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Polyphenolic constituents and antioxidant/antiradical activity in different extracts of *Alstonia scholaris* (Linn.)

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***Alstonia scholaris* (Linn.) leaves extracted in aqueous, dichloromethane (DCM), methanolic and ethanolic solvents were assessed for different polyphenolic constituents endowed with antioxidant/antiradical activity. Total phenolic, flavonoids and tannin contents were significantly (P<0.05) higher in ethanolic extract as compared to other extracts. The antiradical activity and efficiency was significantly (P<0.05) higher in ethanolic extract followed by methanolic, DCM and aqueous extracts. The ethanolic extract as compared to other extracts also exhibited high total antioxidant capacity, superoxide and nitric oxide anion scavenging activity. However, aqueous extract has shown high hydroxyl radicals scavenging activity as compared to other extracts. Total phenolic content of the ethanolic extract of *A. scholaris* was positively correlated with total antioxidant capacity (r = 0.901), antiradical activity (r = 0.948) and antiradical efficiency (r = 0.891). Therefore, ethanolic extract showed maximum, contents endowed with high total antioxidant capacity, superoxide and nitric oxide radicals scavenging activity as compared to other solvents. Thus, the dietary supplementation of ethanolic extract may provide protection in preventing the free radicals induced damage besides improve the food quality by retarding oxidative degeneration of food lipids.**

Key words: Total phenolics, antioxidant potential, free radicals, *Alstonia scholaris*.

INTRODUCTION

Alstonia species belonging to apocynaceae family, distributed throughout the tropical and subtropical regions of the world is widely used in folklore system of medicine in Asia, America and Africa (Dassanayake, 1982). *Alstonia scholaris* (Linn.) an ever green tree in India has been used to treat dysentery, ulcer, dropsy and fever.

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Abbreviations: TAC, Total antioxidant capacity; **PVPP,** polyvinyl-polypirrolidone; **GAE,** gallic acid equivalents; **DCM,** dichloromethane; **AEAS,** aqueous extracts of *A. scholaris*; **DCMEAS,** dichloromethane extracts of *A. scholaris*; MEAS methanolic extracts of *A. scholaris;* **EEAS,** ethanolic extracts of *A. scholaris;* **NBT,** nitroblue tetrazolium; **PMS,** phenazine methosulfate.

Folklore described use of plant bark has been for carminative action, astringent, spasmolytic, expectorant, stimulant, aphrodisiac actions (Singh and Panda, 2005). The leaves extracts have been used in the treatment of beriberi disease, hepatic disorders and act as a powerful galactogogue. The crushed leaves boiled in the edible oil have been used for dropsy (Chopra et al., 1956; Channa et al., 2005). Methanolic leaf extract of *A. scholaris* has been reported to have antidiabetic potential in animal models (Bandawane et al., 2011) and this may be due to its inhibitory activity of intestinal α-glucosidase (Anurakkun et al., 2007) which delays the digestion and absorption of carbohydrates in intestine. The plant is a rich source of flavonoid glycosides (Desoky, 1999), steroids (Atta-ur-Rahman et al., 1987), terpenoids (Dung et al., 2001), indole and other alkaloids (Rahman and Alvi, 1987; Abe et al., 1989). The flowers of *A. scholaris* contain n-hexacosane, palmitic acid and ursolic acid (Biswas and Saharia, 1978; Rahman and Alvi, 1987; Kam et al., 1997). Experimental validation of different parts of plant has demonstrated that it is bestowed with different pharmacological properties (Channa et al., 2005; Bandawane et al., 2011; Arulmozhi et al., 2012). Considerable experimental evidence has contributed to support a key role of free radicals ($OH^{\cdot}$, $O_2^{\cdot}$, $NO^{\cdot}$, H_2O_2, etc) in development of oxidative damage and degenerative disorders in mammals (Halliwell and Gutterridge, 1990; Sohal et al., 2002; Valko et al., 2007). Reduced antioxidant defense and increased oxidant production is primarily responsible for the cancer, aging, neurodegenerative disorders in man and animals. The increased free radicals levels are responsible for alterations in the biochemical processes and directly damage the essential macromolecules like transporter and enzymatic proteins, nucleic acid, membrane lipids, etc (Halliwell and Gutterridge, 1990; Valko et al., 2007).

Most of phytochemical constituents possess strong quenching ability of free radicals like superoxide, peroxide, hydroxyl, nitro and other free radicals. To determine the phytochemical constituent having high radicals scavenging potential different parts of plant were extracted with different solvents to maximize their utility. These reactive radicals are enormously generated in the mammalian body during oxidative stress predisposing individual to many chronic diseases like diabetes, hypertension, atherosclerosis, cancer, aging, etc when antioxidant system of the mammalian body fail to encounter the onslaught of these reactive species (Sohal et al., 2002; Valko et al., 2007). The generation of free radicals during processing of food is also responsible for food deterioration affecting the shelf life of different foods. Thus, there is a scope for identifying and developing effective antioxidants from natural sources to prevent the free radicals implicated diseases in mammals and also minimizes extent of lipid peroxidation in foods during manufacturing processes (Aruoma, 1993). Therefore, the present study was aimed to determine the different polyphenolic constituents endowed with high antioxidant/ antiradical activity in different extracts of *A. scholaris* leaves.

MATERIALS AND METHODS

Collection and preparation of extracts

The leaves of *A. scholaris* (Linn.) were collected from the University Campus at R S Pura, Jammu (India). Plant sample was taxonomically identified by Taxonomist, Department of Botany, University of Jammu. Sufficient fresh leaves were collected and air-dried in shade (temperature not exceeding 40°C) for 3 to 4 weeks. Air dried leaves were pre-crushed and later pulverized into fine powder using electric blender. The aqueous extract was prepared by soaking dry powder in 1:10 ratio in distilled water for 72 h with intermittent shaking. After 72 h of soaking, the contents were filtered through filter paper (0.45 µm) and filtrate was concentrated under reduced pressure using rotatory evaporator (temperature of 50 to 55°C; 10 to 15 rpm). The methanolic and ethanolic extracts were prepared by using methanol and ethyl alcohol in extract container of soxhlet apparatus according to method described by Harborne (1984). Similarly, mixture of dichloromethane and methanol (1:1) was used as a solvent for the extraction of dichloromethane extract. The dried aqueous (AEAS), dichloromethane (DCMEAS), methanolic (MEAS) and ethanolic (EEAS) leaf extracts of *A. scholaris* (Linn.) were stored in air tight containers.

Phytochemical constituents of plant extracts

Determination of total phenolic content

Total phenolics in extracts were determined by Folin-Ciocalteu method (Ranalli et al., 2006). Briefly, 0.5 mL (0.1%, w/v) of each sample was mixed with 2.5 mL of a 10 fold diluted Folin-Ciocalteu reagent followed by 2 mL of 7.5% sodium carbonate. The tubes were covered with parafilm (American National Can, Chicago) and allowed to stand for 30 min at room temperature before the absorbance was recorded at 760 nm (U-1800, Spectrophotometer, Hitachi, Japan). Different concentrations of gallic acid (0.1 to 0.60 mg/mL) were prepared in methanol for preparation of standard curve. All determinations were analyzed in triplicate and results expressed in mg gallic acid equivalents (GAE)/g dried extract.

Determination of total flavonoid content

The total flavonoid content of plant extracts were estimated according to method described by Zhishen et al. (1999). 1.0 mL (0.1%, w/v) of sample was mixed with 4 mL of distilled water and subsequently with 0.3 mL of $NaNO_2$ solution (10%, w/v). After allowing the mixture to stand for 5 min, 0.3 mL $AlCl_3$ solution (10%, w/v) was added followed by 2.0 mL of (1%, w/v) NaOH solution. The mixture was thoroughly mixed immediately and absorbance was determined against blank at 510 nm. Standard curve of quercetin (Sigma Aldrich, USA) was prepared in a concentration ranging from 0 to 12 mg/mL and the results were expressed as quercetin equivalents (mg quercetin equivalents/g dried extract).

Determination of tannin and non-tannin contents

Tannin content in sample was determined using insoluble polyvinyl-polypirrolidone (PVPP) which binds tannins (Makkar et al., 1993).

Briefly, 1 mL of extract (0.1%, w/v) dissolved in methanol in which the total phenolics were determined, was mixed with 100 mg PVPP, vortexed, kept for 15 min at 4°C and then centrifuged for 10 min at 3000 rpm. In the clear supernatant the non-tannin phenolics were determined in the same manner as the total phenolics (Velioglu et al., 1998). Tannin content was calculated as a difference between total and non-tannin phenolic content.

Antioxidant assay

Total antioxidant capacity

The total antioxidant capacity (TAC) of plant extracts was determined according to the method of Re et al. (1999). ABTS [2,2'-azonobis (3-ethylene benzothiazoline-6-sulphonic acid)] radical cations were produced by reacting 7 mM solution of ABTS (Sigma Aldrich, USA) with 2.45 mM potassium sulfate. The mixture was allowed to stand in the dark at room temperature for 12 to 16 h before use. The resulting $ABTS^+$ solution was diluted with phosphate buffer (pH 7.4) to obtain optical density of 0.70 ± 0.05 at 734 nm. Radical scavenging analysis was performed by mixing 50 µL of the sample solution or standard of different concentrations into 950 µL of $ABTS^+$ solution and after 3 min the absorbance was recorded at 734 nm. A blank solution of 50 µL methanol in 950 µL of $ABTS^+$ solution was prepared and analyzed in similar manner. Final TAC values were calculated using regression equation between ascorbic acid concentrations (0 to 100 µg/mL) and per cent inhibition of ABTS radical cations and expressed as mg ascorbic acid equivalents/g dried extract. % $ABTS^+$ inhibition = [1 – (A_{734}nm Sample/ A_{734}nm Blank)] x 100.

Radical scavenging assay

The molecule of 1,1-diphenyl-2-picrylhydrazyl (α,α-diphenyl-β-picrylhydrazyl) (DPPH, Sigma Aldrich, USA) is a stable free radical in methanol. The capacity of the extracts to scavenge the stable free radical DPPH was monitored according to the procedure described by Amarowicz et al. (2002). 1.0 mM solution of DPPH in methanol served as stock and was diluted four times in order to obtain optical density below 1.0 at 517 nm. 800 µL of diluted DPPH solution mixed with 200 µL (0.1%, w/v) of different concentration of sample were vortexed and incubated for 30 min at room temperature in dark. After incubation absorbance was read at 517 nm against blank. Control was run in a similar fashion with exception of replacing sample volume with methanol. The samples were run in triplicate and mean values were plotted in graph in order to calculate the concentration required for 50% inhibition (IC_{50}) of DPPH radical. The antiradical activity (ARA) was expressed as percentage inhibition of DPPH radical determined by the following equation ARA = 100 x [1 – (A_{517}nm Sample/ A_{517}nm Blank)] (Yen and Duh, 1994).

Reducing power (RP) assay

Reducing power of plant extracts was determined according to method described by Oyaizu (1986). Briefly, 1.0 mL (0.1%, w/v) of sample was mixed with 2.5 mL 0.2 M phosphate buffer solution (pH 6.6) and 2.5 mL (0.25%, w/v) of potassium ferricyanide. The final mixture was properly mixed and incubated at 50°C for 20 min. After incubation, the reaction mixture rapidly cooled, mixed with 2.5 mL (10%, w/v) trichloroacetic acid and centrifuged at 3000 rpm for 10 min. To 2.5 mL of the supernatant an equal volume of distilled water and 0.5 mL of ferric chloride (0.025%, w/v) were added to it, mixed thoroughly and allowed to stand for 10 min. Control sample was prepared in similar manner and distilled water was used instead of sample solution. The absorbance was measured at 700 nm against control. Ascorbic acid (SD Fine Chem Ltd. Mumbai, India) was used as reference standard and results expressed as µg ascorbic acid equivalents antioxidant capacity (AEAC)/g of extracts. The assays were carried out in triplicate and the results are expressed as mean value ± SD.

Superoxide anion radical scavenging assay

The superoxide anion radical scavenging ability of extract was determined as per method described earlier (Gulcin et al., 2004). Superoxide anion radicals were generated in Phenazine methosulfate (PMS)–NADH systems by oxidation of NADH (Nicotineamide adenine dinucleotide hydrogen salt) and assayed by the reduction of Nitroblue tetrazolium (NBT). In the process, superoxide anion radicals were generated in 2.5 mL of Tris HCl buffer (16 mM, pH 8.0) containing 0.5 mL of NBT (300 µM) solution, 0.5 mL of NADH (468 µM) solution and 1.0 mL of plant extracts in concentration ranges from 0.05 to 1.00 mg/mL. The reaction was started by adding 0.5 mL of PMS (60 µM) solution to the mixture. The reaction mixture was incubated at room temperature for 5 min and the absorbance was read at 560 nm. The decreased in absorbance of the reaction mixture indicated the superoxide anion-scavenging activity. The capability of scavenging of the superoxide anion radicals was calculated using the equation: % Superoxide radical scavenging activity = [1 – (A_{560}nm Sample/ A_{560}nm Blank)] x 100.

Hydroxyl radical scavenging assay

The potential of different concentrations of plant extract to scavenge the hydroxyl radical generated by the Fenton reaction was measured (Halliwell et al., 1987). The hydroxyl radical attacks deoxyribose, which eventually results in thiobarbituric acid reacting substance (TBARS) formation. The reaction mixture contained in a final volume of 1.0 mL [100 µL of 2- deoxy-D-ribose (28 mM in KH_2PO_4-KOH buffer, 20 mM, pH 7.4), 100 µL of different concentrations of extracts (1.25 to 40 µg/mL) in KH_2PO_4- KOH buffer (800 µL 20 mM, pH 7.4)] was mixed with 200 µL of 1.04 mM EDTA and 200 µM of $FeCl_3$ (1:1 v/v), 100 µL of 1.0 mM of H_2O_2 and 100 µL of 1.0 mM ascorbic acid and incubated at 37°C for 1 h. After incubation, thiobarbituric acid (1%) (1 mL) and trichloroacetic acid (2.8%) (1 mL) were added to the reaction mixture and incubated at 100°C for 20 min. After cooling, absorbance was measured at 532 nm. The control sample was also prepared having no extract but instead of 800 µL had 900 µL KH_2PO_4- KOH buffer and the rest of the procedure was the same. From the absorbance the % scavenging activity was calculated using the formula: % Hydroxyl radical scavenging activity = [1– (A_{532}nm sample / A_{532}nm control)] x 100.

Nitric oxide radical scavenging activity

Nitric oxide radical scavenging activity was measured by the method of Green et al. (1982) with slight modification by using Griess' reagent. 250 µL sample of different concentrations of the extracts (10 to 60 µg/mL) and control sample (250 µL distilled water instead of extract) were mixed with an equal volume (250 µL) of sodium nitroprusside solution (5 mM in standard phosphate buffer pH 7.4) and incubated at 25°C for 1 h. After incubation, the incubation mixture was mixed with 0.5 mL of Griess' reagent (sulphanilamide 1%, o-phosphoric acid 2% and naphthyl ethylene diamine dihydrochloride 0.1%) and diluted with 2 mL of distilled

Table 1. Polyphenolic constituents present in aqueous (AEAS), dichloromethane (DCMEAS), methanolic (MEAS) and ethanolic (EEAS) extracts of *A. Scholaris* Linn.

Parameter (unit)	Leaf extracts of *A. scholaris*			
	AEAS	DCMEAS	MEAS	EEAS
Total phenolic content (mg of GAE/gm extract)	$1.03^{a} \pm 0.09$	$4.68^{b} \pm 0.61$	$1.97^{a} \pm 0.23$	$10.87^{b} \pm 0.96$
Total flavonoids content (mg Quercetin/gm extract)	$13.93^{a} \pm 4.20$	$69.57^{b} \pm 9.56$	$40.67^{a} \pm 5.92$	$94.77^{b} \pm 4.46$
Non-tannin content (mg of GAE/gm extract)	$0.20^{a} \pm 0.08$	$0.45^{a} \pm 0.37$	$0.37^{a} \pm 0.27$	$0.56^{b} \pm 0.22$
Tannin content (mg of GAE/gm extract)	$0.83^{a} \pm 0.09$	$4.22^{b} \pm 0.78$	$1.53^{a} \pm 0.48$	$10.30^{b} \pm 0.76$

Values are expressed as mean ± SD of three replicates. Different superscripted (a, b) values differ significantly (P<0.05) from other extract.

Table 2. Total antioxidant capacity (TAC), antiradical efficiency (AEAC), antiradical activity (ARA) and phenol antioxidant index (PAOXI) of aqueous (AEAS), dichloromethane (DCMEAS), methanolic (MEAS) and ethanolic (EEAS) extracts of *A. Scholaris* Linn.

Extract	TAC (mg ascorbic acid equivalents/g extract)	AEAC (mg ascorbic acid equivalents/g extract)	ARA (% inhibition of the DPPH radicals)	PAOXI (unit contribution of total phenolics to TAC)
AEAS	21.21 [b]	5.75 [b]	33.31 [b]	20.59 [a]
DCMEAS	74.87 [a]	39.98 [a]	75.70 [a]	14.17 [a]
MEAS	73.38 [a]	41.97 [a]	77.51 [a]	9.97 [b]
EEAS	77.15 [a]	43.79 [a]	78.17 [a]	7.09 [b]

Mean in a column with different letters are significantly different (P<0.05).

water. Finally, the absorbance was measured at 546 nm. From the absorbance the % scavenging activity was calculated using the following formula. % nitric oxide radical scavenging activity = [1–(A_{546} nm sample / A_{546} nm control)] x 100.

Statistical analysis

All determinations were made in triplicate for all assays. The results were subjected to analysis of variance (ANOVA) with statistical significance at P<0.05 being tested using the Duncan's Test and Pearson correlation.

RESULTS AND DISCUSSION

Plants are regarded as chemical libraries of structurally diverse phytochemical compounds and constitute a promising approach in drug discovery. Due to diverse chemical nature these compounds have different polarity and solubility in the solvent (Harborne, 1984). To maximize extraction of polyphenolic constituents endowed with high antioxidant/antiradical potential, different solvents were used for the extraction. These extracts were screened for polyphenolic constituents, antioxidant potential, superoxide, nitric oxide and hydroxyl radical scavenging activity. Total phenolics, flavonoid and tannin contents were significantly (P<0.05) higher in EEAS as compared to MEAS and DCMEAS extracts. The AEAS was significantly (P<0.05) low in total phenolics, flavonoid and tannin contents compared to other extracts (Table 1). Total phenolic content was 10 times in EEAS, 7 times in MEAS and 5 times in DCMEAS more than that of the AEAS. Similarly, total flavonoid content was 5 to 8 folds higher in other extracts than the AEAS.

Presence of high flavonoid and phenolic compounds in ethanolic leaf extract of *A. scholaris* has therapeutic importance (Sinnathambi et al., 2010; Shang et al., 2010; Subraya et al., 2012) besides its potential use in the food industry as natural antioxidant from plant origin. The efficiency of antioxidant potential of different extracts depends on its ability to scavenge free radicals either by donating hydrogen atom to the oxidizing free radical or to decrease the energy of the antioxidant radical that prevents the autoxidation of the antioxidant radical into additional free radicals. Therefore, in order to assess the antioxidant potential of different extracts of *A. scholaris* total antioxidant capacity, antiradical efficiency and antiradical activity was determined (Boudjou et al., 2013). The TAC value depends on extent of decolorization of ABTS radical cations by reductants present in different extracts. In the present study, EEAS was observed to have significant *(*P<0.05) higher TAC value as compared to AEAS. However, such activity was not significantly different from MEAS and DCMEAS. A dose dependant increase in the scavenging activity of ABTS radical cations was observed after increasing the concentration of extracts. The median effective concentration (EC_{50}) value of AEAS was significantly (P<0.05) higher than DCMEAS (75.5%), MEAS (72.9%) and EEAS (75.7%) (Table 2). Such difference may be attributed to the solvent used for the extraction of active ingredients from

Table 3. The median effective concentration (EC_{50}) of aqueous (AEAS), dichloromethane (DCMEAS), methanolic (MEAS) and ethanolic (EEAS) extracts of *A. Scholaris* Linn. for scavenging different types of radicals.

Assay	Ascorbic acid (mg/mL)	Leaf extracts of *A. scholaris*			
		AEAS (mg/mL)	DCMEAS (mg/mL)	MEAS (mg/mL)	EEAS (mg/mL)
ABTS radicals	0.085±0.047	2.94±0.45	0.617±0.086	0.681±0.086	0.638±0.054
Free radicals	0.042±0.020	1.82±0.25	0.540±0.070	0.595±0.086	0.595±0.031
Superoxide radicals	0.141±0.015	1.01±0.06	1.275±0.127	0.740±0.086	0.704±0.015
Nitric oxide radicals	0.135±0.036	2.85±0.26	0.975±0.047	0.636±0.015	0.634±0.025
Hydroxyl radicals	0.067±0.015	1.11±0.21	1.107±0.015	1.193±0.127	0.970±0.086
Reducing power [a]	0.091±0.015	6.24±1.06	0.478±0.015	0.503±0.105	1.226±0.185

Values are expressed as mean ± SD of three replicates. [a] Concentration at which optical density (OD) of sample is 0.50 at 700 nm.

A. scholaris.

The antiradical activity is based on the exchange of hydrogen atoms between the antioxidant and the stable DPPH free radical. Intensity of change in color from violet to yellow indicates reduction of DPPH radicals to the corresponding hydrazine (Gulcin et al., 2007). The DPPH scavenging was significantly ($P<0.05$) higher in EEAS, MEAS and DCMEAS extracts as compared to aqueous extracts of *A. scholaris.* EC_{50} value of AEAS, DCMEAS, MEAS and EEAS were: 1.79, 0.57, 0.59 and 0.57 mg/mL, respectively (Figure 2). The lower the EC_{50} value the higher is the antioxidant potential and with increasing the dose, the free radicals scavenging activity get enhanced. The antiradical efficiency is the reductive ability of the extract determined by transformation of the ferric (Fe^{3+}) to ferrous (Fe^{2+}) state in the presence of extract. The presence of reductants such as antioxidant substances in the extract(s) causes the reduction of ferricyanide complex to the ferrous form (Chung et al., 2002). In the present study AEAC value of EEAS was 4.3 and 9.5% higher than the MEAS and DCMEAS, respectively. Total phenolic content of extract was positively correlated with TAC ($r = 0.901$), antiradical activity ($r = 0.948$) and AEAC value ($r = 0.891$). The phenol antioxidant index (PAOXI) is one of the most comprehensive parameters for comparing food antioxidants (Vinson et al., 2001; Boudjou et al., 2013). In the present study the PAOXI value was significantly ($P<0.05$) higher for AEAS and DCMEAS as compared to MEAS and EEAS. Though, the total phenolic content in AEAS was less than the other extracts but significant ($P<0.05$) high value of PAOXI in AEAS indicative of quantitatively better antioxidant phenolics of aqueous medium as compared to other extracts (Table 2).

Similarly, hydroxyl radicals ($OH^{\cdot}$), superoxide ($O_2^{\cdot-}$) and nitric oxide anions ($NO^{\cdot}$) are the most common free radicals generated by auto-oxidation processes or enzymatic reactions in mammalian system. Hydroxyl radical is the most reactive free radical and can also form from the superoxide anion ($O_2^{\cdot-}$) and hydrogen peroxide (H_2O_2) in the presence of metal ions such as copper and iron (Halliwell and Gutterridge, 1990; Moncada et al., 1991). Different extracts of plant exhibit high superoxide, hydroxyl and nitric oxide scavenging activity and their graphical representations are shown in Figure 1, 2 and 3, respectively. The IC_{50} value of different extracts for the scavenging hydroxyl, superoxide and nitric oxide anions radicals were shown in Table 3. Out of different extracts EEAS has high superoxide anion scavenging activity as compared to other extracts. With increase in concentration of extract a dose dependant increase in superoxide radicals scavenging activity was observed (Figure 1). However, AEAS has high hydroxyl radicals scavenging activity as compared to other extracts (Figure 2). The present study reveals that extracts exhibited $NO^{\cdot}$ scavenging activity which leads to the reduction of nitric concentration in the assay medium, a possible protective effect against oxidative damage induced by nitric oxide. The IC_{50} value for nitric oxide scavenging activity was lowest for EEAS indicating that the extract has better scavenger of nitric oxide radicals (Figure 3).

Recently, much attention has been paid on dietary supplementation of phytochemicals having potent radicals scavenging capacity and reducing lipid peroxidation during oxidative damage in mammals (Ghorbel et al., 2011; Shirzad et al., 2011; Yoon et al., 2012). Studies have shown that *A. scholaris* extract supplementation inhibit radiation induced lipid peroxidation and glutathione level (Gupta et al., 2008) by protecting the hematopoietic system against radiation induced alterations in mice (Gupta et al., 2013). Thus, therapeutic potential of different polyphenolic constituents in leaf extract will need to be evaluated in various experimental models.

Conclusion

Ethanolic extract of *A. scholaris* showed maximum extraction of total phenolic and flavonoids contents endowed with high total antioxidant capacity, superoxide and nitric oxide radicals scavenging activity as compared

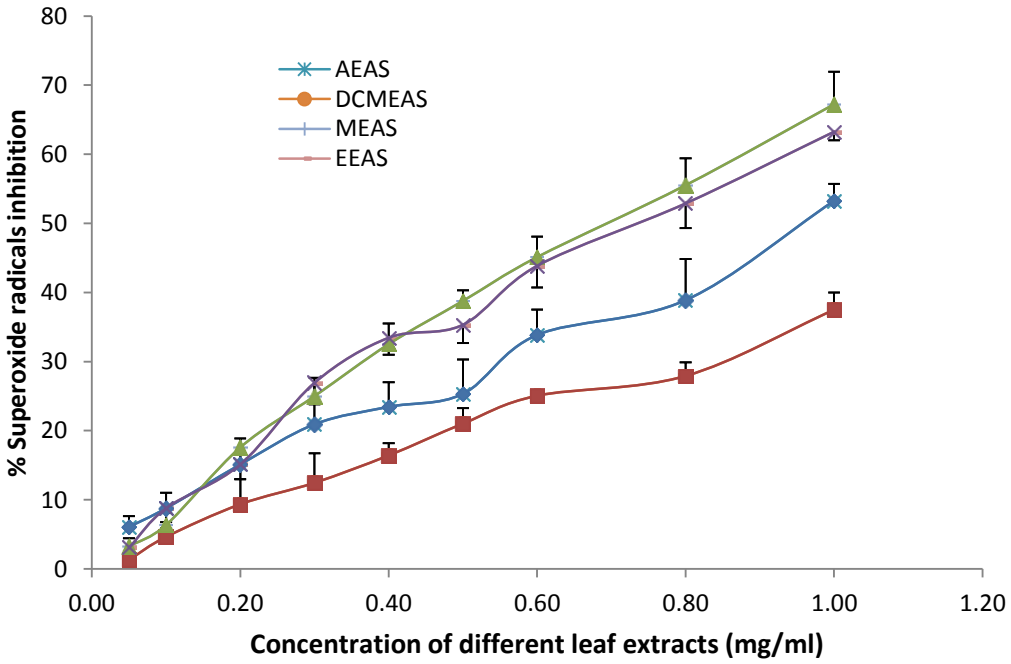

Figure 1. Superoxide radicals scavenging activity (mean value ± SD) of different concentrations of aqueous (AEAS), dichloromethane (DCMEAS), methanolic (MEAS) and ethanolic (EEAS) extracts of *A. scholaris*.

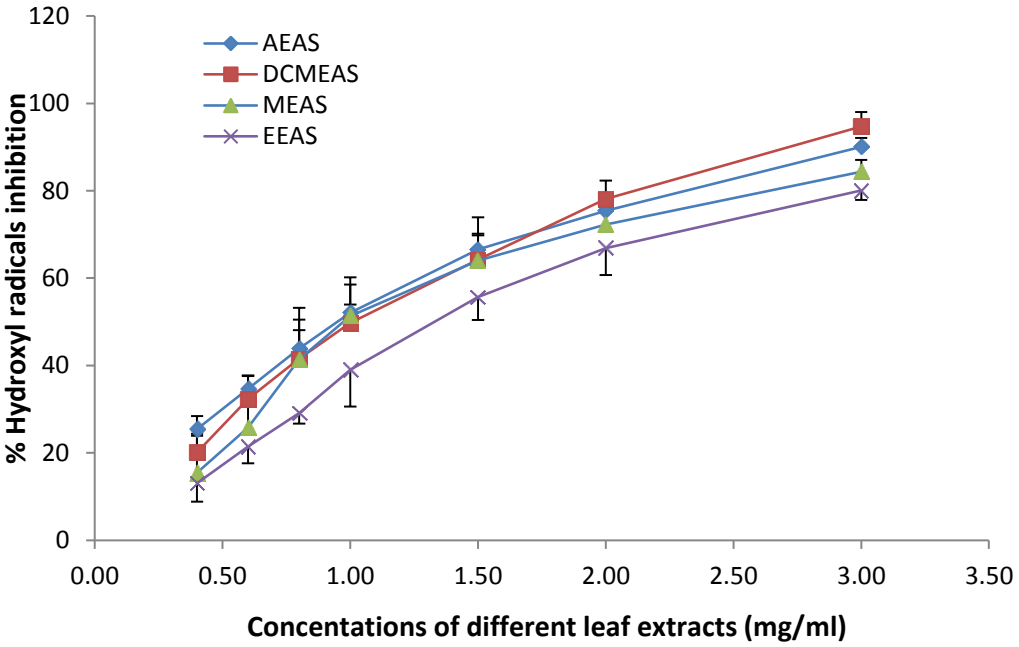

Figure 2. Hydroxyl radicals scavenging activity (mean value ± SD) of the aqueous (AEAS), dichloromethane (DCMEAS), methanolic (MEAS) and ethanolic (EEAS) extracts of *A. Scholaris*.

to other extracts. Thus, dietary supplementation of EEAS may provide protection in preventing the free radicals induced damage associated with aging and other disorders besides improve the food quality by retarding

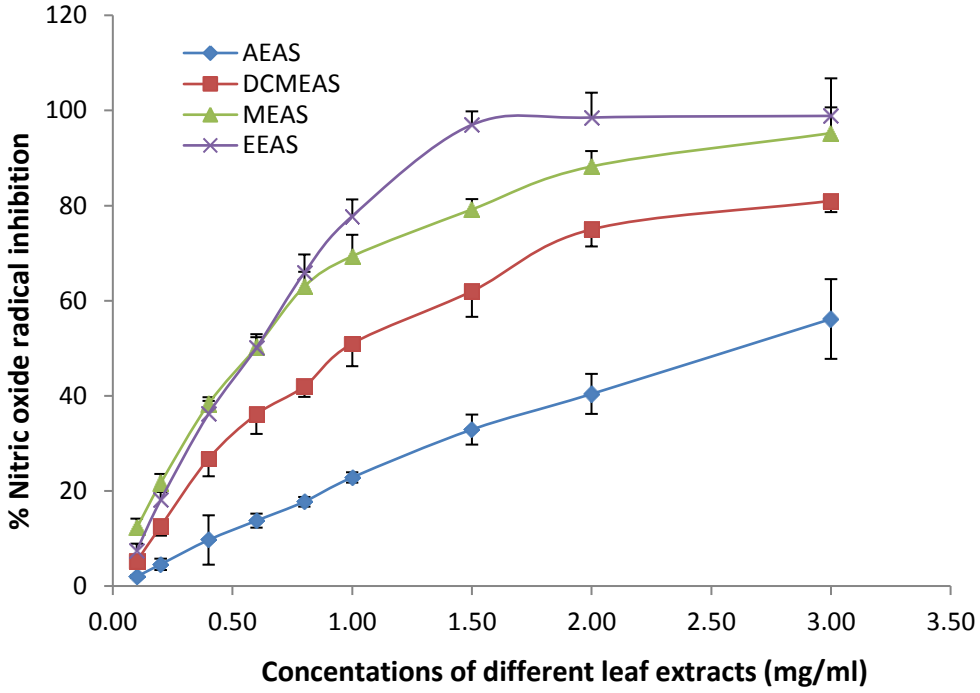

Figure 3. Nitric oxide radicals scavenging activity (mean value ± SD) of aqueous (AEAS), dichloromethane (DCMEAS), methanolic (MEAS) and ethanolic (EEAS) extracts of *A. Scholaris*.

oxidative degeneration of food lipids.

Conflict of interests

The author(s) did not declare any conflict of interest.

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