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Phytochemical screening and anti-microbial and anti-oxidant studies of dehydrated tender tamarind (*Tamarindus indica*) leaves

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Abstract
Tamarind (*Tamarindus indica)* is a multipurpose tropical tree used for the eatable properties of its fruit and leaves. Both parts of the plant have medicinal uses and are known to be used in folk medicine. This work is mainly concerned with the identification of the preservative properties of *Tamarindus indica*. The ethanolic extract of tender tamarind dried leaves was used for its anti-oxidant and antimicrobial activity. The phytochemical constituents of the dried powdered plant parts were extracted using aqueous and organic solvents (acetone and ethanol). The antimicrobial activity of the concentrated extracts was evaluated by determination of the diameter of zone of inhibition against both gram negative and gram positive bacteria using the paper disc diffusion method. Results of the phytochemical studies revealed the presence of tannins, saponins, sesquiterpenes, alkaloids and tri terpinoidal saponins and the extracts were active against both gram positive and gram negative bacteria. The extracts were evaluated for their antimicrobial activity against pathogenic bacteria such as *Clostridium botulinum*, *Pseudomonas, salmonella and Klebsiella.* which can be used as an alternative source of artificial antimicrobials in food. The ethanolic extracts of the leaves were also tested for its antioxidant activity against BHT an artificial antioxidant for its use as an alternative source of artificial antioxidants to prevent lipid oxidation in foods.

Keywords: *tamarindus indica*, antioxidant activity, anti-microbial activity, phytochemical screening

Introduction
The tamarind is a long-lived, medium-growth, bushy tree, which attains a maximum crown height of 12 to 18 meters (39 to 59 ft). The crown has an irregular, vase-shaped outline of dense foliage [1]. The tree grows well in full sun in clay, loam, sandy, and acidic soil types, with a high resistance to drought and aerosol salt [2, 3]. Leaves are evergreen, bright green in color, elliptical ovular, arrangement is alternate, of the pinnately compound type, with pinnate venation and less than 5 cm (2.0 in) in length. The fruit is an indehiscent legume, sometimes called a pod, 12 to 15 cm (4.7 to 5.9 in) in length, with a hard, brown shell [8, 10]. The fruit has a fleshy, juicy, acidulous pulp. It is mature when the flesh is colored brown or reddish-brown. The tamarind is best described as sweet and sour in taste, and is high in tartaric acid, sugar, B vitamins and, oddly for a fruit, calcium. A 2002 diet control study where subjects were fed tamarind paste, concluded that: "tamarind intake is likely to help in delaying progression of skeletal fluorosis by enhancing urinary excretion of fluoride". Based on a 2012 human study, supplementation of tender tamarind leaves improved disturbances to carbohydrate, lipid and antioxidant metabolism caused by chronic fluoride intake. Flavonoids and polyphenols the metabolites found in leaves have recorded as antimicrobial agents in many other plants. Many studies have shown the antimicrobial activity of tamarind leaves against gram positive and negative bacteria. The present study was designed to evaluate the phytochemical, antimicrobial and antioxidant activity of dehydrated tender tamarind leaves extracts.

Material and Methods
Collection of Plant Materials
Fresh tender tamarind leaves were procured from the local market during season and dehydrated in a tray drier to a constant moisture level. The dried tender leaves were packed in polyethylene pouches and stored in a condition condition till further use.

Preparation of Leaf Extract of *Tamarindus indica*
The extraction of dried leaves of *Tamarindus indica* was carried out using known standard procedures. The dried leaves were dried in shade and powdered in a mechanical grinder. The powder (10.0 g) was initially defatted with ethyl alcohol by using a Soxhlet extractor for 72 hours at a temperature not exceeding the boiling point of the solvent. The extracts were filtered using Whatman filter paper (No.1) while hot, concentrated in vacuum under reduced pressure using rotary flask evaporator, and dried in a desiccator. The ethyl alcoholic extract yields a dark reddish residue weighing 4.50 g (45.0% w/w). This crude extracts of ethylalcohol was used for further investigation for potential of antimicrobial and antioxidant properties.

Preliminary Phytochemical Screening
The dried leaves extract were subjected to preliminary phytochemical testing to detect for the presence of different chemical groups of compounds. Air-dried and powdered plant material was screened for the presence of saponins, tannins,

alkaloids, flavonoids, triterpenoids, steroids, glycosides, anthraquinones, coumarin, saponins, gum, mucilage, carbohydrates, reducing sugars, starch, protein, and amino acids.

DPPH radical scavenging activity

The hydrogen atom or electron donation ability of the leaves was measured from the bleaching of the purple-colored methanol solution of 2, 2-diphenly-1-picrylhydrazyl (DPPH). This spectrophotometric assay uses the stable radical DPPH as a reagent (11). 1ml of various concentrations of the seed coat and leaves (25, 50, 75 and 100 µg/ml) was added to 4ml of 0.004 %(w/v) methanol solution of DPPH. After a 30 min incubation period at room temperature, the absorbance was read against blank at 517nm. The ability of MSF to scavenge DPPH radicals was calculated by the following equation

DPPH radical scavenging activity (%)
= [(A control – A sample) / A blank] × 100

Where A control is the absorbance of the control reaction (containing all reagents except the test compound) and A sample is the absorbance of the test compound. Tests were carried out in triplicate. IC 50 values for both seed coat and leaves and BHT were calculated by plotting a graph concentration vs. percent of scavenging activity. IC50 value denotes the concentration of the seed coat and leaves which is required to scavenge 50% of DPPH free radicals.

Test Microorganisms and Growth Media

Clostridium botulinum (ATCC 3502) and Pseudomonas aeruginosa (ATCC 27853) salmonella (ATCC 14028) and klebsiela (ATCC 700603) were chosen. The bacterial strains obtained from Department of Microbiology, Osmania University, were used for evaluating antimicrobial activity. The bacterial stock cultures were incubated for 24 hours at 37°C on nutrient agar medium, following refrigeration storage at 4°C. The bacterial strains were grown in Mueller-Hinton agar (MHA) plates at 37°C (the bacteria were grown in the nutrient broth at 37°C and maintained on nutrient agar slants at 4°C. The stock cultures were maintained at 4°C.

Antimicrobial Activity

Whatman No: 1 filter paper discs of 6mm diameter are prepared and autoclaved by keeping in a clean and dry Petri plate. The filter paper discs were soaked in plant extracts for 6 hours are taken as test material. After 6 hours the discs were shade dried. The concentrations of leaves extracts per disc are accounted for 0.1 grams/1ml. Subsequently they are carefully transferred to spread on cultured Petri plates. Filter paper discs immersed in ethanol, benzene, distilled water are prepared and used as control.

Medium for bacterial cultures

For testing bacterial cultures the above mentioned Nutrient Agar Medium was used. The medium was steamed for 30 min. neutralized at 37^0 c and steamed for half an hour and filtered.

Testing of antimicrobial activity

To test the antimicrobial activity on agar plates, LB agar medium was prepared as mentioned. The medium was sterilized at 121°c for 30 min's. The agar test plates were prepared by pouring about 15ml of the medium into 10cm Petri dishes under aseptic condition and left undisturbed for 2hrs to solidify the medium. 1ml of inocculum (containing suspension) of *Clostridium botulinum*, *Pseudomonas putida*, *Klebsiella pneumoniae and Salmonella* was poured to the respective plates separately containing solidified agar media. Six replicates were maintained. The prepared sterile whatman no :1 filter paper discs of 6mm diameter were impregnated with the extracts and shaken thoroughly and this test plates incubated for a period of 48 hrs in BOD at 37ºc for the development of inhibitory zones and the average of 2 independent readings for each organism in different extracts were recorded. The control Petri plates and also maintained above respective cultures

Measuring the diameter of inhibition zone

The inhibition zones were lead after 1 day at 37ºc for bacteria. The diameter of the inhibition zone was measured and recorded with the aid of plastic ruler. 7 paper discs placed in 1 Petri plate.

Table 1: Dried Leaves phytochemical screening

S. No.	Secondary metabolites	Hexane	Ethyl acetate	Ethanolic	Aqueous
1	Steroids	++	+	+	++
2	Triterpenes	-	+	+	-
3	Saponins	+	-	-	+
4	Tri terpinoidalsaponins	++	-	+	-
5	Alkaloids	+	++	+	++
6	Carbohydrates	+	+	++	+
7	Flavonoids	+	+	+	++
8	Tannins	+	+	+	+
9	Glycosides	++	++	+	+
10	Polyphenols	+	+	+	+

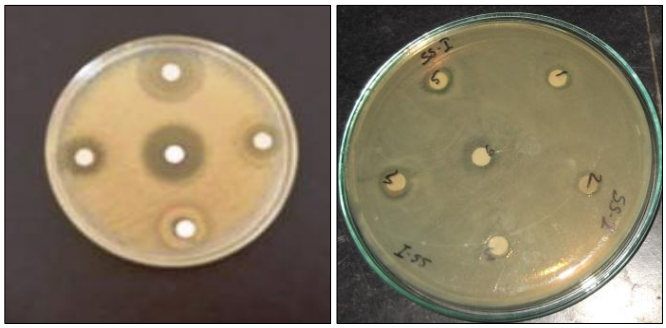
Table 2: Anti-Oxidant Property

S. No.	Concentration of sample µg/ml	Dried tender leaves % of inhibition	BHT % of inhibition
1	25	23.8	37
2	50	38	54
3	75	58.6	68
4	100	67.5	83

The antioxidant activity was determined by IC50. The antioxidant activity of dried leaves of IC 50 value were 63.99µg/ml

Table 3: Inhibitory activities of tender leaf extract of *Tamarindus indica*on microorganisms

Plants	Zone of inhibition			
	Clostridium botulinum (mm)	***Salmonella* Enterica (mm)**	**Pseudomonas *putida* (mm)**	***Klebsiela* Granulomatis (mm)**
Dried Leaves	5.0	4.0	3.0	3.2



Clostridium botulinum *Salmonella*

Pseudomonas *Klebsiella*

Fig 1: Anti-microbial studies of dried tender leaves of *Tamarindus indica* on *Clostridium botulinum, Salmonella, pseudomonas and klebsiella*

Conclusion

In the present study it was found that *Tamarindus indica tender* leaf extract has an excellent antimicrobial and antioxidant activity. The foodborne pathogenic bacteria were inhibited in presence of the dried leaf extracts of *Tamarindus indica.* The antioxidant activity of the tender leaves showed that it can be used in place of artificial antioxidants. Therefore future studies should be aimed to exploit this plant to be used as one of the best alternative source of natural antimicrobials and antioxidants.

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