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Evaluation of Antioxidant Activity of Some Indian Spices using Different In-Vitro Methods

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ABSTRACT

Antioxidants are substances that prevent oxidation of other compounds or neutralize free radicals. Spices are rich sources of antioxidants. They have been used in food and beverages to enhance flavor, aroma and color. Due to their excellent antioxidant activity, spices have also been used to treat some diseases. In this research article, phytochemical estimation, evaluation of antioxidant activity of spices and their correlation with total phenolics content was carried out. The highest concentration of total phenolics (225.3 mg/100 g of DW) and protein (560 mg/g of DW) was present in *S. aromaticum*. The highest concentration of ascorbic acid (24.8 µg/g of FW) and carbohydrate (236.4 mg/g of DW) was present in *Z. officinale*, whereas highest carotenoid in *M. koenigii* (16.9 mg/100g of FW). Lowest IC_{50} for FRSA (1.02 mg/mL), FTC (0.07 mg/mL) and HRSA (0.46 mg/mL) was showed by *S. aromaticum* whereas lowest IC_{50} for SARSA (3.21 mg/mL) by *M. koenigii*. *S. aromaticum* (5.44 mg/mL) and *M. koenigii* (5.42 mg/mL) showed almost equal IC_{50} value for LPO. In conclusion ethanolic extracts of *S. aromaticum* and *Z. officinale* exhibit better antioxidant activity in comparison to other spices and it could be a good source of natural antioxidant for food and pharmaceutical industries.

1. Introduction

Oxidative stress, which is caused by high concentration of free radicals in cells and tissues, can be induced by various negative factors, such as gamma, UV and X-ray radiation, psycho-emotional stress, polluted food, adverse environmental conditions, intensive physical exertion, smoking, alcoholism, and drug addiction [1]. Antioxidants are substances that neutralize or remove free radicals by donating an electron. The neutralizing effect of oxidants helps protect the body from oxidative stress. Antioxidants are widely used as ingredients in dietary supplements, which are used in the hope of maintaining health and preventing diseases such as cancer and coronary heart disease. When added to food, antioxidants control rancidity development, retard the formation of toxic oxidation products, maintain nutritional quality, and extend the shelf-life of products [2].

Various natural and synthetic antioxidants are available in the market. Synthetic antioxidants are chemically synthesized short life compounds since they do not occur in nature and are added to food as preservatives to help prevent lipid oxidation. Butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) were originally developed synthetic antioxidant. BHT and BHA have recently been reported that they can cause damage and can be dangerous to human health, so there is a need, focus and search for effective, nontoxic natural compounds with antioxidant activities [3]. Because of safety concerns, synthetic antioxidants are limited to be used as food preservatives.

Natural antioxidants obtained from edible materials such as spices and herbs, have been of increasing interest. Natural antioxidants contained in spices help to reduce oxidative stress. Therefore, spices could potentially be used as preventive agents for some health issues [4-8].

Since the ancient times, spices and herbs have been added to food to enhance flavor and improve their organoleptic properties. Spices and herbs have also been widely used as preservatives and medicine [9,10]. They have been extensively studied because of the high antioxidant activity in certain spices and their beneficial effects on human health [11]. Reported studies proofed antioxidant activity of spices in methanol [12-14], aqueous [15], ether, acetone, and chloroform [16] extracts mainly through DPPH radical assay.

To have a better understanding of the antioxidant activity from spices, the present study focused on quantification of phytochemicals and evaluation of antioxidant activity of ethanolic extracts by using different *in vitro* antioxidant assays. Further, correlation between antioxidant activities and phenolic content was carried out.

2. Experimental Methods

2.1 Sample Materials

The spices (Table 1) were purchased from local market in Faizabad, Uttar Pradesh, India.

Table 1 Indian spices used in study

Spice name	Abbr.	Common name	Family	Part used
Cuminum cyminum	CC	Cumin	Umbellifers	Seed
Curcuma longa	CL	Turmeric	Zingiberaceae	Rhizome
Coriadrum sativum	CS	Coriander	Umbellifers	Seed
Murraya koenigii	MK	Karipatta	Rutaceae	Leaf
Piper nigrum	PN	Black pepper	Piperaceae	Seed
Syzygium aromaticum	SA	Clove	Myrtaceae	Seed
Zingiber officinale	ZO	Ginger	Zingiberaceae	Rhizome

2.2 Chemicals and Reagents

Quercetin, gallic acid, 1, 1-diphenyl-2-picrylhydrazyl (DPPH) and thiobarbituric acid (TBA), ethidium bromide was purchased from Sigma-Aldrich, St. Louis, USA. Ascorbic acid, Folin Ciocalteau's phenol reagents were the product of E. Merck, Mumbai, India. Nitro blue tetrazolium (NBT), phenazine methosulphate (PMS), reduced nicotinamide adenine dinucleotide (NADH), potassium ferricyanide, trichloroacetic acid (TCA), ferric chloride ($FeCl_3$), ferrous sulphate ($FeSO_4$) and sodium dodecyl sulphate (SDS) were purchased from SRL India. All other reagents and chemicals used were of analytical grade.

2.3 Estimation of Phytochemicals

Ascorbic acid content of plants was estimated by the method of AOAC [17] and reported as mg/ 100 g of fresh weight (FW) of tissues. Carotenoids were estimated by the method of Jensen [18] and reported as µg/g of FW. Total phenolic content (TPC) was measured using the method

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of Ragazzi and Veronese [19] and reported in terms of mg of gallic acid equivalent (GAE)/g of dry weight (DW). Protein content was estimated by the method of Lowry et al. [20] and reported as mg/g of DW. Carbohydrate content was estimated by method of Anthrone [21] and reported as mg/g of DW.

2.4 Extraction Procedure

Twenty grams of the dried and powdered sample of spices (Table 1) were extracted with 70% ethanolic solvent (in distilled water) for overnight at room temperature in an orbital shaker. The extracts were separated from the residues by filtering through Whatman No. 1 filter paper. The residues were extracted until decoloration with the same fresh solvent and extracts combined. The combined extracts were concentrated and freed of solvent under reduced pressure at 40 °C by using a rotary evaporator and lyophilized till dryness. The dried crude concentrated extracts were stored at -4 °C and used for the antioxidant activity determination.

2.5 Antioxidant Studies

2.5.1 Free Radical Scavenging Activity (FRSA)

FRSA of the extracts was measured by using DPPH stable radical according to the method of Yen and Duh [22]. Each extract (0.1 mL) was added to freshly prepared DPPH solution (6×10^{-5} M in HPLC grade 2.9 mL methanol) and mixed vigorously. The reduction of the DPPH radical (DPPH•) was measured by continuous monitoring of the decrease in absorbance at 515 nm until a stable value was obtained.

Inhibition (%) = [(blank absorbance - sample absorbance)/blank absorbance] × 100

The inhibitory concentration (IC_{50}) which represents the amount of antioxidant necessary to decrease the initial DPPH concentration by 50%, representing a parameter widely used to measure the antioxidant activity, was calculated from a calibration curve by linear regression. EC_{50} was calculated as IC_{50} (µg/mL)/concentration of DPPH/mL and expressed as µg/mg DPPH. For rational reasons of clarity, the anti-radical power (ARP) was determined as the reciprocal value of the effective concentration (EC_{50}), representing a comparable term for the effectiveness of antioxidant and radical scavenging capacity, ARP = $1/EC_{50} \times 100$. The larger the ARP, the more efficient the antioxidant

2.5.2 Superoxide Anion Radical Scavenging Activity (SARSA)

This assay was based on the capacity of the extract to inhibit the reduction of nitro blue tetrazolium (NBT) by the method of Nishikimi et al. [23]. Three milliliters reaction mixture containing different aliquot of plant extracts (50, 100, 150 and 200 µl) with 0.1 M phosphate buffer (pH 7.8), 60 µM PMS, 468 µM nicotinamide adenine dinucleotide reduced (NADH) and 150 µM NBT was incubated for 5 min at ambient temperature. Absorbance was read after 6 min at 560 nm using UV-Vis spectrophotometer. The percentage inhibition (PI) of superoxide ($O_2^{\bullet-}$) generation was measured by comparing the absorbance of the control and those of the reaction mixture containing test sample.

2.5.3 Lipid Peroxidation (LPO)

A modified thiobarbituric acid-reactive species (TBARS) assay method of Ohkawa et al. [24] was applied to measure the LPO formation, using egg homogenate as lipid rich media. Egg homogenate (10% in 0.2 M PBS, 0.5 mL), test extract (0.1 mL) and DW (0.85 mL) were mixed in a test tube. Finally, $FeSO_4$ (0.07 M, 0.05 mL) was added to the reaction mixture and incubated at 37°C temperature for 30 min to induce LPO. Thereafter, acetic acid (20%, 1.5 mL), TBA (0.8% prepared in 1.1% sodium dodecyl sulphate, 1.5 mL) and TCA (20%, 0.05 mL) were added, vortexed and then heated in a boiling water bath for 60 min. After cooling, butanol (5 mL) was added to each tube and centrifuged for 10 min at 3000 rpm. The absorbance of the upper organic layer was measured at 532 nm by UV-Vis spectrophotometer (Labtronics, model LT-2910).

2.5.4 Ferric Thiocyanate Assay (FTC)

The reaction mixture containing 400 µL of different concentration of ethanolic plant extracts, 200 µL of diluted linoleic acid (25 mg/mL in 99% ethanol) and 400 µL of 50 mM phosphate buffer (pH 7.4) was incubated for 15 min at 40 °C. A 100 µL aliquot of this was then mixed with a reaction mixture containing 3 mL of 70% ethanol, 100 µL of ammonium thiocyanate (300 mg/mL in DW) and 100 µL of ferrous sulphate. Red color developed was measured at 535 nm [25].

2.5.5 Hydroxyl Radical Scavenging Activity (HRSA)

OH• were generated by a mixture of Fe^{3+}-EDTA, H_2O_2 and ascorbic acid and assessed by monitoring the degraded fragments of deoxyribose, through malondialdehyde (MDA) formation [26]. The reaction mixtures contained ascorbic acid (50 µM), $FeCl_3$ (20 µM), EDTA (2 mM), H_2O_2 (1.42 mM), deoxyribose (2.8 mM) with different concentrations of the plant extracts in a final volume of 1 mL, was incubated at 37 °C for 1 hour and then 1 mL of 2.8% TCA (w/v in water) and 1 mL of 1% TBA (w/v) were added. The mixture was heated in a boiling water bath for 30 min. It was cooled and absorbance was taken at 532 nm.

2.5.6 Reducing Power (RP)

RP of the extracts was determined by using a slightly modified method of ferric reducing-antioxidant power assay [27]. Each extract (1.0 mL) was mixed with 2.5 mL of phosphate buffer (0.1 M, pH 6.6) and 2.5 mL of 1% (w/v) potassium ferricyanide and was incubated at 50 °C for 20 min. After completion of the incubation period, 2.5 mL of 10% (w/v) trichloro acetic acid (TCA) was added to terminate the reaction. The upper layer (2.5 mL) was diluted with equal volume of deionized water. Finally, 0.5 mL of 0.1% (w/v) $FeCl_3$ was added and after 10 min the absorbance was measured at 700 nm against a blank. RP was expressed as ascorbic acid equivalents (1 ASE = 1 mM ascorbic acid). ASE value is inversely proportional to RP.

2.6 Statistical Analysis

Statistical analysis was done using prism software. Values from *in vitro* antioxidant activities were reported as mean± standard deviation (SD) of three determinations. The r^2 value and regression equation were calculated through plotting graph between TPC on x-axis and antioxidant deciding parameters on y axis with the help MS office excel 2007 [28].

3. Results and Discussion

3.1 Phytochemical Estimations

In order to find out the concentration of phytochemicals which impart antioxidant activity, spices were examined for their ascorbic acid (vitamin C), carotene ids, total phenolics, protein and carbohydrate contents (Table 2).

Table 2 Phytochemical contents in Indian spices

Spice name	Ascorbic acid µg/g of FW	Carotenoid mg/100 g of FW	TPC mg/100 g of DW	Protein mg/g of DW	Carbohydrate mg/g of DW
CC	16.3±0.22	4.11±0.11	80.64±1.2	198±1.4	72.80±1.6
CL	16.4±0.31	4.46±0.24	65.92±1.6	400±1.3	127.3±2.5
CS	13.5±0.44	9.80±0.44	42.88±2.5	194±1.3	40.00±1.4
MK	20.4±0.41	16.9±0.40	83.60±1.2	183±1.5	74.72±1.4
PN	8.16±0.44	8.88±0.21	51.84±1.3	205±1.5	105.4±1.6
SA	12.4±0.21	1.16±0.44	225.3±1.2	560±1.2	90.90±1.5
ZO	24.8±0.14	4.19±0.21	59.92±1.5	286±1.5	236.4±2.4

Values are mean ± SD of three replications, TPC: Total phenolic content, GAE: Gallic acid equivalent, FW: Fresh weight, DW: Dry weight, CC: C. cyminum, CL: C. longa, CS: C. sativum, MK: M. koenigii, PN: P. nigrum, SA: S. aromaticum, ZO: Z. officinale

3.1.1 Vitamin C (Ascorbic Acid)

The ascorbic acid in the tested spices ranged between 8.16 to 24.8 mg/g of GAE of DW, which has been summarized in Table 2. Results showed that ZO had highest value of TPC followed by MK (20.4), CL (16.4), CC (16.3), CS (13.5), SA (12.4), PN (8.16) mg/g of GAE of DW.

3.1.2 Carotenoids

Carotenoid content of the tested spices are presented in Table 2. Among the tested extracts, MK had the highest concentration of carotenoids i.e. 16.9 µg/g of fresh weight (FW) followed by CS (9.80) > PN (8.88) > CL (4.46) > ZO (4.19) > CC (4.11) > SA (1.16 µg/g of FW).

3.1.3 Total Phenolic Content (TPC)

Tested spices extract showed varying level of TPC ranging from 42.88 to 225.3 mg/100 g of DW (Table 2). Among the spices, the highest value of TPC was present in SA (225.3 mg/100 g of DW) followed by MK (83.60) > CC (80.64) > CL (65.92) > ZO (59.92) > PN (51.84) > CS (42.88 mg/100 g of DW).

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3.1.4 Protein

Protein content of tested spices is ranged from 183 to 560 mg/g of DW (Table 2). The highest value of protein was present in SA (560 mg/g of DW) fallowed by CL (400) > ZO (286) > PN (205) > CC (198) > CS (194) > MK (183 mg/g of DW).

3.1.5 Carbohydrate

The carbohydrate content in different parts of plants ranged from 40 to 236.4 mg/g of DW (Table 2). The highest carbohydrate content was observed in ZO (236.4) followed by CL (127.3) > PN (105.4) > SA (90.90) > MK (74.72) > CC (72.72) > CS (40.00 mg/g of DW).

3.2 Antioxidant Studies

3.2.1 Free Radical Scavenging Activity (FRSA)

Ethanolic extracts of spices were examined for their potential to scavenge free radicals and measured as percentage inhibition (PI). SA was considered to be most potent free radical scavenger followed by MK, CC, CL, ZO, PN and CS and its value of inhibition at 100 µg/mL was 80.53% (Fig. 1). The IC_{50} value of SA was 1.02 mg/mL whereas, in the case of other spices were MK (1.80) > CC (1.95) > CL (2.21) > ZO (2.41) > CS (3.31) mg/mL, respectively (Table 3). According to Begum et al. [29] and Zahin et al. [30] methanolic and aqueous extracts of SA and acetone extract of MK exhibited inhibition potential at much higher IC_{50} value of 93.75, 187.50 and 638.7 µg/mL than present study. Kumari et al. [31] showed methanolic extract of SA exhibited higher IC_{50} 43.18 µg/mL at 100 µg/mL than ethanolic extract in present study. Reported studies proved that phenolics were better extracted in ethanolic solvents than other due to easily miscible with water.

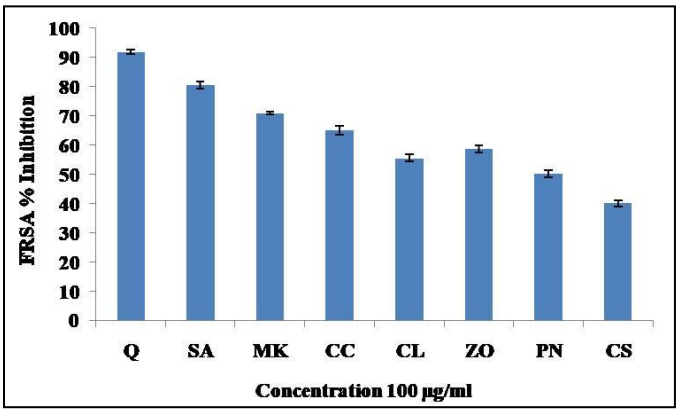

Fig. 1 Free radical scavenging activity of ethanolic extracts of spices in comparison to standard quercetin against DPPH radicals at 100 µg/mL concentration. Values are mean±SD of three replications (n=3). SA: (*S. aromaticum*); MK: *M. koenigii;* CC: *C. cyminum;* CL: *C. longa;* ZO: *Z. officinale;* PN: *P. nigrum;* CS: *C. sativum*

The DPPH•, at its maximum wavelength at 517 nm, can easily receive an electron or hydrogen from antioxidant molecules to become a stable diamagnetic molecule as DPPH-H [32]. Owing to the DPPH• ability to bind H, it is considered to have a radical scavenging property. Discoloration occurs due to the decreasing quantity of DPPH• into reaction mixture which reflects the FRSA of tested extract [33].

3.2.2 Superoxide Anion Radical Scavenging Activity (SARSA)

The antioxidant potential of ethanolic extracts of spices against $O_2^{\bullet-}$ was considered to be significant in comparison to the standard quercetin. The result presented in Fig. 2 shows that MK extract inhibited NBT reduction higher than other tested spice extracts. The inhibition potential of MK against $O_2^{\cdot-}$ was 77.90% in comparison to standard quercetin (85.47%) at 100 µg/mL concentration. The IC_{50} values at which spices extracts showed significant SARSA were found as MK (3.21) > SA (4.23) > CC (5.34) > CL (6.29) > PM (7.55) > ZO (7.98) and CS (8.02) mg/mL, respectively (Table 3).

The $O_2^{\bullet-}$ is a precursor to active free radicals that have the potential of reacting with biological macromolecules and thereby inducing tissue damage [34]. It has been implicated in several pathophysiological processes due to its transformation into more ROS such as OH•, H_2O_2, $^1O_2$ and oxidizing agents that initiate LPO [35,36] and damage to protein and DNA [37]. $O_2^{\bullet-}$ derived from dissolved oxygen by PMS-NADH coupling reaction and reduces NBT in this system. In this method, $O_2^{\bullet-}$ reduces the yellow dye (NBT^{2+}) to produce the blue formazan which is measured spectrophotometrically at 560 nm. Plant extracts containing antioxidants are able to inhibit the formation of blue tetrazolium complex [38,39]. The decrease of absorbance at 560 nm with antioxidants indicates the consumption of $O_2^{\bullet-}$ in the reaction mixture. Fig. 2 clearly indicates that Indian spices are potent $O_2^{\bullet-}$ scavenger. According to Kumari et al. [40] methanolic extracts of SA exhibited 40.30% $O_2^{\bullet-}$ inhibition at 100 µg/mL concentration which is much lower than present study.

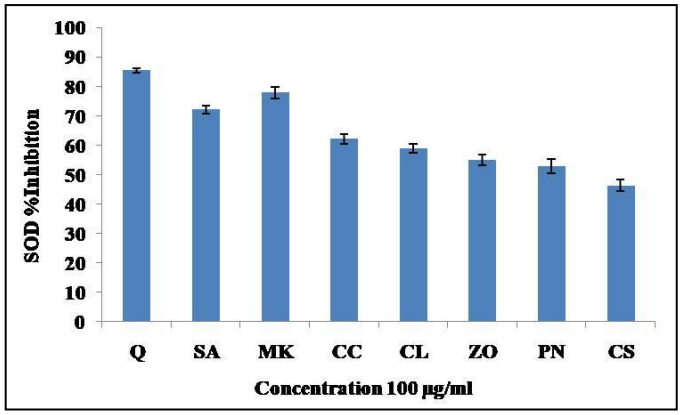

Fig. 2 Inhibitory effects of ethanolic extracts of spices in comparison to standard quercetin against superoxide anion at 100 µg/mL concentration. Values are mean±SD of three replications (n=3). SA: *S. aromaticum*; MK: *M. koenigii;* CC: *C. cyminum;* CL: *C. longa;* ZO: *Z. officinale;* PN: *P. nigrum;* CS: *C. sativum*

3.2.3 Lipid Peroxidation (LPO)

Studies on the inhibition of LPO in the presence of extracts were carried out and expressed as percentage inhibition. The ethanolic extracts of spices prevent LPO induced by $FeSO_4$ and PI varied from 40.20 to 71.00% respectively (Fig. 3). Maximum LPO inhibition was shown by SA (71.00%) than other spices at 100 µg/mL in comparison to standard (81.78%). MK and SA exhibited anti-LPO activity with almost equal IC_{50} values 5.42 and 5.44 mg/mL followed by CC (6.66) > CL (7.40) > ZO (7.42) > PM (8.19) and CS (9.68) mg/mL, respectively (Table 3).

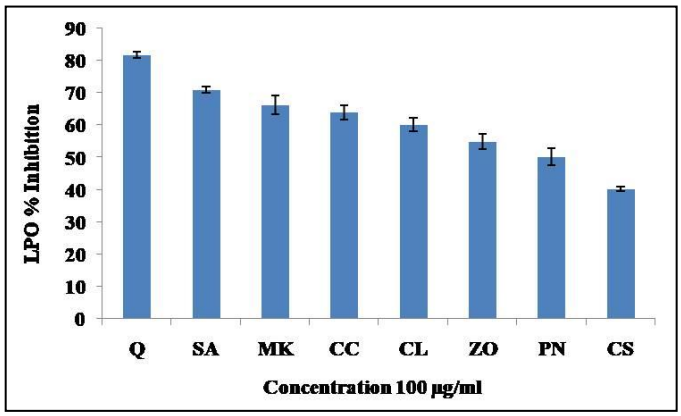

Fig. 3 Inhibitory effects of ethanolic extracts of spices in comparison to standard quercetin on LPO using egg homogenate as a lipid-rich source at 100 µg/mL concentration. Values are mean ± SD of three replications (n=3). SA: (*S. aromaticum*); MK: *M. koenigii;* CC: *C. cyminum;* CL: *C. longa;* ZO: *Z. officinale;* PN: *P. nigrum;* CS: *C. sativum*

In LPO assay antioxidant potential is measured by evaluating the capability of the test sample to hamper the oxidation of polyunsaturated fatty acids (PUFA) into TBARS. Peroxidation generates peroxyl radicals which decompose to MDA. It forms a stable product with TBA, which serve as a mean to quantify the level of peroxidation [41]. This assay is a very useful mean to assess LPO *in vitro* due to its simplicity and reproducibility.

According to Goswami et al. [42] aqueous extracts of SA, CL and CS exhibited anti-LPO activity at IC_{50} of 9.54, 2.50 and 6.10 g/tissue which is much higher in comparison to ethanolic extracts in present study.

3.2.4 Hydroxyl Radical Scavenging Activity (HRSA)

Spices extracts were further studied for their ability to chelate iron and/or to scavenge OH• by using deoxyribose degradation assay. The ethanolic extracts of spices were found to be potent OH• scavenger with inhibition ranged from 48.78 to 79.70% (Fig. 4). The IC_{50} value at which spices extracts showed HRSA was found to be CS (3.53), CL (0.46), ZO (3.39), CC (5.50), SA (4.96), PM (5.96) and MK (7.92) mg/mL, respectively (Table 3).

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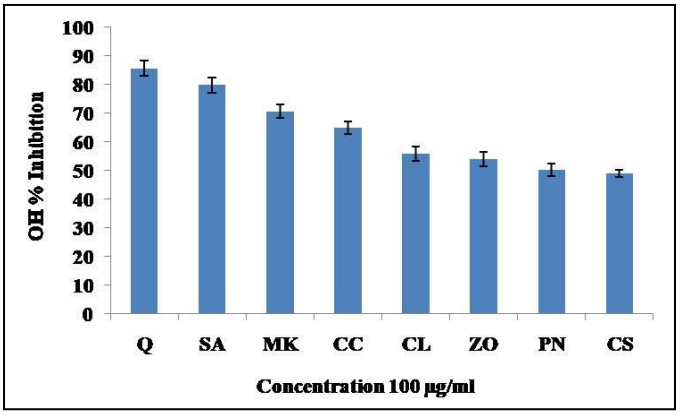

Fig. 4 Inhibitory effects of ethanolic extracts of spices in comparison to standard quercetin on hydroxyl radical mediated deoxyribose degradation at 100 µg/mL concentration. Values are mean ± SD of three replications (n=3). SA: (*S. aromaticum*); MK: *M. koenigii;* CC: *C. cyminum;* CL: *C. longa;* ZO: *Z. officinale;* PN: *P. nigrum;* CS: *C. sativum*

According to Kim et al. [43] aqueous extracts of CC, CS and SA showed 2.95%, 21.94% and 10.78% OH• scavenging activity which is very less in comparison to our ethanolic extracts in present study. The OH• induced oxidative damage to DNA, lipids and proteins are involved in various neurodegenerative and cardiovascular diseases [44]. The HRSA of the extracts was determined by its ability to compete with deoxyribose for OH•. In this assay, 2-deoxy-2-ribose was oxidized when exposed to OH• generated by the fenton-type reaction. The oxidative degradation can be detected by heating the products with TBA under acidic conditions to develop a pink chromogen with a maximum absorbance at 532 nm [45].

3.2.5 Ferric Thiocyanate (FTC)

SA extract exhibited PI to inhibit the production of free radicals those initiate the oxidation of lipids and proteins. The maximum inhibition was shown by SA (69.50%) in comparison to quercetin (78.45%) (Fig. 5). The IC_{50} of extracts was found to be in the range 0.07 to 3.10 mg/mL. According to Tanvir et al. [46] aqueous extracts of two different varieties of CL showed ferric thiocyanate activity at IC_{50} of 646.7 and 1015 µg/mL concentration which is much higher than ethanolic extracts in present study (Table 3).

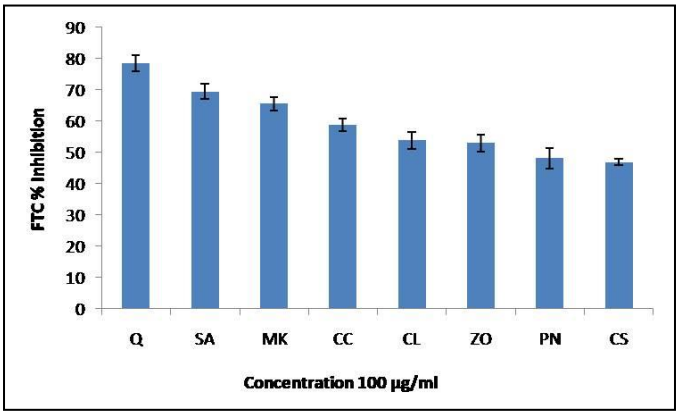

Fig. 5 Inhibitory effects of ethanolic extract of *T. bellerica* in comparison to standard quercetin on ferric ion chelation by ferric thiocyanate assay method at varying concentrations. Values are mean ± SD of three replications (n=3). SA: (*S. aromaticum*); MK: *M. koenigii;* CC: *C. cyminum;* CL: *C. longa;* ZO: *Z. officinale;* PN: *P. nigrum;* CS: *C. sativum*.

The antioxidant activity of plant extracts was further determined by the inhibition of peroxidation of linoleic acid system using thiocyanate method [47]. Linoleic acid is a poly unsaturated fatty acid which upon oxidation forms peroxides those oxidize Fe^{2+} to Fe^{3+}. Fe^{3+} forms complex with thiocyanate ion (SCN^-), whose concentration is determined spectrophotometrically by measuring the absorbance at 535 nm. Higher absorbance denotes higher concentration of peroxides formed during reaction, consequently lower will the antioxidant activity.

3.2.6 Reducing Power (RP)

The RP of a compound may act as a significant indicator of its potential antioxidant activity. With regards to RP, higher reducing capacity might be attributed to the higher number of phenolic compounds. SA exhibited significantly high Fe^{3+} to Fe^{2+} transformation capacity (0.92 ASE/mL) compared to other (Fig. 6). According to Moghadam [48] methanolic extracts of CC showed ferric reducing power at IC_{50} 341.65 µg/mL which is much higher than ethanolic extracts in present study. According to Mashkor [49] fruits and stem of SA exhibited Fe^{3+} to Fe^{2+} transformation capacity at 437.29 and 306.42 IC_{50}, respectively which is much higher than ethanolic extracts in our study (Table 3). The results suggested that phenolics were better extracted in ethanol rather than in other solvents.

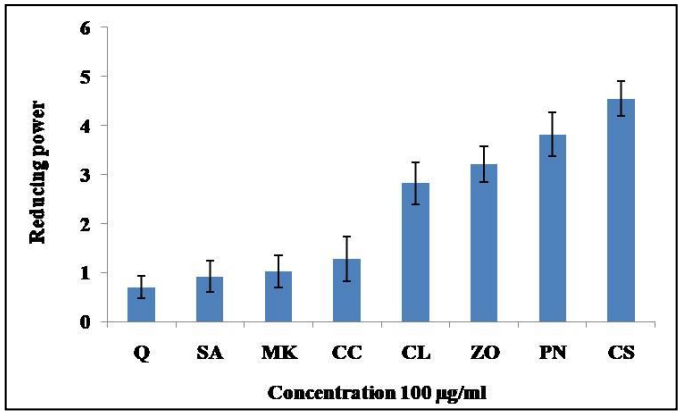

Fig. 6 Reducing power (ASE/mL) of ethanolic extracts of spices in comparison to standard quercetin. Values are mean ± SD of three replications (n=3). SA: *S. aromaticum*; MK: *M. koenigii;* CC: *C. cyminum;* CL: *C. longa;* ZO: *Z. officinale;* PN: *P. nigrum;* CS: *C. sativum*

In the RP assay, the presence of antioxidants in the samples would result in the reduction of Fe^{3+} to Fe^{2+} by donating an electron. Amount of Fe^{3+} reduction can be then monitored by measuring the formation of $(Fe^{3+})_4[Fe^{2+}(CN)_6]_3$ complex (pearl's Prussian blue) at 700 nm, indicates an increase in reductive ability [50]. Fe^{3+} reduction is often used as a significant indicator of electron donating activity which is an important mechanism of phenolic antioxidant action [51].

Table 3 IC_{50} (inhibitory concentration) values (mg/mL) of Indian spices

Spices	[1]IC50 mg/mL	[2]IC50 mg/mL	[3]IC50 mg/mL	[4]IC50 mg/mL	[5]IC50 mg/mL	RP (ASE/mL)
CC	1.95±0.52	5.34±3.29	6.66±0.65	1.07±0.02	3.53±1.83	1.28±0.46
CL	2.21±0.05	6.29±0.59	7.40±0.06	1.90±0.06	4.96±0.98	2.82±0.43
CS	3.31±0.03	8.02±0.33	9.68±0.46	3.10±0.04	7.92±0.75	4.55±0.35
MK	1.80±0.44	3.21±0.44	5.42±0.21	1.07±0.02	3.39±2.08	1.02±0.33
PM	2.41±1.54	7.55±2.70	8.19±0.21	1.07±0.02	5.50±1.45	3.82±0.45
SA	1.02±0.44	4.23±2.70	5.44±0.75	0.07±0.02	0.46±1.45	0.92±0.34
ZO	2.41±0.34	7.98±1.53	7.42±2.26	0.92±0.01	5.96±0.80	3.21±0.36
Q	0.04±0.01	2.33±0.05	0.07±0.021	0.45±0.001	0.06±0.06	0.70±0.23

[1]IC_{50} (Free radical scavenging), [2]IC_{50} (super oxide anion radical scavenging), [3]IC_{50} (lipid peroxidation), [4]IC_{50} (ferric thiocyanate), [5]IC_{50} (hydroxyl radical scavenging activity) and reducing power (RP). CS: C. sativum; CL: C. longa; ZO: Z. officinale; CC: C. cyminum; SA: S. aromaticum; PN: P. nigrum; MK: M. koenigii; Q: Quercetin

3.3 Total Phenolic Content (TPC) in Ethanolic Extracts of Spices

The TPC in ethanolic extracts of spices ranged from 114.11 to 240.23 mg/g GAE (Table 4).

Phenols and polyphenols are the most abundant chemical constituents in plants. The antioxidant properties of phenolic compounds originate from their properties of donating e^- to free radicals to stabilize them. Therefore, we determined the quantity of TPC in ethanolic extracts of Indian spices in order to determine the antioxidant activities [52,53]. El-Serehy et al. [54] showed aqueous extracts of CC contains 22.08 mg/g of GAE TPC which is much lower than ethanolic extracts (Table 4). Results were significant with the study of Ishtiaque et al. & Sivakumar and Meera [55,56]. Phenolics are better extracted in ethanolic solvent than other. This was in significant with the study of Sasidharan and Menon [57].

Table 4 Total phenolic content of ethanolic extracts of Indian spices

Spice	Crude amount (g) after extraction	TPC (mg/g of GAE of DW
CC	5.16	165.75±0.85
CL	5.90	157.81±0.49
CS	7.02	114.11±0.50
MK	5.42	175.24±0.63
PN	5.30	143.31±0.48
SA	4.38	240.23±0.50
ZO	3.24	147.11±1.03

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3.3.1 Correlation between TPC in Ethanolic Extracts in Relation to Their Antioxidant Activity

The linear correlation between total phenolic content and antioxidant assays indicated that these phenolics contribute to the strong antioxidant activities of spices. Correlation between TPC and antioxidant assays showed that 90% FRSA, 91% SARSA, 78% LPO, 87% HARSA and 83% FTC of extracts are contributed by phenolic compounds (Fig. 7). The remaining antioxidant activity is due to non-phenolics compounds. Activity may also come from the presence of other secondary metabolites such as volatiles oils, flavonoids, metalloprotein, vitamins, etc.,.

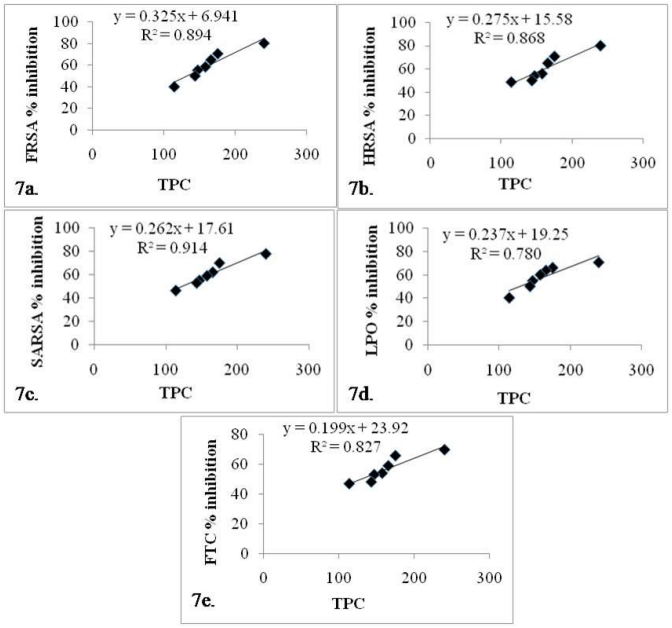

Fig. 7 Linear correlation between TPC (x axis) in the spices extracts in relation to their antioxidant activity (y axis). (7a) TPC versus FRSA, (7b) TPC versus SARSA, (7c) TPC versus LPO, (7d) TPC versus HRSA, (7e)TPC versus FTC assay

4. Conclusion

It is well-known that ROS have significant positive correlation with several diseases such as ageing, atherosclerosis, inflammatory injury, cancer and cardiovascular diseases. The results obtained by us are with respect to the antioxidant activities of the ethanolic extract of spices. Spice extract containing higher phenolic compound showed maximum antioxidant activity. The antioxidant activities of ethanolic extracts of spices may be attributed to their strong hydrogen donating and metal chelating ability, reducing potential, effective hydroxyl and free radical scavenging activity and high levels of phenols that might be responsible for its efficacy as pharmaceuticals.

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