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Original Article

SYNERGISTIC ANTIOXIDANT ACTIVITY OF TEA WITH GINGER, BLACK PEPPER AND TULSI

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ABSTRACT

Food nutrients having antioxidant potential have been a major focus of research in recent years because they were regarded as magic bullets that could help to prevent many human diseases such as cancer, arthritis, Alzheimer, Parkinson and cardiovascular diseases, etc. In the present research, the four plants namely- tea (*Camellia sinensis*), ginger (*Zingiber officinale*), black pepper (*Piper nigrum*) and tulsi (*Ocimum sanctum*) have been selected as all these plants show antioxidant potential individually. People used to drink tea often by boiling it with ginger, black pepper and tulsi. However there is no data available on the antioxidant activity of these four plants in synergism. The aim of the present study was to study the antioxidant potential of the different combinations of above plants by 1,1,-Diphenyl-2-picrylhydrazyl assay. Appropriate combinations of all the four plants extract showed best antioxidant activity.

Keywords: Tea, Tulsi, Ginger, Black pepper, Herbs, Antioxidant and Synergistic effect.

INTRODUCTION

According to the modern theory of free radical biology, reactive oxygen species are involved in several disorders. The main free radicals are superoxide radicals (SOR), hydroxyl radical (OHR), hydroperoxyl radical (HPR), alkoxyl radical (AR), peroxyl radical (PR) and nitric oxide radical (NOR). These free radicals are associated with DNA damage, attack lipids, causes carcinogenesis, cardiovascular and neuro degenerative diseases [1,2]. These harmful actions of free radicals can be blocked by antioxidant substances which scavenge the free radicals and detoxify the organism [3]. The food rich in antioxidants play an essential role in the prevention of many diseases. Therefore plant derived antioxidants [4, 5] and their derivatives [6] are now receiving a special attention. A large number of phenolic compounds present in foods, vegetables, fruits, herbs and spices have been reported to possess good antioxidant properties. We have selected to study the synergistic antioxidant activity of tea with ginger, black pepper and tulsi.

Tea (*Camellia sinensis*) is the most widely consumed beverage in the world. Tea is particularly rich in polyphenols for example catechins, epicatechins, epigallocatechin, epicatechin gallate, theoflavins and theorubigins. Tea polyphenols have high radical scavenging activity. Because of its radical scavenging activity, it is an excellent antioxidant [7]. The rhizome of the ginger (*Zingiber officinale*) is generally used as a spice in food. Gingerols and their corresponding dehydration products shogaols were considered as the active principles of ginger and these constituents are responsible for strong antioxidant activity [8]. Black pepper (*Piper nigrum*) is one of the most popular spice product in oriental countries, largely used as flavoring agent in food and tea. The principal bioactive constituent of black pepper is piperine which increases the bioavailability [9, 10] of many phytochemicals present in food items. Black pepper extract shows good antioxidant activity [11].

Tulsi (*Ocimum sanctum*) of family Labiateae is known as "Queen of plants". It is good source of linalool, eugenol, methyl charicol and cineole. Presence of eugenol contributes to its antioxidant property and is also thought to be responsible for inhibition of lipid peroxidation [12].

Thus the present study was undertaken to show the synergistic effect of commercially available black tea with ginger, black pepper and tulsi which are commonly consumed by natives as herbal drinks for herbal therapies. We have determined the antioxidant activity of individual plant and their combinations by DPPH* (1,1-diphenyl-2-picryl-hydrazyl) free radical assay.

MATERIALS AND METHODS

Chemical such as gallic acid was purchased from CDH Pvt. Ltd. New Delhi, India, 1,1-diphenyl-2-picrylhydrazyl (DPPH*) was purchased from HiMedia Pvt. Ltd. Mumbai, India and Methanol AR was purchased from Loba Chemie Pvt. Ltd. Mumbai, India. Tea (Tata Tea Premium, Batch No. 11KK34), ginger and black pepper were purchased from the local market. Leaves of tulsi were collected and identified from botany department of our College. Extracts were dried in vacuum rotary evaporator (Perfit, Model No. VP 50 D) and UV-Visible spectrophotometer (Systronic, Model No. 1302) was used for recording the absorbance of all samples.

Preparation of aqueous and methanolic extract

Commercially available black tea (10 g) was refluxed with 50 ml distilled water and methanol separately on water bath for 20 minutes. The extracts were filtered and solvent was evaporated by vacuum rotary evaporator under pressure to obtain in powder form. Similar procedure was followed for ginger, black pepper and tulsi. Now the aqueous and methanolic extracts of above herbs and their polyherbal combinations (Table-1) were tested for their antioxidant potential.

1,1,-Diphenyl-2-picrylhydrazyl assay (DPPH* Assay)

The hydrogen atom or electron donating ability of the plant extract was determined from bleaching of purple colored methanol solution of DPPH* [13, 14], gallic acid was used as standard. This spectrophotometric assay uses the stable radical DPPH* as a reagent. The procedure involves measurement of decrease in absorbance of DPPH* at its absorption maxima of 517 nm. DPPH* was prepared at a concentration of 0.002%. The stock solutions of the extracts were prepared in methanol (1 mg/10ml). Different volume (1.0, 0.5, 0.25, 0.125 and 0.062 ml) of extracts was taken in separate test tube and volume was made up to 2 ml with methanol. Now 2ml of DPPH* solution was added in each test tube and kept in dark for 30 minutes. The same procedure was followed for Gallic acid as well. Later optical absorbance was recorded at 517 nm using UV- Visible spectrophotometer. Methanol with DPPH* was used as a control. All the samples were tested in triplicate. The formula used for the calculation is:

% Inhibition of DPPH* activity = (A - B / A) X 100

Where A = absorbance of control;

B = absorbance of sample.

RESULTS

Determination of radical scavenging activity by DPPH* is a rapid and reliable method to evaluate the antioxidant activity. With this method, it is possible to determine the radical scavenging potential of antioxidants by measuring the absorbance at 517nm. Among all the herbs examined, tea exhibited radical scavenging activity with 91.83, 94.64; ginger 30.36, 84.18; black pepper 35.20, 45.66 and tulsi 96.17 and 94.90% for both aqueous and methanolic extract respectively with compare to gallic acid (Table - 1). The combination of tea and ginger; tea and black pepper; tea and tulsi; tea, ginger and black pepper; tea, black pepper and tulsi; tea, ginger and tulsi; tea, ginger, black pepper and tulsi exhibited radical scavenging activity with 94.64, 95.15; 87.50, 86.73; 96.68, 93.11; 84.44, 88.78; 96.68, 86.22; 98.46, 92.35 and 98.78, 91.33 % in that order for aqueous and methanolic extract in comparison to standard (gallic acid). The results showed that selected polyherbal combinations of all the extracts with tea were found to produce best antioxidant activity in comparison to their individual extracts.

Table1: Antioxidant potential of aqueous and methanolic extracts and their polyherbal combinations of some herbs.

Extracts and their polyherbal combinations	% Inhibition by DPPH* Assay	
	Aqueous Extract	Methanol Extract
Tea	91.83	94.64
Ginger	30.36	84.18
Black Pepper	35.20	45.66
Tulsi	96.17	94.90
Tea: ginger (1:1) (a)	94.64	95.15
Tea: black pepper (1:1) (b)	87.50	86.73
Tea: tulsi (1:1) (c)	96.68	93.11
Tea : ginger: black pepper (1:1:1) (d)	84.44	88.78
Tea : black pepper: tulsi (1:1:1) (e)	96.68	86.22
Tea: ginger : tulsi (1:1:1) (f)	98.46	92.35
Tea: ginger: black pepper : tulsi (1:1:1:1) (g)	98.72	91.33
Gallic acid (Standard) (h)	95.66	

DISCUSSION

Different herbs have different bioactive constituents with different antioxidant activities. When they are taken together, the mixtures shows higher or lower antioxidant capacity than their individual extracts. It may be due to synergistic, additive or antagonistic interaction among different compounds. The principal aim of this research is to identify the synergistic mixtures. As antioxidant potential depends upon the presence of phenolic compounds and flavonoids thus the higher antioxidant potential of tulsi and tea can be attributed to the high amounts of polyphenols like eugenol, cardinene, cubenol, and flavon-3-ols, catechin, epicatechin, epicatechin gallate, and their fermentative products–theoflavins, thearubigin dissolvable in water [15, 16], while polyphenols like gingerols and their corresponding dehydration products shogaols in ginger and piperine in black pepper are responsible for higher antioxidant activity in methanol extract [17].

The individual herbs (tea, ginger, black pepper and tulsi) and seven mixtures of tea with other herbs have been evaluated for synergistic antioxidant potential. The results indicated that the best synergistic combination is g (tea, ginger, black pepper, tulsi) followed by f, c = e, a, b and d (aqueous extracts). But it has been observed that among the mixture of methanolic extract of different herbs, the best result is obtained in the synergistic combination; a (tea: ginger). This clearly shows that the amount of antioxidant compounds present in methanolic extract are more than the aqueous extracts of above herbs. The next mixture of methanolic extracts having high antioxidant potential is c followed by f, g, d, b and e. The findings of above experiments clearly shows that instead of drinking tea alone, the mixture of tea, ginger, black pepper and tulsi will be more fruitful for health as it has the highest antioxidant potential among the mixtures studied.

CONCLUSION

Thus it can be concluded that the aqueous and methanolic extracts of all the herbs have good antioxidant capacity excluding the aqueous extracts of ginger and black pepper and methanolic extract of black pepper. On the other hand selected combinations of extracts showed very good antioxidant potential in the synergistic manner. The mixture of aqueous extracts of tea, ginger, black pepper and tulsi (1:1:1:1); tea, ginger and tulsi (1:1:1) exhibited the outstanding antioxidant activity as compared to all other extracts and mixtures. These herbal drinks can be considered as powerful antioxidants sources.

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