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ORIGINAL ARTICLE

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Ethanol as a solvent and hot extraction technique preserved the antioxidant properties of tamarind (*Tamarindus indica*) seed

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ABSTRACT

The influence of two extraction solvents (ethanol and acetone) and two extraction techniques *i.e.*, hot extraction at 40^{0}C and cold extraction at 26^{0}C were investigated on the phenolic content and antioxidant activity of extracts from *Tamarindus indica* seed. The antioxidant activity of *T. indica* was determined by evaluating 1,1-Diphenyl-2-picrylhydrazyl (DPPH) scavenging activity, ferric reducing power assay (FRAP) and ascorbic acid equivalent content (AAC). The tested sample showed appreciable amounts of total phenolic contents (51.45-71.68 mg GAE/gm of dry extract), DPPH scavenging capacity (61.18-71.17%), IC_{50} values (98.30-248.60), reducing power (0.6377-0.7702) and total antioxidant capacity (22.75-43.80 AAE/gm) at different solvents and techniques. Current study data shown higher extract yields, phenolic contents, scavenging activity, reducing power and antioxidant activity using ethanol solvent compared to the respective acetone solvent. In addition, higher extract yields and other properties were obtained by hot extraction at 40^{0}C compared to the cold extraction at 26^{0}C. Present study suggests that ethanol as a solvent and hot extraction technique could be better to preserve the antioxidant properties of tamarind seed.

Keywords

Antioxidant activity, Extraction effect, DPPH, FRAP, Tamarind, Total phenolic content

ARTICLE HISTORY

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INTRODUCTION

Fruits those are rich with antioxidants help in lowering incidence of degenerative diseases like cancer, arthritis, arteriosclerosis, heart disease, inflammation, brain dysfunction (Feskanich et al., 2000). Oxidative mechanisms that lead to degenerative diseases can be inhibited by antioxidant compounds like polyphenols, phenolic acids and flavonoids which scavenge free radicals such as peroxide, hydroperoxide of lipid hydroxyl (Subramanion et al., 2011). This scavenging action takes place by inhibiting initiation and breaking of chain propagation or reducing formation of free radicals by binding to the metal ions, reducing hydrogen peroxide, quenching superoxide and singlet oxygen (Shi et al., 2001).

Considering the chemistry and uneven distribution of antioxidant compounds in the plant matrix, recovery of antioxidant from plant materials is generally performed through different extraction techniques. Normally soluble phenolics are present comparatively in higher concentrations in the outer tissues (epidermal and sub-epidermal portions) of grains and fruits than to the inner tissues (pulp and mesocarp) (Antolovich et al., 2000).

For the isolation of plant antioxidant compounds, solvent extraction is most commonly used technique. In general, the extract yields and antioxidant activities of the plant materials are strongly dependent on the nature of extracting solvent because of different antioxidant compounds with several chemical characteristics and polarities which may or may not be soluble in a particular solvent used. For the recovery of polyphenols from a plant matrix polar solvents are frequently employed. In this sense, the most suitable solvents are (hot or cold) ethanol, methanol, acetone, and ethyl acetate. Ethanol and methanol have been extensively used to extract antioxidant compounds from various plants and plant-based foods such as plum, strawberry, pomegranate, broccoli, rosemary,

sage, sumac, rice bran, wheat grain and bran, mango seed kernel, citrus peel and peel of many other fruits (Bonoli et al., 2004).

Tamarind *(Tamarindus indica*) is a perennial herb belonging to the dicotyledonous family of Leguminosae. It is originally from Africa but today, tamarind grows widely in most tropical and subtropical regions of the world with an average annual temperature of 25^{0}C. In Bangladesh two species of tamarind are found in abundance, namely sweet and sour tamarind. About 150-250 Kg of fruit can be produced per year from 4 to 6 years after planting (Sinchaiyakit et al., 2011). Tamarind is a nutritious fruit, high in vitamins B_1, B_2 and B_3. These vitamins work together to help the body converting food into energy. They also help to provide a healthy immune system to fight off disease and help the body to be strong. Tamarind is a fair source of vitamin C and B vitamins, helps to provide a healthy immune system. Vitamin C also helps to provide strong bones, teeth and skin. An ethanol extract prepared from the seed coat exhibited anti-oxidative activity as measured by the thiocyanate and thiobarbituric (TBA) method. Ethyl acetate extracts prepared from the seed coat also possess a strong antioxidant activity. This suggests that tamarind seed coat, a byproduct of the tamarind gum industry, could be used as a safe and low-cost source of anti-oxidant (Shinmoto et al., 2007). Therefore, a suitable solvent and extraction technique are needed to preserve the antioxidant properties of tamarind seed. Thus, the purpose of this study was to determine the effect of extracting solvents and extracting techniques on the antioxidant activity of tamarind seed.

MATERIALS AND METHODS

Preparation of Materials: Tamarind seeds were collected from local market, Mymensingh, Bangladesh. As the seeds were very hard to smash so those were made sundried properly for two days and then grounded with lab grinder. After then, they were taken out and transformed in to a very fine powder by sieving method through sieve number 25. The powder sample was then stored in a poly bag and kept in a refrigerator (4^{0}C) until use.

Chemicals and equipment: Absolute ethanol and acetone, 1,1-Diphenyl-2-picrylhydrazyl (DPPH), L-Ascorbic acid, Butylated Hydroxyanisole (BHA), Di-sodium hydrogen phosphate (Na_2HPO_4), $FeCl_2.4H_2O$, $FeCl_3$, sodium carbonate (Na_2CO_3), potassium di-hydrogen phosphate (KH_2PO_4), potassium ferricyanide [$K_3Fe(CN)_6$], and trichloroacetic acid (TCAA), Ammonium molybdate, sodium phosphate were obtained from Sigma Chemical Co. (St. Louis, MO, USA). All other reagents were of analytical grade.

Extraction: For cold extraction, 25 gm of dried *T. indica* seed powder was extracted separately with 200 mL of acetone and ethanol in continuous shaker machine at 26^{0}C for 48 h and for hot extraction, the above procedure was followed using temperature controlled shaker machine at 40^{0}C for 48 h. After then all the obtained acetone and ethanol extract were then filtered by Buckner funnel using filter paper and then dried using vacuum oven at reduced pressure and low temperature (40^{0}C). The dried extract of *T. indica* sample was weighed in an electric balance, and stored in vial for further analysis.

Determination of Total Phenolic Content (TPC): The total phenolic content of the extracts was determined by the modified Folin-Ciocaltu method (Imran and Khan, 2014). In short, 1.0 mL of each extract (1 mg/mL) was mixed with 5 mL Folin-Ciocaltu reagent (1:10 v/v in distilled water) and 4 mL of 7.5% Sodium carbonate. The mixture was vortexed for 15 sec and allowed to stand for 30 min at 40°C for color development. Then the absorbance was measured at 765 nm with a spectrophotometer (Specord 205, Germany). Gallic acid was used as a standard for the calibration curve. The estimation was carried out in triplicate then the results were averaged and expressed as GAE (mg/gm of dry extract).

DPPH (1,1-diphenyl-2-picrylhydrazyl) Radical Scavenging Activity: The stable DPPH radical-scavenging activity was measured using the modified method described by Ukwueze et al. (2014). In this assay, 2 mL of 0.1 mM methanolic DPPH solutions was added to 2 mL of acetone and ethanol extracts solution at different concentrations and the contents were stirred vigorously for 15 sec. Then the solutions were allowed to stand at dark place at room temperature for 30 min for reaction to occur. Absorbance was measured against a blank at 517 nm with a double beam Analykjena UV/Visible spectrophotometer (Model 205, Germany). The percentage of DPPH radical-scavenging activity was calculated as:

$$\frac{A_0 - A}{A_0} \times 100$$

Where, A_0 is the absorbance of the control solution (containing all reagents except seed extracts); A is the absorbance of the DPPH solution containing seed extract.

The DPPH radical-scavenging activity (%) was plotted against the plant extract concentration to determine the concentration of extract necessary to decrease DPPH

radical-scavenging by 50% (called IC_{50}). Ascorbic acid and BHA (Butylated Hydroxyanisole) were used as positive control standards.

Total Antioxidant Capacity Determination: Total antioxidant activity of the extract was calculated by the phosphomolybdenum assay method which is based on the reduction of Mo (VI) to Mo (V) by the extract and subsequent formation of a green phosphate-Mo (V) complex in acidic condition (Matthias et al., 2015). The extract (2.0 mg/mL, 0.3 mL) was allowed to mix up with 3.0 mL of reagent solution (0.6 M H_2SO_4, 28 mM Na_3PO_4, 4 mM ammonium molybdate) and the reaction mixture was incubated at 95^{0}C for 90 minutes. After cooling at room temperature, the absorbance of the solution was measured at 695 nm using a UV-Visible spectrophotometer against an appropriate blank. The antioxidant activity was expressed as the number of gram equivalents of ascorbic acid.

Reducing Power Assay: This assay was determined according to the method reported by Dehpour et al. (2009). Briefly, 10 mL of acetone and ethanol extracts solution of different concentrations was mixed with 2.5 mL of potassium ferricyanide [$K_3Fe(CN)_6$] (1%; w/v) and 2.5 mL of phosphate buffer (0.2 M; pH 6.6) . The mixture was incubated at 50°C for 20 min. After that the reaction was terminated by adding 2.5 mL of trichloroacetic acid (10%; w/v), then the mixture was centrifuged at 3000 rpm for 10 min. Finally the supernatant solution (2.5 mL) was mixed with distilled water (2.5 mL) and ferric chloride (0.5 mL; 0.1%, w/v) solution. Then the absorbance was measured at 700 nm against a blank reading. Increased absorbance value of the reaction mixture indicates increased of reducing power of the extracts.

RESULTS AND DISCUSSION

Recovery percent of seed extract: Yields of extracts from tamarind seed sample using two solvents: absolute ethanol and absolute acetone and two extraction techniques: hot extraction and cold extraction are shown in **Table 1**. Maximum yield was obtained for the HEE of the sample (from 25 gm of T. seeds). From the table, HEE was found to be 6.43% while minimum yield was obtained from cold acetone extract (CAE) as 4.20%. This higher yield of extract using ethanol was in accordance with Bushra et al. (2007) and Milan and Stankovic (2011). This variation might be due to the different availability of extractable components, resulting from various chemical compositions of solvent used (Hsu and Coupar, 2006). Again the amount of the antioxidant components extracted from plant sample is affected by different extraction procedure which is in agreement with the findings of Shon et al. (2004) who investigated that alcoholic solvent and hot extraction method are more efficient to extract antioxidant compounds from *Phellinus baumii*.

Table 1. Recovery percent of seed extract of *T. indica*

Sample	Extract recovery (g)	Percentage of extract obtained (%)
CAE	1.05	4.20
HAE	1.07	4.28
CEE	1.54	6.16
HEE	1.60	6.43

CAE, Cold acetone extract (26^{0}C); HAE, Hot acetone extract (40^{0}C); CEE, Cold ethanol extract (26^{0}C); HEE, Hot ethanol extract (40^{0}C).

Table 2. Amount of total phenolic content in different extracts of *T. indica* seed

T. indica	Total phenolic content (mg GAE/ gm of dry extract)
CAE	51.45±1.13
HAE	54.28±1.18
CEE	70.44±2.33
HEE	71.68±2.73

Values are expressed as mean± SD (n=3); GAE: Gallic acid equivalent; CAE, Cold acetone extract (26^{0}C);HAE, Hot acetone extract (40^{0}C); CEE, Cold ethanol extract (26^{0}C);HEE, Hot ethanol extract (40^{0}C).

Table 3. Amount of total antioxidant capacity in different solvent extracts of *T. indica* seed

Name of extract	Total Antioxidant Capacity (AAE/gm)
CAE	22.75±0.016
HAE	27.55±0.021
CEE	37.75±0.028
HEE	43.80±0.035

Values are expressed as mean± SD (n=3); AAE: Ascorbic acid equivalent; CAE, Cold acetone extract (26^{0}C); HAE, Hot acetone extract (40^{0}C); CEE, Cold ethanol extract (26^{0}C);HEE, Hot ethanol extract (40^{0}C).

Total phenolic content: The TPC values in **Table 2** were obtained from Folin-Ciocalteu's reagents with correction. The calibration curve of gallic acid has the equation y=6.9103x–0.0936 (R^2=0.997), where y is absorbance at 765 nm and x is the concentration of gallic acid in µg/mL. Results revealed that HEE was the best solvent and extraction method for extracting phenolic compounds followed by CEE, HAE, CAE. From **Table 2** it is seen that HEE gave the highest phenolic content 71.68 mg GAE/gm of dry extract, and is closely followed by CEE with 70.44 mg GAE/gm of dry extract. HAE gave the total phenolic content with 54.28 mg GAE/gm of dry extract and the lowest phenolic content (51.45 mg GAE/gm of dry extract)

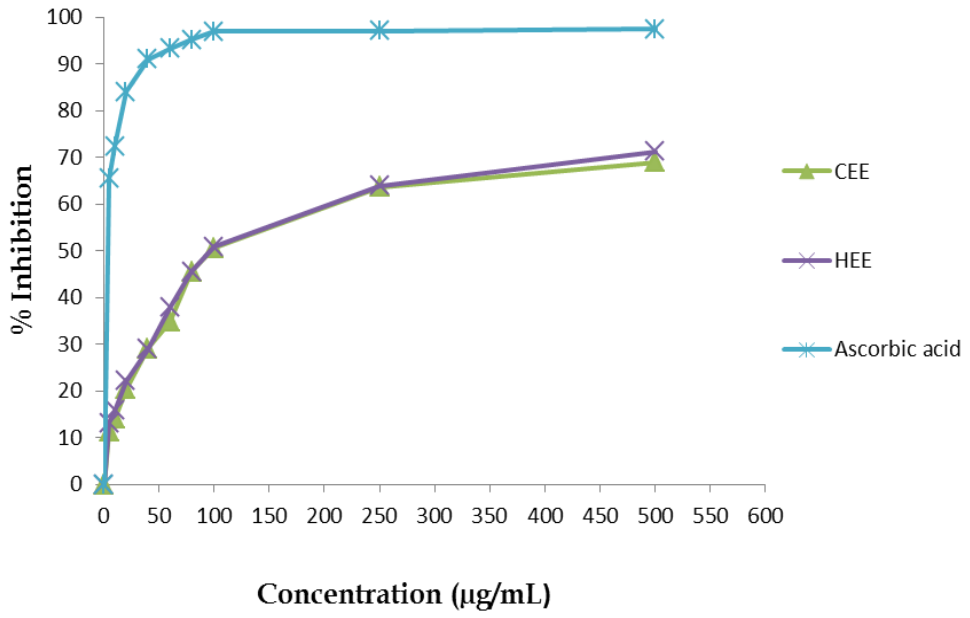

Figure 1. Comparison of ethanol extracts and standard for DPPH scavenging activity. CEE- Cold ethanol extract (26^{0}C); HEE- Hot ethanol extract (40^{0}C).

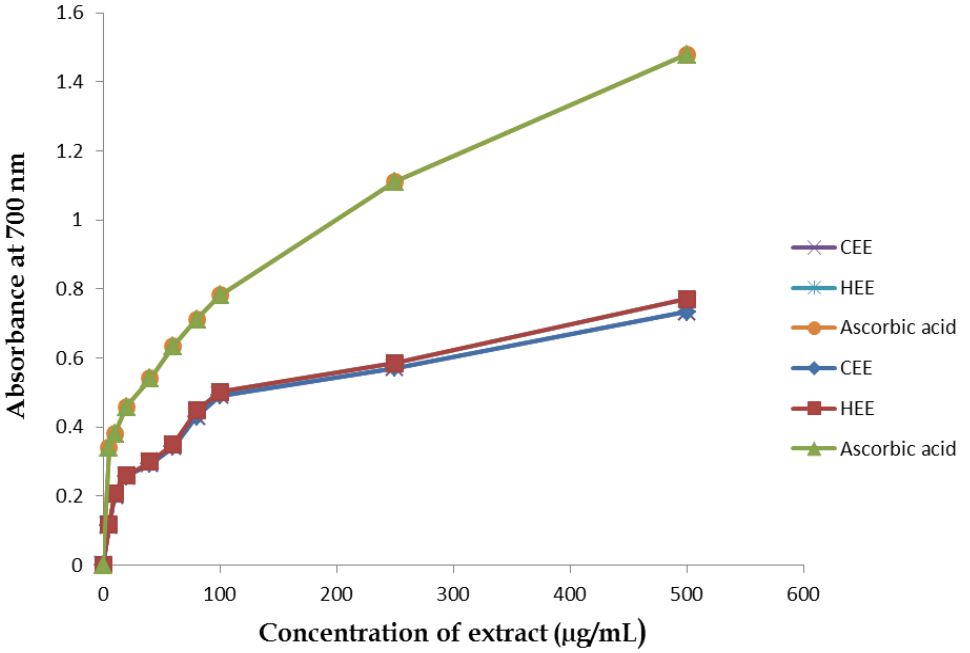

Figure 2.Comparative studies on reducing power assay of ethanol extracts and standard. CEE- Cold ethanol extract (26^{0}C); HEE- Hot ethanol extract (40^{0}C).

was given by CAE which can be compared with the observations of Umesh et al. (2010) and Deepika et al. (2011). This may be due to the fact that, phenolics are often extracted in better amounts in more polar solvents such methanol, ethanol than other solvents such as acetone (Siddhuraju and Becker, 2003; Anwar et al., 2006; Sultana et al., 2007).

DPPH Scavenging Activity: Generally DPPH is a relatively stable free radical scavenger which converts the unpaired electrons to paired ones by donating hydrogen proton to it. In the quantification of free radical scavenging activity, this method has proven the effectiveness of the extracts in a concentration-dependent manner and also used worldwide (Zhou and Yu, 2004).The total antioxidant capacity was

measured as the cumulative capacity of the compounds present in the sample to scavenge stable organic free radicals with a deep violet color by giving the absorbance ranging from 515-528 nm, using the DPPH reaction. Presence of antioxidant in the sample leads to disappearance of DPPH radical chromogens which can be identified spectrophotometrically at 517 nm (Ozcelik et al., 2003). The radical scavenging effects of tamarind seed are represented in **Figure 1**, where for both cold and hot ethanol extracts, the higher the concentration of extracts the higher is the %inhibition (DPPH scavenging activity). In the present study, the order of scavenging activity of seed extracts is as follows: HEE>CEE>HAE>CAE. Here maximum %inhibition for ascorbic acid is 97.40 compared to 68.87 and 71.17 for ethanol extract (cold and hot respectively) at concentration level of 500 µg/mL and the minimum and maximum IC_{50} value was 98.30±0.95 (HEE) and 248.60±0.90 (CAE). These results are in agreement with the previous findings of Bushra et al. (2006), Cheng et al. (2007) and Milan and Stankovic (2011).

Total antioxidant capacity determination: The calibration equation for ascorbic acid for the determination of total antioxidant capacity in different solvent extracts of T. *indica* was determined to be y=0.002x+0.015 (R^2=0.997) where y is absorbance at 695 nm and x is concentration in µg/ml. Using the equation TAC values are summarized in **Table 3** showed that highest TAC was obtained from HEE (43.80 AAE/g) while CAE gave lowest value (22.75 AAE/g). These results are in concordance with the previous findings of Soong and Barlow (2004).

Ferric reducing power assay (FRAP): FRAP assay is one of the most simple, rapid, very useful and inexpensive test for analysis. The FRAP assay was developed for direct antioxidant power of a sample. The FRAP measures the antioxidant effect of any substance in the reaction medium as reducing ability. Antioxidant potential of the *T. indica* seed extracts of tamarind was estimated from their ability to reduce Fe^{3+} complex to Fe^{2+} complex. The results of antioxidant capacities of raw and processed seed coat extracts are given in **Figure 2**. The FRAP of *T. indica* extracts increased with increasing concentration. Here HEE showed higher ability to reduce $Fe3^{+}$ to Fe^{2+} with absorbance 0.7702 and can be compared with ascorbic acid whose absorbance was 1.4785. The order of FRAP activity of respective seed sample extracts was as follow: HEE>CEE>HAE>CAE. Siddhuraju and Becker (2003), Cheng et al. (2006) and Sultana et al. (2007) reported that, the plant extracts with higher levels of total phenolic content also exhibited greater reducing power. Bushra et al. (2007) found reducing power (at absorbance 700nm) of ethanol extract of *Aloe barbadensis* bark as 1.56 under cold extraction and 1.72 under hot extraction.

CONCLUSION

Ethanol extract of tamarind seed, prepared through both hot (at 40^0C) and cold (at 26^oC) extraction techniques exhibited better antioxidant activities and phenolic contents compared to acetone extract of tamarind seed. In addition, higher antioxidant extract yield of the tested samples were obtained using hot extraction technique compared to cold extraction technique, in spite of any solvent used. Further researches are needed to identify individual components responsible for antioxidative activities and develop their application for food industries and other sectors.

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