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RESEARCH ARTICLE

ANTIBACTERIAL EFFECTS AND ESSENTIAL OILS OF *PILIOSTIGMA RETICULATUM (*BENTH)

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ABSTRACT

Ethyl acetate extract of the Stem bark of *Piliostigma reticulatum* was tested against clinical bacterial strains including; *Escherichia coli, Klebsiella aerogenes, Proteus vulgaris, Pseudomonas aeruginosa, Salmonella typhi, Streptococcus pyogenes, Staphylococcus aureus, Serratia marscences, Shigella dysenterie* and *Yersinia enterocolitica* using the agar well diffusion method of Perez, (1990). Purified extract were subjected to spectra analysis using NMR and GCMS.Result obtained revealed that the plant extract has antibacterial properties *against E. coli, Str. Pyogenes, K. aerogenes, S.aureus, and Sh. dysenteriae* with zones of inhibition ranging from 10mm – 20mm at 100mg/ml of extracts. Lower zones were observed for lower concentrations. Essential oils discovered in purified extract include sesquiterpenes, esters, fatty acids, alcohols and palmitic acids. However, sesquiterpenes were found to be most abundant. *Piliostigma reticulatum* justifies its use in traditional medicine and some of the mode of action include destroying the microorganism' ability to produce catalase and the ability to stimulate non-opsonic phagocytosis.

INTRODUCTION

Plants represent an inexhaustible and extensivesource of novel compounds for recent antimicrobial agent development. Plants extracts and their active constituents have beenused in the traditional treatment of disease from time immemorial (Reghu *et al.,* 2017). More than 9000 native plants have been identified and recorded for their pharmacological properties. The interest in using natural resources or medicinal plants is increasing worldwide due to their perceived safety, efficacy, cultural acceptability and lesser side effects as compared to synthetic drugs (Aktar and Foyzun, 2017). The entire natural resources are being screened in a rapid pace to identify potential drug leads (Reghu *et al.,* 2017). The family fabaceae or leguminonaceae are a large and economically important family of flowering plants. It includes trees,shrubs, and herbaceous plants, perennial or annual which are really recognized by their fruits and their compounds, stipulated leaves (Rahman *et al*., 2014). This group is widely distributed and is the third largest land plant family in terms of numbers of species (Judd *et al*., 2002; Stevens, 2006). *Piliostigma reticulatum*Benth (fabaceae*)* is a plant used extensively in traditional medicine. It is medicinal plant belonging to the family fabaceae (leguminosae). In Nigeria (Yoruba tribe), it is commonly called Abafe. The plant healing properties are usually extracted from the leaves, pods, stem, twigs and roots (The editor, 2015). The plant is used for the treatment of ulcer, fever, rheumatism, cough, toothache, hookworms, diuretic, and diabetes (The editor, 2015). In this study, the antibacterial property, and essential oils present in *P. reticulatum* were investigated.

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MATERIALS AND METHODS

Sampling, identification and preparation of plant material: Stem bark of *Piliostigma reticulatum* was sampled from a private garden of a traditional practitioner in Ibadan, Oyo State. The sample was collected in the morning between 9.00am and 11.a.m. The plant was identified by Mr F.O. Omotayo of the Department of Plants Science and Biotechnology, Ekiti StateUniversity, Ekiti State. Voucher specimen with voucher number WHAE2017/00 was deposited in the herbarium of the Ekiti State University, Ado-Ekiti.Plants nomenclature and uses are depicted in table 2. The plant material was washed with distilled water, air dried at room temperature, milled into powder and kept in sterile containers until use as described by Fabricant and Farnsworth, (2001).

Extraction procedure: The ethyl acetate extract was prepared by steeping 200gm of ground powder of stembark of *Piliostigma reticulatum* in 250 ml of ethyl acetate. The solution was allowed to stand for 5days under constant agitation on a magnetic stirrer after which the solution was filtered and then evaporated to dryness in the fume cupboard under sterile condition. The crude extract was stored in sterile bottle until use.

Evaluation of the antibacterial properties of plant extract: A 5gm portion of the crude extract was reconstituted in 50ml of 50% DMSO (Dimethylsulphoxide). The agar well diffusion method of Perez, (1990) was employed in the antibacterial test. Nutrient agar plates were prepared and were inoculated with test inoculum standardized with the Mac Farland's constant using the pour plate method.

The plates were allowed to set and wells of 6mm in diameter were punched on the agar and 0.1ml aliquot of the extract was placed in the hole. The plates were allowed to stand for 5 min to allow the extract to diffuse into the agar and the plates were the incubated at 37^0C for 24hrs. The diameter zones of inhibition were measured with a ruler and the results recorded. The experiments were carried out in triplicate and the average values recorded.

Purification of plant extract

Column chromatography Procedure: The crude extract of *Piliostigma reticulatum*(1.5 g) was suspended in 10mls of petroleum ether and allowed to stand. 90gm of 60-120 mesh silica gel was used to pack the column by wet packing. The silica gel was used as the stationary phase while varying solvent combinations of increasing polarity were used as the mobile phase (Cosa *et al.,* 2006). In the setting up of column chromatography, the lower part of the glass column was stocked with glass wool with the aid of glass rod. The slurry prepared by mixing 90 g of silica gel and 100 ml of Petroleum ether was poured down carefully into the column. The tap of the glass column was left open to allow free flow of solvent into a conical flask below. The set up was seen to be in order when the solvent drained freely without carrying either the silica gel or glass wool into the tap. At the end of the packing process, the tap was locked. The column was allowed 24 h to stabilize, after which, the clear solvent on top of the silica gel was allowed to drain down to the silica gel meniscus. The wet packing method was used in preparing the silica gel column.

The plant's extract was gently layered on top of the column. The column tap was opened to allow the eluent to flow at the rate of 40 drops per minute. Elution of the extract was done with solvent systems of gradually increasing polarity using Petroleum ether, chloroform, ethyl acetate and methanol. A measured volume (100ml) of each solvent combination was collected gradually with a 10 ml syringe and sprayed uniformly by the sides of the glass into the column each time. This measure prevented solvent droplets from falling directly and disturbing the topmost layer of the column. Distortion of this layer would result in non-uniform drain of the fractions. The eluted fractions were collected in aliquots of 100 ml in conical flasks. Fractions with the same RF values were pooled.The relative Retention factor (Rf) value was calculated using the expression:

$$Rf = \frac{\text{Distance traveled by sample}}{\text{Distance traveled by the solvent}}$$

The fractions were kept at 4^0C in the refrigerator for further works**.**

Evaluation of antimicrobial activity of purified fractions of plant's extract: The disk diffusion method of Kirby-Bauer *et al,* (1966), using Nutrient agar was employed. The culture was prepared by the pour plate method. About 1ml of the fresh culture was pipetted into sterile petri-dishes and about 20ml of molten nutrient agar was poured on the culture and the plates were swirled gently to ensure even distribution of the culture. The plates were allowed to set. Disks of about 5mm in diameter were loaded with 100mg/ml extracts and placed equidistant from each other on the plates. A disk soaked with DMSO as the positive control was placed at the center of the plate and the plates were incubated right way up at 37^oC for 24hr after which activities were observed and corresponding zones of inhibition measured in mm and recorded. The experiment was performed in triplicates.

Evaluation of the Nuclear Magnetic Resonance (NMR) of purified fractions: The purified sample was placed in an inert solvent (deuterochloroform ($CDCl_3$), deuteriumoxide (D2O), carbon tetrachloride (CCl_4) or deuterated dimethyl sulphoxide (DMSO) and the solution was placed between the poles of a powerful magnet. The different chemical shifts of the proton according to their molecular environments within the molecule were measured in the NMR apparatus relative to a standard, usually tetramethyl silane (TMS). Chemical shifts are measured in ppm units, where

$$\delta = \Delta V X\ 10^6/V_{op}$$

ΔV being the difference in absorption frequency of the sample and the reference compound (TMS) in Hertz units and V_{op} in the operating frequency. The intensity of the signals may be integrated to show the number of protons resonating at any one frequency. Each chemical shift value corresponds to a set of protons in a particular environment. The intensity of each signal signifies the number of protons of each type.

Gas Chromatography and Mass spectra (GC-MS) analysis of purified fractions: Ethyl acetate extracts of Stem bark of *Piliostigma reticulatum* was analyzed with the help of GC- MS analyzer (Perkin Elmer Gas Chromatography- Mass Spectrum). On Elite-1 column the date was generated. The carrier gas helium (99.999%) was used at flow rate of 1 ml per min in split mode (10:1). 8µ of sample was injected to column at 250^oC injector temperature. Temperature of oven starts at 60^oC and hold for 6min and than it was raised at rate of 10^oC per min to 300^oC without holding. Holding was allowed for 6 min at program rate of 5^oC per min. temperature of ion sources was maintained at 240^oC. The injector temperature was set at 250^oC and detector temperature was set at 260^oC.The mass Spectrum of compounds present in samples was obtained by electron ionization at 70eV and detector operates in scan mode 50 to 600Da atomic units. A 0.5 seconds of scan interval and fragments from 50 to 600Da was maintained. Total running was 40 minutes.

RESULTS AND DISCUSSION

Antibacterial activity of crude extract: Table 1 shows the antibacterial activity of crude and the purified extract against the test organisms. *P.reticulatum* was active against *E.coli* with a zone of inhibition of 16mm, *Str. pyogenes* with zone of inhibition of 18mm, *K. aerogenes* with zone of inhibition of 10mm*, S. aureus* with zone of inhibition of 16mm and *Sh. dysenteriae* with zone of inhibition of 20mm at 100mg/ml of extracts.

Antibacterial activity of partially purified extract: The separation procedure for *Piliostigma reticulatum* yielded 10 fractions and after compounds with the same Rf values were pooled, two (2) compounds coded $Pr3_6$ and $Pr5_6$ were subjected to antimicrobial test. Fraction Pr 3_6 was active against five (5) of the test organisms (Table 1) which include *E. coli* with zone of inhibition of 4mm, *Str. pyogenes* with zone of inhibition of 9mm, *S.aureus* (10mm) and *Sh. dysenteriae* (6mm) while it was not effective against *K. aerogenes*.

Table 1. Antibacterial activities of crude and purified extracts of *P. reticulatum*

Test organisms	Antibacterial activity at 100mg/ml (mm)		
	Crude fraction	Purified fraction	
		$Pr3_6$	$Pr5_6$
Pseudomonas aeruginosa	-	-	-
Salmonella typhi	-	-	-
Escherichia coli	16	4	8
Proteus vulgaris	-	-	--
Streptococcus pyogenes	18	9	10
Klebsiella aerogenes	10	-	4
Staphylococcus aureus	16	10	08
Serratia marscences	-	-	-
Yersinia enterocolitica	-	-	-
Shigella dysentariea	20	6	8

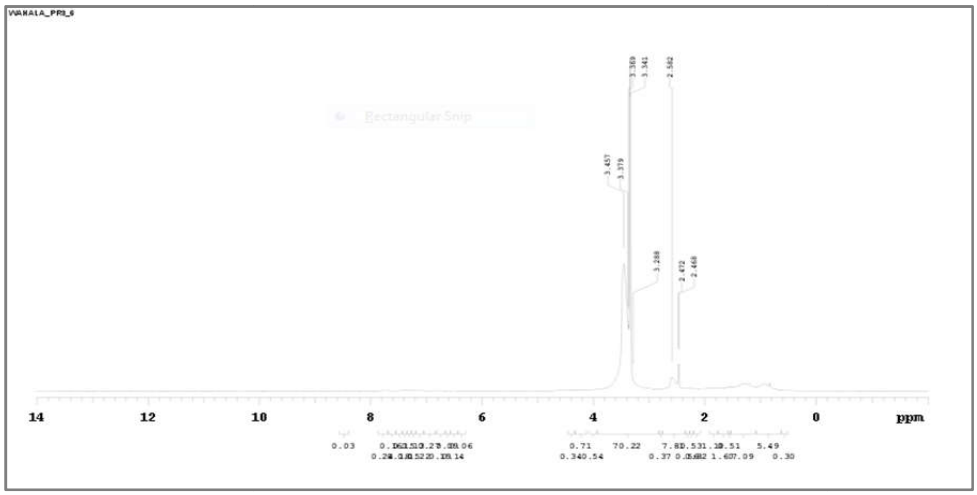


Figure 3. NMR spectra of $Pr5_6$

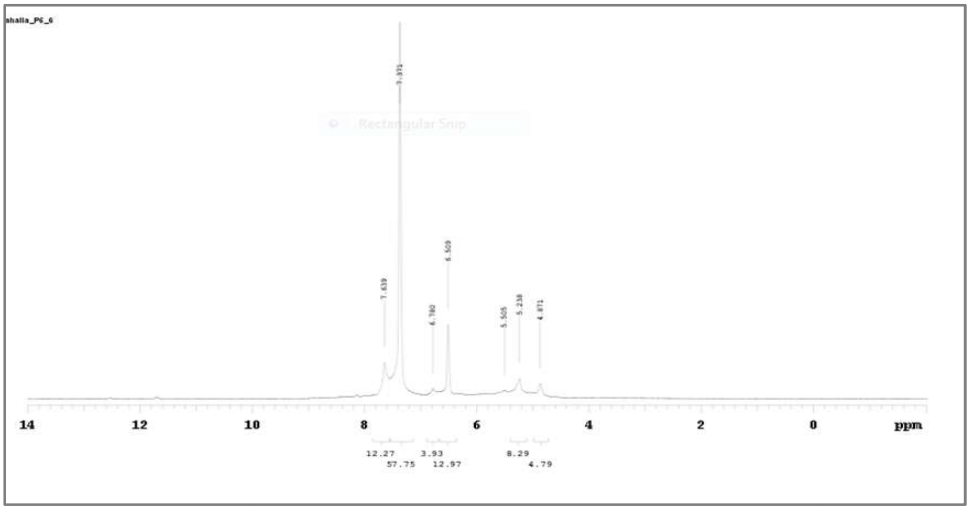


Figure 4. NMR spectra of $Pr3_6$

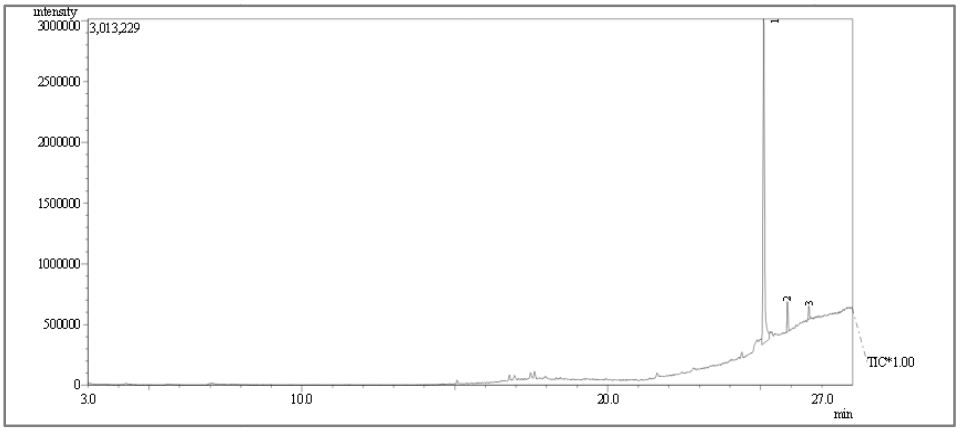


Figure 5. The GC-MS analysis of Pr3 fraction of *Piliostigma reticulatum*

Table 2. Phytocomponents identified in the ethyl acetate extract of *Piliostigma reticulatum* (Fraction $Pr3_6$) by GC-MS Peak Report TIC

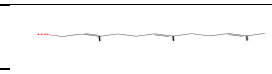
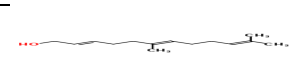
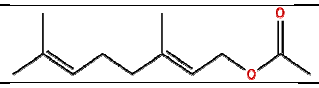
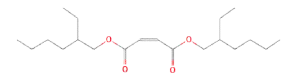
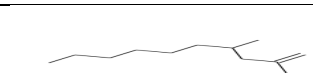
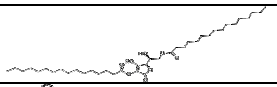
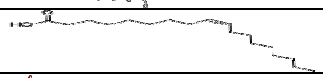
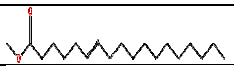
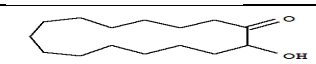
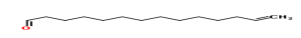
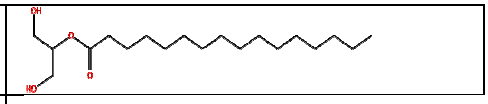
Peaks	R time	IUPAC name, chemical formula and common name	Mol weight	% CONC	Family	Chemical structure
56	25.103	2,6,10-dodecatrien-1-ol, 3,7,11,trimethyl $C_{15}H_{26}O$ Farnesol	222	91.37%	Sesquiterpene	
		6,11,-dimethyl-2,6,10-dodecatrien-1-ol: $C_{14}H_{24}O$ Farnesol	208		sesquiterpene	
37	25.833	2,6, -octadiene-1-ol,3,7,-dimethylacetate $C_{12}H_{20}O_2$: Geraniol acetate	196	5.99%	monoterpene	
34	26.583	Decanoic acid,2-ethylhexylester $C_{18}H_{36}O_2$	284	2.64	Esters	
		Bis(2-ethyhexyl)maleate C20H36O4	340		Ester	
		1-decene2,4 dimethyl $C_{12}H_{24}$ **Alkene**	168			
		N, octyl ether $C_{16}H_{34}O$ Caprylic ether	240		Fatty acid	

Table 3. Compounds identified in the ethyl acetate extract of *Piliostigma reticulatum* (Fraction $Pr5_6$) by GC-MS Peak Report TIC

Peaks	R time	IUPAC name, chemical formula and common name	Mol weight	% CONC	Class	Chemical structure
49	22.383	N, hexadecanoic acid (Palmitic acid) $C_{16}H_{32}O_2$	256	10.78%	Fatty acid	
		Octadecanoic acid $C_{18}H_{36}O_2$; Stearic acid Hydrofol	284		Fatty acid	
		1-(+)-Ascorbic acid,2,6-dihexadecanoate;Dipalmitate $C_{38}H_{68}O_8$	652		Fatty acid	
75	24.525	Oleic acid $C_{18}H_{34}O_2$ Octadeer	282	50.80%	Fatty acid	
		6- Octadecanoic $C_{18}H_{34}O_2$	282		Fatty acid	
		9-Octadecenal (z) $C_{18}H_{34}O$	266		Fatty acid	
		Cyclopentadecanone $C_{15}H_{28}O_2$	298		Fatty acid	
	24.708	Stearic acid $C_{18}H_{36}O_2$	284	25.74	Fatty acid	
71	27.033	9,12-Octadecadienoylchloride, (z) $C_{18}H_{31}ClO$	298	6.66%	Fatty acid chloride	
		7, Tetradecenal (z) $C_{14}H_{26}O$	210		Fatty Acyls	
		13, Tetradecenal ; Megastima $C_{14}H_{26}O$	210		Fatty Acyls	
		Cis-9-hexadecenal $C_{16}H_{30}O$	238		Fatty Acyls	
		8-Cyclohexadecen-1-one $C_{16}H_{28}O$	236			
55	27.183	Octadecanoic acid,2-hydroxyl-1,3, propanediylester. $C_{39}H_{76}O_5$	624	2.87%	Ester	
		Hexadecanoic acid,2-hydroxy-1,3-propanediylester. $C_{35}H_{68}O_5$	568		Ester	
		15-Hydroxypentadecanoic acid $C_{15}H_{30}O_3$	258		Ester	
		Hexadecanoic acid,1-(hydroxymethyl)-1,2-ethanediylester. Palmityl. $C_{53}H_{68}O_5$	568		Ester	
		Docosanoic anhydride $C_{44}H_{86}O_3$	662		Fatty acid	

Continue…….

56	27.433	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethylester $C_{19}H_{38}O_4$	330	3.15%	Ester	
		Hexadecanoic acid, 2,3- dihydroxypropyl ester $C_{19}H_{38}O_4$	330		Ester	
		Hexadecanoic acid,1-(hydroxymetyl)-1,2-ethanediyl ester $C_{35}H_{68}O_5$	568		Ester	
		Pentadecanoic acid,2-hydroxy-1-(hydroxymethyl)ethyl ester $C_{18}H_{36}O_4$	316		Ester	

The $Pr5_6$ fraction was active against *E.coli* (8mm), *Str. pyogenes* (10mm), *K. aerogenes* (4mm), *S. aureus* (8mm) and *Sh. dysenteriae* (8mm) (Table 1). The two Fractions (Pr 3_6 and Pr 5_6) were subjected to spectra analysis using NMR and GCMS

NMR spectra of purified fractions: $Pr3_6$; Aklyl esters and amides were found at peaks 3.457 and 3.379 peak 2.582 showed the presence of benzylic protons; alkanes, alcohols and alkyl ethers were found at peak3.288. Peak 2.472 presented benzyl protons while peak 2.468 presented benzyllic protons (Fig 1).

GC-MS spectra of purified fractions: The total ion chromatogram (TIC) of ethyl acetate extract of *P. reticulatum* showing the GC-MS profile of the compounds identified is given in the figures 4 and 5.The peaks in the chromatogram were integrated and were compared with the database of spectrum of known components stored in the GC-MS library. The detailed tabulations of compounds identified in the GC-MS analysis of the extract are given as shown in the table 2-3 as presented. The major compounds observed in this fraction are; Farnesol, Geranoil and Caprilic ether. These compounds were identified at different peaks.

Figure 4 shows the spectra observed in fraction Pr 5_6 of *Piliostigma reticulatum*. Six peaks were observed in fraction $Pr5_6$and twenty nine compounds were identified. The major compound found in this fraction are Palmitic acid, stearic acid, Dipalmitate, octadeer, megastima, Palmityl and esters. The individual compounds are as presented in table 34. At peak 49 and R-time 22.383, N, hexadecanoic acid (Palmitic acid), Octadecanoic acid (stearic acid), 1-(+)-Ascorbic acid,2,6-dihexadecanoate (Dipalmitate) were identified. At peak 75 and R-time 24.525, Oleic acid, 6- Octadecanoic, 9-Octadecenal (Z), Cyclopentadecanone were identified. At peak 71 and R-time 27.033 9, 12-Octadecadienoylchloride, (z), 7, Tetradecenal (z), 13, Tetradecenal (Megastima) and Cis-9-hexadecenal and 8-Cyclohexadecen-1-one were identified. At peak 55 and R-time 27.183, Octadecanoic acid, 2-hydroxyl-1,3, propanediylester; Hexadecanoic acid – 2 - hydroxy,1,3-propanediylester, Octadecanoic acid,2-hydroxyl-1,3, propanediylester, Hexadecanoic acid, 2-hydroxy-1,3-propanediylester, 15-Hydroxypentadecanoic acid; Dosocanoic anhydride were identified. At peak 56 and R-time 27.433, Hexadeanoic acids-2,3 dihydroxypropyl ester, hexadecanoic acid,1-9hydroxymethyl)1,2 ethanediyl esters and pentadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester were identified.

$Pr5_6$: Figure 7 shows the spectra observed in fraction $Pr5_6$ of *Piliostigma reticulatum*. Six peaks were observed in fraction $Pr5_6$and twenty nine compounds were identified. The major compound found in this fraction are Palmitic acid, stearic acid, Dipalmitate, octadeer, megastima, Palmityl and esters.

The individual compounds are as presented in table 3.At peak 49 and R-time 22.383, N, hexadecanoic acid(Palmitic acid), Octadecanoic acid (stearic acid),1-(+)-Ascorbic acid,2,6-dihexadecanoate (Dipalmitate) were identified.At peak 75 and R-time 24.525,Oleic acid, 6- Octadecanoic, 9-Octadecenal (Z), Cyclopentadecanone were identified. At peak 71 and R-time 27.0339,12-Octadecadienoylchloride, (z), 7, Tetradecenal (z), 13, Tetradecenal (Megastima) and Cis-9-hexadecenal and 8-Cyclohexadecen-1-one were identified. At peak 55 and R-time 27.183, Octadecanoic acid,2-hydroxyl-1,3, propanediylester; Hexadecanoic acid – 2 - hydroxy,1,3-propanediylester, Octadecanoic acid, 2-hydroxy l-1,3, propanediylester, Hexadecanoic acid, 2-hydroxy-1,3-propanediylester, 15-Hydroxypentadecanoic acid; Dosocanoic anhydride were identified. At peak 56 and R-time 27.433, Hexadeanoic acids-2,3 dihydroxypropyl ester, hexadecanoic acid,1-9hydroxymethyl)1,2 ethanediyl esters and pentadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester were identified

DISCUSSION

In recent years, there has been a rise in the search for plants with phytochemicalsand possessing antimicrobial properties in treating chronic infectious diseases. This study has shown that *P.reticulatum* possesses antibacterial properties against some of the test bacteria used. Some of these organisms have been indicated in resistance to antimicrobials such as *Shigella dysenteriae* which is implicated in multidrug resistant shigellosis and dysentery. *S. dysenteriae* is known to be resistant to third generation cephalosporin and Fluoroquinone (Taneja and Mewara, 2016). This plant is also effective against *Str. pyogenes*which is implicated in sepsis, Strept throat, toxic shock syndrome, glomerulonephritis amongst other 600 million infections (Lyskey *et al*., 2011). *P. reticulatum* has been shown to be active against broad range of bacteria, especially those implicated in enteric infections. It is used as antiplasmodic and are usually prescribed traditionally for gastrointestinal diseases (Jawaila *et al.,* 2000).

The antibacterial activity of the plant in this work confirms its use in the treatment of enteric infections.The comparative antibacterial activities of the crude and purified extracts showed marked difference in the values. These findings may suggest that the constituents in combination may have synergistic effects. This is in agreement with many reports that have shown higher antibacterial potency for crude extracts as compared to a fraction (Martins *et al*, 2013; Solatanian *et al*, 2016). Essential oils are complex volatile compounds naturally synthesized by plants during the process of secondary metabolism. Essential oils have great potentials in the field of biomedicine as they effectively destroy several bacterial, fungal and viral pathogens. The presence of different types of aldehydes, phenolic, terpenes and other antimicrobial compounds means that the essential oils are effective against diverse range of pathogens (Swarmy, 2016).

Essential oils in addition to their aromatic properties have biological activities closely linked to their chemical composition and related with environment and human health, their use to treat respiratory tract and skin related infections have been confirmed (Blazquez, 2014). Fraction $Pr3_6$ was found to contain 2,6, 10,dodecatrien-1-ol; $C_{15}H_{26}O$ (Farnesol); 3,7,11- Trimethyl; 6,11-dimethyl-2,6,10-dodecatriene-1-ol $C_{16}H_{26}O$ (Farnesol); 2,6- octadiene-1-ol, 3,7-dimethylacetate $C_{12}H_{20}O_2$. (Geranyl)(Table 3).Gonclave *et al*., (2012) discovered that these essential oils are active against fungi (*Cryptococcus neoformis, Candida guillermondii*). They explained that the antifungal activity could be associated with the significant contribution of oxygenated monoterpines geranyl acetate. Farnesol, a sesquiterpene alcohol is widely used as antiinfammatory and antiallergic (Ku and Lin, 2015).

The most abundant essential oil in this plant is farnesol (91.37%). Brilhante *et al*., (2012) noted in their work that farnesol was effective when tested against *Burkholderis pseudomallei*. Several authors have also reported the antimicrobial effect of farnesol against a wide range of microorganisms and the synergistic effect with β- lactam antibiotics on microbes (Koo *et al.,*2003; Inoue, 2004;Derengowski *et al.,*2009). This probably explains the activity of this plant against a wide range of bacteria. 6,11,-dimethyl-2,6,10-dodecatrien-1-ol; 2,6,-octadiene-1-ol,3,7,-dimethylacetate (Geraniol acetate); also discovered, is known to possess pharmaceutical and biological activities like antifungal, antibacterial, anti-inflammatory properties against a wide range of microbes (Raut *et al*., 2014). Also discovered were; 6,11,-dimethyl-2,6,10-dodecatrien-1-ol; Decanoic acid, 2-ethylhexylester.

The major compound found in fraction $Pr5_6$are Palmitic acid, stearic acid, Dipalmitate, octadeer, megastima, Palmityl and esters. Palmitic acid; a fatty acid has been reported to be beneficial against many diseases and has a wide range of antibacterial (Sofowora, 2013; McGraw *et al*., 2002), anionic surfactant (Rauha *et al*., 2000) and antifungal properties (Rauha *et al*., 2000). It also has a great antioxidant property (Karimi *et al*., 2015). Stearic acid is known to exhibit antibacterial properties (Da Silva *et al.,* 2002); 1-(+)-Ascorbic acid, 2, 6-dihexadecanoate is used as hyaluronidase inhibitor. Hyaluronidase in bacteria is known to aid in spreading of bacteria in host tissues (Botzkit, 2004). It also used as antioxidant, cardio protective, cancer preventive, flavour and anti-infertility (Haidi *et al*., 2015). Octadecanoic acid is used as ;5-Alpha-Reductase-Inhibitor, Allergenic, Alpha-Reductase-Inhibitor, Anemiagenic, Antialopecic, Antiandrogenic, Antiinflammatory, Antileukotriene-D4 (Anti-platelet activating factor), Cancer-Preventive, Choleretic, Dermatitigenic Flavor, Hypocholesterolemic, Insectifuge Irritant, Percutaneostimulant, Perfumery, and Propecic (Rajeswari and Srinivasan, 2015). Previous works revealed the essential oils in *Piliostigma reticulatum* to be dominated by monoterpenes, and sesquiterpenoid hydrocarbon. Other oils include γ-muurolene, α-pinene, tricyclene, δ-cadinen and α- terpenol. This result is also reflected in the spectra analysis of the plant *P. reticulatum* used in this work. Most commonly found classes of essential constituents found in plant include monoterpene, sesquiterpenes, diterpenes, alcohol, phenols, aldehydes, ketones, esters and oxides. The antimicrobial and the oxidative abilities of this plant correlates with the presence of phytochemicals revealed by the chromatographic analyses of the plant's extracts.

Conclusion

The essential oils present in *P. reticulatum* could be responsible for the antibacterial activity of the plant. This supports the traditional uses of the plant evidenced by its antibacterial activity against selected bacteria strains.

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