INVESTIGATION OF TOTAL PHENOL CONTENT AND ANTIOXIDANT POTENCY OF TAMARIND LEAVES

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Abstract

This research deals with the study of total phenol content and antioxidant potency of leaves of *Tamarindus indica* L. (Magyi). The phytochemical investigation of tamarind leaves indicated that alkaloids, α-amino acids, carbohydrates, flavonoids, glycosides, organic acids, phenolic compounds, reducing sugars, saponins, steroids, terpenoids and tannins were found whereas starch was not detected. Nutritional values of tamarind leaves determined by AOAC methods showed that tamarind leaves consist of 11.12 % of moisture, 3.56 % of fat, 18.67 % of fiber, 5.38 % of ash, 11.63 % of protein and 49.64 % of carbohydrate. The energy value of tamarind leaves was found to be 277.12 kcal/100 g. The extractable matter contents of tamarind leaves in petroleum ether, ethyl acetate, acetone, ethanol and water determined by WHO standard method. The total phenol contents of crude extracts were analyzed by UV-visible spectrophotometric method. The total phenol contents of watery and ethanol extracts of tamarind leaves were 24.70 ± 3.38 μg GAE/mg extract and 13.30 ± 3.30 μg GAE/mg extract. Antioxidant activity of tamarind leaves was also investigated by using DPPH assay. The IC_{50} values of watery and ethanol extracts of tamarind leaves were observed to be 57.13 μg/mL and 34.47 μg/mL, respectively. The ethanol extract is more effective than watery extracts in antioxidant activity. All extracts showed mild activity when compared to the standard ascorbic acid (7.97 μg/mL).

Keywords: *Tamarindus indica* L., nutritional values, extractable matter contents, total phenol content, antioxidant activity

Introduction

Tamarind is indigenous to tropical Central Africa and is a very adaptable species, drought hardy, preferring semi-arid areas and woody grasslands, tolerating salty conditions, coastal winds, and even monsoon climates.

Tamarind has been used for a large number of purposes e.g. animal fodder, food for people and medicinal uses. The first medicinal use of tamarind was reported from India. Tamarind products, leaves, fruits and seeds have been extensively used in traditional Indian and African medicines. Tamarind leaves are usually ground into powder and used in lotions or infusions (Bhadoriya *et al*., 2015).

The leaves, mixed with salt and water, are used to treat throat infection, coughs, fever, intestinal worms, urinary troubles and liver ailments. Leaf extracts also exhibit antioxidant activity in the liver. Young leaves are reported to cure other eye infections, sprains and wounds (El-Siddig *et al.,* 2000). Leaves are useful in fevers, scalding of urine, gastropadthy, helminthiases, wound, ulcers, jaundice, scabies, tumors, ringworm, boil, smallpox, otalgia and conjunctivitis (Tariq *et al.,* 2013). Leaves are used as astringent, as gargle, and also made into a poultice to treat inflammatory swellings (Shah, 2014).

Antioxidants are substances that may protect cells from the damage caused by unstable molecules known as free radicals. Antioxidants interact with and stabilize free radicals and may prevent some of the damage free radicals might otherwise cause. Antioxidants are also widely

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used us ingredients in dietary supplements to maintain health and preventing diseases such as cancer and coronary heart disease. Plant based antioxidants are preferred to the synthetic ones because of their multiple mechanisms of actions and non-toxic nature (Hamid *et al.*, 2010). The aim of this research was to investigate total phenol contents and antioxidant potency of tamarind leaves.

Materials and Methods

Sample Collection and Preparation

Tamarind leaves sample was collected from Kyaunggon Township. Tamarind leaves sample was washed with water and air dried at room temperature. This sample was ground into powder in an electric blender and stored in an airtight container to prevent the moisture and other contaminations.

Phytochemical Investigation on Tamarind Leaves Sample

In order to find out the types of organic constituents present in tamarind leaves sample, preliminary photochemical investigation was carried out by the test tube methods (Vogel, 1989).

Determination of Nutritional Values of Tamarind Leaves

In the present study, some nutritional values such as moisture, fat, fiber, ash, protein, carbohydrate and energy value of tamarind leaves were determined by AOAC method at Food Industries Development Supporting Laboratory (FIDSL), Yangon.

Determination of Extractable Matter Contents

This method determines the amount of active constituent extracted with different solvents from a given amount of medicinal plant material. The extractive values provide an indication of the extract of polar, moderately polar and non-polar components present in the medicinal plant material (WHO, 1998).

The powdered tamarind leaves (2 g) was placed in a conical flask. 50 mL of the solvent was macerated for the tamarind leaves sample concerned for 6 hours shaking frequently then allowed to stand for 18 hours. The extract was filtered rapidly taking care not to lose any solvent and transferred to a porcelain basin and evaporated to dryness on water bath. The dried filtrate was then placed in oven maintained until constant weight, at 105 ° C.

Determination of Total Phenol Content of Tamarind Crude Extracts by Folin-Ciocalteu Method

(a) Construction of gallic acid standard calibration curve

Firstly, 0.5 mL each of different concentrations of gallic acid solutions (100, 50, 25, 12.5, 6.25 and 3.125 mg/mL) was mixed with 5 mL of 10 % FC reagent in each test tube and incubated for 5 min. To each tube, 4 mL of 1 M Na_2CO_3 was added and the tubes were kept at room temperature for 15 min and absorbance of reaction mixture was read at 765 nm. A standard calibration curve was obtained by plotting the absorbance against concentration of standard gallic acid.

(b) Determination of gallic acid equivalent in crude extracts sample

The total phenol content in the watery and ethanol extracts of tamarind leaves was determined by Folin-Ciocalteu (FC) method according to the procedure described by Rekha *et al.* (Rekha *et al*., 2012). Each extract sample solution (0.5 mL) was added into 5 mL of 10 % FC reagent and incubated for 5 min. To each tube, 4 mL of 1 M Na_2CO_3 was added and the tubes were kept at room temperature for 15 min and the absorbance of reaction mixture was read at 765 nm. The blank solution was prepared as the above mentioned procedure by using distilled water instead of sample solution. Total phenol content was estimated as mg gallic acid equivalents per milligram (mg GAE/mg) of sample.

Screening of Antioxidant Activity by DPPH Assay Method

The antioxidant activity of 95 % EtOH and H_2O crude extracts are by studied by DPPH Assay Method (Marinova, 2011).

DPPH radical scavenging activity was determined by spectrophotometric method. The control solution was prepared by mixing 1.5 mL of 0.002 % (w/v) DPPH solution and 1.5 mL of 95 % ethanol with vortex mixer. The sample solution was also prepared by mixing thoroughly 1.5 mL of 0.002 % (w/v) DPPH solutions and 1.5 mL of test sample solution. The solutions were allowed to stand at room temperature for 30 min. After 30 min, measurement of absorbance at 517 nm was made by using UV-visible Spectrophotometer (UV-7504, KWF, China). Absorbance measurements were done in triplicate for each solution and the mean value was obtained, and then used to calculate % inhibition of oxidation by the following equation,

$$\%\,\text{oxidative inhibition of test sample} = \frac{A_c - (A - A_b)}{A_c} \times 100\,\%$$

A_c = absorbance of the control (DPPH + EtOH)
A_b = absorbance of the blank (EtOH + Test sample solution)
A = absorbance of test sample solution

Then, IC_{50} (inhibitory concentration) values were calculated by linear regressive excel program.

Results and Discussion

Phytochemical Investigation of Tamarind Leaves

Phytochemicals are biologically active, naturally occurring chemical compounds found in plants which provide health benefits for human further than those attributed to macronutrients and micronutrients. The phytochemicals present in the sample can be tested by test tube method (Saxena *et al*., 2013).

Phytochemical investigation was carried out to know the types of phytoorganic constituents present in the Tamarind (*Tamarindus indica* L.) leaves. According to these results alkaloids, α-amino acids, carbohydrates, flavonoids, glycosides, organic acids, phenolic compounds, reducing sugars, saponins, steroids, terpenoids and tannins were found to be present while starch was not present in tamarind.

Nutritional Values of Tamarind Leaves

The nutritional values such as moisture, protein, fat, ash, fiber, carbohydrate and energy contents of the leaves of tamarind were determined according to the WHO procedure. It was found that carbohydrate, protein, and fiber were present as major nutrients in the leaves of tamarind and the results are shown in Table 1.

Table 1 Nutritional Values of the Leaves of *Tamarindus indica* L.

No.	Parameters	Content (%)
1	Moisture	11.12
2	Fat	3.56
3	Fiber	18.67
4	Ash	5.38
5	Protein	11.63
6	Carbohydrate	49.64
7	Energy value (kcal/100 g)	277.12

Extractable Matter Contents of Tamarind Leaves

The extractable matter contents of leaves of Tamarind were determined by the WHO standard method (WHO, 1998). In this experiment, the solvents used were water, ethanol, acetone, ethyl acetate and petroleum ether. The extractable matter contents of Tamarind leaves are shown in Table 2.

The water, ethanol, acetone, ethylacetate and petroleum ether soluble matters were observed to be 68.05, 69.40, 42.85, 44.85 and 14.00 mg/g in tamarind leaves, respectively.

According to these results, it was observed that the ethanol crude extract of tamarind leaves (69.40 mg/g) was higher than the other crude extracts. Aqueous extract had maximum yield followed by ethanol extract. Generally the amount of polar constituents was higher than that of non polar constituents in the leaves of tamarind.

Table 2 Extractable Matter Contents of the Tamarind Leaves

No.	Solvents used	Extractable matter (mg/g)
1	Petroleum ether	14.00
2	Ethylacetate	44.85
3	Acetone	42.85
4	Ethanol	69.40
5	Water	68.05

Total Phenol Content of Tamarind Crude Extract by FC (Folin-Ciocalteu Reagent) Method

Total phenolic compounds prevent from damage of nutrients contain double bonds such as fatty acids, flavour compounds even proteins and amino acids and other compounds.

The antioxidant activity of phenolic compounds increases with increasing degree of hydroxylation, as is the case of the trihydroxylated gallic acid, which shows a high antioxidant activity. However, substitution of the hydroxyl groups at the 3- and 5- positions with methoxyl groups as in syringic acid reduces the activity. The antioxidant activity of phenolic compounds is

due to their ability to scavenge free radicals, donate hydrogen atoms or electrons, or chelate metal cations (Aberoumand, 2008).

Phenols react with an oxidizing agent phosphomolybdate in FC reagent under alkaline conditions and results in the formation of blue coloured complex, molybdenum blue which is measured at 765 nm colorimetrically. In the present study, the total phenolic content of tamarind extracts were estimated by Folin-Ciocalteu method according to the procedure described. Gallic acid (3,4,5- trihydroxybenzoic acid) was used to construct standard calibration curve. The standard calibration curve of gallic acid was prepared by varying the different concentrations of gallic acid (100, 50, 25, 12.5, 6.25, 3.125 µg/mL) (Figure 1). Total phenolic content was expressed as micro gram of gallic acid equivalent per milligram/milligram of extract (mg GAE/mg extract). Total phenol content of watery extract was found to be higher than that of ethanol extract. This means that phenolic compounds were more soluble in water. The result of phenolic content of tamarind extracts are presented in Table 4 and Figure 2.

Table 3 The Absorbance of Gallic Acid Standard Solution at λ_{max} 765 nm

Sr. No.	Concentration (µg/mL)	Absorbance at λ_{max} 765 nm
1	3.12	0.057
2	6.25	0.065
3	12.5	0.080
4	25	0.109
5	50	0.170
6	100	0.286

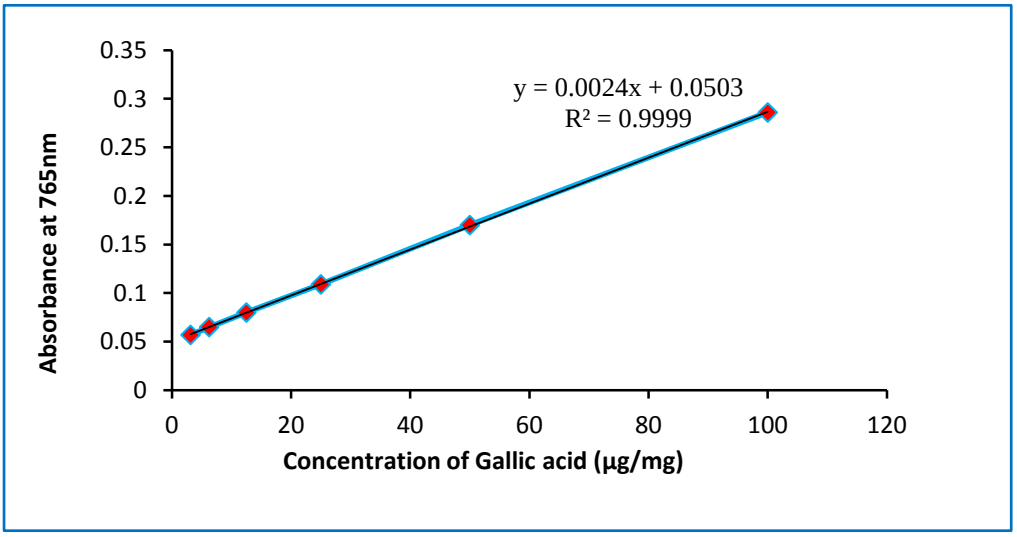

Figure 1 Plot of absorbance vs concentration of standard gallic acid

Table 4 Total Phenol Contents of Watery and Ethanol Extracts from the Tamarind Leaves

Sample	TPC (µg GAE/ mg ± SD)
Water extract	24.70 ± 3.38
Ethanol extract	13.30 ± 3.30

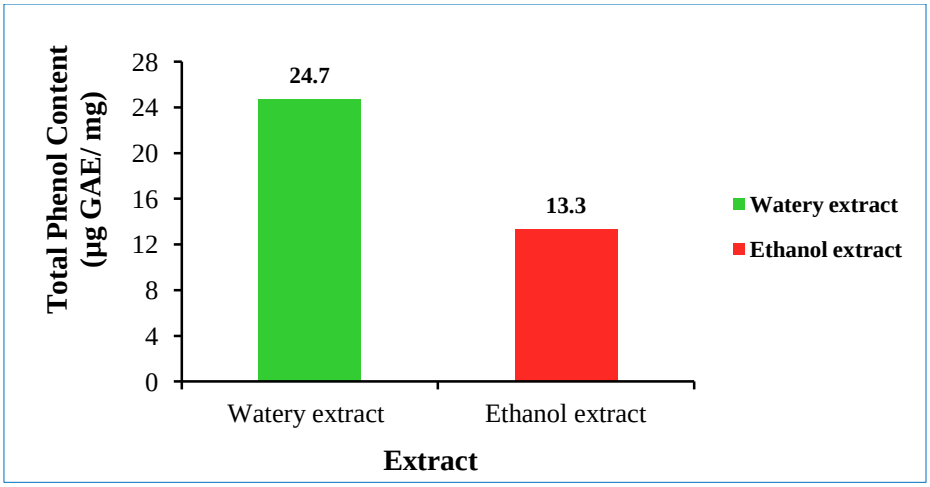

Figure 2 Total Phenol Contents of the Tamarind Leaves Crude Extracts

Antioxidant Activity of Tamarind Leaves by Radical Scavenging DPPH Assay

Antioxidants play an important role as health protecting factor. Most of the antioxidant compounds in a typical diet are derived from plant sources and belong to various classes of compound with a wide variety of physical and chemical properties.

A lot of different procedures have been developed to test the antioxidant activity of foodstuffs. A rapid simple and inexpensive method to measure antioxidant capacity of food involves the use of the free radical, 2,2- diphenyl-1- picrylhydrazyl (DPPH) which is widely used to test the ability of compound to act as free radical scavengers or hydrogen donor and to evaluate antioxidant activity. The DPPH assay method is based on the reduction of DPPH, a stable free radical. The free radical DPPH with an odd electron gives a maximum absorption at 517 nm (purple colour). When antioxidants react with DPPH, which is a stable free radical becomes paired off in the presence of a hydrogen donor (e.g., a free radical scavenging antioxidant) and is reduced to the DPPH and as consequence the absorbances decreased from the DPPH. Radical to the DPPH-H form, results in decolorization (yellow colour) with respect to the number of electrons captured. More the decolorization more is the reducing ability. IC_{50} (50 % inhibition concentration) values calculated after linear regression analysis of the observed inhibition percentages Vs concentration of sample, where lower IC_{50} values indicate higher antioxidant activity (Shekhar and Anju, 2014). The results are shown in Table 5 and Figure 3.

The IC_{50} values for crude extracts and standard ascorbic acid are tabulated in Table 3.9 and Figure 3.3. The IC_{50} values of watery extract, ethanol extract and standard ascorbic acid were observed 57.13, 34.47 and 7.97 µg/mL, respectively. Since the lower the IC_{50} values indicates the higher the antioxidative property, ethanol extract is more effective than water extract. All extracts showed mild antioxidant activity when compared to the standard ascorbic acid.

Table 5 Percent Oxidative Inhibition of Different Concentrations and IC_{50} Values of

Test samples	Percent Oxidative Inhibition of Different Concentrations (µg/mL)						IC_{50} (µg/mL)
	6.25	12.5	25	50	100	200	
Watery extract	18.52 ± 0.39	29.81 ± 1.24	37.12 ± 0.47	48.08 ± 2.49	61.54 ± 0.27	76.34 ± 1.25	57.13
EtOH extract	24.81 ± 1.36	38.46 ± 0.16	45.19 ± 0.16	57.88 ± 5.31	64.81 ± 2.31	71.15 ± 1.93	34.47
Standard ascorbic acid	49.06 ± 1.15	52.48 ± 0.52	53.91 ± 0.23	55.83 ± 0.55	59.73 ± 0.36	65.25 ± 0.33	7.97

Tamarind Leaves Extracts and Standard Ascorbic Acid

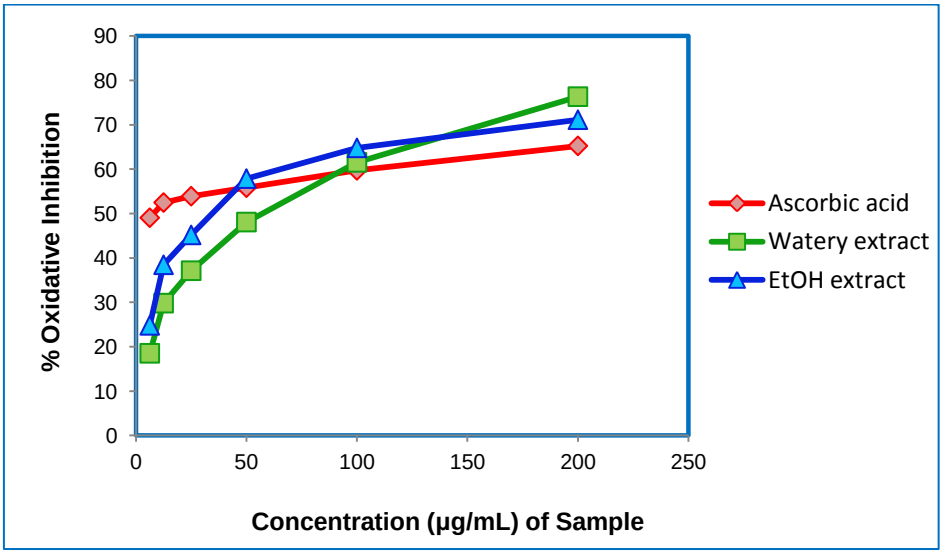

Figure 3 A plot of % oxidative inhibition vs concentrations of different tamarind leaves extracts and standard ascorbic acid

Conclusion

From the overall assessment of the present research work, the following conclusion can be drawn.

Preliminary phytochemical investigation of tamarind leaves sample indicated the presence of alkaloids, α-amino acids, carbohydrates, flavonoids, glycosides, organic acids, phenolic compounds, reducing sugars, saponins, steroids, terpenoids and tannins whereas starch was not found.

Nutritional values analyses showed that tamarind was found to contain 11.12 % of moisture, 3.56 % of fat, 18.67 % of fiber, 5.38 % of ash, 11.63 % of protein, 49.64 % of carbohydrate and 277.12 kcal/100 g of energy values, respectively.

The extractable matter contents were determined by WHO standard method using different polarity of solvents such as petroleum ether, ethyl acetate, acetone, ethanol and water. In the tamarind leaves, the value of extractable matter of ethanol extract is the highest among the extracts.

The total phenol content (TPC) in crude extracts was investigated by UV spectrophotometric method by using Folin – Ciocalteu reagent (FCR). In the determination of total phenol content, gallic acid (3,4,5-trihydroxy benzoic acid) was used for the preparation of standard curve. The TPC content of ethanol and watery extracts were respectively found to be 13.30 ± 3.30 µg GAE/ mg and 24.70 ± 3.38 µg GAE/mg.

The antioxidant activities of tamarind leaves extracts were investigated by using DPPH assay. In DPPH assay, 50 % inhibition concentration IC_{50} (µg/mL or mg) whereas the reducing power ability was presented as absorbance at 517 nm. The IC_{50} values of ethanol and watery extracts of tamarind leaves were to be 34.74 and 57.13 μgmL^{-1}, respectively. The ethanol extract is more effective than watery extract. All extracts showed mild activity when compared to the standard ascorbic acid (7.97 µg/mL).

According to these observations, tamarind leaves possess not only phytochemicals and phenolic compounds, but also antioxidant activity that have proven health benefits.

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