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Comparative Evaluation of the Antioxidant Activity of Some commonly used Spices

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Abstract: The antioxidant activity, total phenolic content (TPC) and total flavonoid content (TFC) of four natural spices namely fennel fruits (*Foeneculum vulgare*), fenugreek seeds (*Trigonella foenum-graecum*), coriander seeds (*Coriandrum sativum* Linn.) and black pepper fruits (*Piper nigrum*) were investigated. In antioxidant assay, DPPH (2,2-diphenyl-1-picrylhydrazyl) scavenging activity and ferric reducing activity studies were performed using ascorbic acid and gallic acid as standard antioxidants, respectively. *In-vitro* antioxidant study of the methanolic extracts of the tested condiments showed significant activity in DPPH method with appreciable lowIC_{50}values.The ferric reducing properties of these spices were also found to be appreciable. The TFC and TPC revealed that the spices had high contents of phenolics and flavonoids. These studies suggested that these spices can be used as rich sources of natural antioxidants against various oxidative stress related diseases.
Keywords: Spices, Flavonoid content, phenolic content, DPPH assay, Ferric reducing power.

Introduction

Antioxidants are referred to as compounds which are able to inhibit the oxidation of different biomolecules and helps in repairing the damages caused to the body tissues due to oxidation processes. Various study has focused on use of antioxidants from natural sources so that they could be used as healthy additives and potential antioxidantin our daily food intake habits[1]. Antioxidants either synthetic or natural are potent scavengers of free radicals and have beneficial effects on human health and disease prevention[2,20-22].

Oxidative metabolism is essential for the survival of cells. A side effect of this dependence is the production of free radicals and other reactive oxygen species that cause oxidative changes. The most widely used synthetic antioxidants in food i.e., butylated hydroxyl toluene (BHT) and butylated hydroxyl anisole (BHA) are very effective in their role as antioxidants. However, their use in food products has been failing off due to their instability or their suspected action as promoters of carcinogenesis. For this reason, there is a growing interest in the studies of natural healthy (nontoxic) additives as potential antioxidants[1].It is known that constituents of plants are associated in reducing the risk of many chronic diseases, in which antioxidants play a major role in their protective effects[3].

Herbs and spices are considered an important part of human diet and have been used for thousands of years in traditional medicine and also to enhance color, flavor and aroma of the food[4].The presence of active components in spices has been demonstrated over the last 30 years and their therapeutic properties have been demonstrated by the presence of many substances, including some vitamins, flavonoids, terpenoids, carotenoids, phytoestrogens and minerals[5].

Spices are common food additives, which are always used as flavoring, seasoning, and coloring agents, and sometimes as preservatives, but they may also be used as medicines. Many spices have been recognized to have medicinal properties and possess many beneficial effects on health such as antioxidant activity, digestive stimulant action, anti-inflammatory, antimicrobial, hypolipidemic, antimutagenic, anticarcinogenic potential etc. The presence of antioxidative and antimicrobial phenolic constituents in spices gives food-preserving properties[6].

Black pepper (*Piper nigrum*) is one of the most popular spice products in oriental countries, largely used as a flavoring agent in foods. The components of the pepper's extract that contribute to its valueas a food additive are the volatile oil for its aroma andthe alkaloid compounds for the pungency[7].Blackpepperis used in skin care, muscle and joint pains, and in improving blood circulation and respiratory systems. The seeds of coriander (*Coriandrum sativum* Linn.)are mainly responsible for the medical use of coriander and have been used as a drug for indigestion, against worms, rheumatism and pain in the joints[8].Fenugreek (*Trigonella foenum-graecum*) has been reported to exhibit pharmacological properties such as antitumor, antiviral, antimicrobial, anti-inflammatory, hypotensive and antioxidant activity[9].Fennel fruit (*Foeneculum vulgare*), a dry seed, is traditionally used as anti-inflammatory, analgesic, carminative, diuretic, antispasmodic agent, in treatment of glaucoma and hypertension[10].These ingredients also have been used in ayurvedic medicines for ailment of various disorders majorly gastrointestinal imbalances which may have been attributed due to their high antioxidant activity[11].

Considering the beneficial effects of spices in treating diseases, methanolic extracts of these commonly used spices i.e. coriander seeds, fenugreek seeds, black pepper fruits and fennel fruit were evaluated for their comparative antioxidant activity evaluation by various *in-vitro* antioxidant models.

Experimental

Chemicals

Aluminum chloride($AlCl_3$), Sodium hydroxide(NaOH), Sodium nitrite($NaNO_2$),DPPH(1,1-Diphenyl–2-picrylhydrazyl), Folin-ciocateau reagent, Sodium carbonate(Na_2CO_3), Trichloroacetic acid(TCA), Potassium ferricyanide, Sodium phosphate buffer, Ferricchloride, Ascorbic acid, Gallic acid and Quercetin.

Collection and authentication

The spices were collected from the local market in Bhubaneswar, India and authenticated by a local botanist.

Extraction

The extraction procedure was done by the process of maceration in which the spices were soaked in methanol solvent for three days with occasional shaking for proper extraction and followed by filtration. The filtrates were evaporated under vacuum and dried to a constant weight using a freeze-drier.

In vitro antioxidant activity evaluation

DPPH radical scavenging activity method

Stock solutions (1 mg/ml) for each ingredient extract was prepared in methanol and diluted subsequently to various concentrations with methanol in time of assay. For the assay, 1ml of different concentration of spice extracts were mixed with 500µl of DPPH solution and 1.5 ml of methanol in sequence and then the samples' were scanned against blank at 517 nm.Similarly readings were taken for standard ascorbic acid[12].IC_{50}values of DPPH radical scavenging activity were determined by the following formula: % radical scavenging activity = $(Abs_{control} - Abs_{sample})/Abs_{control} \times 100$

Reducing power assay

2.5 ml of the spices extracts (1 mg/ml) were taken and mixed with 2.5 ml sodium phosphate buffer (0.2M, pH 6.6) and 2.5 ml of 1 %potassium ferricyanide solution. The mixtures were incubated at 50°C for 20

minutes. Then 2.5 ml of trichloroacetic acid (10%) were added to them and the solutions were centrifuged at 650 rpm for 10 mins. Then 5ml of the supernatents were mixed with 5 ml of distilled water and 1 ml of ferric chloride solution (0.1%) and the absorbances were measured at 700 nm. Higher absorbances of the reaction mixtures indicate a higher reducing power[2].

Total flavonoid content (TFC)

Ina 10 ml test tube, 0.3 ml of extracts (1 mg/ml), 3.4 ml of 30% aqueous methanol, 0.15 ml of sodium nitrite (0.5 M) and 0.15 ml of aluminum chloride (0.3 M) were mixed. After five minutes 1 ml of sodium hydroxide (1M) was added, mixed well and absorbance was taken at 506 nm. The standard curve was constructed using quercetinin concentration range of 50-250 µg/ml. Total flavonoids were expressed as quercetin equivalents(mcg) per gram of extract[13].

Total phenolic content (TPC)

As per the standard protocol, 40 µl of a methanolic extract (1mg/ml) of individual spice, 3.16 ml of distilled water and 200 µl of Folin – Ciocalteau reagent were mixed. After incubation of 8minutes, 600 µl of sodium carbonate solution was added and mixed. The mixture was incubated at 40^0C for 30 min before taking its absorbance at 765 nm. The calibration curve was prepared using Gallic acid as standard from 100 to 200 µg/ml. TPC were expressed as gallic acid equivalent(mcg) per gram of extracts[13].

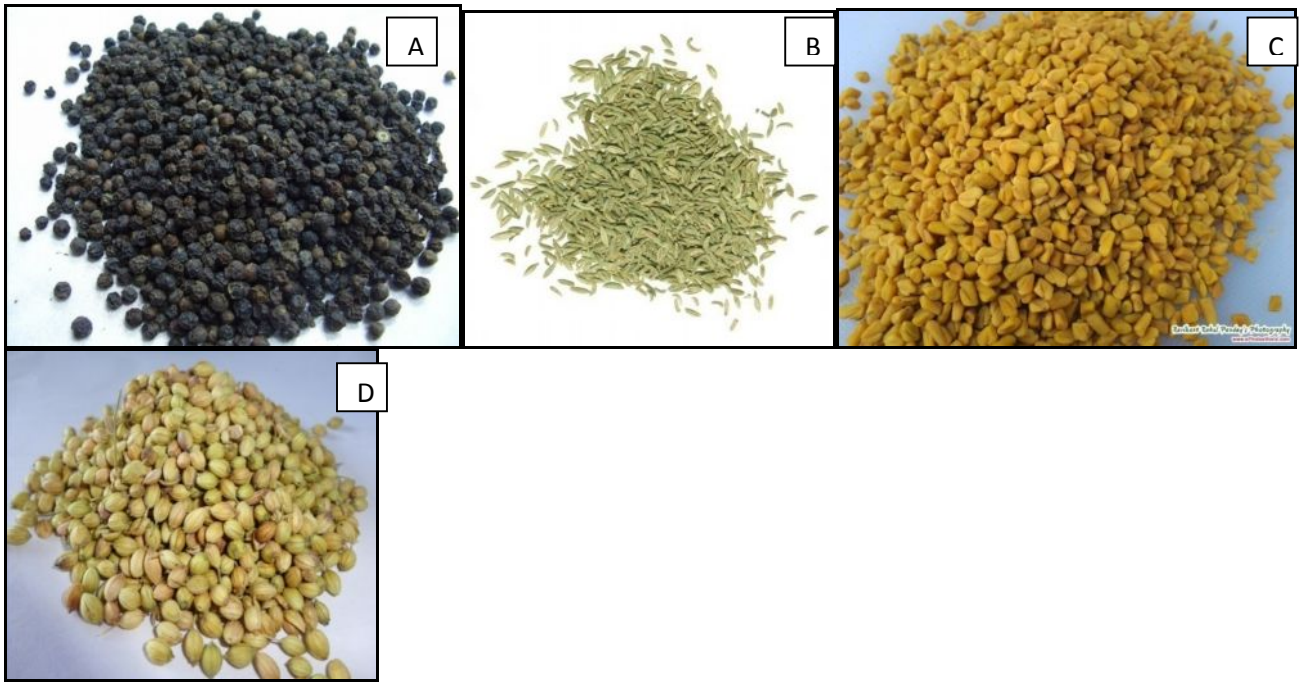

Fig.1: A-Fruits of black pepper, B-Fennel fruit, C-Fenugreek seeds, D-Coriander seeds

Results

Total phenolic and flavonoid contents

Total flavonoids and total phenolic contents were determined as quercetin equivalents in micrograms per gram of extract (QE/gm of extract), while total phenolic contents were calculated as gallic acid equivalents in micrograms per gram of extract (GAE/gm of extract).Results from the quantitative determination of total phenolics and flavonoids of the methanolic extracts of the different spices were summarized in Table 1 and Table2.All the calculations were done using standard equation obtained from standard calibration curves of quercetin and gallic acid as shown in Figure 2 and Figure 3respectively. All the spices showed high total flavonoid content and total phenolic content. TFC of *Coriandrum sativum* Linn.was 23.87 ± 0.81,*Foeneculumvulgare*was 23.41 ± 0.656,*Piper nigrum* was 20.83 ± 1.649 and *Trigonellafoenum- graecum* was10.58 ± 0.46mcg QE/gm of extract. Again, TPC of *Coriandrum sativum* Linn. was 76.376 ±

0.99,*Foeneculumvulgare* was 51.56 ± 3.22, Piper nigrum was 119.39 ± 0.99and *Trigonella foenum- graecum* was59.18 ± 5.79mcg GAE/gm of extract. These results indicated that the phenolic and flavonoid compounds had a major contribution to the antioxidant capacity of herbal spices.

Table 1: **TFC** (**Total flavonoids content) of the Spices**

Sl No.	Spices	Total Flavonoid Content(QE/g of extract)*
1	*Coriandrum sativum L.*	23.87 ± 0.81
2	*Foeneculum vulgare*	23.41 ± 0.656
3	*Piper nigrum*	20.83 ± 1.649
4	*Trigonellafoenum-graecum*	10.58 ± 0.46

*(Values are expressed as Mean ± S.D.)

Table 2: TPC (Total phenolic content) of the Spices

Sl No.	Spices	Total Phenolic Content(mcg/ml of GA)*
1	*Coriandrum sativum Linn.*	76.376 ± 0.99
2	*Foeneculum vulgare*	51.56 ± 3.22
3	*Piper nigrum*	119.39 ± 0.99
4	*Trigonella foenumgraecum*	59.18 ± 5.79

*(Values are expressed as Mean ± S.D.)

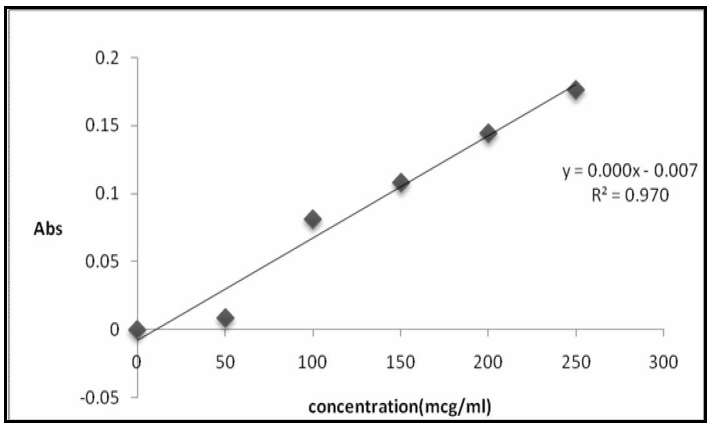

Fig .2: Calibration curve of quercetin standard

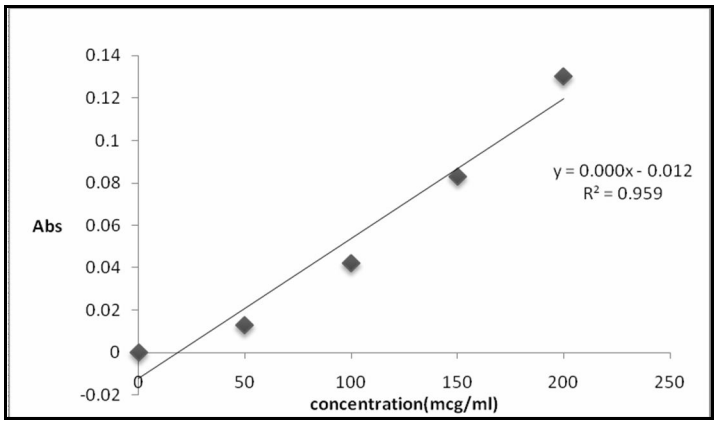

Fig .3: Calibration curve of Gallic acid standard

DPPH radical scavenging activity

The parameter IC_{50} is used for the interpretation of the results from the DPPH method and is defined as the concentration of substrate that causes 50% loss of the DPPH activity. Spice extracts examined in relation to their IC_{50} value and IC_{50} values of *Coriandrum sativum* Linn. was 751± 1.414, *Foeneculum vulgare* was 746.9±2.81,*Piper nigrum* was 444.62±3.38and *Trigonellafoenum- graecum* was772±2.45 (mcg /ml). The methanolic extract of *Piper nigrum* has the highest scavenging activity as compared to the other natural spices used in this study due to its less IC_{50} value as shown in Table3.

Table 3: IC_{50} values of the spices and standard ascorbic acid in DPPH radical scavenging assay

Sl No.	Ingredients and Standard	IC_{50} Value(mcg/ml)*
1	*Coriandrumsativum Linn.*	751± 1.414
2	*Foeneculumvulgare*	746.9±2.81
3	*Piper nigrum*	444.62±3.38
4	*Trigonellafoenumgraecum*	772±2.45
5	Ascorbic acid	19.83± 0.11

*(Values are expressed as Mean ± S.D.)

Reducing power assay

In this assay, the yellow color of the test solution changes to green depending on the reducing power of test specimen. The presence of reductants in the solution causes the reduction of the $Fe^{3+/}$ferricyanide complex to the ferrous form. Therefore, Fe^{2+} can be monitored by the measurement of the absorbance at 700 nm[14].The order of ferric reducing property of tested spice samples were found to be *Foeneculum vulgare>Piper nigrum>Coriandrum sativum L.>Trigonella foenum-graecum*(Figure 4).

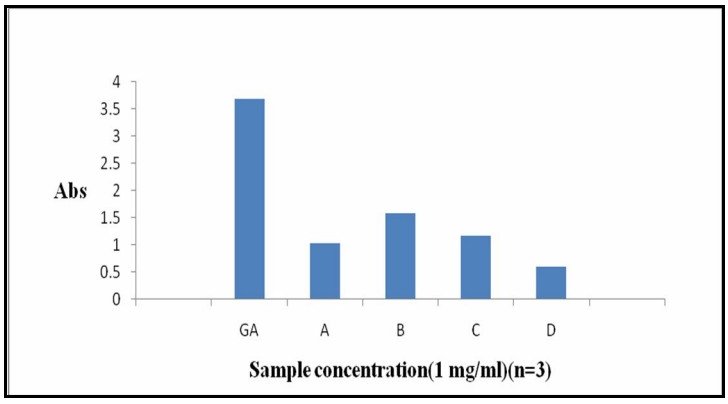

Fig.4: Shows the dose response plot for reducing powers of the spice extract samples (A- *Coriandrum sativum L.*, B- *Foeneculum vulgare*, C- *Piper nigrum*, and D- *Trigonella foenum-graecum*) and standard Gallic acid (GA)

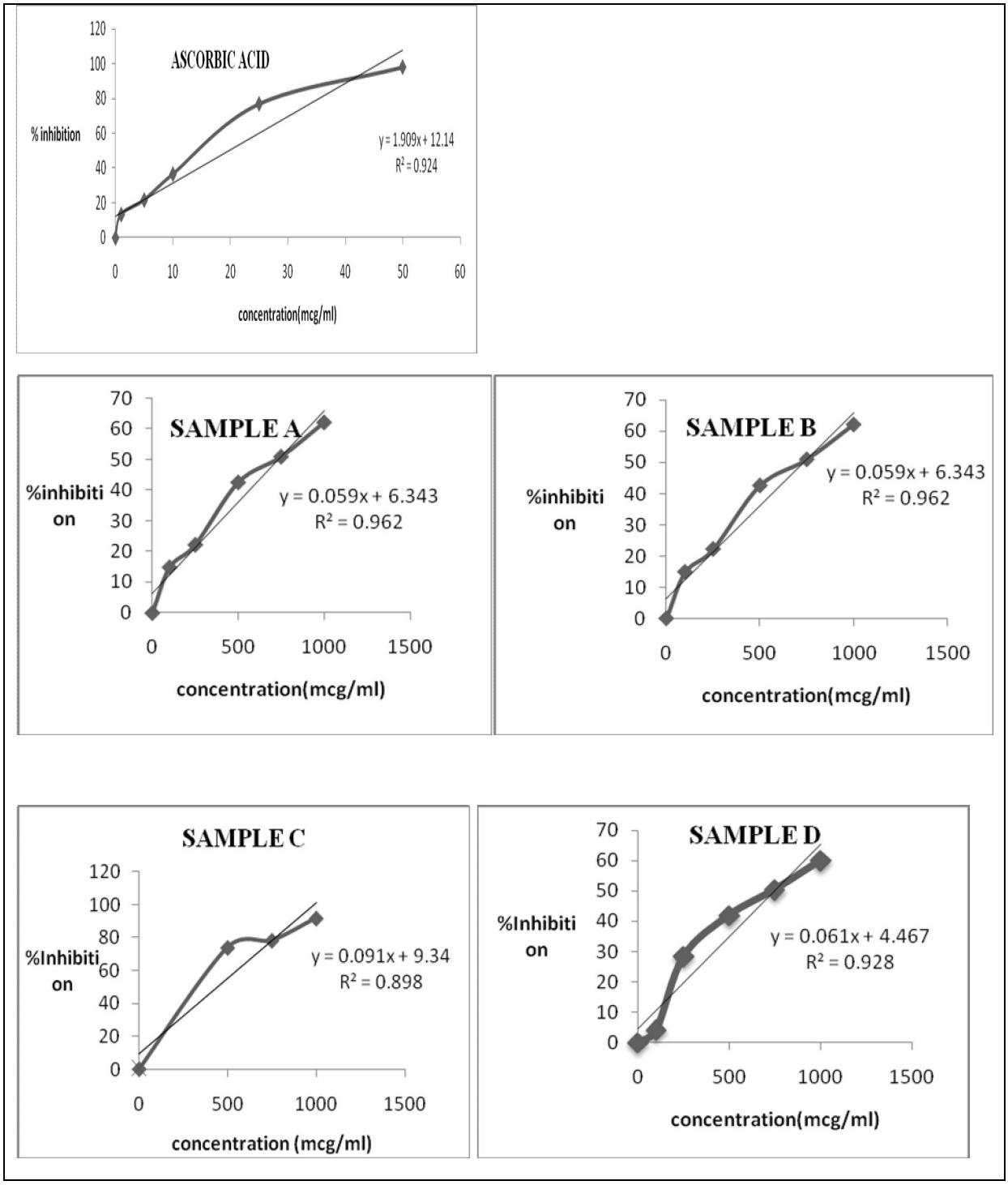

Fig.5: Percentage Inhibition versus concentration plot of samples (A- *Coriandrum sativum L.*, B- *Foeneculum vulgare*, C- *Piper nigrum*, D- *Trigonella foenum-graecum*) and standard ascorbic acid in DPPH radical scavenging activity study.

Discussion

Naturally antioxidants present in many plants, food, sand beverages offer health benefits in preventing various diseases by fighting cellular damage caused by free radicals in the body[15]. Free radicals have been implicated in many disease conditions, the important ones being superoxide radical, hydroxy radical, peroxyl radical and singlet oxygen. Herbal drugs containing radical scavengers are gaining importance in treating such diseases[16].The reducing properties are generally associated with the presence of reductones, which have been shown to exert antioxidant action by breaking the free radical chain by donating a hydrogen atom and may have great relevance in the prevention and treatment of diseases associated with oxidants orfree radicals.

The DPPH assay is based on the ability of the antioxidants present in the sample to decolorize DPPH free radical by virtue of their scavenging activities. Ascorbic acid was chosen as the reference antioxidant for this test. The DPPH method is widely used to test the ability of compounds to act as free radical scavengers or hydrogen donors, and to evaluate antioxidant capacity. The parameter IC50 (efficient concentration value), is used for the interpretation of the results from the DPPH method and is defined as the concentration of substrate that causes 50% loss of the DPPH activity (color)[17].

Phenols are very important plant constituent because of their scavenging ability due to their hydroxyl group. It has been recognized that flavonoids show antioxidant activity and their effects on human health are considerable. The mechanisms of action of flavonoids are through scavenging or chelating process. The flavonoids which contain hydroxyls are responsible for the radical scavenging effect in the plants. It has been reported that the antioxidant effect of plant products is mainly due toradical scavenging activity of phenolic compounds such as flavonoids, polyphenols, tannins, and phenolicterpenes[18].Thehigh content of flavonoids and polyphenolic compounds in the tested spices may have contributed in their antioxidant activity may be due to their redox properties, which can play an important role in adsorbing and neutralizing free radicals, quenching singlet and triplet oxygen, or decomposing peroxides[19].

Conclusion

In the present study the methanolic extracts of the selected spices have been evaluated for potential antioxidant activities. Antioxidant activities of the spices were high enough for the spices to be known as new and natural sources of antioxidant substances for use as natural additives in food. The amount of phenolic content in the extract of *piper nigrum* may be the reason for its potent antioxidant activity whereas other spices have significant phenolic and flavonoids contents which assure their antioxidant properties. It could be concluded that methanolic extracts of these spices could be used as natural means of antioxidants. Hence, inclusion of these spices in the cuisine will be helpful in preventing or slowing the progress of various oxidative stress related diseases.

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