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Research Article

ANTIOXIDANT ACTIVITY SCREENING OF SOME COMMON INDIAN APIACEAE FAMILY SPICE PLANTS

***Riddhi M. Patel and Yogesh T. Jasrai**
Department of Botany, University School of Sciences, Gujarat University, Ahmedabad, Gujarat, India- 380 009
**Author for Correspondence*

ABSTRACT
Herbs and spices have been used since ancient times for the preparation of foodstuffs to enhance their flavor and organoleptic properties. Essential oils and secondary metabolites of such plants besides having a strong and pleasant aroma, possess bio-active antioxidant property. Plant-based antioxidant constituent's act as radical scavengers, and helps in converting the reactive free radicals to less reactive species. The antioxidant role of aromatic principles also have medicinal effects and thus when included in regular diet, contributes long term health benefits by preventing cellular damage and reducing the risk of chronic and degenerative diseases. Present work is an effort to find the health benefits associated with the consumption of spices and their antioxidant/antiradical role in our body. In the present investigation, hexane extracts of seeds of five commonly used Indian spices were screened using Total Phenol Content estimation, % FICA (Ferrous Ion Chelating Activity) and % DPPH RSA (2,2-diphenyl-1-picrylhydrazyl Radical Scavenging Activity). In the study, spice extracts demonstrated an excellent antioxidant activity and good amount of activity recorded with *Trachyspermum ammi* for % phenol content, and *Cuminum cyminum*; *Coriandrum sativum* extracts for % FICA and *Trachyspermum ammi*, *Coriandrum sativum* and *Anethum sowa* extracts for % DPPH RSA.

Keywords: *Plant Extracts, Essential Oils, Antioxidant, Free Radicals, Chronic and Degenerative Diseases*

INTRODUCTION
Antioxidant compounds: During the normal metabolism in a living system, oxidation reactions produce free radicals. Free radicals are a group of bioactive compounds that possess unpaired electrons which start off chain reactions and they are able to react and harm other stable molecules in a living system. When the free radicals damage continues for a long time, it may lead to disease development. While Antioxidants are compounds that protect cells against reactive oxygen or free radicals in the body. Antioxidants are free radical scavengers and they terminate these chain reactions by being oxidizing themselves and acting as reducing agents. More precisely, antioxidants can interfere with oxidation process by reacting with free radicals, chelating metals and also by acting as oxygen scavengers, triplet as well as singlet form and transferring hydrogen atoms to the free radical structure (Aluyor and Ori-Jesu, 2008).

For the screening of antioxidant activity; various asssays are used as various phyto-chemical have its own way to counteract free radicals. Thus different assays are designed in order to find the mechanism of antioxidant action and responsible components behind. Some of the important assays routinely used for evaluations are, (DPPH) Radical Scavenging method, Lipid peroxidation assay, Ferric Reducing Antioxidant Power (FRAP), Thiobarbituric acid Reactive Species (TBARS), Hydroxyl radical scavenging assay in the Fenton reaction, Total phenolic content (TPC), ABTS radical scavenging activity, Determination of antioxidant activity in Linoleic acid system, Thiobarbituric acid radical scavenging (TBARS) activity, CUPRAC assay, β-Carotene bleaching method, Luminol-Photochemiluminescence (PCL) assay, Automatic Determination of the Oxidative Stability of Fat (RANCIMAT) etc.

Plants as source of Antioxidant Phyto-chemicals: The plants are susceptible to damage caused by active oxygen and thus develop numerous antioxidant defense systems resulting in formation of numerous potent antioxidant components, also referred as plant secondary metabolites. Fruits, vegetables and herbs,

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are known to contain large amount of antioxidants (Misharina *et al.*, 2009). With the development in techniques and recent researches, it has been proved that certain non-nutritive chemicals in plants such as terpenoids and flavonoids which were earlier thought to be of no importance to human diet, possess antioxidant properties. Many aromatic plants are medicinally important due to presence of rich variety of secondary metabolite content and thus their usage in regular diet, not only serve as source of fragrance and flavoring agent, but also provides dietary antioxidants. Plant secondary metabolites like flavonoids, isoflavones, flavones, anthocyanins, coumarins, lignans, catechins, isocatechins, and also vitamins C and E, β-carotene, and α-tocopherol are found to possess antioxidative property (Aqil *et al.*, 2006).

The aromatic principles or essential oils are made of a complex mixture of organic substances with different functional groups like phenolic compounds containing hydroxyl groups (-OH) and the low molecular volatile Terpenoids mainly mono- and sesquiterpenes (Kitazurua *et al.*, 2004; Misharina *et al.*, 2009). Phenolic antioxidants in herbs are mainly composed of phenolic acids, alkaloids, polyphenols, phenylpropanoids flavonoids, isoflavones, flavones, anthocyanins, coumarins, lignans, catechins, isocatechins and tannins (Aqil *et al.*, 2006; Yoo *et al.*, 2008). These phenolic compounds have been studied for substitution of synthetic antioxidants.

Table 1: Spices and % extract yield in Hexane

Plants	Common name	% Extract yield*
Anethum sowa Roxb ex Fleming	Dill	5.40
Coriandrum sativum L	Coriander	2.24
Cuminum cyminum L	Cumin	5.98
Foeniculum vulgare Mill	Finnochio	5.97
Trachyspermum ammi (L) Sprague ex Turrill	The bishops weed	12.35

*[**Note: *** represents g extract/100g dry powder]*

Biochemically antioxidants possess diverse physiological roles in the body and are a key for good health. Antioxidants are likely to prevent several chronic diseases caused by free radicals such as atherosclerosis, cancer, diabetes, arthritis, inflammation, cardiovascular and ageing related problems (Kaur and Kapoor, 2001; Apak *et al.*, 2007). From the food preservation perspective, they prevent lipid peroxidation and microbial spoilage of food and works as a natural preservative agent. Thus nowadays, herbs and spices have great potential in a growing Nutrition industry (Juki *et al.*, 2006; Markowicz *et al.*, 2007).

Table 2: Phyto-chemicals present in the seeds of Selected plants

Plants	Phyto-chemicals
Anethum sowa	Carvone, apiole, dillapiole, limonene, dihydrocarvone, eugenol, thymol, isoeugenol, β-phellandrene, α-selinene, phytol, myristicine, o-cymene, α-thujene, exo-2-hydroxycineole, dihydroumbellulone and pinene.
Coriandrum sativum	Coriandrol, linalool, coriandrinonediol, α- and β -pinene, α- and γ-terpinene, myrcene, camphene, m-cymene, citronellal, citronellol, citral, limonene, β-phellandrene, eucalyptol, borneol, β- caryophyllene, geraniol, sabinene, thymol, α-cedrene, α- farnesene, linalyl acetate, geranyl acetate, neryl acetate, elemol and methyl heptenol.
Cuminum cyminum	Cumin aldehyde, cuminal, cuminic alcohol, limonene, geranyl acetate, eugenol, α-and β-pinene, perillaldehyde, sabinene, c-terpinene, safranal, p-cymene and β-phellandrene.
Foeniculum vulgare	Fenchone, methyl chavicol, anethole, estragole, α-and β-pinene, camphene, α- and β-phellandrene, myrcene, limonene, γ-terpinene, cis-ocimene, terpinolene, carvacrol, camphor, borneol, cineol and p-cymene.
Trachyspermum ammi	Thymol, carvacrol, p-cymene, limonene with γ-and β-terpinenes, α- and β-pinene and terpinene-4-ol.

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Apiaceae family is also known as Umbelliferae. This family mostly includes aromatic herbs. The seeds possess aromatic principles, Terpenoid derivatives and high volatile oil content. The aroma of the seeds is because of the volatile oil present in the seeds. Due to the distinct taste and aroma the seeds of plants are broadly used as spices and flavoring of food dishes, drinks etc. The present study was initiated to find the antioxidant property of the oil content present in the seed. Seeds of selected plants were extracted in laboratory using the solvent Hexane and a concentrated extract was prepared (Table 1).

MATERIALS AND METHODS

Plant Material Extraction: Spices *Anethum sowa, Coriandrum sativum, Cuminum cyminum, Foeniculum vulgare* and *Trachyspermum ammi* seeds were purchased from the local markets of Gujarat State, India. Material was grinded in to a fine powder using domestic mixture grinder and subjected for the solvent extraction in a non-polar solvent hexane to extract the bioactive terpenoid and other soluble volatile oil compounds. Plant material extracted in the ratio of 10 gm powder vs. 100 ml solvent with occasional shaking and overnight soaking in the air tight erlenmeyer flask. The content then filtered through the whatman filter paper no.1 and concentrated in open air until all the solvent gets evaporated. The concentrated extract was subsequently collected in the glass vial and dry weight of each extract was recorded (Table 1). The prepared extracts were used for the antioxidant assay.

Antioxidant Activity Analysis: All the extracts were subjected for the screening of Antioxidant activity following standardized protocols. The chemicals utilized were of pure and analytical grade. OD Readings were taken using UV-VIS Spectrophotometer (Elico), in six replicates for each sample. The detailed procedure of the *in vitro* assay was mentioned below. IC_{50} value was calculated for each standard, representing the concentration of the compounds that caused 50% inhibition/ antioxidant activity.

(a) Estimation of Total Phenol Content by Folin-Ciocalteau method

To 6 ml of double dist. water added 2 mg sample, 0.5 ml Folin-Ciocalteau reagent and 1.5 ml 20% Na_2Co_3 solution. Then the total volume made up to 10 ml by addition of dist. water. The mixture incubated for 30 min. at 25ºC and then OD taken at 760 nm. The presence of antioxidant activity indicated by a color change from light yellow to blue. The intensity of blue color is directly correlated with the extant of the antioxidant activity or the amount of phenol present in the sample. The IC_{50} value recorded for the Gallic acid standard was 0.50 mg/10ml. % phenol content calculated using % extract yield with reference to Gallic acid standard curve and referred as Gallic acid equivalents (GAE) (Ghasemi *et al.*, 2009).

(b) Determination of Ferrous Ion Chelating Activity:

3 mg extract mixed with the 2 ml of 0.04 mM $Fecl_2$ and 2 ml of 0.5 mM aqueous Ferrozine solution. The mixture shaken vigorously and left standing at room temperature for 10 min. Extent of ferrous ion chelating activity indicated by turning of mixture colour from dark purple to light purple or pink. Here correlation exists as higher the chelating activity; lighter the color of the solution. OD is taken at 562 nm. Ascorbic acid (vitamin C) used as a reference compound and it's IC_{50} value (concentration for 50% inhibition) was calculated. The calculation is performed using following formula (Dinis *et al.*, 1994).

$$\% \text{ Inhibition of Ferrozine - } Fe^{2+} \text{ complex} = \left(\frac{1\text{-}A_1 \text{ sample}}{A_0 \text{ control}} \right) \times 100$$

A_0 control = OD of $Fecl_2$ and Ferrozine solution without Extract or Standard

A_1 sample = OD of $Fecl_2$ and Ferrozine solution with Extract or Standard

(c) DPPH radical scavenging assay:

To 2 ml 0.5 mM methanolic solution of DPPH (1,1-Diphenyl-2-picrylhydrazyl) was mixed with the 2 ml methanolic solution containing 3 mg extract. The mixture was shaken vigorously and allowed to incubate in dark for 30 min and OD was taken at 517 nm. BHT (Butylated Hydroxy Toluene) was used as a reference compound with IC50 at 0.35 mg/4 ml. The calculation was performed using the formula (Ghasemi *et al.*, 2009).

$$\% \text{ DPPH radical scavenging activity (RSA)} = \left(\frac{\text{A control -A sample}}{} \right) \times 100$$

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A control

[**Note:** A control = OD of DPPH solution without extract or standard, A sample = OD of DPPH solution with extract or standard]

RESULTS AND DISCUSSION

The importance of conducing phyto-chemical studies, is to not only used for the chemical characterization but also important for correlating the chemical contents with specific functional properties (Sacchetti *et al.*, 2005). The spices used in the present study are routinely used for the flavoring of food dishes. They indirectly helps the body from the free radicals damage, which are produced during the normal metabolic processes.

In the present research work, the extracts prepared were weighed (Table 1) and stored in a glass vials until use. Individual extract then screened for antioxidant activity through three different bioassays. Standard compounds were used to as a reference to quantify the activity. The amount of antioxidant activity is mentioned in the Table 3.

Table 3: Antioxidant Activity exhibited by extracts in the Assays

Plants	% Phenol	% DPPH RSA	% FICA
Anethum sowa	0.0675 ± 0.0082	29.391 ± 0.477	44.57 ± 0.15
Coriandrum sativum	0.0051 ± 0.0005	30.405 ± 0.405	86.30 ± 0.09
Cuminum cyminum	0.0657 ± 0.0034	26.590 ± 0.489	91.58 ± 0.15
Foeniculum vulgare	0.0865 ± 0.0061	27.316 ± 0.282	43.45 ± 0.18
Trachyspermum ammi	0.7147 ± 0.0836	45.556 ± 0.377	47.78 ± 0.18

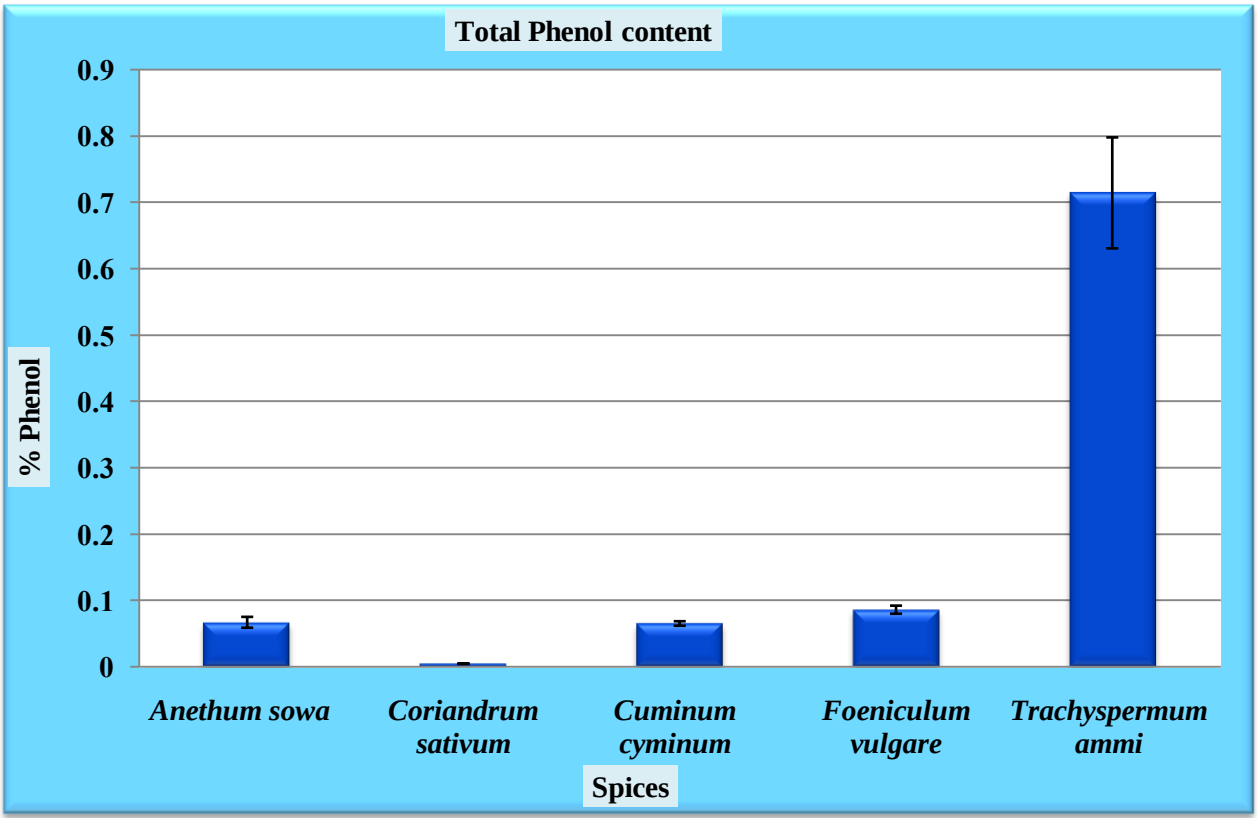

Figure 1: % Phenol content of Hexane extracts of Spices seeds

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(a) Total Phenol Content of Spices:

The Phosphomolybdenum method or Folin-Ciocalteu method usually detects antioxidants such as ascorbic acid, some phenolics, polyphenols, aromatic amines, glutathione, cysteine, α-tocopherol, carotenoids etc. by their hydrogen and electrons donating ability. These compounds undergo a complex redox reaction with the phosphotungstic and phosphomolybdic acids present in the Folin-Ciocalteu reagent. Ethanol and methanol are favourable solvents for extracting various groups of phenols and flavonoids from plant samples due to its more lipophilic affinity and interaction (Sangeetha *et al.*, 2010).

In the present study, Gallic acid standard at 0.01 mg/ml concentration exhibited 0.00010% phenol content (0.50 mg/10 ml). With reference to this GAE, highest amount of % Phenol content was recorded with the *Trachyspermum ammi* extract (0.7147 ± 0.0836). Other spices demonstrated minor quantity of phenol in the following order of efficacy in *Foeniculum vulgare* (0.0865 ± 0.0061), *Anethum sowa* (0.0675 ± 0.0082), *Cuminum cyminum* (0.0657 ± 0.0034) and *Coriandrum sativum* (0.0051 ± 0.0005) (Table 3, Figure 1). The total polyphenol content thus varies with the solvent system used for the extraction. Phenols are polar compounds and can be well extracted in polar solvents. Plant Secondary Metabolites many times exhibits good antioxidant effect due to synergistic effect of group of active principles and other minor metabolites. Biochemically, antioxidative capacity of phenols depends on the volume, electronic characteristics and number of phenol groups occupying 1,2 or 1,4 positions in an aromatic ring (Aluyor and Ori-Jesu, 2008).

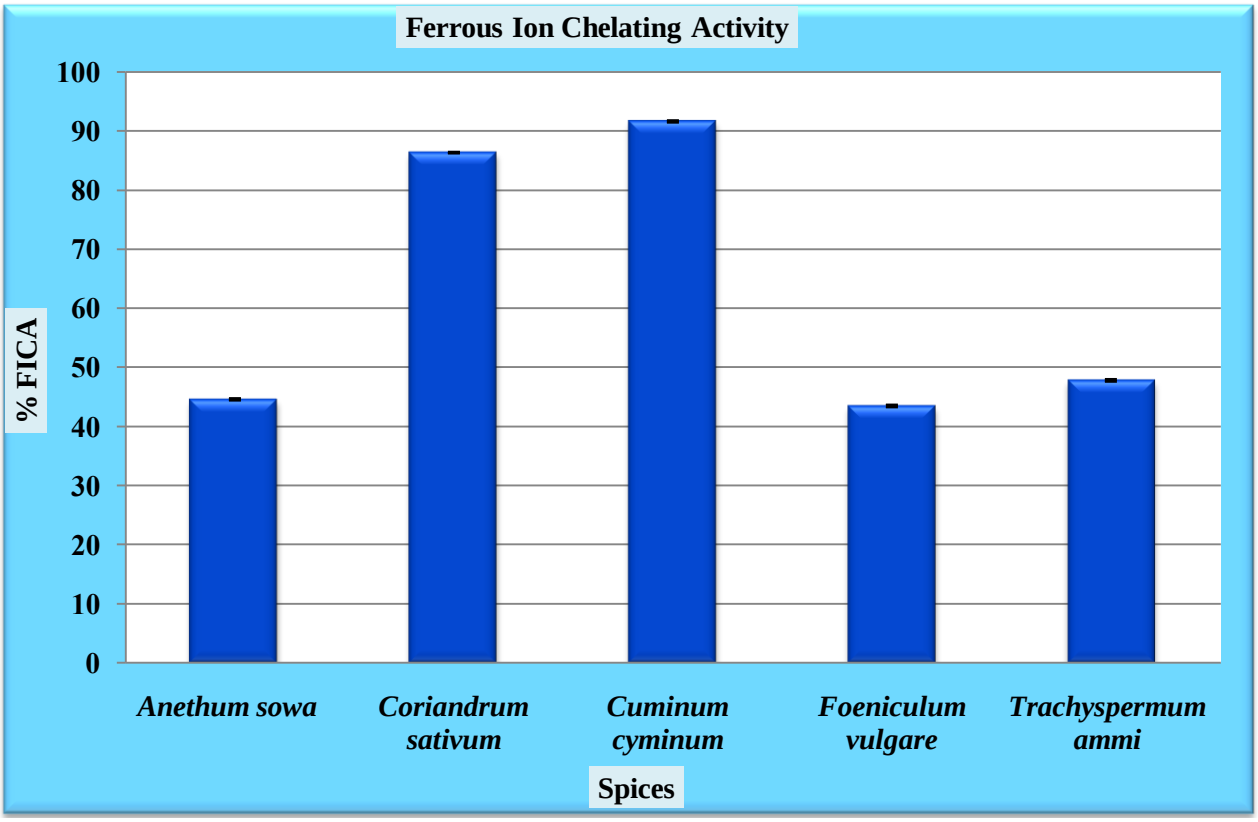

Figure 2: % FICA of Hexane extracts of Spices seeds

(b) % FICA of Hexane extracts of Spices

Transition elements like iron and copper are the free radicals having one or more unpaired electrons and act as powerful catalyst of oxidation reactions because they contain that can enable to participate in electron transfer reactions. Metal ion chelating activity of an antioxidant molecule can inactivate, catalyze and inhibit the harmful transition metal ions responsible for the generation of oxygen free radicals in

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living organisms and potentially (Ghimire *et al.*, 2009). In metal ion chelating assay, the extract and standard compounds interferes with the formation of ferrous and ferrozine complex and are able to capture ferrous ion before the formation of ferrozine by their chelating activity. Ferrozine can quantitatively form complexes with Fe^{2+}. In the presence of chelating agents, the complex formation is disrupted, resulting in decrease of the red colored complex. Thus reduction of the color is equal to the metal chelating activity (Singh *et al.*, 2009).

In the present study, % FICA (Ferrous ion chelating activity) IC_{50} value for standard Ascorbic acid observed at 1.5 mg/ml concentration. In the Ferrous ion chelating assay, hexane extracts of *Cuminum cyminum* (91.58 ± 0.15) and *Coriandrum sativum* (86.30 ± 0.09) demonstrated appreciable quantity of antioxidant activity. Rest of the spices demonstrated activity in the following order of efficacy *Trachyspermum ammi* (47.78 ± 0.18), *Anethum sowa* (44.57 ± 0.15) and *Foeniculum vulgare* (43.45 ± 0.18) (Table 3, Figure 2).

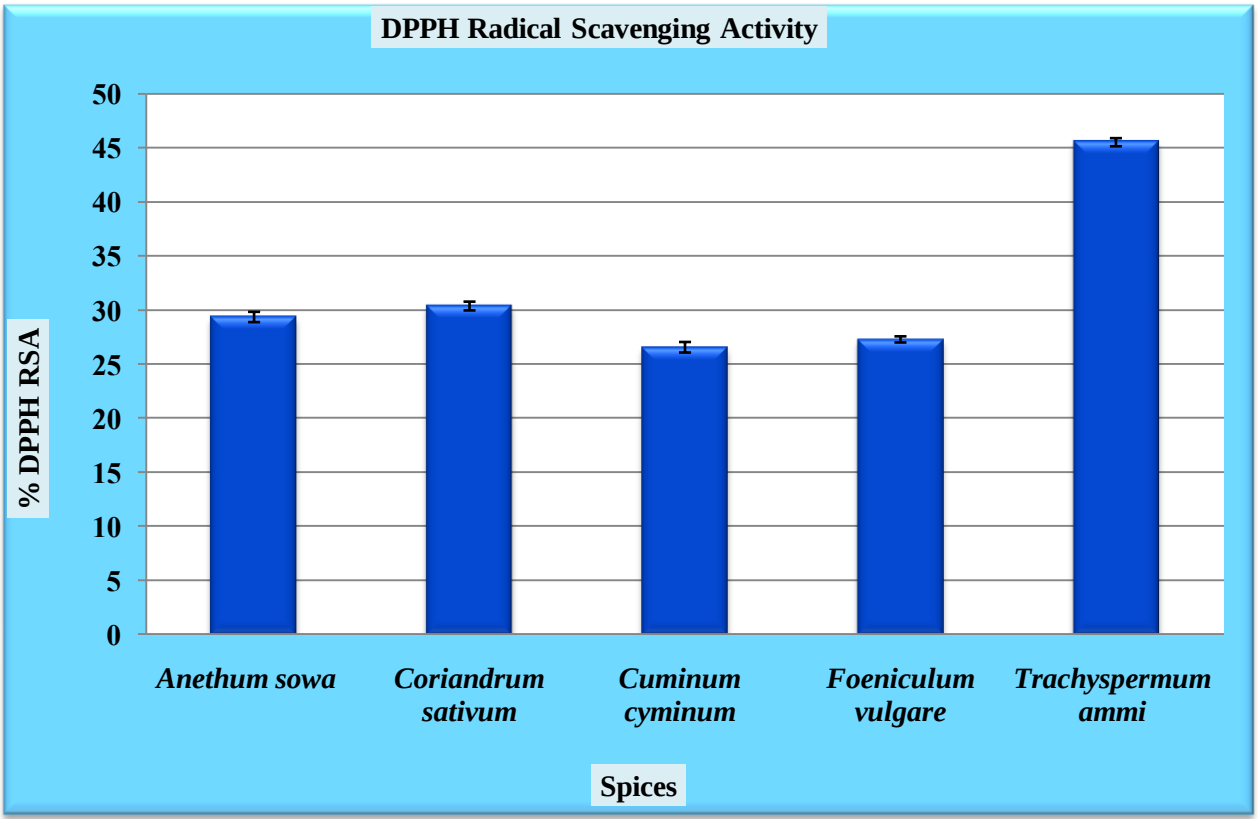

Figure 3: % DPPH RSA of Hexane extracts of Spices seeds

(c) % DPPH RSA of Hexane extracts of Spices

DPPH (2, 2-diphenyl-1-picrylhydrazyl) is a nitrogen-centered free radical that accepts an electron or hydrogen radical to become a stable diamagnetic molecule. The DPPH scavenging ability and reducing power assays provides preliminary information on the reactivity of the test compound with a free radical and its hydrogen-donating propensity and the reduction capability of the DPPH radical is determined by the decrease in its absorbance at 517 nm (Rathee *et al.*, 2006). During this free radical scavenging assay, methanolic solution of DPPH changes its color from deep violet to a pale yellow by antioxidants. In the process, odd electron of the radical becomes paired off with hydrogen donated by the extract, resulting in the reduction of absorption strength. The resulting decolorization from purple to yellow is correlated with respect to the number of electrons captured by the extract (Markowicz *et al.*, 2007). In the present study, amongst the five spice hexane extracts screened; a good amount of % DPPH radical scavenging activity

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was demonstrated by the extract of *Trachyspermum ammi* (45.556 ± 0.377), followed by *Coriandrum sativum* (30.405 ± 0.405), *Anethum sowa* (29.391 ± 0.477), *Foeniculum vulgare* (27.316 ± 0.282) and *Cuminum cyminum* (26.590 ± 0.489). Thus all extracts showed presence of potential free radical scavenging effect in the assay (Table 3, Figure 3).

Conclusion

Vegetables, fruits, spices and aromatic plants are the primary source of natural antioxidant compounds. Epidemiological studies have also supported the positive association between the consumption of antioxidant rich foods and beverages with the prevention of diseases. Thus herbs and spices nowadays have a great potential in a growing nutrition industry because of their dual functionality in preventing oxidative damage and a microbial spoilage. Overall in the present research work, all five Apiaceae family aromatic spices extracts screened demonstrated sufficiently good amount of Ferrous ion chelating activity, DPPH radical scavenging activity, and a moderate quantity of % Phenol content. Thus the present work has contributed in the direction of using the spices not only as a flavoring agent but also to be used as a good phyto-medicine to prevent the harmful free radical damage and prevention of some chronic diseases onset in the body.

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