INTERNATIONAL RESEARCH JOURNAL OF PHARMACY
www.irjponline.com
ISSN 2230 – 8407

Research Article

ANTIOXIDANT LEVELS OF COMMON HERBS AND SPICES USED IN INDIAN TEA: BLACK PEPPER (*PIPER NIGRUM*), FENNEL (*FOENICULUM VULGARE*), CLOVE (*SYGYZIUM AROMATICUM*), MINT (MENTHA), CINNAMON (*CINNAMOMUM VERUM*) AND TULSI (*OCIMUM TENUIFLORUM*)

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Article Received on: 09/09/18 Approved for publication: 28/10/18

DOI: 10.7897/2230-8407.0911270

ABSTRACT

Antioxidants are substances that prevent oxidation of other compounds or neutralize free radicals. Spices and herbs are rich sources of antioxidants which are used in food and beverages such as tea to enhance flavor, aroma and color. Antioxidant potential of the spices and herbs extracts was analyzed as contents of total phenols and flavonoids; and reducing power by the ferric reducing antioxidant power assay (FRAP). For the 4 spices and 2 herbs evaluated, crude protein was highest in clove (46.94 ± 1.15mg/ml) followed by tulsi (15.36 ± 0.31mg/ml), fennel (14.17 ± 1.05mg/ml), black pepper (13.43 ± 2.12mg/ml), cinnamon (7.62 ± 0.22mg/ml) and mint (3.07 ± 0.12mg/ml). Carbohydrates levels differed between all spices and herbs with tulsi (10.02 ± 0.84mg/ml) having high carbohydrate levels followed by fennel (9.99 ± 0.88mg/ml) and black pepper (8.19 ± 0.mg/ml 79) whereas mint (5.89 ± 0.37mg/ml), cinnamon (4.68 ± 0.44mg/ml) and clove (4.46 ± 0.76mg/ml) had low carbohydrate levels.The significantly higher level of phenolics (118.3 mg ± 21.21mg TAE/gm) and flavonoids (57.16 ± 2.12mg AAE/gm) was reported in clove ($p \leq 0.05$) as compared to other spices and herbs studied. The clove has significantly high antioxidative capacity (32.73 ± 0.84mg AAE/gm) ($p \leq 0.05$) than all other spices and herbs studied in the present study.

Keywords: Antioxidant levels, Herbs, India, clove, tulsi, mint, fennel, black pepper, cinnamon.

INTRODUCTION

Traditionally, in India herbs and spices are used in the diet for their aromatic properties but presence of various phytochemicals in herbs and spices may play a role in antioxidant defense and redox signaling[1]. In *Ayurveda* and *Siddha,* the traditional Indian system of medicine, various spices and herbs are used in routine for use in medicines.

Various medicinal benefits of various herbs/spices can be attributed to antioxidants which form a large group of bioactive compounds consisting of flavonoids, phenolic compounds, sulfur-containing compounds, tannins, alkaloids, phenolic diterpenes, and vitamins. Various phytochemical present in herbs/ spices are resveratrol, curcumin, genistein, capsaicin, epigallocatechingallate (EGCG), quercetin, β-carotene, and lycopene. These phytochemicals can alter the activity of several cell signaling pathways, leading to the modulation of inflammatory processes, regulation of cytoprotective mechanism and regulation of cell growth and differentiation[2]. The antioxidants obtained from foods such as herbs and spices are biochemically active due to their easy absorption, transport and excretion, cellular uptake and metabolism, and eventually their effects on oxidative stress in various cellular compartments. Clove, ginger, mint, cinnamon, tulsi are all among the commercially available spices with the highest total antioxidant capacity. Eugenol and gallic acid in clove have been identified as inhibitors of NF-κB, a transcription factor which is crucial in the orchestration of immune and inflammatory responses.[3]

Antioxidants from spices and herbs possess desirable properties such as being natural, non-GMO and having clean label ingredients (as spice or herb or flavoring). They play a role in functional signaling within tissues and cells, involving various transcription factors, protein kinases, phosphatases, and other metabolic enzymes[4]. It has also been reported that various spice-derived ingredients are potent inhibitors of lipid peroxidation in cell[5].

Since the large intake antioxidant supplement such as high-dose vitamin C, vitamin E, or β-carotene supplement are not able to scavenge reactive oxygen species (ROS), dietary antioxidants taken in their usual form of food/ beverage may decrease risk of chronic diseases without compromising the normal functions of ROS[6]. Spices have been reported to have various beneficial effects on human health which include anti-sclerotic, antithrombotic, anti-carcinogenic, anti-inflammatory, antiarrhythmic, anti-rheumatic, gastroprotective, and lipid-lowering action. In addition, spices have radioprotective (protects against radiation), anti-allergic and antimalarial effects. Spices inhibit the oxidation of low-density lipoprotein and protein glycation[7].

India is the largest producer of spices. About 80% of people in developing countries still rely on traditional medicine-based therapy for their primary healthcare. Very few studies are being carried out to study the traditional spices and herbs used in Indian cuisine and black tea, an attempt was made to evaluate the carbohydrate, protein, ascorbic acid, phenolics and flavonoid content and antioxidant levels in spices such as black pepper (*Piper nigrum*), fennel (*Foeniculum vulgare*), clove (*Syzygium aromaticum*), mint (*Mentha*), cinnamon (*Cinnamomum verum*) and Tulsi (*Ocimum tenuiflorum*). As these spices are usually added as flavoring agents in Indian masala tea and boiled at high temperatures, the present study was undertaken to evaluate the

crude aqueous extracts of selected individual spices and herbs for thermo-stability of antioxidant potential of extracts.

MATERIAL AND METHODS

Processing of Spices

Spices like black pepper (*Piper nigrum*), fennel (*Foeniculum vulgare*), clove (*Syzygium aromaticum*), cinnamon (*Cinnamomum verum*) and herbs like mint (*Mentha*) and tulsi (*Ocimum tenuiflorum*) were collected separately from local market. The herbs were dried in hot air oven at 70 degrees for 3-5 days. The dried spices and herbs were powered and stored at room temperature.

Sample extraction

A sample of 250 gm of dried black pepper, fennel, clove, mint, cinnamon and Tulsi was weighed (±0.001 g) into a beaker and 100 ml of boiling distilled water was added. After brewing for 5 min the blend was removed, and the extract was cooled down. All analyses of aqueous spice and herb extracts were done in triplicate.

Estimation of Protein and Carbohydrate Content

The protein concentration determination was done by the Lowry protein assay method.[8] Carbohydrates are assayed by anthrone method in which carbohydrates are dehydrated with concentrated H_2SO_4 to form **"**Furfural**",** which condenses with anthrone to form a green color complex which can be measured by using colorimetrically at 620nm (or) by using a red filter.[9] Anthrone react with dextrins, monosaccharides, disaccharides, polysaccharides, starch, gums and glycosides.

Determination of Ascorbic Acid

The 2,4- dinitrophenylhydrazine (DNP) method is used to determine the ascorbic acid levels in respective samples. The procedure is an adaptation of an analytical method in which reduced ascorbic acid is oxidised and dehydroascorbic acid, followed by coupling with 2,4- dinitrophenylhydrazine under controlled conditions to give red colouredosazones[10]. To 0.6 ml of spice and herb extract, distilled water was added to make volume up to 3ml. Then, 1ml of 2,4-DNP was added to each tube followed by incubation at 37°C for 3 hours. 7ml of 80% H_2SO_4 was then added to each tube in order to completely dissolve the red osazones crystals. Total amount of ascorbic acid was calculated using standard curve of ascorbic acid at wavelength of 540nm.

Estimation of total phenol content (TPC)

The total phenol content (TPC) was determined by spectrophotometer using tannic acid as standard, according to the method described by the International Organization for Standardization (ISO) 14502-1. 1.0 ml of the diluted sample extract (in triplicate) was added to tubes containing 5.0 ml of a 1/10 dilution of Folin-Ciocalteu's reagent in water. Then, 4.0 ml of a sodium carbonate solution (7.5% w/v) was added and incubated at room temperature for one hour. The absorbance was measured at 765 nm. The TPC was expressed as mg tannic acid equivalent per gram (mg TAE/g). The concentration of polyphenols in samples was derived from a standard curve of tannic acid.

Determination of Total flavonoid content

Total flavonoid content was measured by the modified aluminum chloride colorimetric assay. The reaction mixture consists of 1 ml of extract and 4 ml of distilled water was taken in a 10 ml volumetric flask. To the flask, 0.30 ml of 5 % sodium nitrite was added and after 5 minutes, 0.3 ml of 10 % aluminum chloride was mixed. After 5 minutes, 2 ml of 1M Sodium hydroxide was treated and diluted to 10 ml with distilled water. A set of reference standard solutions of ascorbic acid (20, 40, 60, 80 and 100 µg/ml) were prepared. The absorbance for test and standard solutions were determined against the reagent blank at 510 nm with an UV/Visible spectrophotometer. The total flavonoid content was expressed as mg AAE/g of extract.

Determination of antioxidant power by using modified ferric ion reducing antioxidant power assay (FRAP)

The total antioxidant capacity was determined spectrophotometry, using ascorbic acid as standard, according to the modified FRAP assay. 0.1 ml of extract was taken and to it 0.9 ml of ethanol, 5 ml of distilled water, 1.5 ml of HCl, 1.5 ml of potassium ferricyanide, 0.5 ml of 1% SDS and 0.5 ml of 0.2% of ferric chloride was added. This mixture was boiled in water bath at 50°C for 20 minutes and cooled rapidly. Absorbance was measured at 750 nm to measure the reducing power of the spices and herb extract. The antioxidants in samples were derived from a standard curve of ascorbic acid. The total antioxidant power was expressed as mg ascorbic acid equivalent (AAE)/ g.

Statistical analysis: The assays were carried out in triplicate, and the results were expressed as mean values and the standard deviation (SD). The statistical differences were done by one-way ANOVA ($p \leq 0.05$).

RESULTS

The nutritional value of herbs and spices is dependent on its nutrient compositions such as carbohydrates, protein levels, ascorbic acid, and tannins. For the 4 spices and 2 herbs evaluated, crude protein was highest in clove (46.94 ± 1.15mg/ml) followed by tulsi (15.36 ± 0.31mg/ml), fennel (14.17 ± 1.05mg/ml), black pepper (13.43 ± 2.12mg/ml), cinnamon (7.62 ± 0.22mg/ml) and mint (3.07 ± 0.12mg/ml) as shown in Table1. The protein levels ranged from 46.94 ± 1.15 mg/ml to 3.07 ± 0.12mg/ml. The protein level was high in clove as compared to other herbs and spices studied. Tulsi had significantly higher protein levels than mint ($p \leq 0.05$) (table 1). Carbohydrates levels differed between all spices and herbs with tulsi (10.02 ± 0.84 mg/ml) having high carbohydrate levels followed by fennel (9.99 ± 0.88mg/ml) and black pepper (8.19 ± 0.79mg/ml) as reported earlier by[11].The mint (5.89 ± 0.37mg/ml), cinnamon (4.68 ± 0.44mg/ml) and clove(4.46 ± 0.76mg/ml) had low carbohydrate levels as shown in Table 1. The levels of carbohydrates in tulsi, fennel and black pepper were significantly higher than mint, cinnamon and clove ($p \leq 0.05$). The highest amount of vitamin C was found in clove (71.93 ± 7.82mg/ml) followed by black pepper (40.35 ± 1.83mg/ml), fennel (27.66 ± 7.82mg/ml), cinnamon (22.09 ± 0.55mg/ml), mint (12.81 ± 2.47mg/ml) and least was found in tulsi (10.65 ± 1.65mg/ml) as shown in Table 1.

Among the herbs and spices studied, the highest amount of phenolics was present in clove (118.3 ± 21.21mg TAE/gm) followed by mint (35.33 ± 1.69mg TAE/gm), cinnamon (28.13 ± 3.95mg TAE/gm), fennel (21.43 ± 0.14mg TAE/gm), black pepper (3.33 ± 2.82mg TAE/gm) and tulsi (3.33 gm ± 0.56mg TAE/gm) as given in Fig 1. The clove had significantly higher phenolics than all other herbs and spices studied ($p \leq 0.05$). The flavonoids was present in high amount in clove (57.16 ± 2.12mg AAE/gm) followed by mint (53.66 ± 1.41mg AAE/gm), fennel (50.66 ± 2.82mg AAE/gm),tulsi (50.66 gm ± 2.01mg AAE/gm), black pepper (45.16 ± 0.70mg AAE/gm) and cinnamon (43.31 ± 3.74mg AAE/gm) as shown in Fig 2.The flavonoids levels in clove were significantly higher than black pepper and cinnamon ($p \leq 0.05$).

Table 1: Protein, carbohydrate and ascorbic acid levels in herbs and spices undertaken in the present study

HERBS/SPICES	PROTEIN (mg/ml)	CARBOHYDRATES (mg/ml)	ASCORBIC ACID (mg/ml)
BLACK PEPPER	13.43 ± 2.12	8.19 ± 0.79*	40.35 ± 1.83 [a]
FENNEL	14.17 ± 1.05	9.99 ± 0.88*	27.66 ± 7.82 [a]
CLOVE	46.94 ± 1.15	4.68 ± 0.44 [a]	71.93 ± 7.82*
MINT	3.07 ± 0.12[a]	5.89 ± 0.37 [a]	12.81 ± 2.47 [a]
CINNAMON	7.62 ± 0.22	4.46 ± 0.76 [a]	22.09 ± 0.55 [a]
TULSI	15.36 ± 0.31*	10.02 ± 0.84*	10.65 ± 1.69 [a]

* denotes significance at ($p \leq 0.05$) with respect to a

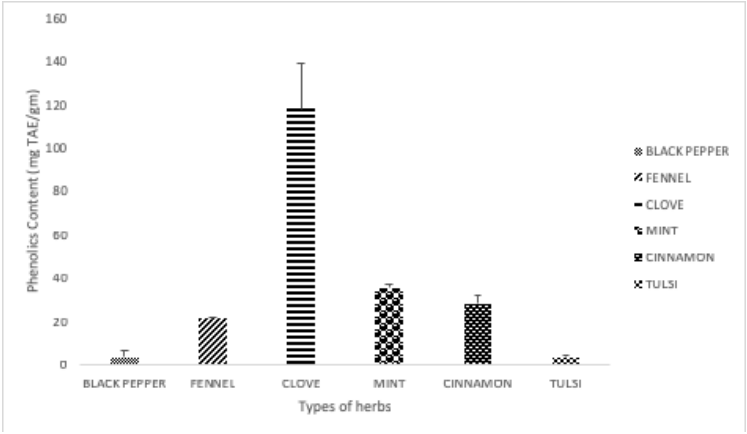

Fig. 1: Phenolic content in Black pepper, Fennel, Clove, Mint, Cinnamon and Tulsi

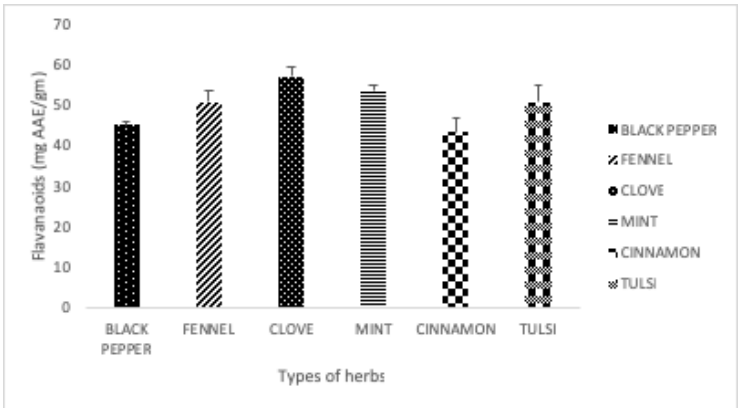

Fig. 2: Levels of flavonoids in Black pepper, Fennel, Clove, Mint, Cinnamon and Tulsi

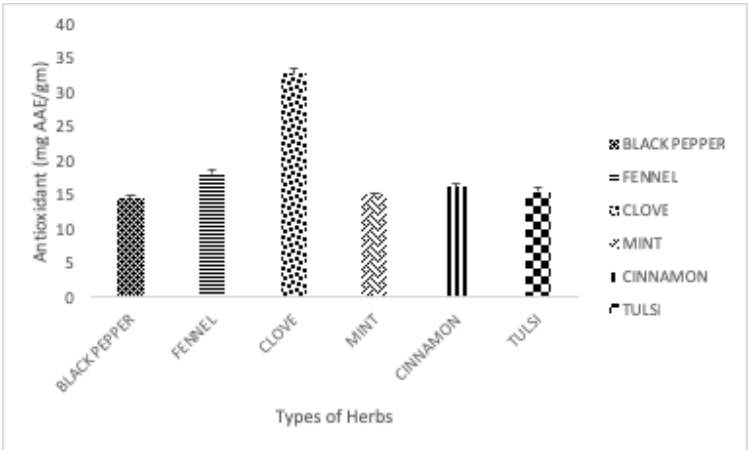

Fig. 3: Antioxidative capacity of Black pepper, Fennel, Clove, Mint, Cinnamon and Tulsi

The aqueous extract of clove (32.73 ± 0.84mg AAE/gm) showed powerful antioxidant activities among fennel (18.13 ± 0.56mg AAE/gm), cinnamon (16.13 ± 0.49mg AAE/gm), tulsi (15.43 ± 0.70mg AAE/gm), mint (15.03 ± 0.14mg AAE/gm) and black pepper (14.43 ± 0.42mg AAE/gm) when tested using ferric ion reducing antioxidant power (FRAP) methods as reported earlier[12,13] (Fig 3).

DISCUSSION

Traditionally spices, as part of the diets have holistic effects on human health so efforts should be directed at increasing the consumption of herbs and spices in common beverages consumed by Indians such as green tea which posses a package of protective phytonutrients. In the present study, the spices and herbs (cloves, black pepper, fennel, cinnamon, mint and tulsi) had significant amount of carbohydrates and protein and thus, provides all nutrients in small quantities. The high amount of carbohydrates in tulsi could be due to the presence of sugars, xylose and polysaccharides in mucilage. Although some of these spices contain some dietary sugars, they cannot be considered as carbohydrate sources as compared to tubers and cereals which are spread throughout the world[14].The spices such as cinnamon, tulsi and cloves have low-glycemic-index and displays insulin-potentiating activity. Cinnamon, clove and tulsi supplementation reduces postprandial hyperglycemia and can be important unexplored source of glycemia control in patients with diabetes mellitus type 2.

The clove had significantly higher ascorbic acid than black pepper, fennel, mint, tulsi and cinnamon. There was reported considerable variation in ascorbic content among the different spices studied with cloves having higher ascorbic acid than cinnamon spice[15]. Vitamin C, in particular, helps iron absorption by making iron less susceptible to phytate complexation, thus increasing its bioavailability [16].

The flavonoid levels in clove were significantly higher than black pepper and cinnamon ($p \leq 0.05$). The cloves have the highest amount of flavonoids among dill, caraway, coriander, oregano, rosemary, mint, basil, and sage [17]. The clove had significantly higher phenolics than all other herbs and spices studied (black pepper, fennel, clove, cinnamon, mint and tulsi) ($p \leq 0.05$) as observed in earlier studies [18, 19] which reported highest antioxidant activity in clove which decreased in the order of cinnamon, pepper, ginger, garlic, mint and onion. The principal polyphenolic compound present in clove (Myrtaceae family) is eugenol which has a strong antioxidant potential. The major types of phenolic compounds found in clove are phenolic acids (gallic acid), flavonol glucosides, phenolic volatile oils and tannins which act as radical scavenger and as a commercial source of polyphenols. Various other phenolic acids found in clove are caffeic, ferulic, elagic and salicylic acids. Flavonoids such as kaempferol, quercetin and its derivates (glycosilated) are also found in clove in lower concentrations. Cloves act as an iron chelator and showed high hydroxyl radical scavenging activity and thus can be used as a strong natural antioxidant to reduce lipid oxidation[20]. In the present study, the mint leaves (Lamiaceae family) also had high total phenolic and total flavonoid content which could be due to high levels of polyphenolic compounds such as caffeic acid, rosmarinic acid and various flavonoids glycoside e.g. Narirutin, Luteolin-7-o-rutinoside, Isorhoifolin and Hesperidin, etc.,[21].

The strong antioxidant activity of cinnamon (Lauraceae family) might be attributed to its high cinnamaldehyde content in addition to eugenol. Piperine present in black pepper contributes to the phenolic levels of pepper and also enhances the levels of circulating thyroid hormones[22] and even stimulates the release of epinephrine[23].The phenolic compounds such as cirsilineol, circimaritin, isothymusin, apigenin and rosameric acid and appreciable quantities of eugenol and flavonoids such as orientin and vicenin are present in tulsi (Lamiaceae)[24]. The various polyphenols present in these spices also inhibit fructose-mediated albumin glycation involved in the pathogenesis of diabetes[25]. The flavonoids present in fennel are eriodictyol-7-rutinoside, quercetin-3-rutinoside, and rosmarinic acid. The *fennel* has been reported to contain phenolic acids like 3-*O*-caffeoylquinic acid, 4-*O*-caffeoylquinic acid, 5-*O*-caffeoylquinic acid, 1,3-*O*-di-caffeoylquinic acid, 1,4-*O*-di-caffeoylquinic acid, and 1,5-*O*-di-caffeoylquinic acid[26] .

The aqueous extract of clove showed powerful antioxidant activities among, cinnamon tulsi, mint and black pepper when tested using ferric ion reducing antioxidant power (FRAP) methods as reported earlier [12,13] (Fig 3). Cloves act as an iron chelator and effectively inhibit the hydroxyl radical and showed high hydroxyl radical scavenging activity and thus can be used as a strong natural antioxidant to reduce lipid oxidation. Tulsi exerts its antioxidant effect by elevating the glutathione and antioxidant enzyme levels (SOD) and decrease lipid peroxidation [24]**.**

CONCLUSION

The present study concluded that water extracts of spices and herbs at high temperature exhibited significant antioxidant activity, indicating that the spice constituents were resistant to thermal denaturation. All the spices and herbs had significant amount of phenolic and flavonoids with clove having the highest levels. The highest antioxidant activity was shown by clove followed by fennel, cinnamon, tulsi, mint and black pepper. Thus the spices and herbs can be used as a good phyto-medicine and natural antioxidants to prevent the harmful free radical damage and prevention of onset of some chronic diseases in the body.

ACKNOWLEDGEMENT

We are highly grateful to the principal GGDSD College, Chandigarh for providing us the infrastructure to carry out this research project. It is declared that there is no commercial or financial conflict of interest.

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Cite this article as:

Avneet Kaur *et al*. Antioxidant levels of common herbs and spices used in Indian tea: Black pepper (*Piper nigrum*), Fennel (*Foeniculum vulgare*), Clove (*Sygyzium aromaticum*), Mint (mentha), Cinnamon (*Cinnamomum verum*) and Tulsi (*Ocimum tenuiflorum*). Int. Res. J. Pharm. 2018;9(11):119-123 http://dx.doi.org/10.7897/2230-8407.0911270

Source of support: Nil, Conflict of interest: None Declared