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Effect of the leaf extracts of *Funtumia africana* (Benth.) Stapf. against selected pathogens

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Abstract
This study investigates the phytochemical and antimicrobial properties of the leaf extracts of *Funtumia africana*. Powdered leaves of *F. africana* were extracted with 20 % aqueous methanol, concentrated *in vacuo* and successively extracted with different solvents. The most active n-hexane fraction was further profiled using FTIR and GC-MS. Effectiveness of the extracts against microorganisms was assessed using agar dilution methods. Phytochemical analysis revealed the presence reducing sugars, alkaloids, terpenoids, steroids, tannins, flavonoids and cardiac glycosides. The ethyl acetate and n-hexane fractions displayed MIC values of ≤ 0.63 mg/ml against 53% and 82% of the test pathogens respectively and exhibited the lowest MBC value (0.63 mg/ml) against *Staphylococcus aureus* and *Klebsiella pneumoniae*, respectively. GC-MS analysis reveals the presence of methyl hexadecanoate at 45.96% and 6,10,14 trimethyl-2-pentadecanone at 17.10% as well as n-hexadecanoic acid/palmitic acid (14.12%), methyl stearate (10.14%) and (Z,Z)-9,12-octadecadienoic acid/linoleic acid (2.09%). Results from this study demonstrate that the leaf of *F. africana* could be a good source of antibiotic agents with broad spectrum.

Keywords: Bacteriostatic, bactericidal, fatty acids, *Funtumia africana*

Introduction
Medicinal plants produce certain bioactive secondary metabolites such as phenolic compounds which can inhibit the growth of bacteria and fungi [1, 2]. Experimental based studies and epidemiological surveys have suggested that consumption of edibles which are rich in phenolic compounds significantly reduce many health challenges because of their inherent anti-mutagenic, and antibacterial properties [3]. Phytochemicals in plant extracts and microbial metabolites are increasingly attracting attentions for their potential usage as prophylactic and therapeutic agents in the combat against degenerative diseases like diabetes, malignancy and aging among others [4]. Bioactive principles of plant origin are widely being employed as drug templates in most pharmaceutical establishments and several ethno-botanical records indicate that medicinal plants could generate an inexhaustible source of affordable drugs that may be readily available to all and sundry [5].
Funtumia africana (Benth.) Stapf. −*Apocynaceae*, is predominantly rich in alkaloids and polyphenolic compounds [6, 7]. Infusion from its leaves is used in traditional healing system of the people in Niger Delta region of Nigeria against diabetes and abdominal diseases. However, there is insufficient information on the antimicrobial properties of this plant species. This present study therefore aims at screening for possible phytochemicals and investigates antibacterial property of the leaf extract of *F. africana*.

Materials and Methods
Samples Collection
Fresh leaves of *F. africana* were sourced from Amatolo town, Wilberforce Island, in the Niger Delta region of Nigeria and air-dried until constant weight. The plant was identified by Prof. B.O Nyannanyo, Department of Botany and Plant Biotechnology, University of Port-Harcourt, Nigeria. A specimen sample of this collection was deposited at the Niger Delta University herbarium with voucher number NDUP 112.

Extraction Methods

Air-dried leaves were ground into fine powder and 500 g was extracted with 20% aqueous methanol (1000 mL) solution by maceration at room temperature and then filtered after 24 h. The filtrate was concentrated to 300 mL *in vacuo* and then lyophilized. The resultant crude methanolic extract (CME) was successively extracted into n-hexane (3 × 300 mL, HEF), dichloromethane (3 × 300 mL, DCF), ethyl acetate (3 × 300 mL, EAF) and n-butanol (3 × 100 mL, BUF).

Qualitative phytochemical tests

Qualitative phytochemical screening of the plant extract was done following standard procedure [8]. A Fourier transform infrared spectrometer, Bruker Alpha-P instrument (Billerica, MA, USA) was used for the FTIR analysis of the samples.

Gas Chromatography-Mass Spectrometry (GC-MS) analysis of the n-hexane fraction

N-hexane fraction of the leaf extract was subjected to GC-MS analysis using an Agilent technologies GC System (7890 model) with an EI detector (70eV). The mass spectra of the components of the n-hexane extract were identified by comparing with the NIST Standard Reference mass spectra Database 1A (NIST/EPA/NIH Mass Spectral Database (NIST 11). The constituents of the n- hexane fraction was also identified by comparison of their Kovats index (KI) in relation to C5–C24 n-alkanes obtained on a non-polar DB-5MS column with constituents known from literature [9].

Antimicrobial activity evaluation

Microbial pathogens utilised in this work were collected from Department of Microbiology, Obafemi Awolowo University, Ile-Ife. The organisms consists of typed cultures (NCIB) and locally isolated organisms (LIO), gram-positive: *Bacillus stearothermophilus* (NCIB 8222), *Bacillus cereus* (NCIB 6349), *Bacillus polymyxa* (LIO), *Clostridium sporogenes* (NCIB 532), *Corynebactarium pyogenes* (LIO), *Enterococcus faecalis* (NCIB 775), *Micrococcus luteus* (NCIB 196), *Proteus vulgaris* (LIO) and *Staphylococcus aureus* (NCIB 8588). Gram-negative: *Klebsiella pneumoniae* (NCIB 418), *Pseudomonas flourescens* (NCIB 3756), *Pseudomonas aeruginosa* (NCIB 950) and *Escherichia coli* (NCIB 86). Yeast isolates: *Candida albicans* and *Candida pseudotropicalis*

Determination of Minimum Inhibitory Concentrations (MICs)

MIC was assessed using agar dilution methods following standard procedure [10]. Two-fold dilution of the test bioactive agent was prepared. 2 ml of different concentrations of the solution was added to 18 ml of molten nutrient agar for the bacteria and malt extract agar for the yeast to give final concentrations regimes of 0.31 -20.0 mg/ml respectively. Surfaces of the media were allowed to dry before streaked with 24 hours old standard inoculums in broth cultures. These were incubated at 37 ºC for 48 h (bacteria) and 25 ºC for 72 h (yeast). The plates were then examined for presence of growth. The MICs were recorded as the lowest concentrations that prevented the growth of the isolates. The experiment was carried out in replicates of two.

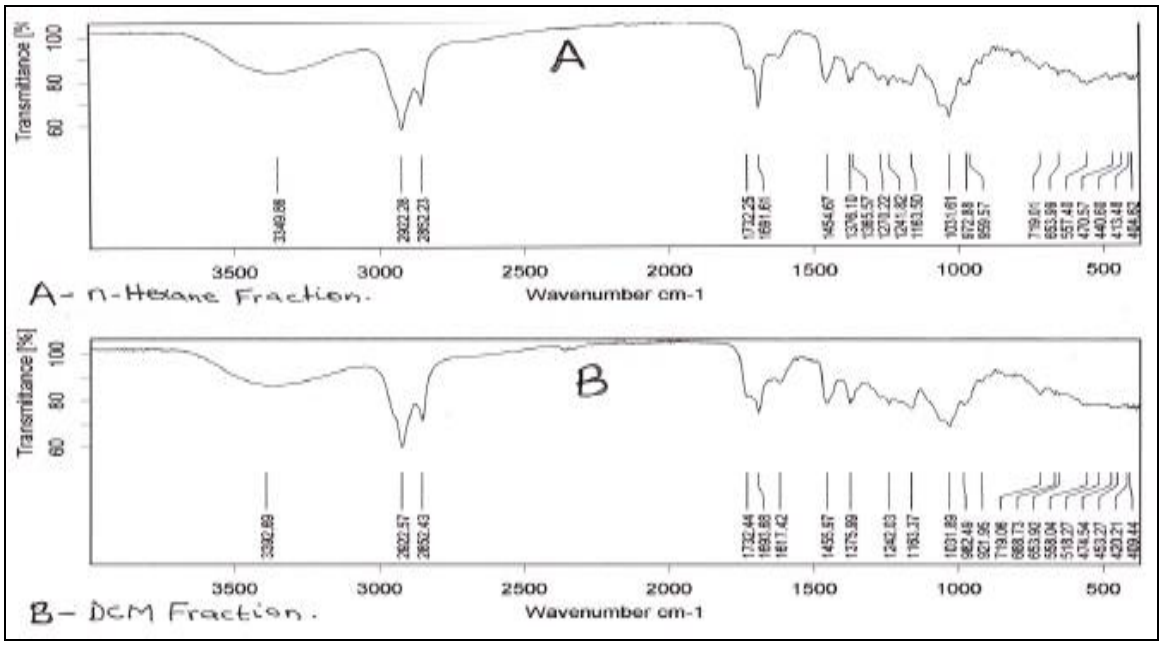
Determination of Minimum Bactericidal/fungicidal Concentrations (MBCs/MFCs)

These were determined as previously described [11, 12] with slight modifications. Samples were obtained from MIC plates with no visible growth on its line of streaks and then inoculated onto nutrient agar and malt extract agar plates respectively for the bacterial and yeast isolates. The plates were incubated at 37 ºC and 25 ºC for 48 hours respectively. The MBCs/MFCs were taken as the lowest concentration of the extract with no visible growth on the new set of Agar plates. The experiment was carried out in duplicates.

Results

Phytochemicals, FT-IR and GC-MS Analyses

Screening of the extracts indicates the presence of tannins, alkaloids, terpenoids, steroids, flavonoids, cardiac glycosides and reducing sugars [13]. In addition, aromatic, phenolic, hydroxyl and carbonyl groups were also found to be present in the fractions as shown in the FTIR spectra in Figure 1[14, 15]. GC-MS analysis of the oily n-hexane fraction presented in Figure 2 and Table 1 indicates presence of nine fatty acids esters among which include methyl hexadecanoate (45.96%), 6,10,14 trimethyl-2-pentadecanone (17.10%), palmitic acid/n-hexadecanoic acid (14.12%), methyl stearate (10.14%) and (Z,Z)-9,12-octadecadienoic acid/linoleic acid (2.09%) as the major components.



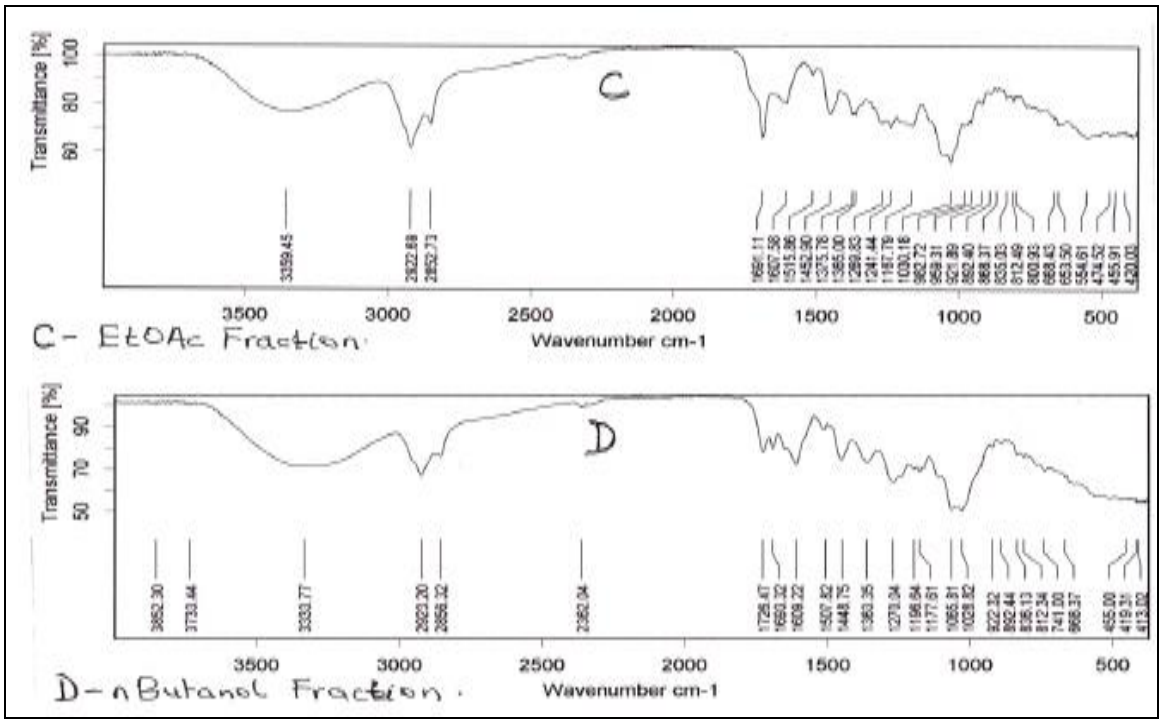


Fig 1: FT-IR spectra of different partitioned fractions of *F. africana* leaf extract

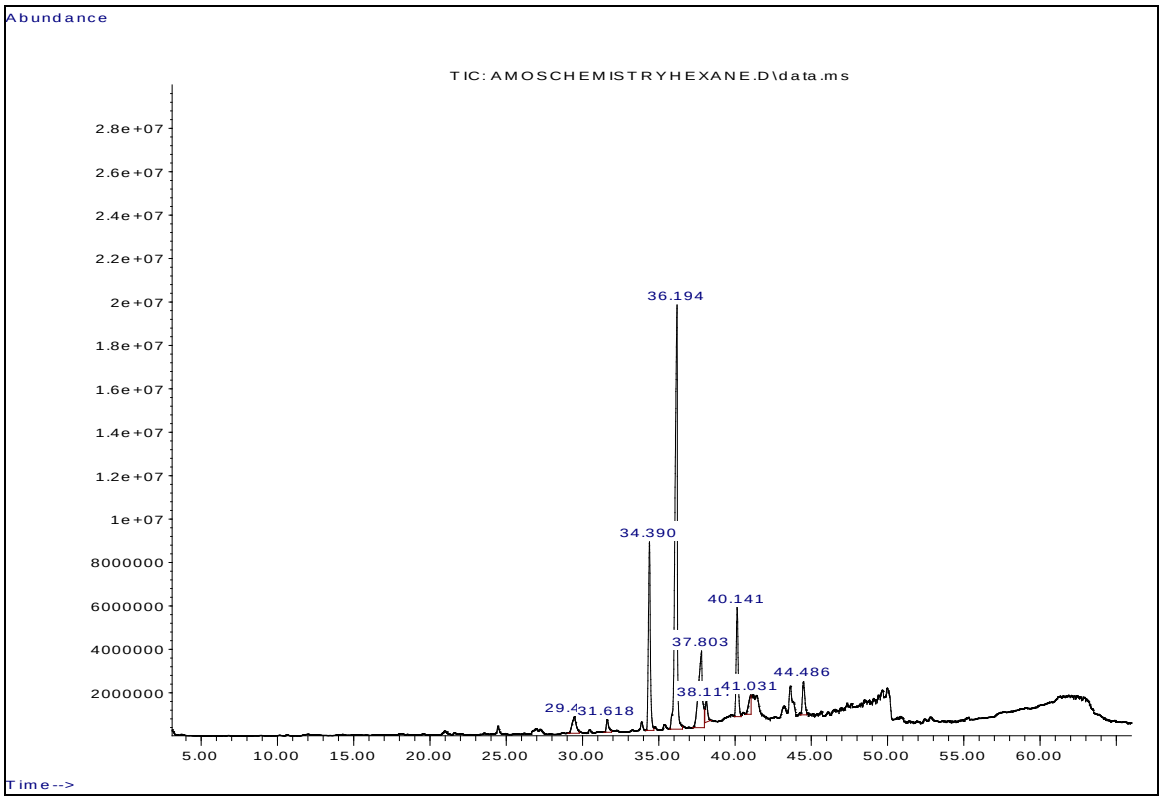


Fig 2: GC-MS spectrum of the oily n-hexane fraction of *F. africana* leaf extract

Table 1: Chemical composition of n-hexane fraction of *F. africana* leaf extract

Elution #	RT (mins)	Compound	[%]	RI (Kovats)
1.	29.475	5-Hexenoic acid, methyl ester	3.12	874
2.	31.618	Methyl tridecanoate	1.48	1631
3.	34.390	6,10,14 trimethyl-2-pentadecanone	17.10	1842
4.	36.194	Hexadecanoic acid, methyl ester	45.96	1927
5.	37.803	n-Hexadecanoicacid[a]	14.12	1984
6.	38.117	Heptadecanoic acid, methyl ester	2.27	2037
7.	40.141	Methyl stearate	10.14	2088
8.	41.031	(Z,Z)-9,12-Octadecadienoic acid[b]	2.09	2173
9.	44.486	Unknown	3.74	-

a **-** palmitic acid; ***b*** **-**Linoleic acid(essential fatty acid)

Antimicrobial activities

Minimum inhibitory concentrations of the crude extract ranges from 1.5 - 1.7 mg/mL (Table 2). The lowest MICs exhibited by the n-hexane and ethyl acetate fraction is 0.31 mg/mL against *Micrococcus luteus* and *Clostridium sporogenes* respectively. Generally, n-hexane fraction expressed MICs of ≤ 0.63 mg/mL against 82% of the entire test isolates followed by ethyl acetate which is 53% and then n-butanol fraction (23%) at the same concentration. None of the bacterial isolates was sensitive to the DCM fraction and only the n-hexane fraction demonstrated anti-fungal activity against all selected yeast isolates with a uniform MICs and MFCs of 0.63 mg/ml and1.25 mg/ml respectively (Table 3).

Table 2: The minimum inhibitory concentrations (MIC) of the extracts of *F. africana* and standard antimicrobials exhibited against susceptible microbial isolates (mg/ml).

Isolates	CME	HEF	DCF	EAF	BUF	STREP	AMP
Bacteria							
Bacillus cereus (NCIB 6349)	1.50	0.63	NS	1.25	NS	0.06	0.06
Bacillus polymyxa (LIO)	1.50	0.63	NS	0.63	1.25	0.13	NS
Bacillus anthracis (LIO)	1.75	0.63	NS	0.63	0.63	0.06	0.06
Bacillus subtilis (NCIB 3610)	1.50	0.63	NS	0.63	1.25	0.06	0.13
Bacillus stearothermophilus (NCIB 8222)	1.50	0.63	NS	0.63	NS	0.13	0.03
Clostridium. sporogenes (NCIB 532)	NS	NS	NS	0.31	NS	0.06	0.03
Corynebactarium pyogenes (LIO)	NS	NS	NS	0.63	NS	0.03	0.06
Staphylococcus aureus (NCIB 8588)	1.75	0.63	NS	1.25	0.63	0.25	0.06
Enterococcus faecalis (NCIB 775)	1.75	0.63	NS	1.25	0.63	0.25	0.25
Micrococcus luteus (NCIB 196)	1.50	0.31	NS	NS	NS	0.50	0.25
Escherichia coli (NCIB 86)	1.75	1.25	NS	1.25	1.25	NS	NS
Klebsiella pneumoniae (NCIB 418)	1.50	0.63	NS	0.63	0.63	0.03	0.13
Pseudomonas aeruginosa (NCIB 950)	1.50	0.63	NS	0.63	NS	0.50	0.25
Pseudomonas flourescens (NCIB 3756)	1.50	0.63	NS	1.25	NS	0.06	0.06
Proteus vulgaris (LIO)	1.50	0.63	NS	0.63	1.25	0.13	NS
Fungi							
Candida albican	NS	0.63	NS	NS	NS	0.06	
Candida pseudotropicalis	NS	0.63	NS	NS	NS	0.13	

Key: NS = Not sensitive, LIO = Locally Isolated Organism, NCIB = National Collection of Industrial Bacteria, CME = Crude Methanol Extract, HEF = n-Hexane fraction, DCF = Dichloromethane fraction, EAF = Ethyl acetate fraction, BUF = n-Butanol fraction, STREP = Streptomycin, AMP = Ampicillin, NYST = Nystatin

Table 3: The minimum bactericidal/fungicidal concentrations (MBCs/MFCs) of the leaf extracts of *F. africana* and standard antimicrobials exhibited against susceptible microbial isolates (mg/ml).

Bacterial isolates	CME	HEF	DCF	EAF	BUF	STREP	AMP
Bacteria							
Bacillus cereus (NCIB 6349)	2.10	1.25	ND	2.50	ND	0.13	0.13
Bacillus polymyxa (LIO)	2.10	1.25	ND	1.25	1.25	0.25	ND
Bacillus stearothermophilus (NCIB 8222)	2.13	1.25	ND	1.25	1.25	0.13	0.13
Clostridium sporogenes (NCIB 532)	2.25	1.25	ND	1.25	2.50	0.13	0.25
Corynebaterium pyogenes (LIO)	ND	1.25	ND	1.25	ND	0.25	0.06
Staphylococcus aureus (NCIB 8588)	5.15	ND	ND	0.63	ND	0.25	0.06
Enterococcus faecalis (NCIB 775)	2.10	ND	ND	1.25	ND	0.06	0.13
Micrococcus luteus (NCIB 196)	5.15	1.25	ND	2.50	1.25	0.25	0.13
Escherichia coli (NCIB 86)	2.25	1.25	ND	1.25	1.25	0.25	0.25
Klebsiella pneumoniae (NCIB 418)	2.10	0.63	ND	ND	ND	0.50	0.25
Pseudomonas aeruginosa (NCIB 950)	2.10	2.50	ND	2.50	2.50	ND	ND
Pseudomonas flourescens (NCIB 3756)	2.10	1.25	ND	1.25	1.25	0.06	0.25
Proteus vulgaris (LIO)	2.10	1.25	ND	1.25	ND	0.50	0.50
Fungi							
Candida albican	ND	1.25	ND	ND	ND	0.06	
Candida pseudotropicalis	ND	1.25	ND	ND	ND	0.13	

Key: ND = Not Determined

Discussion

This study has shown that the extracts of the leaves of *F. africana* has broad spectrum of antimicrobial activities and is mainly composed of methyl hexadecanoate (45.96%), 6,10,14 trimethyl-2-pentadecanone (17.10%), palmitic acid/n-hexadecanoic acid (14.12%), methyl stearate (10.14%) and (Z,Z)-9,12-octadecadienoic acid/linoleic acid (2.09 %).

The antimicrobial potential of the ethyl acetate and n-hexane fractions from the crude extract suggests that bioactive components of the leaf extract reside both in the polar and none polar embedded compounds. Antimicrobial activities of the plant extracts largely depend on the presence of phenolic compounds which have been found to be effective against bacterial pathogens across Grams spectrum [16]. Likewise, the tannins are known to possess antiseptic, anti-inflammatory and anti-haemorrhagic potentials [17] and alkaloids commonly in use against bacterial infection and malarial fever [18]. In addition, the presence of fatty acids (palmitic, linoleic) in the oily n-hexane fraction possibly contributed to its strong antimicrobial activity. Many fatty acids like lauric, palmitic, linolenic, linoleic, oleic, stearic and myristic acids have been described to have antimicrobial properties [19]. It is equally

important to mention that DCM as a solvent lack the ability to extract bioactive components from this plant and hence should not be recommended for such usage.

Conclusion

This study thus affirms the traditional application of *F. africana* leaves in providing remedy against suspected infectious ailment in Niger-Delta region of Nigeria as a form of cheaper and readily available antimicrobial agent.

Conflict of Interest

The authors report no conflicts of interest in this study

Contribution of Authors

All authors contributed equally to the preparation of the manuscript and agreed to the content.

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