Available online at www.pelagiaresearchlibrary.com

Pelagia Research Library

Asian Journal of Plant Science and Research, 2016, 6(4):48-52

ISSN : 2249-7412
CODEN (USA): AJPSKY

Purification of phenolics from defatted tamarind kernel powder

Shlini P.[1]* and K. R. Siddalinga Murthy[2]

[1]Department of Chemistry(PG Biochemistry), Mount Carmel College, Autonomous, Bangalore, India
[2]DOS in Biochemistry, Bangalore University, Bangalore, India

---

ABSTRACT

Tamarind (Tamarindus indica) is an economically important tree, found in many countries of Asia, Africa and South America. It is a tree that is easy to cultivate and requires minimum care. It is generally free of serious pests and diseases, has a life span of 80 – 200 years and can yield 150 – 500 kg of pods per healthy tree/year at 20 years of age. The present study reveals that Tamarindus indica seed contain nutritionally useful quantities of macro and micronutrients. The objective of the study was to extract, isolate, purify and characterize phenolic acids from defatted tamarind kernel powder. However, defatted TKP methanolic extracts contain moderate antioxidant activity, phenolic and protein content. Presented results seem to be the first to determine the partial purification of the phenolic components from TKP by gel permeation chromatography and high performance liquid chromatography (HPLC). The results of the study suggest that the phenolic components were eluted as a single broad peak after void volume in sephadex G-25 column. The HPLC data revealed the presence of gallic acid and ρ-coumaric acid in the methanolic fractions.

Keywords: *Tamarindus indica*, TKP, antioxidant activity, ρ-coumaric acid, gallic acid.

INTRODUCTION

Tamarind (*Tamarindus indica*) is an economically important tree, found in many countries of Asia, Africa and South America. *Tamarindus* belongs to the subfamily Caesalpinioideae. *Tamarindus* itself is a monotypic genus, containing the sole species *T. indica*. Leguminous seeds are important sources of not only nutrient compounds such as protein, starch, dietary fiber, minerals [1] but also for a number of bioactive substances (phytochemicals) including phenolics, which consists of flavonoids, phenolic acids, lignans and tannins that have antioxidant properties [2].

Phenolic compounds commonly found in plants affect their appearance, taste, odor and oxidative stability. The number of antioxidant compounds synthesized by plants as secondary products, mainly phenolics, serving in plant defense mechanisms to counteract reactive oxygen species in order to survive, is currently estimated to be between 4000 and 6000 [3] [4]. A direct relationship has been found between the content of total phenolics and antioxidant capacity of plants [5]. In fact, to counteract deleterious action of ROS, phenolic compounds, naturally distributed in plants are effective. Since purified phenolic compounds are difficult to obtain and plant extracts have better antioxidant activities than those of pure molecules, there is a growing interest for the use of plant extracts [6]. Polyphenols of plant origin like catechins exert antimutagenic, anticarcinogenic and cardioprotective effects, which is attributed to their free radical scavenging activity.

In cereal grains, these compounds are located mainly in the pericarp [7]. The major phenolic acids in cereals are ferulic and p-coumaric acids [8] [9] [10] [11]. Anthocyanins are water-soluble pigments mostly studied in cereals [12].

The type of solvent used for extraction of various classes of phenolic compounds from legumes is very broad and includes water, methanol, ethanol, methanol/water, ethanol/water and acetone/water[13] [14] [15]. The dominant phenolic compounds present in leguminous seeds are flavonoids, phenolic acids and procyanidins and seeds with colored coats are rich in anthocyanidins [16].

The present investigation was carried out to determine the levels of phenolic compounds and proteins in defatted tamarind kernel powder by extracting with various solvent systems. The phenolic compounds were fractionated by gel filtration chromatography on Sephadex G-25. The phenolic compounds were also separated by HPLC and the peak fractions were identified.

MATERIALS AND METHODS

Chemicals :
All Chemicals used in this study were of analytical grade.

Monobasic di-hydrogen phosphate, potassium phosphate dibasic, sodium chloride, sodium carbonate, copper sulphate, sodium tungstate, methanol, ethanol, acetone, acetonitrile, tannic acid, bromine, gallic acid, Folin-ciocalteau reagent were purchased from Sisco Research Laboratories, Mumbai, India..

Plant Material :
The seeds of *Tamarindus indica* were collected using random sampling technique (RST) from local areas of Bangalore district, Karnataka State, India. After dehulling the fruits, equal samples of seeds were combined to give one bulk population sample from which sub samples were taken. The seed samples were dried in the sunlight for 24 hrs. After removing immature and damaged seeds, the matured seeds were washed under tap water, dried and stored in refrigerator until further use.

Sample preparation :
The seeds were broken into small pieces (2 – 4 mm), moistened with water, flaked and dried. The flakes were then extracted with n-hexane to remove the lipids and the defatted flakes were dried at 60° C and powdered (40 – 60 µ) to obtain defatted tamarind kernel powder (defatted TKP).

Extraction of defatted TKP using different aqueous organic solvents:
A 10 % extract of defatted TKP was prepared using 80 % aqueous solutions of methanol, ethanol and acetone. The sample was extracted for 30 mins, centrifuged at 10000 rpm for 30 mins and the supernatants were collected. The residue was reextracted with five volumes of respective solvents and the supernatants were pooled and evaporated to dryness. The residue obtained after evaporation was dissolved in 10 ml distilled water and the volume made up to 25 ml. The solutions were used for the estimation of antioxidants, phenolics and proteins.

Estimation of proteins :
The estimation of proteins was carried out by Lowry's method [17]. Protein is expressed as bovin serum albumin equivalent in gm / 100 gm dry weight of sample.

Estimation of phenolics :
Total soluble phenolics in the fraction was estimated by Folin-ciocalteaus method with slight modifications [18]. To the extract (0.1 ml), 7.9 ml of distilled water and 2.0 ml of 20 % sodium carbonate were added and the tubes were allowed to stand for 3 mins. To this 0.5 ml of Folin-ciocalteau reagent was added and allowed to stand for 60 mins. The absorbance was read at 650 nm. The total soluble phenolics was expressed as gallic acid equivalents (GAE) in gm / 100 gm dry weight of sample.

Estimation of antioxidants by Reducing power assay :
The total reducing power of the fractions were determined according to the method of Hinneburg *et al*., [19] with slight modifications. The extract (0.1 ml) was mixed with 0.9 ml of water and 0.5 ml of potassium ferricyanide,

incubated for 20 mins at 50° C. 0.5 ml of 10 % tricholoroacetic acid was added to the mixture and centrifuged at 6000 rpm for 10 mins. The pellet was discarded. To the supernatant 0.1 ml of water and 0.1 ml of 0.1 % $FeCl_3$ was added and the absorbance measured at 700 nm. Higher the absorbance of the reaction mixture greater the reducing power. Antioxidant activity is expressed as tannic acid equivalents (TAE) in gm / 100 gm dry weight of sample.

Purification of phenolics by gel permeation chromatography :
Sephadex G-25 in distilled water was allowed to swell by heating over a boiling water bath for 5 hours. The gel was washed thoroughly with distilled water and then equilibrated with 0.01 M phosphate buffer, pH 7.0, stirred, allowed to settle and the fines were decanted intermittently during equilibration. The gel was packed into a column of size 60.0 cm X 1.0 cm under gravity. The column was equilibrated with two bed volumes of 0.01 M phosphate buffer, pH 7.0 at a flow rate of 20 ml / hr. The methanolic fraction was evaporated to dryness and the residue was dissolved in methanol and loaded on to the sephadex column. The phenolics were eluted with start buffer and fractions of 2 ml were collected. The phenolics were estimated at 700 nm.

Identification of potent phenolics by using HPLC:
Shimadzu model LC-10AT VP HPLC chromatograph (Kyoto, Japan) equipped with a pump SCL-10A (Kyoto, Japan), UV-VIS spectrophotometric detector UVD 250 (Kyoto, Japan), and a chromatographic station CSW 32 were used for the separation and identification of the compounds in the obtained fractions.

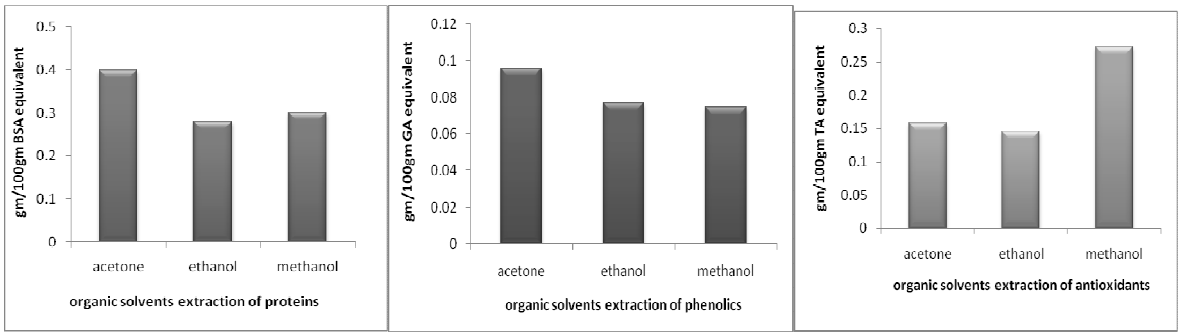
The sample (20 µl) was injected into the reverse-phase 25 cm column (Pheonomenex, C18, 250 mm x 4.6 mm, (Kyoto, Japan) with water-acetonitrile-methanol-acetic acid (79.5:18:2:0.5 v/v/v/v) as the mobile phase at a flow rate of 1 ml / min, at 25°C. The phenolics were detected at 280 nm (Amarowicz and Shahidi 1994).

RESULTS AND DISCUSSION

Phenolic compounds are commonly found in plants and have been reported to have several biological activities including antioxidant properties and are useful indicator of potential nutritional benefit. The total phenolic content (TPC) in leguminous seeds or extracts prepared from such plant materials is one of the main parameters dictating the potential antioxidant capacity of seeds or the antioxidant activity of extracts therefrom.

An important feature of the leguminous seeds is the high content of antioxidant in the seed coats. The seeds are usually processed and extracted with different solvent systems to maximize the extractability of antioxidants, phenolics and proteins.

Extraction of defatted TKP using different aqueous organic solvents and Estimation of proteins, phenolics and antioxidants :
The figure 1 depicts the extractions (10 % extract of the sample) carried out with 80 % aqueous solutions of organic solvents (acetone, ethanol and methanol). Proteins (0.4 gm/100 gm), phenolics (0.1 gm/100 gm) and antioxidants (0.28 gm/100 gm) were extracted with organic solvents. Further the methanolic fraction was used for further assay.



(a) **(b)** **(c)**

Fig 1 : Extraction and estimation of (a) Proteins, (b) Phenolics and (c) Antioxidants from defatted tamarind kernel powder (10% extract) using different organic solvents (80%)

However, Zulkhairi *et. al*., [20] have reported extraction of very small amount of phenolics (< 20 mg/100 gm) from the tamarind seeds using water at 60° C/ 6 hours and 100° C/15 minutes. Details pertaining to the application of different solvents for the extraction of phenolics from plant material have been reviewed. A comparative study of phenolic profiles and antioxidant activities of legumes, as affected by different extraction solvents, has been reported and the results of their study showed that 50% acetone (vol/vol) extracts exhibited the highest TPC for yelow pea, green pea, and chickpea. Acetone / water system extracted greater quantities of phenolic compounds from lentil seeds compared with methanol / water or ethanol / water systems.

Purification of phenolics by gel permeation chromatography and HPLC :

The fractions obtained from 80 % methanol extraction of defatted TKP was concentrated and loaded onto Sephadex G – 25 gel permeation chromatography column. Phenolic compounds were eluted after the void volume and resolved into a single broad peak (Fig 2). Sephadex G – 25 peak fractions were concentrated, separated by HPLC and based on the retention time, phenolic compound was identified as gallic acid and p-coumaric acid (Table 1).

Recent phytochemical examination of plants which have a suitable history of use in folklore for the treatment of cancer has often resulted in the isolation of principles with antitumor activity. Polyphenols of plant origin like catechins exert anticarcinogenic, antimutagenic and cardioprotective effects, which is attributed to their free radical scavenging activity. Gallic acid in carob seed has antibacterial, antifungal and antioxidant properties (Nabel A Negm, 2013).

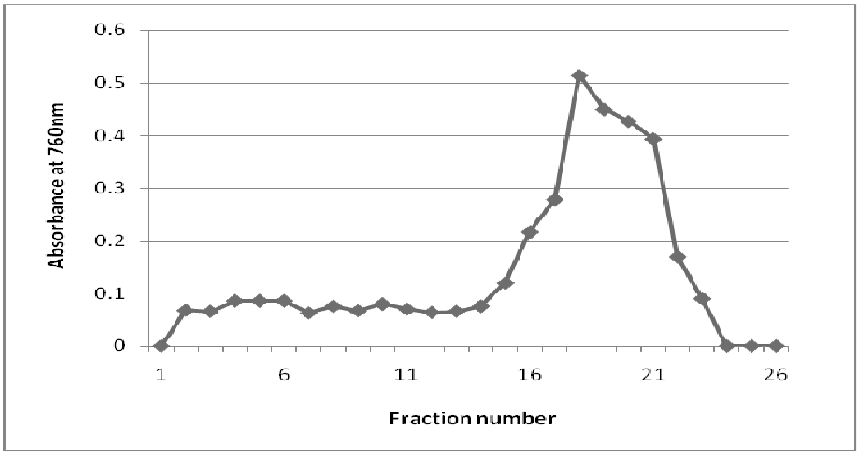


Fig 2 : Gel filtration profile of phenolics fractions on Sephadex G-2

Pumthong [21] reported that tamarind seed coat is composed of polyphenols including tannins, anthrocyanidin, and oligomeric anthrocyanidins. Gu *et al*. [22] found that the whole seed of tamarind contains procyanidin oligomers and 101.89 g/kg high molecular weight tannins, respectively. Moreover, Tsuda *et al*. [23] reported that tamarind seed coat contains phenolic antioxidants, such as 2 – hydroxyl – 30, 40 – dihydroxyacetophenone, methyl 3,4 – dihydroxybenzoate, 3, 4-dihydroxyphenyl acetate and epicatechin. Extracts exhibit antioxidant potential by reducing lipid peroxidation in vitro, and anti-microbial activity.

Table 1 : Standard phenolic acids and their retention time using HPLC.

Peak No.	Phenolic standards	Retention time
1.	P-coumaric acid	4.7
2.	Gallic acid	4.0
3.	Tannic acid	4.4
4.	Salicylic acid	2.6
5.	Benzoic acid	2.9
6.	2,5-dihydroxy benzoic acid	12.5
7.	Sephadex G – 25 fraction	4.68, 3.9

CONCLUSION

The present study reveals that *Tamarindus indica* seed contain nutritionally useful quantities of macro and micronutrients. However, defatted TKP methanolic extracts contain moderate antioxidant activity, phenolic and protein content. Presented results seem to be the first to determine the partial purification of the phenolic components from TKP by gel permeation and HPLC. Our results suggest that the phenolic components were eluted as a single broad peak after void volume in sephadex G-25 column. The HPLC data revealed the presence of gallic acid and ρ-coumaric acid. The further purification and more detailed characterization of the tamarind kernel powder can be used for large scale production of health beneficial phenolic concentrates.

Acknowledgement
The study was supported by UGC under UGC – Major Research Project (2010 – 2012) New Delhi. The authors wish to acknowledge DOS in Biochemistry, Bangalore University for offering their facilities for the analysis. The authors would also like to thank Mount Carmel College Autonomous, Bengaluru for their patronage.

REFERENCES

[1] Geil PB and Anderson JW. *J Am Coll Nutr* **1994**; 13(6): 549-558.
[2] Ryszard Amarowicz and Ronald B Pegg. *European Journal of Lipid Science and Technology* **2008**; 110(10): 865-878.
[3] Havsteen. *Pharmacol Ther* **2002**; 96(2-3): 67-202.
[4] Robards K, Prenzeler PD, Tucker G, Swatsitang P and Glover W. *Food Chem* **1999**; 66: 401-436.
[5] Ferreira KM. *Neotropical Ichthyology* **2007**; 5(3): 311-326.
[6] Calliste CA, Trouillas P, Allais DP and Duroux JL. *Journal of Agricultural and Food Chemistry* **2005**; 53: 282–288.
[7] Naczk MJ, Williams K, Brennan, Liyanathirana C and Shahidi F. *Food Chem* **2004**; 88: 429-434.
[8] Hahn DH, Faubion JM and Rooney LW. *Cereal Chemistry* **1983**; 60:255–259.
[9] Holtekjølen AK. *Food Chem* **2006**; 94: 348-358.
[10] Mattila J, Omelyanchuk L, Kyttala S, Turunen H and Nokkala S. *Int. J. Dev. Biol* **2005**; 49: 391-399.
[11] Zhou. Molecular Pharmacology Fast Forward. Published as doi: 10.1124/mol .106.029264. **2004**.
[12] Yao N, Eisfelder B, Marvin J and Greenberg JT. *Plant J* **2004**; 40: 596–610.
[13] Amarowicz R and Fornal J. *Probl. Post. Nauk Roln* **1995**; 427: 99–105.
[14] Amarowicz R and Abe S. *Acta Phys. Plant* **2001**; 23: 475–482.
[15] Madhujith T, Naczk M and Shahidi F. *Journal of Food Lipids* **2004**; 11: 220–233.
[16] Choung M, Choi B, An Y, Chu Y and Cho Y. *J. Agric. Food Chem* **2003**; 51: 7040-7043.
[17] Lowry OH, Rosebrough NJ, Farr AL and Randall R J. *Journal of Biological Chemistry* **1951**; 193: 265 – 275.
[18] Malick CP and Singh MB In: Plant Enzymology and HistoEnzymology, Kalyani Publishers, New Delhi, p. 286. **1980**.
[19] Hinneburg I, Dorman HJD and Hiltunen R. *Food Chem* **2006**; 97: 122-129.
[20] Khairunnuur FA, Zulkhairi A, Azrina A, Moklas MAM, Khairullizam S, Zamree MS and Shahidan MA. Nutritional Composition, in vitro Antioxidant Activity and Artemia salina L. *Mal J Nutr* **2009**; 15 (1) : 65-75.
[21] Pumthong G. Antioxidative activity of polyphenolic compounds extracted from seed coat of Tamarindus indicus Linn. Chiangmai Mai University, Thaialnd, IV-V. **1999**.
[22] Gu L, MA Kelm, JF Hammerstone, Ze. Zhang, G. Beecher, J Holden, D Haytowitz and RL Prior. *J. Mass Spec* **2003**; 38: 1272- 1280.
[23] Amarowicz R and Shahidi F. *Food Chemistry* **1994**; 70: 17–26.