

In vitro* Antibacterial Activity of the Extracts Derived from *Terminalia catappa

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Abstract: The extracts derived from *Terminalia catappa* leaves and fruit following antibacterial activity directed isolation, were screened for their antibacterial activity against species of corynebacteria, staphylococci, streptococci, enterococci, escherichia, salmonella and shigella. The results indicated that crude ethanolic extract, aqueous fraction of crude extract and its sub fractions (petroleum ether and ethylacetate) possessed prominent antibacterial activity, therefore supporting the medicinal uses of this species. In addition, further isolation and phytochemical analysis of the five fractions finally obtained led to the isolation of ferulic acid, vanillic acid, pelargonidin, cyanidin, myricetin, quercetin and gallic acid.

Key words: *Terminalia catappa*, leaf, fruit, antibacterial activity, activity directed isolation, phytochemical analysis

INTRODUCTION

Finding healing power in plants is an ancient idea. Today, clinical microbiologists have four basic reasons to be interested in the topic of antimicrobial plant extracts. First, it is likely that these phytochemicals may find their way into the arsenal of antimicrobial drugs prescribed by physicians several of which have already been tested in humans. Second, the public is becoming increasingly aware of problems with the over prescription and misuse of traditional antibiotics. Third, time to time discovery of new pathogens and fourth, remarkable abilities of microbes to develop resistance against antibiotics.

Terminalia catappa is very well known for its therapeutic values since long and has proved by many researchers to be useful as anti-inflammatory (Fan *et al.*, 2004; Jayasinghe *et al.*, 2000), anticancer (Kandil *et al.*, 1999), antihepatotoxic (Lin *et al.*, 2001), antigenotoxic (Chen *et al.*, 2000), anticlastogenic (Liu *et al.*, 1996) and for the treatment of skin aging, irritation, hyperpigmentation and allergy (Renimel *et al.*, 1998) and bronchial asthma in children (Prazeres, 1995). The plant also exhibits antimicrobial activity. Ethanol extract of *Terminalia catappa* showed antimicrobial activity against nine bacterial strains with Minimum Inhibitory Concentrations (MICs) ranging from 0.25 to 16 mg mL⁻¹ (Kloucek *et al.*, 2005). The antimicrobial effect of chloroform methylene chloride and methanolic extracts of dried root of *Terminalia catappa* has also been proved against *S. aureus* and *E. coli* (Goun *et al.*, 2003; Pawar and Pal, 2002). Antibacterial bioassay-guided fractionation of an ethyl acetate root extract of *Terminalia sericea* led to the isolation of anolignan B. Anolignan B showed activity against both Gram-positive and Gram-negative bacteria (Eldeen *et al.*, 2006).

In view of its high medicinal potential and previous findings, the study was designed to isolate various fractions from the fruit and leaves following antibacterial activity against various species so that its further possible uses in medicines, therapeutics and food preservation could be determined.

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MATERIALS AND METHODS

Plant Material

The plant leaves and fruits were collected in the month of February from the nursery in campus of the University of Karachi. The sample was identified by a taxonomist, Department of Botany, University of Karachi and a voucher specimen was deposited in the herbarium, Department of Botany, University of Karachi.

Antibacterial Activity Directed Isolation

Two kilograms of the fruit and leaves (cut into small pieces) were soaked in 5 L of absolute ethanol separately for a week. The samples were continuously stirred on a magnetic stirrer at a constant speed (1400 rpm) during the period and then filtered. The filtrates were dried on a rotary evaporator at 30°C. The dried extract (fruit, 96.8 g and leaves 85.4 g) of each was mixed and shaken thoroughly with 400 mL *n*-Hexane and 400 mL distilled water in a 1 L separating funnel and then the contents were left till complete separation of the layers. Two distinct layers so formed in case of fruit sample were collected separately as F-H (*n*-hexane, upper, yellow layer) and F-W (aqueous, lower, red layer) and in leaf sample as L-H and L-W. The crude ethanolic as well as fractions F-H, F-W, L-H and L-W were screened for their antibacterial activities against various gram- positive and gram-negative species. The Fractions- F-W and L-W which showed antibacterial activity were isolated further.

F-W collected as red powdery mass (66.4 g) was mixed with 500 mL of (4:1) methanol: H₂O, homogenized and then filtered. The filtrate was evaporated to 1/10 of its original volume in a rotary evaporator at <40°C, acidified with 2M H₂SO₄ and then extracted with 250 mL chloroform. Both the chloroform (F-WC) and aqueous layers (F-WA) collected separately were dried and then tested for antibacterial activities. F-WC which showed antibacterial activity was purified further.

F-WC (33.0 g) was hydrolyzed with 10 mL of 2M HCl at 100°C for 30 min. The extract so obtained was cooled and filtered and then divided into two portions. First portion of the filtrate was extracted with petroleum ether, ether layer was then separated and evaporated to dryness. The residue dissolved in ether was then separated on silica gel using acetic acid: chloroform (1: 9). The major band (F-WC_{PE}) obtained in this separation was collected, dried and then analysed for total phenolics and qualitative nature of the constituent compounds. Second portion of the filtrate was washed twice with ethyl acetate, ethyl acetate and aqueous layers were collected separately. Aqueous layer was heated at 80°C for 3 min to remove last traces of ethyl acetate and the residue taken in small volume of amyl alcohol was concentrated to dryness. The dried mass was dissolved in methanolic-HCl and then separated on paper in formic acid: conc. HCl: water:: 5:2:3. into two major fractions (F-WC_{EA1} and F-WC_{EA2}). Ethyl acetate layer was divided into two portions: first was chromatographed directly on paper in BAW and the major band (F-WC_{EA3}) was collected while the second was dried, taken up in ethanol, chromatographed on paper in BAW and the major fraction (F-WC_{EA4}) was collected. All the five fractions obtained (F-WC_{PE}, F-WC_{EA1}, F-WC_{EA2}, F-WC_{EA3} and F-WC_{EA4}) were tested for their antibacterial activity. Phytochemical screening of the fractions was also carried out. The fractions L-WC_{PE}, L-WC_{EA1}, L-WC_{EA2}, L-WC_{EA3} and L-WC_{EA4} were obtained from L-W following the same scheme as for the partition of fraction F-W.

Antibacterial Activity

Antibacterial activity was determined by agar well diffusion method Schillinger and Lucke (1989) and minimum inhibitory concentration was calculated after serial dilution assay (Bhunja *et al.*, 1991).

Phytochemical Analysis

Phytochemical screening of the leaves and fruit extracts was undertaken using described methods (Harborne, 1998). The screening covered mainly alkaloids, saponins, flavonoids, tannins and quinones.

RESULTS AND DISCUSSION

The results obtained indicated that crude ethanolic extract and aqueous fraction of crude extract produced *in vitro* antimicrobial activity against tested bacterial species (Table 1). This can be explained by the widespread occurrence of polyphenolic compounds, which are soluble in water, methanol and ethanol. The mechanisms thought to be responsible for phenolic toxicity to microorganisms include enzyme inhibition by the oxidized compounds, possibly through reaction with sulfhydryl groups, or through more nonspecific interactions with proteins (Mason and Wasserman, 1987) often leading to inactivation of the protein and loss of function. Probable targets in the microbial cells are surface exposed adhesions, cell wall polypeptides and membrane-bound enzymes. Phenols may also render substrates unavailable to microorganisms (Stern *et al.*, 1996).

Antibacterial activities of all the fractions derived from fruit were correspondingly higher than the fractions from leaves. On comparing the activities of the fractions from fruit it was found that the fraction F-WC_{EA3} showed highest activity while F-WC_{PE} showed lowest (Table 2 and 3). This may be due to relatively higher hydrophobicity of F-WC_{EA3} identified as phenylpropanoids, compared to all

Table 1: Activity spectrum of the *n*-hexane and aqueous fractions of *T. catappa* against gram-positive and gram-negative bacteria

Indicator organisms	Inhibition zone (mm)			
	F-H	F-W	L-H	L-W
Gram-positive bacteria				
<i>Bacillus subtilis</i>	-	28	-	20
<i>Corynebacterium diphtheriae</i>	10	30	-	26
<i>Corynebacterium diphtheriticum</i>	10	30	-	30
<i>Micrococcus lysodieticus</i>	10	30	-	28
<i>Staphylococcus aureus</i>	10	30	-	30
<i>Staphylococcus epidermidis</i>	10	28	-	24
<i>Staphylococcus saprophyticus</i>	-	30	-	30
<i>Enterococcus faecalis</i>	-	30	-	27
<i>Enterococcus faecium</i>	-	28	-	26
<i>Streptococcus pneumoniae</i>	-	30	-	24
<i>Streptococcus pyogenes</i>	-	30	-	22
Gram-negative bacteria				
<i>Escherichia coli</i> WT	-	28	-	22
<i>Escherichia coli</i> BU40	-	25	-	22
<i>Escherichia coli</i> FPL5014	-	26	-	22
<i>Klebsiella pneumoniae</i>	-	26	-	20
<i>Proteus mirabilis</i>	-	26	-	25
<i>Pseudomonas aeruginosa</i>	-	28	-	25
<i>Salmonella typhi</i>	-	25	-	25
<i>Salmonella paratyphi</i> A	-	25	-	22
<i>Salmonella paratyphi</i> B	-	26	-	25
<i>Shigella dysenteriae</i>	-	25	-	24
<i>Shigella sonnei</i>	-	28	-	24
<i>Shigella flexneriae</i>	-	25	-	25

Table 2: MIC values ($\mu\text{g mL}^{-1}$) of the extracts derived from *T. catappa* fruit

Bacterial species	F-WC _{PE}	F-WC _{EA1}	F-WC _{EA2}	F-WC _{EA3}	F-WC _{EA4}
	MIC ($\mu\text{g mL}^{-1}$)				
<i>Enterococcus faecalis</i>	31.25	15.625	15.625	7.812	15.625
<i>Staphylococcus aureus</i>	31.25	15.625	15.625	7.812	15.625
<i>Streptococcus pneumoniae</i>	31.25	15.625	15.625	7.812	15.625
<i>Corynebacterium diphtheriae</i>	31.25	15.625	15.625	7.812	15.625
<i>Salmonella typhi</i>	62.50	31.350	31.350	15.625	31.250
<i>Shigella dysenteriae</i>	62.50	31.250	31.250	15.625	31.250
<i>Escherichia coli</i>	62.50	31.250	31.250	15.625	31.250

Table 3: MIC values ($\mu\text{g mL}^{-1}$) of the extracts derived from *T. catappa* leaves

Bacterial species	L-WC _{PE}	L-WC _{EA1}	L-WC _{EA2}	L-WC _{EA3}	L-WC _{EA4}
	MIC ($\mu\text{g mL}^{-1}$)				
<i>Enterococcus faecalis</i>	62.5	31.25	31.25	15.625	31.25
<i>Staphylococcus aureus</i>	62.5	31.25	31.25	15.625	31.25
<i>Streptococcus pneumoniae</i>	62.5	31.25	31.25	15.625	31.25
<i>Corynebacterium diphtheriae</i>	62.5	31.25	31.25	15.625	31.25
<i>Salmonella typhi</i>	125.0	31.25	62.50	31.250	31.25
<i>Shigella dysenteriae</i>	125.0	31.25	62.50	31.250	31.25
<i>Escherichia coli</i>	125.0	31.25	62.50	31.250	31.25

other fractions and least hydrophobicity of F-WC_{PE} identified as simple phenols. The same pattern was observed for the fractions from leaves. The antibacterial activity of any compound against bacteria is partly due to their ability to reach the site of action. In bacteria, various enzymes, especially components of energy-converting systems such as Electron Transport Chains (ETC) and ATPases, are embedded in the plasma membrane. The ETC is a chain of specialized complex molecules (redox agents), which form a conducting path for electrons. The inner and outer surfaces of the membrane are hydrophilic, whereas the interior is hydrophobic, so lipophilicity of a compound affects its movement into the membrane lipid bilayer portion (Franks and Lieb, 1986). Once inside the lipid bilayer portions, the compound may inhibit the ETC, perhaps by interfering with the redox reactions. This may reveal that why F-WC_{EA3} (being more hydrophobic) showed greater activity than F-WC_{PE}.

Our results also validates previous reports on the antimicrobial activity of pet.ether, chloroform and methanolic extracts derived from root of *T. catappa* against *S. aureus* and *E. coli* (Pawar and Pal, 2002).

Preliminary phytochemical analysis of fruit and leaves of *T. catappa* revealed the presence of pigments viz. violanxanthin, lutein and zeaxanthin and β -cryptoxanthine (Lopez-Hernandez *et al.*, 2001), tannins (Rayudu and Rajadurai, 1966; Tanaka *et al.*, 1986; Mustapha, 2001) and flavone glycosides (Lin *et al.*, 2000). However when all the bioactive extracts obtained through the scheme adopted in this study were screened chemically, they were found to be simple phenols, anthocyanins, phenylpropanoids and flavonols. Phytochemical groups of organic compounds detected in these plants have been known to possess antimicrobial properties (Mitscher *et al.*, 1987).

This study supports the concept that *T. catappa* plant may be important in the potential discovery of natural product pharmaceuticals and helps in the scientific validation of the uses of this species as a supplementary support in the treatment of infectious diseases.

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