International Journal of Research in Engineering and Science (IJRES)
ISSN (Online): 2320-9364, ISSN (Print): 2320-9356
www.ijres.org Volume 4 Issue 6 ǁ June. 2016 ǁ PP. 69-73

Purification of Phenolics From Processed Tamarindus Indica Seeds

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ABSTRACT : Tamarind 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. In India, it is most commonly grown in the drier warmer areas of the South and Central region, where it thrives best. The present study reveals that Tamarindus indica seed contain nutritionally useful quantities of macro and micronutrients. However, the tamarind seed contain small amounts of anti-nutritional factors (tannins, phytic acid, trypsin inhibitor activity and phytohaemaglutinating activity). The objective of the study was to extract, isolate, purify and characterize phenolic acids from Tamarindus indica L seeds as influenced by processing treatments such as soaking, dehulling, cooking, autoclaving and germinating the seeds. The HPLC data revealed the presence of ρ-coumaric acid in most of the processed samples. At present low quality tamarind seeds are used as cattle feed. Hence there is a need to study the anti-nutritional factors and minimize their effect on the animals and also to produce better feed.

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

I. 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.

Phenolic compounds are secondary metabolites which are synthesized in plants. They posses biological properties such as: antioxidant, anti-apoptosis, anti-aging, anti-carcinogen, anti-inflammation, anti-artherosclerosis, cardiovascular protection, improvement of the endothelial function, as well as inhibition of angiogenesis and cell proliferation activity. Most of these biological actions have been attributed to their intrinisic reducing capabilities [1].

Legumes have to be processed prior to consumption due to their high content of antinutritional compounds, such as tannins, phytic acid, galactosides and trypsin inhibitors [2]. Processing techniques such as soaking, cooking, germination and fermentation have been found to reduce significantly the levels of phytates and tannins by exogenous and endogenous enzymes formed during processing [3],[4],[5].

Most developing tropical countries depend on major conventional legumes and animal based sources as key protein concentrates for livestock feeding as well as for human nutrition. The demand for major conventional legumes, soybean (Glycine max) and groundnut (Arachis hypogea) has given rise to a disproportionate increase in their prices and consequently on the cost of livestock and feeds. There is a need for identification and exploitation of other novel legumes, which are in abundance in these regions to fulfill the growing need of plant based proteins and underutilized legumes as inexpensive and good sources of protein [6],[7],[8].

Removal of undesired components are very essential in improving the nutritional quality and organoleptic acceptability of legumes. This in turn help to effectively utilize their potential as human food. Methods for reducing antinutrients in food are carried out according to their physical (dehulling / cooking, autoclaving / pressure cooking, dry roasting, soaking, milling, selective extraction, irradiation) or biochemical (enzyme processing, germination and fermentation) character [9].

The application of a single technique is frequently insufficient for effective treatment and so combinations are commonly employed. For example, the most effective method for reducing saponin contents have been reported to be soaking and cooking. Industrial processes, including canning, toasting, fractionation and isolation of protein concentrates have also been shown to be effective in reducing or removing anti-nutritional factors.

The present study was carried out to determine the effect of various processing treatments on the levels of phenolic compounds present in the seeds of tamarind. The phenolic compounds were fractionated and separated by HPLC and the peak fractions were identified.

II. MATERIALS AND METHODS

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

Ethyl alcohol, methanol, petroleum ether, sodium hydroxide, hydrochloric acid, sodium carbonate, gallic acid, vanillin, methanol, catechol, phloroglucinol, potassium thiocyanide, sodium chloride, ferric nitrate, monobasic di - hydrogen phosphate, potassium phosphate dibasic, tannic acid, sodium tungstate, sodium molybdate, orthophosphoric acid, sodium potassium tartarate, copper sulphate, acetonitrile, potassium ferricyanide were purchased from Sisco Research Laboratories, Mumbai, India.

2.2: 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.

2.3: Processing treatments : The seeds were subjected to five different types of processing.

2.3.1. Soaking : The seeds were soaked in water for 5 days, dried at 60° C and ground to a fine powder using a blender.

2.3.2. Dehulling : The seeds were soaked in water for 5 days and then hand pounded to separate the hull. The dehulled seeds were then dried at 60° C and ground to a fine powder.

2.3.3. Cooking : The seeds were cooked for 30 minutes, mucus was removed from seed coat and washed. The cooked seeds were then dried at 60° C and ground to a fine powder.

2.3.4. Autoclaving : The seeds were autoclaved, cooled and then dried at 60° C and ground to a fine powder.

2.3.5. Germination : The seeds were treated with 50% H_2SO_4 for 30 minutes. After 30 minutes, it was washed and sowed onto a medium containing coco pith and sand in the ratio 1:1. After 10 days, the seeds were cleaned, dried overnight at 60° C and ground to a fine powder.

2.4: Antinutritional factors :

The anti-nutritional factors – tannins and phenolics – were analyzed in all the five processed samples as described below.

2.4.1.Extraction of tannins :

The tannins were extracted with methanol for 24 hours with occasional swirling and centrifuged at 10000 rpm [10].The supernatants obtained were used for estimation of tannins.

2.4.2.Modified vanillin-hydrochloric acid (MV-HCl) method :

The tannins were determined as described by Robert, E. B.[10]. The vanillin – HCl reagent (just before use mix equal volumes of 8% HCl in methanol and 4% vanillin in methanol) react with any phenol that has an unsaturated resorcinol or pholoroglucinol nucleus to form a colored substituted product which is measured at 500 nm. To 1 ml of the test sample 5 ml of the vanillin hydrochloride reagent is added and incubated at room temperature for 20 mins and the absorbance read at 500 nm. Tannin content was expressed as tannic acid equivalent.

2.4.3.Total phenols determination :

Total phenolics content was determined in all the five processed samples according to Malick, CP and Singh, MB [11]. A standard curve was prepared and phenolics content expressed as phloroglucinol equivalents i.e. amount of (mg per 100g).

2.5: 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 [12].

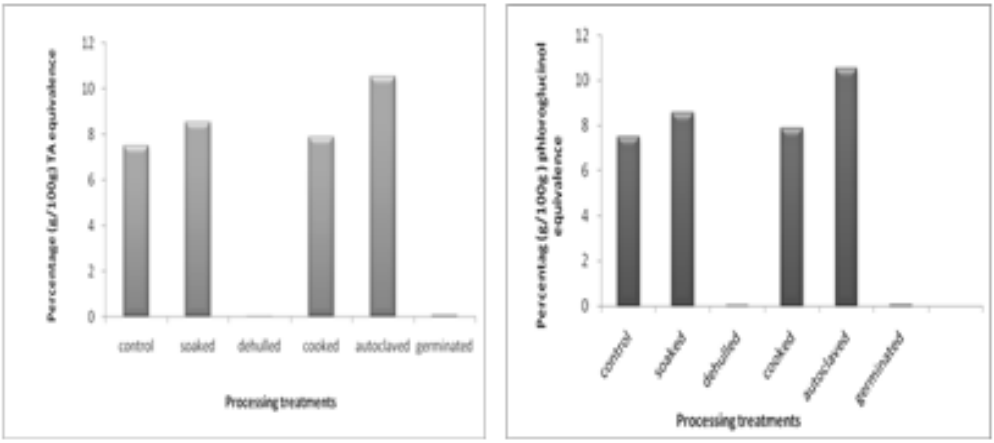
III. RESULTS

Legumes have to be processed prior to consumption due to their high content of antinutritional compounds. Various processing treatments such as soaking, dehulling, cooking, autoclaving and germination have been carried out on the tamarind seed and the proximate composition determined.

3.1.Anti-Nutritional Factors

The effect of processing methods on tannins is illustrated in Fig 1(a). The results indicate that soaking followed by dehulling of seeds and germinated seeds have reduced tannin content in comparison to the control seeds. Tannin content increased with autoclaving.

Fig 1(b) illustrates the effect of processing treatments on total phenolics of tamarind seeds. The results indicate that the autoclaved seeds showed an increase in phenolics while a reduction was noticed in dehulled and germinated seeds.



(a) **(b)**

Figure 1 : Effect of various processing treatments on the levels of (a)tannins and (b)phenolics in Tamarindus indica seeds.

3.2. Identification Of Potent Phenolics By Using HPLC:

The phenolic fractions obtained were loaded onto HPLC along with six standard phenolic acids – gallic acid, tannic acid, benzoic acid, salicylic acid, p-coumaric acid, 2,5-dihydroxy benzoic acid (Table 1). The presence of p-coumaric acid was confirmed in most of the cases (Table 2).

Table 1 : Standard phenolic acids in the processed tamarind seeds with their retention time as determined by 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

Table 2 : Phenolic acids in the processed tamarind seeds with their retention time as determined by HPLC.

Peak No.	Samples	Retention time
1.	Control	4.68
2.	Soaked	4.77
3.	Dehulled	10.32
4.	Cooked	---
5.	Autoclaved	3.3, 4.3, 4.9, 6.2, 7.0, 9.64
6.	Germinated	1.89, 4.6

IV. DISCUSSION

Higher plants produce hundreds to thousands of diverse chemical compounds with different biological activities and important ecological roles. They can be chemical defenses against insects, herbivores and microorganisms [13]. Plants have an almost limitless ability to synthesize aromatic substances, most of which are phenols or their oxygen-substituted derivatives. In many cases these substances serve as plant defense mechanism against predation by micro- and macroorganisms. Among the cereals, sorghum has been found to

contain higher amounts of polyphenols. Even though high polyphenol seeds are immune to attack by birds and diseases, they display impaired nutritional quality, lower digestibility and reduction of food consumption.

Proteins of tamarind seed are of a higher biological value than those of wheat and corn, and lower only than those of millets. But their net utilization is lower than that of others due to the presence of anti-nutritional factors. Germination of seeds improves the nutritive value of seeds by decreasing the level of anti-nutritional factors. Germination studies will be undertaken to study the biochemical changes – the level of anti-nutritional factors and antioxidants, and the effect on the viscosity of the tamarind seed polysaccharide.

Tannins are naturally occurring plant polyphenols which combine with protein and other polymers to form stable complexes. They are fairly large molecules having molecular weights of 500-3000Kd. Several phenolic hydroxyl groups located on the surface of tannin molecules are believed to participate strongly in the properties and biological activities of the tannins. There are several reports on the use of tannins in treating various ailments in humans, including diarrhea, gastric ulcers, snake bites and wounds. However, reports on antimicrobial activity of tannins are scarce.

V. CONCLUSION

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. Studies around the world have identified many new plant constituents with antioxidant activity, among these are the polyphenols. Green plants posses the broadest spectrum of synthetic activity and have been the source of many useful compounds. The use of higher plants and shrubs including some vegetables were originally recognized as antiseptic. For example, thymols, a simple phenol present in essential oil of plants like Thymus vulgaris and Monanda punctoda have both antibacterial and antiviral properties. The results of the present study confirms that the seeds on processing treatments such as soaking followed by dehulling and also germinating will reduce the amount of tannins. Further the presence of phenolic acid ρ-coumaric acid was observed in most of the processed samples.

VI. 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.

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