

Flavanols and Terpenes/Sterols with Antimycobacterial Activity from the Stem Bark of *Pterocarpus erinaceus* Poir (Leguminosae)

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The antimycobacterial activity of parts of *Pterocarpus erinaceus* Poir. (Leguminosae) was studied using the agar proportion method. Phytochemical screening was also carried out to determine the major phytochemical groups responsible for the activity. Only the stem bark of the plant was found to possess varying degrees of activity against four *Mycobacterial* species namely *M. smegmatis* ATCC 607, *M. tuberculosis*, *M. bovis* and *M. avium-complex*. Phytochemical screening of the active fractions revealed the presence of flavanols and terpenes/sterols in the fraction isolated from the hexane crude and tannins and terpenes/sterols in the fraction isolated from the methanol crude. The minimum inhibitory concentrations of the active fractions ranged between 0.25 mg/ml and 2.0 mg/ml.

Key Words: Flavanols, terpenes/sterols, antimycobacterial activities, *Pterocarpus erinaceus*

INTRODUCTION

Pterocarpus erinaceus has many medicinal uses including the use of the leaves as a febrifuge, the bark for tooth and mouth ailments, and bark and resin as astringent for severe diarrhea and dysentery. The grated root is mixed with tobacco and smoked in a pipe as a cough remedy [1, 2]. The plant is used by traditional healers in northern Nigeria for the treatment of tuberculosis and/or some of its symptoms and, despite this use there has not been a study to establish the pharmacological basis for the use of this plant. This paper therefore reports on the antimycobacterial activity of parts of *Pterocarpus erinaceus* and determination of the major chemical groups responsible for the activity.

MATERIALS AND METHODS

Plant Sample

The plant samples were collected from around Bauchi Town in Northern Nigeria. The parts collected were the leaves, stem and root barks.

The plant parts were dried under room temperature for 2 weeks and then ground into fine powder using pestle and mortar. The plant was identified at the herbarium of the National Institute of Pharmaceutical Research and Development (NIPRD), Idu-Abuja, Nigeria where herbarium specimens were deposited (NIPRD 5131).

Test Organisms

Four species of *Mycobacterium* were used for the study namely *Mycobacterium tuberculosis*, *Mycobacterium bovis*, *Mycobacterium avium-complex* and *Mycobacterium smegmatis* ATCC 607. The first three species were isolated from sputum samples obtained from patients at Tuberculosis and Leprosy Hospital Bayara in Bauchi according to standard procedures on Glycerol-Egg and Lowenstein-Jensen (L-J) media [3]. *Mycobacterium smegmatis* ATCC 607 was obtained from Microbiology Department of the National Institute for Pharmaceutical Research and Development, Idu-Abuja, Nigeria.

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Extraction Procedure

Preparation of Crude Methanolic Extract

Dried powdered material of each plant part (500 g) was exhaustively extracted with 2.5 L of methanol at room temperature for 72 hours [4]. The extract was filtered through Whatman No. 1 filter paper and concentrated to dryness using a rotary evaporator at $< 40^{\circ}\text{C}$. The dried methanolic crude extracts were assayed for their *in vitro* antimycobacterial activities.

Sequential Solvent Plant Extraction

Each plant part that exhibited *in vitro* antimycobacterial activity from the initial screening using the methanolic crude extract was subjected to sequential solvent extraction [4-7]. Each plant part (1 kg) was separately soaked in 2.5 L of hexane and allowed to stand for 72 hours with vigorous shaking after every 24 hours. At the end of extraction each extract was filtered through Whatman No. 1 filter paper (England). Subsequently the residues were extracted with chloroform, acetone, methanol and water in that order. All extracts except the aqueous were concentrated to dryness at $<40^{\circ}\text{C}$ using a rotary evaporator (Janke & Kunkee, Labortechnik). The aqueous extracts were filtered through Whatman No. 1 filter paper (England) and the filtrate was allowed to stand at room temperature under a fume cupboard until most of the water was evaporated. The resulting slurry was then concentrated to dryness at reduced pressure. The dried extracts were assessed for *in vitro* antimycobacterial activities.

Determination of Antimycobacterial Activity

Each crude plant extract was tested for antimycobacterial activity using the agar plate (proportion) method [8-10]. The proportion method determines the proportion of the bacterial population that is resistant to the plant extract tested. When 1 % or more of bacteria tested are resistant to the drug (plant extract), the population is considered resistant. The minimum inhibitory concentrations of the plant extracts with antimycobacterial activity were determined by the same method. Varying concentrations of the plant

extracts (10.0, 7.5, 5.0, 4.5, 4.0, 3.5, 3.0, 2.5, 2.0, 1.5, 1.0, 0.5, 0.25 and 0.1 mg/ml) were incorporated into the growth medium.

Preparation of Inoculum for the Agar Plate (Proportion) Method

Standard inoculum was prepared in physiological saline containing 0.5 % Tween 80 to obtain a concentration of 1mg/ml (wet mass) [10]. Growth of the bacilli was picked from the culture slope using a sterile applicator stick. This was transferred into a sterile screw-capped bottle containing 6-8 glass beads and 4 ml of physiological saline containing 0.5 % Tween 80. The mixture was homogenized by shaking for 5 minutes. Large particles were allowed to settle and more broth was added and turbidity adjusted to McFarland no. 1 standard. The suspension was diluted to 1×10^{-2} mg/ml and 1×10^{-4} mg/ml. To each bottle containing plant extract (10 mg/ml), 0.2 ml of the 1×10^{-2} mg/ml inoculum was inoculated. For the control (medium without plant extract), 0.2 ml of each of the 1×10^{-2} and 1×10^{-4} mg/ml were inoculated. All tubes were incubated at 37°C for 8 weeks. The experiments were done in duplicates.

Interpretation of Results

The number of colonies that were growing on the medium with the plant extracts, N^{-2} for the dilution 1×10^{-2} mg/ml, was compared with the growth in the control series, (no plant extracts), NO^{-2} for 1×10^{-2} mg/ml and NO^{-4} for 1×10^{-4} mg/ml. The following criteria were used for the interpretation of results [10]. If:

- (i) $N^{-2} \geq NO^{-2} = \text{Resistant.}$
- (ii) $NO^{-4} \leq N^{-2} \leq NO^{-2} = \text{Partially Susceptible.}$
- (iii) $N^{-2} \leq NO^{-4} = \text{Sensitive (< 1 \% growth).}$

Isolation of Active Fractions from *Pterocarpus erinaceus*

Group testing for the chemical group(s) responsible for the activity was also carried out after isolation of the active fractions. The crude methanolic extract of *Pterocarpus erinaceus* obtained from sequential solvent extraction was subjected to solvent/solvent separation (Figure 1).

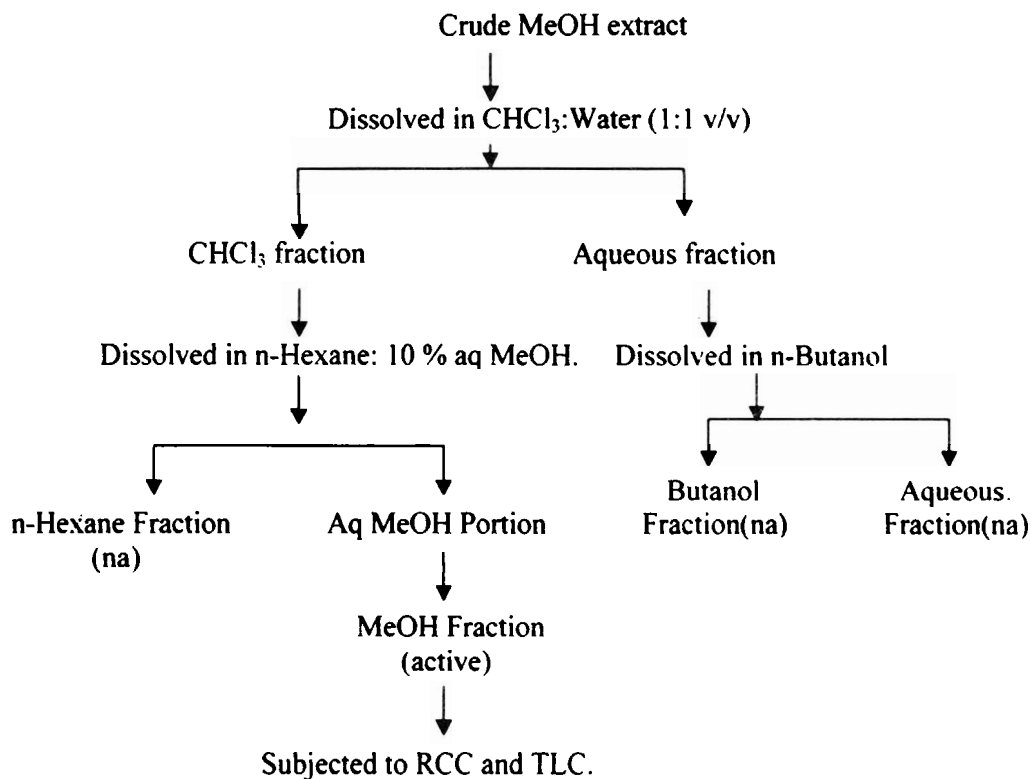


Figure 1: Fractionation of Crude Methanol Extract of *Pterocarpus erinaceus*

na : not active; MeOH : methanol; CHCl₃ :chloroform; RCC : repeated column chromatography; TLC : thin layer chromatography.

Active fractions were further subjected to column chromatography eluting with hexane, ethyl acetate, acetone and methanol. Fractions were monitored by TLC (hexane:ethylacetate:acetone) (15:3:2) using iodoplatinate as spray reagent and ultra violet light (T2202, SIGMA Chemical Company).

The crude hexane extract of *Pterocarpus erinaceus* from sequential extraction also exhibited *in vitro* antimycobacterial activity. This extract was purified by solvent/solvent separation as shown in Figure 2. Fractions were also monitored by TLC (Hexane:Chloroform:Dichloromethane) (4:4:2) using iodoplatinate as spray reagent and ultra violet light (T2202, SIGMA Chemical Company).

RESULTS

The preliminary screening of the crude methanolic extracts of parts of *Pterocarpus erinaceus* indicated that only the stem bark extract exhibited

activity against the test organisms with MICs of 1.0 mg/ml, 2.5 mg/ml, 2.5 mg/ml and 2.5 mg/ml, against *M. smegmatis*, *M. tuberculosis*, *M. bovis* and *M. avium-complex*, respectively. The leaves and root bark extracts showed no *in vitro* antimycobacterial activity. The powdered stem bark material was subjected to sequential solvent plant extraction.

The MICs of crude extracts obtained by sequential extraction and fractions isolated from the crude extracts are shown in Table 1. Only the hexane crude extract inhibited all the 4 test organisms with MICs ranging between 0.5 mg/ml and 1.0 mg/ml. The methanol crude extract inhibited only 3 of the test organisms (MICs ranging between 0.5 mg/ml and 1.0 mg/ml).

The ethyl acetate fraction isolated from the hexane crude inhibited the 4 test organisms with MICs ranging between 0.25 mg/ml and 1.0 g/ml.

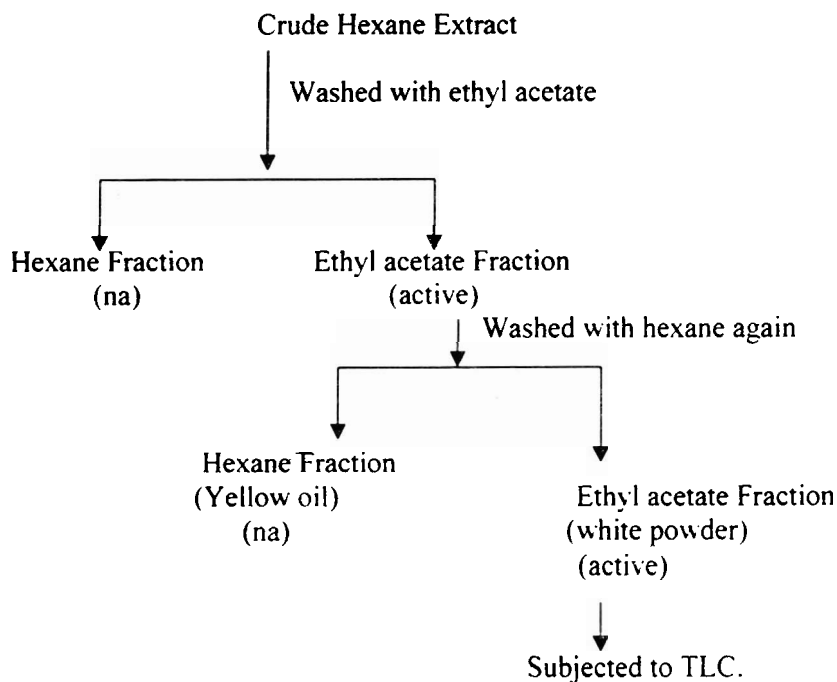


Figure 2: Fractionation of Crude Hexane Extract of *Pterocarpus erinaceus*

na = not active; TLC = thin layer chromatography

Methanol and water fractions isolated from the methanol crude exhibited antimycobacterial activity against the test organisms. The methanol fraction inhibited the 4 test organisms with MICs ranging between 0.25 mg/ml and 2.0 mg/ml, while the water fraction inhibited 2 of the test organisms (MICs ranging between 0.5 mg/ml and 2.0 mg/ml). Note that only the results of fractions with activity are shown in Table 1.

Group testing of the isolated fractions for major chemical groups indicated that flavanols and terpenes/sterols were detected in fraction from hexane crude and, tannins and terpenes/sterols were detected in the methanol fraction isolated from the methanol crude extract.

DISCUSSION

The initial screening of the crude methanolic extracts of *P. erinaceus* (stem bark) exhibited varying degrees of activity against *M. tuberculosis*, *M. bovis*, *M. smegmatis* ATCC 607 and *M. avium-complex*. Literature search has not revealed previous studies on the antimycobacterial activities of this plant. The results of this study show that the stem bark of the plant possesses

antimycobacterial activity and justifies its use by traditional healers in the treatment of TB and /or some of its symptoms. Among the extracts obtained by sequential solvent extraction, the hexane and methanol fractions were found to be the most active against the test organisms (Table 1) inhibiting all and 3 of the test organisms. The MIC values ranged between 0.5 mg/ml and 3.0 mg/ml. The activity of the methanol fractions may be due to the fact that methanol being a polar solvent extracts a wide range of bioactive plant compounds such as terpenoids and tannins [11], quassinoids [12], lactones [13], flavones [14, 15], phenones [16] and polyphenols [17]. This might explain the activity exhibited by the methanol fractions against the test organisms in this study. The MIC values of the hexane fraction on the test organisms ranged between 0.5 mg/ml and 1.0 mg/ml. This correlates with the result of a previous study [4] where hexane extract of *Psoralea corytifolia* from sequential solvent extraction was found to be the most active against *Mycobacterium* species tested (MIC = 31.25 µg/ml). Although hexane is a non-polar solvent, it can extract biologically active components such as terpenes [18].

Table 1: Minimum Inhibitory Concentrations of Crude Extracts and Isolated Fractions from the Stem Bark of *Pterocarpus erinaceus* against four Mycobacterial species (mg/ml).

Test Organisms	^a Extracts from sequential solvent plant extraction					^b Fraction from Hexane crude	^c Fractions from methanol crude	
	Hex	Ch	Acet	Met	Wat	Eacet	Met	Wat2
<i>M. smegmatis</i>	1.0	-	3.5	-	2.5	1.0	2.0	-
<i>M. tuberculosis</i>	0.5	5.0	-	0.5	-	0.25	0.25	0.5
<i>M. bovis</i>	0.5	-	5.0	0.5	3.0	0.5	1.0	-
<i>M. avium-complex</i>	1.0	-	-	1.0	-	1.0	1.0	2.0

a : Extracts obtained by sequential solvent extraction. b : Fraction obtained from hexane crude. c : Fraction obtained from methanol crude. Hex : Hexane crude: Ch : Chloroform crude: Acet : Acetone crude: Met : Methanol crude: Wat : Water crude. Eacet : Ethyl acetate fraction: Met2 : Methanol fraction: Wat2 : Water fraction. Hexane and Methanol crude refer to extracts from sequential solvent extraction

Although water is a polar solvent, the activities of the aqueous extracts in this study indicate that the stem bark extract of *P. erinaceus* exhibited activity against *M. smegmatis* and *M. bovis* only. The possible explanation might be that methanol might have removed most of the active components before water was used [18].

The antimycobacterial activities of fractions obtained through bioassay-guided fractionation are also shown in Table 1. The stem bark of *P. erinaceus* yielded flavanols and terpenes/sterols as active components from the hexane crude and terpenes/sterols and tannins from the methanol crude. Tannins were reported to form a complex with proteins [19, 20] and inactivate microbial adhesins; enzymes and cell wall envelop transport proteins [21]. Terpenes/sterols were also detected in the active fraction isolated from the methanol extract of stem bark of *P. erinaceus*. The presence of terpenes/sterols and flavanols might be responsible for the activity of the fraction on the test organisms. In addition to the biological activity of terpenes as previously described, the fraction from hexane extract also contains flavanols. These compounds were reported to possess activity against a wide range of microorganisms including bacteria [22].

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