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Separation of polyphenol rich fraction from dried ginger rhizomes

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ABSTRACT

Ginger is the rhizome of Zingiber officinale Roscoe. Phenolic compounds are the major constituents of ginger, and possess health-promoting effects. The effects of different solvents of varying polarities on the extraction efficiency and total polyphenols (TPP) content were evaluated. Also, the antioxidant activity of the extracts was evaluated using radical scavenging assay, reducing power, and antioxidant capacity. The yield of the extract increased with increase in the polarity and dielectric constant of the respective solvent used. However, highest yield (16.5%) and greater release of total polyphenol content (TPP, 1.10% GAE) was obtained with 50% aqueous ethanol, due to its higher dielectric constant. Since, aqueous ethanol being considered as green and cheaper solvent compared to all other solvents used for the extraction, it may find application as a supplement in various food as well as pharmaceutical formulations / products.

Keywords: *Zingiber officinale*, polyphenols, antioxidant, extraction.

INTRODUCTION

Plant materials contain different phenolic antioxidant compounds. Phenolic compounds are dietary constituents widely spread in plant kingdom and include thousands of compounds with a variety of chemical structures. Over the last decade, awareness on the importance of antioxidant has been increased among consumers. Phenolic compounds in foods have been considered powerful antioxidants *in vitro* [1-4] and have been proved to be more potent antioxidants than vitamins E, C and carotenoids [5].

Free radicals are generated during oxidative metabolism and energy production in the body. Generation of free radical species beyond the antioxidant capacity of a biological system leads to oxidative stress. There are evidences that oxidative stress plays a role in heart diseases, neurodegenerative disorders, cancer and

the aging process. Therefore, lowering oxidative stress in body tissues by maintaining a healthy level of antioxidants can slow down or prevent the onset of these diseases. In addition, antioxidants are used as preservatives during food manufacture to minimize peroxidation of lipids and prevent rancidity of foods. For example, synthetic antioxidants, such as butylated hydroxy toluene (BHT), butylated hydroxy anisole (BHA) or tertiary butylated hydroxy quinone (TBHQ) possess good antioxidant capacity but have been restricted due to possible toxic side effects for human health [6, 7].

The main structural feature responsible for the antioxidant and free radical scavenging activities of phenolic derivatives is the presence of hydroxyl group on aromatic ring. Their antioxidant activity depends on their ability to donate hydrogen or electron and to delocalize the unpaired electrons within the aromatic structure. Phenols are able to donate the hydrogen atom of the phenolic hydroxyl to the free radicals, thus stopping the propagation chain during the oxidation process. These can also protect biological molecules against oxidation. The antioxidant activity of the spices plays a role in suppression of cell growth, viral replication, inflammation, inhibition of allergy, arthritis, prevention of cancer, heart diseases, and in abrogation of several other pathological conditions. The antioxidant potential of phenolics depends on the number and arrangement of the hydroxyl groups [5, 8, 9]. Many of the spice-derived ingredients such as carnosic acid, rosmarinic acid, caffeic acid, ferulic acid, eugenol, carnosol, thymol, carvacrol, [6]-gingerol, [6]-shogaol, zingerone, curcumin, capsaicin and vanillin are potent antioxidants and are of great interest to biologists and clinicians because they may help to protect the human body against damage by reactive oxygen species.

Ginger is the underground stem or rhizome of the herbaceous perennial plant *Zingiber officinale* Roscoe. Ginger is used throughout the world as a spice and food additive because of its characteristic pleasant fresh aroma and pungency. The nonvolatile pungent compounds (namely gingerols, shogaols, paradols, and zingerone) are some of the extensively studied phytochemicals and account for the antioxidant, anti-inflammatory, antiemetic, and gastro protective activities [10, 11]. It is well known that phenolic compounds are the major constituents of ginger [10], and these had attracted a great deal of research activity because of their health-promoting effects as antioxidants. The ginger CO_2 extract showed an antioxidant activity comparable with that of BHT in inhibiting the lipid peroxidation both at 37°C, and at a high temperature of 80°C [12]. Singh et al., [13] reported the extraction of ginger oleoresin by soxhlet method using ethanol, methanol, CCl_4 and isooctane. Zingerone was found to be the major component (33.6%, 33.3% and 30.5% for, methanol, CCl_4 and isooctane oleoresins), respectively. In the present investigation, extracts of dried ginger using solvents of different polarity were screened for total polyphenols content and their antioxidant potential in different *in vitro* systems. These included scavenging effect on 1,1'-diphenyl-2-picryl-hydrazyl radical, reducing power and antioxidant capacity. The results were compared with that of BHA and propyl gallate.

MATERIALS AND METHODS

Plant collection : Fresh matured rhizomes of ginger (Kodagu variety) were procured from Devaraja market, Mysore in the month of November 2010 and voucher specimen (CFTRI-PPSFT-Ginger 1) preserved in Plantation Products, Spices and Flavour Technology Department, Central Food Technological Research Institute, Mysore, India. Raw ginger rhizomes (5 kg) were thoroughly washed with water to remove the adhered materials such as organic matter and soil. The spoiled / damaged rhizomes were separated manually. The cleaned rhizomes were sliced (1-2 mm thickness) using the food processor (Robot Coupe CL 50E, Robot Coupe U.S.A., Inc. Jackson, MS). The slices of fresh ginger were uniformly spread in aluminum trays to a thickness of 6-10 mm and dried in a 48-tray convection dryer (Precision products, VATVA, Ahmadabad, India) at 50±4°C for 12 h. The dried flakes were powdered to a particle size of BS 25 mesh (<800microns) using apex mill.

Extraction : The dried ginger powder (100 g each) was loaded in glass columns (600 x 40 mm) and extracted with a series of solvents of different polarity separately [viz., hexane (H), dichloromethane (D),

chloroform (C), acetone (A), ethanol (E), methanol (M) and 50% aqueous ethanol (50% E)] while maintaining a material to solvent ratio of 1:20. The extracts were desolventized using rotatory evaporator at 50±2°C under reduced pressure and stored in desiccator until further use.

Quantification of total polyphenol content (TPP) content : The concentration of total polyphenolic content in the extracts was estimated spectrophotometrically by Folin-Ciocalteu (FC) method [14]. Gallic acid as the reference standard and the results are expressed as gallic acid equivalent (GAE) in milligrams per gram on dry weight basis of ginger. Gallic acid (1 mg/ml) was dissolved in distilled water. To 0.5 ml aliquot of various concentrations (0.01 – 0.05 mg/ml) of gallic acid, FC reagent (0.5ml) and 20% saturated sodium carbonate solution (1.5ml) were added to the tubes and the mixture was made up to 10 ml with distilled water. After 2h of incubation at room temperature, the absorbance was measured at 765 nm using a spectrophotometer. Calibration curve was plotted against concentration versus absorbance. Similarly, the extracts (1 mg/ml) were dissolved in methanol and the reagents were added as mentioned above and made up to 10 ml with distilled water and incubated at room temperature. The absorbance was measured at 765 nm and phenolic contents were estimated using standard gallic acid curve. The results are expressed as gallic acid equivalent (mg GAE per gram of extract as well as % GAE on raw material basis).

Antioxidant assays : The extracts were evaluated for antioxidant potential through in vitro models such as radical scavenging activity, reducing power (iron reducing activity) and antioxidant capacity (phosphomolybdenum complex).

Radical scavenging activity : The extracts were evaluated for radical scavenging ability using the stable radical DPPH• according to the method of Blois [15] with slight modifications [16]. Different concentrations of the extracts and BHA solution (each 1 ml) were taken in different test tubes. Four millilitres of 0.1 mM methanolic solution of DPPH was added to these tubes, shaken vigorously and allowed to stand at 27°C for 20 min in dark. The control was prepared as above without any extract. Methanol was used for the baseline correction during absorbance measurements. Absorbance of the samples was measured at 517 nm against the absorbance of the control. Radical scavenging activity was calculated using the following equation**,**

Inhibition (%) = [(absorbance of control -absorbance of sample) / absorbance of control] X 100.

The IC_{50} value, defined as the amount of antioxidant necessary to decrease the initial DPPH concentration by 50%, was calculated from the results and used for comparison.

Reducing power : The reducing power of the extracts was evaluated according to the method of Oyaizu [17]. One ml of various concentrations (25–200 µg) of standard or extract was mixed with phosphate buffer (0.2 mol/L., pH 6.6, 2.5 ml) and potassium ferricyanide (1%, 2.5 ml). The mixture was incubated at 50°C for 20 min. Trichloroacetic acid (10%, 2.5 ml) was added to the mixture, which was then centrifuged at 3000 rpm for 10 min. The upper layer of the solution (2.5 ml) was mixed with distilled water (2.5 ml) and ferric chloride (0.1%, 0.5 ml), and the absorbance was measured at 700 nm. Increased absorbance of the reaction mixture indicates increased reduction capability.

Antioxidant capacity : The antioxidant activity of the extracts was evaluated by the phosphomolybdenum method according to the procedure of Prieto et al. [18]. The total antioxidant capacities of extracts were evaluated and compared with that of standard propyl gallate. Ascorbic acid (0.1 mg/ml) was dissolved in distilled water. To 0.4 ml aliquot of various concentrations (0.25-5 µg/ml) of ascorbic acid, 4 ml of reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate) was added to the tubes. The tubes were capped and incubated in a boiling water-bath at 95°C for 90 min. The samples were cooled to room temperature and the absorbance was measured at 695 nm against blank. Calibration curve was plotted against concentration versus absorbance. Similarly, aliquot of 0.01-0.2 ml solutions (containing 25-200 µg of extract) was made up to 4 ml using

reagent solution, incubated, and the absorbance was measured at 695 nm against blank. The antioxidant capacity of the extracts was expressed as equivalents of ascorbic acid (µmol/g of extract).

Statistical analysis : All determinations were done at least in triplicate and were averaged, the results were provided as mean values with standard deviation. The obtained data were subjected to statistical analysis and the means, compared by Duncan's New Multiple Range test ($p \leq 0.05$), are presented.

RESULTS AND DISCUSSION

Ginger extracts : Different solvents were used for extraction of non-volatile compounds from ginger. The yields of the extracts were in the range of 2.9-16.5%. The yields of the solvent extracts are presented in Table 1. It was observed that the yield of the extract increased with increase in the polarity and dielectric constant of the respective solvent. Highest yield of extract (16.5%) was obtained with 50% aqueous ethanol followed by methanol (7.3%) and ethanol (5.8%). Lesser yield was found with hexane (2.9%).

Generally, methanol is not practically used in food and pharmaceutical processing due to its toxicity. Also, the solvents derived from petrochemicals such as hexane, pentane, di- and tri-chloromethane and acetone are not used in certifiable organic product. Ethanol is often more preferred in practice due to its several advantages, such as lower cost and being derived from natural sources as per International Federation of Organic Movement (IFOAM) [19, 20].

Table 1. Yield and total polyphenols content extracted from ginger with various solvents

Solvent	Dielectric constant (ε)	Yield (%)	TPP* (mg GAE/g)	TPP** (%GAE)
Hexane	1.9	2.9±0.9	164.2±1.8	0.49
Dichloromethane	8.9	3.2±0.1	136.1±0.9	0.51
Chloroform	4.8	4.0±0.5	172.5±1.5	0.68
Acetone	20.7	4.2±1.1	156.1±1.9	0.66
Ethanol	24.6	5.8±0.7	111.6±2.6	0.55
Methanol	32.7	7.3±0.1	148.4±0.7	1.07
50 % aqueous ethanol	46.7	16.5±0.7	66.8±2.1	1.10

***** Values are mg GAE/g of the extract

** Values are based on the raw material basis

Total polyphenols (TPP) content : Generally, phenolic compounds present in plants are known to act as free radical scavengers and the antioxidant activity of most of the plants produce is mostly due to the presence of phenolic compounds [21]. Basically, antioxidant mechanism of polyphenolic compounds is based on their hydrogen donating and metal ion chelating abilities [22, 23]. The TPP content of the extracts was determined using a gallic acid standard curve ($y = 0.0124\ x$; $R^2 = 0.9971$) and expressed as gallic acid equivalents. According to the obtained results (Table 1), it was evident that 50% ethanol

extract (1.10 % GAE based on raw material) had released highest quantity of total polyphenols followed by methanol (1.07%), chloroform (0.68%) and acetone (0.66%). Overall, considering all the solvent systems, 50% ethanol was found to be the most effective solvent since ethanol and aqueous ethanol are the most suitable solvent for extracting phenolic compounds from plant tissues due to their ability to inhibit the action of polyphenol oxidases that cause the oxidation of phenolic compounds and their ease of evaporation compared to water [24]. These results suggest that the extractability of polyphenols is influenced by the polarity of the solvents used [25, 26].

Antioxidant potential of ginger extracts : Polyphenols have been reported to be responsible for the antioxidant activities of botanical extracts. The antioxidant activities of phenolic compounds are due to their ability to scavenge free radicals, donate hydrogen atoms or electron, or chelated metal cations [27]. It is important to select and employ a stable and rapid method to assay antioxidant activity. Several methods have been developed to assay free radical scavenging capacity and the total antioxidant activity of plant extracts. In the present study, determination of antioxidant activity was evaluated using free radical scavenging ability (DPPH assay), reducing power, and antioxidant capacity (phosphomolybdenum assay).

The radical scavenging activity of the extracts was tested using DPPH model system [28] and the results are presented (Fig 1). The test was based on the reaction of antioxidant substances in the plant extract with the free radical, DPPH (1, 1'-diphenyl-2-picrylhydrazyl radical), converting the latter from a deep violet compound to a colorless 1,1'-diphenyl-2-picrylhydrazine. The extent of discoloration (bleaching) indicates the scavenging potential or antioxidant activity of the extract. In addition, the higher rate of discoloration indicates the more potent the antioxidant activity of the extract in terms of hydrogen-donating capacity. In the present study, the extracts were able to decolorize DPPH and it appears that the extracts from ginger possess hydrogen donating capabilities to act as antioxidant. Also, it was observed that chloroform and dichloromethane extracts were found to exhibit maximum radical scavenging activity (72%) at 50 ppm concentration, followed by acetone (69%) and ethanol (65%). Hexane (56%) showed the least activity. The synthetic antioxidant namely butylated hydroxy anisole (BHA) showed 94% activity at the same concentration. IC_{50} values in ppm are as follows: 22 (C), 30 (E), 32 (A), 35 (D), 44(H), 51 (M) and 85 (50% E). The IC_{50} values are inversely proportional to the TPP content of the extracts.

The antioxidative potential of ginger oleoresin may be due to the presence of various classes of organic compounds, which may produce either synergistic effect. There are many reports to emphasize that the phenolic group plays an important role in antioxidant activity [29, 13]. Silva et al. [30] reported the significant scavenging effects of phenolic compounds against the DPPH free radical. Hence, the presence of phenolic compounds such as gingerols, shogaols, zingerone, gingerdiols, diacetoxy-[6]-gingerdiol, etc., in ginger oleoresins may be responsible for their antioxidant properties.

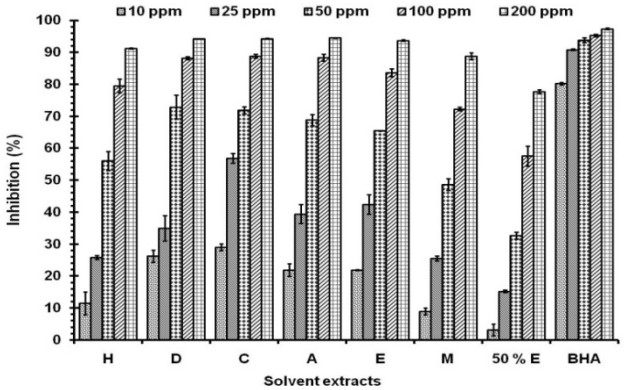

Fig. 1. Radical scavenging activity of solvent extracts of ginger and BHA

H-hexane, D-dichloromethane, C-chloroform, A-acetone, E-ethanol, M-methanol and 50% E- 50% aqueous ethanol; BHA- Butylated hydroxyanisole. Values are expressed as mean of triplicate determinations ± standard deviation; Different letters above the bars for the same concentration indicate statistically significant differences at $P < 0.05$.

The reduction of the ferricyanide (Fe^{3+}) complex to the ferrous (Fe^{2+}) form can be monitored by measuring the formation of Perl's Prussian blue at 700 nm, which occurs in the presence of reductants such as antioxidant substances [31]. In this assay, depending on the reducing power of antioxidant samples, the yellow color of test solution changes into various shades of green and blue colors. The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity. Reducing power of the extracts and standard antioxidant (viz., BHA) using the potassium ferricyanide reduction method were depicted in Figure 2. For the measurement of the reductive ability, the Fe^{3+}–Fe^{2+} transformation was investigated in the presence of extract using the method of Oyaizu [15]. The reducing power of extracts and standards were increased with increase of sample concentrations. At 50 µg/ml concentration, reducing power (absorbance at 700 nm) were in order of BHA > C > D > A > H > E > M > 50%E. Reducing power values are proportional to the TPP content of the extracts.

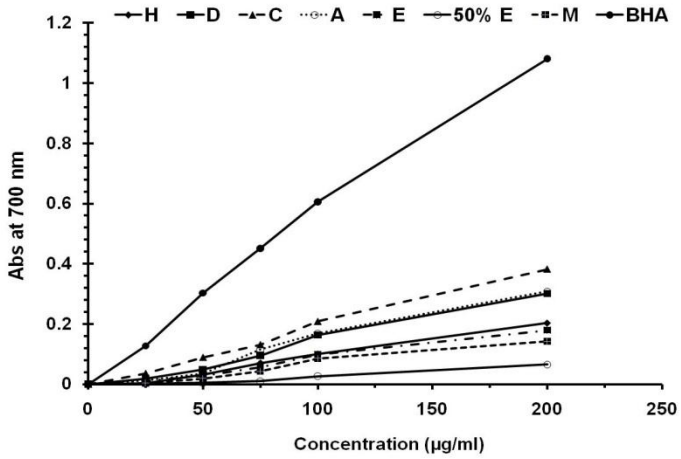

Fig. 2. Reducing power of extracts and standard BHA

H-hexane, D-dichloromethane, C-chloroform, A-acetone, E-ethanol, M-methanol and 50% E- 50% aqueous ethanol. Values are expressed as mean of triplicate determinations ± standard deviation.

The phosphomolybdenum method (PM) was based on the reduction of Mo (VI) to Mo (V) by the antioxidant compound and the formation of a green phosphate Mo (V) complex with the maximal absorption at 695 nm. It has been reported that the assay of phosphomolybdenum method has been used to quantify vitamin E in soybean seeds [18]. The total antioxidant capacity was quantitatively determined spectrophotometrically by measuring the absorbance values of phosphomolybdenum complex formed (Fig. 3). The order of antioxidant capacity as per absorbance values were: PG > C > D > A > H > E > M > 50%E. The antioxidant activity of different extracts depends on the presence of polyphenols which may act as reductones. The antioxidant capacity of the extracts showed 4365±72 (C), 3968±44 (A), 3446±125 (E), 3042±73 (D), 2833±67 (H), 2242±34 (M) and 1366±79 (50% E) µmol ascorbic acid equivalents/g of extract. The antioxidant capacity of the propyl gallate showed 5458 ± 56 µmol ascorbic acid equivalents/g.

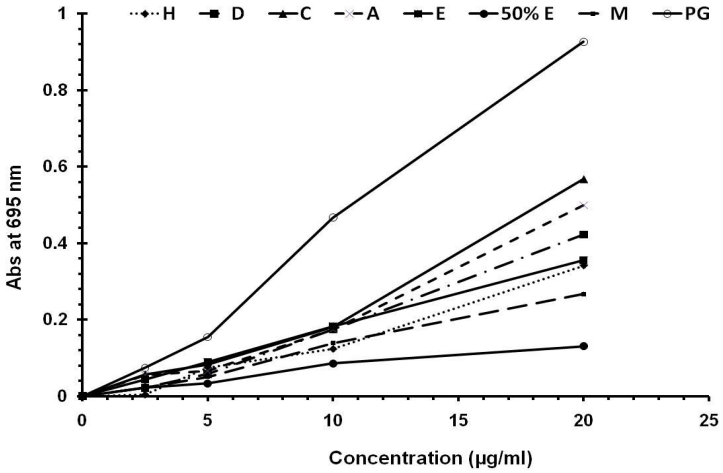

Fig. 3. Antioxidant capacity of the extracts and standard

H-hexane, D-dichloromethane, C-chloroform, A-acetone, E-ethanol, M-methanol and 50% E- 50% aqueous ethanol; PG-propyl gallate. Values are expressed as mean of triplicate determinations ± standard deviation.

APPLICATIONS

Among the various solvents used for the extraction, aqueous ethanol (50%) gave the highest yield and greater release of total polyphenol content (TPP) due to its higher dielectric constant. Moreover, it is considered as green and cheaper solvent compared to all other solvents. Hence, this extract might be used as a supplement in various food as well as pharmaceutical formulations / products. A ready-to-drink beverage using this conserve is developed and patent applied [32]. This extract may also be used for enriching the antioxidant compounds in functional foods.

CONCLUSIONS

The effect of various solvents to release the extracts from ginger was revealed. Extract yield, release of TPP content increased with increase in dielectric constant of the solvent used for the extraction. Highest yield (16.5%) and greater release of total polyphenol content (TPP, 1.10% GAE) was obtained with 50% aqueous ethanol, due to its higher dielectric constant. But the antioxidant capacity wise, it is relatively low. However, it is a green and cheaper solvent compared to all other solvents used for the extraction. Thus, 50% aqueous ethanol extract can be used on pilot scale level for the separation of antioxidant-enriched fraction and identification of antioxidant compounds from dried ginger.

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