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Proximate and phytochemical screening of the seed and pulp of *Tamarind indica*

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Abstract

Tamarind indica L. (Tamarind), an underutilized fruit tree which belongs to the leguminous family, grows wild in the savannah region of Nigeria. This plant is known to have medicinal, cultural and ethno botanical uses and is often used as a therapeutic drink in febrile conditions, anti-diabetic, anti-ulcer, inflammation, skin diseases, laxative, reduction of body temperature in fevers, hepatoprotective, anti-fertility, alleviate sunstroke. Thus knowing the importance of the plant as the therapeutic drug, the present study had taken shape and has been validated to evaluate the phytoconstituents and proximate composition. All the nutrients (moisture, protein, crude fat, fibre) estimated during the study were analyzed by using standard AOAC methods. Carbohydrate was estimated by difference method. Aqueous and ethanolic extracts were prepared from seed and pulp powders of Tamarind. Proximate nutrient analysis revealed that Tamarind reported a higher amount of carbohydrate i.e. (49.5±0.1) in seed and (36.4±1.60) in pulp with observed significance difference ($p \geq 0.05$). Phytochemical screening of the samples revealed the presence of flavonoids and tannins in pulp and saponins in seed which might validate the traditional uses of the plant in the treatment of various diseases, whereas alkaloids and glycosides showed a negative response. Considering that the plant is reasonably safe to use, this plant has not received sufficient promotion for use as herbal medicine. Its development will be important in the improved delivery of primary health in developing countries. The developmental cost of synthetic drugs is increasing tremendously; hence the drug recovery would take an economical path utilizing natural products.

Keywords: phytoconstituents, phytochemical screening, flavonoids, saponins, glycosides, alkaloids, Aqueous and ethanolic extracts

Introduction

Tamarind, commonly called as *Imli* in Hindi, is known as *Chincha* or *Amlika* in Ayurveda (1). It is botanically identified as a *Tamarindus indica* Linn, the member of *Caesalpinaceae* subfamily of *Fabaceae* family. It is distributed throughout India, particularly in the South. The tree averages 20-25 m in height and 1 m in diameter, slow growing, but long lived, with an average life span of 80-200 years (1).

T. indica is rich in nutrients and plays an important role in human nutrition, mainly in the developing countries (2). It contains a high level of protein with many essential amino acids, which help to build strong and efficient muscles. It is also high in carbohydrates, which provides energy, and is rich in minerals, such as potassium, phosphorus, calcium, and magnesium. *T. indica* can also provide smaller amounts of iron and vitamin A (3). It is an excellent source of potassium which is essential for controlling the effect of sodium in the body; thereby regulate the heart rate and blood pressure (6).

Tamarind is valued mostly for its fruit, especially the pulp, which is used for a wide variety of domestic and industrial purposes (4). It is used as a raw material for the manufacture of several industrial products (5). Pulp is also used as a beverage and its seeds are discarded (3).

It is an excellent source of potassium which is essential for controlling the effect of sodium in the body; thereby regulate the heart rate and blood pressure. Tamarind pulp has been reported to be used in the treatment of a number of ailments, including the alleviation of sunstroke and the intoxicating effects of alcohol and cannabis. It can be gargled for sore throats, dressing of wounds and is said to aid the restoration of sensation in cases of paralysis. Tamarind pulp is also said to aid in the cure of malarial fever (6).

The pulp extract has also been shown to enhance the bioavailability of ibuprofen in humans (7). The fruit pulp is used as a digestive, a remedy in bile disorders, to alleviate sunstroke. The acidic pulp is used as a favorite ingredient in culinary preparations, such as curries, chutneys,

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saucers, ice cream, and sherbet in countries where the tree grows naturally [6].

Tamarind (*Tamarindus indica*) seed is a by-product in the tamarind pulp industry. Recently, a large amount of the seed waste is discarded from the tamarind industry [3]. The seed is a rich source of phytochemicals containing a variety of biologically active phytochemical compounds, especially phenolic constituents, flavonoids, anthocyanin INS, vitamin C, and carotenoids. These phytochemicals positively influence human health and indicate high antioxidant activity. However, it has several uses, it is commercially available as a food additive for improving the viscosity and texture of processed foods. [8] Tamarind seeds and kernels are high in protein content, while the seed coat is rich in fibre and tannins (anti-nutritional factors). These proteins have a favorable amino acid composition and could supplement cereals and legumes poor in methionine and cystine. Hence, they can be used as a cheaper source of protein to alleviate protein malnutrition [9]. The powder of tamarind seeds is rich in xyloglycans that works as a prominent ingredient used in pharmaceutical and cosmetic products. It is mostly facilitated for the exposed body parts. Its anti-inflammatory property makes it a great soothing agent in joint pains for those suffering from Arthritis. It boosts the smoothness of the joints and alleviates pain [10].

Tamarind seeds and pulp contain a variety of biologically active phytochemical compounds, especially phenolic constituents, flavonoids, anthocyanin ins, vitamin C, and carotenoids. These phytochemicals positively influence human health and indicate high antioxidant activity. Hence, it is considered crucial to increase the antioxidant intake in the human diet and one way of achieving this is can be through enriching food products with seeds which are rich in phytochemicals. *Tamarind indica* has ameliorative effects on many diseases. It can also be preferred as a nutritious support for malnourished patients [8].

Materials

Fresh tamarind fruits were purchased from the local market in Jaipur, and transported to the Department of Food science and Nutrition, Banasthali University. The equipment and chemicals used in this work were obtained from the laboratory of the Department of Food Science and Nutrition, Banasthali Vidyapith. The fruits were selected for uniformity in shape and color, washed carefully with clean portable water and stored in a refrigerator at 8°C prior to further use. The fruits were divided into two halves. One-half was set for drying under the sun to separate the pulp from the seed. This was done to obtain a powdery form of the pulp, since it was wet, while the other was soaked in tap water for about 20-30 minutes to wash off the pulp to obtain clean, pulp free whole seed. The whole seed were also sun dried, after sun drying, the whole seed and pulp were separately grounded into fine powder.

Methods

Preparation of tamarind seed powder

Tamarind fruit pods were manually cracked and from each pod the pulp containing seeds was separated along with the fibers. The seeds were then washed using clean distilled water. Samples were prepared. Whole seeds of tamarind were sun dried, and the immature and damaged seeds were discarded. The mature whole seeds were then soaked in sodium hydroxide solution (10%) for 30 min then crushed and washed with water several times. The crushed seeds were

dried, grounded into powder to pass through a 60 –mesh size. The powder was then divided into two portions and kept in plastic containers at room temperature. One part was used for proximate analysis where the other part was reserved for phytochemical extraction.

Proximate analysis

Determination of Moisture, Protein, Fat, Ash, and crude fiber were carried out in triplicate. The moisture content was determined by weighing the kernels before and after drying in an oven at 105° for 3 hours. The nitrogen content was estimated by the micro- Kjeldahl methods and then crude protein was calculated by multiplying the N value with the factor 6.25. The fat content was determined by extracting the sample with ether in soxhlet apparatus for 16 hours. The ash content was calculated by the method mentioned in AOAC. The carbohydrate content was obtained by difference.

Total carbohydrate content = 100-(% moisture + % crude protein + % crude fiber + % crude fat + % ash).

All proximate results were expressed as a percentage of the weight of samples analyzed.

Extract preparation of tamarind seed and pulp

Aqueous extract preparation

The aqueous extract of ripe pods of *Tamarindus indica* was prepared following the procedure suggested by Daniyan and Mohammed with some slight modification [11]. The fruit pulp was mixed with warm distilled water (50°C) in the ratio 1:2 and blended in the electric blender. The mixture was shaken on an electric shaker at 200 rpm for 10minutes, filtered through Whatman no. 1 filter paper and evaporated to dryness at ambient temperature. The crude extract obtained was stored at 40°C in the refrigerator for further use [12].

Ethanolic extract preparation

The extract was prepared with ethanol according to the procedure described by Doughari [13]. Fifty grams (50 g) of the pulp was extracted with 100 ml of ethanol and kept on a rotary shaker for 12 hours. The extract was filtered and centrifuged at 500 rpm for 5 minutes on a rotary shaker and the supernatant collected. The supernatant was filtered with a No 1 Whatman filter paper and evaporated to dryness. The crude extract obtained was maintained at 40°C till further use [12].

Phytochemical Screening

The aqueous and ethanolic extracts of the specimen was screened qualitatively for the presence of alkaloids, tannins, flavonoids, saponins, cardiac glycoside and reducing sugars according to the method described by Akinpelu and Kolawole [14]. Quantitative screening of total alkaloid, saponins and tannins was also carried out following standard methods [15, 16].

Statistical analysis

All the results were expressed as mean \pm SEM (Standard Error of mean) for four replicates. The data were statistically evaluated with t-test using excel software. P values < 0.05 were considered to indicate statistical significance.

Results

The result of proximate analysis for the whole seeds and pulp of *Tamarind indica* seed and pulp. The results showed that dry moisture, crude fat, crude fiber, protein and ash content are statistically higher in the pulp as compared to the seed.

The results also showed that the carbohydrate levels to be statistically higher in the seed as compared to the pulp.

Table 1: Result of proximate analysis for the whole seeds and pulp of *Tamarind indica* L.

Parameter in %	Tamarind seed	Tamarind pulp
Moisture(g/100g)	9.33±0.01 ^{ns}	16.82±0.2 ^{ns}
Ash(g/100g)	4.58±0.42 ^{ns}	4.63±0.53 ^{ns}
Crude fiber(g/100g)	14.75±2.1 ^{ns}	18.75±0.56 ^{ns}
Protein(g/100g)	14.0±1.16 ^{ns}	18.84±0.56 ^{ns}
Fat(g/100g)	7.84±0.64 ^{ns}	5.4±0.69 ^{ns}
Carbohydrate(g/100g)	49.5±0.1*	35.56±0.2*

The result of phytochemical screening of the seed and pulp of *Tamarind indica* indicates that Alkaloid and glycosides showed negative response while saponins, tannins and flavonoids showed positive response. Tamarind pulp show positive response for alkaloids, saponins, glycosides.

Table 2: Result of phytochemical screening for the whole seeds and pulp of *Tamarind indica* L seed and pulp.

Phytoconstituents	Seed		Pulp	
	Aqueous	Ethanol	Aqueous	Ethanol
Saponins	+ve	+ve	+ve	+ve
Alkaloids	-ve	-ve	+ve	+ve
Tannins	+ve	+ve	-ve	-ve
Flavonoids	+ve	+ve	-ve	-ve
Glycosides	-ve	-ve	+ve	+ve

The result of nutritional composition of *Tamarind indica* seed are summarized in a Table1.

The result of proximate analysis shows that seed and pulp had 9.33±0.01 and 16.82±0.2% moisture content. The moisture content was higher than those obtained for fluted pumpkin (5.0%).The results show that the moisture of *Tamarindus indica* pulp is similar to that of *Ziziphus spina christi* seed, but values are lower 42.23 than reported for *Nauclea latifolia* fruit by [17].

The ash content which is measure of the mineral content of food gives an idea of the inorganic content of sample wherein the seed and pulp of *Tamarind indica* contained 4.58±0.2% and 4.63±0.53% ash content respectively. The values were in accordance with the earlier studies on some legumes like cream coat (4.63%) and dark red bambara (3.90%) [18].The ash values were in agreement with the range 2.17%-4.2% [19], but greater than 2.8%-4.58% [20].

The crude fiber content of *Tamarindus indica* seed and pulp were non-significantly different ($p \geq 0.05$) from each other values being 14.75±2.1% for seed powder and 18.75±0.56for pulp. *Tamarind indica* seed and pulp sample had high level of crude fiber when compared with *Agave salmiana* (12.7%) [17]. The high fibre and protein content is a further confirmation of its use as vegetable. Fibre is an important part of diet, which decreases serum cholesterol levels, risk of coronary heart disease, hypertension, diabetics, colon and breast cancer [21]. Plants with high fibre are adequate for better rumination and digestion in ruminant animals [22]. The crude fibre of *Z. spin-christi* fruits is lower compared with values obtained for *T. indica* pulp and *Z. spina christi* seed which makes the useful in fibre diet for aiding weight loss normalizing bowel movement. Moreover, slightly similar carbohydrate values are obtained for *T. indica* pulp and *Z. spina christi* fruit indicating that they can be consumed as an alternative source [17].Fiber plays a role in prevention of diseases by reducing the level of cholesterol [19].

Tamarind indica seed and pulp contain high level of protein i.

e 14.0±0.26% and 18.84±0.56 respectively. The high protein value recorded in this study suggests that the seed can be ranked as a potential source of plant protein and therefore could be used as protein supplement. However, the crude protein values obtained for all the samples are lower than 28.60 that were reported for *Adenopus breviflorus* [17].

Fat content of *Tamarind indica* seed was 7.84±0.64. Fat content of *Tamarind indica* seed is found to be more or less equal to that of *prsoipis gladulosa* [23]. The fat content of the samples were found to be in correspondence with an earlier report on the same specie [24].However, these values were lower than the range of (22.8 – 23.5%) reported in soya beans [25]. 75mg/g for *T.indica* seeds reported in [26].

Values obtained for crude fat in all the samples show that they have low fat content compared with 15.60 for *Chrysophyllum albidum* indicating that these fruits cannot be used as fat food formulation [21].

The carbohydrate content of plant seed and pulp was 49.5±0.1 and 35.56±0.2 respectively. Carbohydrate values obtained were found to be higher than that of some earlier investigation of wild pulses like *Azelia africana*. However these values are much lower than carbohydrates value of 87.62 0.04 in *G. africanum* seeds [21].

Proximate analysis is an important index to classify the nutritional value of a food material. A sample with high level of carbohydrates can regulate nerve tissue. Ash contents give an idea about the inorganic content. They are also expected to facilitate the metabolic processes, growth and development while moisture contents display more information about the storage/shelve life and the viability of microorganisms' growth. Proteins, on the other hand, can serve as enzymatic catalyst, growth control and cell differentiation. Based on protein, fat and carbohydrate contents, cladode energy content was 193.1 Kcal/100 g justifying its uses as a good source of nutrient [27].

Phytochemicals are non-nutritive plant chemicals that have protective or disease preventive properties. They play a crucial role in maintaining optimal immune response, such that deficient or excessive intakes can have negative impact on health. Dietary intake of phytochemicals may promote health benefits, protecting against chronic degenerative disorders, such as cancer, cardiovascular and neurodegenerative diseases. protection against numerous diseases or disorders such as cancers, coronary heart disease, diabetes, high blood pressure, inflammation, microbial, viral and parasitic infections, psychotic diseases, spasmodic conditions, ulcers, osteoporosis and associated disorders. This naturally occurring substance is what gives fruits, vegetables and herbs their colours. These same substances have been found to be beneficial for human health as well, as they have antioxidant activity [28].

The phytochemical screening of the plants gives the general idea about the class of compounds present in those plants. Though the utility of plants basically depends on therapeutically effective active principles and therefore chemical tests are essential to identify the various constituents or groups present in the plants [29]. The result of phytochemical screening is shown in table 2

Presence of tannins is most likely to be responsible for the antioxidant and anti-inflammatory properties. Flavonoids are responsible for other medicinal properties. The phytochemical function in plants may further increase our understanding of mechanism by which they benefits human [30].

Alkaloids are a plant derived compound. They are formed as metabolic by products and have been reported to be responsible for antibacterial activity. Alkaloids are powerful

pain relievers, have an antipyretic action, a stimulating effect and can act as topical anesthetic in ophthalmology [31].

K. senegalensis, *V. amygdalina* and *O. gratissimum* have greatest quantity of tannins followed closely by *Tamarind indica*. This confirmed the assertion of Ihekoronye and Ngoddy (1985) that tannins are rarely frequently encountered in food products of plant vegetable origin. The oxidation inhibiting activity of tannins have been known for a long time and it is assumed to be due to the presence of gallic and diagallic acids. Another point of note in this study is the styptic and stringer properties of tannic acid which was used in the treatment of inflammatory skin eruption and bowel conditions. The presences of tannis are responsible for the astringent flavour of tea [32].

Herbs that have tannins as the main components are astringent in nature and are used for training intestinal disorders such as diarrhea and dysentery reviewed the biological activity of tannins and observed that tannins have anticancer activity and can be used in cancer activity and can be used in cancer prevention, thus suggesting that *Tamarindus indica* have potential as a source of important bioactive molecule for the treatment and prevention of cancer [33].

Flavonoid is the most active component of *E.uniflora* and poly phenolic compounds like flavonoids have enormous range of biological activity and are known to inhibit oxidative damage in-vivo better than the classical vitamin and antioxidants. They also reported that "in plants, they protect against lipid peroxidation and UV damage that can affect tropical fruits growing under severe conditions including high heat and intense sunlight". Flavonoids are also responsible for colour in plants. The stem also contains phenol, tannins and flavonoid, while the root according to the tests conducted in this work contains only saponins [34].

Saponin it was strongly present in *O. gratissimum*, *k. senegalensis* *V. amygdalina* and *A. satium* while it was mildly found in *Z. officinale*. The presence of saponin in most of the samples. Saponins are glycoside of both triterpenes and sterols and are used as expectorant and emulsifying agent [35] in agreement with [36] confirmed the presence of saponin in *O. gratissimum*. Hence, saponin as sugar derivatives may be steroidal or triterpenoids. The occurrence of steroidal saponins from numerous studies showed their importance and interest in pharmacy due to relationship with such compounds as sex hormones mostly in the development of female contraceptive pills. Additionally, saponin is equally used in medicine and pharmaceutical industries because of its foaming ability with the production of frothy effect. Saponin is used in the preparation of insecticides, various drugs and synthesis of steroid hormones [37].

Glycosides are nonvolatile and lack fragrance. Glycosides serve as defense mechanisms against predation of against microorganism, insects and herbivores. This may therefore explain the demonstration of antimicrobial activity by the seed and pulp of *Tamarind indica*. Plants containing glycosides are known to exert a beneficial action on immune system by increasing body strength and hence are valuable as dietary supplements. It can be suggested to be beneficial for hyper proliferative skin diseases on the basis of their antimicrobial and anti-inflammatory effects. Glycosides also have vast therapeutic efficacy as they are found in almost every medicinal plant [38].

Hence the presence of these components in *Tamarindus indica* corroborates the anti-microbial activities observed. The success of ethanobotanical approach to drug discovery can no longer be questioned historical and current discoveries

demonstrated its power accompanied study conducted with the purpose of finding these chemical as worthwhile. The optimal effectiveness of a medicinal plant may not be due to one main active constituent, but may be due to the combined action of different compound originally in the plant [39]. The result of phytochemical analysis comprehensively validates the presence of therapeutically importance and valuable secondary plant metabolisms [40].

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