C.A. and Packer L., (1998) Flavanoids in health and disease. Marcel dekkar Newyork, 25-29.
40. Siddhuraju, P. (2006) "The antioxidant activity and free radical scavenging capacity of phenolics of raw and dry heated moth bean (Vigna aconitifolia) (Jacq.) Marechal seed extracts". Food Chemistry,. 99, 149–157.
41. Sedlak, J., Lindsay, R.H. (1968) "Estimation of total protein-bound and nonprotein sulfhydryl groups in tissue with Ellman's reagent". Analytical Biochemistry 25, 192–205.
42. Su, J. D., Osawa, T., Kawakishi, S., & Namiki, M. (1998) "Tannin antioxidants from Osbeckia chinensis". Phytochemistry,: 27, 1315– 1319.
43. The Wealth of India Raw Material, CSIR, New Delhi, 2003, 1: A, 233.
44. Talalay P. (2000) "Chemoprotection against cancer by induction of phase 2 enzymes", Biofactors; 12: 5–11.
45. Yen, G. C., & Hsieh, P. P. (1995). "Antioxidative activity and scavenging effects on active oxygen of xylose-lysine Maillard reaction products". Journal of the Science of Food and Agriculture, 67, 415–420.
46. Yen, G. C., & Hsieh, C. L. (1998) "Antioxidant activity of extracts from Du-zhong (Eucommia ulmoides) toward various lipid peroxidation models in vitro". Journal of Agricultural and Food Chemistry,. 46, 3952–3957.
47. Zhishen, J., Mengcheng, T., & Jianming, W. (1999) "The determination of flavonoid contents on mulberry and their scavenging effects on superoxide radical." Food Chemistry,. 64, 555–559.

Table 1: Recovery percent, total phenolics, tannins and condensed tannins of *Amorphophallus campanulatus* tubers extracts (g /100 g DM)

Sample	Extract Recovery		Total Phenolics (Pyrogallol equivalents)		Flavonoid content(Quercetin equivalents)		Tannins	
	Value	SD	Value	SD	Value	SD	Value	SD
ME	4.9	0.28	12.72	0.09	22.25	0.56	2.23	0.21
HA	3.6	0.35	9.9	1.41	21.1	0.23	1.12	0.09
AE	3.7	0.21	7.1	1.12	13.1	0.45	0.9	0.12
EA	3.1	0.35	5.56	2.18	16.2	0.67	0.89	0.81

Values are means of three replicate determinations; SD, standard deviation.
Mean values followed by different superscript in the same column are significantly ($P < 0.05$) different.

Table 2: Superoxide anion radical scavenging activities of *Amorphophallus campanulatus* extracts

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Sample	200[A]	SD	400[A]	SD	600[A]	SD	800[A]	SD
ME	20	3.1	38.3	1.9	42.4	2.5	49.2	3.5
HA	12.4	1.7	20.1	2.5	34.7	1.2	47.5	4.2
AE	7.7	1.4	14.6	1.2	18.6	3.1	27	2
EA	10	1.2	17	0.7	26.3	1.4	36.2	2
Tannic acid[B]	19	1.8	32.9	1.3	54.2	3.6	70.3	1
Quercetin[B]	18.3	1.4	30.1	2.6	39.2	0.9	53.7	2.5

Values are means of three independent determinations; SD, standard deviation.
Values followed by different superscript in a column are significantly different (P < 0.05).
ME,methanolic extract,HA,hydroalcoholic extact,AE acetone extact,EA ehyl alcohol extact
[A]Concentration in µg.
[B]Concentration was 20, 40, 60 and 80 µg, respectively.

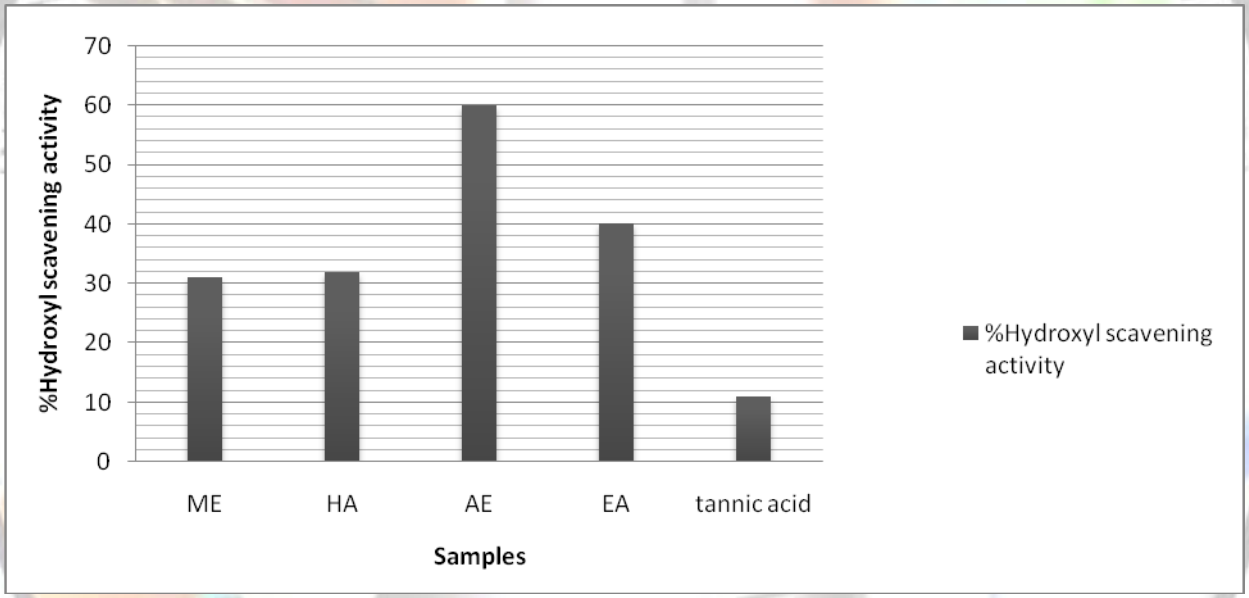

Fig. 1: Hydroxyl radical scavenging activity of four different extracts and tannic acid at a concentration of 200 µg in the reaction mixture. ME,methanolic extract,HA,hydroalcoholic extact,AE acetone extact,EA ethyl alcohol extact

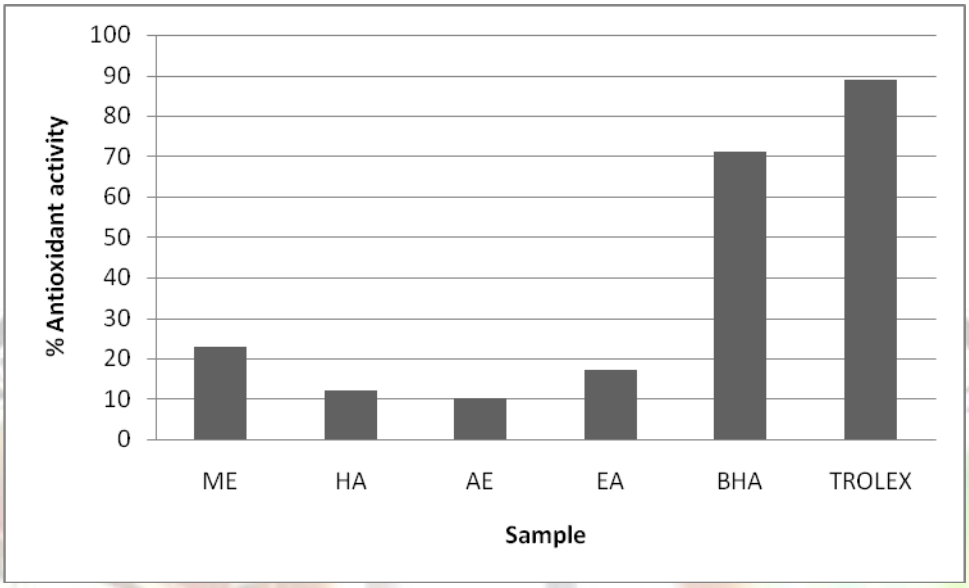

Fig. 3. Antioxidant activity of two varieties of AC extracts and other antioxidants in b-carotene/linoleic acid system. ME,methanolic extract,HA,hydroalcoholic extact,AE acetone extact,EA ethyl alcohol extact, BHA, butylated hydroxyanisole; TRO, Trolox

Table 3: Radical-scavenging activities on α, α-diphenyl- β picrylhydrazyl (DPPH$^{\circ}$) and ABTS^{+}

Sample	DPPH EC_{50}(mgDM/g DPPH)†		TAA (mmol/kg DM) C††		FRAP(mgDM/mmol Fe(II)) †††	
	Mean	SD	Mean	SD	Mean	SD
ME	405	12	391	7.5	445	34.7
HA	418	18	432	4.4	387	4.6
AE	1380	24	231	7.3	1180	16.5
EA	720	18	350	14.9	884	21.8
Quercetin	84.7	12				
BHA	103	9			60.4	3.1
Trolex					90.3	5

Values are means of three independent determinations; SD, standard deviation.
Values followed by different superscript in a column are significantly different ($P < 0.05$).
ME-methanolic extract, HA-hydroalcoholic extract, AE-acetone extract, EA- ethyl alcohol extract
†mg of sample required to decrease one g of the initial DPPH_ concentration by 50%.
†† Total antioxidant activity.
††† Ferric reducing/antioxidant power assay (concentration of substance having ferric-TPTZ reducing ability equivalent to that of 1 mmol Fe(II)).