In-vitro Anti-Microbial Studies and GC/MS Analysis of the Leaf Extract and Fractions of *Polyalthia longifolia* (Engl. & Diels) Verde

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In-vitro Anti-Microbial Studies and GC/MS Analysis of the Leaf Extract and Fractions of *Polyalthia longifolia* (Engl. & Diels) Verde

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Abstract. Extensive studies show that secondary metabolites in plants, used for centuries in traditional medicine, offer new sources of drugs. In the traditional setting, extracts from various parts of the plant *Polyalthia longifolia* (mast tree) are used in treating several ailments but the components of these extracts, which would allow for meaningful dosage, are not known. We therefore decided to examine the antimicrobial activity by testing on selected microorganisms and identify the volatile components by gas chromatography-mass spectrometry of the leaf extracts of *Polyalthia longifolia* (mast tree). The crude leaf extract and fractions derived from the crude exhibited anti-microbial activity against two (2) bacteria and two (2) fungi. The chloroform fraction was very active against *Salmonella typhi* (13.00±0.82) when compared to fractions in other solvents. The GC-MS analysis showed that the extracts were composed fatty acids and their ester along with some long chain aldehydes, like hexadecenal and tetradecenal, and Caryophyllene and Aromandendrene. These chemical constituents may be responsible for the pharmacological and therapeutic activities of this plant.

1. Introduction

Historically, plant has been nature's gift to man-kind. This is because plants and their products have always been explored as sources of drugs in the treatment of various ailments and diseases. According, to the World Health Organisation, over 80% of the world's population use plants and their products traditionally as a primary source of health care [1]. The threat to the treatment of infectious diseases caused by bacteria and fungi has become a major health concern globally due to increased resistance to antimicrobial agents such as antibiotic drugs and other new variety of strains which are multi-drug resistant [2]. It is therefore necessary that steps be taken to tackle these concerns and to reduce the problems caused by the various strains of bacteria and fungi.

Polyalthia longifolia is an ethno-medicinal plant that has been used for traditional therapeutic purposes [3]. The plant is of the genus *Polyalthia* and belongs to the family of Annonaceae. The genus *Polyalthia* has about 120 species in existence and can be found in the tropical parts of Africa, America, Asia and the Islands of Oceania. In India [4], almost all parts of the *P. longifolia* var pendula (*Polyalthia* genus) are used traditionally in the treatment of different types of diseases such

as diarrhoea, cough, skin infection, sore throat and cold. In Nigeria the stem and the leaves are used traditionally in treating diseases caused by kinetoplastid protozoa such as sleeping sickness and leishmaniosis; hypertension and diabetes [11, 12]. While in other geographical regions, research has been found that the herbal drugs are used as tonic and febrifuge. The bark of this plant is the more popular as the helpful portion of this plant utilized in treating diabetes, rheumatism, pyrexia, hypertension and menorrhagia and scorpion sting. It also helps in treating constipation, urinary system, digestive system, antipyretic activity and circulatory system movements [13]. It is thought that the presence of clerodane diterpenoids and alkaloids in all parts is responsible for its use in treatment of these ailments [14]. Therefore, this study sought to investigate the antimicrobial properties against Gram-positive bacteria *(Staphylococcus aureus*), Gram-negative bacteria (*Salmonella typhi*) and fungi (*Trichophyton rubrum* and *Candida albican*) and identify the volatile chemical constituents in leaf extract and fractions of *P. longifolia* (Engl. & Diels) Verde.

2. Material and Methods

2.1. Plant Material and Extraction

Polyalthia longifolia (Engl. & Diels) Verde plant grows wild in Covenant University campus and the required parts were taken as needed. The mature leaves of *Polyalthia longifolia* (Engl. & Diels) Verde were collected from the campus of Covenant University Ota, Ogun State, Nigeria (latitude 6.672065 north and longitude 3.1598830 east) in the early hours of the morning (between 6 and 7 am) of 15th July, 2015 and the temperature was about 27 ± 2^{o}C. The plants were identified at the Department of Biological Sciences, Covenant University, Nigeria and authenticated at the Forestry Research Institute of Nigeria (FRIN) herbarium, Ibadan with the voucher number FHI: 110014 for reference purpose. The extraction of *Polyalthia longifolia* leaves was carried out using the procedure described by Okoronkwo et al. [5]. Air-dried leaves were pulverised and extracted in methanol by cold maceration technique. This was then fractionated in chloroform and hexane by separating funnel respectively. The fractions obtained were separated, concentrated and stored at 2^{o}C for further use.

2.2. Antimicrobial Studies

2.2.1. Agar-well diffusion Assay. Antibacterial activities of fractions were determined using agar-well diffusion method [6, 7]. The micro-organisms were incubated at 37^{o}C for 24 hours and the microbial cultures were adjusted by comparing them against 0.5 McFarland before transferring to the plate. A sterile cork borer was used to make 9 mm diameter wells on the agar. The extract and fractions were diluted with ethanol and screened for antibacterial activity using 100 mg/mL concentration extracts. These were then applied to each well in the culture plates previously inoculated with the test organisms. The plates were incubated at 37^{o}C for 24 hours for bacteria and at 28^{o}C for 72 hours for fungi. Antimicrobial activity was determined by measuring the zone of inhibition in 'mm' around each well for extract and fractions. This was done in triplicate with ciprofloxacin as a positive control for bacteria and fluconazole for fungi.

2.2.2. Determination of Minimum Inhibitory Concentration (MIC's). The MIC was determined when the' least concentration of the extracts inhibited (stop) the growth of the test organisms after 24 hours. This was accomplished using the tube dilution method as described by the Clinical and Laboratory Standards Institute. Where, 1 mL of different concentrates (3.13 mg/mL, 6.25 mg/mL, 12.50 mg/mL, 25.00 mg/mL, 50.00 mg/mL and 100.00 mg/mL) of extract and fractions in nutrient broth was placed in different test tubes. They were further incubated at 37^{o}C for 24 hours after adding bacteria and observed for turbidity. The least concentration where no turbidity was observed was noted as the Minimum Inhibitory Concentration (MIC) value. The experiment was carried out in triplicates for accuracy [8].

2.3. Gas Chromatography-Mass spectrometry

GC-MS analyses were performed on Agilent 19091S-433UI system equipped with HP-5MS ultra inert capillary column (30 m × 0.25 mm × 0.25 µm). The oven temperature was programmed from 50°C to 325°C at 10°C/min for 5 min. The carrier gas was helium with a flow rate 0.73677 mL/min. The volume of sample injected was 2 µL of diluted in benzene in a spit mode of 10:1. The mass spectrometer was in the EI mode at 70 eV in m/z range 50 -550 amu. It had a run time of 47 min. The identification of components present in the extract and fractions was based on direct comparison of the retention times and mass spectral data with those for standard compounds and by computer matching with the NIST14.L GC-MS Library [15].

3. Results and Discussion

The *in vitro* antimicrobial activity of methanol extract, chloroform fraction and *n*-hexane fraction of the leaves of *P. longifolia* showed activity against the human pathogenic micro-organism (*S. aureus*, *S. typhi*, *C. albican* and *T. rubrum*) used in this study.

Table 1. Anti-microbial Activity of Extract and Fractions *P. longifolia* Leaf

Samples	*S. aureus*	*S. typhi*	*T. rubrum*	*C. albican*
Methanol extract (mm)	9.67 ± 1.25	12.33 ± 0.47	7.33 ± 0.47	13.33 ± 0.94
Chloroform fraction (mm)	12.00 ± 0.82	13.00 ± 0.82	10.33 ± 1.24	10.00 ± 0.82
Hexane fraction (mm)	9.33 ± 0.47	11.00 ± 0.82	8.33 ± 0.47	10.67 ± 0.47
Standards (mm)	22.00	23.00	22.00	26.00

Positive control for bacteria is ciprofloxacin; for fungi is fluconazole at 100 mg/mL; all data are reported in triplicate mean ± standard deviation

The results suggested that extract and fractions from the leaves of *P. longifolia* possess toxic activity against Gram-positive bacteria *(Staphylococcus aureus*), Gram-negative bacteria (*Salmonella typhi*) and fungi (*Trichophyton rubrum* and *Candida albican*) as shown in Table 1. The activity index, Figure 1, shows the effectiveness of methanol extract, chloroform fraction and *n*-hexane fractions against the micro-organisms used as compared to the standards (ciprofloxacin and fluconazole) used. This is an indication that the active component in extracts can be isolated as antimicrobial agents and can then be used in the treatment of diseases caused by such organisms as used in this study.

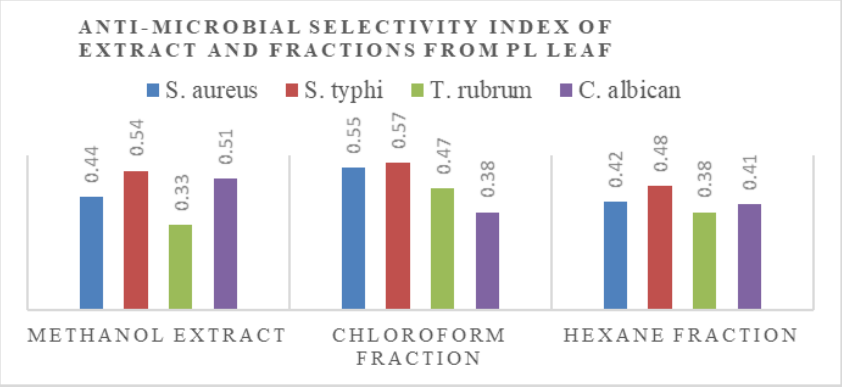

Figure 1: Selectivity Index of Polyalthia longifolia leaf extracts and fractions

The minimum inhibitory concentration ranged from 12.50 mg/mL the chloroform fraction with *S. typhi* to 100 mg/mL as shown in Table 2 below. Some of the extracts can be used as drug boaster on already existing drug used in treatment of diseases caused by such organisms.

Table 2. Minimum Inhibitory Concentration (mg/mL) of *P. longifolia* Extract and Fractions

Samples	*S. aureus*	*S. typhi*	*C. albican*	*T. rubrum*
Methanol extract	50.00	25.00	25.00	100.00
Chloroform fraction	25.00	12.50	25.00	50.00
Hexane fraction	50.00	50.00	50.00	100.00
Standards	6.25	6.25	3.13	6.25

Positive control for bacteria is ciprofloxacin; for fungi is fluconazole at 100 mg/mL

The constituents of the leaf oil have been structurally identified and elucidated by GC-MS. Identification of components was based on comparison with matching library spectral using their retention time (RT). GC-MS analysis of *P. longifolia* revealed that active component consists of a mixture of nine compounds present in methanol extract, three compounds in the chloroform fraction and ten compounds in the *n*-hexane fraction; as shown on Table 3. The volatile chemical constituent in the methanol leaf extract and solvent fractions (chloroform and *n*-hexane) are composed of essential oils (*Z*)–7–hexadecenal, caryophyllene, aromandendrene, methyl palmitate, ethyl palmitate, methyl stearate, oleic acid and 9-tetradecenal.

These volatile compounds are known to possess enormous potential to exhibit microbial pathogens. For instance, caryophyllene and aromandendrene are known for their anti-microbial potential as reported in other studies [3]. Other compounds identified such as Hexadecanoic acid have antioxidant, hypocholesterolemic and haemolytic properties [16]. Octadecanoic acid is used in cosmetic, flavour, lubricant and perfumery [17]. It has been reported that the compound, phytol contains phytochemicals such as terpenoids, flavonoids, alkaloids, tannins etc. In killing bacteria, anti-cancer agents and treatment of diseases. Phytol is also known as an acrylic diterpene alcohol which is used to produce synthetic Vitamin K (for improving bone health and treatment of gastrointestinal illness) and Vitamin E (for stronger immune system, healthy skin, and reduced cell aging) which have essential roles of the human system. It is essential in reducing blood cholesterol and effective in enzymes activation helping in the production of insulin. When phytol in plants are digested in the intestine, they are released, converted to phytanic acid which is stored in the body's plant tissue [18]. Studies from literatures suggest that *P. longifolia* contains diterpenoids, alkaloids and sesquiterpene derivatives [9]. Hydro-distillation by Clevenger type apparatus, also led to identification of some similar compounds in leaf extract of *Polyalthia longifolia* sonnerat (Thwait) [10]. The difference in the composition of the plant extracts could be as a result of different mode of extraction and environmental factors.

Table 3. GC-MS Results of Identified Compounds in *P. longifolia* Leaf Extract and Fractions

	Methanol extract		**Chloroform fraction**		**N-Hexane fraction**	
S/N	t_R (min)	Name of Compound	t_R (min)	Name of Compound	t_R (min)	Name of Compound
1	1.1957	(Z)-7-Hexadecenal	24.9018	Ethyl palmitate	1.1955	cis-9-Hexadecenal
2	18.1957	Caryophyllene	31.6079	2,2'-methylenebis[6-(1,1-dimethylethyl)-4-methyl-Phenol	18.7391	Aromandendrene
3	18.7390	Aromandendrene	35.6476	Oleic Acid	18.9222	α-Curcumene
4	18.9723	α-Curcumene			20.4957	Caryophyllene oxide
5	24.2210	Methyl 14-methyl Pentadecanoate			24.1578	Methyl palmitate
6	26.1721	Methyl trans-13-Octadecenoate			25.1935	2-Methyl-Z,Z-3,13-octadecadienol
7	26.3896	Phytol			26.0976	9-Tetradecenal, (Z)-
8	26.4354	Methyl stearate			26.3608	Methyl stearate
9	36.0139	9-Octadecenoic acid (Z)-, 2-hydroxy-1-(hydroxymethyl)ethyl ester			31.3103	2-Methyl-Z,Z-3,13-octadecadienol
10					36.0141	9-Octadecenoic acid (Z)-, 2-hydroxy-1-(hydroxymethyl)ethyl ester

4. Conclusion

The bio-activity of leaf extract in methanol and the fractions in chloroform and *n*-hexane of *P. longifolia* has been established at least for the microorganisms used in this work. We have also been able to identify some components in the extract that may be contributing to the bioactivity of the extracts. The presence of these compounds in the extracts confirms a potential role for the use of *P. longifolia* (Engl. & Diels) Verde in pharmaceuticals.

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6. Disclosure Statement:

The authors wish to declare there is no potential conflict of interest.

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