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ORIGINAL PAPER

Effect of different solvents on volatile and non-volatile constituents of red bell pepper (*Capsicum annuum* L.) and their in vitro antioxidant activity

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Abstract The fresh fruit of *Capsicum annuum* L. (red bell pepper) was successively extracted using n-hexane, chloroform, ethyl acetate, ethanol and their percentage yield was calculated. The effectiveness of each extract on chemical composition and antioxidant activity was studied. The qualitative phytochemical evaluation of each extract of bell pepper was done by HPTLC and Gas chromatography–mass spectroscopy (GC-MS) analysis. The total content of phenols, flavonoids and carotenoids were estimated by standard chemical methods. Further, the antioxidant potential of each extract was measured via DPPH and reducing power assays. Gas chromatography–mass spectroscopy analysis showed that the majority of compounds were related to phenols and flavonoids. Further analysis of the extract by HPTLC verified the presence of different types of phenolic compounds in addition to flavonoids and carotenoids. Among the different solvent extracts analyzed, total phenolic content was higher in ethanol extract ($7.136 \pm 0.03\%$, w/w) whereas ethyl acetate extract showed the presence of higher flavonoid content ($4.0521 \pm 0.03\%$, w/w). The ethanol and ethyl acetate extracts of the fruit of *C. annuum* exhibited the highest radical scavenging activity with inhibition percentage of 53.66 and 49.55% at a concentration of 254 µg/ml. Based on the biochemical analysis and phytochemical screening, we conclude that *C. annuum* possess potent antioxidant potential and this ability of the extract is attributed to the presence of rich polyphenolic compounds.

Keywords Antioxidant activity · Bell pepper · GC-MS analysis · HPTLC

Introduction

Natural antioxidants in food and other biological materials have attracted considerable interest because of their safety and potential nutritional and therapeutic value. The increased interest in natural antioxidants has led to the antioxidant evaluation of many species of fruits, vegetables, herbs, spices and cereals [1, 2]. Antioxidant capacity of foods depends on the synergistic action of various antioxidant compounds .It is necessary to combine more than one method in order to determine the in vitro antioxidant capacity of food stuffs [3]. It is also important to note that even within a method small difference in solvent polarity may afford different responses. Therefore, extraction is an important step in the investigation of bio-active compounds of food stuffs. Different solvents have been used in earlier studies to extract antioxidant compounds from plant materials. Biological activities of plant extracts showed significant differences depending upon the different extraction methods, emphasizing the importance of selecting the suitable extraction method for the given sample [4]. Successive extraction is the most convenient, exhaustive and time saving extraction technique and would be helpful in the isolation and characterization of pharmacologically active principles of plant materials [5].

Fruits and vegetables normally contain phytochemicals that possess potent free radical scavenging ability of molecules. The biologically active compounds such as

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polyphenols, carotenoids (α-carotene, β-carotene, lycopene and lutein) and vitamins A, B, C and E contain in fruits and vegetables were reported to exhibit protective effects against cellular oxidation [6]. Intake of these bioactive compounds via food in adequate amount is an important health protecting factor, and being beneficial for the prevention of widespread human diseases including, cancer and cardio-vascular diseases [7].

Bell pepper belongs to the genus *Capsicum* of Solanaceae family and the genus includes 25 species. The bell pepper cultivated in temperate and tropical areas belongs to the species *Capsicum annuum* and was originated from Mexico and Central America [8]. This crop is widely consumed as fresh vegetables or condiment and also used for pharmaceutical and cosmetic purposes [9]. Fresh red bell pepper is rich in vitamin C and vitamin A. In addition, it contains antioxidant compounds such as β-carotene, lutein, zea-xanthin and cryptoxanthin [10]. The noticeable levels of phenolic compounds attribute to the antioxidant properties of red bell pepper. In addition to other nutrients, the red bell pepper has lycopene, which is antioxidant, known for preventing certain types of cancer such as breast and prostate cancer [11–13]. Natural polyphenolic compounds are water soluble due to the hydrophilicity arising from their hydroxyl groups. This may compromise their effective application as antioxidant in lipophilic systems such as fats, oils, lipid based food on cosmetic formulas and emulsion. As a sequence, the development of compounds with better antioxidants capacity and less toxicity is desirable in health, in food, in pharmaceutical and cosmetic field to improve shelf life of respective consumer products.

Lycopene present in bell pepper, a naturally available colouring agent is used to colour various pharmaceutical products such as tablets, capsules, syrups and also is an important ingredient of anti-oxidants. Lycopene is the best organic alternative to give red colour for food products such as beverages, dietary supplements, dairy products and also in the cosmetic industry for the preparation of creams and lotions.

The aim of the present research work is to evaluate the effect of extracting solvent on the bioactive compounds of *C. annuum* (Red Bell Pepper) and also to analyze their antioxidant activity of using DPPH and reducing power assay.

Materials and methods

Chemicals

DPPH (2,2-diphenyl-1-picrylhydrazyl) was purchased from Sigma–Aldrich Chemicals, India. All other reagents are of AR grade obtained from Merck, India.

Collection of plant material

Fresh fruit of bell pepper was purchased from local market, Trichy, Tamilnadu, India. The bright red colour fruit of bell pepper were collected with uniform shape, size and weight. The samples were authenticated by Dr. N. Ravichandran, Botanist, Department of CARISM, SASTRA University, Thanjavur, India. The loss of weight of samples was determined to check the initial raw material quality.

Extraction procedure

Fresh bell pepper was cut into half and the seeds were removed. About 150 g bell pepper was taken in a conical flask and successively extracted after maceration at room temperature with organic solvents such as *n*-hexane, chloroform, ethyl acetate and ethanol and kept aside for 72 h. The extraction was repeated until complete extraction was taken place and the solids were filtered using whatman no.1 filter paper. The filtrate was concentrated using rotary vacuum evaporator (Make: Buchi, Switzerland, Model: R-300) at 60 °C. Finally the dried crude extracts were weighed and the percentage yield of each extract was calculated on dried basis, using the formula.

$$\text{Extract yield}\,(\%) = \text{weight of dried residue in g}/\text{weight of sample in g} \times 100$$

Chromatographic analysis of extracts

Gas chromatography–mass spectroscopy (GC–MS) analysis

Different crude extracts from fruits of *C. annuum* L. was performed using a gas chromatograph (Make: PerkinElmer, Model: Clarus 500, USA) equipped with Turbomass Gold Quadrapole Mass Spectrometer fitted with Elite-5 capillary non-polar column (30 m × 0.25 mm internal diameter, film thickness 0.25 µm). An electron ionization system with ionization energy of 70ev was used for the detection of chemical constituents. Helium gas with purity 99.999% was used as a carrier gas at constant flow rate of 1 ml/min. The injector temperature was set at 280 °C and the oven temperature was set initially at 60–150 °C at 8 °C/min and finally raised to 280 °C at 7 °C/min with a holding time of 5 min. The diluted samples (1 µl) were injected in the split mode with the ratio of 1:10. The mass spectrum was scanned from 40 to 450 amu with scan time interval of 0.2 s. The resulted mass spectrum was matched with inbuilt NIST library database version 2.0. The percentage of the

chemical constituents in the crude extract of *C. annuum* was expressed as % by peak area normalization.

High performance thin layer chromatography (HPTLC) analysis

HPTLC was performed on 10 cm × 10 cm aluminium sheets coated with silica gel $60F_{254}$ (E-Merck, Germany). Sample solutions were applied to the plates as bands of 7 mm wide, 15 mm apart and 15 mm from the bottom of the plate by means of Camag Linomat5 (Muttenz, Switzerland) sample applicator equipped with a 100 µl Hamilton syringe. The mobile phase used for the development of phenols was chloroform: ethyl acetate: formic acid (5:4:1, v/v/v), for flavonoids, toluene: ethyl acetate: formic acid (5:4:1, v/v/v) and for carotenoids, benzene: isopropanol (9:1, v/v/v) [14] in a Camag twin-trough chamber previously saturated with mobile phase vapour for 30 min. After development, the plates were dried in a hot air oven at 105 °C. The developed plates were scanned at 254 nm using Camag TLC scanner3 with winCATS software version 1.3.4. The images were captured using Camag Reprostar3 under UV 254 and 366 nm.

Determination of total phenolic content

Folin–Ciocalteu reagent was used to determine the total phenolic content of the different extracts [15]. In brief, 1 ml of extracts (1 mg/ml) and standard solution of gallic acid (0.5–2.5 ml) was added to a 25 ml volumetric flask containing 10 ml of distilled water. 1.5 ml of Folin–Ciocalteu reagent was added to the mixture and shaken well. After 5 min, 4 ml of 20% sodium carbonate solution was added and make up the volume to 25 ml with distilled water. After 30 min of incubation at room temperature, the absorbance was taken at 765 nm using UV–Visible spectrometer (Make: PerkinElmer, Model: Lambda25, USA).

Determination of total flavonoid content

The total flavonoid content of the extracts was determined by aluminum chloride method according to Chang et al. [16]. In brief, 1 ml of extract (10 mg/ml) or a standard solution of quercetin was mixed with 3 ml of distilled water and 0.3 ml of 5% $NaNO_2$ solution; after 5 min of incubation 0.3 ml of 10% $AlCl_3$ solution was added and the mixture was allowed to stand for 6 min. Finally, 2 ml of 1 mM NaOH solution was added to the mixture, the final volume was made to 10 ml with distilled water. After 15 min of incubation at room temperature, the absorbance was measured at 510 nm using UV–Visible spectrometer.

Determination of lycopene

Lycopene content of the extract was determined using UV–VIS spectrometer at 472 nm [17]. Different extracts of bell pepper were dissolved in 5 ml of tetra hydro furan and 40 ml of absolute ethanol was mixed and shaken well until obtained a homogenous solution. Diethyl ether (100 ml) was added and shaken well, allowed to separate and ether layer was collected and evaporated to dryness. The dried residue was dissolved in 25 ml of isopropanol and the absorbance was measured using isopropyl alcohol as blank and the content of lycopene was calculated using E 1%, 1 cm as 3450.

Evaluation of radical scavenging activity by DPPH method

Antioxidant activity of the extracts was evaluated by using DPPH assay [18]. Aliquots (0.1 ml) of various concentrations of the different extracts were added to 3.9 ml of DPPH (0.025 g/l in methanol). After incubation at room temperature for 30 min, the absorbance was measured at 515 nm UV–visible spectrophotometer. Gallic acid was used as a standard. The DPPH scavenging activity was calculated using the formula,

$$\% \text{ Inhibition } = \left(A_0 - A_1/A_0\right) \times 100$$

where A_0 is the absorbance of the control and A_1 is the absorbance of the sample.

Antioxidant activity by reducing assay

The reducing power assay was used to determine the antioxidant activity of extracts according to the method of Oyazu [19]. The reducing assay of a compound may serve as a significant indicator for its antioxidant potential. According to this method, the aliquots of various concentrations of extract and standard ascorbic acid (50–1000 µg/ml) in 1.0 ml of distilled water were mixed with 2.5 ml of phosphate buffer (pH 6.6) and 2.5 ml of 1% potassium ferricyanide. The mixture was incubated at 50 °C in a water bath for 20 min. After cooling, 2.5 ml of 10% trichloroacetic acid was added to the mixture, which was then centrifuged at 3000 rpm for 10 min. Supernatant solution (2.5 ml) was pipette out and mixed with 2.5 ml distilled water and freshly prepared 0.5 ml of 0.1% ferric chloride solution. The absorbance was measured at 700 nm using a UV–Visible spectrometer.

Statistical analysis

The results are expressed as mean ± SD. Student's *t* test and one-way ANOVA were used to analyze the level of statistical significant variation among extracts. $P < 0.05$ was considered as statistically significant.

Results and discussion

Percentage yield of various extracts of bell pepper

In the present study, *n*-hexane, chloroform, ethyl acetate and ethanol were used for the successive extraction of phytochemicals present in the red bell pepper and the yield of each extract was calculated on dried basis, which was found to be 0.25, 0.13, 0.21 and 2.87% w/w respectively. The extraction yield depends on time, solvent used and temperature conditions as well as chemical nature of the sample [20, 21]. From the results of the study, the successive extraction reveals the solubility of definite components in particular solvent, which improve the isolation of bioactive metabolites. *C. annuum* showed better extraction yield in ethanol (2.87%, w/w) than other solvents which are in agreement with Bushra [22], Milan and Stankovic [23] and Bonoli [24]. The effectiveness of ethanol as an extraction solvent could be due to it being a polar solvent has the ability to extract more polar substances such as phenolic and flavonoid compounds.

Chemical composition of extracts by GC-MS and HPTLC analysis

The chemical composition of various extracts of red bell pepper by GC-MS analysis was shown in Table 1 and the chromatogram obtained was shown in Fig. 1. The major constituents of n-hexane extract were found to be (Z) 6, (Z) 9-pentadecadien-1-ol, *n*-hexadecanoic acid, 3-hexen-2-one and tetradecanoic acid. Chloroform extract shows 9, 17-octadecadienal, (Z)-, *n*-hexadecanoic acid and tetradecanoic acid. Ethyl acetate extract shows *n*-hexadecanoic acid, 3-eicosene, (E)-, hydroquinone, 9, 12, 15-octadecatrienoic acid, (Z, Z, Z)-, E-15-heptadecenal and as major constituents. Ethanol extract shows *n*-Hexadecanoic acid, Hydroquinone, 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-, 7,11-hexadecadienal, 2H-Pyran-2-one, tetrahydro-5,6-dimethyl-, trans- and 7,11-hexadecadienal as major compounds. Bioactive constituents such as *n*-hexadecanoic acid and tetradecanoic acid were found in all extracts. Benzophenone was commonly present in chloroform, ethyl acetate and ethanol extracts. Previous studies have shown that red bell pepper containing higher levels of phenolic and carotenoid compounds than other varieties of bell pepper [25]. According to Pieternel Luning [26], GC-MS analysis of *C. annuum* showed the volatile compounds like hexanal, 6-methyl-5-hepten-2-one, β-ionone, 2,3-butadione, 1-penten-3-one, 3-carene, octanal, (e)-2-hexenal and (e)-2-hexenol were rich in red variety bell pepper. In our study, most of the identified compounds were hydrocarbons, fatty acids and some constituents such as hydroquinone, fragmentation of flavonoid like 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-were also detected. Most of these identified compounds have been already been reported as pharmacologically active by Kankeaw [27].

In HPTLC chromatogram, the different Rf values identified from hexane, chloroform, ethyl acetate and ethanol extracts denoted the presence of different kind of phenol (Fig. 2), flavonoid and carotenoid compounds (Table 2; Figs. 3, 4, 5). Band at R_f 0.56 corresponding to quercetin was visible in chloroform and ethyl acetate extracts which was shown in Fig. 2b, e. Previous reports suggested large quantities of neutral phenolic and flavonoids like quercetin and luteolin in bell pepper [28] was in agreement with the present study. Chloroform extract shows multiple peaks than other extracts which indicated the presence of different types of carotenoids (Fig. 5). Band at R_f 0.53 corresponds to lycopene was present in chloroform extract (Table 2).

Total phenols, flavonoids and lycopene content

The antioxidant compounds such as phenols, flavonoids and carotenoids in various extracts of red variety bell pepper were estimated by different chemical methods. The total phenolic content was found to be higher in ethanol extract (7.14 ± 0.03%, w/w) than hexane (0.01%, w/w), chloroform (0.13%, w/w) and ethyl acetate (3.39 ± 0.02%, w/w). Maximum amount of total flavonoids (4.05 ± 0.03%, w/w) was found in ethyl acetate extract of red bell pepper than chloroform (0.89 ± 0.01%, w/w) and ethanol extract (3.26 ± 0.02%, w/w). The carotenoid related compound lycopene was found to be 0.016% in hexane, 0.014% in chloroform, 0.011% in ethyl acetate and 0.0047% in ethanol extract. The lycopene was found in hexane due to its lipophilic nature. Phenolic compounds are often extract with high amounts in more polar solvents [29] and our results also proven that ethanol extract has highest phenolic content. In previous studies, the best preparation of flavonoids

Table 1 Chemical composition of various extracts by GC-MS analysis

S.No	Extract	Compound name	Retention time (min)	Molecular formula	Molecular weight	Area (%)
1	Hexane extract	3-hexen-2-one	5.90	$C_6H_{10}O$	98	6.20
2		2,4-heptadienal, (E,E)-	6.26	$C_7H_{10}O$	110	0.23
3		1,4-pentadien-3-ol	6.41	C_5H_8O	84	0.52
4		Ethanone, 1-(3-methylphenyl)-	10.72	$C_9H_{10}O$	134	0.12
5		2,4-decadienal	13.04	$C_{10}H_{16}O$	152	0.73
6		2,4-decadienal, (E,E)-	13.59	$C_{10}H_{16}O$	152	0.93
7		Dodecanoic acid	20.56	$C_{12}H_{24}O_2$	200	0.72
8		3-hydroxy-7,8-dihydro-á-ionol	23.93	$C_{13}H_{20}O_2$	208	0.19
9		Tetradecanoic acid	26.15	$C_{14}H_{28}O_2$	228	3.03
10		2(4H)-benzofuranone, 5,6,7,7a- tetrahydro-4,4,7a-trimethyl-	26.62	$C_{11}H_{16}O_2$	180	0.49
11		(E,E)-7,11,15-trimethyl-3-methylene- hexadeca-1,6,10,14-tetraene	29.23	$C_{20}H_{32}$	272	0.15
12		*n*-hexadecanoic acid	31.61	$C_{16}H_{32}O_2$	256	25.03
13		(Z)6,(Z)9-pentadecadien-1-ol	36.09	$C_{15}H_{28}O$	224	60.14
14		Hexanoic acid, 3,5-dimethylcyclohexyl ester	38.49	$C_{14}H_{26}O_2$	226	1.39
15	Chloroform extract	2,5-octadiene	6.29	C_8H_{14}	110	0.10
16		2,4-decadienal	13.09	$C_{10}H_{16}O$	152	0.24
17		2,4-decadienal, (E,E)-	13.62	$C_{10}H_{16}O$	152	0.34
18		à,á-D-glucopyranoside, 4-*O*-hexyl-	20.53	$C_{12}H_{24}O_6$	264	0.63
19		1,2-dioxolan-3-one, 5-ethyl-5-methyl-4-methylene-	21.51	$C_7H_{10}O_3$	142	0.38
20		Benzophenone	22.08	$C_{13}H_{10}O$	182	0.26
21		Hexadecanal	23.96	$C_{16}H_{32}O$	240	0.85
22		Tetradecanoic acid	26.08	$C_{14}H_{28}O_2$	228	2.67
23		Cyclohexane, 2,4-diisopropyl-1,1-dimethyl-	26.36	$C_{14}H_{28}$	196	2.53
24		2,6,10-dodecatrien-1-ol, 3,7,11-trimethyl-, (E,E)-	29.25	$C_{15}H_{26}O$	222	0.22
25		*n*-hexadecanoic acid	31.39	$C_{16}H_{32}O_2$	256	24.34
26		9,17-octadecadienal, (Z)-	35.81	$C_{18}H_{32}O$	264	67.41
27	Ethyl acetate extract	Hydroquinone	13.56	$C_6H_6O_2$	110	4.43
28		Phenol, 2,4-bis(1,1-dimethylethyl)-	18.15	$C_{14}H_{22}O$	206	1.68
29		*n*-decanoic acid	19.63	$C_{10}H_{20}O_2$	172	0.87
30		3-hexadecene, (Z)-	19.89	$C_{16}H_{32}$	224	2.63
31		Benzophenone	21.40	$C_{13}H_{10}O$	182	0.57
32		Hexadecanal	23.39	$C_{16}H_{32}O$	240	0.93
33		Tetradecanoic acid	25.07	$C_{14}H_{28}O_2$	228	2.68
34		E-15-heptadecenal	25.39	$C_{17}H_{32}O$	252	15.32
35		Eicosane	25.54	$C_{20}H_{42}$	282	1.19
36		*n*-hexadecanoic acid	30.32	$C_{16}H_{32}O_2$	256	26.63
37		3-eicosene, (E)-	30.46	$C_{20}H_{40}$	280	21.82
38		9,12,15-octadecatrienoic acid, (Z,Z,Z)-	34.55	$C_{18}H_{30}O_2$	278	11.24
39	Ethanol extract	2,4-dihydroxy-2,5-diemthyl2(2H)-furan-3-	5.74	$C_6H_8O_4$	144	3.64
40		2H-Pyran-2-one, tetrahydro-5,6-dimethyl-, trans-	6.77	$C_7H_{12}O_2$	128	9.22
41		Benzene acetaldehyde	7.13	C_8H_8O	120	0.90
42		4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	9.58	$C_6H_8O_4$	144	9.63
43		2-furancarboxaldehyde, 5-(hydroxymethyl)-	11.71	$C_6H_6O_3$	126	1.66
44		Hydroquinone	13.58	$C_6H_6O_2$	110	10.98
45		(S)-3,4-dimethylpentanol	14.68	$C_7H_{16}O$	116	1.50
46		Phenol, 2,4-bis(1,1-dimethylethyl)-	18.14	$C_{14}H_{22}O$	206	4.43

Table 1 (continued)

S.No	Extract	Compound name	Retention time (min)	Molecular formula	Molecular weight	Area (%)
47		3-tetradecene, (Z)-	19.89	$C_{14}H_{28}$	196	5.58
48		Benzophenone	21.41	$C_{13}H_{10}O$	182	4.32
49		Hexadecanal	23.39	$C_{16}H_{32}O$	240	0.59
50		Tetradecanoic acid	25.02	$C_{14}H_{28}O_2$	228	1.96
51		3-hexadecene, (Z)-	25.37	$C_{16}H_{32}$	224	8.28
52		*n*-hexadecanoic acid	30.19	$C_{16}H_{32}O_2$	256	17.98
53		7,11-hexadecadienal	34.53	$C_{16}H_{28}O$	236	9.30

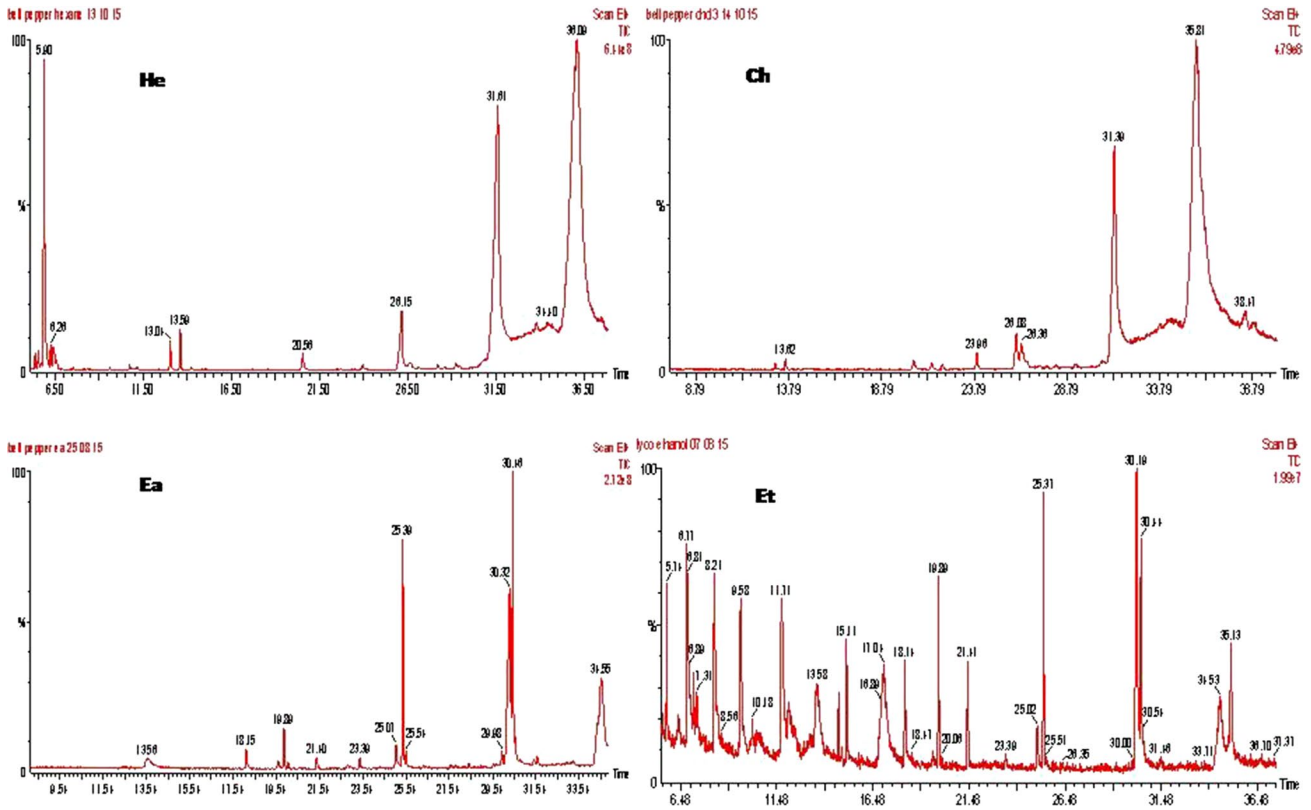

Fig. 1 GC-MS chromatogram of different extracts of bell pepper. *He* Hexane, *Ch* Chloroform, *Ea* Ethyl acetate and *Et* Ethanol extract

Table 2 HPTLC profile of various extracts of bell pepper

Extract	Carotenoids		Phenols		Flavonoids	
	No of spots	R_f value	No of spots	R_f value	No of spots	R_f value
Hexane	9	0.24, 0.62, 0.65, 0.69, 0.73, 0.79, 0.83, 0.94, 0.98	4	0.41, 0.72, 0.92, 0.95	–	–
Chloroform	12	0.14, 0.24, 0.36, 0.40, 0.49, 0.53, 0.58, 0.60, 0.64, 0.72, 0.83, 0.96	12	0.06, 0.14, 0.22, 0.29, 0.38, 0.43, 0.50, 0.71, 0.76, 0.84, 0.93	11	0.01, 0.09, 0.22, 0.30, 0.42, 0.46, 0.53, 0.56, 0.70, 0.79, 0.86
Ethyl acetate	9	0.24, 0.36, 0.58, 0.60, 0.65, 0.73, 0.80, 0.95	8	0.07, 0.12, 0.24, 0.46, 0.61, 0.77, 0.85, 0.95	7	0.03, 0.13, 0.21, 0.30, 0.56, 0.69, 0.76
Ethanol	9	0.04, 0.14, 0.24, 0.37, 0.60, 0.66, 0.74, 0.85, 0.95	3	0.21, 0.79, 0.91	3	0.11, 0.21, 0.63

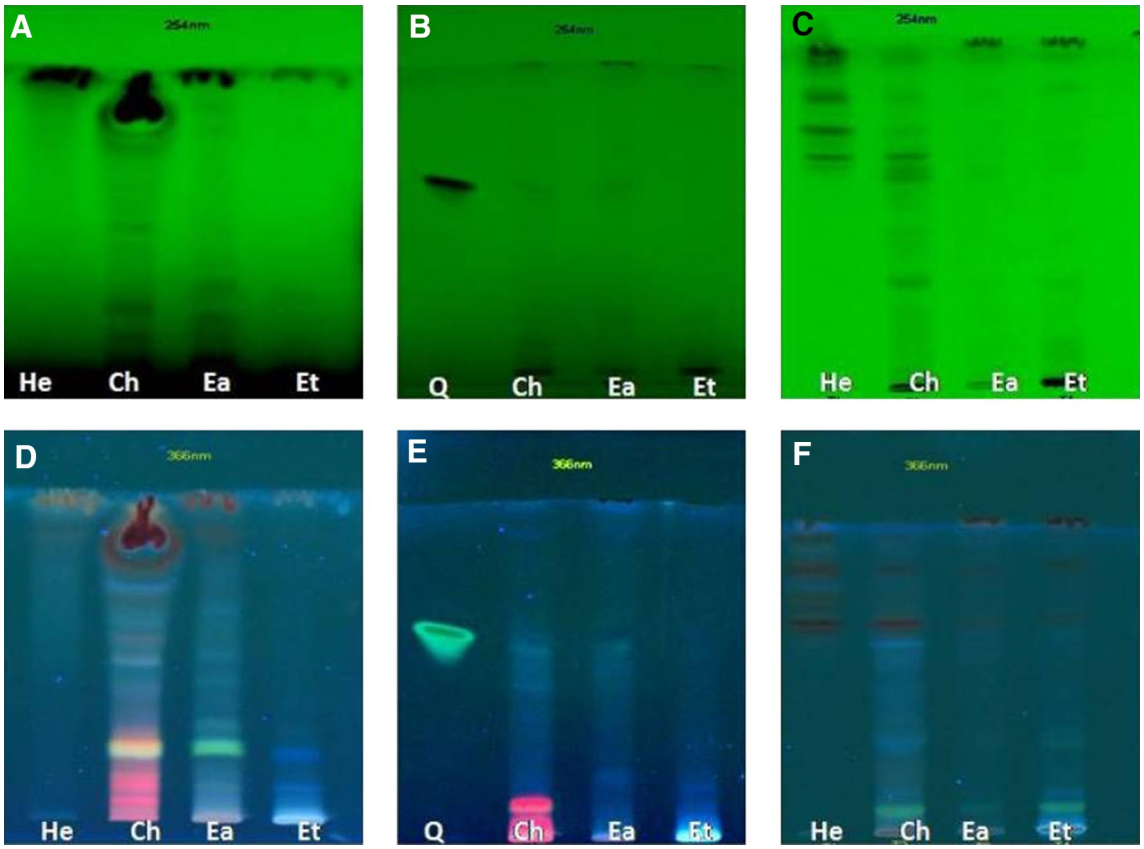

Fig. 2 HPTLC analysis of various extracts of *C. annuum*. **a**, **d** Photo Documentation of bell pepper based on phenols, **b**, **e** based on flavonoids, **c**, **f** based on carotenoids visualized under UV at 254 and 366 nm. *He* hexane, *Ch* chloroform, *Ea* ethyl acetate, *Et* ethanol extract, *Q* quercetin standard

from *Malus domestica, Laumea procumbens, Citrus japonica* and Spanish olive cultivars was obtained with the use of ethyl acetate and also it has been reported that ethyl acetate was the optimal solvent for the isolation of active substances from plant materials [30, 31]. In our study, it is confirmed that among all the employed organic solvents, ethyl acetate as the most effective solvent for the extraction of flavonoids.

Antioxidant activity

The antioxidant activity of bell pepper was determined by using in vitro methods namely DPPH and reducing power assays where the results are shown in Fig. 6a, b. During DPPH free radical reaction, the degree of discolouration (decrease in absorbance) of DPPH indicates the scavenging potential of the extract. Among all the extracts tested, ethyl acetate and ethanol exhibit higher ($p < 0.001$) inhibition percentage than other two extracts. The highest free radical scavenging activity was exerted by ethanolic (53.66%) and ethyl acetate (49.55%) extracts of bell pepper at a concentration of 254 µg/ml, which was higher than chloroform extract (25.03%). These results indicated that the bell pepper has a noticeable scavenging effect on DPPH free radicals. This may be attributed to its highest content of phenolic and flavonoid. It has been documented that radical scavenging activity is mainly due to the presence of hydroxyl groups in aromatic rings of phenolics [32, 33]. Our results suggested that phenolic acids and flavonoids may be the major contributors for the antioxidant activity of red bell pepper extracts.

In reducing power assay (Fig. 6b), the yellow colour of the test solution changed into green depends on the reducing power of the test solution. The presence of reducing compounds in the test solution causes the reduction of Fe^{3+}/ferric cyanide complex to the ferrous form. Therefore, Fe^{2+} can be monitored by measuring the absorbance 700 nm. Kolli et al. [34] and Gordon [35] suggested that the

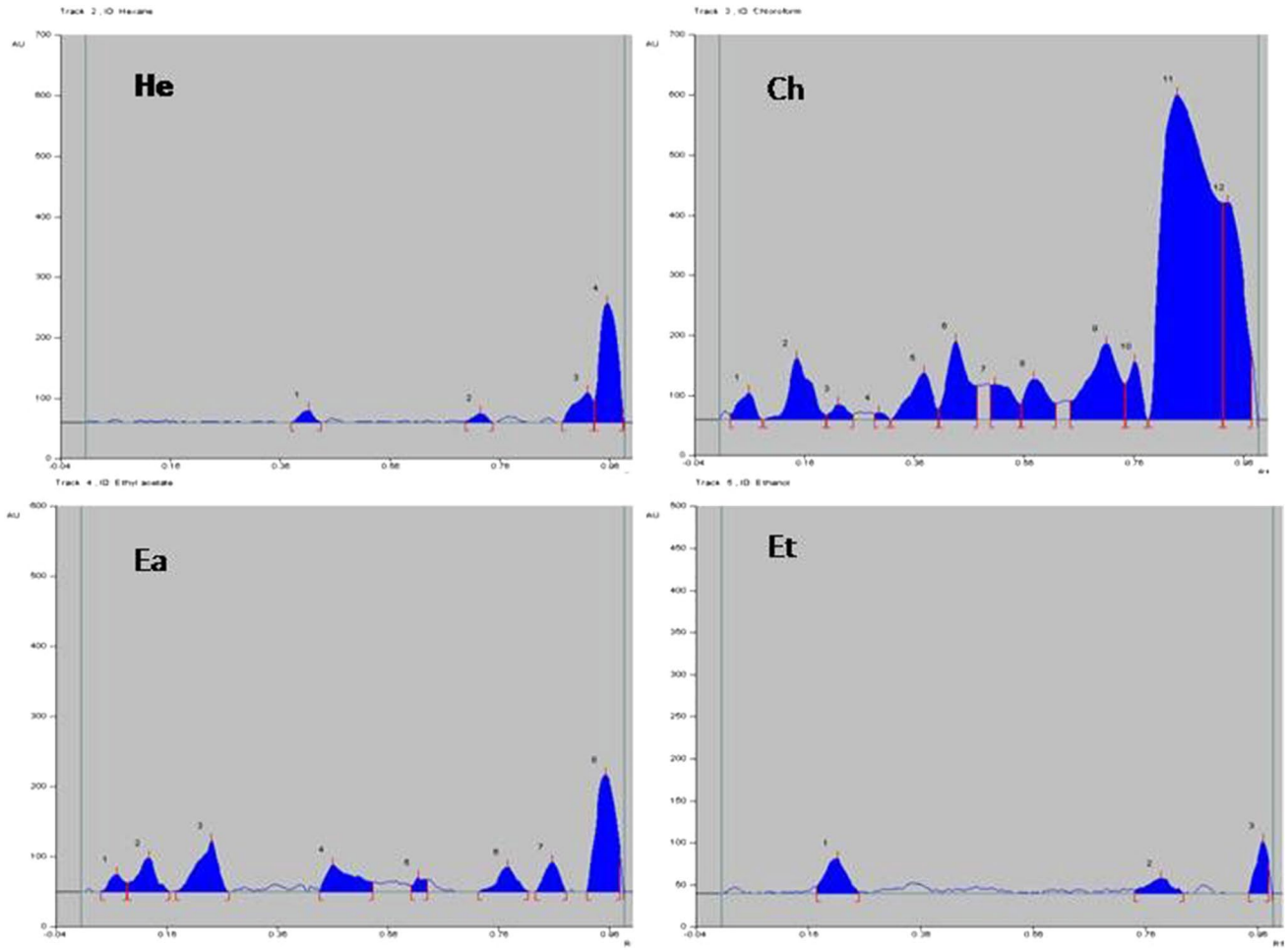

Fig. 3 HPTLC chromatogram of different extract of *C. annuum* based on phenols. *He* hexane, *Ch* chloroform, *Ea* ethyl acetate and *Et* ethanol extract

reducing potential was determined based on the potential to donate a hydrogen atom to break free radical chain. Figure 6b shows that the hexane, chloroform, ethyl acetate and ethanol extracts have higher reducing ability with increase in absorbance at 700 nm. The antioxidants present in all the extracts of bell pepper showed their reduction of Fe^{3+}/ ferric cyanide complex to the ferrous form and thus proved their reducing power. The combined effect of phenols and flavonoids created a great antioxidant capacity along with lycopene. As excellent source of phenols, flavonoids and carotenoids, the bell pepper becomes a healthy vegetable for regular consumption.

Conclusion

The result of the present study focuses on the content of bioactive compounds and antioxidant activity among the

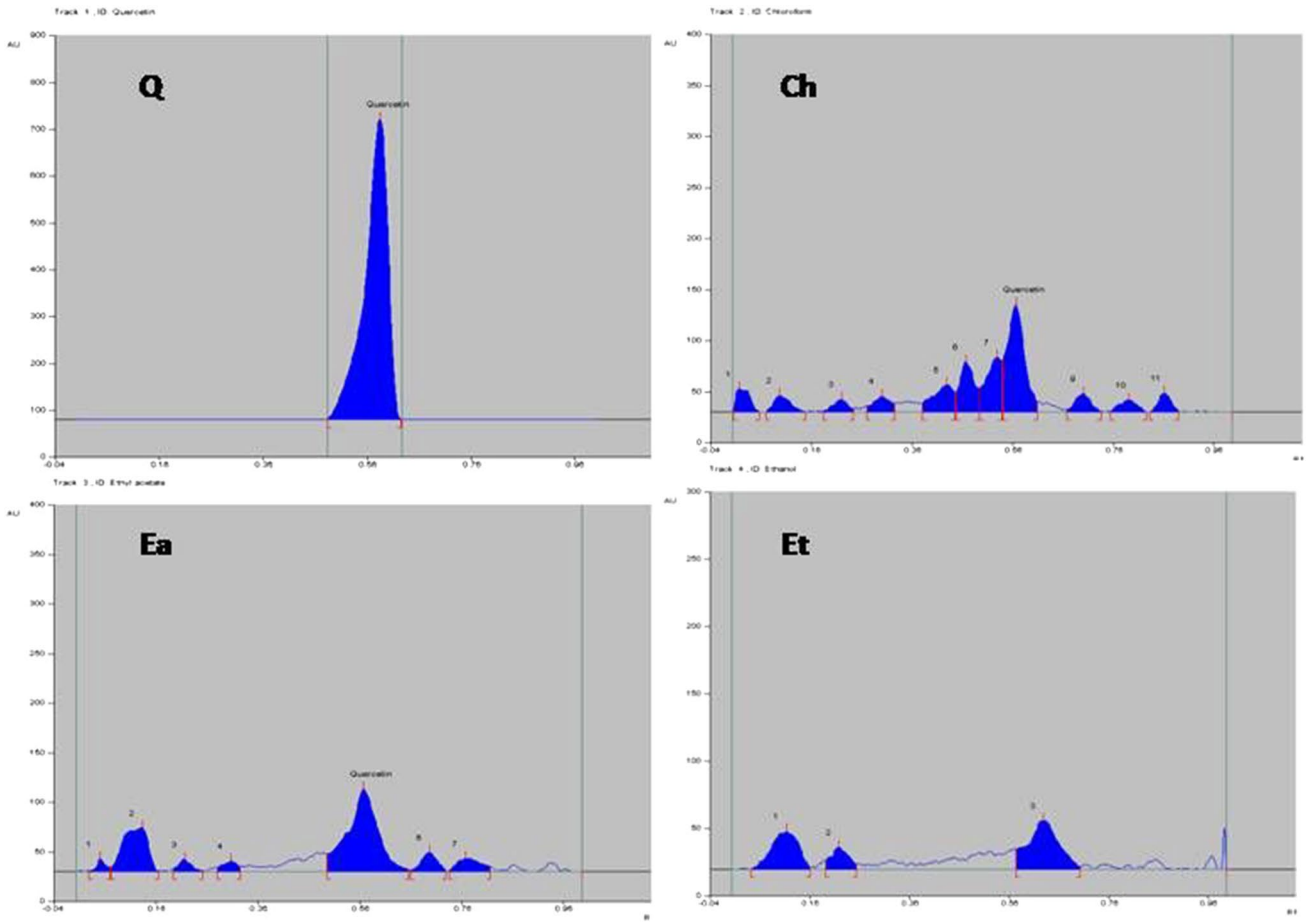

Fig. 4 Chromatogram showing different extract of red bell pepper based on flavonoids. *Q* quercetin standard, *Ch* chloroform, *Ea* ethyl acetate, *Et* ethanol extract

different solvents used for the extraction process. The ethanol and ethyl acetate are the ideal solvents for the extraction of phenolic and flavonoid compounds from *C. annuum* fruit. The secondary metabolites present in the bell pepper are very effective in the prevention of lot of diseases. It also seems to identify and characterize compounds isolated for use in food, pharmaceutical, medicinal and therapeutic industries. We conclude that the ethanol and ethyl acetate extracts of *C. annuum* act as a natural antioxidant in food and pharma industry and also a safe alternative to synthetic antioxidant drugs.

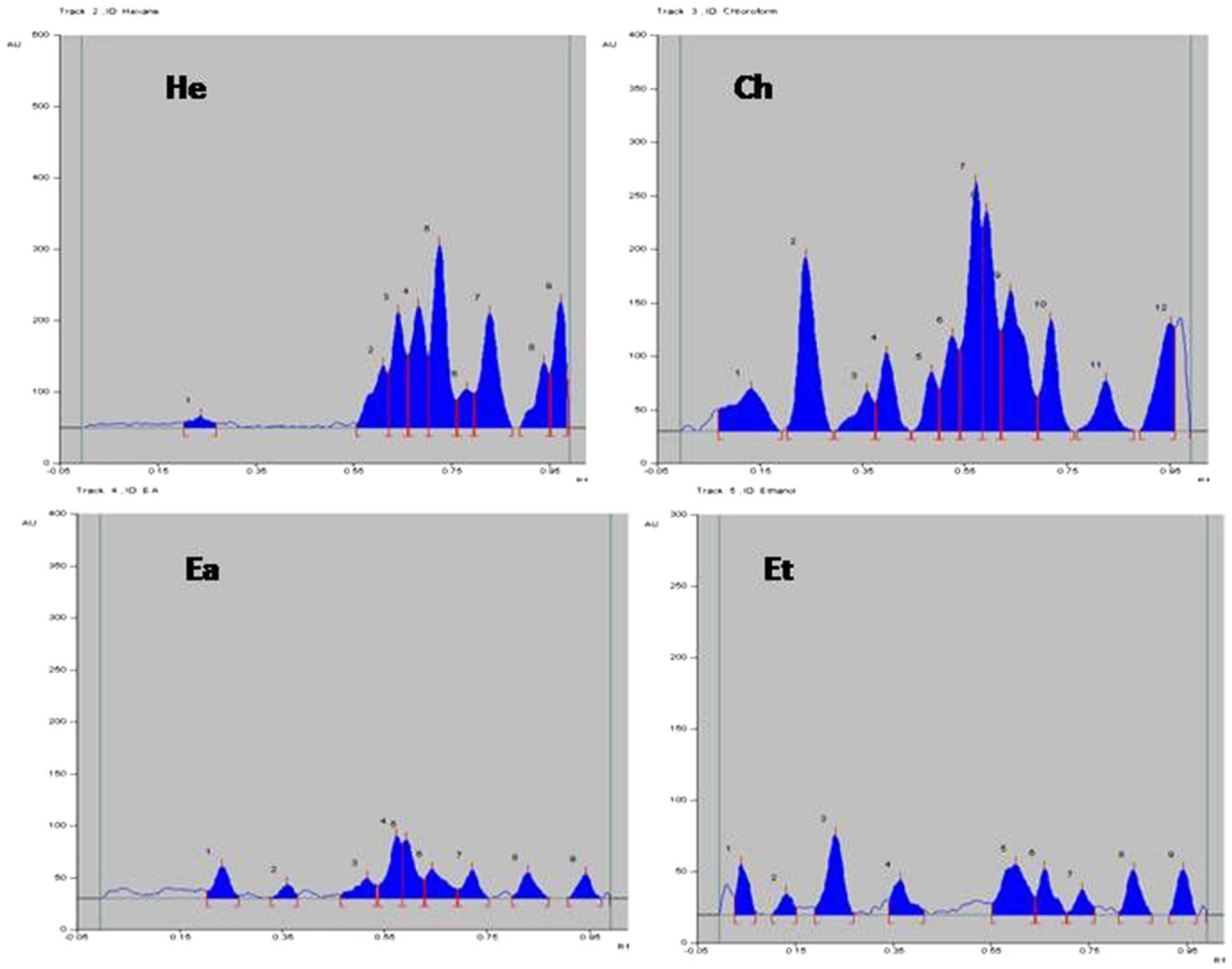

Fig. 5 Chromatogram of different extract of *C. annuum* based on carotenoids. *He* hexane, *Ch* chloroform, *Ea* ethyl acetate, *Et* ethanol extract

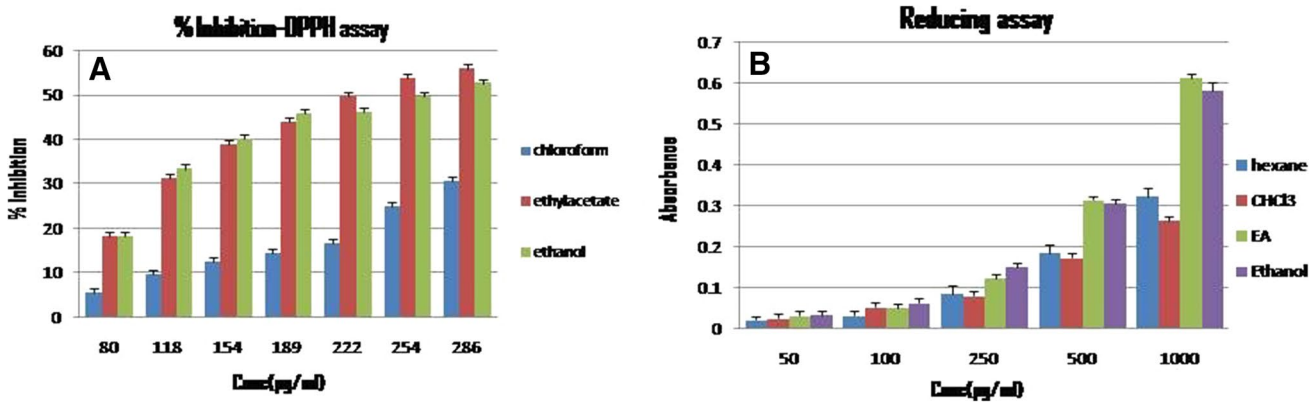

Fig. 6 Histogram showing antioxidant activity of various extracts of bell pepper by **a** DPPH method. **b** Reducing assay. The mean difference is significant at $p \leq 0.05$ level

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References

1. M. Maria do Socorro Rufino, E. Ricardo Alves, S.E. de Brito, J. Perez-Jimenez, F. Saura-Calixto, J. Mancini-Filho, Bioactive compounds and antioxidant capacities of 18 non-traditional tropical fruits from Brazil. Food Chem. **121**, 996–1002 (2010)
2. K.L. Wolfe, R.H. Liu, Apple peels as value-added food ingredient. J. Agric. Food Chem. **51**, 1676–1683 (2003)
3. E.A. Hayouni, M. Abedrabba, M. Bouix, M. Hamdi, The Effects of solvents and extraction method on the phenolic contents and biological activities in vitro of Tunisian *Quercuscoccifera L.* and *Juniperusphoenicea L.*. fruit extracts. Food Chem. **105**, 1126–1134 (2007)
4. L. Abdullah Hussain, A.S. Shahzad Chatha, S. Noor, Z.A. Khan, U. Muhammad Arshad, A. HassaanRathore, Z.A. Munavvar Sattar, Effect of extraction techniques and solvent systems on the extraction of antioxidant components from Peanut (*Arachishypogaea L.*) Hulls. Food Anal. Methods **4**, 890–896 (2012)
5. J. Mital Kaneria, B. Manisha Bapodara, V. Sumitra Chanda, Effect of extraction techniques and solvents on antioxidant activity of Pomegranate (*Punica granatum L.)* leaf and stem. Food Anal. Methods **5**, 396 (2012)
6. D. Evaggelia Tzika, V. Papadimitriou, G. Theodore Sotiroudis, A. Xenakis, Antioxidant properties of fruits and vegetables shots and juices: an electron paramagnetic study. Food Biophys. **1**,48–53 (2008)
7. M. Ghazemnezhad, M. Sherafati, G.A. Payvast, Variation in phenolic compounds, ascorbic acid and antioxidant activity of five coloured bell pepper (*Capsicum annum*) fruits at two different harvest times. J. Funct. Foods **1**, 44–49 (2011)
8. J. Andrews, *Peppers: Botany, production and uses The Domesticated Capsicums*. (University of Texas Press, Austin, 1984), pp. 83
9. P.W Bosland, In *Compendium of Pepper Diseases*, ed. by K Pernezny, PD Roberts, JF Murphy, NP Goldberg (APS Press, St. Paul, 2003), pp. 1–4
10. M.R. Gomez-Garcia, O.-A. Neftali, Biochemistry and molecular biology of carotenoid biosynthesis in chilli peppers (*Capsicum spp.*). Int. J. Mol. Sci. **14**, 19025–19053 (2013)
11. N.P. Holzapfel, B.M. Holzapfel, S. Champ, J. Feldthusen, J. Clements, D. Werner Hutmacher, The potential role of lycopene for the prevention and therapy of prostate cancer: from molecular mechanisms to Clinical evidence. Int. J. Mol. Sci. **14**, 14620–14646 (2013)
12. E.S. Guna, S.P. Cowell, Drug insight: lycopene in the prevention and treatment of prostate cancer. Nat. Clin. Proct. Urol. **2**, 38–43 (2005)
13. D. Howard Sesso, E. Julie Buring, M. Shumin Zhang, P. Edward Norkus, J. Michael Gaziano, Dietary and plasma lycopene and the risk of breast cancer. Cancer Epidemiol. Biomark. Prev. **14**, 1074 (2005)
14. A.K. Gupta, *Quality Standards of Indian Medicinal plants*, (Indian Council of Medical Research, Indraprastha press, New Delhi, 2003) pp, 32
15. A.K. Gupta, *Quality Standards of Indian Medicinal plants*, (Indian Council of Medical Research, Indraprastha press, New Delhi, 2003). pp, 209
16. C. Chang, M. Yang, H. Wen, J. Chern, Estimation of total flavonoid content in propolis by two complementary colorimetric methods. J. Food Drug Anal. **10**, 178–182 (2002)
17. S.K. Sharma, M. Le Maguer, Lycopene in tomatoes and tomato pulp fractions. Italian J. Food Sci. **2**, 107–113 (1996)
18. W. Brand-Williams, M.E. Cuvelier, C. Berset, Use of a free radical method to evaluate antioxidant activity. Food Sci. Technol. **28**, 25–30(1995)
19. M. Oyaizu, Studies on product of browning reaction prepared from glucosamine. Jpn. J. Nutr. **44**, 307–315 (1986)
20. D.-M. Yang, Q.-S. Wang, K. Le-Qin, J.-M. Jiang, T.-J. Ying, Antioxidant activities of various types of lotus (*Nelumbo nicifera Gaertn*) rhizome. Asian Pac. J. Clin. Nutr. **04**, 158–163 (2007)
21. Y. Baravalia, M. Kaneria, Y. Vaghasiya, J. Parekh, S. Chanda, Antioxidant and antibacterial activity of *Diospyros ebenum* Roxb leaf extracts. Turk. J. Biol. **33**, 159–164 (2009)
22. S. Bushra, A. Farooq, P. Roman, Antioxidant activity of phenolic components present in barks of *Azadirachta indica, Terminalia arjuna, Acacia nilotica* and *Eugenia jambolana Lam* trees. Food Chem. **104**, 1106–1114 (2007)
23. S. Milan Stankovic, Total phenolic content, flavonoid concentration and antioxidant activity of *Marrubium peregrinum L.* extracts. Kragujev. J. Sci. **33**, 63–72 (2011)
24. M. Bonoli, A. Bendini, L. Cerretanietal, Qualitative and semi quantitative analysis of phenolic compounds in extra virgin olive oils as a function of the ripening degree of olive fruits by different analytical techniques. J. Agric. Food Chem. **52**, 7026–7032 (2004)
25. T. Sun, Z. Xu, C.T. Wu, M. Janes, W. Prinyawiwatkul, H.K. No, Antioxidant activities of different colored sweet bell peppers (*Capsicum annuum* L.). J. Food Sci. **72**, 98–102 (2007)
26. A. Pieternel Luning, Ria van der Vuurst de Vries, D. Yuksel, T. Ebbenhorst-Seller, J. Harry Wichers, P. Jacques Roozen, Combined instrumental and sensory evaluation of flavor of fresh bell pepper (*Capsicum annuum*) harvested at three maturation stages. J. Agric. Food Chem. **42**, 977–983 (1994)
27. U. Kankeaw, E. Masong, The Antioxidant activity from Hydroquinone derivatives by the synthesis of *CinnamomumverumJ. Presl* bark's extracted. Int. J. Chem. Eng. Appl. **6**, 91–94 (2015)
28. C.M. Hasler, Functional foods; their role in disease prevention and health. Food Technol. **52**, 63–69 (1998)
29. B. Simon Iloki Assanga, M. Lidianys Lewis Lujan, L. Claudia Lara-Espinoza, A. Armida Gil-Salido, DanielaFernandez-Angulo, L. Jose Rubio-Pino, D. David Haines, Solvent effects on phytochemical constituent profiles and antioxidant activities, using four different extraction formulations for analysis of *Bucida buceras L.* and *Phoradendron californicum*. BMC Res. Notes **8**, 1–14 (2015)
30. S. Sinimol, A. Sarika, A.J. Nair, Diversity and antagonistic potential of marine microbes collected from south-west coast of india. Biotech **6**, 1–9 (2016)
31. T. Zhang, X. Wei, Z. Miao, H. Hassan, Y. song, M. Fan, Screening for antioxidant and antibacterial activities of phenolics from Golden delicious apple pomace. Chem. Cent. J. **47**, 1–9 (2016)
32. W. Brand-Williams, M.E. Cuvelier, C. Berset, Use of a free radical method to evaluate antioxidant activity. Food Sci. Technol. **28**, 25–30 (1995)
33. W.J. Yen, L.W. Chang, P.D. Duh, Antioxidant activity of peanut seed testa and its antioxidative component, ethyl protocatechuate. LWT Food Sci. Technol. **38**, 193–200 (2005)
34. D. Kolli, K.R. Amperayani, U. Parimi, Total Phenolic content and antioxidant activity of *Morindatinctoria* leaves. Indian J. Pharm. Sci. **77**, 226–330 (2015)
35. M.H. Gordon, The mechanism of antioxidant action in vitro. in *Food Antioxidants*, ed. by B.J.F. Hudson (Elsevier Applied science, London, 1990), pp 1–18