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Research Article

Phytochemical investigation and antioxidant activities of tamarind (*Tamarindus indica* L.)

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Abstract

Tamarind (*Tamarindus indica*) is a common worldwide medicinal plant. Due to high medical importance, tamarind seed and pulp fraction and sub fractions were compared. The present study was aimed to investigate the phytochemical investigation and antioxidant activities of different extracts of tamarind by demonstrating different extraction methods and then selection of best and less time taking method. Biological activities including 2, 2-diphenyl-1-picrylhydrazyl (DPPH) showed maximum inhibition for seed (74.09±0.76) as compare to pulp (72.09±0.43) at 300 µg/ml for butanol fraction, 2, 2'-azino-Bis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS) showed maximum inhibition for seed (79.19±0.36) as compare to pulp (75.69±0.23) at 300 µg/ml for butanol. Metal chelation showed maximum inhibition for seed (81.39±0.70) as compare to pulp (77.49±0.93) at 300 µg/ml for butanol fraction, whereas lipid-peroxidation of thiobarbituric acid reactive substances (TBARS) inhibition showed maximum value for seed at 120 µg/ml as compare to pulp with $FeSO_4$ for butanol fraction and with single nucleotide polymorphisms (SNP), TBARS inhibition showed maximum values for seed at 120 µg/ml as compare to pulp for butanol faction. The total antioxidant activity phosphomolybdenum assay was performed, which showed maximum values for seed at 120 µg/ml as compare to seed for butanol fraction. Total phenolic contents of seed for butanol fraction were 1.83 ± 0.31 mg/g for seed and 2 .83 ± 0.44 mg/g for pulp. Similarly, high amount of flavonoid content for seed was 1.31 ± 0.09 mg/g was given for dichloromethane and for pulp it was given as 1.91 ± 0.96 mg/g for butanol fraction. The results suggested that the extract of *T. indica* is potential source of the phytochemical investigation and antioxidant activity and utilized in diseases arising from oxidative stress in near future by using ultra-sonication method which is precise and time-consuming method.

Keywords: phytochemical and antioxidant; pulp; seed; *Tamarindus indica*

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From Volume 49, Issue 1, 2021, Notulae Botanicae Horti Agrobotanici Cluj-Napoca journal uses article numbers in place of the traditional method of continuous pagination through the volume. The journal will continue to appear quarterly, as before, with four annual numbers.

Abbreviations: DPPH: 2, 2-diphenyl-1-picrylhydrazyl; TBARS: Thiobarbituric acid reactive substances; ABTS: 2, 2'-Azino-Bis-3-Ethylbenzothiazoline-6-Sulfonic Acid; SNP: Single nucleotide polymorphisms; TFE: tamarind leaf fluid extract; LP: lipid peroxidation

Introduction

The *Tamarindus indica* is a fruit tree belonging to the Magnoliophyta, order Fabales, family Fabaceae (subfamily Caesalpinioideae). It is native to tropical Africa and its cultivation was widespread, developing well in all tropical continents (Van, 2015; Nayak *et al*., 2015). Due to their potential to cure many diseases there is a growing trend in researches about medicinal plants, because of lower frequency of side effects and low costs when compared to synthetic drugs (Amir *et al*., 2016). *T. indica* is divided into different varieties such as acidic and sweet fruit. The sweet and sour at the same time in the fruit is unique and it is used popularly in cooking. In addition to the fruit, its various parts, as roots, wood, bark, and leaves, possess nutritional and pharmaceutical properties (Reis *et al*., 2016).

T. indica leaves contain several phytochemicals such as flavonoids, alkaloids, glycosides, coumarin, and quinone. All these bioactive compounds are reported as anti-bacterial effect. Inhibition zones ranged from 1.65 cm to 1.95 cm and maximum activity is found in aqueous extract of tamarind leaves as compared to the ethanol which is 1.9 (Katare and Hakrovorty, 2019).

Many elements of tamarind plant have been found in traditional medicines for treating a wide selection of diseases such as for instance gonococci jaundice, and gastrointestinal disorders. Polysaccharides and their derivatives have already been the decision of polymers as rate controlling carriers in sustains drug delivery system. Making antimicrobial drug therapy effective, safe and affordable has been the focus of interest during recent years. In the recent study attempts have already been designed to screen mature unripe tamarind fruit pulp extract for possible antimicrobial activity and basis for antimicrobial activities by determining the phytochemicals presence (Dipali *et al*., 2010).

Medicinal values of plants are due to their phytochemical's secondary metabolites, which are biosynthetically derivative of primary metabolism, stored by plants in a very minor quantity. The ingredients which are present in *T. indica* have extraordinary medicinal values, therefore these plants are used for treatment purpose. These medicinal plants are familiar source of medicines from ancient history of human beings to the date (Newman and Cragg, 2007). An estimate of world health organization (WHO) almost 80% peoples who are belonging to developing world are dependent upon herbal medicines (Ranilla *et al*., 2010).

The objective of this research was to investigate the pulp and seed of *T. indica* for phytochemical studies and to determine and compare antioxidant activity of different fractions and sub fractions of seed and pulp of *T. indica*. This research article was also demonstrated the biological potential as well as the anti-lipid peroxidation activity of pulp and seed of *T. indica.*

Materials and Methods

Plant materials

The plant material (seeds) of Imali (*Tamarindus indica*) was purchased from local market were stored in refrigerator at desired temperature until needed for the experiment.

Processing of plant material

Processing was carried out in following steps which are given below.

Selection of solvents

The solvent should have a broad range to extract secondary metabolites from the plant parts in order to yield a rich extract with high yield for soaking the dried material. In this study ethanol water mixture was used for the said purpose, for the fractionation those solvents were employed which have almost no miscibility with water (Van-Dyk and Nieuwoudt, 2000).

Simple soaking method of extraction

Total 300 grams of dried seeds were taken and grinded to powder was soaked in ethanol water mixture for 15 days, 500 g of the peeled pulp was taken and soaked in the same solvent for 15 days. The suspension was filtered and concentrated by using rotatory evaporator. Then it was dried and weighed (Krishna *et al*., 2001).

Formation of fractions of crude extract

The crude extract of seeds was subjected to solvent/solvent extraction. First the weighted amount of crude material was suspended in minimum amount of distilled water. Then various sub fractions were produced ranging from hexane to methanol in order of increasing polarity (Ghosh *et al*., 2010).

Hexane fraction

100 mL of hexane was poured into suspension and then shaken vigorously in a 500 mL of separating flask. Flasks were kept for enough time to produce hexane and aqueous layer. Hexane layer was concentrated, process was repeated thrice and obtained extract was weighed (Doughari, 2006).

Dichloromethane fraction

The remnant aqueous fraction after removal of hexane fraction was subjected to an extraction by dichloromethane. 100 mL of dichloromethane was added and after formation of two layers dichloromethane layer become dense, collected and concentrated again. Procedure was repeated thrice, and fraction extract was weighed (Santra*et al*., 2017).

Ethyl acetate fraction

The remnant aqueous fraction after removal of hexane and dichloromethane faction was subjected to solvent–solvent extraction by more polar solvent ethyl acetate. This process was repeated thrice, and ethyl acetate fraction was obtained (Belik-Alexei *et al*., 2007).

Butanol fraction

Aqueous fraction was subjected to solvent-solvent extraction by butanol. The process was repeated to obtain butanol fraction (Etuk, 2010).

DPPH radical scavenging activity

Scavenging of the stable DPPH radical (ethanolic solution of 0.25 mM) was assayed in vitro by the method described by (Annabi *et al*., 2004). Briey, a 0.25 mM solution of the DPPH radical (0.5 mL) was added to extract sample solution in ethanol (1 mL) at concentrations from 25-200 g/mL. The mixture was shaken vigorously and left to stand for 30 min in the dark, after which the absorbance was measured (Spectronic D-20; ThermoScientic) at 517 nm. The capacity to scavenge the DPPH radical was calculated as: DPPH radical scavenging (%) = [(Ao-A1)/Ao)] 100 where Ao is the absorbance of the control reaction and A1 is the absorbance of the sample reaction. All determinations were carried out in triplicate.

Production of TBARS from egg yolk by rhizome

Production of TBARS was determined using a modified method (Rana *et al*., 2014). Chloroform was used to anesthetize from egg yolk by rhizome by decapitation. The extract was immediately removed and placed on ice. Tissues (1:10, w/v) were homogenized in cold 100 mM Tris buffer pH 7.4 (1:10 w/v) and centrifuged at 1,000 x g for 10 minutes. The resulting homogenates (100) were incubated with or without 50 of freshly prepared oxidant (iron) and dierent concentrations of the extracts together with the proper volume of deionized water to give a total volume of 300 at 37 °C for 1 h. The color reaction was done by adding 200, 500 and 500 each of the 8.1% Sodium dodecyl sulphate (SDS), acetic acid (pH 3.4) and 0.6% TBA, respectively. The reaction mixtures, including those of serial dilutions of 0.03 mM standard MDA (1.5-9 nM), were incubated at 97 °C for 1 h. The absorbance of tubes was read after cooling at a wavelength of 532 nm in a spectrophotometer.

Metal chelating activity

The iron chelating ability of the extract was determined using a modified method of (Puntel *et al*., 2005). Briey, 150L of freshly prepared 2 mM $FeSO_4{\cdot}7H_2O$ was added to a reaction mixture containing 168L of 0.1 M Tris-HCl (pH 7.4), 218L of saline, and plant extracts at concentrations of 25-200g/ml. The reaction mixture was incubated for 5 min before addition of 13L of 0.25% 1, 10-phenanthroline (w/v). The absorbance was subsequently measured at 510 nm using a spectrophotometer (Spectronic D-20; ThermoScientic).

Qualitative analysis

Aqueous extracts were separated for presence of tannins, alkaloid, saponins, flavonide, terpenoid, quinones, coumarins, Xanthoprotein, cardiac glycosides, glycosides, steroids, phenols, resins and carboxylic acid groups using standard method (Abdullah *et al*., 2018).

Test for finding alkaloids

2ml of extract was added in test tube and putted 1ml of (1% HCl) and treated it with few drops of Mayer's reagent. Precipitate appears with creamy white precipitate showed presence of alkaloids in extract (Jahanvi *et al*., 2017).

Test to find flavonoids

2mL of extract was added in test tube with few drops of 1% NH_3 solution, if the color becomes yellow, it will show the presence of flavonoids. Phenols and flavonoids in extract of seeds and leaves of *T. indica* having different antioxidant activity and therapeutic activities have found a rich present of flavonoids, polyphenols and other biological basics within seed and promoted their anti-stress and antibacterial potential (Adilah *et al*., 2018).

Phenolic groups test

Put 2mL of distilled water in 1ml of extract also added few drops of 10% Ferric chloride, if color changes into blue or black it was indicated presence of phenolic groups. Seeds, leaves and fruits are natural sources of antioxidants. The presence of phenolic compounds and flavonoids allows them to become reducing agents (Escalona *et al*., 2016).

Antioxidant potential assay

The total antioxidant potential of the extracts was estimated using the phosphomolybdenum reduction assay of Prieto (Mohamed *et al.,* 2007). The assay is based on the reduction of molybdenum, Mo (VI)-Mo (V) by the extract and subsequent formation of a green phosphate/Mo (V) complex at acidic pH. The extracts (25-200 g/ml) were mixed with 3 ml of the reagent solution (0.6 M H_2SO_4, 28 mM sodium phosphate and 4 mM

ammonium molybdate). The tubes were incubated at 95 °C for 90mins. The mixture was cooled to room temperature and the absorbance of the solution was measured at 695 nm.

Results

The present study revealed that the aqueous extract of shell of *T. indica* contained some secondary metabolites including alkaloids, phenols, glycosides, terpenoids, flavonoids, tannins, saponins and quinones as shown in Table 1. Carbohydrate, proteins, amino acids, lipids, and steroids were not detectable at the tested assay condition.

Table 1. Results for different phytochemicals tests in *T. indica* obtained by simple soaking method

Sr. No.	**Chemical Constituent**	**Aqueous Extract**
1	Alkaloids	+
2	Phenol	+
3	Glycosides	+
4	Terpenoids	+
5	Flavanoids	+
6	Tannins	+
7	Saponins	+
8	Quinines	+
9	Carbohydrates	-
10	Proteins	-
11	Amino acids	-
12	Lipids	-
13	Steroid	-

+ = Present - =Not detected

Ultra-sonication

The 200 g of powder was soaked in 1.2 litter of ethanol for 45min and 60min at room temperature, the yield was given in both cases the activity was extremely high for extract obtained from ultra-sonication method. In the Table 2, the extract obtained from 45min and 60min were furthers processed to fraction for yield, RSD and W/W activity, all of them were shown significant results.

Table 2. The w/w yield is obtained by taking mean of triplicate of value by ultra-sonication method

		***T. indica* seed**			***T. indica* pulp**		
		Yield(g)	RSD%	w/w%	Yield(g)	RSD%	w/w%
Crude extracts	45 minutes	1.02	5.33	0.65	0.44	4.66	0.52
Ultra-sonication	60 minutes	3.21	3.77	1.71	2.89	3.61	1.51

Comparison of DPPH radical scavenging activity of T. indica seed fraction and pulp fraction by soaking method

The DPPH activity of *T. indica* seed and pulp of different fraction by soaking method was compared. it was observed that activity of seed is maximum on 300 µg/ml of butanol fraction as compared to pulp butanol fraction were shown in Figure 1.

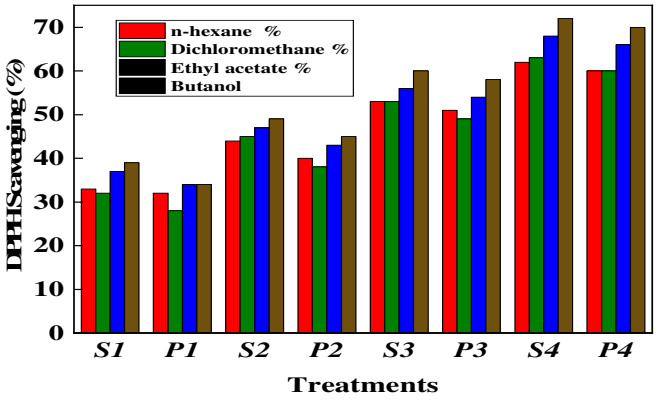

Figure 1. Comparison of DPPH radical scavenging activity of *T. indica* seed fraction and pulp fraction by soaking methods
Where S1=seed 37.5, P1=pulp 37.5, S2=seed75, P2=pulp75, S3=seed150, P3=pulp150, S4=seed300, P4=pulp300

Comparison of ABTS radical scavenging activity of T. indica seed and pulp by ultrasonic method.

The ABTS activity of *T. indica* for seed and pulp of different fraction by ultrasonic method was compared. It was observed that activity of seed is maximum on 250µg/ml of butanol fraction as compare to pulp fraction as shown in Figure 2.

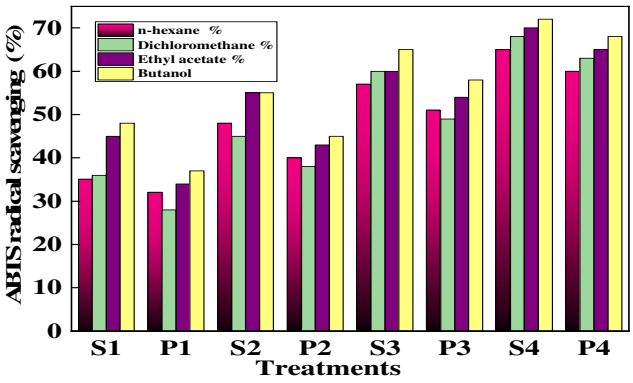

Figure 2. Comparison of DPPH radical scavenging activity of *T. indica* seed and pulp by ultrasonic method
Where S1=seed 37.5, P1=pulp 37.5, S2=seed75, P2=pulp75, S3=seed150, P3=pulp150, S4=seed300, P4=pulp300

Comparison of metal chelating activity of T. indica seed and pulp by soaking method

The chelating activity of *T. indica* seed and pulp of different fraction by soaking method was compared. It was observed that activity of pulp was maximum on 250µg/ml of butanol fraction as compare to seed on 250µg/ml as shown in Figure 3.

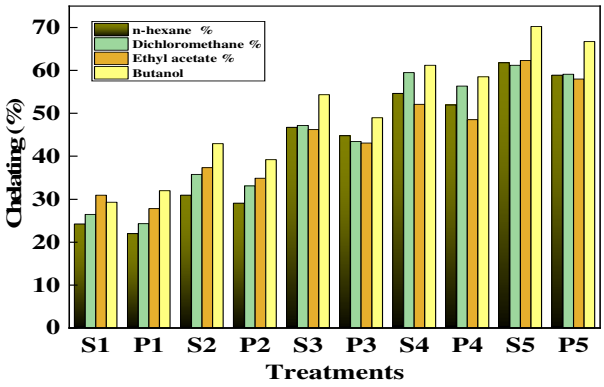

Figure 3. Comparison of metal chelating activity of *T. indica* seed and pulp by soaking method Where S1=seed25, P1=pulp25, S2=seed50, P2=pulp50, S3=seed100, P3=pulp100, S4=seed200, P4=pulp200, S5= seed250, P5=pulp250

Comparison of metal chelating activity of T. indica seed and pulp by ultrasonic method

The Chelating activity of *T. indica* seed and pulp of different fraction by sultrasonic method was compared. It was observed that activity of pulp was maximum on 250 µg/ml of butanol fraction as compare to seed on 250 µg/ml as shown in Figure 4.

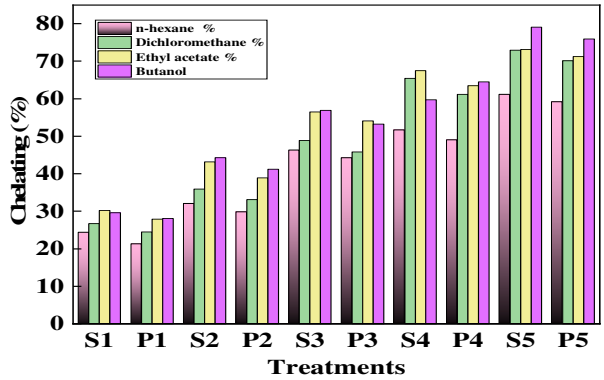

Figure 4. Comparison of Metal Chelating activity of *T. indica* seed and pulp by ultrasonic method Where S1=seed25, P1=pulp25, S2=seed50, P2=pulp50, S3=seed100, P3=pulp100, S4=seed200, P4=pulp200, S5= seed250, P5=pulp250

Comparison of TBARS inhibition in egg yolk by rhizome extract of T. indica seed and pulp with $FeSO_4$

The TBARS inhibition of *T. indica* seed and pulp with $FeSO_4$ with different fraction by soaking method was compared. It was observed that TBARS inhibition mole/g tissue of pulp was maximum on 90µg/ml of butanol fraction as compare to seed on 90µg/ml of butanol as shown in Figure 5.

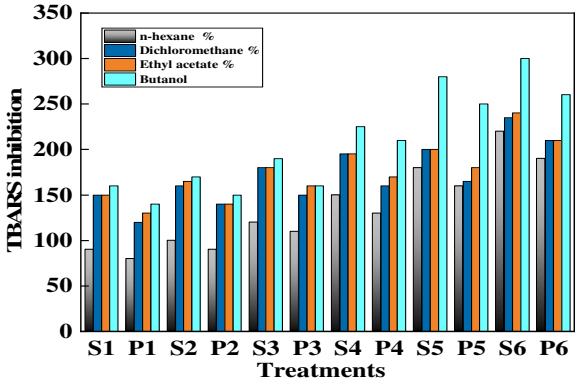

Figure 5. Comparison of TBARS inhibition in egg yolk by rhizome extract of *T. indica* seed and pulp with $FeSO_4$

Where S1=seed control, P1=pulp control, S2=seed15, P2=pulp15, S3=seed30, P3=pulp30, S4=seed60, P4=pulp60, S5= seed90, P5=pulp90, S6= seed120, P6=pulp120

Comparison of TBARS inhibition in egg yolk by rhizome extract of T. indica seed and pulp with SNP

The TBARS inhibition of *T. indica* seed and pulp with SNP of different fraction by soaking method was compared. It was observed that TBARS inhibition n mole/g tissue of pulp was maximum on 90µg/ml of butanol fraction as compare to seed on 90µg/ml of butanol as shown in Figure 6.

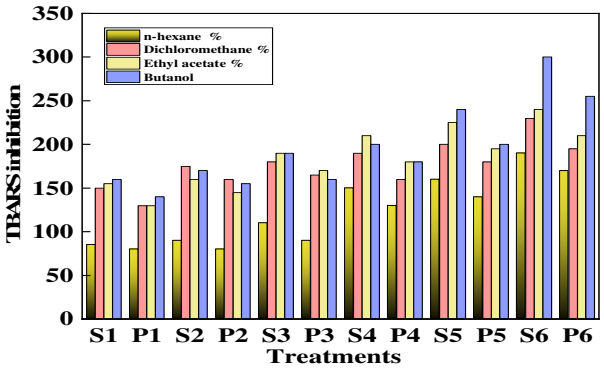

Figure 6. Comparison of TBARS inhibition in egg yolk by rhizome extract of *T. indica* seed and pulp with SNP

Where S1=seed control, P1=pulp control, S2=seed15, P2=pulp15, S3=seed30, P3=pulp30, S4=seed60, P4=pulp60, S5= seed90, P5=pulp90, S6= seed120, P6=pulp120

Comparison of total anti-oxidant activity of T. indica seed and pulp extract

It was observed that antioxidant activity for seed and pulp extract comparison was maximum on 120µg/ml of butanol fraction for seed as compare to pulp on 120 µg/ml of butanol as shown in Figure 7.

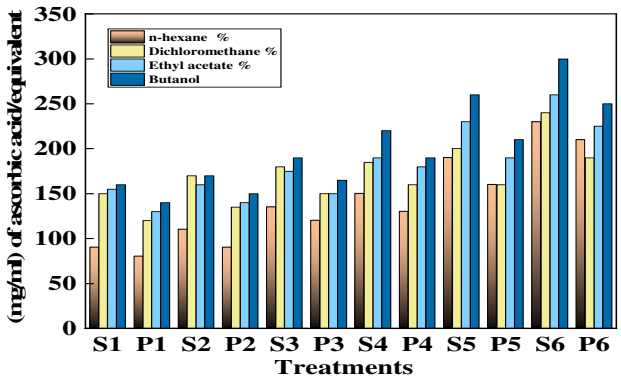

Figure 7. Comparison of total anti-oxidant activity of *T. indica* seed and pulp extract
Where S1=seed control, P1=pulp control, S2=seed15, P2=pulp15, S3=seed30, P3=pulp30, S4=seed60, P4=pulp60, S5= seed90, P5=pulp90, S6= seed120, P6=pulp120

Comparison of total phenolic and flavonoid content of T. indica seed and pulp extract

It was shown in Figure 8 that the comparison of flavonoid and phenolic contents present in both ethyl acetate and butanol fractions for pulp and seed. It was noticed that both the flavonoid and phenolic contents present in the fractions were grater for seeds as compared to the pulp.

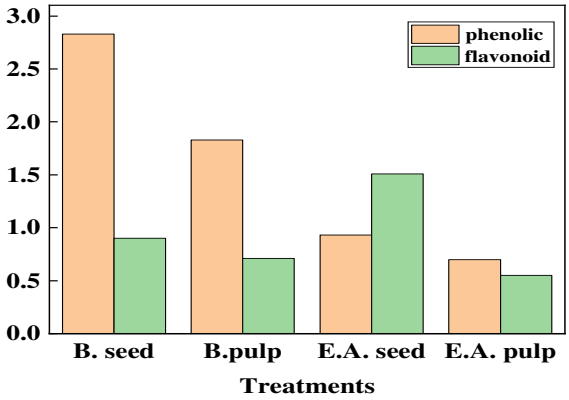

Figure 8. Comparison of total phenolic and flavonoid of *T. indica* seed and pulp extract
Where, B. seed= butanol fraction for seed, B. pulp= butanol fraction for pulp, E.A. seed = ethylene acetate fraction for seed, and E.A. pulp= ethylene acetate fraction for pulp.

Discussion

The present study revealed that the aqueous extract of shell of *T. indica* contained some secondary metabolites including alkaloids, phenols, glycosides, terpenoids, flavonoids, tannins, saponins and quinones as shown in Table 1. Carbohydrate, proteins, amino acids and lipids were not detectable at the tested assay condition. The related study was conducted with the extract of seed and pulp of *T. indica* and reported the presence of some of secondary metabolites. Antibacterial activity in the leaves of Tamarind which was attributed to the presence of flavonoids, alkaloids, tannins, cyanogenic glycosides and anthroquinones (Escalona-Arranz*et al*., 2010). The phytochemical screening demonstrated the presence of different types of compounds like alkaloids, flavonoids and steroids which could be responsible for the antibacterial activities.

For both plant extracts and for pure compounds DPPH assay can apply (Kedare and Singh, 2011). The DPPH activity of *T. indica* seed and pulp of different fraction by soaking method was compared. it was observed that activity of seed was maximum on 300 µg/ml of butanol fraction as compared to pulp butanol fraction were shown in Figures 1-2, by simple soaking as well as ultrasonic method. Same results for seed and pulp activity were also shown at 300 µg/ml of butanol fraction for tamarind (Waqas *et al*., 2013).

When metal like iron increases in our body than it may be toxic. Oxidative stress in liver usually associated with the iron Fe (II) content. So, for the Fe (II) management a popular therapy is used which is called iron chelation. When Fe^{2+} and phenanthroline joins than complex formed. But because of existence of chelating agent complex formation is disrupted. Color reduction shows the chelating capacity of the chelating agent. In Figures 3-4, it was shown that the metal chelating activity for seed was greater at 250 µg/ml as compare to pulp for both simple soaking and ultra-sonication**.** In another research a tamarind leaf fluid extract (TFE) wholly characterized was evaluated for its anti-DPPH activity (IC50 = 44.36 µg/mL) and its reducing power activity (IC50 = 60.87 µg/mL) (Escalona-Arranz *et al*., 2015).

Through the concept of oxidative stress, the relationship between disease and free radical can be determined. In normal and healthy body of human, RNS and ROS produced effectively. But due to harmful environmental and pathogenic agents such as ultraviolet rays, over nutrition, toxic chemicals, cigarette smoking and radiation etc. results in oxidative stress. It may cause many human diseases. Due to this free radical attack, every biological molecule which is found in body, is in risk. These destroyed molecules involve in cell death and results in disease start. Free radical damage the lipid which is present in organelles. Lipid peroxidation (LP) chain reaction forms, when free radical and lipid react with each other. This chain results in both indirect and direct effects. More than two hundred diseases caused by oxidative stress. Aging process also linked with oxidative stress (Aengwanich and Suttajit, 2012). Our results showed high performance for seed at control than pulp in both cases when we used TBARS inhibition in egg yolk by rhizome extract of *T. indica* seeds with $FeSO_4$ as well as TBARS inhibition in egg yolk by rhizome extract of *T. indica* seeds with SNP as shown in Figures 5-6. Similarly, in another document *T. indica* seed coat (TSCE) at 50 mg/kg (as tannic acid equivalents) followed by CCl_4 treatment, caused restoration of superoxide dismutase, catalase and lipid peroxidation to values close to control while peroxidase was restored to 67 % of the control (Sandesh *et al*., 2014).

Phosphomolybdenum assay measure the reduction of the phosphate-molybdenum (VI) into phosphate-molybdenum (V). It is quantitative method used to examine the rate of reduction reaction among oxidant, molybdenum ligand and antioxidant. PM assay describes the antioxidant reduction capacity (Phatak and Handre, 2014). Presence of antioxidant in the extract describe by incubation of 56 extract with the MO (VI). This can be assessed by taking the absorbance, at 695nm. At 695nm reduced green color complex was detected. In this research paper comparison of anti-oxidant activity of *T. indica* seed and pulp extract was estimated as shown in Figure 7 the seed extract on 120µg/ml of butanol fraction shown high value than the pulp extract. Different studies were carried out by comparing *Kalanchoe pinnata* extract with antioxidant references such as gallic acid; ascorbic acid. All these antioxidant activities increased with increasing concentrations in a dose dependent manner (Phatak and Handre, 2014).

Phytochemical analysis revealed that plant extract of *T. indica* seeds have high amount of phenolic content (Dey and De, 2014), as well as flavonoid content (Muanda *et al*., 2011). Presence of these contents contribute in extracts antioxidant activity. Plants are main source of bioactive compounds like antioxidants and secondary metabolites. In plants more frequent secondary metabolites are phenolic compounds are present. These compounds have important role in reproduction and growth of plants. They have different activities like anti-allergic, anti-inflammatory, anti-carcinogenic, antiviral and antioxidant. Total phenolics (Folin–Ciocalteu assay) and flavonoid (aluminum chloride assay was determined by total seed powder) were increased and values are given as for total phenolic content (6.84 ± 0.21 to 88.44 ± 0.8 mg GAE/100 mL) and for flavonoid (4.64 ± 0.03–21.7 ± 0.36 mg CE/100 mL), (Natukunda *et al*., 2015; Yadav *et al*., 2016).

Conclusions

This study was conducted to examine the antioxidant, phytochemical, and xanthine oxidase inhibitory effect of *T. indica*. This medicinal plant is used in many parts of the world for health purposes. To check the antioxidant potential of the plant extract different methods were used. Most effective method for the scavenging of free radicals is DPPH assay. Scavenging capacity of plant was shown by DPPH assay. To check antioxidant potential of plant ABTS assay is also used, better results were obtained. To check the binding ability of plant extract with iron, metal chelation activity was performed and this activity showed significant results. To check the peroxidation by lipid peroxidation method TBARS assay was used. This assay was performed by using per-oxidants like Sodium nitro-pruside and iron sulphate. Phenolic and flavonoid content was also detected in plant. To check the total antioxidant activity phosphomolybdenum assay was performed, which showed positive and significant results. These activities are due to the presence of high phenolic and flavonoid content. So, this study revealed that *T. indica* have various beneficial effect such as in food and pharmaceutical fields.

Authors' Contributions

All authors contributed equally in this research article. All authors read and approved the final manuscript.

Ethical approval (for researches involving animals or humans)

Not applicable.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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