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GC-MS ANALYSIS OF *PAVETTA CORYMBOSA* LIPOPHILIC EXTRACT AND ITS ANTIMICROBIAL ACTIVITY

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ABSTRACT

Pavetta corymbosa is a medicinal plant used as remedy for infectious diseases in Nigerian traditional medicine. In this study, analysis of chemical composition of lipophilic extract and the antimicrobial activity of the extract were carried out with a view to discovering effective phytomedicine. The gas chromatography-mass spectrometric (GC-MS) analysis carried out showed that 9-methyl octadecanoic acid- ethyl ester (22.6%), cyclotetracosane (15.3%), 2,4,6-trimethyl hexacosanoic acid- methyl ester (12.7%) and 9,12,15-octadecatrienoic acid- methyl ester (12.1%) were present as the major chemical components at the indicated percent composition. The antimicrobial activity on clinical susceptible, standard and resistant species was carried out using disc diffusion and broth-micro-dilution techniques as recommended by CLSI. The lipophilic extract showed significant antimicrobial activity against *Bacillus subtilis* and *Streptococcus faecalis* with minimum inhibitory concentration (MIC) of 0.624 mg/ml, whilst fungal species such as *Candida albicans* and *Candida krusei* had MIC of 1.25 mg/ml. The antimicrobial potency of the lipophilic extract is attributed to fatty acids/esters chemo types present therein. It was concluded that *P. corymbosa* lipophilic extract could be exploited as complimentary source of antimicrobial agents.

Keywords: *P. corymbosa*, antibacterial, antifungal activities, fatty acid methyl esters, GC-MS.

INTRODUCTION

Plants have been used for centuries as sources of therapeutic agents in traditional medicine. This is due to organic bioactive components such as alkaloids, flavonoids, triterpenoids, saponins and phenolics among others, which they contain. In recent years, phytochemicals from herbal plants have attracted attention as alternative or complementary sources of antimicrobial agents to modern chemotherapeutic drugs. The increasing incidence of drug resistant pathogens such as methicillin resistant *Staphylococcus aureus* (MRSA) (Siegel *et al.*, 2006), vancomycin resistant enterococci (VRE) (O'Driscoll and Crank, 2015) and multi drug resistant tuberculosis (MDR-TB) (WHO, 2016) necessitate the search for effective therapeutic agents from plants. *Pavetta corymbosa* **(**Rubiaceae) is a small tree found in dry forest, grass savanna across West Africa from Senegal to Cameroon. The whole plant is used for treatment of leprosy (Joshi, 2000), the root decoction as anti-inflammatory agent and the leaves as remedy for several bacterial infections (Vasudevan, 1997). Because of the widespread use of *P. corymbosa* in traditional medicine, it is desirable to identify the bioactive components and evaluate their efficacy as antimicrobial phytomedicine. Previous antibacterial activities of leaf ethanolic extract and antimycobacterial activity of aqueous extract have been reported (Anago *et al.*, 2011; Nvau *et al.*, 2011). Phytochemical investigations on the ethyl acetate and dichloromethane leaf extracts have resulted to isolation of some flavonoids and triterpenoids, respectively (Ahmadu *et al.*, 2011).

However, no phytochemical screening or biological study on the lipophilic extract of *P. corymbosa* have previously been reported. But plants bioactive constituents have been shown to be distributed between polar and non-polar regions (Aliyu *et al.*, 2011; Ibrahim *et al.*, 2011). Thus, the study of bioactive phytochemicals of various solvent components is necessary to understanding therapeutic profiles of the plant as sources of antibacterial agents. In this study, the *P. corymbosa* lipophilic extract was subjected to gas

chromatography-mass spectrometry (GC-MS) analysis to identify the bioactive components. In addition, the antimicrobial activity was investigated on standard, clinical susceptible and resistant bacterial species as well as fungal isolates.

MATERIALS AND METHODS

Collection of plant material and extraction

The leaves of *Pavetta corymbosa* were collected in February, 2015 in Zaria, Kaduna State, Nigeria. The sample was authenticated at the Herbarium Unit, Department of Biological Sciences, Ahmadu Bello University, Zaria, Nigeria. Voucher specimen (No. 263) was deposited. The sample was air-dried and pulverized to powder. One hundred gram (100 g) of it was extracted with 500 ml of hexane by simple percolation on a shaker (Labcon, South Africa) for 24 h. The extract was filtered using whatman filter paper No. 2, and concentrated on a rotary evaporator (Büchi Rota vapor R-124) at 40 ºC to give the hexane extract (12.5 g) labeled as *Pavetta corymbosa* lipophilic extract (PCLE).

Test organisms

Bacterial clinical isolates: methicillin resistant *Staphylococcus aureus* (MRSA), vancomycin resistant *Enterococcus fecalis* (VRE), *Proteus vulgaris, Streptococcus pyogenes, Streptococcus faecalis* and standard strains including *Staphylococcus aureus* NCTC 6571*, Escherichia coli* NCTC 10418*, Bacillus subtilis* NCTC 8236*, Salmonella typhi* ATCC 9184, *Pseudomonas aeruginosa* NCTC 6750 and four yeast isolates (*Candida tropicalis, Candida stellatoidea, Candida krusei* and *Candida albicans*) were used in this study. They were all obtained from the Department of Microbiology, Ahmadu Bello University Teaching Hospital (ABUTH) Shika. Isolates were purified on nutrient agar plates (Oxoid, UK) and characterized using standard microbiological and biochemical procedures (Cowan and Steel, 1974; McFadden, 1977).

Gas chromatography-mass spectrometric analysis (GC-MS)

GC-MS analysis of the PCLE was conducted on Agilent technologies 6890 series GC coupled with an Agilent 5973 mass selective detector and driven by Agilent Chemstation software. A HP-5MS capillary column was used (30 m × 0.25 mm internal diameter ×0.25 µm film thickness). The carrier gas was ultra-pure helium at a flow rate of 1.0 ml/min and a linear velocity of 37 cm/sec. The injector temperature was set at 250 °C. The initial oven temperature was 60 °C and programmed to 280 °C at the rate of 10 °C/min with a hold time of 3 min. Injection of 1 µl was made in split less mode with a split ratio of 20:1. The mass spectrometer was operated in the electron ionization mode at 70 eV and electron multiplier voltage at 1859 V. Other MS operating parameters were as follows: ion source temperature 230 °C, quadrupole temperature 150 °C, solvent delay 4 min and scan range 50-700 amu. Compounds were identified by direct comparison of the retention times and mass spectral data with those in the National Institute of Standards and Technology (NIST) library and confirmation from published data in the literature was used (Masada, 1976). The relative percentage of each component was calculated by comparing its peak to the total areas.

Antimicrobial assay, MIC, MBC and MFC

The antimicrobial activity of the PCLE against the test microorganisms was determined using the disc diffusion method as described by Nostro *et al.* (2000). Stock solution of extract (100 mg/ml) was prepared in 5% DMSO. The blank sterile disc (6 mm) was placed on the inoculated Mueller Hinton agar (MHA) surface and impregnated with 15µl of stock solution. Standard antibiotics disc of Sparfloxacin (30 µg/disc) and Fluconazole (30 µg/disc) were used as positive control for bacteria and fungi respectively. Plates were incubated at 37 ºC for 24 h and the activities were expressed as the mean diameter of inhibition zones (mm) from duplicate test produced by the extract. The minimum inhibitory concentration (MIC) was determined using micro broth dilution in accordance with CLSI, 2007. Serial dilution of sample extract was prepared between 0.1 mg/ml to 6.50 mg/ml concentration. The test tubes were inoculated with the suspension of the standardized inocula. These were incubated at 37 ºC for 24 h and observed for growth. The minimum inhibitory concentration (MICs) for each test organism was regarded as the lowest concentration that inhibited visible growth of the test organisms. The minimum bactericidal concentration (MBC) and minimum fungicidal

concentration (MFC) were carried out via aseptic inoculation of cultures (100 µl) from growth-free MIC tubes on sterile nutrient agar plates and incubating at 37 ºC for 24 h for bacteria and 48 h for fungi. The MBC/MFC was recorded as the lowest concentration of extract showing no bacterial or fungal growth.

RESULTS

The chemical composition of the PCLE constitute largely fatty acid esters and the major compounds identified include octadecanoic acid, 9-methyl- ethyl ester (22.6%), cyclotetracosane (15.3%), hexacosanoic acid, 2,4,6-trimethyl-methyl ester (12.7%) and 9,12,15-octadecatrienoic acid- methyl ester (12.1%). Other compounds including terpenes/terpenoids, steroids, aldehydes and ketones were identified in trace quantities as less than 1% (Table 1). The sensitivity of the test organisms to the PCLE was measured by zones of inhibition with a range between 22-29 mm. PCLE demonstrated significant antibacterial activity on *B. subtilis* (29 mm), *S. faecalis* (28 mm) *S. aureus* (25 mm) and *S. pyogenes* (25 mm). But organisms such as *E. coli*, *S. typhi*, *P. aeruginosa*, *C. stellatoidea* and VRE were resistant to the extract. The MIC (0.625 mg/ml) and MBC (1.25 mg/ml) on *B. subtilis* were the lowest amongst the bacterial species tested. Similarly, fungal isolates such as *C. krusei* (25 mm; MIC 1.25 mg/ml) also demonstrated the lowest MFC. Despite bacterial virulence associated to drug resistant strains, MRSA (24 mm, MIC 1.25 mg/ml; MBC 5.0 mg/ml) was susceptible to PCLE.

Table 1: Chemical composition of lipophilic extract of *Pavetta corymbosa*

Compound no.	Compounds	[a]RT (min)	% composition
1	Tetradecanoic acid, propyl ester	15.8	1.67
2	Cyclotetracosane	17.7	15.3
3	Phytol	18.0	10.0
4	9,12,15-octadecatrienoic acid, methyl ester, (Z, Z, Z) -	19.3	12.1
5	Octadecanoic acid, 9-methyl-, ethyl ester	19.6	22.6
6	Hexadecanoic acid, 2-hydroxy-, methyl ester	21.0	1.89
7	p-menth-8(10)-en-9-ol, cis	21.3	1.46
8	Octadecane	22.2	7.20
9	Hexacosanoic acid, 2,4,6-trimethyl-, methyl ester, (2R, 4S, 6R) -(-)-	23.9	12.7
10	Campesterol	24.6	2.19
11	N-carbethoxy-N-methoxymethylamine	24.8	1.62
	Major compounds identified		88.73%
	*Compounds identified in traces		7.27%
Total			**96%**

[a]Retention time (in minutes) as eluted from DB-5 column, *Compounds identified as <1%

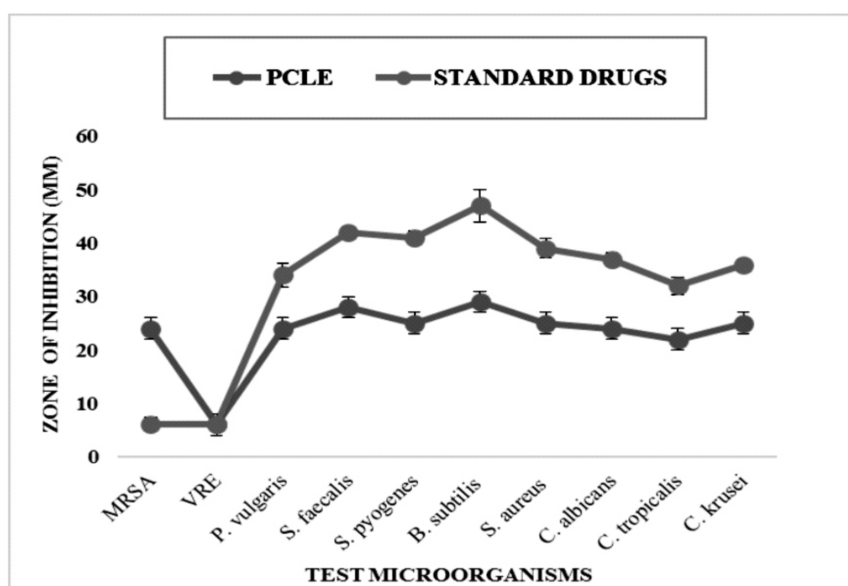

Figure 1: Antimicrobial activity of *P. corymbosa* lipophilic extract (PCLE)

DISCUSSION

The polar extracts of *P. corymbosa* have previously been reported for preliminary phytochemical and biological studies (Anago *et al.*, 2011; Nvau *et al.*, 2011). But in this study, the lipophilic components of *P. corymbosa* extract have been identified by GC-MS analysis (Table 1) and evaluated to be highly potent antimicrobial agents. Out of the thirty-four (34) components analyzed by the GC-MS, twenty-seven (27) were identified which constitutes 96% of total composition. They are largely fatty acid/esters, steroid and hydrocarbons (Table 1). Some of them including campesterol have been identified as active component of ethyl acetate extract of *Cenchrus setigerus* on bacterial pathogens (Singariya *et al.*, 2012). In addition, an analogue of octadecanoic acid 9-methyl-ethyl ester isolated from neem oil had antibacterial activity on *S. aureus*, *E. coli* and *S. typhi* (Zhong *et al.*, 2010). Gram-positive bacteria such as *B. subtilis* and *S. aureus* are more susceptible to drug molecules than the Gram-negative bacteria. This could be linked to the high cell wall permeability which allows easy penetration of bioactive compounds into cytoplasm of Gram-positive bacteria. But in contrast, Gram-negative bacterial cell wall is a barrier to penetration of molecules thus becoming resistant to treatment (Lambert, 2002). However, despite the preferential inhibition of Gram-positive bacteria; the PCLE demonstrated fungi static activity on *candida* strains indicating some level of broad-spectrum activity of the extract.

Generally, fatty acid/ester chemo-types are ubiquitous in higher plants with several functions as membrane metabolites (Chandrasekaran *et al.*, 2011). But their chemical content and composition varies largely from amongst species probably due to environmental differences as well as plants genetics (Figueiredo *et al.*, 2008). Hence, the chemical or structural motifs of fatty acids and their aggregate composition have enormous implications to antibacterial activity.

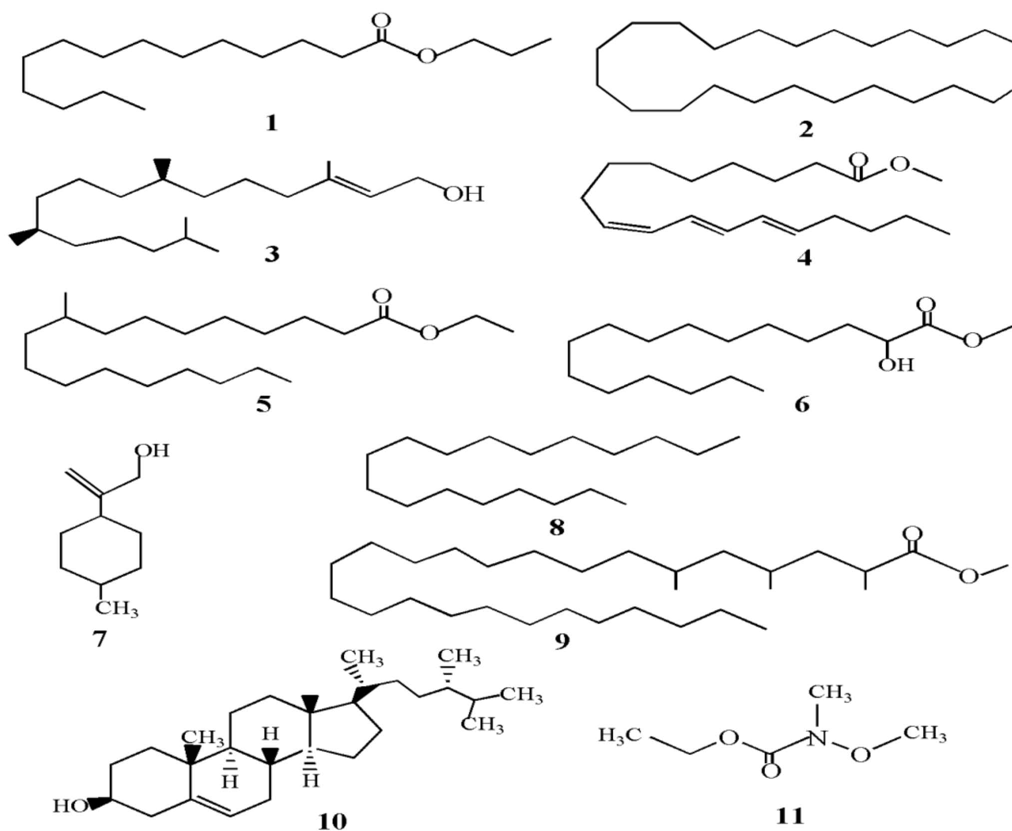

Figure 2: Chemical structures of major compounds of PCLE identified by GC-MS

1.Tetradecanoic acid- propyl ester, **2**. Cyclotetracosane, **3**. Phytol, **4**. 9,12,15-octadecatrienoic acid- methyl ester, **5**. 9-methyl octadecanoic acid- ethyl ester, **6**. 2-hydroxy hexadecanoic acid- methyl ester, **7**. *Cis*-p-menth-8(10)-en-9-ol, **8**. Octadecane, **9**. 2,4,6-trimethyl hexacosanoic acid- methyl ester, **10**. Campesterol and **11**. N-carbethoxy-N-methoxymethylamine

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Previous study of fatty acids (FA) and their methyl esters (ME) from *Sesuvium portulacastrum* in relation to antibacterial and antifungal activities, showed that free carboxy-OH group in medium chain unsaturated fatty acids resulted to strong activity against Gram positive bacteria (Desbois and Smith, 2010). In contrast to our findings, the major compounds identified in this study are mostly long chain saturated fatty acid methyl esters (FAME), yet remarkable antibacterial activity was recorded especially on the MRSA compared to standard drug used (Figure 1). This may suggest that the efficacy of PCLE depends largely on aggregate mixture of major compounds constituting about 89% of the identified components (Figure 2). Thus, it is also suggestive that the antimicrobial activity of PCLE was mediated by either additive or synergistic interactions of the FAME, hydrocarbons, terpenes and steroid. We concluded that the lipophilic compounds of *P. corymbosa* leaf possess potent antimicrobial activities *in vitro* which lend credence to the use of this plant for treating various bacterial and fungal diseases. It will be beneficial to further isolate and characterize some bioactive components from the plant which may serve as adjuncts in the treatment of infectious diseases.

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