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Antibacterial activity and chemical profiling of *Bombax glabrum* (Pasq.) A. Robyns leaves

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ABSTRACT

Background & Aim: *Bombax glabrum* is used in traditional medicine for the relief of general pain and digestive problems. The aim of this study was to establish the antibacterial activity, to characterize and identify the bioactive compounds in the leaves of the plant.

Experimental: The powdered leave samples were sequentially extracted with n-hexane and chloroform using a soxhlet apparatus. Phytochemical screening was done using standard analytical procedures and the antibacterial activity of both extracts was tested against *Bacillus subtills, Staphylococcus aureus, Escherichia coli, Klebsiella pneumonia* using modified agar disc diffusion method. The chloroform extract was subjected to column chromatography separation in combination with TLC. Contact autobiography was carried out on two active spot(s) while purified fractions of these spots were analyzed using GC-MS.

Results: Phytochemical screening shows the presence of saponins, flavonoids, tannins, alkaloids, and sterols. The plant has inhibitory activity against *S. aureus* and *E. coli*; while the GC-MS of two active spots of the chloroform extract (with Rf of 0.60 and 0.82) yielded 12 and 17 compounds for band C3 and C5, respectively, which were active against *S. aureus* only. Most abundant compounds are bis (2-ethylhexyl) phthalate (34.54) and 2-pentadecanone, 6,10,14-trimethyl (41.81 %), respectively.

Recommended applications/industries: The presence of bioactive compounds could account for the plant's inhibitory action against *S. aureus* and *E. coli* which may justify its use in managing infections.

1. Introduction

Bombax glabrum (Pasq.) A. Robyns (synonyms *Bombacopsis glabrum* (Pasq.) A. Robyns, *Bombax glabra, Pachira glabra*) with the common name French peanut, is classified in the sub-family Bombacoideae of the family Malvaceae, formerly Bombacaceae (Ogunlade *et al.*, 2011). It is known among the Yoruba ethnic group in Nigeria as epa oyinbo. It is a medium-sized tree (9-18 m tall). The leaves are shiny, bright green, and alternate 15-28 cm in length and palmate compound with 5-9 leaflets and are quickly shed. The large and showy flowers are perfect and terminate with a long peduncle. The petals and numerous stamens are white. They are also fragrant with a sort of vanilla aroma. The fruit is a smooth green capsule 4-8 inches (10-20 cm) and it splits open longitudinally when ripe. The seeds are rounded but irregular with about 10-25

seeds per fruit. They are about 1inch (2-5 cm) in diameter, with a light brown testa (Oni *et al.*, 2015). Few studies had been conducted to identify chemical constituents of certain parts of the plant. The chemical characterization of the essential oil from the leaves of the plant and its activity as an antimicrobial and insecticidal agent had been reported (Lawal *et al.*, 2014), the seed was shown to be a good source of protein and fats (Adeleke and Abiodun, 2010) while the chemical characterization and stability of the oil produced by the nut as well as the potential production of biodiesel from the oil has also been documented (Chaves *et al.*, 2012; Araujo *et al.*, 2017).

Natural product extraction from medicinal plants such as *B. glabrum* whether it is present as pure compounds or it is made instandardizedextracts, offer limitless prospects for novel drug pointers as a result of the unrivalled presence of chemical variety. Due to the growing need for chemical diversity in testing protocols, searching for beneficial drugs from plants and specific interest inedible nutraceutical plants has increased worldwide (Sasidharan *et al.*, 2011). Plants are to known contain a wide range of chemical compounds that can be used to treat chronic, and infectious diseases (Krishnananda *et al.*, 2017); plants and herbal concoctions for medicinal use contain various types of bioactive compounds. The resistance of some pathogenic microorganisms like bacteria and fungi to chemically synthesized drugs has induced researchers to move towards ethnopharmacognosy in the search for new drug lead with significant activity against these microbes. To this end, thousands of phytochemicals have been found to have a beneficial and biological activity such as anticancer, antimicrobial, antioxidant, antidiarrheal, analgesic, and wound healing activity (Krishnananda *et al.*, 2017). Many members of the Bombacaceae have been shown to possess a wide range of phytochemical compound in various parts of the plants (Refaat *et al.*, 2013), however, little has been documented in terms of the chemical characterization of the leaves of *B. glabrum*. *B. glabrum* is a neglected fruit tree in Nigeria. The plant can be classified as an underutilized crop and there is limited research work done on its parts. It is reportedly used in traditional medicine for the relief of general pain and digestive problems (Ogunlade *et al.*, 2011). Its use in the traditional remedies is not popularly known in many parts of the world. This has hindered the development of the plant and also made its cultivation, recognition, acceptance, and utilization remain locally restricted, however, other members of the same genus such as Bombax ceiba (Rehman *et al.*, 2017) and Bombax buonopozense (Akuodor *et al.*, 2012; Abdu and Ammani, 2017) have been demonstrated to possess antibacterial activity. The aim of this study was therefore to establish the antibacterial activity and to characterize and identify the bioactive compounds in the leaves of the *B. glabrum.*

2. Materials and Methods

2.1. Plant collection, authentication, and extraction

The leaves of the plant were collected from Block 32, Plot 2, Awolowo Avenue, G.R.A. Quarters, Sagamu; the plant was identified at the Forestry Research Institute of Nigeria, Ibadan, as *Bombax glabrum* (Pasq.) A. Robyns with voucher specimen deposited at the Forest Herbarium Ibadan (FHI: 110698).

The leaves were air-dried for 14 days. The dry leaves were ground into a fine powder using a milling grinder. The powdered plant material (200 g) was sequentially extracted with n-Hexane, Chloroform, and Methanol using the Soxhlet extraction method until complete extraction was achieved. The extracts were concentrated using a rotary evaporator at 40 ^{o}C. The concentrated extracts were kept tightly for further analysis.

2.2. Phytochemical screening

Phytochemical screening was carried out on the ground leaves to determine the presence of flavonoids, phenols, tannins, saponins, and sterols in the plant. it was also screened for alkaloids and combined Anthraquinone (Trease and Evans, 2008).

2.3. Testing for antibacterial activity

The antimicrobial activity of the chloroform and n-hexane extract was tested against four bacteria, two Gram-positive and two Gram negative; *Bacillus subtills*, *Staphylococcus aureus*, *Escherichia coli*, and *Klebsiella pneumonia* which were all obtained from the medical microbiology laboratory of the Olabisi Onabanjo University Teaching Hospital, Sagamu. Antibacterial activity testing of the plant extracts was done using the modified agar disc diffusion method

(Baris *et al.*, 2006). Muller Hinton agar was prepared according to manufacturer's standard, it was sterilized in an autoclave at 121 ^{o}C for 15 min and then cooled to 45 ^{o}C. Suspensions of the bacteria were prepared using the nutrient broth. Pure cultures of the isolates were inoculated into the sterile nutrient broth and incubated at 37 ^{o}C for 24 h to grow the bacteria organism. Bacteria suspensions were inoculated into prepared Petri dishes and using sterile metal cork borer (6 mm in diameter), four wells were carefully made on the agar plate without distorting the media, the wells contain the test extract. A separate plate was used to test the control drug Ciprotab and Gentamycin against the bacteria while another plate was used as a negative control and all other plates contained the extracts in various serial dilutions, each test was done in triplicate. The plates were left on the bench for 60 min to allow the inoculated microorganisms to get acclimatized to the new environment. The culture plates were then incubated at 37 ^{o}C for 24 h. By using a metric ruler, the diameter of the zone of inhibition (the area of no growth of the microorganism around the disc) was measured for n-Hexane and chloroform extracts of the plant. Extracts that had more than 12 mm were considered to exhibit moderate activity while those below 12 mm were considered to have little or no activity. The most active extract was selected for fractionation and further analysis.

2.4. Column chromatography

The most active crude extract (chloroform extract) was subjected to silica gel column chromatography. The extract was first run on TLC with varying solvent mixtures to predict the best solvent combination to use for the isocratic elution. 100 g of silica gel was mixed with n-Hexane to form a homogenous suspension or slurry and stirred using a glass stirring rod to remove bubbles. The silica gel slurry was then poured into a glass column. The chloroform extract mixture was then loaded on the column. The solvents used are n-hexane, chloroform, ethylacetate, and methanol (4:3:2:1) (which gave better separation on the TLC earlier). The 10 % methanol was added in other to enhance the separation of the polar constituent of the plant. The eluent was collected in fractions as viewed on the column and 137 pet bottles of 20 ml each (labelled serially from 1-150) were used for the collection of the eluent. The collected fractions were concentrated.

2.5. Thin layer chromatography

The concentrated eluents/fractions collected from the column chromatography were further subjected to thin-layer chromatography (TLC) in other to pool together those with similar constituents. A drop of each fraction was carefully spotted on a thin layer chromatography plate (pre-coated with silica) and left to dry. After drying the plate was dipped into a mixture of solvents which are supposed to allow the compounds on the spot to move upwards by capillary action and further separate the constituent of each eluent. The plate was then removed from the solvent and left to dry and different solvent mixtures were tried to know which one separates the fraction better. The positions of different compounds as separated by the mixture of solvents were observed by fluorescence under ultraviolet light at 254 nm and 365 nm to properly see the migration distances and intensities of the characteristic zone, followed by staining with iodine vapour for the detection of spots. Different solvent systems were tested to study the resolution and the number of components in each fraction and consequently, the solvent mixture including n-hexane: chloroform: ethylacetate: methanol (4:3:2:1) which gave the best separation resolution was used for all the eluents and those with a similar array of compounds were pooled together and the resultant fractions were labelled A to G in the order of their elusion from the column chromatography. These were then further separated again on TLC as described above in preparation for the bioassay and the retention factors (R_f) of each spot was calculated.

2.6. Bioassay of the spots (bioautography)

Bioautography was determined using the contact method described by Valle *et al.* (2016) with modifications. Muller Hinton agar was used as the agar media. It was prepared following the manufacturer's instruction and sterilized by autoclaving at 121 ^{o}C for 15 min at 15 psi. Suspensions of *S. aureus* and *E. coli* were prepared using nutrient broth (13 g/L). Pure cultures of the isolates were inoculated into 10 ml of sterile nutrient broth. Tubes were incubated at 37 ^{o}C for 18 h. The bacterial suspensions were standardized (after the period of incubation), using the 0.5 McFarland standard which is equivalent to 1×10^{8} CFU/ml of the bacteria.

20 ml of sterile Muller Hinton agar was dispensed in sterile Petri dishes and allowed to solidify. Exactly, 0.1 ml of the bacterial suspension (*S. aureus* and *E. coli*) were individually inoculated into separate Petri dishes and the inoculum was spread on the surfaces each of the plates using a sterile glass rod. The plates were allowed to stand for about 30 min.

Each spot, that is A-G was run on the TLC plate, and the separation was carried out using the best solvent mixture. After the separation, the plates were left to dry and each spot was marked with pencil and labelled. Then each separated spots: C_1, C_2, C_3, C_4, C_5, C_6, D_1, D_2, D_4, D_5, E_1, E_2, E_3, E_4, E_5, F_1, and F_2 was cut out and placed on the inoculated plate aseptically and left for a few minutes before being incubated at 37 ^oC for 18 h, after which the zones of inhibition were read. To have a better comparison of the zones of inhibition for each compound or spot on the TLC, and to get a better analysis of the data, the size of the TLC plate bearing each spot was deducted since the plates were cut into the size of each band which varied from one to the other. A blank plate was used as a control.

2.7. GC-MS analysis

For the identification of the bioactive compounds, the two of the most active spot (C_3 and C_5) were further fractionated using column chromatography and some of the fractions after being pulled together, the ones with a similar array of compounds were subjected to GC-MS analysis following the method previously used by Wintola and Afolayan, (2017). The samples (1 µl) were injected into the column HP5MS Agilent technologies of length 30 m, internal diameter of 0.320 mm, and thickness of 0.2 µm of the GC-MS (model Agilent technologies 7890) coupled with mass spectrometer model 5975 Agilent technologies. The oven temperature-programmed, the initial temperature was 80 ^oC to hold for 2 min at 10^o C/ min to the temperature of 240 ^oC to hold for 60 min. The carrier gas was helium with 2 ml/ min flow rate. The injector temperature was 250 ^oC. The database of the National Institute Standard and Technology (NIST) was used to interpret analyzed data. The comparison of the mass spectrum of the unidentified components in the tested spots was done against the mass spectrum of already known components available in the NIST library. The name, molecular weight, and peak area percentage of unknown compounds were evaluated by the software as observed from the chromatogram. The name and structure of the components of the test materials were confirmed and tabulated.

3. Results and discussion

As presented in the previous section, little information is available on the pharmacological activities of *B. glabrum*, thus, the objective of this study was to assess the antibacterial activity of the plant against gram-positive and negative bacteria while also carrying out the chemical characterization of the bioactive component of the plant extract using GC-MS. *B. glabrum* which happens to be underutilized has proven to have bioactivity similar to that of the plants in the same family, Malvaceae, that are being used in the treatment of diarrhoea, stomach pain, and dysentery. The results of the phytochemical screening of the powdered leave samples of *B. glabrum* showing the presence of saponins, tannins, sterols, flavonoids, and alkaloids is presented in Table 1. This shows a correlation with the findings of Paula *et al.*, (2002), which reported the presence of one flavone and three triterpenes in the stem bark of *B. glabrum*, similarly, different plant parts of the closely related plants of the same family such as *B. buonopozense* (Abdu and Ammani, 2017), *B. ceiba* (Shah *et al.*, 2018) and *Ceiba petandra* (Parulekar, 2017; Osuntokun, 2017) have also been reported to have these range of phytochemicals in the leaves. Saponins from various plants have been shown to exhibit antimicrobial properties, guarding the body against fungi, bacteria, and viruses (Soetan *et al.*, 2006; Tagousop *et al.*, 2018). In medicine, tannin is administered internally to treat diarrhoea and intestinal bleeding while some flavonoids have been reported to be important sources of antioxidants in human diets (Kasim *et al.*, 2011).

Table 1. Result for phytochemical screening of the powdered plant sample

Test	Result
Saponins	Present
Flavonoids	Present
Tannins	Present
Anthraquinone	Absent
Alkaloids	Present
Sterols	Present

The antimicrobial activity of the crude extract n-hexane and chloroform extract of *B. glabrum* was measured using the agar well diffusion technique, the mean zone of inhibition obtained is presented in Tables 2 and 3 respectively.

The zone of inhibition (ZI) produced by the n-hexane extract of the plant against *B. subtillis* ranged from 10-12 mm; while it was 12-14 mm, 12-15 mm, and 13-14 mm against *S. aureus, K. pneumonia*, and *E. Coli*, respectively. For the chloroform extract, the ZI produced against *B. subtillis* ranged from 10-12 mm; while it was 10-12 mm, 10-12 mm, and 14-17 mm against *S. aureus, K. pneumonia* and *E. Coli*, respectively. Both crude extracts were found to have lower sensitivity to the bacteria samples compared to the standard drugs Ciprotab and Gentamycin.

Table 2. Antibacterial activity of various concentrations of n-hexane extract of *B. glabrum***.**

Concentration (mg/ml)	*B. subtillis*	*S. aureus*	*K. pneumonia*	*E. coli*
	Zones of Inhibition (mm)			
250	12	14	15	14
125	12	14	15	14
62.5	11	14	14	14
31.25	11	13	13	13
15.625	10	12	12	13
-ve Control	10	11	10	11
Ciprotab	30	30	28	30
Gentamycin	23	25	25	25

Table 3. Antibacterial activity of various concentrations of chloroform extract of *B. glabrum***.**

Concentration (mg/ml)	*B. subtillis*	*S. aureus*	*K. pneumonia*	*E. coli*
	Zones of Inhibition (mm)			
250	12	12	12	17
125	12	12	12	16
62.5	11	11	11	15
31.25	11	11	11	15
15.625	10	10	10	14
-ve Control	10	10	11	12
Ciprotab	30	30	30	30
Gentamycin	25	23	22	25

The antimicrobial test of the n-hexane extract and chloroform extracts proved that the plant has significant antibacterial activity against the tested gram-positive and negative strains. The n-hexane had better antimicrobial activity against *E. coli* and the lower inhibition effect was observed for other bacteria strains (*K. pneumonia, B. subtills, S. aureus*) while the chloroform extract showed better and greater antimicrobial potency against *S. aureus* than the others. This observation compares favourably with previous studies reporting the antibacterial activity of *B. ceiba* (Paruleka, 2017; Kamble *et al.*, 2017), *B. buonopozence* (Abdu and Ammani, 2017) and *Ceiba petandra* (Doughari and Ioryue, 2009; Osuntokun, 2017).

The results from the chromatographic separation of the chloroform extracts of *B. glabrum* show that 137 fractions were obtained from the column chromatography, these were further resolved using TLC where fractions that gave similar components were pooled together to give Fraction A-G. Further resolution of these fractions from TLC with the respective R_f is shown in Table 4 which shows that six spots were obtained in Fraction C where five were visible to the naked eyes and one visible at 365 nm, five spots were observed in Fraction D, all of which produced visible light green and yellow colour, and two spots in F which were only visible under the UV light, while no spot was found in Fraction A, B, and G after exposure to UV light at 254 nm and 365 nm and iodine vapour.

The result of the bioautography is presented in Table 4. The band C_3 exhibited the greatest zone of inhibition against *S. aureus* while the least zone of inhibition was observed in the negative control. However, against *E. coli*, no activity was found by all the TLC spots as no zone of inhibition was observed.

Table 4. Bioassay of spots on TLC against *Staphylococcus aureus*.

Spot Identification Number	Zone of inhibition (mm)	R_f
C1	21	0.22
C2	18	0.34
C3	23	0.60
C4	20	0.69
C5	20	0.82
C6	20	0.90
D1	20	0.25
D2	21	0.37
D3	18	0.60
D4	20	0.67
D5	22	0.82
E1	19	0.18
E2	19	0.25
E3	20	0.36
E4	18	0.69
E5	17	0.76
F1	18	0.42
F2	18	0.09
Negative Control	14	n/a

Thus, the bioassay of the spots on the TLC further proved the susceptibility of *S. aureus* to the separated spots thus, implying the activity of the chloroform extracts against the organism with most of the spots having high inhibition. This activity may be attributed to the phytochemical compounds identified in the plants.

The results from the GC-MS chromatograms of two of the active spots of the chloroform extract of *B. glabrum* (Fig. 1 and Fig. 2) revealed various peaks that show the presence of 12 compounds in the spot C_3 and 17 compounds in the spot C_5. The chemical components were ascertained and identified by the comparison of the obtained mass spectra with the NIST database using their retention time and peak areas.

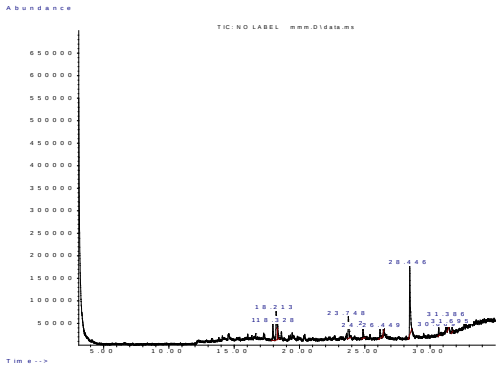

Figure 1. GC-MS chromatogram of the spot C_3 of the most active spot of the chloroform extract of *B. glabrum.*

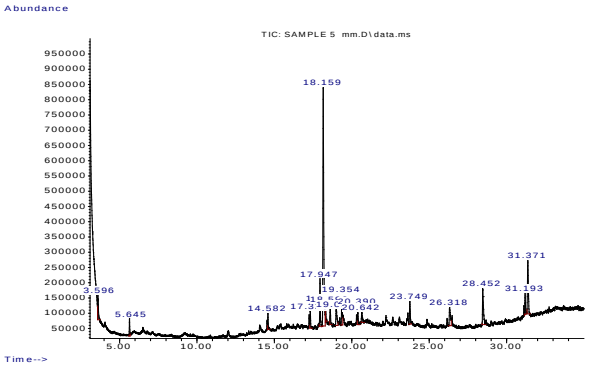

Figure 2. GC-MS chromatogram of the spot C_5 of the most active spot of the chloroform extract of *B. glabrum.*

The identified compound in spot C_3 and C_5 are presented in Tables 5 and 6. The most abundant component of spot C_3 and C_5 identified with the highest peaks are bis (2-ethylhexyl) phthalate and 2-pentadecanone, 6,10,14-trimethyl with the respective peak areas of 34.54 and 41.81 %, respectively.

Table 5. Chemical composition of fraction C_3 chloroform extract of *B. glabrum***.**

RT	Compound Name	Peak area (%)	Molecular formula	Molecular weight
17.964	Bicyclo (3.1.1) heptane 2,6,6-trimethyl, (1R (1.alphaα., 2.alpha., 5.alpha.))-	9.02	$C_{10}H_{18}$	138.25
18.216	2-Pentadecanone, 6,10,14-trimethyl	17.43	$C_{18}H_{36}O$	268.48
18.330	Bicyclo[3.1.1]heptane, 2,6,6-trimethyl-, (1alpha,2beta,5alpha)-	7.27	$C_{10}H_{18}$	138.25
18.330	i-Propyl 11-octadecenoate	11.28	$C_{21}H_{40}O_2$	324.5
23.749	Cis-Vaccenic acid	4.52	$C_{18}H_{34}O_2$	282.5
24.859	n-Propyl 11-octadecenoate	5.33	$C_{21}H_{40}O_2$	324.5
26.158	Fumaramic acid	0.41	$C_4H_5NO_3$	115.09
26.450	Bis(2-ethylhexyl) phthalate	34.54	$C_{24}H_{38}O_4$	390.6
28.447	Hexasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11-dodecamethyl-	2.67	$C_{12}H_{36}O_5Si_6$	428.92
30.667	.alpha.-Tocospiro B	5.42	$C_{29}H_{50}O_4$	462.70
31.338	Cyclononasiloxane, octadecamethyl-	2.10	$C_{18}H_{54}O_9Si_9$	667.39
31.697	Bicyclo (3.1.1) heptane 2,6,6-trimethyl, (1S (1.alpha., 2.beta., 5. alpha.))-	7.27	$C_{10}H_{18}$	138.25

Table 6. Chemical composition of spot C_5 chloroform extract of *B. glabrum*.

RT	Compound Name	Peak area %	Molecular formula	Molecular weight	
3.596	Cyclotrisiloxane, hexamethyl-	3.41	$C_6H_{18}O_3Si_3$	222.46	Organosiloxane
5.645	Cyclotetrasiloxane, octamethyl-	1.65	$C_8H_{24}O_4Si_4$	296.62	Organosiloxane
14.582	Hexadecane	1.78	$C_{16}H_{34}$	226.44	Saturated hydrocarbon
17.323	Octadecane	2.08	$C_{18}H_{38}$	254.5	Saturated hydrocarbon
17.947	Neophytadiene	5.61	$C_{20}H_{38}$	278.52	Sesquiterpenoids
18.159	2-Pentadecanone, 6,10,14-trimethyl	41.81	$C_{18}H_{36}O$	268.48	Sesquiterpenoids
18.313	Cyclopentaneethanol, 2-(hydroxymethyl)-.beta.,3-dimethyl-	1.72	$C_{10}H_{20}O_2$	172.26	Terpenes
18.599	Neophytadiene	2.34	$C_{20}H_{38}$	278.52	Sesquiterpenoids
19.000	Phthalic acid, 2 methoxybenzyl pentyl ester	3.65	$C_{21}H_{24}O_5$	356.41	
19.354	1-Nonadecene	4.62	$C_{19}H_{38}$	266.51	Terpene
20.390	Eicosane	2.27	$_{20}H_{42}C$	282.55	Saturated hydrocarbon
20.642	Octadecanamide, N,N'-1,2-ethanediylbis-	1.93	$C_{42}H_{84}N_2O_2$	649.1	Amide
23.749	i-Propyl 11-octadecenoate	3.66	$C_{21}H_{40}O_2$	324.5	Fatty acid
26.318	4,8,12,16-Tetramethylheptadecan-4-olide	4.40	$C_{21}H_{40}O_2$	324.54	
28.452	Bis (2-ethylhexyl) phthalate	6.89	$C_{24}H_{38}O_4$	390.6	Phthalate
31.193	alpha.-Tocospiro A	2.65	$C_{29}H_{50}O_4$	462.7	
31.371	alpha.-Tocospiro B	9.54	$_{29}H_{50}O_4C$	462.7	Tocopherol

Consequently, the chemical characterization of two of the active fractions in the GC-MS analysis confirmed the presence and identity of some of the compounds present in the active spots, which ranged from aromatic to aliphatic hydrocarbons, esters, and organic acids.

The spots C_3 and C_5 contain the phytochemical constituents such as hexadecane; octadecane; noephythadiene; 2-pentadecanone, 6, 10, 14-trimethyl; cyclopentaneethanol, 2-(hydroxymethyl)-B, 2-methoxybenzyl pentyl ester, and octadecane. Many of the compounds identified a have been reported in essential oils may medicinal plants. For instance, 2-pentadecanone, 6, 10, 14-trimethyl which is the major component in the spot C_5 and also present in significant quantity in spot C_3 and neophytadiene were previously reported to be present in the essential oils of *Jatropha curcas* where they are among the bioactive component exhibiting anti-inflammatory activity (Adeosun *et al.*, 2017) and in *Ocimum obovatum* essential oil where the antimicrobial activity was demonstrated (Naidoo *et al.*, 2014). These compounds are also reported to be present in the leaf extracts of *Cenchrus ciliaris* (Arora *et al.*, 2017). The most abundant compound identified in band C_3 is a phthalate ester called bis (2-ethylhexyl) phthalate with a peak area of 34.54 %. These groups of compound are known as environmental contaminants whose potential toxic effect have been widely reported in living organisms. For example, the exposure of adult and juvenile *Daphnia magna* to certain levels of di-(2-ethylhexyl) phthalate was reported to cause biochemical and physiological effects by inhibiting enzymes, increasing lipid peroxidation levels and pattern of gene expression (Wang *et al.*, 2018). On the other hand, the biological activity of Di-(2-ethylhexyl) phthalate isolated from *Penicillium janthinellum* was reported where it showed significant cytotoxicity against human breast carcinoma cell line, human alveolar basal epithelial cell line and also antiviral activity against human influenza virus at different concentrations (El-Sayed *et al.*, 2015). This suggests that these compounds present in the plant may be harnessed for various pharmacological activities including antimicrobial activity. The most abundant compound identified in the spot C_5 is 2-pentadecanone, 6, 10, 14-trimethyl- (17.43 %), which according to Amudha *et al.*, (2018) is an antioxidant and bio-lubricant. Nonadecene is a long-chain fatty acid which shows anticancer, antioxidant, and antimicrobial activity (Amudha *et al.*, 2018). All these phyto-compounds may individually or collectively act to elicit the observed antibacterial activity of *B. glabrum*.

4. Conclusion

The results suggest that the identified compounds in the plant may be responsible for the antimicrobial activity of the plant. Therefore, the present observation may justify the plant use in the treatment of stomach pain, dysentery, diarrhoea, and even food poising because *S. aureus* and *E. coli*, which the plant has shown activity against, are found to be some of the causative organisms for food poisoning and some other digestive system disorder. Among others, the bis (2-ethylhexyl) phthalate, i-propyl 11-octadecenoate and 2-pentacanone, 6, 10, 14-trimethyl-, which are common in both fractions, may be responsible for the antimicrobial activity of the extract which buttresses the use of the plant in the treatment of food poisoning.

5. Acknowledgements

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6. References

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