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Phytochemical, Anti-microbial and *In-vitro* Antioxidant activity of *Terminalia catappa*

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Abstract

Phytochemical, anti microbial and invitro antioxidant activity of *Terminalia catappa* bark was determined. Phytochemicals such as Steroids, Triterpenes, Tri terpinoidal saponins Alkaloids, Carbohydrates, Flavonoids, Tannins, Glycosides and Polyphenols were present in the methonolic extract of T.catappa bark. In the anti microbial activity test *Bacillus subtilis* *Escherichia coli* and *Staphylococcus aureus* were studied, among the three strains *Bacillus subtilis* *Escherichia coli* showed highest zone of inhibition at 200mg/ml concentration, all results were compared with standard anti biotech. For anti fungal activity *Aspergillus Niger*, *Penicillium Notatum* and *Chlamydomonas* were studied under the work. *Aspergillus Niger* and *Penicillium Notatum* at 200mg/ml concentration showed effective activity. DPPH radical scavenging activity of *T. catappa* bark was showed similar antioxidant activity as that of ascorbic acid a standard antioxidant. Based on our results we have concluded that *Terminalia catappa* bark is very potent antimicrobial and antioxidant activity, further studies are underway to understand the medicinal importance of *Terminalia catappa*

Key-Words: *Terminalia catappa*, Phytochemical, anti microbial and DPPH activity

Introduction

Traditional medicine has been practiced from many centuries, especially in India tribal and rural areas people were used to treat several diseases, due to availability and low cost and negligible side effects it occupies great importance in many formulations and ayurvedic drugs. Nature has provided a source of medicinal agents for thousands of years and an impressive number of modern drugs have been isolated from natural sources, many based on their use in traditional medicine¹. There has been an increasing the incidence of multiple resistances in human pathogenic microbial strains, largely due to the indiscriminate use of synthetic antimicrobial drugs commonly used in the treatment of infectious diseases². The development of bacterial resistance to presently available antibiotics has necessitated the search for new antibacterial agents, to overcome this problem studies have been conducted with the various medicinal plants, screening antimicrobial activity as well as for the discovery of new antimicrobial compounds³⁻⁴ and antioxidant activity.

The efforts of researchers in establishing natural compounds with promising antimicrobial and *invitro* antioxidant property resulted positive and effective results as a number of plants with high antimicrobial and invitro anti oxidant activities have been elucidated⁵⁻⁶.

Terminalia catappa L. belongs to the family Combretaceae. *T. catappa* is used primarily as an ornamental shade, and salt-tolerant street tree, but the leaves provide food for the Tasar silkworm, and the seeds are edible like almonds with similar oils. On the Malay peninsular and through the Canary islands this tree is known as the tropical almond. *T. catappa* has been claimed to have therapeutic effects for liver related diseases⁷. In India, it is used as cardiac stimulant. Its leaves are widely used as a folk medicine in Southeast Asia for the treatment of dermatitis and hepatitis⁸. More and more pharmacological studies have reported that the extract of *T. catappa* leaves and fruits have anticancer, antioxidant, anti-HIV reverse transcriptase, anti-inflammatory, antidiabetic effects and hepatoprotective activities⁹⁻¹⁰ but the effective components and related mechanisms remain unknown. In the present work, phytochemical, antimicrobial and *invitro* antioxidant activities were carried out from *T. catappa* bark methanol extract. Antimicrobial activity

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of *T. catappa* bark extract was investigated against bacterial and fungal cultures where as *in vivo* antioxidant activity was performed with DPPH method.

Material and Methods

Collection and Extraction of Plant Material: *Terminalia catappa* bark was collected from local market in Tirupati, Andhra Pradesh, India. *Terminalia catappa* bark was shade dried pulverized to a coarse powder and extracted with methanol. The filtrate obtained was evaporated to dryness at 50-65°C in distillation process to obtain a dark colored molten mass.

Phytochemical Analysis of Extract: The methods described by Harborne, (1998) with slight modifications were used to screen the presence of the active ingredients in the bark extracts.

Test for Steroids: 10 ml of the extract was evaporated to dry mass and dissolved in 0.5 ml of solvent. Acetic anhydride (0.5 ml) and 2 ml of concentrated sulphuric acid were added. A blue or green colour or a mixture of these two shades was regarded as positive for the presence of steroidal compounds (Harborne, 1998).

Test for Terpenoids: The presence of terpenoids was determined as described for steroids except that red, pink or violet colour indicates the presence of terpenoids (Harborne, 1998).

Test for Tannins: 1 cm³ of freshly prepared 10% KOH was added to 1 cm³ of the extract. A dirty white precipitate indicated the presence of tannins (Harborne, 1998). ii) Powdered stem bark of the test plant (1.0 g) was weighed into a beaker and 10 ml of distilled water added. The mixture was boiled for five minutes. Two drops of 5% FeCl₃ were then added. Production of greenish precipitate indicated the presence of tannins (Harborne, 1998).

Test for Flavonoids: A small piece of magnesium ribbon was added to extract of the plant material, this was followed by the drop wise addition of concentrated hydrochloric acid. Colours varying from orange to red indicated flavones, red to crimson indicated flavonols, crimson to magenta indicated flavonones (Harborne, 1998).

Test for Alkaloids: The extract of the plant sample (0.5 g) was stirred with 5 ml of 1% HCl on a steam bath. The solution obtained was filtered and 1 ml of the filtrate was treated with two drops of Mayer's reagent. The two solutions were mixed and made up to 100 ml with distilled water. Turbidity of the extract filtrate on addition of Mayer's reagent was regarded as evidence for the presence of alkaloids in the extract (Harborne, 1998).

Test for Saponins: Extract of the test plant was ground into powder form and 0.5 g of the powdered stem bark was introduced into a tube containing 5.0 ml of distilled water, the mixture was vigorously shaken for 2 min, formation of froth indicated the presence of Saponins (Harborne, 1998).

Test for Glycosides: Coarsely powdered extract (1g) was added into two separate beakers. To one of the beakers was added 5 ml of dilute sulphuric acid while 5 ml of water was added to the other beaker. The two beakers were heated for 3 – 5 min and the contents filtered into labeled test tubes. The filtrate was made alkaline with 5% sodium hydroxide and heated with Fehling's solution for 3 min. The presence of reddish precipitate in the acid filtrate and the absence of such precipitate in the aqueous filtrate were regarded as positive for glycosides (Harborne, 1998).

Antimicrobial Activity

Growth and Maintenance of Test Microorganism for Antimicrobial Studies: *Bacillus subtilis* (*B. subtilis*), *Escherichia coli* (*E. coli*), *staphylococcus aureus* (*S. aureus*) and fungal cultures of *Aspergillus niger*, *penicillium notatum*, *Chlamydomonas* were used to study antimicrobial anti microbial activity.. The bacteria were maintained on nutrient broth (NB) at 37°C and fungus were maintained on Potato dextrose agar (PDA) at 28°C.

The agar disc diffusion method was used to determine the antimicrobial activity of the different plant extracts (Cruickshank 1968). The discs (6 mm diameter) impregnated with different concentrations of the extracts were placed on the surface of the petri plates containing 20 ml of nutrient agar media for bacterial strains and potato dextrose agar media for fungal strains respectively, seeded with 100µl of microbial cultures (5 x 10⁵ CFU/ml). The plates were incubated for 24 hrs at 35 ±2⁰C for bacteria and for 72 hrs for fungi at 30⁰C. The inhibition zones formed around the discs were measured and expressed in millimeter. Three independent trials were conducted for each concentration and the average values calculated and given in Table 2 and 3. The microbial activity was confirmed by transferring a subculture from the clear zone of inhibition to a fresh broth media and observed for the growth of microbes.

Anti Oxidant activity: DPPH (1, 1-Diphenyl-2-picrylhydrazyl) assay. The scavenging activity of *Terminalia catappa* bark on DPPH was determined using the method described by⁵. This method depends on the reduction of purple DPPH to a yellow colored diphenyl picrylhydrazine. The determination of the disappearance of free radicals was done using

spectrophotometer. The remaining DPPH which showed maximum absorption at 518 nm was measured. Each plant extract sample's stock solution (1.0 mg/mL) was diluted to final concentrations of (0.6, 0.5, 0.4, 0.3, 0.2, 0.1, 0.05 and 0.01) mg/mL, in ethanol. One mL of a 0.3 mM DPPH ethanol solution was added to 2.5 mL of sample solution of different concentrations. These are test solutions. One ml of ethanol was added to 2.5 mL of sample solution of different concentration. These are blank solutions. One mL DPPH solution plus 2.5 mL of ethanol was used as a negative control. The blank for this solution is ethanol. As DPPH is sensitive to light, it is exposed to the minimum possible light. These solutions were allowed to react at room temperature for 30 minutes. The absorbance values were measured at 518 nm and converted into the percentage antioxidant activity using the following equation:

$$\text{Scavenging capacity (\%)} = 100 - \left[\frac{\text{absorbance of sample} - \text{absorbance of blank}}{\text{absorbance of control}} \right] \times 100$$

The tests were done in triplicate. The IC50 values were calculated by linear regression of plots, where the abscissa represents the concentration of the tested plant extracts and the ordinate the average percent of scavenging capacity. The concentration of sample required to scavenge 50% of DPPH (IC50) were determined.

Results and Discussion

Phytochemical studies reveal that the presence of Steroids, Tri terpinoidal saponins, Alkaloids, flavonoids, glycosides and polyphenols in methanolic extract of *Terminalia catappa* bark (Table 1). Polyphenols, glycosides, Flavanoids, alkaloids and Steroids were present in high amount.

Table 1: Phytochemical Screening of methanolic Extract of *Terminalia catappa* bark

Secondary metabolites	Methanolic extract
Steroids	++
Triterpenes	+
Saponins	-
Triterpinoidal saponins	+
Alkaloids	+++
Carbohydrates	+
Flavonoids	++
Tannins	+
Glycosides	++
Polyphenols	+++

+++ Present in high quantity, ++ Present in appreciable quantity, + Present in low quantity, - absent

Antimicrobial activity

The methanol extract of *Terminalia catappa* bark checked for anti bacterial and anti fungal activity. Three bacterial culture *Bacillus subtilis*, *Escherichia coli*, *Staphylococcus aureus* were used in this study to know the efficacy of extract among all cultures *Bacillus subtilis* shown highest inhibition (14 mm) at a dose of 200mg/ml concentration, *Escherichia coli* shown 11mm and, *Staphylococcus aureus* shown 8mm zone of inhibition at a dose of 200mg/ml concentration, In all cultures Tetracycline, *Ampicillin* were used as standard control.

Table 2: *Invitro* antimicrobial activity of methanolic extracts of *Terminalia catappa* bark

Micro organisms	Zone of Inhibition (mm)			
	Methanol extract(mg/ml)		Standard mg/ml Tetracycline	Standard mg/ml Ampicillin
	100	200		
<i>Bacillus subtilis</i>	10	14	17	20
<i>Escherichia coli</i>	8	11	14	17
<i>Staphylococcus aureus</i>	6	8	11	16

Anti fungal activity

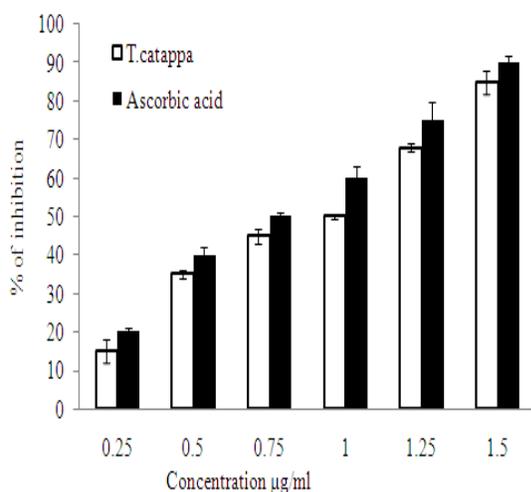
For anti fungal activity *Aspergillus Niger*, *Penicillium Notatum*, *Chlamydomonas* cultures were used in this study. Among all the cultures *Penicillium Notatum* shown highest zone of inhibition (13mm) at dose of 200mg/ml concentration. *Aspergillus Niger* and *Chlamydomonas* shown zone of inhibition 12mm and 9mm at a dose concentration of 200mg/ml.

Table 2: *Invitro* antifungal activity of methanolic extracts of *Terminalia catappa* bark

Micro organisms	Zone of Inhibition (mm)	
	Methanol extract(mg/ml)	
	100	200
<i>Aspergillus Niger</i>	8	13
<i>Penicillium Notatum</i>	7	12
<i>Chlamydomonas</i>	5	9

DPPH Radical Scavenging Activity

DPPH test, which is based on the ability of DPPH, a stable free radical, to decolorize in the presence of antioxidants, is a direct and reliable method for determining radical scavenging action. Ascorbic acid was chosen as the reference antioxidant for this test. The DPPH radical contains an odd electron, which is responsible for the absorbance at 515-517 nm and also for a visible deep purple color. When DPPH accepts an electron donated by an antioxidant compound, the DPPH is decolorized, which can be quantitatively measured from the changes in absorbance. The Scavenging effect of methanol extract of *Terminalia catappa* bark and vitamin C on the DPPH radical is illustrated in Fig1. Methanol extract of *Terminalia catappa* bark extract have significant scavenging effect on DPPH, it was increased with the increasing concentration from 0.25- 20 μ g/ml but the % of inhibition concentration was higher than that of standard.



TC: *Terminalia catappa*, Vit C: Vitamin C

Fig. 1: DPPH Radical Scavenging Activity of methanolic extracts of *Terminalia catappa* bark

Herbal medicine in developing countries is commonly used for the traditional treatment of health problems¹¹. In recent years multiple drug resistance in human pathogenic microorganisms have developed due to the indiscriminate use of commercial antimicrobial drugs commonly used in the treatment of infectious diseases¹². In addition to this problem, antibiotics are sometimes associated with adverse effects on host including hyper-sensitivity, immune suppression and allergic reactions¹⁴. Therefore there is a need to develop alternative antimicrobial drugs for the treatment of infections obtained from various sources

such as medicinal plants¹⁵⁻¹⁷. In the present study *T. catappa* bark extracts extracted in methanol (TME) were investigated for their phytochemical, antimicrobial potentiality against microbial strains and determine the antioxidant activity.

The bacterial strains namely *Bacillus subtilis*, *Escherichia coli*, *Staphylococcus aureus* were used in this study among all culture *Bacillus subtilis* shown highest sensitive nature treated with 200mg concentration of bark extract.

For anti fungal activity *Aspergillus Niger*, *Penicillium Notatum*, *Chlamydomonas cultures* were used, among all fungal strains *Aspergillus Niger* shown highest zone of inhibition at a dose of 200mg concentration.

Conclusion

Present study evaluated the presence of various secondary metabolites in the methanol extract of *Terminalia catappa* which may be responsible for the antimicrobial and antioxidant efficacy.

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