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ORIGINAL ARTICLE

ANTIOXIDANT PHENOLIC ACIDS FROM SOME SELECTED MEDICINAL PLANTS OF SOUTH INDIA

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ABSTRACT

Polyphenols are the heterogeneous class of compounds possessing a variety of effects towards antioxidant behaviour. In the search of plants as a source of natural antioxidants, four medicinal plants namely *Anisomeles malabarica* (L.) R. Br. ex Sims (Lamiaceae), *Erythrina variegata* L., (Leguminosae) and *Merremia gangetica* Cufod., (Convolvulaceae) and *Operculina turpethum* (L.) Silva Manso (Convolvulaceae) were selected for the present study. In the present study, four bioactive phenolic acids were isolated from methanolic extract of the selected plants viz., tannic acid (*A. malabarica*), ferulic acid (*E. variegata*), chlorogenic acid (*M. gangetica*), *p*-coumaric acid (*O. turpethum*) and their identities were confirmed with previous reports. The isolated molecules were subjected to determine the antioxidant activity by different *in vitro* assays such as DPPH, FRAP, phosphomolybdenum, hydrogen peroxide scavenging, hydroxyl scavenging, and superoxide dismutase assays. In the present study, tannic acid showed the highest percentage of inhibition by DPPH, FRAP and superoxide dismutase methods while *p*-coumaric acid showed highest value of absorbance/inhibition by phosphomolybdenum assay and hydrogen persoxide method and chlorogenic acid showed highest value of inhibition by hydroxyl scavenging activity method. Thus all the phenolic acids showed significant antioxidant activities. From the present study, it is concluded that all the phenolic acids exhibited significant activity and thus it proved as a potent antioxidant therapeutic agent to develop an antioxidant plant based drug from these bioactive molecules.

Keywords: Medicinal plants, phenolic acids, antioxidant, drug development

1.INDRODUCTION

There is a growing body of evidence suggesting that free radicals play an important role in the development of tissue damage and pathological events in living organisms (Aruoma, 1998; Lefer and Granger, 2000; Smith et al., 2000; Bhatia et al., 2003; Olinski et al., 2003; Peuchant et al., 2004). Lipid peroxidation of fats and fatty acids in foods results not only in their spoilage but is also a source of peroxyl and hydroxyl radicals that are associated with carcinogenesis, mutagenesis and aging (Yagi, 1987; Finkel and Holbrook, 2000). Therefore, antioxidants that scavenge reactive oxygen species (ROS) and reactive nitrogen species (RNS) may be of major importance in preventing the onset and/or the progression of oxidative pathologies and provide anticancer, antiviral, antimicrobial, anti-inflammatory activities, antioxidant properties, effects on capillary fragility, and an ability to inhibit human platelet aggregation have been ascribed to phenolics (McGregor et al., 1999; Spignoli, 2000). The physiological benefits of the plant phenolics have been attributed to their potential role in inhibiting lipid peroxidation, modulating cell signal transduction pathways and inducing apoptosis (Lin et al., 1999; Hou et al., 2004; Wiswedel et al., 2004).

Increasing experimental evidence has suggested that these compounds can affect a wide range of cell biological functions by virtue of their radical scavenging properties (Aruoma, 1998; Kinsella et al., 1993; Lai et al., 2001). The intake of antioxidants such as polyphenols has been effective in the prevention of these diseases (Cao et al., 1997; Vinson et al., 1995). Polyphenols belong to a heterogeneous class of compounds possessing a variety of effects towards antioxidant behaviour. In the search of plants as a source of

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natural antioxidants, some medicinal plants and fruits have been extensively studied for their antioxidant activity and radical scavenging in the last few decades (Singh et al., 2002). Among the various natural antioxidants, phenolic compounds are reported to have the character of quenching oxygen-derived free radicals by donating a hydrogen atom or an electron to the free radical (Wanasundara and Shahidi, 1996; Yuting et al., 1990). Also, phenolic compounds of plant materials have been shown to neutralize free radicals in various model systems (Zhang et al., 1996). Polyphenols, including vitamins, pigments and flavonoids, possess antimutagenic properties as well as blood glucose decreasing activity (Thompson et al., 1984). A great number of aromatic, spicy, medicinal and other plants contain chemical compounds exhibiting antioxidant properties. Numerous studies were carried out on some of these plants, e.g. rosemary, sage and oregano, which resulted in a development of natural antioxidant formulations for food, cosmetic and other applications. However, scientific information on antioxidant properties of various plants, particularly those which are less widely used in culinary and medicine is still rather scarce. Therefore, the assessment of such properties remains an interesting and useful task, particularly for finding new sources for natural antioxidants, functional foods and neutraceuticals. With this background, in this paper, the isolated bioactive molecules from the four important selected medicinal plants were subjected for antioxidant potential.

2. MATERIALS AND METHODS

Plant material

Four medicinal plants namely *Anisomeles malabarica* (L.) R. Br. ex Sims (Lamiaceae), *Erythrina variegata* L., (Leguminosae) and *Merremia gangetica* Cufod., (Convolvulaceae) and *Operculina turpethum* (L.) Silva Manso (Convolvulaceae) were selected for the present study to determine the antioxidant activity of the isolated bioactive molecules. Fresh aerial parts of all the plants were collected from Marakanam forest vicinity of Villupuram district, Tamil Nadu. The herbarium specimens were prepared for each plant, botanically identified and submitted at the Department of Botany, Kanchi Mamunivar Centre for Post Graduate Studies, Puducherry.

Extraction and Isolation

The air dried aerial parts of the plant (1 kg) were extracted thrice with boiling 95% EtOH (3×5L) and concentrated in vacuo to 500ml. The aqueous alcoholic concentrate was fractionated into benzene, ether, ethyl acetate, ethyl methyl ketone solubles. Benzene, fraction on paper chromatography gave (15% AcOH) no characteristic spots for polyphenolics and was not worked up further. The ether fraction was column chromatographed over sephadex LH-20. 20 fractions each of 10 ml were collected.

Methods

DPPH method

The antioxidant activity of the plant extracts and the standard was assessed on the basis of the radical scavenging effect of the stable 1, 1-diphenyl-2-picrylhydrazyl (DPPH)-free radical activity by modified method (Braca, 2002). The diluted working solutions of the test extracts were prepared in methanol. 0.002% of DPPH was prepared in methanol and 1 ml of this solution was mixed with 0.005 ml of sample solution and control solution separately. These solution mixtures were kept in dark for 30 min and optical density was measured at 517 nm. Methanol (1 ml) with DPPH

$$\% \text{ of Scavenging Activity} = \frac{\text{Absorbance of Control} - \text{Absorbance of test}}{\text{Absorbance of control}} \times 100$$

Ferric Reducing Antioxidant Power Assay

FRAP assay was performed according to the methods of Benzie and Strain (1999) with slightly modification. A known volume of the sample was made up to 3 ml with phosphate buffer, 1% potassium ferric cyanide and incubate in water bath for 20 minutes at 50°C. Cool it and add 10% TCA, 2.5 ml distilled water and 0.5 ml ferric chloride. Keep it for 10 minutes in room temperature and read the absorbance at 700 nm against standard ascorbic acid equivalent.

Hydrogen Peroxide Scavenging Capacity

The ability to scavenge hydrogen peroxide was determined according to the method of Ruch et al (1989). A solution of hydrogen peroxide (40 mM) was prepared in phosphate buffer (pH 7.4). 5 µl sample made to3 ml with Phosphate buffer and 1ml of 40mM hydrogen peroxide solution was mixed. Absorbance of hydrogen peroxide at 230 nm was determined 10 minutes later against a blank solution containing the phosphate buffer without hydrogen peroxide. The percentages of hydrogen peroxide scavenging were calculated:

$\%\ \text{Scavenged}\ [H_2O_2] = [(AC - AS)/AC] \times 100$

Where AC is the absorbance of the control and AS is the absorbance in the presence of the extract.

Hydroxyl Scavenging Activity

The Hydroxyl radical scavenging activity (HRSA) of the sample was determined by the method given by Klein et al., (1991). The intensity of the color formed was measured at 412 nm against reagent blank using spectrophotometer. The percentage hydroxyl radical scavenging activity was calculated by the following formula:

% HRSA = 1-(Absorbance of sample / Absorbance control) x 100

Phosphomolybdenum Method

The antioxidant activity of the extract was evaluated by the phosphomolybdenum method according to the procedure described by Prieto et al., (1999). A 0.04 ml extract was combined with 3 ml of reagent solution (0.6 M sulfuric acid,

28 mM sodium phosphate and 4 mM ammonium molybdate). In case of blank 0.3mL of methanol was used in place of extracts. The tubes containing the reaction solution were capped and incubated in a boiling water bath at 95°C for 90 min. After cooling to room temperature, the absorbance of the solution was measured at 695 nm using a spectrophotometer. The antioxidant capacity of each sample was expressed as ascorbic acid equivalent.

Superoxide Dismutase Activity

The antioxidant activity of the extract was evaluated by the Superoxide Dismuatse Activity according to the procedure described by Beauchamp and Fedovich (1976). 50 µl of the sample, 75 mM of Tris-HCl buffer (pH 8.2), 30 mM EDTA and 2 mM of pyrogallol were added. An increase in absorbance was recorded at 470 nm for 3 min by spectrophotometer. The rate of autoxidation of pyrogallol as determined by change in absorbance/min at 470 nm..

% inhibition of pyrogallol autoxidation= $[1-(\Delta A/\Delta A_{max})] \times 100$

where

ΔA= Absorbance change due to pyrogallol autoxidation in the sample reaction system

ΔA_{max} = Absorbance change due to pyrogallol autoxidation in the control

3. RESULTS

In the present study, four bioactive phenolic acids were isolated from methanolic extract of the selected plants viz., tannic acid (*A. malabarica*), ferulic acid (*E. variegata*), chlorogenic acid (*M. gangetica*), *p*-coumaric acid (*O. turpethum*) and their identities were confirmed with previous reports. The isolated molecules were subjected to determine the antioxidant activity by different *in vitro* assays such as DPPH, FRAP, phosphomolybdenum, hydrogen peroxide scavenging, hydroxyl scavenging, and superoxide dismutase assays. In DPPH method, tannic acid showed the highest percentage of inhibition 05.86 % of inhibition followed by chlorogenic acid (90.49 %), ferulic acid (51.42 %) and *p*-Coumaric acid (45.39 %) respectively. In FRAP method, tannic acid showed the absorbance value as highest value of absorbance as 0.0522 % followed by chlorogenic acid (0.0032 %), ferulic acid (0.0076 %) and *p*-coumaric acid (0.032 %) respectively. In phosphomolybdenum assay, *p*-coumaric acid showed highest value of absorbance as 0.1792, followed by tannic acid (0.1072), chlorogenic acid (0.149) and ferulic acid (0.0367) respectively. In Hydroxyl scavenging activity method, chlorogenic acid showed 53.47 % followed by tannic acid (44.91 %), *p*-coumaric acid (20.85 %), ferulic acid (17.64 %) respectively. In hydrogen peroxide scavenging method, *p*-coumaric acid showed the highest percentage of inhibition as 59.02 % of inhibition followed by ferulic acid (39.89 %), tannic acid (26.24 %) and chlorogenic acid (16.57 %) respectively. In superoxide dismutase method, tannic acid showed the highest percentage of inhibition (89.34 %), *p*-coumaric acid (50.21 %), ferulic acid (29.13 %) and chlorogenic acid (28.69 %) respectively.

Table 1. Antioxidant activity by DPPH method

Compound	Absorbance	% of Inhibition
Control	1.567	***
p-Coumaric acid	0.8557	45.39
Ferulic acid	0.7612	51.42
Chlorogenic acid	0.149	90.49
Tannic acid	0.0648	95.86

Table 2. Antioxidant activity by FRAP method

Compound	Concentration (µg)	Absorbance
Ascorbic acid		
p-Coumaric acid	10	0.032
Ferulic acid	10	0.0076
Chlorogenic acid	10	0.0032
Tannic acid	10	0.0522

Table 3. Antioxidant activity by Phosphomolybdenum assay method

Compound	Concentration(µg)	Absorbance
Standard 1		
p-Coumaric acid	10	0.1792
Ferulic acid	10	0.0367
Chlorogenic acid	10	0.149
Tannic acid	10	0.1072

Table 4. Antioxidant activity by Hydroxyl scavenging assay method

Compound	Absorbance	% of Inhibition
Standard	0.0187	***
p-Coumaric acid	0.0148	20.85
Ferulic acid	0.0154	17.64
Chlorogenic acid	0.0087	53.47
Tannic acid	0.0103	44.91

Table 5. Antioxidant activity by Hydrogen persoxide assay method

Compound	Absorbance	% of Inhibition
Standard	1.9706	***
p-Coumaric acid	0.8074	59.02
Ferulic acid	1.1844	39.89
Chlorogenic acid	1.644	16.57
Tannic acid	1.4535	26.24

Table 6. Antioxidant activity by superoxide dismutase assay method

Compound	Absorbance	% of Inhibition
Standard	0.046	***
p-Coumaric acid	0.0229	50.21
Ferulic acid	0.0326	29.13
Chlorogenic acid	0.0328	28.69
Tannic acid	0.0049	89.34

4. DISCUSSION

Antioxidants may be defined as compounds that inhibit or delay the oxidation of other molecules by inhibiting the initiation or propagation of oxidizing chain reactions (Velioglu et al., 1998). Antioxidants can also protect the

human body from free radicals and ROS effects. They retard the progress of many chronic diseases as well as lipid peroxidation (Pryor, 1991; Plant phenolics present in the fruit and vegetables have received considerable attention because of their potential antioxidant activity (Lopez-Velez et al., 2003). All the compounds investigated in the present study are phenolic compounds which are the major contributors of antioxidant activity in vegetable juices and the phenolic compounds are effective hydrogen donors which make them good antioxidant. In the present study, tannic acid showed the highest percentage of inhibition by DPPH, FRAP and superoxide dismutase methods while *p*-coumaric acid showed highest value of absorbance/inhibition by phosphomolybdenum assay and hydrogen persoxide method and chlorogenic acid showed highest value of inhibition by hydroxyl scavenging activity method. The activity of tannic acid by DPPH assay may be attributd due to hydrogen donating ability (Lee and Shibamoto, 2001; Siddaraju and Dharmesh, 2007). It was reported that the decrease in the absorbance of the DPPH caused by phenolic compound is due to scavenging of the radical by hydrogen donation, which is visualized as a discoloration from purple to yellow (Meir et al., 1995; Lee and Shibamoto, 2001). Similarly in FRAP method, the tannic acid were reported to reduce Fe^{3+} to Fe^{2+} in the presence of tripyridyltriazine (TPTZ), whereby an intense blue Fe2+–TPTZ complex with an absorbance maximum at 593 nm is formed (Benzie and Strain, 1996). Increasing absorbance indicates an increase in reductive ability. It was reported that the polyphenolic nature of tannic acid, it's relatively hydrophobic ''core'' and hydrophilic ''shell'' are the features responsible for its antioxidant action (Isenburg et al., 2006) and reported to possess antioxidant activity. The activity of *p*-coumaric acid by phosphomolybdenum assay is particularly based on the reduction of Mo (VI) to Mo (V) by the sample analyte and subsequent formation of a green phosphate Mo (V) complex at acidic pH (Strazˇisˇar et al., 2008; Kannan et al., 2013). In addition, it has been reported that the –OH of coumaric acid contributed to antioxidant activity (Kannan et al., 2013). Chlorogenic acid by hydroxyl scavenging activity method showed the highest percentage of inhibition than other phenolic compounds. Chlorogenic acid has been reported to possess antioxidant activity (Medini et al., 2014). Hydroxyl radical is one of the potent reactive oxygen species in the biological system that reacts with polyunsaturated fatty acid moieties of cell membrane phospholipids and causes damage to cell. Thus in the present study, it is concluded that all the phenolic acids exhibited significant activity and thus it proved as a potent antiooxidant therapeutic agent. Therefore, it is more opt to develop an antioxidant therapeutic from these bioactive molecules.

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