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Differential Antioxidant Composition and Potential of some commonly used Indian Spices

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ABSTRACT

Spices and herbs have been added to Indian foods not only as flavoring agents but also as natural supplements of medicines and food preservatives. The purpose of this study was to study the composition and antioxidant potential of some commonly used seed spices and derive a correlation between their phytochemical components and antioxidant capacity. Crushed seeds of cumin (*Cuminum cyminum*), coriander (*Coriandrum sativum*), fennel (*Foeniculumvulgare*), fenugreek (*Trigonellafoenum-graecum*), ajwain (*Trachyspermum ammi*) and turmeric (*Curcuma longa*)were usedto prepare methanolic extract and iron (III) reduction, DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging, hydrogen peroxide , superoxide and nitric oxide radical scavenging, reducing power were assayed as an index of the antioxidant capacity along with phenolics and flavonoids were also estimated. In addition to turmeric, ajwain, fennel and coriander showed higher phenolic, flavonoid content, and antioxidant potential in comparison to fenugreek and cumin. The extracts of these spices are promising sources of alternative medicine with high free radical scavenging ability of synthetic antioxidants and can also be used for therapeutic purposes as well.

Keywords: Ajwain, Antioxidant, Coriander, Cumin, Fennel, Fenugreek, Seed spices

INTRODUCTION

Free radicals and ROS (reactive oxygen species) are charged molecules that are continuously produced by human body as a consequence of both enzymatic and non-enzymatic reactions. The accumulation of ROS and free radicals leads to oxidative stress and accounts for the pathophysiology of highly prevalent human diseases like atherosclerosis, diabetes, hypertension, Alzheimer and Parkinson diseases. Antioxidants on the contrary also called "free radical scavengers" are chemicals that block the activity and neutralize the free radicals, thus preventing them from causing damage. Hence a balance between the ratio of antioxidants and free radicals is necessary for the proper metabolic function. A minor shift in balance can lead to oxidative stress. In the recent years, there has been an increasing interest in the role of synthetic and natural antioxidants for therapeutic purposes. They are widely used in dietary supplements and their role has been investigated in the prevention of diseases such as coronary heart disease, cancer, aging and altitude sickness. Ascorbic acid, cysteine, flavonoids, phenolic acids, anthocyanins, α-tocopherol and glutathione are the different that nutrients act as antioxidants for cardio protective action (Vinson *et al*., 1995a and b; Wang *et al*., 1997). Several experiments have focused on natural sources of antioxidants and their applications in food systems to cure many human diseases. BHT (butylated-hydroxytoluene) and BHA (butylated hydroxyanisole) are the most widely used supplementary artificial antioxidants in diet and are effective as antioxidants, but are slowly compromised owing to their instability and suspected action as promoters of carcinogenesis (Namiki, 1990). To address this issue, there is a growing interest in the studies of natural healthy (nontoxic) additives as potential antioxidants (Tomaino *et al*., 2005).

Herbs and spices are the rich natural source of antioxidants. Most of the antioxidant potential of herbs and spices is due to the redox potential of phenolic moieties, which allow them to act as reducing agents, hydrogen donators and singlet oxygen quenchers (Caragay, 1992). Seed spices are the major flavoring and fragrance agents of the Asian dishes. They can be added to foods in several forms, i: whole seeds ii: as ground seeds and iii: as isolates from their extracts. Seed spices are aromatic and pungent food ingredients with significant antioxidative effects (Suhaj, 2006) due to the presence of antioxidants like vitamin A, vitamin C, vitamin E, glutathione as well as some enzymes like catalase, superoxide dismutase and various peroxidases. Previously many investigations have been done to analyze the antioxidant properties of herbs (Wojdyło *et al*., 2007), cumin (Thippeswamy and Naidu, 2005), coriander (Wangensteen *et al*., 2004), fenugreek (Bukhari *et al*., 2008), fennel (Oktay *et al*., 2003), and Ajwain (Singh *et al*., 2004). However, there has been no previous reports comparing the level of antioxidants and their potential in major edible Indian seed spices in spite of having great medicinal and antioxidant properties.

Cumin (*Cuminum cyminum*) seeds contain numerous phyto-chemicals that are known to have antioxidant, carminative and anti-flatulent properties. Cumin seeds contain flavonoid phenolic anti-oxidants such as carotenes, zea-xanthin, and lutein and are an excellent source of dietary fiber (Norman, 1990). Coriander (*Coriandrum sativum*) seeds are rich source of antioxidants, essential oils, vitamins, and dietary fiber that

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help in reducing LDL (Low Density Lipoproteins) while increasing HDL (High Density Lipoproteins) levels. Coriander seed oil works as analgesic, aphrodisiac, anti-spasmodic, deodorant, digestive, carminative, fungicidal, lipolytic (weight loss), stimulant and stomachic medicine. (Husain *et al.,* 2008). Fenugreek (*Trigonellafoenum-graecum*) contains non-starch polysaccharides (NSP) which constitutes major fiber content that includes saponins, hemicellulose, mucilage, tannin, and pectin. These compounds help lower blood LDL-cholesterol levels by inhibiting bile salts re-absorption in the colon. They also bind to toxins in the food and help to protect the colon mucus membrane from cancers (Puri, 1998). Fennel (*Foeniculum vulgare*) seeds contain numerous flavonoids, and antioxidant compounds like kaempferol and quercetin. These compounds function as powerful antioxidants by removing harmful free radicals from the body thus protect from cancers, infection, aging and degenerative neurological diseases (Oktay *et al.,* 2003). Ajwain (*Trachyspermum ammi*) an erect annual herb originating from Persia and India, has been used by medical practitioners for its medicinal and pharmacological effects. In Ayurvedic medicines, it is used as a medicinal plant for its antispasmodic, stimulant, tonic, and carminative properties. Turmeric (*Curcuma longa)* rhizome is crushed to a yellow colored powder and consumed in amount upto one g/day since a long time in many Asian countries especially India. Previous researches have demonstrated the anti-tumor, anti-inflammatory, anti-infectious and antioxidant activity of turmeric with very low toxicity (Shalini and Srinivas, 1987 and 1990). Hence, the present research was designed to identify and compare the phytochemical and antioxidant potential of methanolic extracts of these commonly used seed spices from India by number of testing methods. To conclude the present study entails the beneficial role of antioxidants in major Indian spices like cumin, coriander, ajwain, turmeric, fennel and fenugreek in the light of its phytochemical content and free radical scavenging assays like DPPH, superoxide, nitric oxide and H_2O_2.

MATERIALS AND METHODS

Material

Seeds of commonly used spices namely cumin, coriander, ajwain, fenugreek, fennel and rhizomes of turmeric were purchased from Indian store in Bentonville, Arkansas, USA. The seeds were dried, crushed to powder in liquid nitrogen and extracted with methanol. The methanolic extracts were aliquoted and stored at -70°C for further use.

Estimation of phenolic content and flavonoids

Total phenolic content was estimated from the seed spices and turmeric rhizomes following the protocol of Jayaprakasha *et al.* (2001) with minor modifications. An aliquot of 100µl of methanolic extract was mixed with 500µl of ten-fold diluted Folin-Ciocalteu reagent and 400µl of 7.5% (w/v) sodium carbonate. The reaction mixture was incubated at room temperature (25ºC) for 30 min, following absorbance measurement at 760 nm. The phenolic content was finally expressed as tannic acid equivalents.

Flavonoid content was estimated by following a modified spectrophotometric method (Basu *et al.,* 2012). 500mg of each sample of seed spices under investigation was grounded to fine powder in 10ml methanol and extracted thrice with 10ml of n-butanol. All the extracts were pooled together and concentrated under vacuum at 60°C. The filtrate was re-suspended in 5ml of 60% (v/v) ethanol and washed twice with 5ml of 30% (v/v) ethanol and filtered. The filtrate was diluted upto 25ml with 30% (v/v) ethanol. An aliquot of 100 µl of the extract was mixed with 900µl of 30% (v/v) ethanol and 60 µl of 5% (w/v) sodium nitrite for 5min followed by the addition of 60 µl of 10% (w/v) aluminium nitrate. The reaction was stopped after 6min, by adding 2ml of 1M NaOH. The reaction mixture was further diluted upto 3ml with 30% (v/v) ethanol. The absorbance of the mixture was measured immediately at 510nm and the flavonoid content was calculated and expressed as rutin equivalents.

Total antioxidant capacity

Assessment of total antioxidant capacity is based on the color formed resulting from the reduction of Mo (VI) to Mo (V) by the methanolic extract resulting in a green phosphate/Mo (V) complex at acidic pH (Prieto *et al*., 1999). The reaction mixture comprising of methanolic extract (50µl) and 1.25ml reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate and 4mM ammonium molybdate) was incubated at 95º for 90 minutes and cooled down to room temperature. The absorbance was measured at 695nm and the results are expressed as ascorbic acid equivalents.

Determination of reducing power

Methanolic extract (100 µl) was mixed with 2.5 ml of 0.2M phosphate buffer (pH 6.6) and 2.5 ml of 1 %(v/v) potassium ferricyanide and incubated at 50˚C for 20 min. Following incubation, 2.5ml of 10% trichloroacetic acid (TCA) was added and the mixture was centrifuged at 8000 rpm for 5 min. The aqueous layer (2.5 ml) of the solution was mixed with 2.5ml distilled water and 0.5ml of 0.1% (w/v) ferric chloride and then absorbance was measured at 700 nm. Increment of absorbance of different methanolic extracts indicates increased reducing power (Syeda *et al*., 2008).

DPPH radical scavenging activity

Scavenging of DPPH radical by methanolic extract is the most commonly used procedure to assay the antioxidant potential. The free radical scavenging abilities of the samples were measured from the bleaching of the purple-colored methanolic solution of 1, 1-diphenly-2-picrylhydrazyl (DPPH) (Brand-williams *et al.,* 1995; Wojdyło *et al.,* 2007). Methanolic extract (100µl) was added to 1ml of 0.004% (v/v) methanolic solution of DPPH in the dark. After 30 minutes incubation the absorbance was measured at 517 nm. The % inhibition exhibited by the extracts was calculated as $\{(A_{Control}-A_{sample})/A_{control}\}$ x100 where A is the absorbance. Control here refers to the reaction mixture without the methanolic extract.

Assay of superoxide radical (O_2^-) scavenging activity

Scavenging of superoxide radical by the methanolic extract was carried out using the riboflavin-light-nitrobluetet-razolium (NBT) system (Martinez *et al.,* 2001). The reaction mixture (3ml) was prepared by mixing 50mM phosphate buffer (pH 7.8), 13 mM methionine, 2µM riboflavin, 100µM EDTA, NBT (75µM) and 1ml of methanolic extracts. Formation of blue colored formazan was measured by monitoring the increase in absorbance at 560nm after 10 mins of illumination from a fluorescent lamp. Scavenging ability of the extracts were calculated as $\{(A_{Control}\text{-}A_{sample})/A_{control}\}$ x100 where A is the absorbance. Control here refers to the reaction mixture without the methanolic extract.

Assay of NO radical scavenging activity

Generation of nitric oxide radical from sodium nitroprusside was measured using Griess reagent (Marcocci *et al.*, 1994). 100µl of methanolic extracts was mixed with sodium nitroprusside (5mM) in phosphate buffered saline (PSB) in a final volume of 1.5ml and incubated at 25ºC for 150 minutes. After incubation, 500µl of samples were removed and diluted with same amount of Griess reagent {1% (w/v) sulphanilamide and 0.2% (w/v) N-(1-naphthyl) ethylene diaminedihydrochloride (NEDH)}. The nitrite produced was measured colorimetrically at 540nm, after 15minutes of incubation. The inhibition percentage was calculated as $\{(A_{Control}\text{-}A_{sample})/A_{control}\}$ x100 where A is the absorbance. Control here refers to the reaction mixture without the methanolic extract.

Measurement of Fe (II) chelating activity

Measurement of Fe (II) chelating activity was done following the method of Carter (Carter, 1971) with modifications. Methanolic extract (50µl) was added to 100µl of 2mM $FeCl_2$, 200 µl of 5mM ferrozine and mixed thoroughly. The reaction mixture was kept at 25ºC for 10minutes and then absorbance was measured at 562 nm. The % Fe(II) chelating activity was calculated as $\{(A_{Control}\text{-}A_{Sample})/A_{Control}\}$x100 where A is the absorbance and expressed as µg EDTA equivalents g^{-1} of defatted material using a standard curve prepared with EDTA. Control here refers to the reaction mixture without the methanolic extract

Inhibition of conjugated diene formation in linoleic acid emulsion

Methanolic extract (100µl) was added to 500µl of 10mM linoleic acid emulsion (pH 6.6) and incubated for 15h at 37ºC with shaking. Following incubation, 100 µl of the solution was added to1.75ml of 80 %(v/v) methanol and absorbance was recorded at 234 nm and the conjugated diene formation (%) was calculated as $\{(A_{Control}\text{-}A_{sample})/A_{control}\}$ x100 where A is the absorbance (Basu *et al.*, 2012). Control here refers to the reaction mixture without the methanolic extract.

Measurement of H_2O_2 scavenging activity

Methanolic extract (100µl) was added to 750µl of 0.1M phosphate buffer (pH 7.4) and mixed with 150µl of 43mM H_2O_2 solution (prepared in phosphate buffer) for H2O2 scavenging activity assay. The absorbance value was recorded twice after 0min and 40min and the concentration of H_2O_2 in the assay mixture was determined using a standard curve. A separate blank was used (devoid of H_2O_2) for each time point for background subtraction (Ruch *et al.*, 1989).

Statistical analyses

All the analysis was carried out in triplicate, and the obtained experimental results were expressed as means ± Standard Error (n = 3). The statistical significance was evaluated at $P \leqslant 0.05$ by two-sided Student's t-test using Microsoft excel package.

RESULTS AND DISCUSSION

Oxidative stress resulting from ROS accumulation are implicated in many clinical disorders affecting human health such as cancer, diabetes, heart diseases and cerebrovascular diseases through multiple mechanisms (Yoshikaw *et al.*, 2000). Consequently, antioxidants can help in therapies for these diseases. The higher level of antioxidant in various herbs and spices has a significant role in the prevention of various neurodegenerative diseases (Hu and Willett, 2002). To meet the growing demand of the consumers for naturally occurring antioxidants in food, researchers have started investigating the antioxidative potential of various plant sources. Seed spices such as cumin, turmeric and coriander which not only excite our taste buds but also have a major role in maintaining human health owing to their impressive list of phyto-nutrients, essential oils, antioxidants, minerals, and vitamins that are essential for overall human health. Coriander and fennel has been used as medicine for the relief of anxiety, insomnia (Deepa *et al.*, 2013) and also finds a potential role in therapeutics as a result of diuretic, analgesic, anti-inflammatory and antioxidant activities (Choi and Hwang, 2004). Similarly ajwain, cumin, fenugreek, turmeric have also been reported to have medicinal and hepatoprotective effects (Anilakumar *et al.*, 2009; Gilani *et al.*, 2005). Consequently, in the current study we have compared the antioxidant properties of the commonly used Indian seed spices in Asian food.

Total phenolic content and flavonoids

Results showed that ajwain had the highest phenolic content (22.1mg/g) followed by fennel and coriander (12.1 and 11.22 mg/g respectively) while the others followed the pattern fenugreek>cumin> turmeric (Fig.1). Turmeric and Fennel had the highest flavonoid content (27.3 and 19.3 mg/g respectively) followed by ajwain which followed the pattern ajwain> cumin> coriander> fenugreek (Fig. 2). Previously it

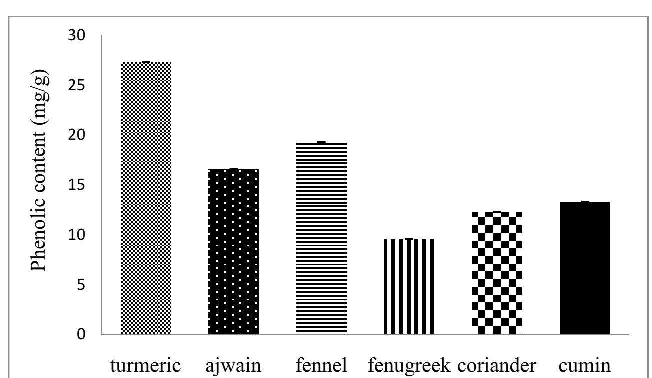

Fig. 1: Estimation of total phenolics (A) in the methanolic extract of different Indian spices

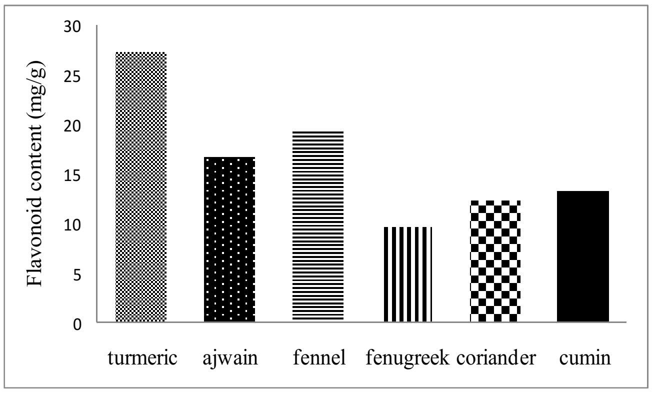

Fig. 2: Estimation of total flavonoids in the methanolic extract from different Indian spices

has been shown that the flavonoids, polyphenols, and anthocyanins contribute significantly to the antioxidant properties (Basu *et al.*, 2012) owing to the presence of conjugated electron systems that allows easy donation of electrons or H atoms from hydroxyl moieties to neutralize the free radicals. Polyphenolic compounds are effective scavengers of hydroxyl and peroxyl radicals and can stabilize membrane lipid peroxidation as well.

Total antioxidant capacity and DPPH radical scavenging activity

Methanolic extracts of turmeric, ajwain, cumin, coriander,

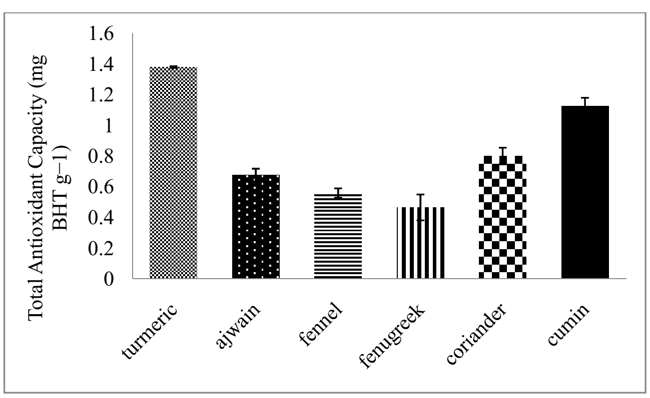

Fig. 3: Total antioxidant capacity by the methanolic extract of different Indian spices

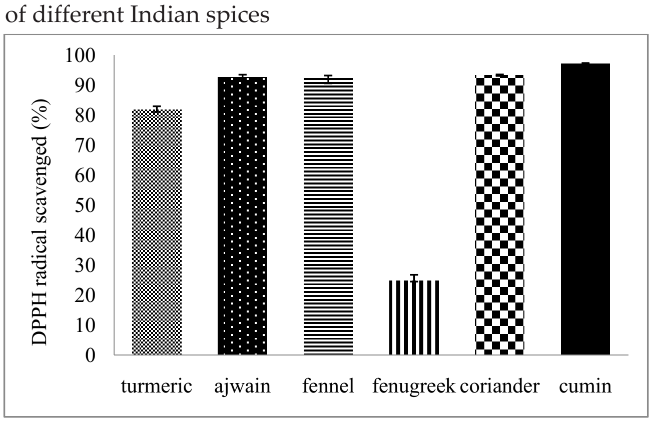

Fig. 4: Percentage DPPH radical scavenged by the methanolic extract of the different Indian spices

and fennel had a high DPPH radical scavenging activity (>80%) except for fenugreek which showed 24% DPPH radical scavenging ability (Fig. 3). Similar pattern was observed for total antioxidant capacity as well with turmeric being the highest followed by cumin (Fig. 4).In recent years, several in vitro methods for evaluating the antioxidant activity have been developed to measure the efficiency of natural antioxidants either as pure compounds or as plant extracts. DPPH is a stable free radical that is widely used for evaluating the antioxidant potential of plant extracts which changes to a stable diamagnetic molecule on accepting an electron or hydrogen radical (Thaipong *et al*., 2006; Khalaf *et al*., 2008).

Nitric oxide radical scavenging assay

The methanolic extract of ajwain and fennel showed the highest NO scavenging ability (>55%) followed by coriander> fenugreek> turmeric> cumin in descending order (Fig. 5). In addition to ROS, another key mediator of various physiological processes is nitric oxide, which is a potent diffusible free radical generated by the endothelial cells and macrophages. The observation from the current study can be correlated to the presence of phenolic compounds (Sonawane *et al*., 2010).

Reducing power and superoxide radical scavenging assay

Turmeric and ajwain had the highest reducing power while fennel had the highest superoxide radical scavenging activity correlating their ability to the increased flavonoid content

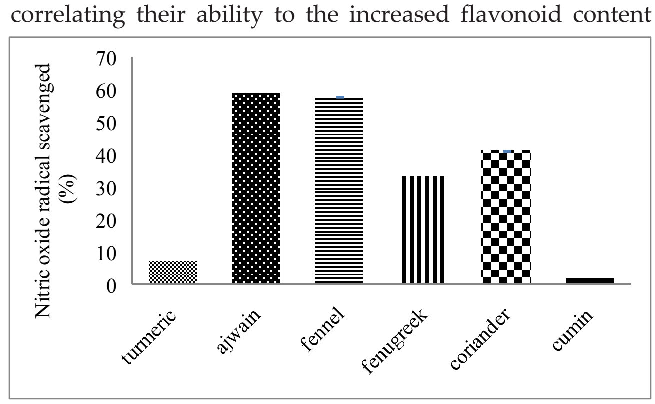

Fig. 5: Percentage of NO radical scavenged in different India spices

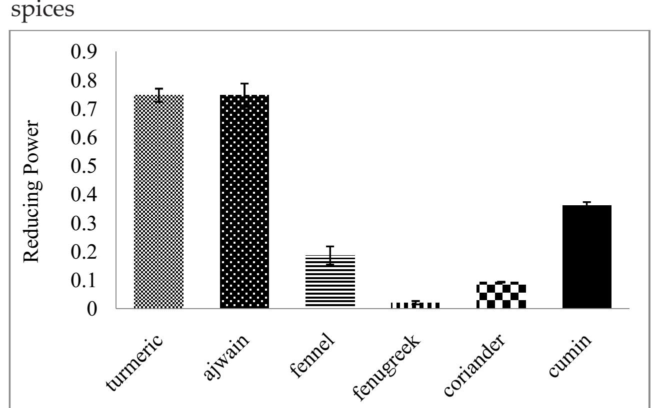

Fig. 6: Reducing power ability in different India spices

(Fig. 5 and 6). The reducing power of plant extracts is attributed to the presence of reductones which is shown to exert antioxidant action by breaking the free radical chain by donating the hydrogen atom (Duan *et al*., 2007). Superoxide anion creates oxidative stress by generating powerful and dangerous hydroxyl radicals as well as singlet oxygen and damages cells by causing lipid peroxidation (Basu *et al*., 2012; Chanda and Dave, 2009).

Previous researches has been reported that flavonoid content is associated with superoxide radical scavenging activity primarily due to the presence of polyhydroxylated substitution on ring A or Band a free 3-hydroxyl substitution (Bravo, 1998; Chanda and Dave, 2009; Chen and Yen, 2006; Siddhuraju *et al*., 2002) which is consistent to our observation from the study under consideration.

Fe (II) chelation capacity

Neutralizing and minimizing the level of Fe (II) might be helpful in removing the ROS mediated oxidative damage and improving the level of antioxidant action by inhibiting the metal catalyzed reaction. Hence the extent of chelation of ferrous sulphate by the methanolic extract of the seed spices was analysed. In this assay, coriander, turmeric, and ajwain had the highest iron chelation capacity (>75%) followed by the others in the order of cumin > fennel > fenugreek (Fig. 7).

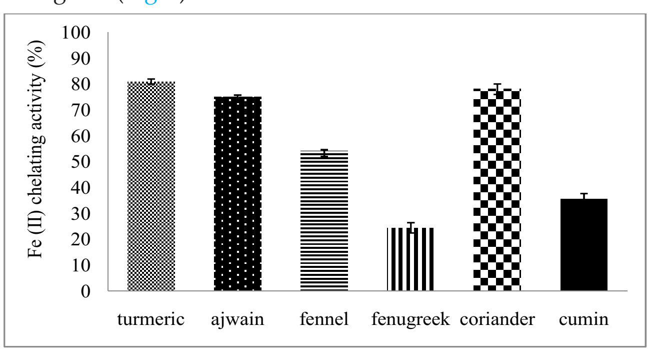

Fig. 7: Percentage of Fe (II) chelation activity in India spices

Inhibition of conjugated dienes and H_2O_2 radical scavenging activity

Increased inhibition of conjugated diene formation might be an indicator of prooxident formation. Experiments conducted with the methanolic extracts showed that all the seed spices had an inhibition of conjugated diene formation >55% while cumin and turmeric had the lowest of 28% (Fig. 8). Oxidative stress is caused chiefly due to the accumulation of ROS. The most common form of ROS produced by the mitochondria is H_2O_2 that is cytotoxic and can alter protein structure or function. Consequently, the issue of scavenging peroxide radical is of exceeding importance. Turmeric a had the highest scavenging ability (45%) followed by cumin (35%) while the others had a very lower ability (>15%) (Fig. 9). Increased phenolic content may be attributed to the peroxide scavenging ability which can donate electrons thereby neutralizing it to water (Nabavi *et al*., 2009; Ebrahimzadeh *et al*., 2009).

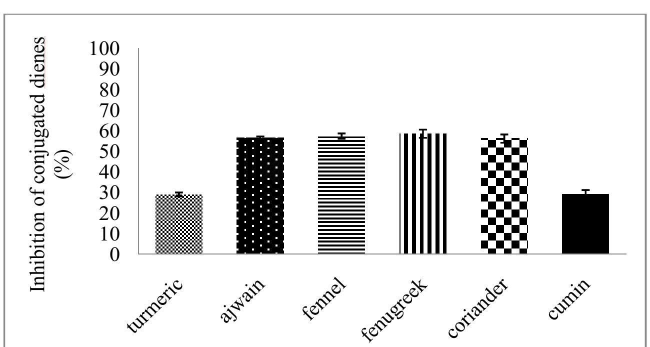

Fig. 8: Percentage of inhibition of conjugated diene formation in India spices

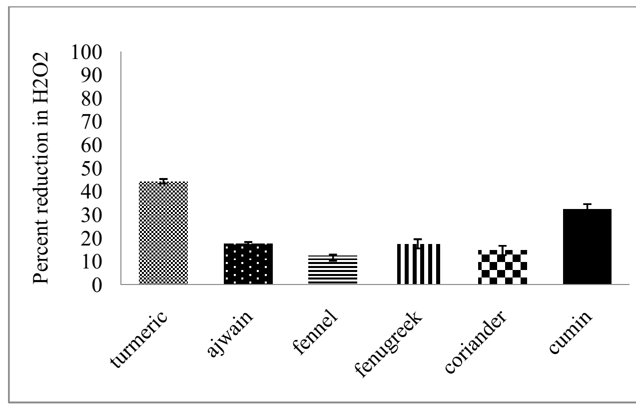

Fig. 9:Percentage of reduction in H_2O_2 concentration in the methanolic extract of the Indian spices

CONCLUSION

Increased antioxidant potential might be due to the accumulation of substances, including some vitamins, flavonoids, terpenoids, carotenoids, phytoestrogens, minerals, etc. In the present study, a linear correlation was observed between the accumulation of phenolic compounds and flavonoids to the antioxidant potential of the seed spices. Furthermore, this is the first time where we have reported on the differential antioxidant potential of the commonly used seed spices in Indian food. Here, we have seen that turmeric; ajwain, fennel, coriander, and cumin had the highest phenolic, flavonoid content in comparison to fenugreek. Moreover they also showed increased DPPH radical scavenging activity and increased ability to scavenge peroxide, nitric oxide, and superoxide radicals.

In the light of the current observation, it can well be concluded that antioxidant properties associated with the seed spices can be a rich source of therapeutic purposes instead of the commercially available antioxidant compounds. However, the further analysis of identification of individual antioxidant compounds occurring in the Indian seed spices and characterization of their antioxidant potential is warranted.

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