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SHORT COMMUNICATION

Isolation and Characterization of Friedelin and 5-DodecylResorcinol from the Stem Bark Extract of *Pterocarpus erinaceus*

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Abstract

Pterocarpus erinaceus (Fabaceae) is a medicinal plant used in the treatment of several diseases such as, fever, diarrhea, and dysentery. The aim of this research work is to isolate phytochemicals, evaluate antimicrobial and cytotoxic activities of the extract/isolated compounds from the stem bark of *Pterocarpus erinaceus*. The plant material was air dried and extracted with a polarity gradient of petroleum ether, ethyl acetate, and methanol. Purification of the methanol extract using column chromatography yielded two compounds: friedelin (**1**) and 5-dodecylresorcinol (**2**). Antimicrobial screening of the extracts and isolated compounds was conducted using the disc diffusion method, while the cytotoxicity was tested on the extracts using Brine Shrimp larvae. The methanol extract was more toxic (LC_{50} of 0.93 µg/mL) than petroleum ether (PER-PE) and ethyl acetate (PER-EA) extracts. Antimicrobial screening of the plant extracts and compounds revealed a moderate activity at 1000 µg/mL.

Keywords: *Pterocarpus erinaceus*, friedelin, resorcinol, cytotoxicity, microbes

INTRODUCTION

Plants have formed the basis of a traditional medicine system that has been used for thousands of years. They represent the principal means of therapy in traditional medicine, and the plant kingdom has long served as a prolific source of useful drugs. African indigenous herbal medicines are widely used throughout the African continent, despite the apparent lack of scientific evidence for their quality, safety, and efficacy (Amos *et al.*, 1998). The chemical constituents in medicinal plants usually explain the rationale for the efficacy of the plants in traditional medicine (Benzie and Wachtel-Galor, 2011). Phytochemists exploit medicinal plants and isolate bioactive compounds from which different analogues are synthesised with the aim of obtaining agents with better activity or even different biological properties (Dawurung *et al.*, 2021; Chikezie *et al.*, 2015).

Pterocarpus erinaceus is a deciduous legume tree of Africa's savannahs and dry forests, famous for producing one of the finest woods in its native region. It also produces leafy fodder high in protein, which makes it an excellent animal feed crucial for survival of livestock during the dry season (Hutchinson *et al.*, 1958). Medicinal uses of the plant include the use of the leaves as a febrifuge, the bark for tooth and mouth diseases, and the bark resin as an astringent for severe diarrhoea and dysentery. The grated root is mixed with tobacco and smoked in a pipe as a cough remedy. It has also been found useful in the treatment of fever (Hutchinson *et al.*, 1958, Sandrine, 2006). In this work, the stem bark of *Pterocarpus erinaceus* is investigated for its cytotoxic and antimicrobial activities including the isolation and characterization of friedelin (**1**) and 5-dodecylresorcinol (**2**) from the stem bark of the plant.

MATERIALS AND METHODS

General Experimental Procedures

Petroleum ether (60-80°C) was redistilled before being used. Distilled petroleum ether (PE), chloroform ($CHCl_3$), ethyl acetate (EtOAc) and methanol (MeOH) were used as solvent systems in the chromatographic method. A cold extraction technique was used for the extraction of the sample. Gravity column chromatography (CC) was carried out using Merck silica gel 60 (70-230 mesh). Thin layer chromatography (TLC) was performed on 0.20 mm precoated silica gel aluminium sheets (Merck Kieselgel 60 F_{254}). Spots were visualised with UV light (254 nm and 365 nm) and exposed to the iodine crystal. The ^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz) spectra were recorded on a Bruker Avance 400 spectrometer. Chemical shifts were recorded in ppm relative to tetramethylsilane (TMS) in deuterated chloroform ($CDCl_3$). The infrared spectra were measured using ATR-FTIR. The melting point was measured on a Leica Gallen III Kofler micromelting point apparatus and was uncorrected.

Plant Material

The stem bark of *P. erinaceus* was collected at Alkaleri, Bauchi State, Nigeria in August 2015. A voucher specimen (BUKHAN 0369) was deposited in the herbarium of the Department of Plant Biology, Bayero University Kano, Nigeria.

Extraction and Isolation of Compounds

The coarse powder of the stem bark of *P. erinaceus* (800 g) was sequentially extracted with solvents of increasing polarities of PE, EtOAc, and MeOH, each for two weeks. A portion of the MeOH extract (5.0 g) was purified over a silica gel column (150 g silica gel; column size 121×2.5 cm) eluted with solvent mixtures of increasing polarity of PE:EtOAc, EtOAc: MeOH to afford 150 fractions. PER-ME-06 was obtained as a white solid (20 mg) that melted between 245-246°C with an R_f value of 0.43 (PE:EtOAc, 9:1). Meanwhile, further purification of fraction 106 (PER-ME-106) over silica gel column chromatography, (15 g silica, column size 30.0×2.8 cm) by elution with a gradient of $CHCl_3$:MeOH led to the isolation of PER-ME106-43 a brownish sticky substance (0.02 g) and an R_f value 0.72, ($CHCl_3$:MeOH, 4:1).

Spectral Data of Friedelin (1)

IR (ATR) v_{max} cm^{-1}: 2924, 2859, 1713; ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 0.77 (3H, s, H-28), 0.87 (3H, s, H-30), 0.88 (3H, s, H-29), 1.00 (3H, s, H-26), 1.01 (3H, s. H-27), 1.18 (3H, s, H-25) 1.25 (3H, s, H-24) 1.70 (3H, d, H-23), 1.90 (1H, t, H-6), 2.25 (2H, m, H-1), 2.31 (1H, s, H-4), 2.41 (2H, t, H-2); ^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm) 22.2 (C-1), 41.5 (C-2),

213.2 (C-3), 58.1 (C-4), 42.1 (C-5), 41.3 (C-6), 18.2 (C-7), 53.1 (C-8), 37.4 (C-9), 59.4 (C-10), 35.6 (C-11), 30.5 (C-12), 39.7 (C-13), 38.3 (C-14), 32.4 (C-15), 35.6 (C-16), 29.9 (C-17), 42.8 (C-18), 35.3 (C-19), 28.1 (C-20), 32.7 (C-21), 39.2 (C-22), 6.8 (C-23), 14.6 (C-24), 17.9 (C-25), 20.2 (C-26), 18.6 (C-27), 31.9 (C-28), 35.0 (C-29), 31.7 (C-30); EIMS *m/z* (%): 399 (17), 327 (42), 281 (83), 147 (58), 73 (100).

Spectral Data of 5-dodecylresorcinol (2)

IR (ATR) ν_{max} cm^{-1}: 3200, 2924, 1604, 1460, 1365; ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 7.54 (1H, d, J = 1.44 Hz, H-2), 7.34 (1H, d, J = 1.48 Hz, H-4), 7.13 (1H, d, *J* = 1.36 Hz, H-6), 2.63 (2H, t, H-1'), 1.62 (CH_2, m, H-2' to H-11'), 0.99 (3H, t, H-12'); ^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm) 147.1 (C-1), 124.5 (C-2), 147.1 (C-3), 119.1 (C-4), 123.9 (C-5), 119.1 (C-6), 34.8 (C-1'), 34.5 (C-2'), 31.9 (C-3'), 31.4 (C-4'), 30.2 (C-5'), 29.7 (C-6'), 29.3 (C-7'), 29.2 (C-8'), 29.1 (C-9'), 24.7 (C-10'), 22.6 (C-11'), 14.1 (C-12'); EIMS *m/z* (%): 249 ($M\text{-}29^{+}$, 5), 207 (25), 125 (22), 111 (42), 97 (67), 83 (72), 55 (75), 32 (100).

Cytotoxicity Assay against Brine Shrimp

Hatching Shrimp - Brine shrimp eggs (Artemix, GmbH & Co., Germany), *Artemia salina* were hatched in distilled water (16 g in 500 mL). After 24 h of incubation at room temperature, the larvae were attracted to one side of the vessel by a light source and collected by pipette. Larvae were separated from eggs by aliquoting them three times in small beakers containing seawater (Muhammad and Sirat, 2013).

Brine Shrimp Assay - The toxicity of the extract was monitored by the brine shrimp lethality test according to the method of Muhammad and Sirat (2013) with slight modification. Each of the extracts (1 mg/mL) was dissolved in methanol, from which 5 000, 500, and 50 µL of each solution were transferred into vials corresponding to 1000, 100, and 10 µg/mL respectively. This was allowed to evaporate to dryness in about 24 h at room temperature. Each dosage was tested in triplicate (9 per test sample). Seawater (4 mL) and 10 larvae were introduced into each vial. The final volume of solution in each vial was adjusted to 5 mL with seawater immediately after adding the shrimp. A negative control was prepared as a drug-free and potassium dichromate was used as a positive control. Survivors were counted after 24 h, and LC_{50} was determined by probit analysis using SPSS version 16.

Antimicrobial Disc Diffusion Assay

The antimicrobial activities of the test samples were determined by the agar disc diffusion method (Karaman *et al.*, 2003). The suspension (400 µL) of the test bacteria and fungi, which were spread on the nutrient agar and sabouraud dextrose agar, respectively. The disc (6 mm diameter) impregnated with 10 µL of the extracts and DMSO (negative control) was placed on the inoculated agar, which was incubated for either 24 h at 37°C (bacteria) or 48 h at 30°C (fungi). Ciprofloxacin (10 µg/mL) and ketoconazole (100 IU) were used as the positive controls for bacteria and fungi, respectively. Clear inhibition zones around the discs indicate the presence of antimicrobial activity. All tests and analyses were carried out in triplicate.

RESULTS AND DISCUSSION

The methanol extract from the stem bark of the *P. erinaceus* led to the isolation of a white solid (**1**) that melted between 245-246°C and a sticky brownish substance (**2**). Figure 1 shows the chemical structures of the isolated compounds.

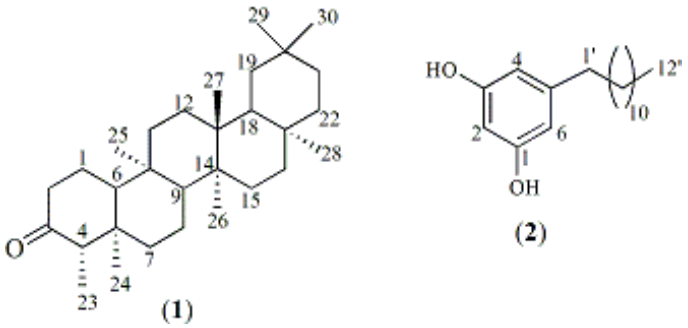

Figure 1. Chemical structures of isolated compounds

The white solid substance shows an IR absorption band at 2924 cm^{-1} associated with C-H stretching, while absorption at 1713 cm^{-1} indicates the presence of a ketonic carbonyl group. ^{1}H NMR data displayed a cluster of signals between 0.72 to 1.56 ppm assigned to triterpenic protons, while the signals observed at 2.31 (s) and 2.41 (t) ppm are associated with hydrogens adjacent to the carbonyl group. Information obtained from the ^{13}C NMR spectrum showed 30 carbon signals, suggesting the presence of a typical triterpene skeleton. An observable signal at 213.2 ppm confirmed the presence of ketonic carbonyl, while other carbon signals (6.8 to 59.5 ppm) were consistent with that of a pentacyclic triterpene as reported by Queiroga et al., (2000). The DEPT spectrum substantiates the compound as a friedelane type through the classification of carbon atoms into 8 methyl, 11 methylene, 4 methine, and 7 quaternary carbon atoms. 2D NMR data confirmed proton couplings in the COSY spectrum, while the proton-carbon connections were also revealed as indicated in HSQC and HMBC spectra. Evidence from mass spectrometry has shown a characteristic friedelane type triterpene fragment ion as presented in Figure 2. The spectral data and literature comparison led to its identification as friedelin (**1**) (Odeh *et al.*, 2016; Queiroga *et al.*, 2000).

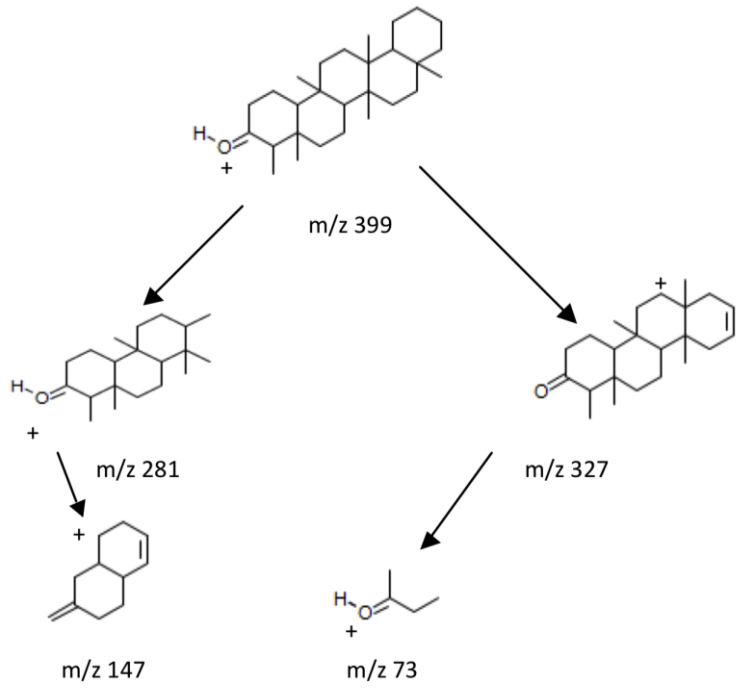

Figure 2. MS fragmentation of PER-ME-06

Further purification of fraction 106, PER-ME-106, from the MeOH extract over silica gel CC afforded the sticky brownish substance (0.02 g) R_f value of 0.72, ($CHCl_3$:MeOH, 4:1). The IR data of the compound shows absorptions at 3200, 2924, and 1605 cm^{-1} that are associated with O-H, C-H, and C=C (aromatic) stretching, respectively. The presence of aromatic compounds was supported by observed signals from ^{1}H NMR at δ 7.54 (1H, d, J = 1.44 Hz, H-2), 7.34 (1H, d, J = 1.48 Hz, H-4), 7.13 (1H, d, *J* = 1.36 Hz, H-6), which corresponded to 124.5 (C-2), 119.1 (C-4), 119.1 (C-6) in the ^{13}C NMR data, respectively. The downfield signals from NMR data indicated the presence of an aromatic group, whereas the upfield signals indicated the presence of an aliphatic group, suggesting an alkylated phenol. The compound revealed the presence of an alkyl group in the range of δ 0.99-2.63 and δ 14.1-34.8 from the ^{1}H NMR and ^{13}C NMR data, respectively. The compound's MS data showed the presence of fragment ions with m/z values of 111 ($C_6H_7O_2^+$), 125 ($C_7H_9O_2^+$), 207 ($C_6H_5O_2C_7H_{15}^+$) and 249 ($C_6H_5O_2C_{10}H_{21}^+$) supporting an alkylated phenol, while m/z value 249 suggested $[M-29]^+$ molecular ion that is associated with the loss of a C_2H_5 fragment. These were further supported by the appearance of 12 signals for carbon atoms from ^{13}C NMR data in the upfield region.

Thus, compound (**2**) was proposed based on the available data from IR, MS, ^{1}H NMR, and ^{13}C NMR as a phenolic compound, 5-dodecylresorcinol. A similar resorcinol were reported from the leaves of *Arbidia sieboldii* (Shahinozzaman *et. al.*, 2019) and the bark of *Myristica fatua* (Megawatta, 2017), however, this is the first report of the resorcinol isolation from the stem bark *P. erinaceus*.

Brine Shrimp Lethality Test

The toxicity of the plant extracts was tested at concentrations of 10, 100, and 1000 µg/mL against ten shrimp in each of three replicates, and the results were expressed as LC_{50} values in µg/mL. Syahmi *et al.* (2010) reported that extracts resulting in LC_{50} values of less than 1000 µg/mL are considered significantly active. Thus, all the tested extracts suggested being active, as indicated in Table 1. The MeOH extract (PER-ME) with 0.93 µg/mL is the most active in comparison with EtOAc (PER-EA) and PE (PER-PE) extracts.

Table 1. Brine Shrimp Lethality Test Result

Samples	**LC_{50} (µg/mL)**
PER-PE	2.89
PER-EA	1.30
PER-ME	0.93

Antimicrobial Screening

The extracts and isolated compounds were investigated on selected bacterial and fungal strains using disc diffusion method. The samples exhibited different degrees of inhibition against the tested organisms in the range of 8-26 mm and 8-20 mm for the bacterial and fungal strains, respectively. The highest inhibitory effect of 26 mm was observed from PER-ME against *S. aureus*, and a similar inhibition was recorded for the same extract against *E. coli* of 25 mm. The isolated compounds have demonstrated remarkable bacterial growth inhibitory effects against *S. aureus* with values of 17 and 14 mm for (**1**) and (**2**), respectively.

The more potent antimicrobial effect of the tested samples was observed from the distribution of their antifungal properties as showed in Table 2. The extracts were found to be active (8-20 mm) at all the tested concentrations, except for PER-EA at 250 µg/mL, while the isolated compounds have indicated a promising inhibitory effect of compound **1** (10 mm) and compound **2** (12 mm) against *A. flavus*.

Table 2. Antimicrobial activity of samples from *Pterocarpus erinaceous*

Samples	Conc. (µg/mL)	Zone of inhibition (mm) against microorganism			
		S	**E**	**A**	**M**
PER-PE	1000	20	-	20	19
	500	18	-	17	18
	250	-	-	08	12
PER-EA	1000	10	-	14	13
	500	-	-	14	09
	250	-	-	-	-
PER-ME	1000	26	25	20	18
	500	12	10	18	19
	250	-	08	17	15
(1)	1000	17	-	10	-
	500	-	-	-	-
	250	-	-	-	-
(2)	1000	14	8	12	11
	500	09	-	-	08
	250	-	-	-	-
Ciprofloxacin		24	24		
Ketoconazole				30	30

PER = *Pterocarpus erinaceous;* PE = petroleum ether; EA = ethyl acetate; ME = methanol; S = *Staphyloccocus aureus*; E = *Escherichia coli*; M = *Mucor spp*; A = *Aspergillus flavus*; - = not active

CONCLUSION

From the results obtained, it can be concluded that the stem bark of *P. erinaceus* contains toxic substances and possesses antibacterial activities against *Staphylococcus aureus*, *Escherichia coli;* and also antifungal activities on *Aspergillus niger*, *mucor spp*. The isolated compounds PER-ME-06, friedelin exhibited a moderate antimicrobial activity against *S. aureus* (17 mm) and *A. niger* (10 mm) at 1000 μg/ml, the same organisms were found to be inhibited by the 5-dodecylresorcinol at 14 mm and 12 mm, respectively.

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