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Research Article

EVALUATION OF BODY-CELL PROTECTING- ANTIOXIDANT ABILITY OF COMMON INDIAN SPICES AND AROMATIC HERBS USING FERROUS ION CHELATING ACTIVITY ASSAY

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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. 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 commonly used Indian spices, condiments and herbs were screened using % FICA (Ferrous Ion Chelating Activity). In the study, all extracts demonstrated good amount of activity especially *Citrus sinensis*, *Elettaria cardamomum*, *Cinnamomum zeylanicum, and Cuminum cyminum* extracts exhibited excellent effect.

Key Words: *Plant extracts, Essential oils, Antioxidant, Free radicals, Chronic and degenerative diseases*

INTRODUCTION
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. 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).
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 (PSMs). Fruits, vegetables and herbs, are known to contain large amount of antioxidants (Misharina *et al.,* 2009; Patel, 2013). 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, found to possess antioxidant properties. PSMs like flavonoids, isoflavones, flavones, anthocyanins, coumarins, lignans, catechins, isocatechins, and also vitamins C and E, β-carotene, and α-tocopherol are reported to possess antioxidative property (Aqil *et al.,* 2006). The aromatic property of herbs and other plants/ plant parts is due to presence of volatile/ essential oils. 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; Patel and Jasrai, 2012). Thus 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.

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). From the food preservation perspective, they also 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). Thus nowadays, there has been increasing interest in screening essential oils and plant extracts to obtain newer sources of natural antioxidants with broad-spectrum actions. However, despite of huge medicinal value of Indian medicinal plants, a rich diversity of plants is yet to be scientifically evaluated for such properties and to understand their mechanism of action.

MATERIALS AND METHODS

Plant Material Extraction: Plants used in the present study were *Anethum sowa* Roxb ex Fleming, *Cinnamomum tamala* Nees & Eberm, *Cinnamomum zeylanicum* Blume, *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 (Figure 1).

Anethum sowa

Cinnamomum tamala

Cinnamomum zeylanicum

Citrus sinensis

Coriandrum sativum

Cuminum cyminum

Cymbopogon caesius

Elettaria cardamomum

Foeniculum vulgare

Illicium verum

Mentha piperita

Myristica fragrans



Ocimum sanctum ***Santalum album*** ***Trachyspermum ammi***

Figure 1: Aromatic herbs and spice plants used for the study

The plant material for the study was purchased from the local markets of Gujarat State, India. Material was grinded in to a fine powder using domestic mixture grinder and further subjected for the solvent extraction in a non-polar solvent hexane to extract the bioactive terpenoid and other soluble 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 (Harborne, 1984). 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).

Table 1: Spices and Extract yield in Hexane

Plants	Common name	Family	Plant part used	%Extract yield*	% FICA
Anethum sowa	Dill	Apiaceae	Seeds	5.40	44.57 ± 0.15
Cinnamomum tamala	Indian cassia lignea	Lauraceae	Leaves	3.53	44.55 ± 0.23
Cinnamomum zeylanicum	Cinnamon	Lauraceae	Bark	2.90	91.62 ± 0.12
Citrus sinensis	Sweet orange	Rutaceae	Fruit peel	1.70	94.37 ± 0.06
Coriandrum sativum	Coriander	Apiaceae	Seeds	2.24	86.30 ± 0.09
Cuminum cyminum	Cumin	Apiaceae	Seeds	5.98	91.58 ± 0.15
Cymbopogon caesius	Kachi grass	Poaceae	Leaves	3.41	59.03 ± 0.27
Elettaria cardamomum	Cardamom	Zingiberaceae	Fruit	5.46	92.31 ± 0.22
Foeniculum vulgare	Finnochio	Apiaceae	Seeds	5.97	43.45 ± 0.18
Illicium verum	Staranise	Illiciaceae	Fruit	7.80	84.64 ± 0.09
Mentha piperita	Mint	Labiatae	Leaves	3.55	71.52 ± 0.13
Myristica fragrans	Nutmeg	Myristicaceae	Fruit	24.52	34.59 ± 0.06
Ocimum sanctum	Basils	Labiatae	Leaves	4.15	58.17 ± 0.16
Santalum album	White Indian sandalwood	Santalaceae	Wood	1.07	85.47 ± 0.17
Trachyspermum ammi	The bishops weed	Apiaceae	Seeds	12.35	47.78 ± 0.18

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

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. IC_{50} value was

calculated for standard, representing the concentration of the compounds that caused 50% inhibition/ antioxidant activity. In Ferrous Ion Chelating Activity assay, 3mg extract mixed with the 2ml of 0.04 mM $Fecl_2$ and 2ml of 0.5mM aqueous Ferrozine solution. The mixture shaken vigorously and left standing at room temperature for 10min. 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 taken at 562nm. Ascorbic acid (vitamin C) used as a reference compound and IC_{50} value was calculated. The calculation was 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

RESULTS AND DISCUSSION

As per Ladoa *et al.*, (2004) and Politeo *et al.*, (2006) studies, the reducing abilities of the components of volatile oils are lower than those of volatile oils. Thus the reducing capacities of volatile oils cannot be attributed solely to individual component of extract, but it is due to synergistic effect. Consequently crude extract is more effective than an individual phyto-chemical component. In the present study, % FICA (Ferrous ion chelating activity) IC_{50} value for standard Ascorbic acid observed at 1.5mg/ml concentration. In this respect, 3mg hexane extract test sample of *Citrus sinensis*, *Elettaria cardamomum*, *Cinnamomum zeylanicum*, *Cuminum cyminum*, *Coriandrum sativum*, *Santalum album*, *Illicium verum* and *Mentha piperita* exhibited an excellent %FICA in the range 95-70%. Likewise *Anethum sowa*, *Cinnamomum tamala*, *Cymbopogon caesius*, *Foeniculum vulgare*, *Ocimum sanctum* and *Trachyspermum ammi* extracts demonstrated appreciable amount of % FICA (Table 1, Figure 2). Hence the test result supports the free radical scavenging ability of the non-polar extract of plants and therefore oil-based recipe prepared using the mentioned plant components, can provide the body with promising antioxidant and harmful free radical scavenging impact.

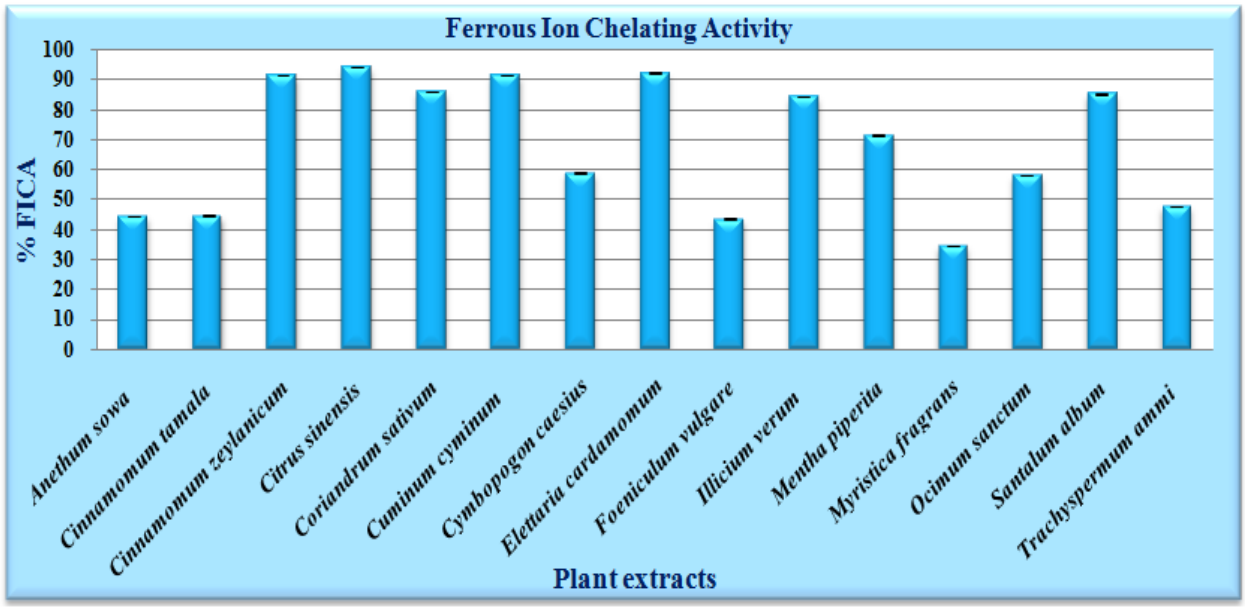

Figure 2: % FICA of Hexane extracts of spices and aromatic herb plants

Plants used for the present investigation are almost routinely used spices and are part of our daily diet. They are not just taste modifiers but the study result indicates their excellent body-cell protecting and free radical scavenging potential. Present finding in terms of ferric ion chelating potential of spices and herbs is the first report and in this context, added contribution for the further development of phyto-medicines and neutraceuticles containing these extracts in form of refined drugs and supplements for the protection of body.

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