REPORT

Cotinus coggyria: A rich source of antioxidants

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Abstract: Methanolic extract of *Cotinus coggyria* Scop. was mixed in distilled water and partitioned first with the *n*-hexane, then with chloroform, then ethyl acetate and at the end with *n*-butanol. The phytochemical screening of plant showed presence of the phenolics, cardiac glycosides and flavonoides in large amount in the chloroform, *n*-butanol and ethyl acetate soluble fraction. Antioxidant activity of these four fractions and the left behind aqueous fraction was measured by four methods such as: 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging activity, ferric thiocyanate assay, ferric reducing antioxidant power (FRAP) assay and total antioxidant activity. Total phenolics were also measured. Noteworthy antioxidant potential was shown by the chloroform, *n*-butanol and ethyl acetate soluble fraction showed. Ethyl acetate fraction showed highest % inhibition of the DPPH radical when compared with the other studied fractions i.e. 81.64 ± 1.29% inhibition of the DPPH radical at the concentration of 30 µg/ml. Its IC_{50} value was found to be 15.58 ± 0.09 µg/ml, comparative to the butylated hydroxytoluene (BHT), which has IC_{50} value 12.6 ± 0.85µg/mL. This fraction also showed the highest lipid peroxidation inhibition (61.41 ± 1.16%), as well as highest values of FRAP (697.76 ± 1.98 µg of trolox equivalents) total antioxidant activity (1.02 ± 0.09) and total phenolic contents (229.34 ± 0.57) comparative to the other studied fractions. The chloroform and *n*-butanol soluble fraction also showed good results for all the studied antioxidant assays.

Keywords: *Cotinus coggyria* Scop., DPPH assay, lipid peroxidation inhibition (%), FRAP value, total antioxidant activity, total phenolics.

INTRODUCTION

Consumption of the vegetables and fruits is inversely and strongly related with the mortality rate and/or incidence of numerous forms of cerebro-vascular diseases, cardiovascular, cancer and neurodegenerative diseases (Riboli and Norat, 2003; Liu, 2003; Bazzano *et al*., 2003). As the oxidative stress leads to the development of all the above mensioned disorders, therefore, the presence of high amounts of antioxidants in vegetables and fruits in general has been considered as a very significant basis for the health-protecting special effects linked with their high consumption (Terry *et al*., 2001; Gate *et al*., 1999). Alongwith vegetables and fruits, various herbs having no specific nutritional value, also constitute vital sources of the antioxidants (Ng *et al*., 2000; Warren, 1999). This term "herb" refers to not only the herbaceous plants as well as the roots, leaves, seeds, bark, flowers and fruits of trees and shrubs. Recent studies on many herbs as potent sources of the antioxidants have been stemmed from the requirement to study and screen the dying out floras, and also have been provoked by increasing the supposition that the antioxidants derived from the plants are harmless compounds. Many synthetic antioxidants which are commonly used to add in various foodstuffs i.e. BHT, BHA and TBHQ are relatively cheap as well as efficient but these exhibit various toxic properties and so disadvantageous. Therefore, nowadays so much attention is being paid in search of the natural antioxidants and identification of the natural compounds which can act as appropriate antioxidants to replace the synthetic antioxidants (Khan *et al*., 2010).

Epidemiological as well as *in vitro* studies on the vegetables, fruits and medicinal plants have strongly supported this idea that the plant constituents having antioxidant potential exert the protection effects in the biological systems against the oxidative stress (Souri *et al*., 2008). *Cotinus coggyria* Scop. is very important medicinal plant belonging to family Anacardiaceae. It is a shrubby tree commonly known as "smoke tree" (Hans *et al*., 2000). It grows in the North West Himalayas at the height of 762-1524 m. The infusion of its leaves is very medicinal and is used internally in the folk medicine as an antiinflammatory, antihaemorragic, antiseptic and antimicrobial. It also has wound healing properties as well as it is used against diarrhea (Demirci *et al*., 2003). Phytochemical studies on the methanolic extract of *Cotinus coggyria* revealed that it contains many important compounds such as 3,4,6-trihydro xyaurone (sulfuretin),

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′,4′,7-trihydroxyflavonol (fisetin), 3′,4′,7-trihydroxyflavanol (fustin), 3′,4′,5,7-tetrahydroxyflavanol (taxifolin), 3′,4′,5,7-tetrahydroxyflavonol (quercetin), 4′,7-dihydroxyflavanol, 3′,4′,5,7-tetrahydroxyflavanol (taxifolin), 3′,4′,7-trihydroxyflavanone (butin), trans-2′,3,4,4′-tetrahydroxychalcone (butein), 4′,7-dihydroxyflavanone (liquiritigenin), 4′,5,7-trihydroxyflavanone, trans-2′,4,4′-trihydroxychalcone (isoliquiritigenin), disulfuretinsulfurein, gallic acid, methyl gallate and pentagalloyl glucose (Hans *et al*., 2008; Valianou *et al*., 2008). To the best of our knowledge, no work is done in detail on the *in vitro* antioxidant activities of organic and aqueous fractions of *Cotinus coggyria*, comparative to each other, so, in the present work, we described the *in vitro* antioxidant activities of *n*-hexane soluble fraction, ethyl acetate soluble fraction, chloroform soluble fraction, *n*-butanol soluble fraction and aqueous fraction of *Cotinus coggyria,* comparatively, by four methods i.e., 1,1-Diphenyl-2-picrylhydrazyl radical (DPPH) scavenging, ferric thiocyanate assay, Ferric Reducing Antioxidant Power (FRAP) assay and total antioxidant activity by phosphomolybdenum complex (PC) method, along with the determination of total phenolic contents, relative to the conventionally used standards (BHT).

MATERIAL AND METHODS

Plant material

Collection of the plant *Cotinus coggyria* Scop. was done from the district Kotli of Azad Kashmir in June 2009, and identification was done by Mr. Muhammad Ajaib, Taxonomist at the Department of Botany, GC University, Lahore. A voucher specimen (GC. Herb. Bot. 966) was deposited in Herbarium of the Department of Botany of the same university.

Extraction and fractionation of antioxidants

The shade-dried whole plant was ground (1 kg) and the antioxidants were extracted in the methanol (5L) on soxchlet apparatus. The residue (141 g) was obtained by evaporation of the above extract. It was then mixed with distilled water (1 L) and partitioned first with the *n*-hexane (1L × 5), then with chloroform (1L × 5), then with ethyl acetate (1L × 5) and at the end with *n*-butanol (1L × 5) sequentially. These four organic fractions and the left behind water fraction were separately concentrated on the rotary evaporator (*n*-hexane at 34°C, chloroform at 38°C, ethyl acetate at 46°C, *n*-butanol at 55°C and water at 58°C). The yields of the *n*-hexane fraction, chloroform fraction, ethyl acetate fraction, *n*-butanol fraction and aqueous fraction were 35g, 22g 27g, 25g and 32g respectively. These five fractions were studied to determine their *in vitro* antioxidant activities.

Chemicals and standards

Trolox, DPPH˙ (1,1-Diphenyl-2-picrylhydrazyl radical), TPTZ (2,4,6-Tripyridyl-s-triazine), BHT (Butylated hydroxytoluene), Gallic acid and Follin Ciocalteu reagent were purchased from Sigma Chemicals Company Ltd. (USA). The organic solvents (ethyl acetate, *n*-hexane, *n*-butanol, chloroform), sodium phosphate, ammonium molybdate, sulphuric acid, ferrous chloride, ammonia, ferric chloride, acetic acid, bismuth nitrate, aluminium chloride, potassium iodide and ceric sulphate from Merck (Pvt.) Ltd. (Germany).

Phytochemical screening

The phytochemical screening was performed for all the studied fractions using reported methods (Sofowara, 1993; Trease and Evans, 1989; Ayoola *et al*., 2008), described as fallows.

Test for alkaloides

“For the test of alkaloides the TLC card having spots of the studied samples was sprayed with Draggendorff’s reagent. Appearance of orange colour indicates the presence of alkaloids”.

Test for terpenoides

“Two methods were adopted to test the presence of terpenoides. First, Ceric sulphate solution was sprayed on TLC card having spots of samples. TLC card was heated on TLC heater. Appearance of brown color indicated the presence of terpenoides. Second, to 0.5 g of each of the extract, 2 ml of chloroform was added .3 ml of concentrated H_2SO_4 was carefully added along the wall of test tube, thus a layer was formed. A reddish brown coloration at the interface of two layers indicated the presence of terpenoides”.

Test for saponins

“5 ml of distilled water was added to 0.5g of extract in a test tube. The solution was shaken vigorously and a stable persistent froth was observed. 3 drops of olive oil were mixed with the frothing and shaken vigorously, after which it was noted that an emulsion was formed”.

Test for tannins

“2 ml of the sample was taken in a test tube and 5 ml of *n*-butanol-HCl solution was added. Mixture was warmed for 1 hour at 95°C in a water bath. Appearance of red colour indicated the presence of tannins”.

Test for sugars

“Fehling’s solution (A and B) was added to the sample solutions (0.5 g in 5ml water), in a test tube, and boiled at water bath for half an hour. Formation of red precipitates indicated the presence of sugars”.

Test for phenolics

“Neutral ferric chloride was added to each fraction. Appearance of bluish green colour indicated presence of phenolics”.

Test for flavonoides

"Four methods were adopted to test for the presence of flavonoides. Firstly, 5ml dilute ammonia was added, to a portion of sample solution in water. Then about 1ml of conc. sulphuric acid was added. A yellow colouration that disappears on standing indicated the presence of flavonoides. Secondly, 3 to 4 drops of 1% aluminium chloride solution were added to sample solution. A yellow colouration indicated the presence of flavonoides. Thirdly, the TLC card having spots of samples was sprayed with Benidict's reagent. Green fluorescence in UV light indicated the presence of flavonoides. Fourth, the TLC card having spots of samples was sprayed with lead acetate solution. Green fluorescence in UV light indicated the presence of flavonoides".

Test for cardiac glycosides (Keller-Killiyani test)

"The 0.5g of each sample was diluted up to 5ml in water and 2ml of the glacial acetic acid having one drop of $FeCl_2$ solution was added. It was underlayed by 1 ml of conc. sulphuric acid. Formation of brown ring at the junction of two solutions showed the presence of deoxysugar which is the characteristic of cardenolides. A violet ring appeared below the brown ring, while a greenish ring appeared just above the brown ring, in the acetic acid layer which slowly spread throughout this layer".

Antioxidant assays

Antioxidant assays were performed as described below, on all the studied fractions.

DPPH radical scavenging activity

"The DPPH radical scavenging activities of various fractions of plant were examined by comparison with that of known antioxidant, butylated hydroxytoluene (BHT) using the reported method (Lee and Shibamoto, 2001). Briefly, various concentrations of the samples (1000 μg/mL, 500 μg/mL, 250 μg/mL, 125 μg/mL, 60 μg/mL, 30 μg/mL, 15 μg/mL) were mixed with 3 ml of methanolic solution of DPPH (0.1mM). The mixture was shaken vigorously and allowed to stand at room temperature for one an hour. Then absorbance was measured at 517 nm against methanol as a blank in the UV-visible spectrophotometer (CECIL Instruments CE 7200 Cambridge England). Lower absorbance of spectrophotometer indicated higher free radical scavenging activity.

The percent of DPPH decoloration of the samples was calculated according to the formula:

$$\text{Antiradical activity} = A_{control} - A_{sample}/ A_{control} \times 100$$

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

Total Antioxidant activity by phosphomolybdenum complex method

"The total antioxidant activities of various fractions of plant were evaluated by phosphomolybdenum complex formation method (Prieto *et al*., 1999). Briefly, 500 μg/mL of 1each sample was mixed with 4 mL of reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM 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^{o}C for 90 minutes. 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".

Ferric reducing antioxidant power (FRAP) assay

"The FRAP assay was done according to Benzie and Strain (1996) with some modifications. The stock solutions included 300 mM acetate buffer (3.1 g $CH_3COONa.3H_2O$ and 16 mL CH_3COOH), pH 3.6, 10 mM TPTZ (2,4,6-Tripyridyl-s-triazine) solution in 40 mM Hydrochloric Acid and 20 mM 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 $FeCl_3.6H_2O$ solution and then warmed at 37^{o}C before using. The solutions of plant samples and that of trolox were formed in methanol (250 μg/mL). 10 μL of each of sample solution and BHT solution were taken in separate test tubes and 2990 μL of FRAP solution was added in each to make total volume up to 3 mL. The plant samples were allowed to react with FRAP solution in the dark for 30 minutes. Readings of the coloured product [ferrous tripyridyltriazine complex] were then taken at 593 nm by UV-visible spectrophotometer. The FRAP values were determined as micromoles of trolox equivalents per mL of sample by computing with standard calibration curve constructed for different concentrations of trolox. Results were expressed in TE μM/mL".

Total phenolic contents

"Total phenolics of various fractions of plant were determined reported method (Makkar *et al*., 1993). The 0.1 mL (0.5 mg/mL) of sample was combined with 2.8 mL of 10% Sodium carbonate and 0.1 mL of 2N Folin-Ciocalteu's phenol reagent. After 40 minutes absorbance at 725 nm was measured by UV-visible spectrophotometer. Total phenolic contents were expressed as micrograms of gallic acid equivalents (GAE) per gram of sample using the standard calibration curve constructed for different concentrations of gallic acid. The curve was linear between 50 μg/mL to 500 μg/mL of gallic acid. Results were expressed in GAE μg/mL".

Ferric thiocyanate (FTC) assay

"The antioxidant activities of various fractions of plant on inhibition of linoleic acid peroxidation were assayed by thiocyanate method (Valentao *et al*., 2002). The 0.1 mL of each of sample solution (0.5 mg/ mL) was mixed with 2.5 mL of linoleic acid emulsion (0.02 M, pH 7.0) and 2.0 mL of phosphate buffer (0.02 M, 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 days at 40^{o}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 mM ferrous chloride in 3.5% HCl and allowed to stand at room temperature. Precisely 3 minutes after addition of ferrous chloride to the reaction mixture, absorbance was recorded at 500 nm. The antioxidant activity was expressed as percentage inhibition of peroxidation (IP%) [IP% = {1-(abs. of sample) / (abs. of control)} × 100]. The antioxidant activity of BHT was assayed for comparison as reference standard".

STATISTICAL ANALYSIS

All measurements were performed in the triplicate and the statistical analysis was done by Microsoft excel 2007. Results have been presented as average ± S.E.M.

RESULTS

The phytochemical screening was done on all the studied fractions. Tests were performed for the detection of alkaloids, terpenoids, saponins, tannins, sugars, phenolics, flavonoids and cardiac glycosides and the results have been shown in table 1. DPPH assay was performed on all the five fractions and percent scavenging of the DPPH radical was calculated along with IC_{50} values. The values of percent scavenging of DPPH radical have been shown in table 2 while the IC_{50} values have been given in table 3. The total antioxidant activities, FRAP values (TE μM/mL), total phenolics (GAE μg/g) and % inhibition of lipid peroxidation of all the five studied fractions was measured and results have been shown in table 3.

DISCUSSION

Phytochemical screening

The phytochemical screening tests were performed on the five studied fractions and the results are shown in the table 1. Flavonoides and phenolics were found in more amounts in ethyl acetate fraction than in chloroform fraction and *n*-butanol fraction, while in very less amount in aqueous fraction and *n*-hexane fraction. Cardiac glycosides were found in more amounts in the ethyl acetate, chloroform and *n*-butanol fraction while in less amount in other two fractions. Alkaloids were found in maximum concentration in the ethyl acetate fraction as comparative to other fractions whereas absent in the aqueous fraction. Terpenoides and saponins were found in all fractions but saponins were very less in *n*-hexane and chloroform fraction. Tannins and sugars were found in all the polar fractions whereas absent in *n*-hexane soluble fraction. Sugars were found in more amounts in the remaining aqueous fraction.

DPPH radical scavenging activity

Phenolic compounds have hydrogen donating ability due to which they take part in DPPH radical scavenging. DPPH is a stable free radical which has ability to accept an electron or the hydrogen radical to become the stable diamagnetic molecule (Siddaraju and Dharmesh, 2007). It has been reported that the decrease in the absorbance of the DPPH radical caused by the phenolic compounds is because of the reaction between the antioxidant molecules and the radicals which results in scavenging of the radical by the donation of hydrogen and can be observed as changing the colour from purple to yellowish (Meir *et al*., 1995). The DPPH is preformed stable radical that is often used to measure radical scavenging activity of the antioxidant samples. The scheme is based on the reaction of the DPPH radical which is characterized as stable free

Table 1: Phytochemical constituents of various fractions of *Cotinus coggyria* Scop

Test	*n*-hexane soluble fraction	Chloroform soluble fraction	Ethyl acetate soluble fraction	*n*-Butanol soluble fraction	Remaining Aqueous fraction
Alkaloides	+	++	++++	+++	−
Terpenoides	++	+++	++	+++	++
Saponins	+	+	++	+++	+++
Tannins	−	+	++	+++	+++
Sugars	−	+	++	++	+++
Phenolics	+	++	+++	++	+
Flavonoides	+	++	+++	++	+
Cardiac Glycosides	+	++	++	++	+

Note: '+' represents presence and '–' represents absence.

radical having deep violet colouration. Its reduction is done by any substance which has ability to donate a hydrogen atom to the DPPH, hence it becomes stable diamagnetic molecule. DPPH radical decolourizes in the presence of the antioxidants. Visible deep purple colour of the DPPH is due to presence of an odd on it (Lee and Shibamoto, 2001). Reduction of the DPPH radical was observed by the decrease in the absorbance at 517 nm, while colour faints from purple to yellowish. The various fractions of *Cotinus coggyria* significantly reduced the DPPH radicals. Values of the percent scavenging of the DPPH radical are presented in the table 2. From the results it was observed that the scavenging activity increased sequentially with increase in the concentration of sample in the assay. The ethyl acetate fraction showed highest % inhibition of the DPPH radical comparative i.e. 81.64 ± 1.29% inhibition of the DPPH radical at the concentration of 30 µg/ml. Lowest % scavenging was shown by *n*- hexane fraction comparative to the other fractions. "IC_{50} value can be defined as concentration of the substrate which causes 50% loss of DPPH activity. It was calculated by the linear regression mentioned of the plots of percentage of the antiradical activity against concentration of tested compounds (Nahak and Sahu, 2010)". The IC_{50} values of studied fractions were calculated and the results are presented in the table 3. Lower IC_{50} values represent higher scavenging power of the antioxidants. Lowest IC_{50} value was exhibited by ethyl acetate fraction i.e. 15.58 ± 0.09 as comparative to the other fractions. Values of IC_{50} shown by *n*-hexane fraction, chloroform fraction, ethyl acetate fraction, *n*-butanol fraction and remaining aqueous fraction were 147.29 ± 1.18, 52.30 ± 0.43, 58.32 ± 0.71 and 59.58 ± 0.84 relative to the butylated hydroxytoluene (BHT) which is a standard reference, having IC_{50} of 12.6 ± 0.85 µg/ml.

Total antioxidant activity by the phosphomolybdenum complex method

The total antioxidant activity of studied fractions was determined by the phosphomolybdenum complex formation method. This process is based on the reduction of the molybdenum (VI) to molybdenum (V) by antioxidants, as a result of which green phosphate Mo (V) complex is formed at the acidic pH. Transfer of electron takes place in this assay, depending upon the structure of the antioxidants (Prieto *et al.*, 1999). This method generally detects the antioxidants such as tocopherols, ascorbic acid, carotenoids and various phenolics. The total antioxidant activities of all the fractions were measured and compared with that of standard antioxidant BHT. Results are presented in table 3. From the results it was inferred that highest value of total antioxidant activity was shown by ethyl acetate fraction i.e. 1.02 ± 1.09 as comparative to the other fractions. The total antioxidant activity of the chloroform fraction was observed to be 0.856 ± 0.99 whereas that of the *n*-butanol fraction and the remaining aqueous fraction was 0.766 ± 0.62 and

Table 2: Free radical scavenging activity of the various fractions of *Cotinus coggyria* Scop. using 1,1-Diphenyl-2-picryl hydrazyl radical (DPPH)

Sr. No.	Sample	Concentration in Assay (µg/mL)	%age Scavenging of DPPH radical ± S.E.M[a)]
1	*n*-Hexane soluble fraction	250	70.08 ± 0.53
		125	52.71 ± 0.48
		60	26.13 ± 0.8
2	Chloroform soluble fraction	125	75.18 ± 1.07
		60	55.71 ± 1.19
		30	37.14 ± 0.86
		15	27.43 ± 0.38
3	Ethyl acetate soluble fraction	30	81.64 ± 1.29
		15	57.14 ± 0.96
		8	26.12 ± 0.37
4	*n*-Butanol soluble fraction	125	79.27 ± 1.28
		60	57.96 ± 1.33
		30	21.76 ± 0.22
5	Aqueous fraction	125	70.71 ± 0.08
		60	53.12 ± 1.66
		30	29.27 ± 0.2
6	BHT[b)]	60	92.36 ± 0.17
		30	74.45 ± 0.28
		15	43.17 ± 0.08
		8	24.46 ± 0.35

[a)] Standard error mean of three assays. [b)] Standard reference antioxidant.

Table 3: *IC_{50}*, total antioxidant activity, FRAP values, total phenolics and lipid peroxidation inhibition values of different fractions of *Cotinus coggyria* Scop.

Sr. No.	Sample	DPPH-radical scavenging activity (*IC_{50}*; µg/mL) ± S.E.M [a)]	Total antioxidant activity ± S.E.M [a)]	FRAP value TE (µM/ml) ± S.E.M [a)]	Total phenolics (GAE µg/g of sample) ± S.E.M [a)]	Inhibition of lipid peroxidation (%) ± S.E.M [a)]
1	*n*-Hexane soluble fraction	147.29 ± 1.18	0.514 ± 0.02	136.57± 1.93	39.41 ± 1.77	12.83 ± 0.79
2	Chloroform soluble fraction	52.30 ± 0.43	0.856 ± 0.03	476.88± 1.89	147.24 ± 0.38	53.52 ± 0.59
3	Ethyl acetate soluble fraction	15.58 ± 0.09	1.02 ± 0.05	697.76± 1.98	229.34 ± 0.57	61.41 ± 1.16
4	*n*-Butanol soluble fraction	58.32 ± 0.71	0.766 ± 0.04	591.39± 1.47	174.83 ± 1.86	49.16 ± 0.73
5	Aqueous fraction	59.58 ± 0.84	0.629 ± 0.03	213.59± 0.29	98.62 ± 0.91	21.3 ± 0.86
6	BHT [b)]	12.6 ± 0.85	1.118 ± 0.05	-	-	62.96 ± 0.99

[a)] Standard error mean of three assays. [b)] Standard reference antioxidant

0.629 ± 0.44. The lowest antioxidant activity was shown by *n*-hexane fraction (0.514 ± 0.71). Results were compared with the BHT, a standard reference, which showed total antioxidant activity 1.118 ± 0.84.

Ferric reducing antioxidant power (FRAP) assay

This assay measures the reduction potential of the antioxidants against the oxidative effects of the reactive oxygen species. The antioxidants which can donate electron are called reductants and the inactivation of the oxidants by the reductants is considered as the redox reactions. In this assay the antioxidants reduce the Fe^{3+} to Fe^{2+}, in the presence of the tripyridyltriazine (TPTZ) as a result of which an intense blue coloured Fe^{2+}–TPTZ complex is formed having the absorption maximum at the 593 nm (Benzie and Strain, 1996). The increase in absorbance indicates the increase in reductive ability. The FRAP values of all the five fractions were measured and the results are presented in table 3. Highest FRAP value was shown by ethyl acetate fraction i.e. 697.76 ± 1.98 TE µM/mL. Chloroform and *n*-butanol fraction also showed good FRAP values i.e. 476.88 ± 1.89 TE µM/mL and 591.39 ± 1.47 TE µM/mL respectively. The FRAP value for aqueous fraction was found to be 213.59 ± 0.29 whereas that of *n*-hexane fraction was found to be lowest (136.57 ± 1.93). The value of blank was observed to be 75. 19 ± 1.64. The higher FRAP values observed for the polar fractions may be attributed partly to the presence of the flavonoid and phenolic compounds.

Total phenolic contents

Flavonoides and Phenolic compounds are especially significant secondary metabolites of plants. Such compounds perform several defense functions in the plants. There are several environmental factors which contribute to their synthesis, such as light, humidity, temperature and various internal factors including nutrients, genetic differences, hormones, etc. (Strack, 1997). Likewise, other factors such as germination of seeds, the degree of ripening, processing, variety and the storage also influence the plant phenolics content (Bravo, 1998). It has been reported that phenolic contents are responsible for the variation in the antioxidant potential of plant (Cai *et al*., 2004). Phenolic compounds exhibit antioxidant potential by inactivating the lipid free radicals and also prevent the decomposition of the hydroperoxides into the free radicals (Pitchaon *et al*., 2007; Pokorney, 2001) or the metal chelate ions and thus save from harmful pathogens and also predators (Balasundram *et al*., 2006). Most frequently encountered flavonoides are the flavonols, flavanols, quercetin and anthocyanins. Table 3 presents the phenolic concentration in the five studied fractions, expressed as the µg of the gallic acid equivalents (GAEs) per g of the fraction. Highest number of the total phenolics were shown by the ethyl acetate soluble fraction showed (229.34 ± 0.57 GAE µg/g) comparative to other five fractions. The total phenolic contents of chloroform soluble fraction, *n*-butanol soluble fraction and aqueous fraction were found to be 147.24 ± 0.38, 174.83 ± 1.86 and 98.62 ± 0.91 GAE µg/g respectively. Lowest number of the total phenolics were shown by the *n*-hexane soluble fraction i.e., 39.41 ± 1.77 GAE µg/g. The value of blank was found to be 25. 69 ± 1.08.

Ferric thiocyanate (FTC) assay

Peroxidation of the lipids occurs both *in vitro* and *in vivo* and gives rise to the cytotoxic and reactive products which disturb the regular functioning of the cell, and as a result can give rise to the damaged or the modified DNA. Oxygen reacts with the unsaturated double bonds on the lipids and as a result generates free radicals and also lipid hydroperoxides. The antioxidants having hydrogen donating ability can react with the lipid peroxyl radicals and thus break the cycle of the generation of new radicals. This assay measures the quantity of peroxide, at the start of the lipid peroxidation. The peroxides form ferric ions by reacting with the ferrous chloride. Ferric ions then combine with the ammonium thiocyanate and as a result a reddish pigment, ferric thiocyanate is produced (Aqil *et al*., 2006). The studied fractions of *C. coggyria* were tested by this method and the results are presented in table 3. *n*-butanol soluble fraction, ethyl acetate soluble fraction and chloroform soluble fraction showed significantly lower absorbances as comparative to the control which showed that these fractions exhibit significant antioxidant potentials. These fractions showing good values for the percent inhibition of the lipid peroxidation, might contain the primary antioxidant compounds that violently react with the free radicals specially the hydroxyl radicals and as a result the radical chain reaction is terminated and thus the formation of the hydroperoxides retards significantly (Ismail *et al*., 2010). The highest percentage of the inhibition of lipid peroxidation was exhibited by ethyl acetate fraction i.e 61.41 ± 1.16% while *n*-hexane soluble fraction showed lowest value (12.83 ± 0.79%). *n*-butanol, chloroform and aqueous fraction showed percent inhibition of the lipid peroxidation as 53.52± 0.59%, 49.16 ± 0.73% and 21.3 ± 0.86% respectively. Inhibition of the lipid peroxidation by the BHT, a standard reference, was 62.96% ± 0.99.

CONCLUSION

The results showed that the *n*-butanol soluble fraction, ethyl acetate soluble fraction and chloroform soluble fraction showed good *in vitro* antioxidant activity because of the presence of considerable amounts of phenolics, cardiac glycosides and flavonoides while remaining aqueous fraction and *n*-hexane fraction showed no activity due to absence of such compounds. Ethyl acetate fraction showed highest percent inhibition of the DPPH radical comparative to the other fractions i.e., 81.64 ± 1.29% inhibition of the DPPH radical at the concentration of 30 µg/ml. Its IC_{50} value was found to be 15.58 ± 0.09 µg/ml, relative to the BHT, which showed the IC_{50} value 12.6 ± 0.85 µg/mL. This fraction also showed the highest lipid peroxidation inhibition (61.41 ± 1.16%), as well as highest values of FRAP (697.76 ± 1.98 µg of trolox equivalents) total antioxidant activity (1.02 ± 0.09) and total phenolic contents (229.34 ± 0.57) comparative to the other studied fractions. So it was concluded that the *n*-butanol fraction, ethyl acetate fraction and chloroform fraction are rich with the strong antioxidants. So, these fractions may be the potentially valuable sources of the natural antioxidants as well as bioactive materials, which are expected to increase the shelf life of the foods and reinforce against the peroxidative damage in the living systems, related to carcinogenesis and aging.

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