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# Antioxidant activity of medicinal spices and aromatic herbs

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## Abstract

Essential oils of many plants besides having a strong and pleasant aroma also are biologically active as an antioxidant. They have an important economic role in the medical and pharmaceutical industries, aromatherapy and cosmetology, production of natural food flavorings and as natural preservative. A variety of free radical scavenging antioxidants are found in dietary sources like fruits, vegetables, aromatic herbs, spices *etc*. The antioxidant role of aromatic principles also have medicinal effects and play important roles in long term health, reduction in the risk of chronic and degenerative diseases and preventing cellular damage, when included in regular diet. Considering the highly significant medicinal role of antioxidant, in the present investigation, effort is made to find out the antioxidant capacity of hexane extracts of 15 aromatic herbs and spices. Antioxidant activity screening assays implemented here is % DPPH radical scavenging activity (RSA). All the plant extracts have shown an excellent activity. The highest activity recorded in the present assay is with *Ocimum sanctum* extract 76.608 ± 0.063 % for DPPH radical scavenging activity.

**Key words:** Plant extracts, essential oils, antioxidant, free radicals, chronic and degenerative diseases

## Introduction

Plants synthesize essential oils to protect from infections and parasites. The essential oils are the secondary metabolites of the aromatic plants which include the fragrant material present in any part from the root, bark, wood, seed, fruit, leaf, flower or in the whole plant (Yoo *et al.,* 2008). Essential oils are made of a complex mixture of organic substances with different functional groups like phenolic compounds containing hydroxyl groups (-OH) and low molecular volatile terpenoids mainly mono- and sesquiterpenes (Kitazurua *et al.*, 2004; Misharina *et al.,* 2009). Many aromatic plants are medicinally important due to these secondary compounds and their usage in regular diet, not only serve as fragrance and flavoring agents but also as dietary antioxidant. Antioxidant inhibits oxidation process even at relatively small concentration by acting as a radical scavenger, oxygen scavenger, triplet and singlet form. They 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; Markowicz *et al.,* 2007). Furthermore, they inhibit other food deterioration affecting its nutritional quality and flavor by preventing lipid peroxidation and microbial spoilage and works as a natural preservative agent (Kaur and Kapoor, 2001; Singh *et al.,* 2004; Juki *et al.,* 2006; Markowicz *et al.,* 2007). Thus, nowadays, herbs and spices have great potential in a growing nutrition industry (Samojlik *et al.,* 2010).

Thus, in recent decades, there has been great interest in screening essential oils and various plant extracts to obtain natural antioxidants with broad-spectrum actions (Wu *et al.,* 2009). However, despite of huge medicinal value of Indian medicinal plants, their rich diversity is yet to be scientifically evaluated for such properties.

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*Cinnamomum zeylanicum*

*Cinnamomum tamala*

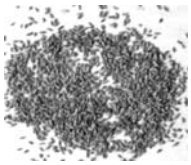
*Citrus sinensis*

*Coriandrum sativum*

*Cuminum cyminum*

*Cymbopogon caesius*

*Elettaria cardamomum*



*Foeniculum vulgare*

*Illicium verum*

*Mentha piperita*

*Myristica fragrans*

*Ocimum sanctum*

**Figure 1.** Plants used for the study

**Table 1.** Active phytochemicals present in plants

| Plants and part | Phytochemicals |
|---|---|
| ***Anethum sowa*** Roxb. ex Fleming. Seeds | Carvone, limonene, phellandrene, pinene, diterpene, dihydrocarvone, cineole, myrcene, paramyrcene, dillapiole, isomyristicin, myristicin, myristin, apiol, dillapiol |
| ***Cinnamomum zeylanicum*** Blume. Bark | Cinnamaldehyde, eugenol, trans-cinnamic acid |
| ***Cinnamomum tamala*** Nees. & Eberm. Leaves | Phellandrene, eugenol, linalool, cinnamaldehyde, α-and β- pinene, p-cymene, limonene, Caryophyllene |
| ***Citrus sinensis*** Osbek. Fruit peel | Limonene, hesperidin, rutoside, sinensetin, nobiletin, tangeretin, polymethoxylated flavones, hydroxycinnamates, gallic acid, elagic acid |
| ***Coriandrum sativum*** L. Seeds | Linalool, α-and β-pinene, limonene,λ-terpinene, p-cymene, anethole, geraniol, camphorgeranyl acetate |
| ***Cuminum cyminum*** L. Seeds | Cuminaldehyde, p-cymene, cuminic aldehyde, cyminol |
| ***Cymbopogon caesius*** (Nees et Hook et Arn) Stapf. Leaves | Carvone, perillyl alcohol, geraniol, limonene |
| ***Elettaria cardamomum*** (L.) Maton. Fruit | Bisabolene, borneol, campesterol, camphene, camphor, 1, 8-cineole, citronellal, citronellol, geraniol, α-terpinyl acetate, α-terpineol, linalool |
| ***Foeniculum vulgare*** Mill. Seeds | Trans-anethole, fenchones, methylchavicol, anisaldehyde, α-and β-pinene, α-phellandrene, limonene, estragole, safrol, camphene, β-myrcene, p-cymen, salicylic acid, hydroquinone, pyrogallic acid, resorcinol, protochatechenic, chlorogenic acid, vanillin, p-coumaric acid, ferulic acid, o-coumaric acid, coumrin, cinnamic acid |
| ***Illicium verum*** Hook. f Fruit | Trans- and cis-anethole, estragole, limonene, p-anisaldehyde, anisylacetone |
| ***Mentha piperita*** L. Leaves | Menthol, menthone, carvone, pulegone, isorhoifolin, menthoside, piperitoside, nevadensin, hymenoxin, menthocubanone |
| ***Myristica fragrans*** Houtt. Fruit | Eugenol, methyl eugenol methyl isoeugenol, *cis*-isoeugenol, terpineol isoeugenol, linalool, geraniol, nerol, benzyl alcohol, 2-phenylethanol, pulegone, thujone, cuminol, isoelimicin, terpinen-4-ol., α-and β-pinene, limonene myristicin, safrole |
| ***Ocimum sanctum*** L. Leaves | Cineole, eugenol, ursolic acid, cirsilineol |
| ***Santalum album*** L. Wood | α-and β-Santalol, α-and β-santenes, teresantalol, santenol, santalone, l-santenone, α-and β-santalic acid |
| ***Trachyspermum ammi*** (L.) Sprague. ex. Turrill. Seeds | Thymol, p-cymene, terpinene, α-pinene, terpinene-4-ol, terpinene, p-cymene |

**Source:** (Singh *et al.*, 2004; Juki *et al.*, 2006; Singh *et al.*, 2006; Khalil *et al.*, 2007; Nickavar *et al.*, 2008; Singh *et al.*, 2008; Farooq *et al.*, 2009; Anwara *et al.*, 2009; Dzamic *et al.*, 2009; Patel and Jasrai, 2009; Kaur and Arora, 2010)

### *Mechanism of action*

During the normal metabolism, oxidation reactions produce free radicals which start off chain reactions. Antioxidants are free radical scavengers and terminate these chain reactions by being oxidized themselves and acting as reducing agents. Antioxidants function as hydrogen donors and inhibit the formation of free alkyl radicals or interrupt the propagation of the free radical chain (Aluyor and Ori-Jesu, 2008; Nahar *et al*., 2009). They interrupt the harmful free radicals in the body and forms a stable radical (Aluyor and Ori-Jesu, 2008). More precisely, biochemically they function as singlet or triplet oxygen quenchers, free radical scavengers, peroxide decomposers, enzyme inhibitors and synergists (Kitazurua *et al.,* 2004; Mandal *et al.,* 2009). This way they help to prevent several chronic diseases and the process of ageing in the body. Thus, the quest for newer and newer finding to find out more sources of natural antioxidants boost their usage and will lead to fit and healthy body in a natural way. Antioxidant activities of essential oils are mainly attributed to the presence of active principles or Plant Secondary Metabolites (PSMs) and many times to synergistic effect of active principles and other minor metabolites (Table 1).

### *Screening* DPPH *radical scavenging activity of plant extracts*

DPPH is a stable nitrogen-centered free radical that accepts an electron or hydrogen radical to become a stable diamagnetic molecule (Sanja *et al*., 2009). 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 (Rathee *et al.,* 2006). During this free radical scavenging assay, the color of DPPH solution changes from deep violet to a pale yellow due to formation of colorless α-Diphenyl-β-picryl hydrazine a stable molecule, via either transfer of an electron or hydrogen atom to DPPH, thus, neutralizing its free radical character (Nickavar *et al.,* 2008; Nahar *et al.,* 2009; Sreelatha and Padma, 2009). Here the odd electron of the radical becomes paired off with hydrogen donated by the extract, resulting in the reduction of absorption strength. DPPH, has characteristic absorbance maxima at 517 nm, which decreases with the scavenging of the radical (Singh *et al.,* 2009; Porwal *et al.,* 2010). The reduction of DPPH radicals can be observed by the decrease in absorbance at 517 nm and on the degree of discoloration due to the radical scavenging ability of antioxidant (Aquil *et al.,* 2006; Sreelatha and Padma, 2009). Many researchers have reported positive correlation and observed that high reduction of DPPH is related to the high scavenging activity and higher amount of antioxidants present in the sample. $EC_{50}$ (Effective concentration of extract needed for 50% free radical inhibition) is the amount of antioxidant present in the sample necessary for 50% DPPH inhibition. Thus, lower the $EC_{50}$ value, higher the antioxidant activity (Ghafar *et al.,* 2010).

## Materials and Methods

### *Collection and extraction of plant material*

Plants used in the present study were *Anethum sowa* Roxb. ex Fleming., *Cinnamomum zeylanicum* Blume., *Cinnamomum tamala* Nees. & Eberm., *Citrus sinensis* Osbek., *Coriandrum sativum* L., *Cuminum cyminum* L., *Cymbopogon caesius* (Nees et Hook et Arn) Stapf., *Elettaria cardamomum* (L.) Maton., *Foeniculum vulgare* Mill., *Illicium verum* Hook. f., *Mentha piperita* L., *Myristica fragrans* Houtt., *Ocimum sanctum* L., *Santalum album* L. and *Trachyspermum ammi* (L.) Sprague ex. Turrill. The plant material for the study was purchased from the local markets of Gujarat state (Figure 1). The collected herbs were shade dried. Dried plant material was grinded in to a fine powder with the help of domestic mixture grinder. The powdered drug was then subjected for the solvent extraction. As hexane is a non-polar solvent, can very well dissolve and extract volatile oil components like Monoterpenes. Thus, all the plant powder was extracted in hexane in the ratio of 10 gm powder vs. 100 ml solvent with occasional shaking. The powder was then allowed to soak in the solvent for over night in the air tight erlenmeyer flask. The content was filtered through the whatman filter paper no.1 and concentrated in the air until all the solvent gets evaporated. The extract was subsequently collected in the glass vial and dry weight of each extract was taken.

### *Antioxidant activity analysis*

All the extracts were then subjected for the screening of antioxidant activity following standardized protocols. The chemicals utilized were of pure and analytical grade. Readings were taken in six replicates for each sample. The detailed procedure of the *in vitro* assay was mentioned below. $IC_{50}$ value was calculated for standard, representing the concentration of the compounds that caused 50% inhibition/ antioxidant activity.

### DPPH *radical scavenging assay*

2ml 0.5 mM methanolic solution of DPPH (1,1-diphenyl-2-picrylhydrazyl) is mixed with the 2 ml methanolic solution containing 3 mg extract. The mixture was shaken vigorously and allowed to incubate in dark for 30 minutes. Presence of antioxidant activity was indicated by turning of dark violet to brownish color or light yellow. More the antioxidant activity, more paler the colour of mixture after incubation. OD was taken at 517 nm. BHA (Butylated hydroxyl Toluene) was used as a reference compound. The calculation was performed using following formula (Ghasemi *et al.,* 2009).

% DPPH Radical Scavenging Activity (RSA)

$$= \frac{\text{A control -A sample} \times 10}{\text{[A control]}}$$

A control = OD of DPPH solution without extract or standard

A sample = OD of DPPH solution with extract or standard

## Results and Discussion

In the DPPH radical scavenging assay, all the hexane extracts at 0.6mg/ml concentration had demonstrated significant antioxidant activity (Figure 2).

The % DPPH RSA $IC_{50}$ value for standard BHA was observed at 0.08mg/ml concentration. In other terms, 0.08 mg/ml concentration of BHA found to inhibit 50% DPPH radical in the assay. In the present study, *Ocimum sanctum* (76.608 ± 0.063) had demonstrated maximum % DPPH radical scavenging activity (RSA) followed by *Myristica fragrans* (58.737 ± 0.598) and *Cinnamomum tamala* (58.704 ± 0.619) extracts. A moderate activity was exhibited by the *Trachyspermum ammi* (45.556 ± 0.377), *Cinnamomum zeylanicum* (34.539 ± 0.432), *Mentha piperita* (32.777 ± 0.169), *Cymbopogon caesius* (31.672 ± 0.475), *Coriandrum sativum* (30.405 ± 0.405) and *Anethum sowa* extract (29.391 ± 0.477). While minimum activity was recorded for *Citrus sinensis* (25.972 ± 0.164). The solvent hexane was used to isolate the aromatic terpenoid compounds which are the main phytochemicals of the spices (Table 1). Thus, as indicated in the experimental results in Figure 2, all hexane crude extracts had demonstrated presence of appreciable free radical scavenging activity. As the volatile fraction of spices is found to possess free radical scavenging and antioxidant effect, it proves the importance of spices in the diet for good health.

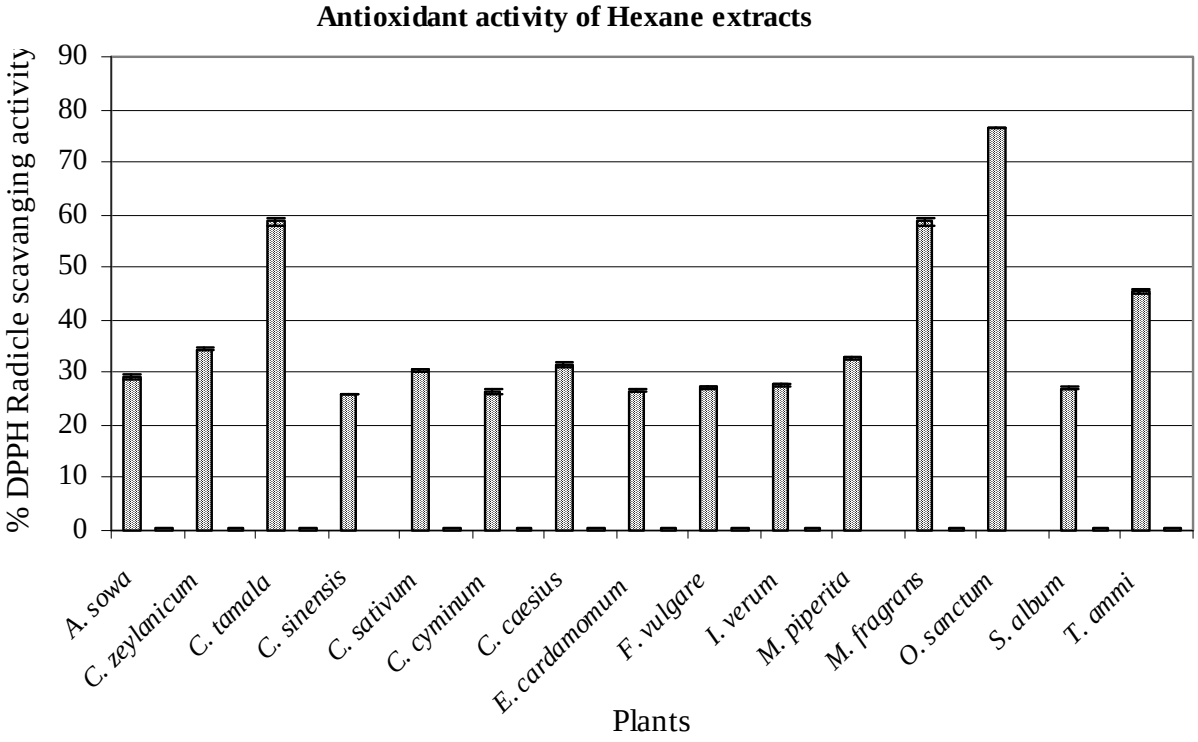



**Figure 2.** Figure showing amount of % DPPH RSA

## Conclusion

Plants used for the present investigation are a routine spices and are part of our daily diet. They are not only taste modifiers but after doing this study, it is proved that they are potential antioxidants. The hydrogen donating tendency of antioxidant molecule neutralizes the damaging free radicals in the body. Thus the present work indicates that we should include spices along with green vegetables and fruit in our daily diet so as to prevent an oxidative damage by free radicals and keep your body fit and fine. Present research work has also contributed for the further development of phytomedicines and nutraceuticles containing these extracts in form of refined drugs and supplements for the protection of body.

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