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Effects of solvent conditions on the antioxidant activities of biologically active compounds extracted from tamarind (*Tamarindus indica* L.) seed

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Abstract
This study researched on the effect of different extraction solvents on the antioxidant activities of biologically active compounds which were extracted from tamarind seeds. Ethanol, methanol solvents with three solvent-to-water proportions of 30%, 40%, 50%; and water were used for extracting at three temperature conditions (30^0C, 40^0C and 50^0C). The Folin-Ciocalteu's method, DPPH radical scavenging method were used for determination antioxidant activity, when ferric reducing antioxidant power assay were used for assessment of reducing ability. The highest TPC value was obtained by using methanol 40% at 40^0C (3.62 ± 0.12 mg GAE/ g TSE powder). The percentages of antioxidant activity inhibition of TSE powder extracts in DPPH radical scavenging method were also highest when using methanol 40% at the temperature of 30^0C (60.71 ± 0.05%). In FRAP assay, reducing ability of different solvents were increased when increasing solvent concentrations (from 30% to 50%) and thermal conditions (from room temperature to 40^0C), in which ethanol 50% showed the highest value (2.09 ± 0.02 mM TE/ g TSE powder). As a result, methanol 40% was suitable for the extraction of biologically active compounds from tamarind seeds. However, methanol needed to be considered for food safety.

Keywords: tamarind seeds, ethanol, methanol, total phenolic content, DPPH scavenging

Introduction
The tamarind seeds are hard, skinny, reddish- brown or purplish- brown, and can be scarified to assist germination. Though the presence of tannin and other coloring matter in the seed coat makes the whole seed inedible, the presence of proteins, lipids, poly/oligosaccharides, procyanidins, triterpenes (such as lupanone and lupeol), xylitol- glycans and antioxidants (such as 2- hydroxy-3, 4-dihydroxyacetophenone, methyl 3, 4-dihydroxybenzoate, 3, 4-dihydroxy-phenylacetate and epicatechin), makes it therapeutically significant. Several therapeutic bioactive compounds have been isolated from different parts of the tree; however, the tamarind seed extract (TSE) has been largely employed in the textile, cosmetic, and pharmaceutical industries (Shankaracharya, 1998) [1].

Though the tamarind seeds seem to have been overlooked in the past, nowadays TSE and the isolated products of tamarind are receiving a great deal of attention due to the presence of therapeutic agents like antioxidants, triterpenes, polyphenolic bioactive compounds, procyanidines, polysaccharides, and other unknown factors, which have heling activities for various human pathophysiological disorders (Marangoni *et al*, 1988; Sudjaroen *et al*, 2005) [2, 3]. Tamarind seed extract has been shown to inhibit strong antioxidant scavenging activity against hydroxyl radicals and superoxide anions and hypoxanthine- xanthine oxidase systems. Four antioxidants have been identified are 2-hydroxy-3, 4-dihydroxyacetophenone, methyl 3, 4-dihydroxybenzoate, 3, 4-dihydroxyphenylacetate, and epicatechin. TSE is recommended for the treatment of snakebites, chronic diarrhea, diabetes, dysentery, jaundice, boils, eye diseases, and ulcers, and as an antiviral, anti-inflammatory, and antirheumatic agent. In addition, TSE is useful in the preparation of facial toners, moisturizers, serums, gels, masks, and anti-ageing formulations.

Antioxidants are defined as substances that inhibit or delay the oxidation of biologically relevant molecules either by specifically quenching free radicals or by chelation of redox metals (Handbook of Arsenic Toxicology, 2015) [4]. Potassium ferricyanide, ferric chloride, 2,2-diphenyl-1-picryl-hydrazyl (DPPH), potassium persulfate, 2, 2′azinobis (3-ethylbenzothiozoline-6-sulfonic acid) disodium salt (ABTS), 6-hydroxy-2, 5, 7, 8-tetramethylchroman-2-carboxylic acid (Trolox), linoleic acid, ferrous chloride, ammonium thiocyanate, hydrogen peroxide, ferrous ammonium sulfate, ethylenediamine tetracetic acid (EDTA) disodium salt, 2, 2′-bipyridyl and hydroxylamine hydrochloride were obtained from Himedia, Merck or Sigma. All other reagents were used of analytical grade.

Biologically active compound is compound that is produced by or extracted from a biological source, such as micro-organisms, organs and tissues of either plant or animal origin; cells or fluids of human or animal origin; and biotechnological cell constructs. This compound is for which a combination of physico- chemical- biological testing and the production process and its control is needed for its characterization and the determination of its quality. It can be classified into three main types. The first one is traditional biologically active compound, such as vaccines, blood components, allergenic, extracted proteins and carbohydrates etc. Biotechnological active compound include proteins produces by recombinant DNA technology. The final type is advanced therapy like cell and gene therapy, tissue engineering.

Extraction is the first step in the estimation of total phenolic compound. In laboratory, as well as in food industry, solvent extraction is the commonly method for extracting material from plant origin. Besides, solid–liquid extraction

method of phenolic compounds with different solvents from vegetable sources are the most commonly used for isolating these compounds. Crude phenolic extracts contain complex mixtures of some classes of phenols, which are selectively soluble in the different solvents. In this sense, solvent polarity plays a key role in increasing phenol solubility. Water, acetone, aqueous methanol and ethanol were widely used in many researches for extraction of bioactive compounds in plant materials. Methanol and ethanol have different polarity which can help them extract different compounds due to their chemical characteristics in various materials (Boeing *et al*., 2014) [5]. So, in this experiment, methanol and ethanol with different ratios with water will be chosen for extraction of phenolic compounds.

Tamarind seeds have been reported to contain polyphenolic compounds like epicatechin, procyanidin polymers and are used in traditional medicine for the management of DM (Iyer SR, 1995) [6]. Furthermore, the aqueous extract of Tamarind seeds was found to have potent antidiabetic and antihyperlipidemic activities in streptozotocin (STZ) induced diabetic male rat (Maiti *et al*., 2004, 2005, Sole *et al*.. 2013) [7, 8, 9]. There was, however, no report which study on the effect of different concentrations of solvents and temperature conditions on the antioxidant activities of biologically active compounds which are extracted from tamarind seeds. It is necessarily important to investigate effects of solvent conditions on antioxidant activities of phenolic compounds extracted from tamarind seeds for a better understanding the roles of those biologically active compounds.

Materials and Methods

Materials

Tamarind (Tamarindus Indica L.) seeds used in this study were collected from Tien Giang Province in the Mekong Delta region of southern Vietnam. The tamarind seeds were cleaned and stored in clean plastic jar before transporting to Ho Chi Minh city, Vietnam.

Methanol, ethanol, acetone, hexane, sodium carbonate, sodium hydroxide, potassium hydroxide, monopotassium phosphate, sodium phosphate dibasic, trichloroacetic acid, ferric chloride, potassium ferricyanide, concentrated sulfuric acid 98%, boric acid, copper sulphate were purchased from chemical agents in Ho Chi Minh city, Vietnam.

Folin ciocalteu's phenol reagent, garlic acid, 2, 2-diphenyl—picryhydrazyl, trolox were purchased from sigma Co.Ltd.

All used equipment was provided by Food Technology Lab (room LA1.102), Food Microbiology and Food Safety Laboratory (room LA1.602), Department of Food Technology, International University of Vietnam National University – Ho Chi Minh City.

Methods

Sample preparation

Tamarind seeds were firstly grinded by grinder mill and sieves were used to obtain a powder particles size of 417µm. The tamarind seed powder was then dried in oven at 60^0C for 24 hours. The moisture content of the powder was expected to be around 10% on the wet basis. The tamarind seeds powder needs to be stored in plastic zip bags. All bags were put into desiccator at room temperature before using.

Extraction of biologically active compounds

Based on the suggested method of Tril *et al*., (2014) [10], the biologically active compounds were extracted with some modifications. 3 grams of tamarind seed powder was added to 100ml beaker. Then 50ml solvent was added. Next, the mixture was shacked in shacking incubator at 200rpm in 2 hours. After having shaken, the extracted liquid was centrifuged at 5000 rpm in 10 minutes for collecting the supernatant. The residues were continuous re-extracted by adding more 50ml solvent to extract all biologically active compounds. All collected supernatant was mixed together to have the final solution. The solution was then evaporated using vacuum rotary evaporator until consistent dryness. Finally, residue was reconstituted by 20 ml methanol and stored at the controlled temperature.

This kind of experiment was intentionally carried out to find the effect of different solvents on the antioxidant activities of biologically active compounds of the Tamarind extracts. So, there were 21 samples which were water; methanol-in-water, ethanol-in-water with three ratios 30%, 40%, 50%; in three temperature conditions 30^0C, 40^0C and 50^0C.

Determination of chemical properties

Moisture content analysis and ash content analysis

The values of moisture content and ash content of the prepared samples were determined using using the methods described by AOAC [11]

Protein content (g protein/100g dry matter)

Protein content was analyzed by using the Kjeldahl method according to the AOAC methods (2012) [11]. 1g of sample was placed in a digestion tube; 0.2g CuSO4, 1g K2SO4, and 20ml concentrated H2SO4 were added to the tube with taro flour. The sample was let digested on digestion block until white fumes can be seen and continue heated for about 60 – 90 minutes until cleared with no charred material remaining. Tube was placed in the distillation apparatus and 50ml NaOH 32% was added. The ammonia in the sample was steam- distilled for 5 minutes into a receiving flash containing 4% boric acid. The sample was titrated with H2SO4 0.1N solution. The protein was calculated by the equation: %Nitrogen x 6.25.

Fat content (g fat/100g dry matter)

The fat content was determined by adopting the method Described by Mohamed, *et al*., (2015) [12]

Total dietary fiber (g TDF/ 100g dry matter)

Total dietary fiber (TDF) was determined following AOAC methods (AOAC, 1997) [13] and calculated using the formula

$$TDF(\%) = \frac{F2 - F1}{Weight\ of\ sample}\ x\ 100$$

Where:

F1 is weight after digested by dietary fiber digesting system,

F2 is weight after heating in the muffle furnace up to 550°C for 3 hours.

Determination of total phenolic content (TPC)

The total phenolic content was estimated by adopting te method described by Singleton and Rossi [14], using the

Folin Ciocalteau reagent (0.5ml) to oxidize 0.5 ml extracted solution in a 50ml falcon. Then 1ml of saturated sodium carbonate solution 20% was added sequentially in each falcon. The volume of mixture was adjusted to 10 ml by adding adequate volume of distilled water. After vortexing the reaction mixture, the test tubes were placed in dark for 40 min. The blue color mixture was next centrifuged at 4000 rpm for 5 minutes. After collected, the supernatant was measured by using spectrophotometer. The absorbance was recorded at 725 nm against the reagent blank. The results were expressed as milligrams of gallic acid equivalents per gram of dry tamarind seed extract powder (mg GAE/g TSE powder), as mean of three replicates. Standard curve was formed using gallic acid as a standard and the calibration curve was made from 0, 20, 40, 60, 80 and 100 µg/ml. Total phenolic content was measured based on standard curve.

Determination of antioxidant activities of the extract using DPPH radical scavenging method

The antioxidant activities of the tamarind extract were adopting the method described by Blois (1958) [15]. Different concentrations of the extracts were taken in separate test tubes. The volume was adjusted to 100 µl with methanol. Five ml of 0.1 mM methanolic solution of DPPH was added to these tubes and shaken vigorously. The tubes were allowed to stand for 20 min at 27°C. The control was prepared as above without any extract, and methanol was used for the baseline correction. Changes in the absorbance of the samples were measured at 517 nm. Radical scavenging activity was expressed as the inhibition concentration (IC50) as mean of three replicates.

Determination of antioxidant activities by using ferric reducing antioxidant power

The reducing power was determined following the method of Oyaizu (1986) [16]. Different concentrations of the extract sample were adjusted to 1 ml of phosphate buffer in a test tube and 5 ml of 0.2 M phosphate buffer, pH 6.6 was added. At this stage, 5 ml of 1% potassium ferricyanide solution was added. The mixture was incubated at 50°C for 20 min. After the incubation, 5 ml of 10% TCA was added and the content was centrifuged at 1,000 rpm for 10 minutes. The upper layer of the supernatant (5 mL) was mixed with 5 mL of distilled water. To this, 1 mL of ferric chloride (0.1%) was added and vortexed. Then, the absorbance of the reaction mixture was read spectrophotometrically at 700 nm against water blank. The FRAP of the sample was estimated in term of mM Trolox equivalent antioxidant capacity per gram of dry sample (mM Trolox/g TSE powder) as mean of three replicates.

Statistical analysis

Conventional statistical methods were used to calculate means and standard deviations of three simultaneous assays carried out with the different methods. Statistical analysis (ANOVA) was applied to the data to determine differences ($p<0.05$) for each analysis. The statistical analyses were made using SPSS version 22 software.

Results and discussion

Physiochemical analysis

Table 1: Chemical composition of tamarind (Tamarindus indica L.) seed powder extracts

Sample	Moisture content (%)	Ash (%)	Fat (%)	Protein (%)	TDF (%)
TSP	11.23 ± 0.23	4.11 ± 0.22	3.76 ± 0.84	8.88 ± 0.13	8.20 ± 0.66

%: Values expressed as g/100g dry matter. *All data was presented as means ± standard deviation.

The chemical composition of TSE was shown on table 1. The moisture content of TSE was 11.23 g/ 100g dry matter. The content of fat was 3.76 g/ 100g dry matter. The tamarind seed contained less fat when compared to the tamarind pulp which was 8.95 g/ 100g dry matter (Tril *et al*., 2014) [10]. The lipid content was depended on the part of tamarind fruit. The content of protein was 8.88 g/ 100g dry matter. This value was much higher than the protein content in tamarind pulp (2.06 g/ 100g dry matter) (Tril *et al*., 2014) [10]. So, consuming tamarind seeds yielded 4 times more protein than tamarind pulp. TSE contained 4.11 g/ 100g dry matter of ash and it was higher than that found in tamarind pulp (3.03 g/ 100g dry matter) (Tril *et al*., 2014) [10]. In case of application of fruit extract in food industry, high amount of ash may accelerate negative oxidation changes in food stuff (Tril *et al*., 2014) [10].

Table 1 also showed the percentage of total dietary fiber (TDF) (8.20 g/ 100g dry matter) which was less than half the amount of TDF in the tamarind pulp (Tril *et al*., 2014). In food industry, fiber plays a number of roles as improving texture, bulking agent in reduced-sugar applications, to manage moisture in the replacement of fat, to add color, and natural antioxidant (Viuda-Martos *et al*., 2010; Ramirez-Santiago et a., 2010) [16]. So, the TSE may be used as an additional role in fiber content in some foods.

Effect of solvents on extraction of total phenolic content

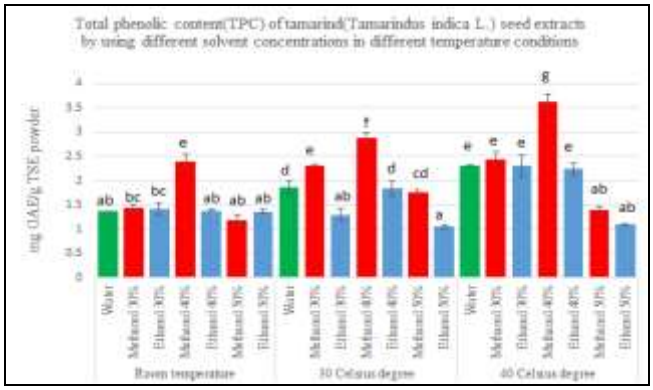

Fig 3: Total phenolic content (TPC) of tamarind seed extracts by using different solvents with different concentrations and temperature conditions.

*All data was presented as means ± standard deviation. Means followed by different lower case letters were significantly different ($p< 0.05$). The error bars were presented for standard deviation.

The comparisons of the total phenolic (TPC) content of TSP extracted with different solvents in different concentrations and temperature conditions were presented in figure 3. The TPC of TSP extracts, expressed as gallic acid equivalent, ranged from 1.05 to 3.62 mg GAE/g TSE powder, with not much significant differences between methanol and ethanol.

At room temperature, The TPC ranged from 1.18 to 2.39 mg GAE/g TSE powder, with no statistically differences ($p > 0.05$) between the samples extracted with water; methanol and ethanol in different concentrations, excepted methanol 40%. The TSP extracted with methanol 40% showed the highest ($p<0.05$) TPC value. This value was higher than 1.7 times when compared to ethanol 40%.

At $30^{O}C$, The TPC ranged from 1.05 to 2.89 mg GAE/g TSE powder, with significantly differences ($p > 0.05$) between the samples extracted with water; methanol and ethanol in different concentrations. Methanol 40% was also shown the highest ($p>0.05$) TPC value which was higher than 1.6 times when compared to ethanol 40%.

At $40^{O}C$, The TPC ranged from 1.09 to 3.62 mg GAE/g TSE powder, with no significantly differences ($p > 0.05$) between samples extracted with water, methanol 30%, ethanol 30%, ethanol 40%; with methanol 50%, ethanol 50%; excepted methanol 40%. The methanol 40% also showed the highest ($p>0.05$) TPC value which was higher than 1.6 times when compared to ethanol 40%.

In short, the temperature conditions were not much significant effect to the TPC values which was determined by different solvents in different concentrations. The solvent showed highest TPC value was methanol 40% which was higher than ethanol with the same concentrations. Based on the "like dissolved like" principle, the compounds were dissolved well in solvents which have the similar polarity with them. So, the phenolic compounds in tamarind seed may dissolved well in methanol 40%. However, the methanol was considered in food industry to its safety.

Phenolic content is corrected greatly with antioxidant activity of plant extracts. These constituents are very important to human health thank to its free-radical scavenging activity and against oxidative stress (Xu and Chang, 2007) [17]. In report of Tril *et al*., 2014, the TPC of tamarind seed extract using methanol in room temperature was 3.35 mg GAE/g TSE powder. There was nearly matched with the result above. When comparing with another seeds, the TPC of tamarind seed was lower than chia seed (5.78 mg GAE/ g dry matter), sunflower seeds (16.01 mg FAE/g dry matter) (Velioglu *et al*., 1998) [18].

DPPH radical scavenging

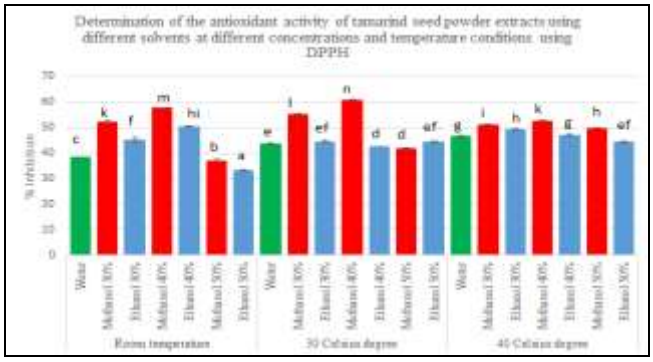

Fig 4: Determination of the antioxidant activity of tamarind seed powder extracts using different solvents at different concentrations and temperature conditions using DPPH radical scavenging method.

*All data was presented as means ± standard deviation. Means followed by different lower-case letters were significantly different ($p< 0.05$). The error bars were presented for standard deviation.

DPPH radical scavenging method was applied to determine the antioxidant activity of biologically active compounds extracted from tamarind seed. The percentages of DPPH inhibition of tamarind seed were presented in figure 4, ranged from 33.2 to 60.71%, with the significantly differences between water, methanol and ethanol.

At room temperature, the percentage inhibitions of DPPH radicals of TSE powder were in range from 37.18 to 57.71%, with the significantly differences between water, methanol and ethanol. Methanol showed the higher ($p<0.05$) inhibition than ethanol and water, in which, methanol 40% showed the highest inhibition (57.71%, respectively).

At 300C, the percentage inhibitions of DPPH radicals of TSE powder were in range from 41.80 to 60.71%, with no significantly differences between water, ethanol 30%, ethanol 50%; and between ethanol 40%, methanol 50%. Methanol 40% also showed the highest percentage inhibition of DPPH radical (60.71%, respectively).

At 400C, the percentage inhibitions of DPPH radicals of TSE powder were in range from 44.49 to 52.64%, with no significantly differences between water and ethanol 40%; ethanol 30% and methanol 50%. Methanol 40% also showed the highest percentage inhibition of DPPH radical (52.64%, respectively).

In short, in three temperature conditions, methanol 40% showed the highest percentage inhibition of DPPH radicals. This result corresponded to the TPC which was extracted by methanol 40%. When compared to another seeds, the antioxidant activity of tamarind seed was higher than chia seed (53.71%), and sunflower seed (23.19%) (Hyeun Sung Song, 2013). Because tamarind seed contained vitamins, phenolic acids and flavonoids, so, those compounds led to the high antioxidant activity of tamarind seed.

Ferric reducing antioxidant power (FRAP) assay

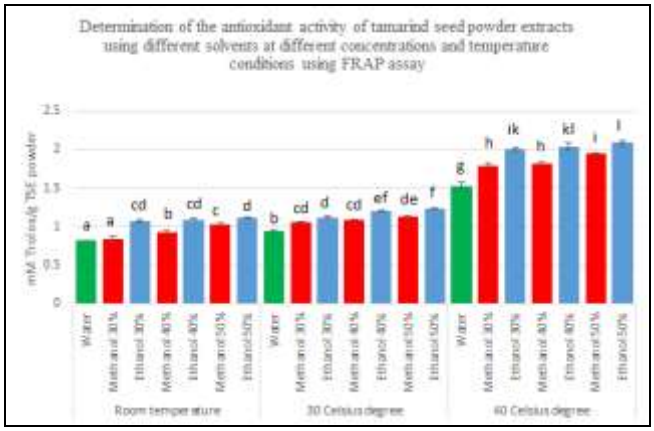

Fig 5: Determination of the antioxidant activity of tamarind seed powder extracts using different solvents at different concentrations and temperature concentrations using Ferric reducing antioxidant power assay.

*All data was presented as means ± standard deviation. Means followed by different lower-case letters were significantly different ($p< 0.05$). The error bars were presented for standard deviation.

The basic of ferric reducing antioxidant assay is the ability to donate electrons to reactive radical cation, reducing them to more stable or unreactive compound (Tril *et al*., 2014) [10]. Figure showed the comparisons of the antioxidant activity of tamarind seed powder extracts using different

solvents with different concentrations and temperature concentrations. The ability to reduce Fe3+ to Fe2+ of solvents were in range from 0.82 to 2.09 mM TE/g TSE powder.

At room temperature, the reducing power gradually increased from 0.82 to 1.11 mM TE/g TSE powder, with no significant different between water and solvents with each other. The highest reducing capacity corresponded to ethanol at 50% (1.11 mM TE/g TSE powder, respectively). Ethanol 30% and 40% also showed higher reducing power than methanol with the same concentrations.

At 300C, reducing power also increased from 0.94 to 1.23 mM TE/g TSE powder with no significant different between ethanol and methanol, excepted water sample. Ethanol showed higher reducing power when compared to methanol which were same concentration. The highest value corresponded to methanol 50% (1.23 mM TE/g TSE powder, respectively).

At 400C, the value of reducing power of TSE powder was in range from 1.53 to 2.09 mM TE/g TSE powder. There were not much significant different between methanol 30% and 40%; ethanol 30% and 40%; ethanol 30% and methanol 50%; ethanol 40% and 50%. Excepted water sample. The highest value also was extracted by ethanol 50% (2.09 mM TE/g TSE powder, respectively).

To sum up, the ability to reduce Fe3+ to Fe2+ of solvents were increased by temperature conditions from room temperature to 400C. Ethanol seemed to have higher reducing power than methanol with the same concentrations and water. This result was due to samples, extracted by ethanol, had substances that may exhibit metal-ion chelating properties at different concentrations. This meant that TSE could act as antioxidant agent which took an important role during the storage of food when the chemical changes.

Conclusions

In this study, the effects of solvent extraction by using water; methanol, ethanol with three proportions in water (30, 40, 50%) in different temperature conditions on the antioxidant activities of biologically active compounds of tamarind seeds were successfully and thoroughly investigated.

The tamarind seed extracts showed that they contained low fat, but high in protein content and total dietary fiber. They were also high in TPC value and antioxidant activities. The highest TPC value of TSE was determined by methanol 40%. There were not much significant differences between water, methanol and ethanol in TPC determination. However, there were significant different between water, methanol and ethanol in DPPH radical scavenging and FRAP assay. The highest percentage inhibition of TSE was also extracted by methanol 40%, while, ethanol 50% showed most significantly reducing ability in FRAP assay. For collecting high yield of biologically active compounds of tamarind seed powder, methanol 40% showed the most effective result in this study. However, methanol needs to be considered due to the safety of food preparing materials.

The results of this work also proved that TSE powder should be considered for using in food industry because of its good chemical properties as well as higher phenolic content. However, further research is needed for study on the applications of tamarind, as well as, tamarind seeds in food industry.

References

1. Shankaracharya NB. Tamarind – chemistry, technology and uses: a critical appraisal, J Food Sci. Technol. 1998; 35(3):193-208.
2. Shankaracharya NB. Tamarind – chemistry, technology and uses: a critical appraisal, J Food Sci. Technol. 1998; 35(3):193-208.
3. Shankaracharya NB. Tamarind – chemistry, technology and uses: a critical appraisal, J Food Sci. Technol. 1998; 35(3):193-208.
4. Shankaracharya NB. Tamarind – chemistry, technology and uses: a critical appraisal, J Food Sci. Technol. 1998; 35(3):193-208.
5. Shankaracharya NB. Tamarind – chemistry, technology and uses: a critical appraisal, J Food Sci. Technol. 1998; 35(3):193-208.
6. Marangoni A, Ali I, Kermasha S. Composition and properties of seeds of the true legume Tamarindus indica. Journal of water Health, A, 1988, 453-461.
7. Sudjaroen Y, Haubner R, Wurtele G, Hull WE, Erben G, Spiegelnalder B, *et al*. Isolation and structure elucidation of phenolic antioxidants from tamarind (Tamarindus indica L.) seeds and pericarp. Food and Chemical Toxicology. 2005; (43):1673-1682
8. Flora SJS. Handbook of arsenic toxicology. Academic Press, 2015.
9. Boeing JS, Barizão ÉO, E Silva BC, Montanher PF, Almeida VDC, Visentainer JV, *et al*. Evaluation of solvent effect on the extraction of phenolic compounds and antioxidant capacities from the berries: application of principal component analysis. Chem. Cent. J, 2014, 8:48.
10. Iyer SR. Tamarindus indica Linn. In: Warrier, PK, Nambiar VPK, Kutty CR (Eds.). Indian Medicinal Plants, vol. V. Orient Longman Limited, Madras, 1995, 235-236.
11. Maiti R, Jana D, Das U, Ghosh D. Antidiabetic effect of aqueous extract of seed of Tamarindus indica in streptozotocin-induced diabetic rats J Ethnopharmacol. 2004; 92(1):85-91
12. Maiti R, Jana D, Das U, Ghosh D. Attenuation of hyperglycemia and hyperlipidemia in streptozotocin-induced diabetic rats by aqueous extract of seed of Tamarindus indica. Biol Pharm Bull. 2005; 28(7):1172-1176.
13. Sole SS, Srinivasan BP, Akarte AS. Anti-inflammatory action of tamarind seeds reduces hyperglycemic excursion by repressing pancreatic beta-cell damage and normalizing SREBP-1c concentration Pharm Biol. 2013; 51(3):350-360
14. Tril U, Fernandez-Lopez J, Alvarez JAP, Viuda-Martos M. Chemical, physicochemical, technological, antibacterial and antioxidant properties of rich fibre powder extraxt obtained from tamarind (Tamarindus indica L.). Industrial Crops and Products, 2014; 55:155-162.
15. Latimer G. Official Methods of Analysis of AOAC International. Gaithersburg, Md.: AOAC International, 2012. ISBN: 978-0-935584-83-7
16. Arangoni A, Alli I, Kermasha S. Composition and properties of seeds of the tree legume, Tamarindus indica, J. Food Sci, 1988; 53:1452-5.
17. Arangoni A, Alli I, Kermasha S. Composition and properties of seeds of the tree legume, Tamarindus

indica, J Food Sci, 1988; 53:1452-5.
18. Marangoni A, Alli I, Kermasha S. Composition and properties of seeds of the tree legume, Tamarindus indica, J Food Sci, 1988; 53:1452.
19. Marangoni A, Alli I, Kermasha S. Composition and properties of seeds of the tree legume, Tamarindus indica, J. Food Sci, 1988; 53:1452.
20. Marangoni A, Alli I, Kermasha S. Composition and properties of seeds of the tree legume, Tamarindus indica, J Food Sci, 1988; 53:1452.
21. Marangoni A, Alli I, Kermasha S. Composition and properties of seeds of the tree legume, Tamarindus indica, J Food Sci, 1988; 53:1452-5.
22. Marangoni A, Alli I, Kermasha S. Composition and properties of seeds of the tree legume, Tamarindus indica, J Food Sci, 1988; 53:1452-5.
23. Marangoni A, Alli I, Kermasha S. Composition and properties of seeds of the tree legume, Tamarindus indica, J Food Sci, 1988; 53:1452-5530.
24. Handbook of herbs and spices © Woodhead Publishing Limited, 2012.
25. Marangoni A, Alli I, Kermasha S. Composition and properties of seeds of the tree legume, Tamarindus indica, J Food Sci, 1988; 53:1452-530.
26. Handbook of herbs and spices © Woodhead Publishing Limited, 2012.
27. Marangoni A, Alli I, Kermasha S. Composition and properties of seeds of the tree legume, Tamarindus indica, J Food Sci, 1988; 53:1452-530.
28. Handbook of herbs and spices © Woodhead Publishing Limited, 2012.
29. Marangoni A, Alli I, Kermasha S. Composition and properties of seeds of the tree legume, Tamarindus indica, J Food Sci, 1988; 53:1452.
30. Mohamed HA, Mohamed BE, Ahmed KE. Physicochemical Properties of Tamarind (Tamarindus indica) Seed Polysaccharides. J Food Process Technol, 2015; 6:452. doi:10.4172/2157-7110.1000452
31. AOAC-Association of Official Analytical Chemists International Official Methods of Analysis. 16th Edition, 1997. AOAC, Arlington
32. Singleton VL, Rossi JA. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. Am J Enol Vitic, 1965; 16:144-158.
33. Blois MS. Antioxidant determinations by the use of a stable free radical. Nature, 1958; 29:1199-1200.
34. Viuda-Martos M, El Gendy NG, Sendra E, Fernandez-Lopez J, El-Razik KAA, El-Sayed A, *et al*. Chemical composition and antioxidant and anti-listeria activities of essential oils obtained from some Egyptian plants. Journal of Agricultural and Food Chemistry, 2010; 58:9063e9070.
35. Xu B, Chang KCS. A Comparative Study on Phenolic Profiles and Antioxidant Activities of Legumes as Affected by Extraction Solvents. Journal of Food Science. 2007; 72(2):S159-66.
36. Velioglu YS, Mazza G, Gao L, Oomah BD. Antioxidant activity and total phenolics in selected fruits, vegetables, and grain products. Journal of Agricultural and Food Chemistry, 1998; 46:4113- 4117.