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Phytochemical and *in vitro* antioxidant evaluation of different fractions of *Amaranthus graecizans* subsp. *silvestris* (Vill.) Brenan.

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ABSTRACT

Objective: To evaluate the phytochemical and *in vitro* antioxidant ability of methanolic extract and different fractions of *Amaranthus graecizans* subsp. *silvestris* (*A. graecizans* subsp. *silvestris*).

Methods: Methanolic extract of *A. graecizans* subsp. *silvestris* was obtained by cold maceration and then methanolic extract was subjected to fractionation and different fractions *i.e. n*-hexane, chloroform, ethyl acetate, *n*-butanol and aqueous fractions were obtained. Methanolic extract and all other fractions were subjected to phytochemical investigation by performing different phytochemical group tests like alkaloid, tannins, carbohydrates, lipids, proteins, *etc. In vitro* antioxidant activity of *A. graecizans* subsp. *silvestris* was evaluated by using 1, 1-diphenyl-2-picrylhydrazyl radical (DPPH), ferric thiocyanate assay, total antioxidant activity by phosphomolybdenum, ferric reducing antioxidant power, total phenolic content and lipid peroxidation methods.

Results: Maximum antioxidant activity was shown by *n*-hexane fraction of the extract by carrying out DPPH (86.44±0.23), ethyl acetate fraction by total antioxidant (0.95±0.06) and ferric reducing antioxidant power (299.45±1.48) methods, while by employing total phenolic contents and inhibition of lipid per oxidation assays, methanolic extract (92.88±4.16) and *n*-hexane fraction (69.47 ±0.68) exhibited maximal activity. Ethyl acetate fraction showed the least IC_{50} values by DPPH assay, hence a more pronounced potential for antioxidant activity.

Conclusions: The results indicate that *A. graecizans* subsp. *silvestris* has antioxidant potential and can be utilized as a natural source of antioxidant.

1. Introduction

All living organisms utilize aerobic metabolism as a source of energy, but this process results in the formation of reactive oxygen derivatives. Such free radicals are usually short-lived species but they possess a single unpaired electron, rendering them highly reactive against biologically important macromolecules including DNA, proteins and membrane lipids[1]. These reactive oxygen species (ROS) can damage DNA which causes mutation and chromosomal damage and also oxidizes cellular thiols and extracts hydrogen atoms from unsaturated fatty acids to initiate the peroxidation of membranes lipids[2]. In spite of this metabolism, human body has multiple mechanisms particularly enzymatic and non-enzymatic antioxidant systems to protect the cellular molecules against ROS induced damage. The ROS include superoxide anions (O_2), hydrogen peroxide (H_2O_2) and hydroxyl radicals (OH•). Lipid peroxidation, which involves a series of free radical mediated chain reaction processes, is associated with several types of biological damage[3]. Oxidative stress produced as a result of ROS may be the root cause of different diseases like atherosclerosis, stroke, diabetes, Alzheimer's disease and cancer[4]. In order to avoid the oxidative stress, prevention and treatment of complex diseases antioxidant-based drug formulations are used. Fruits and vegetables are rich

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in natural oxidants and have been health promoting effects and these positive effects have been related with their antioxidant activity[5]. Flavonoids and alkaloids which are usually found in medicinal plants have been accounted to have high antioxidant activity as well as multiple biological effects[6]. Ingestion of several synthetic antioxidants such as butylated hydroxytoluene (BHT) and butylated hydroxyanisole has been reported toxic to man[7]. Therefore, the use of natural antioxidant has gained much attention from consumers because they are considered safer than synthetic antioxidants. Recently, there has been a worldwide trend towards the use and ingestion of natural antioxidants present in different parts of plants due to their phytochemical constituents[8,9]. There has also been an upsurge of interest in the therapeutic potentials of medicinal plants as antioxidants in reducing such free radical induced tissue injury. Besides well-known and traditionally used natural antioxidants from tea, wine, fruits, vegetables and spices, some natural antioxidant (*e.g.* rosemary and sage) are already exploited commercially either as antioxidant additives or nutritional supplements[10]. *Amaranthus graecizans* subsp. *silvestris* (Vill.) Brenan (*A. graecizans* subsp. *silvestris*) is a locally occurring herb and is used as a folk medicine in the treatment of inflammation, gonorrhea and piles. The present work is to evaluate the antioxidant potential of this medicinal plant that will justify its use as a folk medicine[11].

2. Materials and methods

2.1. Extraction and fractionation

The shade-dried ground whole plant (1 kg) was exhaustively extracted with methanol (5 L) on the Soxhlet apparatus. The extract was evaporated in rotary evaporator (Laborta 4000-efficent Heidolph) at 40-45 °C under vacuum to yield the residue (130 g), which was dissolved in distilled water (1 L) and partitioned with *n*-hexane (1 L×5), chloroform (1 L×4), ethyl acetate (1 L×4) and *n*-butanol (1 L×4) respectively. These four organic fractions and remaining water fraction were concentrated separately on rotary evaporator (*n*-hexane at 38 °C, chloroform at 40 °C, ethyl acetate at 45 °C, *n*-butanol 50 °C and water at 60 °C under vacuum) and the residues thus obtained were used to evaluate their *in vitro* antioxidant potential.

2.2. Chemicals and standards

1, 1-Diphenyl-2-picrylhydrazyl radical (DPPH), 2, 4, 6-tripyridyl-s-triazine (TPTZ), trolox, gallic acid, Folin-Ciocalteu's phenol reagent and BHT were from Sigma Chemical Company Ltd., (USA). Organic solvents (*n*-hexane, chloroform, ethyl acetate, *n*-butanol), sulphuric acid, sodium phosphate, ammonium molybdate, ferric chloride, ferrous chloride, ceric sulphate, hydrochloric acid, copper sulphate, aluminium chloride, lead acetate, acetic acid, linoleic acid, Tween 20 and ammonia were purchased from Merck (Pvt.) Ltd. (Germany).

2.3. Phytochemical screening

Qualitative phytochemical screening were performed to identify the phytochemical constituents, *i.e.*, alkaloids, terpenoids, saponins, tannins, sugars, phenolics, flavonoids and cardiac glycosides, using standard procedures[12,13].

2.3.1. Test for terpenoids (Salkowski test)

A total of 2 mL of chloroform was added to 0.5 g each extract followed by 3 mL of concentrated H_2SO_4 that was added carefully to form a layer. Presence of terpenoids was indicated by preparation of a reddish brown layer at the interface of the organic and aqueous layer.

2.3.2. Test for flavonoids

We used two methods for flavonoids. In the first test few drops of 1% aluminium chloride solution were added to plant extracts, a persistent yellow coloration indicated the presence of flavonoids. While in the second method, Benedict's reagent was sprayed on thin layer chromatography card which have spots of samples. In UV light green fluorescence showed flavonoids presence.

2.3.3. Test for saponins

A total of 0.5 g of plant extracts were added to 5 mL distilled water in a test tube. Stable persistent froth that is observed after vigorous shaking mixed with 3 drops of olive oil forms an emulsion indicates presence of saponins.

2.3.4. Test for tannins

For indicating presence of tannins in plant extracts, 0.5 g of the extracts were boiled in 10 mL of water and then filtered, then added few drops of 0.1% ferric chloride, brownish-green or blue-black coloration indicates presence of tannins in the extracts.

2.3.5. Test for alkaloids

Dragendorff's reagent was sprayed on thin layer chromatography card on which sample spots are present. If orange color has appeared then alkaloids were confirmed.

2.3.6. Test for phenolics

Neutral solution of ferric chloride was added to 0.5 g of each extract. The bluish green color indicates the presence of phenolics.

2.3.7. Test for reducing sugars (Fehling's test)

One milliliter of water and 5-8 drops of Fehling's solution (A and B) was added to 0.5 g of each plant extract. It is heated and observed for brick red precipitate, indicating the presence of sugars.

2.3.8. Test for cardiac glycosides (Keller-Killiani test)

In aqueous solution (0.5 g each sample added in 5 mL

water) added glacial acetic acid (2 mL) having ferric chloride (1 drop). Concentrated H_2SO_4 was added in this solution. At the interface brown ring shows the deoxysugar characteristic of cardenolides presence. Below brown ring violet ring may be formed. Just above the brown ring a greenish ring may be formed in the aceticacid layer and this ring spread gradually throughout this layer.

2.4. Evaluation of antioxidant activity

Following antioxidant assays were performed on all the studied fractions.

2.4.1. DPPH radical scavenging activity

The DPPH radical scavenging activities of each crude extract of plant were examined by comparison with that of known antioxidant, BHT, using the reported method[14]. Briefly, various amounts of the samples (1 000, 500, 250, 125, 60, 30, and 15 μg/mL) were mixed with 3 mL of methanolic solution of DPPH (0.1 mmol/L). The mixture was shaken vigorously and allowed to stand at room temperature for 1 h. Then absorbance was measured at 517 nm against methanol as a blank in the spectrophotometer (Shimadzu UV1650). Lower absorbance of reaction mixture indicated higher free radical scavenging activity. The percent of DPPH discoloration of the samples was calculated according to the formula:

Antiradical activity (%)=$(A_{control}-A_{sample})/A_{control}\times100$

Each sample was assayed in triplicate and mean values were calculated.

2.4.2. Total antioxidant activity by phosphomolybdenum method

The total antioxidant activities of various fractions of plant were evaluated by phosphomolybdenum complex formation method[15]. Briefly, 500 μg/mL of each crude extract was mixed with 4 mL of reagent solution (0.6 mol/L sulphuric acid, 28 mmol/L sodium phosphate and 4 mmol/L ammonium molybdate) in sample vials. The blank solution contained 4 mL of reagent solution. The vials were capped and incubated in water bath at 95 °C for 90 min. After the samples had been cooled to room temperature, the absorbance of mixture was measured at 695 nm against blank. The antioxidant activity was expressed relative to that of BHT. All determinations were assayed in triplicate and mean values were calculated.

2.4.3. Ferric reducing antioxidant power (FRAP) assay

The FRAP assay was done according to Benzie and Strain with some modifications[16]. The stock solutions included 300 mmol/L acetate buffer (pH 3.6), 10 mmol/L TPTZ solution in 40 mmol/L hydrochloric acid, and 20 mmol/L ferric chloride hexahydrate solution. The fresh working solution was prepared by mixing 25 mL acetate buffer, 2.5 mL TPTZ solution and 2.5 mL ferric chloride hexahydrate solution and then warmed at 37 °C before using. The solutions of plant samples and that of trolox were prepared in methanol (500 μM/mL). A total of 10 μL of each of crude extract was taken in separate test tubes and 2 990 μL of FRAP solution was added in each to make total volume up to 3 mL. The plant crude extracts were allowed to react with FRAP solution in the dark for 30 min. Absorbance of the coloured product [ferrous tripyridyltriazine complex] was checked at 593 nm. The FRAP values were expressed as micromoles of trolox equivalents (TE) per mL of the sample solution using the standard curve constructed for different concentrations of trolox. Results were expressed in TE μM/mL.

2.4.4. Total phenolic content

Total phenolics of various fractions of plant were determined by the reported method[17]. An aliquot of 0.1 mL of each crude extract (0.5 mg/mL) was combined with 2.8 mL of 10% sodium carbonate and 0.1 mL of 0.2 mol/L Folin-Ciocalteu reagent. After 40 min absorbance at 725 nm was checked by UV-visible spectrophotometer. Total phenolics were expressed as milligrams of gallic acid equivalents (GAE) per gram of sample using the standard calibration curve constructed for different concentrations of gallic acid. Results were expressed in GAE mg/g.

2.4.5. Ferric thiocyanate (FTC) assay

The antioxidant activities of various fractions of plant on inhibition of linoleic acid peroxidation were assayed by thiocyanate method[18]. Each crude extract (0.1 mL, 0.5 mg/mL) was mixed with 2.5 mL of linoleic acid emulsion (0.02 mol/L, pH 7.0) and 2.0 mL of phosphate buffer (0.02 mol/L, pH 7.0). The linoleic emulsion was prepared by mixing 0.28 g of linoleic acid, 0.28 g of Tween-20 as emulsifier and 50.0 mL of phosphate buffer. The reaction mixture was incubated for 5 d at 40 °C. The mixture without extract was used as control. The mixture 0.1 mL was taken and mixed with 5.0 mL of 75% ethanol, 0.1 mL of 30% ammonium thiocyanate and 0.1 mL of 20 mmol/L ferrous chloride in 3.5% hydrochloric acid and allowed to stand at room temperature. Precisely, 3 min after addition of ferrous chloride to the reaction mixture, absorbance was recorded at 500 nm. The antioxidant activity was expressed as follows:

Inhibition of lipid peroxidation (%)=$[1-(A_{sample})/(A_{control})]\times100$

The antioxidant activity of BHT as reference standard was assayed for comparison.

2.5. Statistical analysis

All the measurements were done in triplicate and statistical analysis was performed by statistical software. All the data were expressed as mean±SEM. Statistical analysis were determined using One-way ANOVA followed by *post hoc* Tukey's test.

3. Results

3.1. Phytochemical analysis

The phytochemical analysis conducted on *A. graecizans*

subsp. *silvestris* (Vill.) Brenan extracts revealed the presence of tannins, flavonoids, sugars, alkaloids, terpenoids, phenols and saponins in methanolic, *n*-hexane, chloroform, ethyl acetate and *n*-butanol soluble fractions (Table 1).

Table 1

Phytochemical screening of various fractions of *A. graecizans* subsp. *silvestris* (Vill.) Brenan.

Test	Methanolic extract	*n*-Hexane fraction	Chloroform fraction	Ethyl acetate fraction	*n*-Butanol fraction	Aqueous fraction
Terpenoids	−	++	−	++		−
Flavonoids	++	−	++	+++	+++	+
Tannins	++	−	++	+++	+++	+++
Alkaloids	+++	+++	+++	++	+++	−
Carbohydrates	+++	−	++	−	+++	+++
Sterols	+++	+	++	+		−
Cardiac glycosides	+	−	+++	+++		−
Saponins	+	+++	+++	+	−	−
Proteins	−	−	−	−	+++	+

+: Presence; −: Absence.

Table 2

DPPH radical scavenging activity of the various fractions of *A. graecizans* subsp. *silvestris*.

Sample	Concentration in assay (μg/mL)	% Scavenging of DPPH ±SEM
Methanolic extract	250	59.10±0.11
	120	41.05±0.05
	60	37.20±0.01
	30	32.76±0.38
	15	30.03±0.14
n-Hexane fraction	1 000	86.44±0.23*
	500	61.16±0.03*
	250	45.27±0.04
	120	33.60±0.03
Chloroform fraction	250	51.16±0.02*
	120	37.05±0.04*
	60	34.18±0.02
	30	28.95±0.03
	15	28.05±0.03
Ethyl acetate fraction	250	85.10±0.01*
	120	65.18±0.01
	60	62.57±0.06*
	30	52.70±0.03
	15	45.04±0.03
n-butanol fraction	250	46.51±0.03
	12	38.93±0.07
	60	33.21±0.01
	30	28.10±0.01
	15	29.30±0.02
Aqueous fraction	1 000	80.30±0.03*
	500	62.15±0.02*
	250	55.28±0.02
	120	45.70±0.03
BHT[a]	60	91.28±0.02
	30	75.39±0.01
	15	42.53±0.02

All results are presented as mean±SEM of three assays; [a]: Standard antioxidant; *$P<0.05$ when compared with negative control *i.e.* blank/solvent.

3.2. Antioxidant assay

3.2.1. DPPH radical scavenging activity

The DPPH radical is widely used in the model system to evaluate antioxidant activities in a relatively short time. In our results the *n*-hexane fraction showed the highest DPPH scavenging activity (86.44±0.23)% at concentration of 1 000 μg/mL while the lowest scavenging value was of chloroform fraction (28.05±0.03)% at 15 μg/mL as presented in Table 2. For each fraction, the IC_{50} value was calculated from the curves plotted. Lower IC_{50} value indicates better DPPH radical scavenging activity. Ethyl acetate fraction of *A. graecizans* subsp. *silvestris* (Vill.) Brenan exhibited the lowest IC_{50} value [(16.02±1.80) μg/mL]. On the other hand, *n*-hexane fraction exhibited the highest IC_{50} [(356.18±1.50) μg/mL] as shown in Table 3.

3.2.2. Total antioxidant activity by phosphomolybdenum method

Total antioxidant activities of all fractions were evaluated spectrophotometrically by the phosphomolybdenum method. The antioxidant activities of the fractions were compared with the reference standard antioxidant BHT (0.96±0.06). The ethyl acetate fraction displayed the highest total antioxidant activity value (0.95±0.06), followed by the slightly less antioxidant of methanol, *n*-butanol, chloroform, remaining aqueous fraction and *n*-hexane fraction. while the *n*-hexane soluble fraction had the lowest total antioxidant activity (Table 3).

3.2.3. FRAP assay

The FRAP assay is a simple and inexpensive procedure that measures the total antioxidant levels in a sample by taking into account of their oxidation-reduction potential. Among all the *A. graecizans* subsp. *silvestris* (Vill.) Brenan fractions, the ethyl acetate fraction showed the highest FRAP value [(299.45±1.48) μg/mL] and other fractions in the decreasing order, *i.e.* *n*-butanol [(230.93±1.66) μg/mL], chloroform [(118.45±1.95) μg/mL], aqueous [(78.22±1.21) μg/mL], methanolic [(34.41±1.29) μg/mL] and *n*-hexane fraction [(24.99±1.45) μg/mL] (Table 3).

3.2.4. FTC assay

The ferric thiocyanate method measures the amount of peroxide generated at the initial stage of linoleic acid emulsion during incubation. Our results revealed that *n*-hexane displayed the maximum inhibition of lipid peroxidation [(69.47±0.68)%] and the lowest actvity was exhibited by aqeuous fraction [(19.28±0.95)%]. The inhibition of lipid peroxidation by BHT (standard) was (62.48±1.07)%.

3.2.5. Total phenolic content

The antioxidant activity of phenolic compounds is mainly due to their redox properties and chemical structure, which allow them to act as reducing agent, hydrogen donators and singlet quenchers[19], and chelating transitional metals, inhibiting lipoxygenase and scavenging free radical. Folin-Ciocalteu reagent was used to determine total polyphenols

Table 3

IC_{50}, total phenolics, total antioxidant activity, FRAP values and lipid peroxidation inhibition values of different fractions of *A. graecizans* subsp. *silvestris*.

Sample	DPPH radical scavenging activity (IC_{50}: μg/mL)	Total antioxidant activity (Absorbance at 695 nm)	FRAP value (TE μM/mL)	Total phenolics (GAE mg/g)	Inhibition of lipid peroxidation (FTC) (%)[c]
Methanolic extract	178.58±0.76	0.81±0.04	34.41±1.29	92.88±4.16	50.12±0.20
n-Hexane fraction	356.18±1.50	0.30±0.02	24.99±1.45	24.66±1.06	69.47±0.68
Chloroform fraction	240.91±1.04*	0.70±0.03*	118.45±1.95*	53.44±1.04*	21.06±1.53
Ethyl acetate fraction	16.02±1.80	0.95±0.06	299.45±1.48	89.90±1.98	55.04±1.46**
n-Butanol fraction	284.47±0.76	0.73±0.05**	230.93±1.66**	78.43±1.03	43.91±0.95**
Aqueous fraction	176.29±1.00	0.57±0.04	78.22±1.21	28.08±0.39	19.28±0.95
BHT	15.36±0.87	0.96±0.06	–	–	62.48±1.07
Blank[a]	–	–	18.64	16.49	–

All results execpt blank control are presented as mean±SEM of three assays; [a]: Standard antioxidant; *$P<0.05$ when compared with negative control *i.e.*. blank/solvent.

in samples[20]. The standard curve was constructed using gallic acid. Gallic acid is a water soluble polyhydroxy phenolic compound which can be found in various natural plants. The equation obtained from standard curve is y (absorbance)=0.006x+0.139. The absorbance value of the sample was inserted in the above equation and the total amount of phenolic compound was calculated. In present result methanolic extract possessed the highest amount of total phenolic compounds, having value [(92.88±4.16) μg/g] followed by ethyl acetate fraction [(89.90±1.98) μg/g], *n*-butanol fraction [(78.43±1.03) μg/g], chloroform fraction [(53.44±1.04) μg/g], aqueous fraction [(28.08±0.39) μg/g], while *n*-hexane fraction [(24.66±1.06) μg/g] exhibited the lowest total phenolic contents as shown in Table 3.

4. Discussion

The phytochemical analysis revealed that the amount of common bioactive components like terpenoids, alkaloids, phenolics, flavonoids and saponins were concentrated in medium polar and polar fractions (*i.e.*, chloroform, ethyl acetate and butanol), while the quantity of sugars was good in remaining aqueous fraction. DPPH scavenging activity is based on the ability of sample to donate hydrogen which reacts with the DPPH radical. When a solution of DPPH is mixed with a substance that can donate a hydrogen atom or transfer electron to DPPH, thus neutralize the free radical character and then this gives rise to the reduced form DPPH (non-radical) with the loss of the violet color. Radical scavenging activity increases with increasing percentage of the free radical inhibition[21]. The color change from violet to yellow and fall in absorbance of the stable radical DPPH was measured for different concentration. In the present study the methanolic extract and polar fraction (ethyl acetate) showed higher or similar antioxidant activities as compared to the standard BHT. This is due to most bioactive compounds such as polyphenols including tannins, flavonoids existed in higher polar fraction[22].

The total antioxidant activity was analyzed by phosphomolybdenum method which is based on the reduction of Mo (VI) to Mo (V) by the sample analyte and the subsequent formation of green phosphate Mo (V) complex at acidic pH. Electron transfer occurs in this assay which depends upon the structure of the antioxidant[23]. In our study the polar fraction showed highest antioxidant activity, whereas the non-polar fraction didn't reduce Mo (VI) to Mo (V) when compared with BHT. This shows that ethyl acetate fraction which contains more polar compounds than hexane and chloroform possess more antioxidant activity.

The FRAP method measures the ferric reducing ability of plasma. It utilizes the reducing potential of the antioxidants to react with the ferric tripyridyltriazine complex and produce the intense blue color ferrous tripyridyltriazine complex[24]. The reaction conditions favor reduction of the complex and thereby color development provided that a reductant (antioxidant) is present. In this study the ethyl acetate fraction revealed the highest ferric reducing potential as observed with other antioxidant tests.

In ferric thiocyanate test the ethyl acetate fraction showed maximum inhibition of the amount of peroxide generated at the initial step when compared with BHT. This peroxide generated then reacts with ferrous chloride to form ferric chloride, which in turn reacts with ammonium thiocyanate to produce ferric thiocyanate, a reddish pigment. Low absorbance values measured via the FTC method indicated high antioxidant activity[25].

In this study the chloroform fraction revealed the highest amount of phenolic contents estimated by the addition of Folin-Ciocalteu reagent. Folin-Ciocalteu reagent consists of a yellow acidic solution containing complex polymeric ions formed from phosphomolybdic and phosphotungsticheteropoly acids. These reagents oxidizes phenolates resulted in the production of complex molybdenum-tungsten blue which can be detected spectrophotometrically at 725 nm and calculated as GAE.

According to the results of this study, the methanolic extract of *A. graecizans* subsp. *silvestris* (Vill.) Brenan may be suggested as a potential source of natural antioxidant. It was concluded that as *n*-hexane fraction of this plant exhibited the highest percentage inhibition of DPPH radical [(86.44±0.23) μg/mL] and ethyl acetate fraction showed lowest

IC_{50} value. Ethyl acetate fraction also showed highest total antioxidant, FRAP activity but methanolic extract is rich in total phenolics and *n*-hexane possess high value of inhibition of lipid peroxidation. Thus further phytochemical investigations may bring new natural antioxidants into the food industry that might provide good protection against the oxidative damage which occurs in the body and our daily foods.

Conflict of interest statement

We declare that we have no conflict of interest.

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References

[1] Xia DZ, Yu XF, Zhu ZY, Zou ZD. Antioxidant and antibacterial activity of six edible wild plants (*Sonchus* spp.) in China. *Nat Prod Res* 2011; **25**(20): 1893-1901.

[2] Abalaka ME, Mann A, Adeyemo SO. Studies on *in-vitro* antioxidant and free radical scavenging potential and phytochemical screening of leaves of *Ziziphus mauritiana* L. and *Ziziphus spina-christi* L. compared with ascorbic acid. *J Med Genet Genomics* 2011; **3**(2): 28-34.

[3] Lobo V, Patil A, Phatak A, Chandra N. Free radicals, antioxidants and functional foods: impact on human health. *Pharmacogn Rev* 2010; **4**(8): 118-126.

[4] Jothy SL, Zuraini Z, Sasidharan S. Phytochemicals screening, DPPH free radical scavenging and xanthine oxidase inhibitory activities of *Cassia fistula* seeds extract. *J Med Plants Res* 2011; **5**: 1941-1947.

[5] Patel DK, Kumar R, Laloo D, Hemalatha S. Evaluation of phytochemical and antioxidant activities of the different fractions of *Hybanthus enneaspermus* (Linn.) F. Muell. (Violaceae). *Asian Pac J Trop Med* 2011; **4**(5): 391-396.

[6] Mbaebie BO, Edeoga HO, Afolayan AJ. Phytochemical analysis and antioxidants activities of aqueous stem bark extract of *Schotia latifolia* Jacq. *Asian Pac J Trop Biomed* 2012; **2**(2): 118-124.

[7] Barros L, Carvalho AM, Ferreira IC. Leaves, flowers, immature fruits and leafy flowered stems of *Malva sylvestris*: a comparative study of the nutraceutical potential and composition. *Food Chem Toxicol* 2010; **48**(6): 1466-1472.

[8] Martins S, Mussatto SI, Martínez-Avila G, Montañez-Saenz J, Aguilar CN, Teixeira JA. Bioactive phenolic compounds: production and extraction by solid-state fermentation. A review. *Biotechnol Adv* 2011; **29**(3): 365-373.

[9] Mandegary A, Pournamdari M, Sharififar F, Pournourmohammadi S, Fardiar R, Shooli S. Alkaloid and flavonoid rich fractions of fenugreek seeds (*Trigonella foenum-graecum* L.) with antinociceptive and anti-inflammatory effects. *Food Chem Toxicol* 2012; **50**(7): 2503-2507.

[10] Patel VR, Patel PR, Kajal SS. Antioxidant activity of some selected medicinal plants in western region of India. *Adv Biol Res* 2010; **4**(1): 23-26.

[11] Nisar MF, Ismail S, Arshad M, Majeed A, Arfan M. Ethnomedicinal flora of district Mandi Bahaudin, Pakistan. *Middle-East J Sci Res* 2011; **9**(2): 233-238.

[12] Weremfo A, Adinortey MB, Pappoe ANM. Haemostatic effect of the stem juice of *Musa paradisiaca* L. (Musaceae) in guinea pigs. *Adv Biol Res* 2011; **5**(4): 190-192.

[13] Evans WC. *Trease and Evans' pharmacognosy*. Amsterdam: Elsevier Health Sciences; 2009.

[14] Ebrahimzadeh MA, Nabavi SM, Nabavi SF, Bahramian F, Bekhradnia AR. Antioxidant and free radical scavenging activity of *H. officinalis* L. var. *angustifolius*, *V. odorata*, *B. hyrcana* and *C. speciosum*. *Pak J Pharm Sci* 2010; **23**(1): 29-34.

[15] Baydar NG, Özkan G, Yaşar S. Evaluation of the antiradical and antioxidant potential of grape extracts. *Food Control* 2007; **18**(9): 1131-1136.

[16] Thaipong K, Boonprakob U, Crosby K, Cisneros-Zevallos L, Byrne DH. Comparison of ABTS, DPPH, FRAP, and ORAC assays for estimating antioxidant activity from guava fruit extracts. *J Food Compost Anal* 2006; **19**(6-7): 669-675.

[17] Sarneckis CJ, Dambergs RG, Jones P, Mercurio M, Herderich MJ, Smith PA. Quantification of condensed tannins by precipitation with methyl cellulose: development and validation of an optimised tool for grape and wine analysis. *Aust J Grape Wine Res* 2006; **12**(1): 39-49.

[18] Zhang LL, Lin YM. Antioxidant tannins from *Syzygium cumini* fruit. *Afr J Biotechnol* 2009; **8**(10): 2301-2309.

[19] Babbar N, Oberoi HS, Uppal DS, Patil RT. Total phenolic content and antioxidant capacity of extracts obtained from six important fruit residues. *Food Res Int* 2011; **44**(1): 391-396.

[20] Boivin D, Lamy S, Lord-Dufour S, Jackson J, Beaulieu E, Côté M, et al. Antiproliferative and antioxidant activities of common vegetables: a comparative study. *Food Chem* 2009; **112**(2): 374-380.

[21] Schreiner M, Beyene B, Krumbein A, Stutzel H. Ontogenetic changes of 2-propenyl and 3-indolylmethyl glucosinolates in *Brassica carinata* leaves as affected by water supply. *J Agric Food Chem* 2009; **57**(16): 7259-7263.

[22] El-Shemy HA, Aboul-Soud MA, Nassr-Allah AA, Aboul-Enein KM, Kabash A, Yagi A. Antitumor properties and modulation of antioxidant enzymes' activity by *Aloe vera* leaf active principles isolated via supercritical carbon dioxide extraction. *Curr Med Chem* 2010; **17**(2): 129-138.

[23] Kammoun M, Miladi S, Ben Ali Y, Damak M, Gargouri Y, Bezzine S. *In vitro* antioxidant study of the PLA2 inhibition and antioxidant activities of *Aloe vera* leaf skin extracts. *Lipids Health Dis* 2011; doi: 10.1186/1476-511X-10-30.

[24] Gülçin İ. Antioxidant activity of food constituents: an overview. *Arch Toxicol* 2012; **86**(3): 345-391.

[25] Djeridane A, Yousfi M, Nadjemi B, Boutassouna D, Stocker P, Vidal N. Antioxidant activity of some algerian medicinal plants extracts containing phenolic compounds. *Food Chem* 2006; **97**(4): 654-660.