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Antioxidant and Antimicrobial Activity of *Tamarindus Indica L* Leaves

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A B S T R A C T

The aim of this study was to characterize the phytochemical composition and evaluation of antioxidant and antibacterial potentials of *Tamarindus indica* leaves in different *in vitro* assays.The antioxidant activity of different solvent extracts of leaves of *Tamarindus indica* were studied using FRAP assay, DPPH free radical scavenging activity and Superoxide peroxidase activity.Antibacterial activity against two Gram-positive (*Bacillus subtilis,*and, *Staphylococcus aureus*) and three Gram negative (*Klebsiella pneumonia,Proteus vulgaris and Escherichia coli*) bacterial species was investigated using agar well diffusion method. Significant activity was observed against the tested microorganisms. The results of antibacterial screening assaydisplayed broad spectrum antibacterial activity by *Tamarindus indica*.

Introduction:

Increased bacterial resistance to currently used antibiotics initiated search for new and innovative antimicrobial agents. This lead to the screening of several medicinal plants for their potential antimicrobial activity. Phytochemical constituents such as tannins, flavonoids, alkaloids and several other aromatic compounds are secondary metabolites of plants that serve as defense mechanisms against predation by many microorganisms, insects and herbivores[(1), (2)] Medicinal plants are therefore represent a rich source of antimicrobial agents and are also potential source of antioxidant compounds.. Oxidative stress generate reactive oxygen species which can damage important bio-molecules, leading to several disease conditions Antioxidants act by decreasing oxygen concentration, intercepting singlet oxygen, preventing first chain initiation by scavenging initial radicals, binding metal ion catalysts, decomposing primary products to non-radical compounds and chain breaking to prevent continued hydrogen abstraction from substrates. In vitro experiments on antioxidant compounds in higher plants help us to know how they protect against oxidation damage by inhibiting or quenching free radicals and reactive oxygen species. Therefore, it is very important to find out new sources of safe and inexpensive antioxidants of natural origin.

Material and Methods:

Plant collection:

Test Plant leaves were collected from two different areas of Visakhapatnam viz. S. Kota area and Akkayyapalem area. The taxonomic identity of test plants was confirmed by comparing with the authentic certified specimens at the Andhra University herbarium, department of Botany and Central National Herbarium Howrah. Fresh plant leaves were washed with tap water, air dried, homogenized to a fine powder and stored in air-tight bottles.

Microorganisms for determining anti-microbial activity:

Bacterial cultures of *Bacillus subtilis (B.subtilis), staphylococcus aureus (S. aureus, Klebsiellapneumonia (K.pneumonia), Proteus vulgaris (P.vulgaris),)and Escherichia coli (E.coli) were* obtained from Microbial Type Culture Collection Center (MTCC), Chandigarh, India. They were used as antimicrobial test organisms. These bacteria were maintained on nutrient broth at 37°c and were cultured on nutrient agar to perform identification tests.

Extraction Preparation:

The shade dried leaves were finely powdered and25 g of powder was filled in the thimble and extracted successively with aqueous, chloroform and ethyl alcohol solvents in Soxhlet extractor for 48h.The solvent extracts were concentrated under reduced pressure and preserved at 5°C in airtight bottle until further use.

Phytochemical screening:

The extracts were subjected to qualitative phytochemical analysis for the presence of various

constituents like alkaloids, glycosides, sapponins, phytosteroids and flavonoids.Chemical tests were carried out on the aqueous extract and on the powdered specimens using standard procedures to identify the phytoconstituents as described by [3], [4].

Antioxidant activity:

Diphenyl Picryl hydrazyl radical scavenging assay (DPPH):

The DPPH assay was carried out as described by Cuendet 1997[5]. 5.0ml of DPPH solution in methanol was added to 50µl of sample. After 30min of incubation period at room temperature, the absorbance was read against a blank sample and methanol at 517nm.Control containing the buffer and reagent was carried out. Similarly positive controls are treated in the same way as test sample except sample replaced by positive standard. Butylated hydroxy toluene (BHT) and Rutins were used as positive controls. The percent inhibition of DPPH radical is calculated by the formula A_o–A x 100/ A_o. Where, A_o is Absorbance of control and A is Absorbance of test sample. IC_{50} values denote the concentration of sample, which is required to scavenge 50% of DPPH free radicals.

Superoxide radical scavenging assay:

The assay of superoxide dismutase was carried by the method of Beauchamp and Fridovich 1971[6]. based on the reduction of Nitroblue tetrazolium (NBT). To 0.5ml of plant extract, 1ml of sodium carbonate, 0.4ml of NBT and 0.2ml of EDTA were added. The reaction was initiated by adding 0.4ml of Hydroxylamine hydrochloride. Zero time absorbance was taken at 560nm using Hitachi spectrophotometer, Germany, followed by recording the absorbance after 5min at 25^0 C. The control was simultaneously run without plant extract. Units of SOD were expressed as amount of enzyme required inhibiting the reduction of NBT by 50%. The specific activity was expressed in terms of units per milligram of protein.

Ferric reducing or antioxidant power assay (FRAP):

The total antioxidant power of the sample was assayed by the method of Berzie IFF and J.J.Strain 1996[7].3.0ml of FRAP working reagent was taken in a test tube then 100µl of plant extract was added, this is vortex mixed, and the absorbance was read at 593nm against a reagent blank at a predetermined time after sample–reagent mixture. The results are expressed as ascorbic acid equivalents (µ moles/ml) or FRAP units. An Ascorbic acid standard of 1000µM, is equivalent to 2000µM of antioxidant power as FRAP. For standard curve, aqueous Ascorbic acid solutions were prepared in the range from 100 to 1000µM (equivalent to 200 to 2000µM FRAP) prepared freshly.

Total phenolic compound analysis

The total phenolic content was determined by the spectrophotometric method. In brief, a 1 ml of sample (1 mg/ml) was mixed with 1 ml of Folin-Ciocalteu's phenol reagent. After 5 min, 10 ml of a 7% Na2CO3 solution was added to the mixture followed by the addition of 13 ml of deionized distilled water and mixed thoroughly. The mixture was kept in the dark for 90 min at 23°C, after which the absorbance was read at 750 nm. The TPC was determined from extrapolation of calibration curve which was made by preparing Gallic acid solution. The estimation of the phenolic compounds was carried out in triplicate. The TPC was expressed as milligrams of Gallic acid equivalents (GAE) per g of dried sample

Antibacterial activity of leaf extracts:

The antibacterial assay of aqueous, chloroform and ethanolic extracts was performed by agar well diffusion method. Mueller Hinton Agar media was poured into the Petri plate (Hi-Media), and inoculated with bacterial cultures. For the agar well diffusion method, a well was prepared in the plates with a cup-borer (0.85 cm) and 100 µl of the test compound was pipetted directly into the well. The plates were incubated overnight at 37°C. Antibacterial activity was determined by measuring the diameter of the zone of inhibition (mm) surrounding bacterial growth. The experiments were repeated three times and the mean values are presented.

Estimation of minimum inhibitory concentration of samples by using Well diffusion method:

300 ml of nutrient agar was adjusted to P^H 7. Sterilized in an autoclave cooled 45^0C and poured into labeled Petri plates under sterile conditions. The plates were allowed to solidify and inoculated with 100µl (microliters) bacterial culture by spread plate technique. The wells were loaded with the different diluted samples and incubated at 37^0C for 24-48 hours.

Estimation of minimum inhibitory concentration of samples by using Well diffusion method:

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Results and Discussion:

Phytochemical screening reveals that the major constituents of ethanolic extract of *Tamarindusindica* leaves were phytosteroids and glycosides; for aqueous extract phytosteroid, tannins and phenolics; for chloroform extract phytosteroids and tannins and phenols (Table 1).

The ethanolic leaf extract was found to have highest phenolic content 319±0.07 µg /ml GAE followed by 103±0.12 µg /ml GAE for chloroform extract and 68±0.07 µg /ml GAE for aqueous extract (Fig. 1). Phenolic compounds play significant role in antioxidant activity because of their unique structure. Phenolics have

aromatic rings bearing single or multiple hydroxyl groups which enable them to quench free radicals by forming resonance-stabilized phenoxyl radical[8], [9].The antioxidant activity was determined by FRAP assay and represented as 1300±0.07 FRAP units in µM. The DPPH radical is a widely used model to evaluate the antioxidant property of plant extracts[10]. The DPPH radical scavenging activity of the leaf extracts was highest for ethanolic extract 87.280±0.17 followed by 31.886±0.12 for chloroform extract and 28.215±0.15 for aqueous extract. The Super Oxide Scavenging Activity for ethanolic extracts was highest85.350±0.05, for chloroform were 78.545±0.06 and for aqueous extract 56.284±0.07 (Fig. 2).

Table: 1. Phytochemical screening of *Tamarindus indica.*

Test for Phytochemical	Aqueous	Ethanol	Chloroform
Alkaloids	-ve	-ve	-ve
Phytosteroids	+ve	+ve	+ve
Flavonoids	-ve	-ve	-ve
Tannins andphenolics	+ve	-ve	+ve
Saponins	-ve	-ve	-ve
Glycosides	-ve	+ve	-ve
Oils and fats	-ve	-ve	-ve

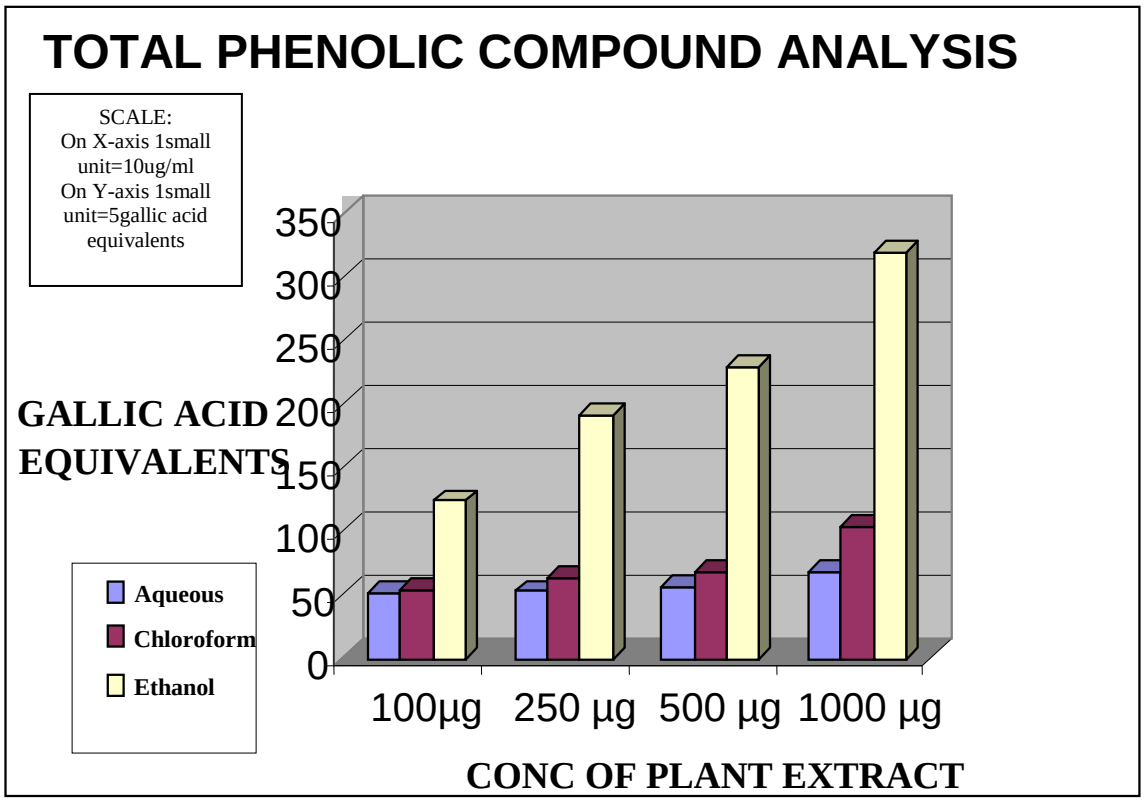


Fig. 1: Total Phenolic Content of *Tamarindus indica*.

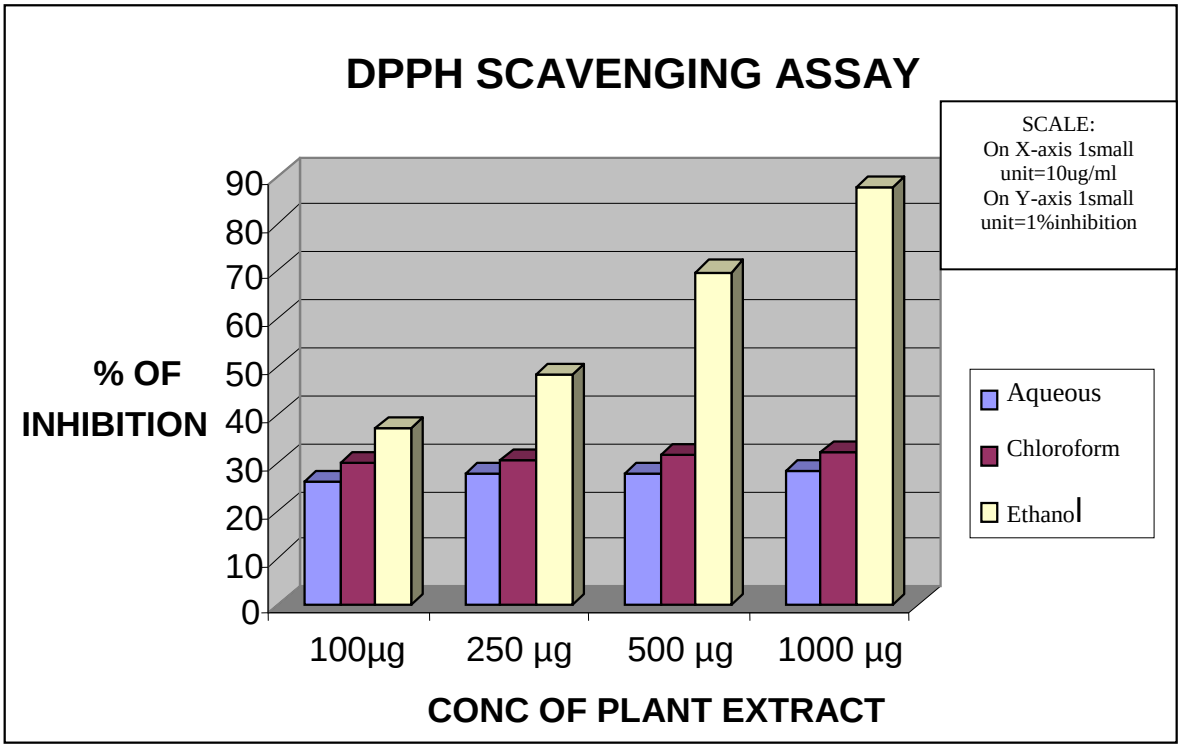


Fig. 2: DPPH free radical scavenging activity of *Tamarindus indica*

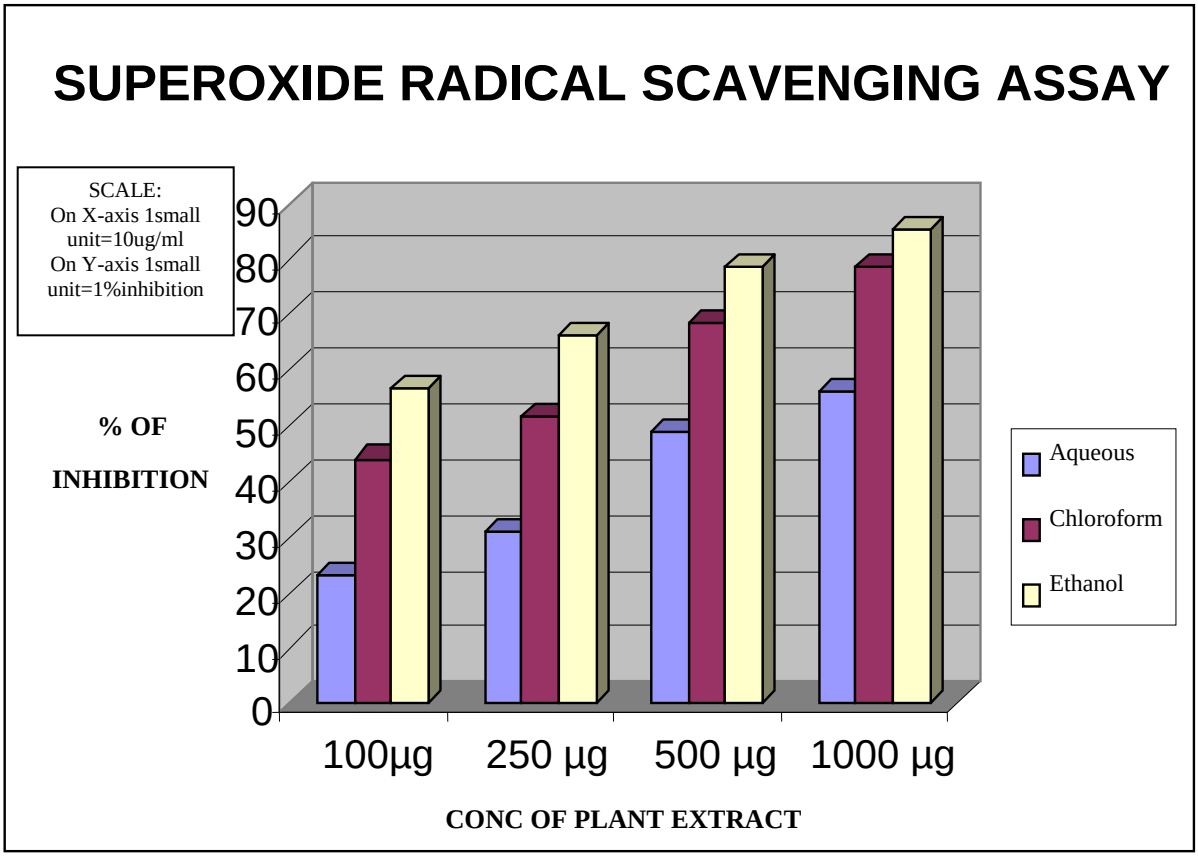


Fig. 3: Super oxide radical scavenging activity of *Tamarindus indica* Anti-bacterial activity

*T. Indica*showed significant antibacterial action againstboth Gram positive and Gram negative tested organisms. Ethanol extracts showed maximum activityagainst *Bacillus subtilis(27mm), Escherichia coli(22mm),Staphylococcus* aureus(22mm), *Proteus vulgaris (20mm), klebsiella pneumonia (19mm) and chloroform extracts showed maximum activity against, Bacillus subtilis(10mm) Escherichia coli(4mm) followed by Staphylococcus aureus (4mm)*and, *Proteus vulgaris(4 mm.).*Antibacterial activity of the ethanolic extracts wasevaluated against pathogens and expressed in terms of minimum inhibitoryconcentration. The MIC of ethanolicextracts againstdifferent pathogens varied depending on pathogen.Among the pathogens tested, *Escherichia coli* was the most sensitive species andshowed the least MIC value(0.019mg/ml)followed by, *Bacillus subtilis*(0.078), *klebsiella pneumonia (*0.312mg/ml), *proteus vulgaris*(0.624mg/ml) and*Staphylococcus aureus* (1.248mg/ml)

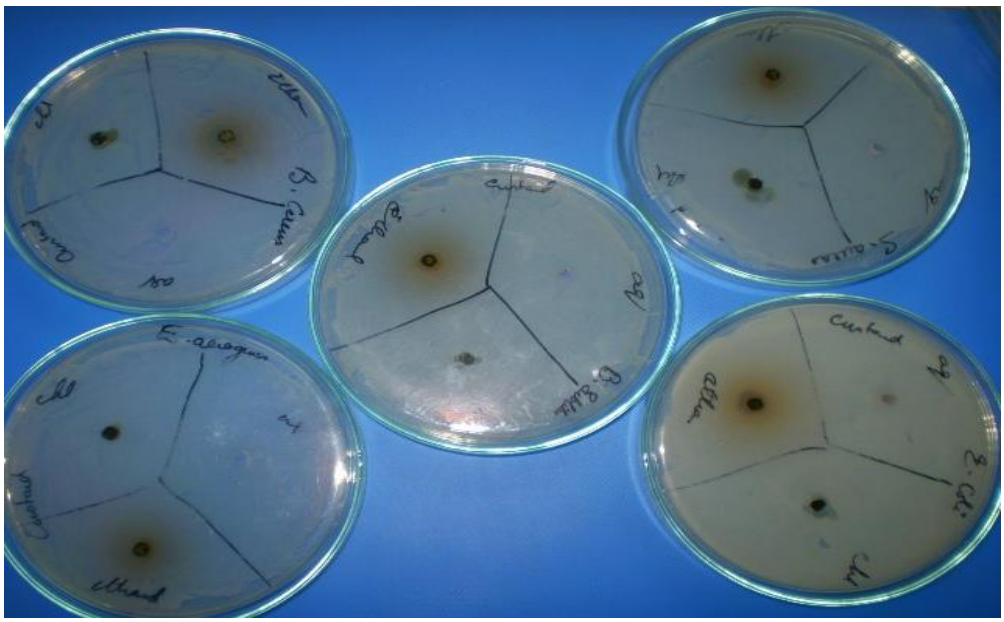


Fig. 4: Zone of Inhibition of *Tamarindud indica* (Akkayyapalem) ethanolic extracts for anti-bacterial activity.

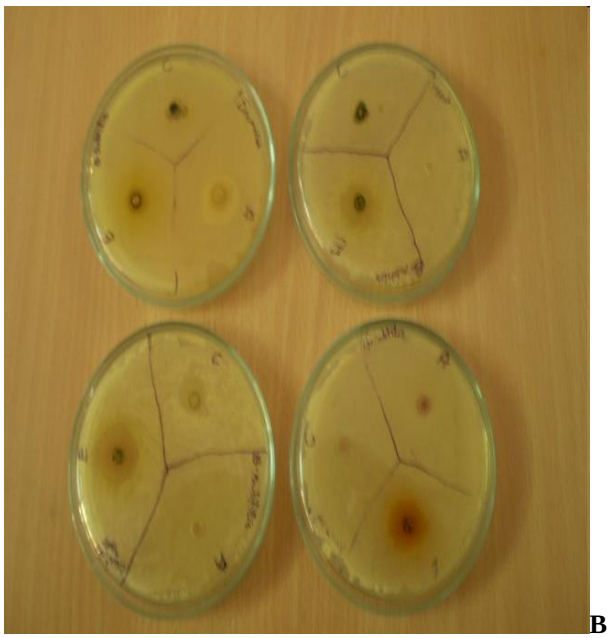
A  **B** 

Fig. 5: Inhibition effect of *T.indica* extracts against (A-*Streptococcus aureus* and B-*E.coli*)

Table: 2. Antibacterial activity of *Tamarindus indica* samples collected from Skota area.

Test Organism	Diameters of zones of inhibition in mm		
	Aqueous	Chloroform	Ethanol
E.coli	7	4	22
B.subtilis	2	10	27
S.aureus	9	4	22
K. pneumoniae	0	0	19
P.vulgaris	0	4	20

Different solvents have been reported to have the capacity to extract different phytoconstituents depending on their solubility or polarity in the solvent. Increase in activity of phyotoconstituents in the presence of acidic medium has earlier been reported [(11)]. The demonstration of antibacterial activity against both gram positive and gram negative bacteria may be indicative of the presence of broad spectrum antibiotic compounds [(12)]. The demonstration of broad spectrum of antibacterial activity by *Tamarindus indica* may help to discover new chemical classes of antibiotic substances that could serve as selective agents for infectious disease chemotherapy and control.

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