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Phytochemical screening and anti-microbial and anti-oxidant studies of tamarind (*Tamarindus indica*) seed coat

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Abstract
Tamarindus indica L. of the family Fabaceae is a plant that is used in traditional medicine for the treatment of cold, fever, stomach disorder, diarrhea and jaundice and as skin cleanser. To evaluate the scientific basis for the use of the plant, the antimicrobial activities of extracts of the seed coat were evaluated against some common gram negative and gram positive bacteria and fungi. The study also investigated the chemical constituents of the plant and the effect of temperature and pH on its 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 which can be used as an alternative source of artificial antimicrobials in food. The ethanolic extracts of the seed coat 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
Plants remain the most common source of antimicrobial agents. Their usage as traditional health remedies is the most popular for 80% of world population in Asia, Latin America and Africa and is reported to have minimal side effects.1, 2. In recent years, pharmaceutical companies have spent a lot of time and money in developing natural products extracted from plants, to produce more cost effective remedies that are affordable to the population. The rising incidence in multidrug resistance amongst pathogenic microbes has further necessitated the need to search for newer antibiotic sources. Tamarindus indica (commonly called Tamarind), family Fabaceae, subfamily caesalpiniaceae is a tropical evergreen tree native to fertile areas throughout Africa and Southern Asia. It is widely cultivated as an ornamental tree and for its acidic fruits used in making drinks and a popular component of many decoctions used as health remedies. Because of its wide usage and availability, this study was set out to evaluate the phytochemical, antimicrobial and antioxidant activity of the seed coat, a by-product of the tamarind gum industry, which may have potential as a low cost source of antimicrobials and antioxidants and to determine the effect of temperature and pH on the efficacy of the plant as an antimicrobial agent.

Material and Methods
Collection of Plant Materials
Tamarind seeds were purchased from local people and dried in a tray drier to loosen the seed coat. The seed coat was collected and stored in a dry condition till further use.

Preparation of Seed coat Extract of *Tamarindus indica*
The extraction of seed coat of *Tamarindus indica* was carried out using known standard procedures. The seed coat 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 ethyl alcohol was used for further investigation for potential of antimicrobial properties.

Preliminary Phytochemical Screening
The seed coat extract were subjected to preliminary phytochemical testing to detect for the presence of different chemical groups of compounds. Air-dried and powder plant material was screened for the presence of saponins, tannins, alkaloids, flavonoids, triterpenoids, stroids, 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 seed coat 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 (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 samples 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 BHT were calculated by plotting a graph concentration vs. percent of scavenging activity. IC50 value denotes the concentration of the seed coat which is required to scavenge 50% of DPPH free radicals.

Test Microorganisms and Growth Media

Clostridium botulinum (ATCC 3502), Pseudomonas putida (ATCC 12633), 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, 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

Preparation of Discs

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 seed coat 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 above. 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* and *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 37 ºC 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 are also maintained above respective cultures

Measuring the diameter of inhibition zone

The inhibition zones were read after 1 day at 37ºc for bacteria. The diameter of the inhibition zone was measured and recorded with the aid of plastic ruler.

Table 1: phytochemical screening

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

Fig 2: Anti-Oxidant Property

S. NO	Concentration of sample µg/ml	Seed coat % of inhibition	BHT % of inhibition
1	25	31.2	37
2	50	42.6	54
3	75	64.8	68
4	100	74.9	83

The antioxidant activity was determined by IC50. The antioxidant activity of seed coat of IC 50 value were 58.68µg/ml

Antibacterial activity of seed coat

Plants	Zone of inhibition			
	Clostridium botulinum (mm)	*Salmonella* Enterica (mm)	Pseudomonas *putida* (mm)	*Klebsiela* Granulomatis (mm)
Seed Coat	2.2	3.0	1.0	4.0

Fig 1-4: Anti-microbial studies of seed coat of *Tamarindus indica*

Fig 1: Clostridium botulinum

Fig 2: Salmonella

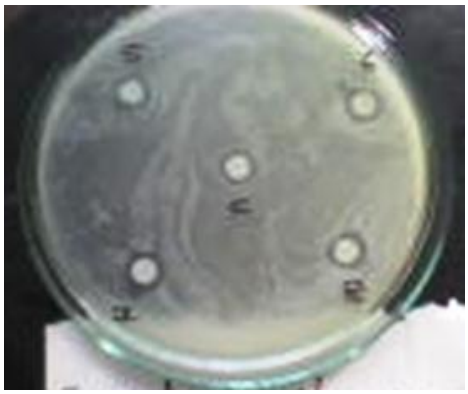

Fig 3: Pseudomonas

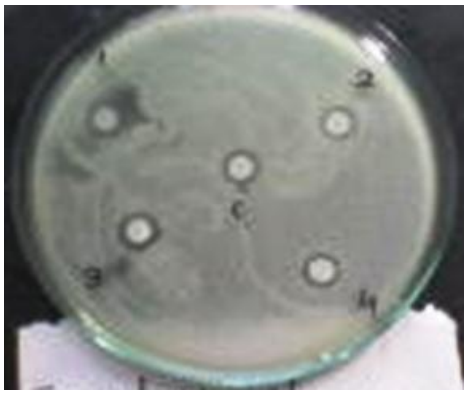

Fig 4: Klebsiella

Conclusion

In the present study it was found that *Tamarindus indica* seed coat extract has an excellent antimicrobial and antioxidant activity. The foodborne pathogenic bacteria were inhibited in presence of the dried seed coat extracts of *Tamarindus indica*. The antioxidant activity of the seed coat 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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