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Phytochemical and Antimicrobial Properties of the Hexane and Ethanol Leaf Extract of *Pterocarpus Erinaceous* poir (Fabaceae)

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Authors' contributions

This work was carried out in collaboration between all authors. Authors UMJ, GI and UHD designed the study, wrote the protocol and the first draft of the manuscript. Authors UMJ, MM, AA and SII managed the literature searches, analyses of the study performed the phytochemical and antimicrobial analysis in this study. Authors UMJ and AA identified the species of plant. All authors read and approved the final manuscript.

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ABSTRACT

This study evaluated the phytochemical and antimicrobial properties of the hexane and ethanol leaf extract of *Pterocarpus erinaceous* poir (Fabaceae). Ethno medicinally, the leaves are used in treatment of conjunctivitis, diarrhea, malnutrition, inflammation and scabies. The phytochemical screening using standard methods revealed the presence of alkaloids, flavonoids, tannins, triterpenes and steroids. The antibacterial and antifungal activities of the extracts were tested against some gram positives and gram negatives clinical isolates and the activity was determined using well diffusion method with zones of inhibition ranges from 20-30 mm for hexane and 18-25 mm for ethanol. Minimum Inhibitory Concentration (MIC) of the extracts was determined at 0.5 Mc-farland's turbidity standard. The extracts at 2.5-50 mg/ml (Hexane extract) and 5.0-10 mg/ml (Ethanol extract) inhibited the growth of the clinical isolates. Minimum bactericidal and fungicidal

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concentration of the extracts range from 5-20 mg/ml (Hexane extract) and 5-10 mg/ml (Ethanol extract) Zones of inhibition of the extracts were comparable with that of standard drugs, Ciprofloxacin (30-42 mm) against bacteria and fluconazole (35-37 mm) against fungal isolates. In conclusion, the extracts have great potential antimicrobial activity and support the claimed traditional uses of the leaves in the treatment of bacterial and fungal infections.

Keywords: Phytochemicals; antimicrobial; zones of inhibitions; MIC; MBC; P. erinaceous poir.

1. INTRODUCTION

Plants role in the maintenance of good health cannot be overemphasized. Studies have shown that plants play a good role in the maintenance of good health [1]. Many modern pharmaceutical companies today use plants and plant-based products [2] and Plants have been generally utilized today for the treatment of disease worldwide according to [3], over 80% of the world populations depend on plants based medicine for their primary healthcare. [4] Observed that the majority of the populations in the developing countries still rely on herbal medicine to meet their health needs.

There is need to develop alternative antimicrobial medicine from plants for the treatment of infectious disease, because antimicrobial agents from plants have been found to have enormous advantage and therapeutics potentials [5]. Furthermore, the studies by [6] showed that antimicrobial agents from plants origin are effective in the treatment of infectious diseases and on the other hand mitigate many of the side effects that are linked with synthetic antimicrobial drugs.

The usefulness of the plant based products in medicine is due to the presence of bioactive compounds such as alkaloids, flavonoids, saponins, tannins, oils, triterpenes and steroids and other secondary metabolites which they contain and are capable of producing definite physiological actions in the body [7].

Pterocarpus erinaceous poir is a member of a plant family fabaceae. It has diameter of 1.2-1.8 m, it's commonly known as 'African rosewood''. In northern Nigeria, it is called 'Madobiya' or Shajini (Hausa), while in the South it is called 'Apepe' (Igbo) and ''Osun dudu'' (Yoruba). It's synonymous to *P. angolensis* and *P. echinatus* DC.

The aims of this research was to established phytochemical constituents of the leaves of *P. erinaceous* and their antimicrobial properties with the view to either support or debunk the traditional claims of using the leaves in the treatment of infectious diseases.

2. MATERIALS AND METHODS

2.1 Plant Collection, Identification and Preparation

Fresh leaves of *P. erinaceous* were collected around Samaru village in Sabongari local government area of Kaduna state, Nigeria, in July, 2013. It was identified by the taxonomist (Mallam Umar Gallah) at the herbarium section of the Department of Biological Sciences, Ahmadu Bello University, Zaria, Nigeria. It was assigned a voucher specimen number of 900063 and deposited for future reference. The plant was dried at room temperature, powdered and sieved to a suitable size and stored at a room temperature in a closed container for future use.

2.2 Extraction of Plant Materials

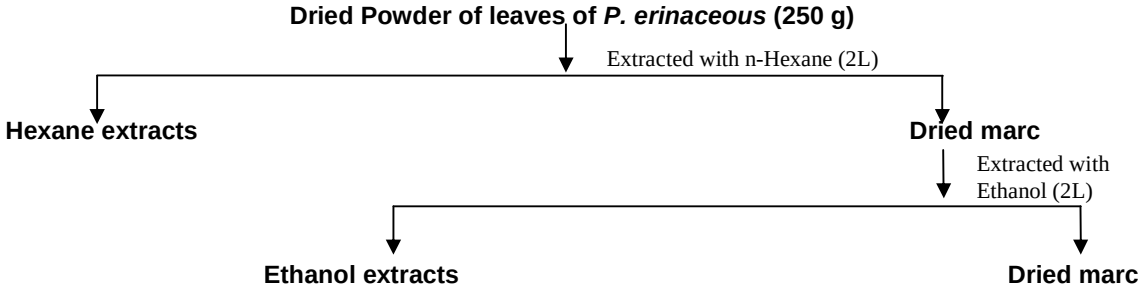

Fig. 1. Flowchart of extraction profile of *P. erinaceous* leaves

2.3 Phytochemical Screening of the Leaf Extract

The leaf extract of both hexane and ethanol were subjected to phytochemical screening in order to identify their chemical constituents by using the methods described by [8,9].

2.4 Collection and Preparation of Clinical Isolates

Eight (8) antimicrobial clinical isolates were collected from Department of Medical Microbiology, Ahmadu Bello University Hospital (ABUTH), Shika. The clinical isolates used include: *Staphylococcus aureus, Escherichia coli, Proteus mirabilis, Klebsiella pneumonia, Pseudomonas aeruginosa, Bacillus subtilis, Candida albicans* and *Candida krusei*. Viability test of each isolate was carried out by resuscitating the buffered peptone broth and thereafter sub-cultured into nutrient agar medium and incubated at 37℃ for 24 hours.

2.5 Preparation of Stock Solution

Hexane and ethanol extracts (0.2 g) each of *P. erinaceous* were weighed and dissolved in 10 ml of DMSO to obtain a concentration of 20 mg/ml as an initial concentration. From the stock solution, two-fold serial dilution was made to obtain 10 mg/ml, 5 mg/ml, 2.5 mg/ml and 1.25 mg/ml concentration of each extracts. Standard drugs Ciprofloxacin and Fluconazole were used as the positive control for antibacterial and antifungal agents respectively [10].

2.6 Preparation of Culture Media

Culture media for both antibacterial and antifungal was prepared using Muller Hinton agar. Sensitivity test of the organism to the hexane and ethanol extract was carried out according to [10,11].

2.7 Determination of Zone of Inhibition

Solution of 0.5 ml of the 20 mg/ml of each extract was introduced in to each of the wells made on the medium. All the media were then inoculated and incubated at 37℃ for 24 hours and the zone of inhibition was measured using a transparent ruler and the result was recorded in millimeter and the results were recorded in Table 3.2.

2.8 Minimum Inhibition Concentration (MIC)

The minimum inhibitory concentration of the extracts was carried out using the broth dilution method [12]. Mueller Hinton broth was prepared, 10 ml was dispensed into test tubes and sterilized at 121℃ for 15 minutes, and the broth was allowed to cool. Mc-farland's turbidity scale number 0.5 was prepared to give turbid solution. Normal saline was prepared and 10 ml was dispensed into sterilized test tube and the test microbes was then inoculated and incubated at 37℃ for 6 hours. Dilution of the test micro organism was done in the normal saline until the turbidity marched with that of the Mc farland standard by visual comparison and the concentration was determined at that point. Two-fold serial dilution of the extracts in the sterile broth was prepared and concentrations were obtained as 20 mg/ml, 10 mg/ml, 5 mg/ml, 2.5 mg/ml, and 1.25 mg/ml and the initial concentration was obtained by dissolving 0.2 mg of the extract in 10 ml of the sterile broth.

2.9 Minimum Bactericidal and Fungicidal Concentration (MBC/MFC)

Minimum bactericidal and fungicidal concentration was carried out to determine whether the test microbes were killed or their growth was inhibited. Mueller Hinton agar was prepared, sterilized at 121℃ for 15 minutes and poured into sterilized Petri dishes and was allowed to cool and solidify. Then contents of the MIC in the serial dilution was then sub-cultured onto the prepared medium and the medium was incubated at 37℃ for 24 hours, after each plate of the medium was observed for colony growth, MBC/MFC was determined as the plates with lowest concentration of the extracts without colony growth [12].

2.10 Statistical Analysis of Data

Data collected were statistically analyzed using Student t-test at $P < 0.05$ to compare the zones of inhibition displayed by each extract with the standard drugs against each clinical isolates.

3. RESULTS

3.1 Extraction of Plant Materials

The mass and percentage yield of hexane extract was (4.80 g and 1.92%) and that of the

ethanol extract (9.60 g and 3.84%) were obtained from the 250 g leaf powder of *P. erinaceous* (Table 3.1).

Table 3.1. Mass and percentage yield of the leaves extracts of *P. erinaceous* (250 g)

S/N	Extract	Mass (g)	Yield (%w/w)
1.	Hexane	4.80	1.92
2.	Ethanol	9.60	3.84

3.2 Phytochemical Screening of the Leaves extract of *P. erinaceous*

Phytochemical screening of the extracts indicated the presence of alkaloids, flavonoids, tannins, saponins, triterpenes, steroids and cardiac glycosides in both extracts while flavonoids and saponins were absent in hexane extract but present in ethanol extract.

3.3 Zones of Inhibition (mm)

The hexane and ethanol leaf extract of *P. erinaceous* show antibacterial and antifungal activities on the test organisms used, zones were significant ($P < 0.05$) and ranged from 20-30 mm with hexane extract and 18-25 mm with ethanol extract compared to controls (Ciprofloxacin and Fluconazole) with zone of inhibitions of 30-42 mm and 35-37 mm respectively. Zones of inhibition of the extract against test microorganisms were higher for hexane extract than that of the ethanol extract.

3.4 Minimum Inhibitory Concentration (MIC)

The minimum inhibitory concentration (MIC) was 5mg/ml for the hexane extract against all pathogens except for *B. subtilis* and *Kleb. Pneumoniea* which was observed at 2.5 mg/ml. whereas, MIC of the ethanol extract was observed at 5 mg/ml against all pathogens except for *P. mirabilis* at 10 mg/ml and the fungal isolates *C. albicans* and *C. krusei* which was observed at 10 mg/ml. Hexane extract had exhibited better activity at MIC of 2.5 mg/ml compared to ethanol extract at MIC of 5 mg/ml (Table 3.4).

Table 3.2. Some phytoconstituents from the hexane and ethanol extracts of *P. erinaceous*

Constituents	Tests	Observation	Inferences	
Alkaloids	Dragendorff	Orange precipitate	+	+
	Mayers	Whitish precipitate	+	+
	Wagners	Brown precipitate	+	+
Flavonoids	Shinoda	Pink/Red coloration	-	+
Tannins	Ferric chloride	Greenish coloration	-	+
Saponins	Frothing	Honeycomb-like	-	+
Triterpenes	Liebermann Burchard	Purple to violet ring formation	+	+
Steroids	Salkowski	Red precipitate	+	+
Cardiac glycosides	Kella killiani	Appearance of reddish brown color	-	+
Anthracenes	Bontragers	Pink red to violet color	-	-

+ = Present, - = Absent

Table 3.3. Zones of inhibition (mm) of hexane and ethanol leaf extract of *P erinaceous*

Test organisms	Zones of inhibitions (mm)			
	Hexane	Ethanol	Ciprofloxacin	Fluconazole
Staph. aureus	25*	22*	32	0
Bacillus subtilis	30*	25*	40	0
Escherichia coli	22*	20*	35	0
Kleb. pneumoniea	27*	21*	42	0
Proteus mirabilis	20*	18*	30	0
Pseud. aeruginosa	0	0	31	0
Candida albicans	24*	19*	0	35
Candida krusei	20*	18*	0	37

** Significant at P < 0.05*

Table 3.4. Minimum inhibitory concentration of hexane and ethanol extracts

Organisms	Hexane extract (mg/ml)					Ethanol extract (mg/ml)				
	20 ml	10 ml	5 ml	2.5 ml	1.25 ml	20 ml	10 ml	5.0 ml	2.5 ml	1.25 ml
Staph.aureus	-	-	-	*	++	-	-	*	+	++
B.subtilis	-	-	*	+	++	-	-	*	++	+++
E. coli	-	-	*	+	++	-	-	*	++	+++
K. pneumoni	-	-	-	*	++	-	-	*	+	++
P. mirabilis	-	-	*	-	+	-	*	+	+	++
C. albicans	-	-	*	+	++	-	*	+	++	+++
C. krusei	-	-	*	+	++	-	*	+	+	++

*KEY: - = Clear (No growth), * = MIC, + = Turbid (Light growth), ++ = (Moderate turbid), +++ = (High turbidity) 0 = Resistant*

Table 3.5. Minimum bactericidal/fungicidal concentration of hexane and ethanol extra

Organisms	Hexane extract (mg/ml)					Ethanol extract (mg/ml)				
	20 ml	10 ml	5 ml	2.5 ml	1.25 ml	20 ml	10 ml	5.0 ml	2.5 ml	1.25 ml
Staph.aureus	-	*	+	++	++	*	*	+	++	+++
B. subtilis	-	-	-	*	++	-	*	++	+++	+++
E. coli	*	+	++	+++	+++	*	+	++	+++	+++
K. pneumoni	-	*	+	++	+++	*	+	++	+++	+++
K. pneumo	-	*	+	++	+++	*	+	++	+++	+++
P. mirabilis	*	+	++	++	+++	*	+	++	+++	+++
P. aeruginos	0	0	0	0	0	0	0	0	0	0

*Key: - = No growth, * = MIC/MFC, + = Light growth, ++ = Moderate colonies growth, +++ = Dense colony growth, 0 = Resistant*

3.5 Minimum Bactericidal/ Fungicidal Concentration (MBC/MFC)

Hexane had a better activity against gram positive *B. subtilis* at MBC of 5 mg/ml followed by MBC/MFC of 10 mg/ml against *Staph. aureus, Kleb. Pneumoniea* and *C. albicans* whereas, *E. coli, P. mirabilis* and *C. krusei* were observed at an MBC/MFC of 20 mg/ml. Ethanol extract was general at 20 mg/ml against all isolates except gram positive *Staph. Aureus* and *B. subtilis* with exhibited strong activity at 10 mg/ml (Table 3.5).

4. DISCUSSION

This study indicated that the ethanol had high extractive yield values of (3.84%w/w) compared to hexane which had extractive yield values of (1.92%w/w) depicted in Table 3.1. The composition of chemical constituents depends upon the nature of the drugs and the solvent used. It also gives an indication whether the crude drug is exhausted or not [13].

Phytochemical analysis of the leaves extracts had reveals the presence of some secondary metabolites like alkaloids, tannins, flavonoids, cardiac glycosides, saponins (triterpenes and steroids) whereas; tannins and anthracenes were absent (Table 3.2). This result corresponded with the finding of [14] in the phytochemical screening of *P. erinaceous*.

The presences of phenolic compounds which are known to have antimicrobial activity were revealed in the plant. This therefore, supported the use of the plant in the traditional treatment of conjunctivitis, venereal diseases, urinary tract infection and diarrhea [15].

The Hexane and Ethanolic extracts of plant had inhibited the growth of the test microorganism (Table 3.3). The antimicrobial activity of other plants has been reported by [16,2]. The ability of plant extract to inhibit the growth of the microorganism may be as a result of the bioactive compounds such as flavonoids, saponin, alkaloids, tannins and phenols in their leaves [17]. Some of which were observed in this study. Research findings indicated that the antimicrobial properties of plants are conferred on them by the presence of secondary metabolites [18,7]. Plants are rich in a wide variety of secondary metabolites such as tannins, terpenoids, alkaloids, flavonoids, etc., have been found *in vitro* to have antimicrobial properties [19].

Antibacterial activity was expressed as diameter of zones of inhibition (Table 3.3). A zone of

observable inhibition of growth of each micro-organism served as a criterion for declaring an extract sensitive and was indicated by a clear zone around the well. The extent of antimicrobial activity of the extracts based on the diameter zones of inhibition has been described as low (12-18 mm), moderate (19-22 mm) and strong activity (23-38 mm) by [20]. The diameter of zones of inhibition of extract against test microorganisms (in mm) was highest for hexane extract with zone of 30 mm exhibited against gram positive *B. subtilis* compared to standard drug with the highest zone of inhibition of 40mm, this was also observed in antimicrobial studies of the same plant (*P. erinaceous*) hexane had a zones of inhibition range of 20-30 mm against *B.subtilis* and 30mm against *Staph. aureus* and *C. albicans* [14].

The antimicrobial properties of substances are desirable tools in the control of infections and in food spoilage [21]. Minimum inhibitory concentration (MIC = 2.5 mm) of hexane extract was observed and higher against gram positive *B. subtilis and* gram negative *Kleb. Pneumoniea* this is in lined which the studies carried out by [22] in the antimicrobial studies of the leaves of *Stylochiton loncifolius*. Whereas, (MIC =5 mm) of hexane extract against *Staph. aureus, E. coli, Proteus mirabilis, C. albican and C. krusei.* The minimum inhibitory concentrations (MIC = 5mm) of ethanol extract against *Staph. aureus, B. subtilis, E. coli, and Kleb. Pneumonia* was observed at 5mm while, MIC of ethanol extract against *P. mirabilis, C. albican and C. krusei* was observed at 10 mm. the above MIC was also observed in the antimicrobial screening of *P. santalinoids* ranges from 5.0-10 mg/ml studied by [23] (Table 3.4).

Minimum bactericidal and fungicidal concentration (MBC = 5 mg/ml) of hexane extract was observed with a higher activity against gram positive *B. subtilis* while MBC/MFC= 10 mg/ml was observed against *Staph. aureus, Kleb. Pneumoniea* and *C. albicans.* Ethanol extract was observed at (MBC/MFC= 10 mg/ml) against gram positive *Staph. Aureus* and *B. subtilis* whereas, all the remaining isolates tested in both hexane and ethanol extracts were observed at MBC/MFC =20 mg/ml (Table 3.5).

5. CONCLUSION

Antimicrobial activity ranges from moderate to strong activity displayed by the leaf extracts of *Pterocarpus erinaceous* had shown that the plant leaf has significant antimicrobial activity that may explain and support the traditional use in the treatment of microbial infections.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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