ANTIMICROBIAL ACTIVITY OF SOLVENT EXTRACTS OF *TERMINALIA CATAPPA* LINN LEAVES

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Abstract

The antimicrobial activity of the hexane, ethylacetate and methanol extracts of the leaves of *Terminalia catappa*, and the oil fractions from the hexane extract (TC1-TC5), were examined. Thirteen different bacterial and two fungal isolates were used. The results show that all the solvent extracts exhibited activity at concentrations ranging from 0.625-10.0 mg/ml for Gram-negative and 0.313-5.0 mg/ml for Gram-positive bacteria. All the oily fractions from hexane extract, TC1-TC5, showed inhibitory action on *Candida albicans* and *Aspergillus flavus.*

Keywords: Antibacterial, antifuugal activity, *Terminalia catappa* Linn leaf.

1. Introduction

All over the world, several hundreds of plants are good sources of medicinal agents and are used in traditional medicine for many different purposes, including bacterial and fungal infections. It has been pointed out (Baker *et al.*, 1995) that plants continue to play a prominent role in the primary health care of about 80% of the world's population. In general, conventional drugs (from natural and synthetic sources) are useful antibiotics for the treatment of infections but the problem of antibiotic resistance is increasing and there is the continuous need for the discovery of new and more effective therapeutic agents or new solutions.

Some patients, in many countries, prefer to be treated with herbal medicines although natural products are not necessarily safer than synthetic organic antibiotics.

Terminalia catappan Linn (combretaceae), commonly called "tropical almond", "Indian almond", etc is a large decidous tree and thrives in many tropical areas of the world.

In traditional medicine, the leaves and/or fruits of the plants are used in the treatment of diarrhea in Western Nigeria and Suriname. The dried fallen leaves are used as a herbal drug in the treatment of liver diseases in Taiwan (Chiu and Chang, 1986), while in the Phillipines and India, the leaves are used for treating dermatitis and hepatitis (Lin *et al.*, 1997). In addition, solvent extracts of the leaves and bark of the plant have been reported to show antioxidant and anticancer activities (Masuda *et al.*, 1999; Chyau *et al.*, 2002), anticlastogenic effects (i.e., prevention of the breakage of chromosome) (Liu *et al.*, 1996), anti-inflammatory activity (Lin *et al.*, 1999), HIV-1 reverse transcriptase inhibition (Tan *et al.*, 1991), aphrodisiac effect (Ratnasooriya and Dharmasiri, 2000) and antidiabetic activity (Nagappa *et al.*, 2003).

Various compounds have been isolated from the plant, including many tannins from the leaves and bark (Lina and Hsub, 1999) (some of which are reported to exhibit antidiabetic (Teotia and Singh, 1997) and anti-HIV replication (Tanaka *et. al.*, 1990) properties), ellagic acid (Tan *et al.*, 1991), gallic acid (Dorsch and Warner, 1991), flavonoid glycosides from the leaves (Lin *et al.*, 2000), amongst others.

The pharmacological properties of Nigerian *Terminalia catappa* have not been well established. In the present study, the antimicrobial activity of extracts of *T. catappa* was examined *in-vitro*.

2. Materials and Methods

Melting point was determined with open capillary tubes on a Gallenkamp (variable heater) melting point apparatus (uncorrected). Infrared spectra were recorded, either as neat or KBr pellets, on a Buck spectrometer. NMR spectra were run on a Varian Mercury 200 in $CDCl_3$.

(a) Plant material

Fresh leaves of *Terminalia catappa* were collected from Obafemi Awolowo University, Ile-Ife, Nigeria, in March, 2003 and identified by Dr. H.C. Illoh of the Department of Botany, Obafemi Awolowo University, Ile-Ife, Nigeria, where a voucher

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specimen was deposited in the herbarium. The leaves were air-dried.

(b) Extraction procedure

The dried leaves were powdered with a blender to give a total weight of 1.14 kg. This was successively extracted with hexane, ethyl acetate and methanol (5 liters each) at room temperature. Evaporation of the solvents under vacuum for the different fractions gave 5.9 g (0.5% on dried wt) for hexane; 13.8 g (1.2% on dried wt) for ethyl acetate and 162.1 g (13.6% on dried wt) for methanol. The crude extracts were kept in sample tubes and stored in a refrigerator. They were subsequently used for antimicrobial sensitivity testing and MIC determinations against the microorganisms listed below.

(c) Fractionation of the Hexane Extract

The hexane extract was accelerated gradient chromatographed on silica gel 60 (0.040 mm-0.063 mm, Merck) eluting with hexane followed by gradient mixtures of hexane-CCl_4. Fractions were collected and pooled according to the TLC characteristics (number of spots and rf values), to give five fractions TC1-TC5 which were assayed for activity.

(d) Phytochemical Screening

The crude extracts were subjected to standard chemical tests as described by Trease and Evans (1983) and Farnsworth (1966) to determine the presence or absence of alkaloids, flavonoids, phenols, steroids, saponins and triterpenoids.

(e) Microorganisms

Thirteen bacterial strains, obtained from the laboratory stock of the Department of Microbiology, Obafemi Awolowo University, Ile-Ife, Nigeria, were used. The strains are from the National Collection of Industrial Bacteria (NCIB) or Locally Isolated Organism (LIO): Gram-negative: (*Escherichia coli* (NCIB 86), *Klebsiella pneumoniae* (NCIB 418), *Serratia marcescens* (NCIB 1377), *Shigella dysenteriae* (LIO)) and Gram-positive: (*Bacillus anthracis* (LIO), *Bacillus polymyxa* (LIO), *Bacillus sterothermophilus* (NCIB 8222), *Bacillus subtilis* (NCIB 3610), *Clostridium sporogenes* (NCIB 532), *Corynebacterium pyogenes* (LIO), *Micrococcus luteus* (NCIB 196), *Staphlococcus aureus* (NCIB 8588) and *Streptococcus faecalis* (NCIB 775)). Five fungal strains were also used: *Candida albicans, Aspergillus flavus, Trichophyton mentagrophytes, Trichophyton tonsurans* and *Trichophyton interdigitale.*

(f) Antimicrobial testing

Nutrient broth (Oxoid Ltd) and nutrient agar (Oxoid Ltd) were used for sub-culturing the bacterial isolates, while diagnostic sensitivity test agar (Oxoid Ltd) was used for sensitivity testing. The sensitivity testing of the plant extracts were determined using agar-well diffusion method (Irobi *et al.*, (1996)). The bacterial isolates were first grown in nutrient broth (Oxoid Ltd) for 18 h before use. The inoculum suspension were standardized and then tested against the effect of the extracts at a concentration of 20 mg/ml each (for the solvent extracts) and 2 mg/ml each (for the oil fractions from the hexane extract) in diagnostic sensitivity test agar (Oxoid Ltd). The plates were observed for zones of inhibition after 24 h incubation at 37° C. The effects were compared with that of the streptomycin standard antibiotic at a concentration of 1 mg/ml.

The Minimum Inhibitory Concentration (MIC) of different concentrations of the extracts was determined using two-fold dilutions method (Russell and Furr, 1977). Different concentrations of the crude extracts ranging between 10,000 µg/ml and 156 µg/ml were prepared. Two millimetres of the concentrate from each dilution was added to 18 ml of molten sterile nutrient agar (Oxoid Ltd) asceptically, and thoroughly mixed together in sterile Petri dish. This was allowed to set. The surface of the nutrient agar was allowed to dry properly before streaking with the appropriate bacterial isolate. The plates were then incubated at 37°C for up to 72 h. The lowest concentration of antimicrobial agent that completely prevented the growth of microorganisms was taken as the minimum inhibitory concentration of the extract.

3. Results and Discussion

The results obtained by appropriate chemical tests carried out on the extracts of *T. catappa* leaves show (a) the presence of tannins but absence of alkaloids and saponins in the methanol extract (b) the presence of tannins, flavonoids and steroids but absence of alkaloids and saponins in the ethyl acetate and hexane extracts.

The third fraction (TC3) obtained upon column chromatography of the crude hexane extract, was examined further: Basic hydrolysis has no effect (infrared evidence and the recovered product gave no reaction with aqueous $NaHCO_3$, indicating the absence of carboxylic functional group), hence TC3 does not contain an ester functional group. Also, TC3 tested negative for phenols but positive for presence of sterol, while its infrared spectrum showed absorption bands at 3380 (br, w), 2925,2850 (CH/CH_2/CH_3), 1721, 1595, 1460, 1375 and 1055 cm^{-1}. Heating TC3 in petroleum ether to dissolution and then left to stand gave white solid (mp 80-84 °C) which is insoluble in most organic solvents, but only slightly soluble in chloroform. The ^1H nmr of TC3 shows the presence of mainly hydrocarbon protons in the region δ 2.4 - 0.85 ppm.

The data for the sensitivity testings (inhibition zones (mm)) of the extracts (at 20 mg/ml), streptomycin (a clinical antibiotic used for reference, at 1 mg/ml) and DMSO (solvent) against nine species of Gram-positive and four Gram-negative bacteria, along with

four fungi isolates, are shown in Table 1. The MIC of the extracts was determined and the results are also reported in Table 1. The results show that the extracts have broad-spectrum antimicrobial activity against all the Gram-positive and Gram-negative bacteria, except on *Bacillus polymyxa* (for the methanol extract) *Bacillus anthracis* and *Bacillus polymyxa* (for the ethyl acetate extract). The hexane extract also exhibited activity against two of the tested fungi-*Aspergillus flavus* and *Trichophyton mentagrophytes*. In addition, the hexane extract showed larger zones of inhibition than the ethyl acetate extract, for most of the strains.

The MICs of methanol extract varied between 0.313 and 10 mg/ml; between 1 and 2.5 mg/ml for ethyl acetate extract and between 0.625 and 5 mg/ml for hexane extract. The standard streptomycin had MIC values varying between 0.031 and 0.250 mg/ml. The results indicated that the standard streptomycin has stronger activity than the extracts. However, among the extracts, the non-polar (hexane) extract exerted higher or equal activity on most organisms when compared with the polar (methanol) extract.

The hexane extract was partitioned into five fractions (TC1, TC2, TC3, TC4 and TC5, all oils) via accelerated gradient chromatography and screened for activity at a concentration of 2 mg/ml. The results are reported in Table 2.

The first fraction TC1, eluted with pure hexane, showed activity against all the organisms tested, except on *Corynebacterium pyogenes* (LIO), *Micrococcus luteus* (NCIB 196), *Staphylococcus aureus* (NCIB 8588) (all Gram-positive), *Trichophyton mentagrophytes* and *Trichophyton tensurrans*, with zones of inhibition ranging from 10-25 mm. TC2 and TC3 followed a similar trend in their activity, except an additional inactivity against two Gram-positive strains – *Bacillus polymyxa* (LIO) and *Clostridium sporogenes* (NCIB 532). TC2 showed activity against three of the five

Table 1: Antimicrobial activity of solvent extracts of *Terminalia catappa* leaves.

		Inhibition zone (mm)[a]				
Microorganisms	Gram	M(MIC)[b]	EA (MIC)	Hx (MIC)	S[c] (MIC)	DMSO
Eschericha coli	-	17 (10)	11 (1.25)	20 (2.5)	0 (-)	-
Klebsiella pnemoniae	-	22 (10)	15 (2.5)	20 (5)	0 (-)	-
Serratia marcescens	-	12 (10)	10 (2.5)	10 (5)	22 (0.062)	-
Shigella dysenteriae	-	23 (0.625)	15 (1.25)	20 (1.25)	22 (0.250)	-
Bacillus anthracis	+	18 (1.25)	0 (-)	13 (2.5)	20 (0.031)	-
Bacillus polymyxa	+	0 (-)	0 (-)	10 (5)	15 (0.125)	-
Bacillus stearothermophilus	+	21 (0.625)	15 (1.25)	24 (0.625)	23 (0.062)	-
Bacillus subtilis	+	15 (1.25)	12 (1.25)	19 (0.625)	22 (0.062)	-
Clostridium sporogenes	+	22 (5)	10 (1.25)	11 (2.5)	28 (0.008)	-
Corynebacterium pyogenes	+	17 (5)	21 (2.5)	14 (5)	19 (0.031)	-
Micrococcus luteus	+	15 (0.313)	10 (2.5)	10 (2.5)	0 (-)	-
Staphylococcus aureus	+	20 (1.25)	15 (1.25)	18 (1.25)	21 (0.25)	-
Streptococcus faecalis	+	30 (2.50)	18 (2.50)	13 (5)	24 (0.062)	-
Fungi						
Aspergillus flavus		ND	ND	12	ND	-
Trichophyton mentagrophytes		ND	ND	14	ND	-
Trichophyton tonsurans		ND	ND	0	ND	-
Trichophyton interdigitale		ND	ND	0	ND	-

a: at a concentration of 20 mg/ml
b: MIC values expressed as mg/ml
M = methanol; EA = Ethylacetate; Hx = Hexane
c: S =streptomycin (screening concentration = 1 mg/ml)
ND = Not Determined

fungal cultures, while TC3 showed activity against four of the fungal strains. TC4 did not show activity against seven bacteria strains (2 Gram-negative and 5 Gram positive), but showed activity against all the five fungal cultures.

The dichloromethane eluted fraction, TC5, showed activity against three Gram-negative organisms only - *Klebsiella pneumoniae* (NCIB 418), *Serratia marcescens* (NCIB 1377) and *Pseudomonas aeruginosa* – but showed effectiveness against all the five fungal cultures screened.

The results of this study have implications for the use of oils of *Terminalia catappa* (from non-polar extract) and the more polar solvent extracts as antibacterial and antifungal agents in several applications requiring these properties. Further studies are in progress for isolation/purification of active components and in-vivo antibacterial evaluation.

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Table 2: Antimicrobial effects of oil fractions from the hexane extract of *Terminalia catappa* leaves

		Inhibition zone (mm)[a]				
Microorganisms	Gram	TC1	TC2	TC3	TC4	TC5
		H:D* (100:0)	(96:4)	(90:10)	(60:40)	(0:100)
Eschericha coli	-	15	20	18	0	0
Klebsiella pnemoniae	-	16	18	13	15	14
Psendomonas aeruginosa	-	15	12	16	15	12
Serratia marcescens	-	15	13	15	15	13
Shigella dysenteriae	-	15	14	14	0	0
Bacillus anthracis	+	23	15	20	18	0
Bacillus polymyxa	+	10	0	0	0	0
Bacillus stearothermophilus	+	25	23	28	22	0
Bacillus cereus	+	14	15	15	14	0
Bacillus subtilis	+	17	16	20	18	0
Clostridium sporogenes	+	10	0	0	0	0
Corynebacterium pyogenes	+	0	0	0	0	
Micrococcus luteus	+	0	0	0	0	0
Staphylococcus aureus	+	0	0	0	15	0
Streptococcus faecalis	+	15	12	15	0	0
Fungi						
Candida albicans		14	15	17	12	20
Aspergillus flavus		12	12	12	15	20
Trichophyton mentagrophytes		0	16	15	18	22
Trichophyton tonsurans		0	0	0	12	18
Trichophyton interdigitale		0	0	12	14	18

a = at a concentration of 2 mg/ml
* = Eluting solvent mixture

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