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Full Length Research

Chemical Components of *Piliostigma reticulatum* and *Cleistopholis patens* with Antibacterial Properties of their Crude and Purified Extracts

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The aim of this study is to investigate the antibacterial properties, isolate and characterize the essential oils, especially the sesquiterpenes from two medicinal plants *Piliostigma reticulatum* and *Cleistopholis patens.* Stem bark of both plants were sampled, air-dried and subjected to extraction procedures using ethyl acetate. The extracts were subjected to antibacterial analysis using the agar cup diffusion method against *Shigella dysenteriae and Streptococcus pyogenes.* Fractions from the crude extracts were also assayed for antibacterial efficacy employing the disc diffusion method. Spectrum analysis of the fractions was also done to identify their chemical components using nuclear magnetic resonance (NMR), Fourier transform infrared (FTIR), Gas chromatography and mass spectra (GCMS). Results obtained showed that the extract of *P. reticulatum* was effective against *S. dysenteriae* with zones of inhibition of 14 mm, 12 mm, 8 mm and 6mm at concentrations of 100 mg/ml, 60 mg/ml 40 mg/ml and 20 mg/ml respectively while it showed lesser efficacy against *S. pyogenes* with zones of inhibition of 10mm and 8mm at concentrations of 100mg/ml and 80mg/ml respectively. On the other hand, *C. patens* was active against *S. pyogenes* with zones of inhibition of 18 mm, 16 mm, 14 mm, 13 mm, and 8 mm at concentrations of 100 mm, 60 mm, 40 mm, 20 mm and 10 mm respectively. Crude extract showed higher activity than purified fractions against test organisms. Spectra analyses showed the phytochemicals present to include sesquiterpenes, fatty acids, Abeitic acid, palmitic acid, stearic acid and alcohols.

Keywords: Phytochemicals, Fatty acids, purification, antibacterial, GC-MS, NMR, FTIR.

INTRODUCTION AND LITERATURE REVIEW

Medicinal plants are known to produce phytochemicals that are responsible for their pharmaceutical activities. Sesquiterpenes C15 terpenoid is built from their isoprene units (Awouafack et al., 2013) are phytochemicals found abundant in higher plants and in many living systems. They are essential oils, and they act as irritant when applied topically and when consumed, they irritate the gastrointestinal tract (Anon, 2018). In nature, sesquiterpenes play an important role in plant defense, as antibacterial, antiviral, antifungal and insecticides. The biological activity of sesquiterpenes is connected to the presence of α-β- unsaturated γ-lacton ring (Izbosarov et al., 2000).

Cleistophlis patens is a tree up to 27 m high. The infusion of its leaves is used as febrifuge and vermifuge (Boyom et al., 2011) *Cleistopholis patens* (Benth) Engl and Diels belongs to the family Annonaceae. It is widely distributed in Senegal and Uganda. The plant is used for the construction of door frames, roof-beams, drums, floats and canoes. It is sometimes used as food preservatives (Burkil, 1985). The long narrow leaves held in one plane on slightly drooping branches give this tree a distinctive appearance. The leaves are shiny on their upper surface when fresh. This species can grow to a diameter of 50 cm. Decoction of the bark is taken to treat stomach ache, diarrhea, tuberculosis, bronchitis and hepatitis. Bark pulp is applied against swelling, oedema, withlow and bark sap is dropped into the nose to treat headache and rubbed in to treat rickets in children. In Uganda, the bark is used in preparation to treat malaria and measles. In Nigeria, the bark is used to treat typhoid fever and menstrual irregularities (Bolza and Keating, 1972). The root bark is used as vermifuge, leaf infusion or decoction is administered against hepatitis, fever, trypanosomiasis, and rheumatic arthritis (Atuhe, 2010).

Piliostigma reticulatum (DL.) Hochst. (common name; Yoruba: 'abafe', Hausa: 'kalgo', Igbo: okpo atu') belongs to the family Leguminosae - Caesalpiniaceae and is found in the savannah region of Nigeria. It is a tree, occurring up to 30ft in height with an evergreen, dense spreading crown (Keay, 1989). It is used traditionally in the treatment of diarrhea. Tea from the leaves to treat colds, bark is astringent and used against diarrhoea and dysentery; leaves and bark have haemostatic and antiseptic properties, cures also ulcers, boils, wounds and syphilitic cancer. Other medical uses are against coughs, bronchitis, malaria, hepato-biliary ailments, hydropsy, sterility, rachitis and kwashiorkor. This study investigates the phytochemicals present in *P. reticulatum* and *C. patens*, their antibacterial properties of the crude and purified extracts and also to identify specific biological compounds using spectra analysis such as FTIR, NMR and GC-MS.

MATERIALS AND METHODS

Plant sampling and preparation

The stem bark of both the plants were collected from Ibadan, cleaned, dried and subjected to extraction procedures using Ethyl acetate. The extracts were evaporated to dryness and the yield calculated. The extracts were resuspended in 50% DMSO and used in the antibacterial analysis.

Antibacterial test

The agar well diffusion method of Perez, (2006) was employed in testing the crude extracts against *Shigella dysenteriae* and *Streptococcus pyogenes*, while the purified extracts were tested against the bacteria using the disk diffusion method of Kirby-Baeur et al., (2003). Blank discs were impregnated with 0.5 ml of the purified extracts and placed on the surface of inoculated agar plate and incubated.

The extracts were also allowed to pass through purification procedures employing column chromatography, fractions obtained were subjected to spectra analysis using FTIR, NMR and GC-MS.

FTIR: Infrared spectrometry (Fourier transform infra-red)

Fourier Transform Infrared (FTIR) spectroscopy is a technique used to determine qualitative and quantitative features of IR-active molecules in organic or inorganic solid, liquid or gas samples. It is a rapid and relatively inexpensive method for the analysis of solids that are crystalline, microcrystalline, amorphous, or films. Samples are analyzed on the scale of microns to the scale of kilometers and new advances make sample preparation, where needed, relatively straight-forward. Another advantage of the IR technique is that it also can provide information about the "light elements" (e.g., H and C) in inorganic substances. All spectra were obtained with the aid of OMNI sample attenuated total reflectance accessory on an ASCO FTIR spectrophotometer. (FTIR 4600). Purified extracts of the stem bark of *Cleistopholis patens* suspended in methanol were encapsulated and placed directly on the germanium piece of the infrared spectrometer with constant pressure applied. Data of infrared absorbance were collected over the wave number ranged from 4000/cm to 650/cm. The reference spectra were acquired from the cleaned blank crystal before the presentation of each sample replicate. All spectra were collected with a resolution of 4.0-1.0 cm and to improve the signal-to-noise ratio. Samples were run in triplicates. The FTIR spectra of all samples were analyzed on the basis of peak values in the region of infra-red radiations.

Table 1. Antibacterial activity of the Ethyl acetate extracts of *P. reticulatum* and *C. patens*.

	P. reticulatum **(Zones of inhibition in mm)**					***C. patens*** **(Zones of inhibition in mm)**				
Plants/ Conc (mg/ml)	100	60	40	20	10	100	60	40	20	10
Shigella dysenteriae	14	12	08	06		-	-	-	-	
Strepococcus pyogenes	10	08	-	-	-	18	16	14	13	8

Evaluation of the Nuclear Magnetic Resonance (NMR) of purified fractions

The purified sample was placed in an inert solvent deuterochloroform ($CDCl_3$), deuterium oxide (D_2O), 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.

Where, $\delta = \Delta V \times 10^6 / V_{op}$

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* and *Cleistopholis patens* were 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°C injector temperature. Temperature of oven starts at 60°C and hold for 6 min and then it was raised at rate of 10°C per min to 300°C without holding. Holding was allowed for 6 min at program rate of 5°C per min. temperature of ion sources was maintained at 240°C. The injector temperature was set at 250°C and detector temperature was set at 260°C. 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.

RESULTS

Antibacterial activities of crude extract

The results of the antibacterial test carried out showed that *P. reticulatum* showed considerable antibacterial activity against *S. dysenteria* with zones of inhibition of 14 mm, 12 mm, 08 mm, 06 mm at concentrations of 100, 60, 40, and 20 mm/ml of extracts respectively. *C. patens* had no antibacterial activity against *S. dysenteriae*. *P. reticulatum* showed a lesser activity against *S. pyogenes* with zones of inhibition of 10mm and 8mm at concentrations of 100mg/ml and 60mg/ml of extract while the *C. patens* showed efficacy to a larger extent with zones of inhibition ranging of 18mm, 16mm, 14mm, 13mm and 8mm at concentrations of 100, 60, 40, 20 and 10mg/ml of extract (Table 1).

Antibacterial activity of purified extracts

Two purified fractions from *P. reticulatum* and three fractions from *C. patens* were subjected to antibacterial analysis and result is presented in Table 2. The results showed a marked difference in the result of the crude extracts and the purified fractions. The 100 mg/ml of extracts of *P. reticulatum* showeda zone of inhibition of 10 mm against *Streptococcus pyogenes* as compared to the purified fraction ($Pr3_6$ and $Pr5_6$) which had a zone of inhibition of 6 and 4 mm respectively. The crude extract was active against *S. dyseteriae* with a zone of inhibition of 14mm while the fractions ($Pr3_6$ and $Pr5_6$) showed zones of inhibition of 12 mm and 8 mm respectively. The crude extract of *C. patens* was not active against *S. dysenteriae* but had a zone of inhibition of 18mm against *S. pyogenes* whereas the purified fractions showed inhibition zones of between 6, 8 and 4 mm

Table 2. Antibacterial activity of purified fractions of *C. patens* and *P. reticulatum* at 100 mg/ml.

Organisms	Plant extracts / Zones of inhibition in mm at 100mg/ml of extracts						
	Piliostigma reticulatum			***Cleistopholis patens***			
	Crude	Fraction $Pr3_6$	Fraction $Pr5_6$	Crude	Fraction Cp7	Fraction Cp 12	Fraction $Cp12_3$
Shigella dysenteriae	14	12	8	-	-	-	-
Streptococcus pyogenes	10	6	4	18	6	8	4

- = no activity

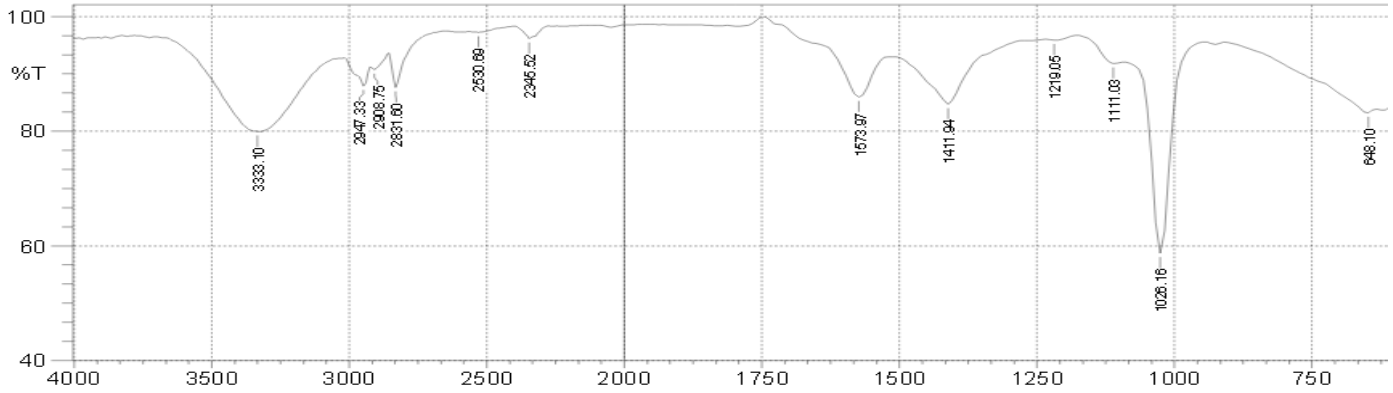

Figure 1. FTIR Spectra of fraction Cp7 of *Cleistopholis patens*.

respectively.

FTIR Spectra for fractions from *C. patens*

Fraction from *Cleistopholis patens*, Sample Cp 7, has the characteristics as presented in Figure 1, twelve peaks were observed. The peak observed at 3333.10cm^{-1} showed primary amines. The peak at 2497.33 cm^{-1} is an alkane with a Sp^3 C-H stretch. The peak at 2831. 60 cm^{-1} showed the presence of aldehyde with C-H. 2345.52 cm^{-1} is a peak showing an overlap of C-H stretch. 1666.55 cm^{-1} is a saturated amide while the peak at 1442.28 cm^{-1} showed a Sp3 C-H bend. The peak observed at 1111.03cm-1 is an aromatic alkoxy (Esters) while that observed at 1026.16 cm-1 is likely to be an Alkoxy medium while the peak at 643.10 shows no active component.

Fraction Cp12

Fourteen (14) peaks were observed for sample Cp12 as presented in Figure 2. Peak 3340.82cm^{-1} is a primary (1^o) amide, peak 2948.33cm^{-1} had Sp3 C-H stretch alkane or an acid with an alkane with Sp^3 C-H stretch. The peak observed at 2901.04 cm^{-1} was an alkane with a Sp^3 C-H stretch. While the peak observed at 2831.6 cm^{-1} was an aldehyde with C-H weak bond while the peaks at 1782.29 cm^{-1} and 1728.28 are unsaturated anhydrides with 2 strong bonds. A nitro compound was found at the 1566.25 cm^{-1} peak. Peaks 1257.63cm^{-1} and 1080.17cm-^{1} contain alkoxy. The peak at 925.89 cm^{-1} is a monosubstituted alkene with Sp2 bend. (Figure 2).

FTIR of $Cp12_3$

The spectra for fraction $Cp12_3$ is presented in Figure 3. Eleven (11) peaks were observed in the spectrum. The peak observed at 3333.10 cm^{-1} showed primary amines. The peak at 2497.33 cm^{-1} was an alkane with a Sp3 C-H stretch. An aldehyde with a C-H was found at the 2831.6 cm^{-1}, While at peak 2530.69 cm^{-1}, a very broad stretch of an acid (thiol) with a very weak bond was present. Peak observed at 2345.52 had an overlap of C-H stretch. Peak at 1666.55 cm^{-1} was a saturated amide while the peak at 1442

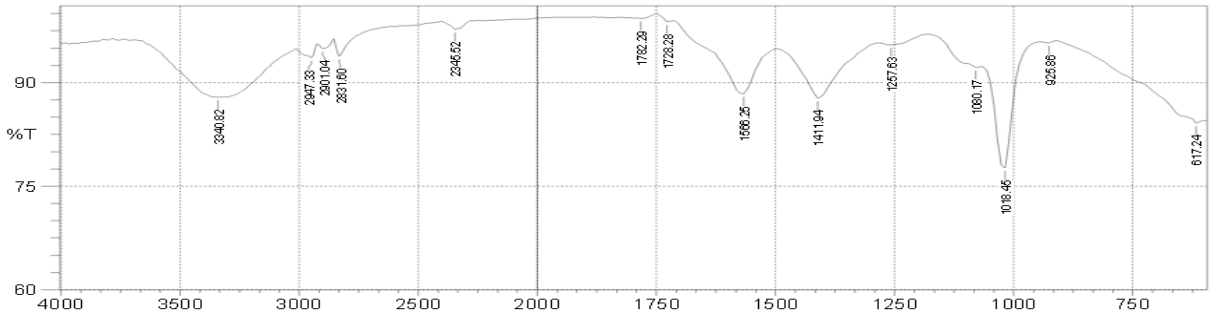

Figure 2. FTIR of fraction Cp12 of *C. patens.*

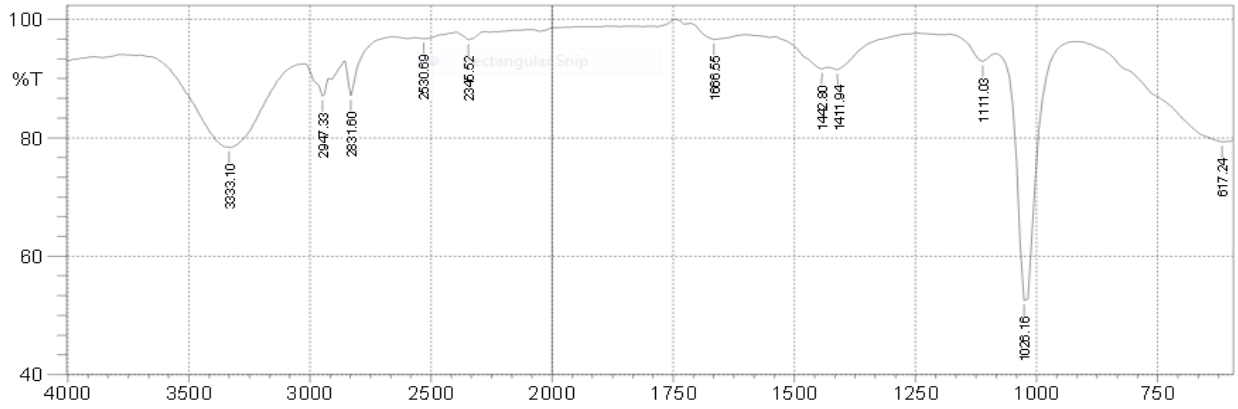

Figure 3. FTIR of fraction $Cp12_3$ from *C. patens.*

has a Sp^3 C-H bend (alkanes).

FTIR of Fractions $Pr3_6$ from *P. reticulatum*

The spetra observed for fraction $Pr3_6$ are presented in Figure 4. Fifteen peaks were observed. Peak at 3595.43 cm^{-1} is an alcohol (O-H). Peak at 3387.11 cm^{-1} was a primary amine (1º). Peaks at 2978.19 cm^{-1} to 2823.88 cm^{-1} are alkanes. An acid with a very broad overlap stretch is observed at the 2522.98 cm^{-1} absorption. Peak at 2252.93 cm^{-1} was a nitrile with a sharp band (C≡N). The peak at 1782.29 cm^{-1} is an anhydride with an unsaturated 2 strong bands. Unsaturated anhydride was observed at peak 1674.27 cm^{-1}. Peak 1519.96 cm^{-1} presented an asymmetric nitro compound. The peak at 1211.34 cm^{-1} is a strong acid.

The spectra observed for fraction $Pr5_6$ is presented in Figure 5. Fourteen (14) peaks were obtained. Peak recorded at 3394.83/cm showed it to be an amine. Peak recorded at 2962.76; cm was an alkane. Peak observed at 2870.17 was an aldehyde. Peak at 1681.98/ cm was an acid. The peak observed at 1589.4 was an amide (N-H bend).

NMR Spectra of purified fractions

Cp7: Cp7 contains alkanes, amides, alkylether and alcohol overlap at peak 3.545. At peak 3.333, aromatic ketones were observed. At peak 2.978, aromatic ketones and amines were discovered. Thiols, alkylether and amines were present at peak 2.469. At peak 2.112, allylic protons and propagylic protons were observed. At peak1.526, epoxides were found (Figure 6).

Cp12: Fraction Cp7 was found to contain at peak 3.490 an alkyl ether, and at peak 2.596, amines were discovered while allylic protons were observed at peak 1.733 (Figure 7).

$Cp12_3$: The fraction $Cp12_3$ was found to contain alkyl

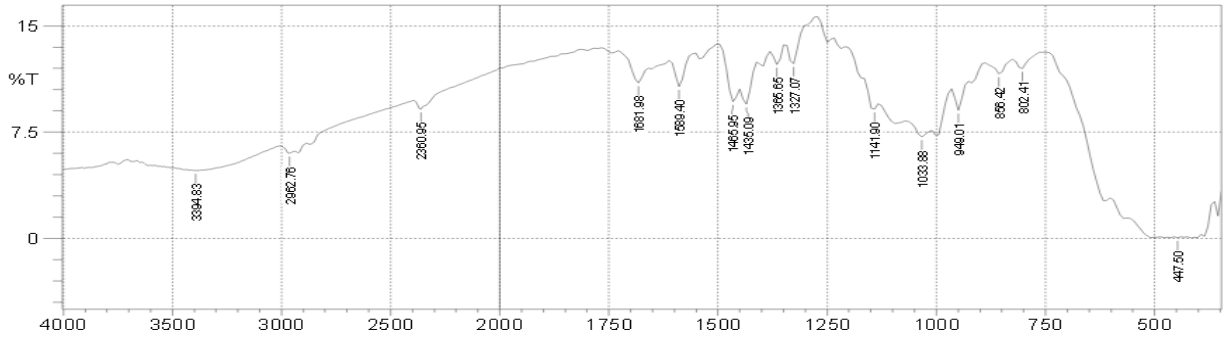

Figure 4. FTIR Spectra of a fraction of *P. reticulatum* ($Pr3_6$).

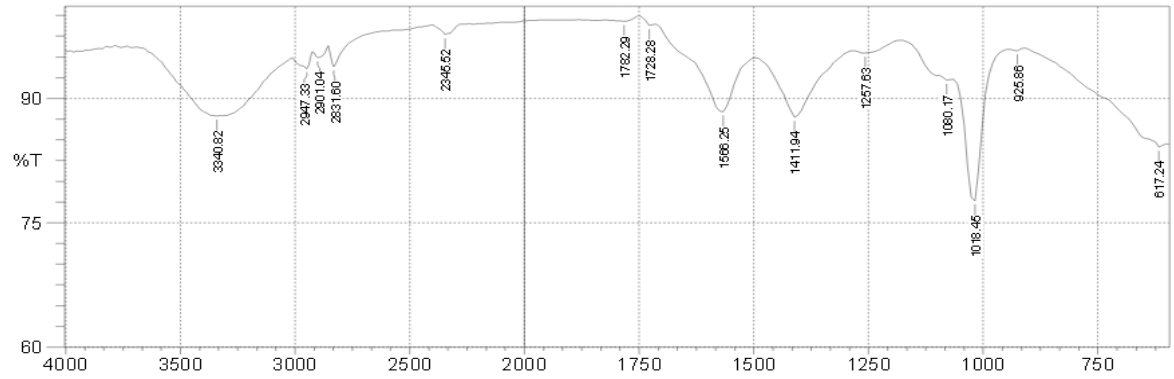

Figure 5. FTIR Spectra of a fraction of *P. reticulatum* ($Pr5_6$).

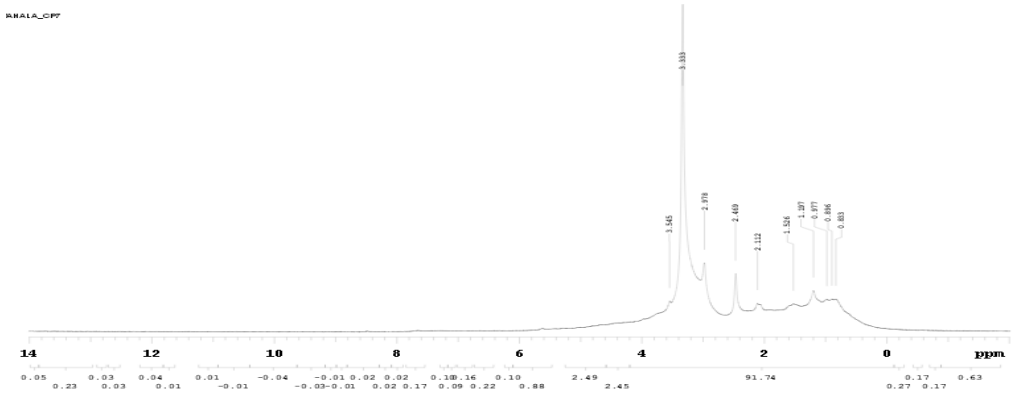

Figure 6. NMR spectra of fraction Cp7 of *Cleistopholis patens*.

esters at peak 3.897 and at peak 2.530, epoxide ether, amines and acetylester thiols were observed (Figure 8).

$Pr3_6$: aklyl esters and amides were found at peaks 3.457 cm^{-1} and 3.379 cm^{-1} peak 2.582 cm^{-1} showed the presence of benzylic protons; alkanes, alcohols and alkyl ethers were found at peak 3.288. Peak2.472 cm^{-1} presented benzyl protons while peak 2.468 cm^{-1} presented benzylic protons (Figure 9).

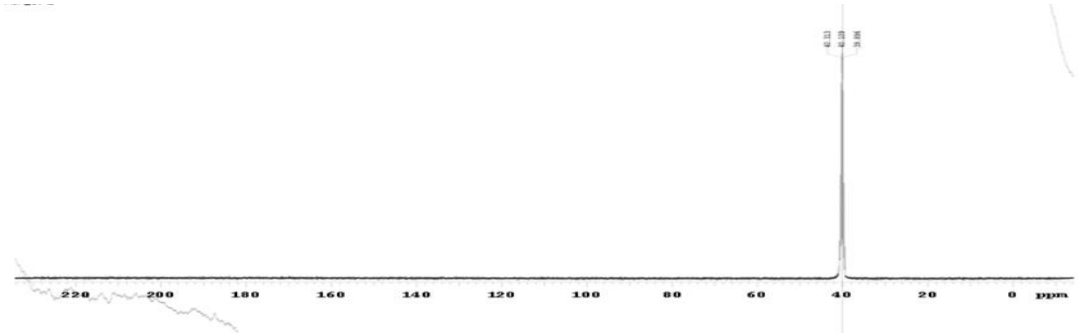

Figure 7. NMR spectra of fraction Cp12 of *C. patens*.

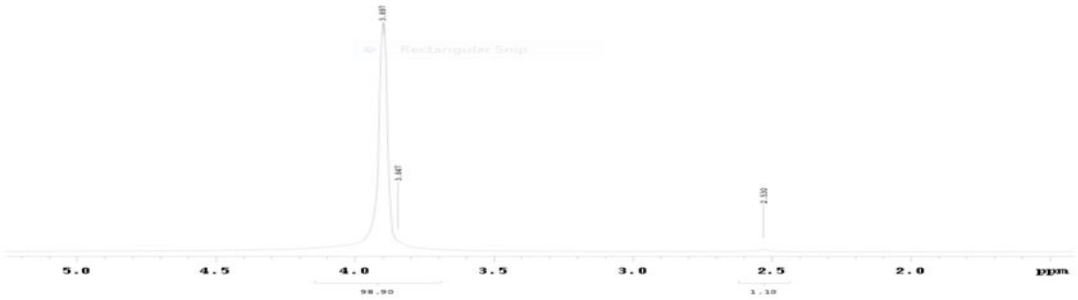

Figure 8. NMR spectra of fraction $Cp12_3$ of *C. patens*.

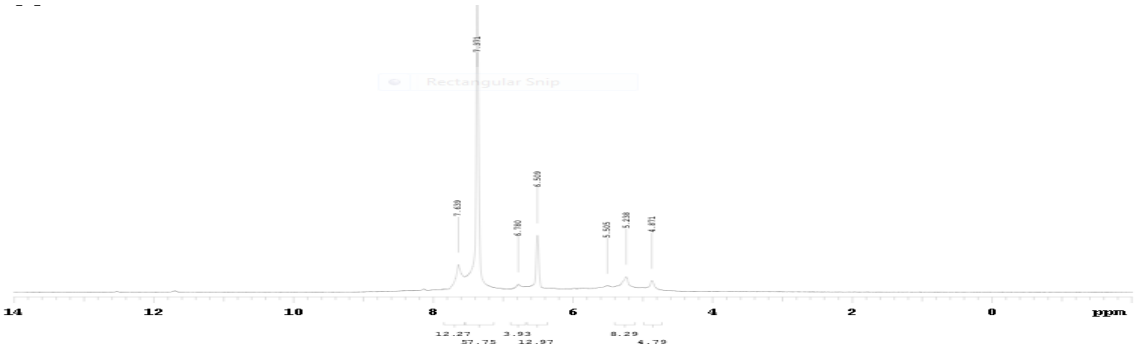

Figure 9. NMR spectra of fraction of *P. reticulatum*.

Pr 5_6: Fig 10 presented the proton NMR of fraction $Pr5_6$. Peak 6.780 cm^{-1} presented vinyl protons, peak 6.509 cm^{-1} presented aromatic protons while peak 5.505 cm^{-1} presented vinylic protons.

GC-MS Spectra

Thirteen (13) compounds were identified in this fraction presented in Table 3. The compounds include majorly sesquiterpenes, acids (fatty acids, palmitic acids, abietic acid, stearic acids), and alcohol. Sesquiterpenes are the most abundant constituting about of the total fractions.

The extract Cp12 contains the following compounds as shown in Figure 5 and Table 4. The compounds include generally monoterpenes, alkaloids,

Table 3. Compound identified in fractions CP_7 of *C. paten* by GC-MS.

S/N	RT	Name of compound	Chemical formula	Molecular weight	Nature of compound
1	4.988	Name:.alpha.-Pinene . Bicyclo[3.1.1]hept-2-ene, 2,6,6-trimethyl	$C_{10}H_{16}$	136	sesquiterpene
2	5.680	beta.-Pinene Bicyclo[3.1.1]heptane,6,6-dimethyl-2-methylene-	$C_{10}H_{16}$	136	Alcohol
3	11.663	Cyclohexene, 3-methyl-6- (1-methyl ethyl diene)-	$C_{10}H_{16}$	136	sesquiterpene
4	14.895	Azulene, 1,2,3,4,5,6,7,8-octahydro-1,4-dimethyl-7-(1-methylethenyl)-, [1S-(1.alpha.,4.alpha.,7.alpha)	$C_{15}H_{24}$	204	sesquiterpene
5	15.140	1,6-Cyclodecadiene, 1-methyl-5-methylene-8-(1-methylethyl)-, [s-(E,E	$C_{15}H_{24}$	204	sesquiterpene
6	16.611	1,2-Ethanediol, 1,2-dimyristoyl-	$C_{20}H_{30}O_2$	302	Abietic acid
7	22.390	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	Palmitic acid
8	24.541	Oleic Acid ; 9-Octadecenoic acid (Z)-	$C_{18}H_{34}O_2$	282	Fatty acid
9	24.723	Octadecanoic acid	$C_{18}H_{36}O_2$	284	Stearic acid
10	24.924	2,6,10-Dodecatrien-1-ol, 3,7,11-trimethyl-	$C_{15}H_{26}O$	222	sesquiterpene
11	25.724	2,6,10-Dodecatrien-1-ol, 3,7,11-trimethyl-	$C_{15}H_{26}O$	222	sesquiterpene
12	27.031	9,12-Octadecadienoyl chloride, (Z,Z)-	$C_{18}H_{31}ClO$	298	Fatty acid
13	27.184	Hexadecanoic acid, 2-hydroxy-1,3-propanediyl ester	$C_{39}H_{76}O_5$	624	Fatty acid

Sesquiterpenes, and fatty acids.

GCMS of Cp12 extracts of *C. patens*

Compounds identified in fraction $CP12_3$ of *C. patens* are presented in Figure 6 and Table 5. The most abundant classes in this fraction are fatty acids and esters.

Compounds identified in fractions from fraction $Pr3_6$ of *P. reticulatum* are listed in Table 4. The most abundant classes include sesquiterpenes, esters., alkanes and dialkyl ethers. Compounds identified in fractions from fraction Pr36 of *P. reticulatum* are shown in Figure 5 and listed in Table 6. The most abundant classes include fatty acids, Fatty aldehydes and cyclic ketones. Phytochemicals found in abundance in PR56 are fatty acids, fatty aldehydes and cyclic ketones (Table 7) which were revealed in the FTIR and NMR spectra (Figures 1-4).

DISCUSSION

A multitude of biological activities have been described for essential oils. In recent years, there has been a rise in the search for plants with phytochemicals possessing antimicrobial and antioxidant potentials in treating chronic infectious diseases. The yield of plant can determine the quantity of bioactive compounds present in the plant. Extraction yield and purity of extracts can be greatly influenced by processing and plant species (Braga et al., 2016). However, the relationship between extraction yield, phenolic content and antioxidant activity may reveal the true biological value of a plant. The extract yield in the plants used in this study are generally low, this could be as a result of the extracting solvent which has a low polarity.

This study has revealed that the *P. reticulatum* is effective against Shigella dysentariae which is a culprit in multidrug resistant Shigellosis and dysentery. *S. dysentariae* is known to be resistant to third generation cephalosporins, and fluoroquinones (Taneja and Mewara, 2016). However, the two plants; *P. reticulatum* and *C. patens* were also to be effective against *Streptococcus pyogenes* which is implicated in sepsis, Strept throat, toxic shock syndrome, glomerulonephritis amongst others

Table 4. Compound identified in fractions CP_{12} of *C. paten* by GC-MS.

S/N	RT	Name of compound	Chemical formula	Molecular weight	Nature of compound
1	4.892	2,6,trimethylbicyclo(3,1)hept-2-ene	$C_{10}H_{16}$	136	monoterpene
2	5.611	Trans-3,7, dimethyl-1,3,6- octatriene	$C_{10}H_{16}$	136	monoterpenes
3	5.608	Bicyclo(3,1,1)heptane,6,6 dimethyl-2-methylene	$C_{10}H_{16}$	136	Bicyclic-monoterpenoids
4	14.892	1,2,3,4,5,6,7,8- octahydro-1,4-dimethyl-7-(1-methylethenyl)	$C_{15}H_{24}$	204	
5	14.892	1R,3Z,9s,-4,11,11,-trimethyl-8-methylenebicyclo(7,2,0)undec-3-ene	$C_{15}H_{24}$	204	Alkaloid
6	15.142	1,6-cyclodecadiene,1-methyl-5-methylene-8-(1-methylethyl).	$C_{15}H_{24}$	204	sesquiterpenoids
7	15.142	Naphtalene, 1,2,3,4,4a, 5,6,8a-octahydro-7methyl-4-methylene-1-(1methylethyl)	$C_{15}H_{24}$	204	Phenol
8	15.142	2-((1E)-1,3-butadienyl)-1,1-dimethyl-3-methylenecyclohexane	$C_{13}H_{20}$	176	Phenol
9	22.383	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	fatty acid methyl esters
10	22.383	Octadecanoic acid	$C_{18}H_{36}O_2$	284	fatty acids

Table 5. Compound identified in fractions $CP12_3$ of *C. paten* by GC-MS.

S/N	RT	Name of compound	Chemical formula	Molecular weight	Nature of compound
1	17.500	Cyclopentaneundecanoic acid, methyl ester	$C_{17}H_{32}O_2$	268	carboxylic acid esters.
2	17.500	Heptacosanoic acid, methyl ester	$C_{28}H_{56}O_2$	424	Fatty esters
3	17.500	Tetradecanoic acid, 12-methyl-, methyl ester	$C_{16}H_{32}O_2$	256	fatty acid
4	17.500	Heneicosanoic acid, methyl ester	$C_{22}H_{44}O_2$	340	carboxylic acid esters
5	17.500	Tetradecynoic acid, methyl ester	$C_{15}H_{26}O_2$	238	fatty acid
6	19.683	Decanoic acid, methyl ester	$C_{11}H_{22}O_2$	186	fatty acid
7	19.683	Octanoic acid, methyl ester	$C_9H_{18}O_2$	158	fatty acid methyl ester
8	19.683	Tridecanoic acid, methyl ester	$C_{14}H_{28}O_2$	228	fatty acid
9	19.683	Hexadecanoic acid, 15-methyl-, methyl ester	$C_{18}H_{36}O_2$	284	fatty acid
10	19.683	Undecanoic acid,11-bromo-, methyl ester	$C_{12}H_{23}BRO_2$	278	Carbonyl esters

causing about 600million infections annually (Lynskey et al., 2011). This organism is resistant mainly to macrolides and teracyclines (Richter et al., 2005). The antibacterial activities of the crude and purified fractions suggest a synergistic relationship between the components of the components of individual plants.

Furthermore, *P. reticulatum* is obviously a broad spectrum antibacterial having activities against both Gram positive and Gram-negative bacterial strain whereas *C. patens* is effective only against Gram-positive *Streptococcus pyogenes*. The broad-spectrum status of *P. reticulatum* makes it a better specimen as a pharmaceutic as compared with *C.*

Table 6. Compound identified in fraction Pr 3_6 of *P. reticulatum* by GC-MS.

S/N	RT	Name of compound	Chemical formula	Molecular weight	Nature of compound
1	25.103	2,6,10-Dodecatrien-1-ol, 3,7,11-trimethyl-, (E,E)-	$C_{15}H_{26}O$	222	Sesquiterpene
2	25.879	2,6,10-Dodecatrien-1-ol, 3,7,11-trimethyl-, (Z,E)-	$C_{15}H_{26}O$	222	Sesquiterpene
3	25.883	2,6,-octadiene-1-ol,3,7,-dimethylacetate	$C_{12}H_20O_2$:	196	fatty alcohol esters
4	26.580	Decanoic acid, 2-ethylhexyl ester	$C_{18}H_{36}O_2$	284	Esters
5	26.580	Bis(2-ethyhexyl)maleate	$C_20H_{36}O_4$	340	Ester ..
6	26.580	1-decene2,4 dimethy	$C_{12}H_{24}$	168	alkene
7	26.580	N, octyl ether	$C_{16}H_{34}O$	240	dialkyl ethers

Table 7. Compound identified in fraction Pr 5_6 of *P. reticulatum* by GC-MS.

S/N	RT	Name of compound	Chemical formula	Molecular weight	Nature of compound
1	22.383	N, hexadecanoic acid	$C_{16}H_{32}O_2$	256	Palmitic acid
2		Octadecanoic acid	$C_{18}H_{36}O_2$	284	Stearic acid
3		1-(+)-Ascorbic acid,2,6-dihexadecanoate	$C_{38}H_{68}O_8$	652	Dipalmitate
4	24.525	Oleic acid	$C_{18}H_{34}O_2$	282	monounsaturated omega-9 fatty acid
5		6- Octadecanoic	$C_{18}H_{34}O_2$	282	fatty acid
6		9-Octadecenal	$C_{18}H_{34}O$	266	Fatty aldehydes
7		Cyclopentadecanone	$C_{15}H_{28}O_2$	298	cyclic ketones
8	27.033	9,12-Octadecadienoylchloride	$C_{18}H_{31}ClO$	298	fatty alcohol
9		7, Tetradecenal (z)	$C_{14}H_{26}O$	210	Fatty aldehyde
10		13, Tetradecenal	$C_{14}H_{26}O$	210	Fatty aldehydes

patens. Although, literature has shown *C. patens* to be more of an antifungal especially candidiasis than antibacterial, this could be the reason behind the narrow antibacterial spectrum. *P. reticulatum* on the other hand is known to be active against a broad range of bacteria, especially those implicated in enteric infections. It is also used as antiplasmodic and are usually prescribed for gastrointestinal diseases (Jailwala and Shakar., 2000).

The widespread use of these compounds has highlighted the need for a systematic characterization of the vibrational frequencies and molecular fingerprints for their identification and discrimination (Ricci et al., 2015). The FT-IR spectrum was used to identify the functional groups of the active components present in extract based on the peak's values in the region of IR radiation. When the extract was passed into the FT-IR, the functional groups of the components were separated based on its peak's ratio. The FTIR result shows to a large extent the presence of hydrocarbons, unsaturated acids, amines aromatic alkoxy (esters) alkenes, nitro-compounds and aldehydes (Figures 1 - 4). Ragavendran et al. (2011) screened the functional groups of carboxylic acids, amines, amides, sulphur derivatives, polysaccharides, organic hydrocarbons, halogens that are responsible for various medicinal properties of *Aerva lanata.* Thangarajan et al. (2012), while analyzing the ethanolic extracts of Ichnocarpus frutescens, by FTIR, revealed functional group components of amino acids, amides, amines, carboxylic acid, carbonyl compounds, organic

hydrocarbons and halogens. The presence of these functional groups possibly indicates the presence of antibacterial compounds in the plants analyzed. In all the extracts subjected to FTIR, Amide, amine and aldehydes were common in extracts of *C. patens* while alcohol was substantial in extracts of *P, reticulatum.* The H1 NMR was used for the retrieval of the number of protons present and the electronic state of the protons in the various compound. The zone under the plots gives information about the number of protons present in the molecule, the circumstance of the signs (the mixture moves) reveals information as for the engineered and electronic state of the protons, and the part configuration gives information about the amount of neighbouring (vicinal or geminal) protons. The NMR spectra also revealed the presence of allylic protons, propagylic protons, aromatic ketones, thiols alkyl esters, alcohols, epoxides ether, acetyl ester thiols, benzyl protons and aromatic protons which possibly responsible for the plants' biological activities. The two plants are rich in sesquiterpenes, on the qualitative basis, the major sesquiterpenes are the α and β pinene, azulene, sativen, cubene and β-ocimen. Boyom et al (2011) in their work discovered that essential oils extracted from the stem bark of *C. patens* was found to contain only terpenoids (97%) and sesquiterpenes (93%). *P. reticulatum* have also been shown by researchers to be dominated by sesquiterpenes (Tira- Pokos et al., 2010). Sesquiterpenes are known to have antimicrobial activities especially antifungal (Watermann and Mohammad, 1985), antioxidant (Tira- Pokos et al., 2010), anti-inflammatory (Jeena et al., 2013) bactericidal (Ishnava et al., 2013) and antitumor (Feraz et al., 2013). The root bark of *C. patens* essential oil was shown by Watermann and Mohammad (1985) in their work to contain two sesquiterpene and five alkaloids. Quattara et al, (2016) however, discovered various sesquiterpenes *C. patens.* Other compounds discovered include; fatty acids and alcohol. Fatty acids (fatty acids, palmitic acids, abietic acid, stearic acids) are saturated fatty acids. Several free fatty acids are known to have an inhibitory effect on fungal germination and sporulation and bacterial growth (Urbanek et al., 2011, Golebiowski et al., 2012,). Some carboxylic acids or their derivatives are also sources of insect pheromones (Lorenzo-Figueiras et al., 2009). Fatty acids are widely occurring in natural fats and dietary oils and they play an important role as nutritious substances and metabolites in living organisms (Cakir, 2004). Many fatty acids are known to have antibacterial and antifungal properties (Russel, 1991). The biological activities of free fatty acids have roles in host defenses against potential opportunistic or pathogenic microorganisms. An important aspect of this is growth inhibition or the quick destroying of bacteria. several studies for understanding the mechanism of the anti-bacterial effects of different fatty acids from a wide range of biological sources such as algae, animals and plants have been done by several researchers (Wille and Kydonieus, 2003; Desbois and Smith, 2010,). Indeed, fatty acids are normally identified as the active ingredients in ethnic and herbal medicines (Yff, 2002).

CONCLUSION

The presence of therapeutically potent antibacterial compounds against pathogenic bacteria was confirmed to be present in *P. reticlatum* and *C. patens.* The results of the crude and purified extracts showed a strong synergistic activity in the components of individual plants. These two plants can be considered as lead plant in the production of pharmaceutics.

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