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Research article

In Vitro Antioxidant Activity of Fresh and Shade Dried *Tamarindus Indica* Leaves using Different Solvents

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Keywords: *Tamarindus indica*, antioxidant activity, DPPH assay method, Folin Ciocalten method.

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Abstract

Objective: The present study was aimed to ascertain the antioxidant status of different solvent leaf extracts of *Tamarindus indica leaves* and its potential for scavenging free radical species in the body and by the determination of their total phenolics content.
Methods: The different solvent plant extract was screened for possible antioxidant activities by the determination of total phenolic content by using Folin – Ciocalten reagent method and 2, 2diphenyl 1 picrylhydrazyl (DPPH) free radical scavenging assay.
Results: The results of this study revealed that, the percentage inhibition of different extracts of water (WE) and hydroalcoholic (HAE) of *Tamarindus indica* was concentration dependent with an effective concentration at fifty percent of 79.49 µg/ml and 82.96 µg/ml compared to that of standard with IC 50 of 77.08 µg/ml, but the petroleum ether (PEE) and chloroform (CE) extracts revealed a very poor antioxidant activity (significantly lower IC 50 values = 144.17µg/ml and 279.74µ/ml).
Conclusion: The antioxidant screening results indicate that exciting DPPH radical scavenging activity was observed in water and hydroalcoholic leaf extract of *Tamarindus indica* in comparison with standard ascorbic acid. This suggests that *T. indica* extracts exhibit a great potential for antioxidant activity and may be useful for their future nutritional and medicinal developments.

Introduction

Natural products especially which are derived from plants have been used as for various therapeutic processes for long times from the human civilization. The most common strategy of drug development from plants is careful observation of use of natural resources in folk medicine in different cultures by ethnopharmacology [1]. In the recent trend, herbal drugs are prescribed abundantly in modern therapy because of the great deal of safety and less toxicity profiles [2].

The free radical induced oxidative stress is a main risk in the numerous human chronic diseases such as diabetes, ischemic heart disease, atherosclerosis, aging and immune suppression [3].

An antioxidant is widely employed substance in order to inhibit or it can delay the chain reaction in a cell due the formation of free radicals, which causes the oxidative damage or death to the target cell [4-8]. Oxidation is a

chemical reaction that transfers electrons or hydrogen from substances to an oxidizing agent. Antioxidants terminate these chain reactions by removing free radical intermediates and inhibit other oxidative reactions. Antioxidants are often reducing agents such as thiols, ascorbic acid or polyphenols. The term antioxidant has been defined in a number of ways like substances that in small quantities are able to preventor greatly retard the oxidation of easily oxidizable materials, or any substance when present in low concentrations compared to those of an oxidizable substrate significantly delays or prevents oxidation of those substances [9-10].

Aging and different chronic diseases including diabetes, cancer and cardiovascular diseases could be caused by oxidative stress. Oxidative stress can arise from the excessive formation of free radicals. Antioxidant constituents of the plant material act as radical scavengers, and help in converting the radicals to less reactive species [11]. It gives a broad information about the bioactive constituents and scientifically claimed medicinal uses of T indica. It possesses large range of medicinal application in human health care it also possesses large amount of vitamin B and vitamin C which is responsible for enhancement of immune system [12-13]. Several, carbohydrate, fat, protein and tannins, acids, minerals have been reported to be present in different part of T. indica. The plant shows various type of antidiabetic, hypolipidemic, antioxidant, hepatoprotective which may be due to presence of the investigated active chemical constituents. It also used as flavoring agent to impart flavor to various dishes and beverages a impart flavor to pharmacological studies so far have been performed in both vitro and vivo. Therefore, there is need for investigation and qualification of different phytoconstituents present and its pharmacological profile [14-15].

The polyphenols in tamarind have antioxidant and anti-inflammatory properties. These can protect against diseases such as heart disease, cancer and diabetes. The seed extract may also help lower blood sugar, while the pulp extract may help you lose body weight and reverse fatty liver disease. Therefore, there is need to study more detailing on tamarind leaves constituents and to know about the extract concentration obtained by using different solvent and their variation in antioxidant activity.

Materials and methods

Sample collection

The fresh leaves of plant, *Tamarindus indica* was collected from the local areas of Wayanad, Kerala in the mid of February 2020on the basis of its wide variety of uses in traditional medicinal history. The plant species was confirmed by a botanist Dr. Deena Meria Jose, Assistant professor & Head, Department of Botany, Providence Women's College, Kozhikode, Kerala (Accession No. 980). The plant species name, family, parts used, traditional uses, solvents used and extract yield was shown in table 1.

Preparation of plant extracts

The collected leaves of plant (*Tamarindus indica*) were firstly thoroughly sieved to remove the unwanted course particles washed with distilled water to remove dirt and it is air dried in a shade area in laboratory at room temperature for 2 weeks. The fresh and dried leaves were then crushed to course powder in a grinder. Then the fixed amount of powder is weighed (20g) and extracted by using various solvents such as Petroleum ether (PEE), Chloroform (CE), Hydroalcoholic (HAE) (7:3) [16]. The 20g of course powder was dissolved in 250ml of distilled water (WE) and kept in room temperature for three successive for maceration process [17]. The resulted extract was filtered through Whatman filter paper no. 4. All the filtrate was collected in a porcelain dish and condensed at 60°C in a rotary evaporator, then dried under vacuum to yield the concentrated crude extract. The concentrated extracts were stored in a refrigerator at -4°C until use.

Percentage yield = Dry weight of plant extract / Dry weight of plant material X 100

The different solvent leaf extracts were subjected to qualitative chemical tests for the detection of various plant constituents like carbohydrates, glycosides, flavonoids, phenolic compounds, tannins, steroids, saponins, coumarins, phytosterols, alkaloids, carbohydrates and triterpenoids [18].

Screening of antioxidant activity (DPPH assay)

The molecule 1, 1-diphenyl-2-picrylhydrazyl (a,a-diphenyl-bpicrylhydrazyl; DPPH) is characterized as a stable free radical by virtue of the delocalization of the spare electron over the molecule as a whole, so that the molecule does not dimerize, as would be the case with most other free radicals. The delocalization of electron also gives rise to the deep violet colour, characterized by an absorption band in ethanol solution centered at about 517 nm. When a solution of DPPH is mixed with that of a substrate that can donate a hydrogen atom, then this gives rise to the reduced form with the loss of this violet colour. In order to evaluate the antioxidant potential through free radical scavenging by the test samples, the change in optical density of DPPH radicals is monitored [19-20].

The ability of scavenging activity of DPPH free radicals by the different solvent plant extract was followed by the procedure, Mahbubur Rahman *et al.*, [21] the different solvent plant extract (1.6 mL) is diluted with methanol at different concentration (12.5 – 150 µg/ml) and 2.4 mL of DPPH solution (0.1 mM) is added. The reaction mixture was mixed thoroughly and left in dark place for 30 minutes at room temperature, the absorbance of mixture was measured spectrophotometrically at 517 nm. Ascorbic acid was used as standard. The percentage of the DPPH radical scavenging is calculated using the equation as given below:

% inhibition of DPPH radical scavenging activity = $\{(A_0 - A_1) / A_0\} \times 100$

where A_0 is the absorbance of control and A_1 is the absorbance after reaction has taken place in samples.
The percentage inhibition was plotted against concentration and IC_{50} was calculated [22].

Folin – Ciocalteu method

Total phenolics were determined using Folin – Ciocalteu reagent (FCR) spectrophotometric method with some modification as described by Badakhshan *et al* [23]. Folin – ciocalteu reagent consists of a yellow acidic solution containing complex polymeric ions formed from phosphomolybdic and phosphotungsticheteropoly acids. Dissociation of a phenolic proton in a basic medium leads to a phenolate anion, which reduces FCR forming blue colored molybdenum oxide. The colour intensity is directly proportional to the phenolic contents [24]. The stock solution (gallic acid – 5mg/ml) was added into 100ml volumetric flasks and then diluted to 1, 2, 3, 5 and 10ml to volume with water. The same procedure was followed for the different solvent leaf extract of *Tamarindus indica* in concentrations: 0.1 mg/ml, 0.5 mg/ml and 1.0 mg/ml. The test was done thrice. From each diluted solution, 0.25 ml was mixed with 1.25 ml (diluted 10-fold in distilled water) and allow to stand for 5 min. Then add 1 ml 7.5% sodium carbonate solution. Allow to stand for 1 hour for reaction in room temperature and the absorbance at 760 nm was determined by spectrophotometrically [25].

Results

The extraction was carried out as per procedure and the plant species name, family, parts used, traditional uses, solvents used and extract yield was shown in table 1.

The results of preliminary phytochemical studies were reported as per the procedure of phytochemical screening tests. The phytochemical studies of different solvent leaf extracts of *Tamarindus indica* showed the presence of phytoconstituents such ascarbohydrates, alkaloids, glycosides, phytosterols, flavonoids, phenolic compounds, tannins, coumarins, steroids and terpenoids and the isolated compound showed the presence of carbohydrate and glycoside.
The results of preliminary phytochemical studies were reported as per the procedure of phytochemical screening tests. The phytochemical studies of different solvent leaf extracts of *Tamarindus indica* showed the presence of phytoconstituents such as carbohydrates, alkaloids, glycosides, phytosterols, flavonoids, polyphenolic compounds, tannins, coumarins, steroids and terpenoids. The polyphenols present in the leaf extracts may be show the antioxidant property.

Determination of quantitative scavenging assay of 1, 1-diphenyl-2-picrylhydrazyl (DPPH) radical

The DPPH radical scavenging activity results are shown in table 2, figure 1 and figure 2 by comparing with the standard drug Ascorbic acid. The ability of plant extracts in different solvents to scavenge DPPH was investigated at various concentration of the extract. From the observed results the percentage inhibition of different extracts of water and hydroalcoholic of *Tamarindus indica* was concentration dependent with an effective concentration at fifty percent of 79.49 µg/ml and 82.96 µg/ml compared to that of standard with IC 50 of 77.08 µg/ml, but the petroleum ether and chloroform extracts revealed a very poor antioxidant activity (significantly lower IC 50 values = 144.17µg/ml and 279.74µ/ml).

Table 1. The ethnobotanical data of employed plant species and their extract percentage yield.

Plant species	Family	Plant part used	Traditional uses	Solvents used	Extract yield (100%)
Tamarindus indica	Fabaceae	Leaves	Used to treat diarrhea, constipation, fever and peptic ulcer	Petroleum ether Chloroform Hydroalcoholic Water (Maceration)	9.74% 3.12% 6.54% 5.26%

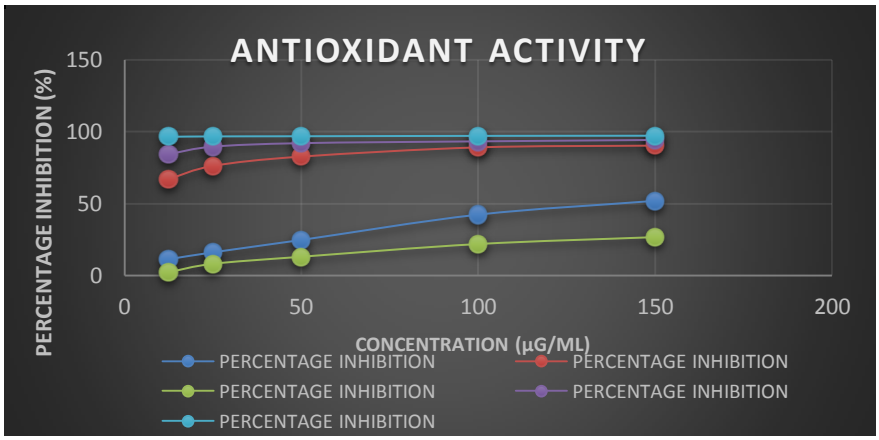


Figure1. Percentage inhibition of DPPH scavenging effect vs.concentration of *T. indica* extracts and standard.

Table 2. The IC 50 values of DPPH scavenging effect of *Tamarindus indica* extracts (µg/ml).

No	Sample in µg/ml	DPPH ASSAY		
		OD	Percentage inhibition (%)	IC_{50}
	Control 0.00	0.813		
1	PEE 1 -12.5	0.720	11.43	
2	PEE 1– 25	0.681	16.23	
3	PEE 1 – 50	0.611	24.84	144.17
4	PEE 1 – 100	0.468	42.43	
5	PEE 1 – 150	0.390	52.02	
1	HAE2– 12.5	0.267	67.15	
2	HAE2– 25	0.193	76.26	
3	HAE2 -50	0.140	82.77	82.96
4	HAE2- 100	0.088	89.17	
5	HAE 2 – 150	0.078	90.40	
1	CE3– 12.5	0.793	2.46	
2	CE3– 25	0.746	8.24	
3	CE3– 50	0.706	13.16	279.74
4	CE3– 100	0.634	22.01	
5	CE 3 – 150	0.595	26.81	
1	WE 4 -12.5	0.126	84.50	
2	WE 4– 25	0.084	89.66	
3	WE 4 – 50	0.064	92.12	79.49
4	WE 4 – 100	0.054	93.35	
5	WE 4 – 150	0.046	94.34	
	Standard	Ascorbic acid		
1	STD – 12.5	0.028	96.55	
2	STD – 25	0.026	96.80	97.29
3	STD– 50	0.025	96.92	
4	STD –100	0.023	97.17	
5	STD – 150	0.022	97.29	

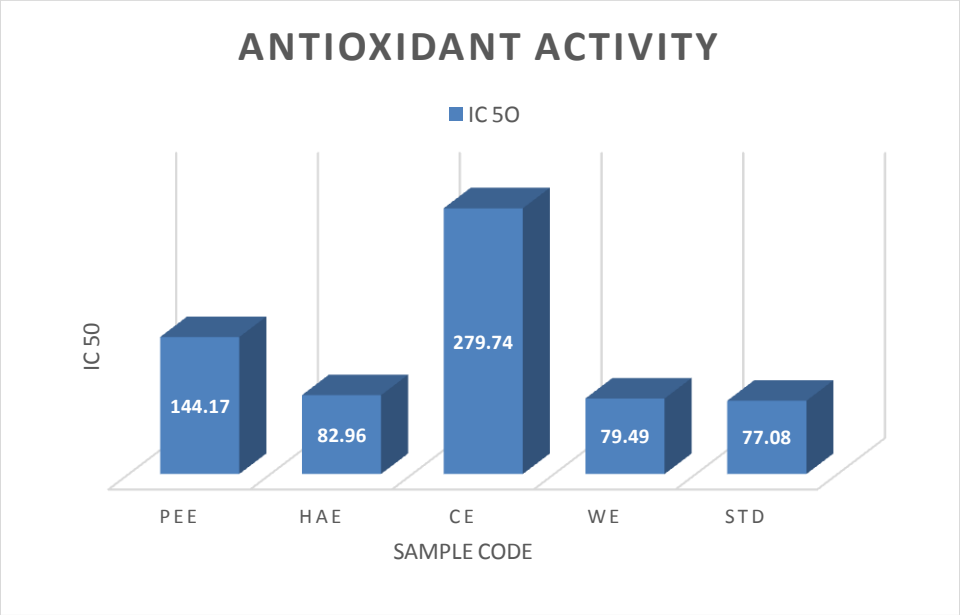


Figure 2. IC_{50} values of DPPH scavenging effect vs final concentration of *T. indica* extracts and Ascorbic acid.

Determination of total phenolic contents of leaf extracts of *Tamarindus indica*

Table 3. Shows the total polyphenols contents in different solvent extracts of PEE, HAE, WE, and CE expressed as GAE.

Polyphenols	PEE	HAE	WE	CE
Phenolics	56.19±1.12	12.85±0.523	12.71±0.974	16.93±0.890

Expressed in terms of GAE, respectively (mg of GA/g of dry extract)
Each value is the average of three analyses ± standard deviation.

Discussion

Antioxidants are an important compound which possess the ability to prevent the damage of the cells from free radical oxidative stress in the body. The antioxidant potential activity of *Tamarindus indica* leaf extract was investigated for a newer biologically active compound. From the results it is clear that aqueous extract of *Tamarindus indica* present the highest antioxidant activity compared with reference. The DPPH radical scavenging activity results are shown in table 2, Figure 1 and figure 2 by comparing with the standard drug Ascorbic acid. The ability of different leaf extracts (PEE, HAE, WE & CE) to scavenge DPPH was investigated at various concentration of the solution. From the observed results the percentage inhibition of water leaf extract (WE) was concentration dependent with an effective concentration at fifty percent of 79.49 µg/ml compared to that of standard with IC 50 of 77.08 µg/ml, the results revealed the values were also remarkably excellent for the aqueous extract (WE) , then the Hydroalcoholic leaf extract shows comparable good result with the IC 50 value 82.96 µg/ml, but in the case of petroleum ether and chloroform leaf extract revealed a very poor antioxidant activity (significantly lower IC 50 values = 144. 17µg/ml and 279.74µ/ml).

Total phenolic content of the extractives showed significant and strong positive correlation (p value ≤ 0.001) with free radical scavenging efficiencies. The polyphenolic constituents of the leaf extracts of *Tamarindus indica* the major contributes to the antioxidant activity in free radical neutralization. The plant polyphenols act as a reducing agent and antioxidants by the hydrogen donating property of their hydroxyl groups.

Conclusions

In conclusion, we have achieved a convenient method for the extraction of leaves of *Tamarindus indica* by using different solvents in good yield and evaluated in vitro antioxidant activity by using DPPH radical scavenger assay and total phenolic contents of leaf extracts by Folin – Ciocalteu method. Our antioxidant screening results indicate that exciting DPPH radical scavenging activity was observed in aqueous extract in comparison with standard ascorbic acid. Since this study used in vitro approach there is compelling need to check the in vivo antioxidant efficacy of the plant for better medicinal knowledge and utilization of plant for further studies.

Conflict of Interest
The authors declare that there are no conflicts of interest.

Authors Contribution
All the authors have contributed equally in designing, drafting the manuscript as per the journal submission format. All authors read and approved the final manuscript.

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