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Evaluation of Antioxidant Activity, Phenol and Flavonoid Contents of Some Selected Indian Medicinal Plants

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ABSTRACT

Phenolic compounds from plants are a class of antioxidant agents which act as free radical terminators. Flavonoids show antioxidant activity and their mechanism of action are through scavenging or chelating process. Our present study, we carried out a systematic record of the relative antioxidant activity is selected Indian medicinal plant species extracts. The total phenol varied from 24.0±1 to 288.5±5 mg/g in the methanol extracts. Flavonoid contents were between 25.05 ± 0.18 and 78.2± 4.5 mg/g. 1, 1-diphenyl-2-picryl hydrazyl (DPPH) radical scavenging effect of the extract was determined spectrophotometrically. The highest radical scavenging effect was observed in *Camellia sinensis* with IC_{50} = 0.017 mg/ml. The potency of radical scavenging effect of *Camellia sinensis* extract was about 4 times greater than synthetic antioxidant butylated hydroxyl toluene (BHT). The greater amount of phenolic compounds leads to more potent radical scavenging effect as shown by *Camellia sinensis* extract.

KEY WORDS: Anti-Oxidant, *Camellia sinensis,* Flavonoids, Medicinal plants, *Thespesia populnea*,

INTRODUCTION

Free radicals contribute to more than one hundred disorders in humans including atherosclerosis, arthritis, ischemia, and reperfusion injury of many tissues, central nervous system injury, gastritis, cancer, and AIDS (1, 2). Free radicals due to environmental pollutants, radiation, chemicals, toxins, deep fried and spicy foods as well as physical stress, cause depletion of immune system antioxidants, change in gene expression and induce abnormal proteins. Oxidation process is one of the most important routes for producing free radicals in food, drugs, and even living systems. Catalase and hydroperoxidase enzymes convert hydrogen peroxide and hydro peroxides to non radical forms and functions as natural antioxidants in human body. Due to depletion of immune system natural antioxidants in different maladies, consuming antioxidants as free radical scavengers may be necessary (3-5).

Currently available synthetic antioxidants like butylated hydroxyl anisole (BHA), butylated hydroxyl toluene (BHT), tertiary butylated hydroquinones and gallic acid esters have been suspected to cause or prompt negative health effects. Hence, strong restrictions have been placed on their application and there is a trend to substitute them with naturally occurring antioxidants. Moreover, these synthetic antioxidants also show low solubility and moderate antioxidant activity (6).

The many plant species have been investigated in the search for novel antioxidants but generally there is still a demand to find more information concerning the antioxidant potential of plant species. It has been mentioned the antioxidant activity of plants might be due to their phenolic compounds (7). Recently there has been an upsurge of interest in the therapeutic potentials of medicinal plants as antioxidants in reducing such free radical induced tissue injury. Besides well

known and traditionally used natural antioxidants are already exploited commercially either as antioxidant additives or a nutritional supplement. Also many other plant species have been investigated in the search for natural antioxidants (8) but generally there is still a demand to find more information concerning the antioxidant potential of plant species.

Flavonoids are a group of poly phenolic compounds with known properties, which include free radical scavenging, inhibition of hydrolytic and oxidative enzymes and anti-inflammatory action (9). Some evidence suggests the biological actions of these compounds are related to their antioxidant activity.

The compounds such as flavonoids, which contain hydroxyls, are responsible for the radical scavenging effect in the plants (10). An easy, rapid and sensitive method for the antioxidant screening of plant extracts is free radical scavenging assay using 1,1,diphenyl-2-picryl hydrazyl (DPPH) stable radical spectrophotometrically. In the presence of an antioxidant, DPPH radical obtains one more electron and the absorbance decreases (11). According to our study, the high contents of these phytochemicals in *C.sinensis* can explain its high radical scavenging activity. Based on the above, our present study, we carried out a systematic record of the relative free radical scavenging activity in selected four Indian medicinal plant species, which are being used traditionally; *Camellia sinensis* (Theaceae), *Sesbania grandiflora* (Leguminosae), *Thespesia populnea* (Malvaceae) *and Cassia auriculata* (Fabaceae).

MATERIALS AND METHODS

Chemicals

1,1-Diphenyl -2-picryl hydrazyl (DPPH) and quercetin were purchased from Sigma chemicals .Co, Vadodara, Gujarat-6. Gallic acid, tert-butyl-4-hydroxy toluene (BHT), Folin Ciocalteu reagent and Methanol were purchased from Merck India Ltd,Mumbai-18.

Plant materials

The leaves of the four plants *Camellia sinensis* (Theaceae), *Sesbania grandiflora* (Leguminosae), *Thespesia populnea* (Malvaceae) and *Cassia auriculata* (Fabaceae) were collected from Erode and its surroundings. Plant materials were identified by Mr.G.V.S.Murthy, Joint director of the Botanical Survey of India (BSI), Coimbatore, Tamilnadu, India and a voucher specimen was deposited (SC5/23). Plant materials were dried at room temperature and ground in a mortar.

Preparation of extracts

50 Gms of each plant powder was extracted in 500ml of methanol by maceration process (48hrs). The solvents were removed under the vacuum at temperature below 50°C and the extracts were stored.

Total flavonoids determination

Aluminum chloride colorimetric method was used for flavonoids determination (12). Each plant extracts (0.5ml of 1:10g/ml) in methanol were separately mixed with 1.5ml of methanol, 0.1ml of 10% Aluminum chloride, 0.1ml of 1M potassium acetate and 2.8ml of distilled water. It remained at room temperature for 30 minutes. The absorbance of the reaction mixture was measured at 415nm. The calibration curve was prepared by preparing quercetin solutions at concentrations 12.5 to 100 µg/ml in methanol.

Total phenols determination

Total phenols were determined by Folin Ciocalteu reagent (13). A dilute extract of each plant extract (0.5ml of 1:10g/ml) or Gallic acid (Std.phenolic compound) was mixed with Folin Ciocalteu reagent (5ml, 1:10 diluted with distilled water) and aqueous Na_2CO_3 (4ml, 1M). The mixtures were allowed to stand for 15 minutes and the total phenols were determined by colorimetry at 765nm. The standard curve was prepared using 0, 50, 100, 150, 200, 250mg/l solutions of Gallic acid in methanol: water (50:50, v/v). Total phenol values are expressed in terms of Gallic acid equivalent (mg/g of dry mass), which is a common reference compound.

Antioxidant activity

1, 1-diphenyl-2-picryl hydrazyl (DPPH) was used for determination of antioxidant potential of the extracts (11). Different concentrations of each herbal extracts were added at an equal volume, to methanolic solution of DPPH. After 15min at room temperature, the absorbance was recorded at 517nm. It was repeated for 3 times, BHT and quercetin were used as standard controls. IC_{50} values denote the concentration of sample, which is required to scavenge 50% of DPPH free radicals.

Statistical analysis

The statistical significance between antioxidant activity values of the extracts was evaluated with a Mann - Whitney U test. P values less than 0.05 were considered to be statistically significant.

RESULTS

Flavonoid and total phenol contents of the extracts

The flavonoids show antioxidant activity and their effects on human nutrition and health care considerable. The mechanisms of action of flavonoids are through scavenging or chelating process (14). Phenolic compounds are a class of antioxidant agents, which act as free radical scavengers (15). The flavonoid contents of the extracts in terms of quercetin equivalent (The std curve equation: $y = 0.0067X + 0.0132$, $r^2 = 0.999$) were between 25.15 ± 0.18 and 78.3 ± 4.5.

The flavonoid contents in the extracts of *Camellia sinensis* (58 ± 5.4mg/g) and *Sesbania grandiflora* (78.2 ± 4.5mg/g) were higher than that in the extracts of *Cassia auriculata* and *Thespesia populnea*. The table no.1 also show the contents of total phenols that were measured by Folin Ciocalteu reagent in terms of Gallic

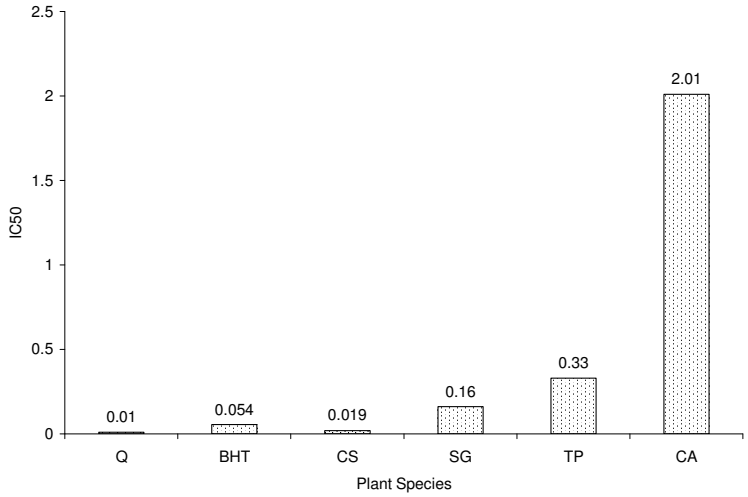

Fig 1. IC_{50} (mg/ml) values of plant extracts for free radical scavenging activity by DPPH radical. Lower IC_{50} values indicates higher antioxidant activity. Extracts: CS = Camellia sinensis, SG = Sesbania grandiflora, TP = Thespesia populnea, CA = Cassia auriculata, Q = Quercetin, BHT = Butylated hydroxyl toluene.

Table 1. Flavonoid and phenol contents in the 4 plant extracts.

Plant species	*Flavonoid (mg/g)*	*Phenol (mg/g)*
Camellia sinensis	*58 ± 5.4[1]*	*288.5 ± 5[1]*
Sesbania grandiflora	*78.2 ± 4.5*	*54.5 ± 7*
Thespesia populnea	*25.05 ± 0.18*	*31.2 ± 4*
Cassia auriculata	*36.2 ± 1.2*	*24.0 ± 1*

[1]Each value in the table was obtained by calculating the average of three experiments ± standard deviation.

Table 2. The comparision of DPPH radical scavenging activity of the plant extracts and those BHT and quercetin.

Plant species	*Concentration (mg/ml)*	*Scavenging (%)*
Camellia sinensis	*0.1*	*94.2 ± 0.6[1]*
Sesbania grandiflora	*0.8*	*89.3 ± 1.5*
Thespesia populnea	*4*	*44.1 ± 1*
Cassia auriculata	*4*	*70.9 ± 1*
BHT	*0.4*	*93 ± 0.5*
Quercetin	*0.025*	*95.6 ± 0.4*

[1]Each value in the table was obtained by calculating the average of three experiments ± standard deviation.

acid equivalent (The std.curve equation: y = 0.05X + 0.0545, r2 =0.9873). In case of total phenol, it was varied from 22.3 ± 3 to 289.5 ± 5mg/g in the extract powder. *Camellia sinensis* with total phenol contents of 288.5 ± 5mg/g had the highest amount among the plants in this study. The compounds such as flavonoids, which contain hydroxyls, are responsible for the radical scavenging effect in the plants (16). According to our study, the high contents of these phytochemicals in *C.sinensis* can explain its high radical scavenging activity.

Antioxidant activity

Free radicals are involved in many disorders, like Neurodegenerative diseases, Cancer and AIDS. Antioxidants through their scavenging power are useful for the management of those diseases. DPPH stable free radical method is an easy, rapid and sensitive way to survey the antioxidant activity of a plant extracts (11). The IC_{50} of the standard compounds, BHT and quercetin were 0.054 and 0.01 mg/ml, respectively. The fig. no 1 shows the amount of each extract needed for 50% inhibition (IC_{50}). The highest radical scavenging activity was showed by *C.sinensis* with IC_{50} = 0.019 mg/ml, which is higher than that of BHT ($P<0.05$). The radical scavenging effect of *C. sinensis* at 0.1 mg/ml was similar to BHT at 0.4 mg/ml. Therefore, the antioxidant effect of *C. sinensis* was four times greater than that of the synthetic antioxidant, BHT. The radical scavenging activity in the plant extracts decreased in the following order; *C.sinensis* > *S.grandiflora* > *T.populnea* > *C.auriculata*.

DISCUSSION

DPPH is relatively stable nitrogen centered free radical that easily accepts an electron or hydrogen radical to become a stable diamagnetic molecule (17). DPPH radicals react with suitable reducing agents as a result of which the electrons become paired off forming the corresponding hydrazine. The solution therefore loses colour stoichometrically depending on the number of electrons taken up (18).

Nitric oxide is an important chemical mediator generated by endothelial cells, macrophages, neurons, and involved in the regulation of various physiological processes. Excess concentration of nitric oxide is implicated in the cytotoxic effects observed in various disorders like AIDS, Cancer, Alzheimer's, and Arthritis (19). Oxygen reacts with the excess of NO to generate nitrite and peroxy nitrite anions, which acts as free radicals.

Free radicals are often generated as by products of biological reactions or from exogenous factors. The involvement of free radicals in the pathogenesis of a large number of diseases are well documented. A potent scavenger of free radicals may serve as a possible preventative intervention for the diseases (20).

The result of the present study showed that the extract of *C.sinensis*, which contain highest amount of flavonoid and phenolic compounds, exhibited the greatest antioxidant activity. The high scavenging activity of *C.sinensis* , may be due to hydroxyl groups existing in the phenolic compounds chemical structure that can provide the necessary component as a radical scavenger.

All of the extracts in this research exhibited different extent on antioxidant activity. *C.Sinensis* extract showed a higher potency than BHT in scavenging of DPPH free radical. This may be related to the high amount of flavonoid and phenolic compounds in this plant extract. In the longer term, plant species (or their active constituents) identified as having high levels of antioxidant activity *in- vitro* may be of value in the design of further studies to unravel novel treatment strategies for disorders associated with free radicals induced tissue damage

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