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Antibacterial and Radical Scavenging Activity of Fatty Acids from *Paullinia pinnata* L.

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ABSTRACT

Twelve fatty acids identified from the methanol extract of *Paullinia pinnata* L. root extract by the use of Gas Chromatography coupled with mass spectrophotometer were screened for antibacterial activity against both Gram positive and Gram negative bacteria as well as three resistant strains of *Staphylococcus aureus SA1199B, XU212* and *RN4220* resistant to norfloxacin, tetracycline and erythromycin respectively. Using the 96-well microtitre plate method, azelaic acid (a dicarboxylic acid), showed the highest activity against all organisms tested with minimum inhibitory concentration range of 32-256 µg/ml while palmitic, oleic, eicosanoic and stearic acids also showed weak to moderate activity against *Staph. aureus*, *B. subtilis*, *E. coli* and *P. aeruginosa*. However, docosanoic, tetradecanoic and eicosenoic acids had no activity against all organisms tested. The results also indicated that while the crude methanol extract possess potent antioxidant activity by bleaching DPPH radical (IC_{50} of 3.8µg/ml), the fatty acids did not show any significant radical scavenging action.

KEYWORDS: Antibacterial, DPPH, Fatty acids, Free radical, *Paullinia pinnata.*

INTRODUCTION

Infectious diseases caused by bacteria, fungi, parasites and viruses are still a major threat to public health, despite the huge progress in human medicine. Their impact is particularly large in developing countries due to unavailability of medicines and the emergence of widespread drug resistance (1). Natural products especially, those used in ethnomedicine provide a major source of innovative therapeutic agents for various conditions including infectious diseases.

P. pinnata L. (Sapindaceae) is used in phytotherapy for the management of various diseases in different parts of the world (2). The whole plant is used in Ghana to treat dysentery. The roots, mashed with seeds of *Piper guineense*, are applied as a styptic to cut veins and to treat leprosy (3). The roots are also chewed for coughs and pulmonary diseases, gonorrhoea, fractures or abscesses or used on open sores. It is also used as aphrodisiac (4). The roots are also reported to be used in ethnomedicine to treat bacterial infections and healing of chronic wounds (5). However, the various compounds previously isolated from *P. pinnata* have not been linked directly to the purported traditional usage. These include triterpene saponins, catechol tannins; Flavone glycosides; paullinoside A, paullinomide A, β-sitosterol and β-amyrin (6,7). In an effort to understand the medicinal role of the fatty acids present in *P. pinnata*, this report focused on their identification, *in vitro* antibacterial and free radical scavenging properties.

MATERIAL AND METHODS

Collection of plant

Plant material was collected in October 2005 and authenticated at the Centre for Scientific Research into Plant Medicine (CSRPM), Akwapim-Mampong, Ghana, where voucher specimen number 10/03/005 have been deposited.

Isolation and identification of fatty acids

Twelve fatty acids were isolated and identified from the methanol extract of the root by the Gas chromatography-mass spectroscopy (GC-MS) method (8). The sample was derivatised into the methyl ester by refluxing with tetramethylsilane (TMS) at 100°C for 30 min. Methyl esters of fatty acids were analysed with a Fisons MD 800 mass (quadrupole)–GC 8000 series instrument equipped with silica column (30 m × 0.25 mm I.D.) coated with OV1. Samples were injected by hot split method, with the injector and detector maintained at 270°C and 250°C respectively. The temperature program was 40°C for 4 min. after injection, then increased to 280°C (8°C/min) with a hold at 280°C for 20 min. Helium (20 psi) was used as a carrier gas with the flow rate of 0.5 ml/min. The gas chromatograph was directly coupled to a mass spectrometer working in electron impact ionization mode at 70 eV, and scanned masses in the range of 40-650 Da.

Data were acquired in full scan mode and processed with the Hewlett Packard Chemstation Sotfware. Compounds were identified by their mass spectra, interpretation of MS fragmentation patterns and comparing retention times of their peaks to those of standard compounds analyzed previously. Quantification of methyl esters of fatty acids was performed by integration of appropriate peak areas in Total Ion Chromatogram (TIC).

Antibacterial assay

The bacteria used for the tests were obtained from the National Culture Type Collection (NCTC), UK and included both Gram positive and Gram negative bacteria. The Gram positive bacteria used were *Bacillus subtilis* (NCTC 10073), *Staphylococcus aureus* (NCTC 4163) *Streptococcus faecalis* (NCTC 775), *Micrococcus flavus* (NCTC 7743), as well as resistant strains of *Staph. aureus SA1199B*, *RN4220* and *XU212*. Gram negative bacteria used were *Escherichia coli* (NCTC 9002) and *Pseudomonas aeruginosa* (NCTC 10662).

Inocula of the microorganisms were prepared from the 24 h Mueller-Hinton broth (Sigma) cultures and suspensions were adjusted to 105CFU/ml. Fatty acid samples were solubilized in 2% DMSO in water. Minimal inhibition concentration (MIC) values of the extracts were determined based on a micro-well dilution method (9,10).

The 96-well sterile plates were prepared by dispensing 180 µl of the inoculated broth plus a 20 µl aliquot of the fatty acid made up in broth or 20 µl broth in the case of negative control in each well. Tetracycline (Sigma) was included as positive control. Plates were incubated for 24 h at 37°C. Bacterial growth was determined after addition of 50 µl p-iodonitrotetrezolium violet (0.2 mg/ml, Sigma).

Antioxidant activity (DPPH radical scavenging activity)

The DPPH scavenging activity of the extract and the identified fatty acids was measured from the bleaching of a purple-coloured methanol solution of 2,2'-diphenylpicrylhydrazyl (DPPH) which was used as a reagent in a spectrophotometric assay (11,12). Fifty microlitres of various concentrations of the extract and fatty acids in methanol were added to 5ml of a 0.004% methanol solution of DPPH. This was incubated at room temperature for 30 minutes after which absorbance was read against a blank at 517nm on a Thermo Spectronic UV spectrophotometer. L-ascorbic acid was used as positive control in these experiments.

Inhibition of free radical DPPH, in percentage was calculated as

Scavenging activity (%) = $A_0 - A_1 \times 100\ A_0$

A_0 is the initial absorbance of methanolic solution of DPPH at 517nm.

The IC_{50} value was obtained through extrapolation from linear analysis, using the Prism Software, and denoted the concentration of sample required to scavenge 50% of DPPH radicals.

RESULTS AND DISCUSSIONS

Twelve fatty acids were identified from the methanolic extract of the root, using the GC-MS. The relative percentage and the retention times of the methyl ester of each fatty acid are reported in Table 1 and were obtained from the peak area in the Total Ion Chromatogram (Fig 1). The two most abundant fatty acids were 9-octadecenoic (oleic) acid [30.8%] and hexadecanoic (palmitic) acid [30.0%]. Two of the identified fatty acids, 9-octadecenoic (oleic) acid and 13-eicosenoic (paullinic) acid are mono unsaturated fatty acids. Nonanedioic (Azelaic) acid [4.4%] is a dicarboxylic acid. Fatty acids, including eicosanoic and eicosenoic acids, and cyanolipids have been reported in the genus *Paullinia*, including *P. elegans, P. meliaefolia* and *P. cupana* (13,14). They are usually found in the seed oils of

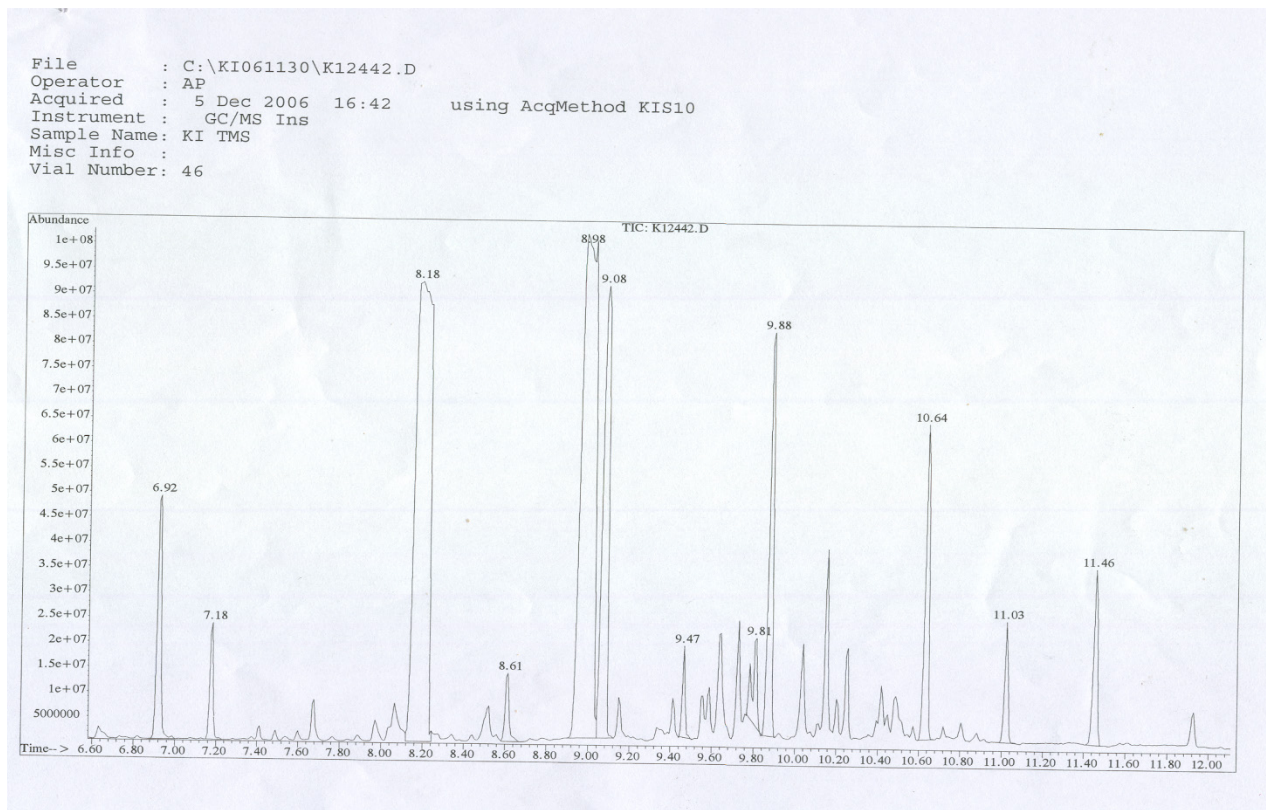

Figure 1. The Total Ion Chromatogram (TIC) of fatty acid mixture

Table 1. Fatty acid composition of *P. pinnata* root extract

RT	Fatty acid	No. of carbon atoms in FA	Rel. percentage of total	Double bonds
6.92	Nonanedioic (Azelaic) acid	C-9	4.4	0
7.18	Tetradecanoic (Myristic) acid	C-14	1.7	0
8.18	Hexadecanoic (Palmitic) acid	C-16	30.0	0
8.61	Heptadecanoic acid	C-17	1.2	0
8.98	9-octadecenoic (Oleic) acid	C-18	30.8	1
9.08	Octadecanoic (Stearic) acid	C-18	11.8	0
9.47	Nonadecanoic acid	C-19	1.1	0
9.73	Eicosenoic (paullinic) acid	C-20	2.0	1
9.88	Eicosanoic (arachidic) acid	C-20	6.9	0
10.64	Docosanoic (behenic) acid	C-22	5.3	0
11.02	Tricosanoic acid	C-23	1.8	0
11.46	Tetracosanoic (lignoceric) acid	C-24	3.0	0

RT- retention time of methyl ester of fatty acid.
Relative percentage was obtained from the peak area in chromatogram.

these plant species. However, this is the first report on the fatty acid composition of *P. pinnata*.

Azelaic acid significantly inhibited the growth of all organisms tested. Palmitic, oleic, eicosanoic and stearic acids also showed weak to moderate activity against *Staph. Aureus, B.subtilis, E.coli* and *P. aeruginosa* (Table 2). However, docosanoic acid, tetradecanoic acid and eicosenoic acid had no activity against all organisms tested. With the exception of azelaic acid, none of the fatty acids had activity against the multi-drug resistant strains of *Staph aureus, SA1199B, XU212* and *RN4220*. These results are in agreement with the previous studied activity of fatty acids where they were found to inhibit the growth of both Gram positive and Gram negative bacteria (15). Fatty acids of various chain lengths are known for their antimicrobial action, particularly against Gram positive bacteria. The observed inhibition is explained as a consequence of the uptake of undissociated fatty acids which dissipate

Table 2. Minimum Inhibitory Concentration (MIC) of identified fatty acids from *P. pinnata* against susceptible organisms

Bacteria	Minimum Inhibitory Concentration (MIC) [µg/ml]									
	pa	oa	ea	sa	da	aa	ta	tc	ec	tet
Staph. aureus	256	256	256	512	NI	64	NI	>1000	NI	4
B. subtilis	512	NI	512	512	NI	64	NI	>1000	NI	2
M. flavus	128	256	256	256	NI	32	NI	>1000	NI	1
E. coli	512	512	512	512	NI	64	NI	>1000	NI	8
P. aeruginosa	512	NI	512	512	NI	64	NI	>1000	NI	8
SA 1199B	NI	NI	NI	>1000	NI	256	NI	>1000	NI	4
XU212	NI	NI	NI	>1000	NI	256	NI	>1000	NI	128
RN4220	NI	NI	NI	>1000	NI	128	NI	>1000	NI	8

Key; NI- no inhibition. pa-palmitic acid; oa- oleic acid; ea- eicosanoic acid; sa-stearic acid; da- docosanoic acid; aa- azelaic acid; ta- tetradecanoic acid; tc- tetracosanoic acid; ec- eicosenoic acid; tet- tetracycline (positive control); n = 5.

Table 3. DPPH scavenging activity of methanol extract and fatty acids of *P. pinnata*

Material	IC_{50} (µg/ml) ±SD
Nonanedioic (Azelaic) acid	>1000
Tetradecanoic (Myristic) acid	>1000
Hexadecanoic (Palmitic) acid	>1000
Heptadecanoic acid	>1000
9-octadecenoic (Oleic) acid	500.8±9.4
Octadecanoic (Stearic) acid	>1000
Nonadecanoic acid	>1000
Eicosenoic (paullinic) acid	284.7±5.3
Eicosanoic (arachidic) acid	>1000
Docosanoic (behenic) acid	>1000
Tricosanoic acid	>1000
Tetracosanoic (lignoceric) acid	>1000
P. pinnata (methanol extract)	3.8±1.2
L-Ascorbic acid	21.1±1.1

The scavenging effects were expressed as IC50 (±S.D., n=5) compared to the blank. L- ascorbic acid was used as a positive control.

the transmembrane proton gradient and thereby affect ATPase activity (16). The undissociated form of fatty acids is highly soluble in membrane phospholipids and has been shown to enter the cell by passive diffusion (17).

In the DPPH assay, the methanol extract exhibited a significant radical scavenging action (Table 3) with an IC_{50} value of 3.8 µg/ml. *P. pinnata* was much more potent than L- ascorbic acid with IC50 of 21.1µg/ml. This compared favorably with the literature value of 21.04µg/ml for L-ascorbic acid (18). The observed strong antioxidant action of *P. pinnata* may be attributed to the presence of phenolic compounds and flavonoids in the plant but these have not been tested for antioxidant activity (2). Such compounds have been found by different workers to be highly antioxidant (19).

CONCLUSIONS

Our investigations have established the presence of twelve fatty acids in the crude methanol extract of *P. pinnata*, some of which contribute to the overall antibacterial action of the plant. Although the total methanol extract exhibit a highly significant antioxidant action, this activity could not be linked with the fatty acids present. We have also justified the ethnomedicinal use of *P. pinnata* in the treatment of bacterial infections.

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ABBREVIATIONS

ATP	Adenosine triphosphate
CFU	Colony forming units
DPPH	1,1-diphenyl-2-picrylhydrazyl
IC_{50}	Concentration that gives 50% inhibition
MIC	Minimum inhibitory concentration
TMS	Tetramethyl silane

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