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Some pharmacological properties of extracts of *Terminalia sericea* roots

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Abstract

Terminalia sericea Burch. Ex. DC (Combretaceae) extracts are used to treat bacterial infections, diarrhea, and diabetes. Intermediate and polar extracts of the roots exhibited antibacterial activity against *Staphylococcus aureus*, *Escherichia coli*, *Bacillus anthracis*, and *Pseudomonas aeruginosa*, while the petroleum ether extract was inactive. The extracts were mildly active against *Bacillus anthracis* and *Pseudomonas aeruginosa* but exhibited the highest activity against *Staphylococcus aureus*. They also exhibited antifungal activity against *Candida albicans* and *Aspergillus niger*. An 80% aqueous ethanol extract of the roots did not have any effect on blood glucose levels during an oral glucose tolerance test (OGTT), in mice ($P > 0.05$). With the exception of the dichloromethane and petroleum ether extracts, all the intermediate and polar extracts were toxic to brine shrimps giving LC_{50} (95% confidence intervals) values ranging from 5.4 (3.5–8.4) to 17.4 (11.4–26.5) μg/ml, while that of cyclophosphamide, a standard anticancer drug, was 16.3 (10.6–25.2) μg/ml. Further work is in progress to isolate and identify active compounds in the extracts.

Keywords: *Terminalia sericea*; Antibacterial; Antifungal; Cytotoxicity; OGTT

1. Introduction

Terminalia sericea Burch. Ex. DC (Combretaceae) is an abundant plant in the tropical and warm temperate regions, especially in Africa (Watt and Breyer-Brandwijk, 1962; Hutchings et al., 1996). Ethnomedical information revealed that this plant, which is commonly known as "mpululu" among the Hehe tribe of Iringa, Tanzania, is used for the treatment of diabetes, diarrhea, and gonorrhea. In Malawi, the dried fruit is used in a multicomponent recipe for the treatment of tuberculosis (Msonthi and Magombo, 1983) and the dried leaves are used for the treatment of dysentery (Msonthi and Magombo, 1983). The Venda of southern Africa use a water extract of the dried leaf to treat menorrhagia (Arnold and Gulumian, 1984), while the powdered dried leaves are used to cover infected wounds (Arnold and Gulumian, 1984). In East Africa a decoction of the ground roots is used for the treatment of bilharzia and stomach troubles (Kokwaro, 1976). A decoction of the dried roots is used to prepare a soft porridge with maize flour for treatment of diarrhea by the Venda (Arnold and Gulumian, 1984). A decoction of the plant is used in a multi-component preparation to enhance virility and for treatment of venereal diseases (Arnold and Gulumian, 1984). It has previously been reported that aqueous and methanol extracts of the dried bark have no antibacterial activity against *Bacillus subtilis*, *Staphylococcus aureus* and *Staphylococcus epidermidis* (Rabe and Van Staden, 1997), but two more recent studies have reported the presence of antibacterial activity in the leaves, roots and stem bark (Eloff, 1999; Fyhrquist et al., 2002), and antifungal activity against *Candida albicans* (Fyhrquist et al., 2002). Another study reported that ethyl acetate extracts of dried leaves, stem bark and wood have topoisomerase II inhibitory activity (Wall et al., 1996). Compounds so far isolated from *Terminalia sericea* include a triterpene sericoside (Maeda and Fukuda, 1996), and resveratrol-3-*O*-β-D-rutinoside, a hydroxystilbene glycoside (Bombardelli et al., 1975). Skin lightening preparations

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containing sericoside have been patented in Japan (Maeda and Fukuda, 1996). Work done in our laboratory showed that the stem bark has antibacterial activity and two compounds active against *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa* (MIC, 31.25–250 μg/ml) were isolated (Innocent, 2002). Based on the wide use of this plant in Tanzania, the present study was carried out to investigate extracts of the roots of the plant for antibacterial, antifungal, cytotoxic and hypoglycemic activity.

2. Materials and methods

2.1. Materials

Petroleum ether, dichloromethane, ethyl acetate, butanol, and ethanol were purchased from Fisher Scientific UK Ltd. (Bishop Meadow Road, Loughborough, Leicestershire, LE 11 5RG, UK). Saboraud's dextrose agar (SDA) and broth and Mueller Hinton agar were purchased from Oxoid Ltd (Basingstoke, Hampshire, England), while dimethylsulfoxide (DMSO) was purchased from Sigma (Poole, Dorset, England). Brine shrimp eggs were bought from Dohse Acquaristic, Bonn (Aus Dem Hause Dohse Acquaristik), Germany. Sea salt was prepared locally by evaporating water collected from the Indian Ocean, along the Dar es Salaam Coast.

2.2. Collection of plant material

Terminalia sericea roots were collected in Ismani village, Iringa region, Tanzania. The plant was identified by Mr. F. Mbago of the Department of Botany, University of Dar es Salaam, and a voucher specimen (no. IMPP 001-0144) is kept in the Herbarium of the Institute of Traditional Medicine, Muhimbili University College of Health Sciences. Recollection was done by Mr. E.B. Mhoro, and the new voucher no. MJ 209 is kept in the same Herbarium.

2.3. Preparation and extraction of plant material

The powder of air-dried roots (2.0 kg) of *Terminalia sericea* was defatted using petroleum ether, by maceration, overnight. After evaporation of the solvent under pressure, 3.0 g of fatty extract was obtained. The plant material was then subjected to sequential organic solvent extraction to afford the dry extracts of dichloromethane (4 g), ethyl acetate (9.5 g), 1:1 dichloromethane:methanol (160.8 g), methanol (127.3 g) and 80% ethanol (85.7 g), respectively. The plant material was further extracted with distilled water, followed by partitioning with butanol twice and both solvents were evaporated to complete dryness, followed by freeze-drying, to afford 30.6 and 24.4 g of powdered aqueous and butanol extracts, respectively. A portion of each dry extract was tested for antibacterial, antifungal and brine shrimp cytotoxic activity. Total root extract, separately prepared using 20% aqueous ethanol, was used to test for the effect on blood glucose.

2.4. Antimicrobial tests

Antibacterial and antifungal activities were tested by the disc-diffusion method (Singh et al., 2002). Six standard bacteria, *Staphylococcus aureus* (NCTC 6571), *Escherichia coli* (NCTC 10418), *Pseudomonas aeruginosa* (NCTC 10662), *Klebsiella pneumoniae* (NCTC 9633), *Salmonella typhi* (NCTC 8385), and *Bacillus anthracis* (NCTC 10073), and the fungi, *Candida albicans* (Strain HG 392), and two local strains of *Aspergillus niger*, and *Aspergillus fumigatus* were used. Filter paper discs (Whatman no. 1; 6 mm diameter) were impregnated with crude extracts (10 mg/disc) or standard drugs (20 μg/disc ampicillin, 10 μg/disc gentamicin; for bacteria) and miconazole (20 μg/disc; for fungi). The discs were overlayed on Mueller Hinton agar plates (for bacteria) and Saborauld's dextrose agar plates (for fungi) and incubated at 37 °C, for 24 h in the case of bacteria and *Candida* and for 48 h in the case of the other fungi. The discs were tested in triplicate, including one with a solvent blank and three for the standard drugs. Inhibition zones were calculated as the difference between disc diameter (6 mm) and the diameters of inhibition (Hewitt and Vincent, 1989). The mean inhibition zones were used to calculate the activity index. Activity index (AI) was calculated as the mean inhibition zone for test sample divided by the mean inhibition zone for the standard drug (Singh et al., 2002).

2.5. Brine shrimp lethality test

The brine shrimp lethality test (BST) was used to predict the presence, in the extracts, of cytotoxic activity (Meyer et al., 1982). Solutions of the extracts were made in DMSO or distilled water, at varying concentrations and incubated in duplicate vials with the brine shrimp larvae. Ten brine shrimp larvae were placed in each of the duplicate vials. Control brine shrimp larvae were placed in a mixture of seawater and DMSO only. After 24 h, the nauplii were examined against a lighted background, with a magnifying glass and the average number of survived larvae was determined. The mean percentage mortality was plotted against the logarithm of concentrations and the concentration killing 50% of the larvae (LC_{50}) was determined from the graph. Cyclophosphamide was used as a standard test drug.

2.5.1. Data analysis

The mean results of brine shrimp mortality against the logarithms of concentrations were plotted using the Fig P computer program (Biosoft Inc., USA), which also gives the regression equations. The regression equations were used to calculate LC_{16}, LC_{50} and LC_{84} values. Confidence intervals (95% CI) were calculated according to the method of Litchfield and Wilcoxon (1949). Extracts giving LC_{50} val-