*European Journal of Medicinal Plants*
*14(3): 1-10, 2016, Article no.EJMP.26156*
*ISSN: 2231-0894, NLM ID: 101583475*

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# Phytochemical Analyzes from the Leaves of *Bryophyllum pinnatum*

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***Authors' contributions***

*This work was carried out in collaboration between all authors. Author EOF designed the study and wrote the first draft of the manuscript. Authors LW, SL, AGM and CAO managed the analyses of the study and edited the manuscript. All authors read and approved the final manuscript.*

***Article Information***

DOI: 10.9734/EJMP/2016/26156
Editor(s):
(1) Marcello Iriti, Professor of Plant Biology and Pathology, Department of Agricultural and Environmental Sciences, Milan State University, Italy.
Reviewers:
(1) Walter Antonio Roman Junior, Regional Community University of Chapeco, Brazil.
(2) Sandra Maria Barbalho, UNIMAR, Brazil.
(3) Mohini Chetan Kuchekar, Pune University, India.
(4) Charu Gupta, Amity University, UP, India.
Complete Peer review History: http://sciencedomain.org/review-history/14321

***Original Research Article***

***Received 3rd April 2016***
***Accepted 19th April 2016***
***Published 25th April 2016***

## ABSTRACT

**Aims:** To identify extractable components in the traditional medicinal plant, *Bryophyllum pinnatum* (*BP*).
**Methodology:** The leaves of *BP* were sequentially extracted with non-polar ($CH_2Cl_2$) and polar ($CH_3OH$) solvents. The extracts were derivatized to their volatile fatty acid methyl esters (FAME) and trimethylsilyl (TMS) esters/ethers and characterized by gas chromatography mass spectrometry (GC-MS).
**Results:** GC-MS analysis revealed the presence of sterols, fatty acids, monoarylphenolics, sugars, alcohols, sugar alcohols, sugar acids, carboxylic acids, dicarboxylic acids, tricarboxylic acids, vitamin, alkanes and alkenes.
**Conclusion:** Compounds identified (e.g. tocopherol) in the extracts are most likely responsible for its antimicrobial, antifungal, anticancer, antitumour and insecticidal activities.

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*Keywords: Bryophyllum pinnatum; extracts; GC-MS; fatty acids.*

## 1. INTRODUCTION

Historically in Africa, people with diseases, ailments and other health conditions were treated by traditional healers who were versed in the use of herbs, (leaves, roots and barks), animals and marine products. In recent times, there has been an increase in the patronage of traditional medicine practitioners in Africa because of the dearth of orthodox medical doctors. For example in Nigeria, the doctor/patient ratio is 1:5000 [1] and this scenario is replicated in other African countries [2]. Another reason why many people are taking to natural medicine is that they are scared of the side effects in orthodox medicine, since it is generally believed that herbs are organic in nature and have no side effects, hence the preference for herbal treatment. Furthermore, the cost of accessing the services of traditional medical practitioners is inexpensive when compared to qualified medical doctors, which results in an increased patronage. Therefore, the use of medicinal plants, such as *Bryophyllum pinnatum* (*BP*), for treating illnesses is common in Africa.

*BP,* also known as *Kalanchoe pinnata,* is a perennial herb that grows up to 0.9-1.5 m tall, succulent dark green leaves and bell like pendulous flowers [3,4]. Common names for *BP* include: Zakham-e-hyat, life plant, air or maternity plant, love plant, Canterbury bells, Cathedral bells, and parnabija. Moreover, *BP* is widely used in traditional medicine to treat ailments such as infections, healing wounds, and cancer. Its wide range of uses in folk medicine justifies its being called “life plant” or “resurrection plant”, promoting researchers interest [5]. It grows in the wild and is used as folk medicine in tropical Africa, India, China, Australia, tropical America, Madagascar, Asia and Hawaii [6,7].

*BP* is astringent, sour in taste, sweet in the post digestive effect and has hot potency [8]. Furthermore, it is well known for its heamostatic and wound healing properties. The plant has gained considerable attention for its medicinal properties and finds application in folk medicine, as well as in the contemporary medicine [8,9]. In Southeastern Nigeria, the herb is used to facilitate the dropping of the placenta of a newly born baby [10,11]. The lightly roasted leaves are used externally for skin fungus and inflammations. The leaf infusion is an internal remedy for fever [12]. Its use in folk medicines is for treatment of hypertension and kidney stones [6], pulmonary infections, and rheumatoid arthritis [13]. The plant has hepatoprotective activity and is also used to increase vascular integrity [7]. Leaf juice is used in the treatment of coughs, bronchial affections, blood dysentery, jaundice and gout [14]. In Madagascar, it is used in anthroposophical medicine to treat psychiatric disorders and as a tocolytic agent to prevent premature labor [15]. The leaf extract of *BP* is also suspected have anticancer, antihypertensive, antidiabetic, anthelmentic, antileishmanial and antioxidant activities [16].

Several classes of compounds have been identified in the extracts of *BP*. Sterols have been identified in *BP* namely; stigmasterol [17] and bryophyllol, bryophollone and bryophollenone [18] and they have been associated with anti-inflammatory and analgesic properties. Furthermore, the leaves contain bufadienolides, a class of steroids, which possess antibacterial and antitumorous actions [8]. Work by Tatsimo et al. [5] found several kaempferol rhamnosides in the methanol extracts of *BP* that have antimicrobial activity. Waxes, alkanes and fatty alcohols have also been found [19,20]. Fatty acids (e.g. palmitic, stearic, arachidic and behenic acids) from *BP* were shown to have an immunosuppressive activity [21]. Di- and tri-acids (e.g. malic, succinic and isocitric acids) have also been observed in extracts and their pharmacological actions are unknown [8,22].

The aim of this study is to identify the yield and composition of the extracts of the leaves of *BP* to identify potential pharmaceutical candidates. Both a non-polar (dichloromethane, $CH_2Cl_2$) and polar (methanol, $CH_3OH$) solvent extracts were characterized by GC-MS as their trimethylsilyl (TMS) and fatty acid methyl esters (FAME) derivatives. FTIR spectroscopy was used to support the chemical functionality of the crude extract compounds.

## 2. MATERIALS AND METHODS

### 2.1 Collection of Plant Material

*Bryophyllum pinnatum (Lam.) Oken* leaves were collected from an open land on the campus of Obafemi Awolowo University (latitude: 7°27' 59.99" N and longitude: 4°33' 59.99" E), Ile-Ife, Nigeria. The leaves were identified and authenticated by Mr. Gabriel Ibhanesebhor and a

voucher specimen deposited (Herbarium Ife number 17090) at the Department of Botany, Obafemi Awolowo University.

### 2.2 Sample Preparation and Extraction

The leaves were rinsed with water, air-dried and pulverized into a powder using a domestic blender and stored in an air-tight container. The moisture content (9.7%) of the sample was determined just before extraction. The sample (5 g, in duplicate) were Soxhlet extracted continuously overnight with $CH_2Cl_2$ (150 mL) for 24 h and then $CH_3OH$ (130 mL) for 48 h. Both the $CH_2Cl_2$ and $CH_3OH$ extracts were rotary evaporated to dryness under vacuum to yield 3.30% and 7.88% on a dry leave basis, respectively.

### 2.3 GC-MS of Extract TMS Derivatives

Extracts (1.0 mg, in duplicate) were weighed into GC vials to which $CH_2Cl_2$ (1 mL) containing anthracene as an internal standard (IS, 50 µg $mL^{-1}$) was added. The samples were silylated with addition of N,O-bis(trimethylsilyl)-trifluoro-acetamide (BSTFA) containing 1% trimethylchlorosilane (TMCS) (50 µL) and pyridine (50 µL) and heated for 30 min at 70°C or continued heating until the solution became clear [23,24]. The prepared TMS derivatives were analyzed by GC-MS (FOCUS-ISQ, Thermoscientific) in the electron ionization mode; temperature profile: 40°C (1 min) ramped to 305°C (10 min) at 5°C $min^{-1}$; GC capillary column (RTx-5MS, 30 x 0.25 mm Φ, Restek). The eluted compounds were identified by their mass spectrum, spectral matching with the 2008 National Institute of Standard and Technology (NIST) spectral library and known standards.

### 2.4 GC-MS of Extract FAME Derivatives

Extracts (5.0 mg, in duplicates) were weighed into 5 mL reacti-vials™ to which $CH_3OH/H_2SO_4/CHCl_3$ (1.7:0.3:2.0 v/v/v, 2 mL) was added to each vial and heated for 90 min at 90°C. $CHCl_3$ contained 1-naphthaleneacetic acid as an internal standard (50 µg/mL) [23,24]. The mixture was allowed to cool after which water (1 mL) was added, shaken vigorously, centrifuged, the $CHCl_3$ layer removed, dried through anhydrous sodium sulfate and transferred to GC vials. The prepared FAME derivatives were analyzed by GC-MS (details given above) using a temperature profile of 40°C (1 min) to 320°C at 5°C $min^{-1}$. The eluted compounds were identified with authentic standards (C14 to C26 fatty acids) and by spectral matching with the NIST spectral library. The concentration of each compound was referenced against the area of internal standard.

### 2.5 FTIR Spectroscopic Analysis

The functional groups in the crude samples were determined by FTIR spectroscopy using a Nicolet iS5 spectrometer using a ZnSe attenuated total reflection (ATR) iD5 probe (ThermoScientific). Spectra were collected in duplicate; the absorbance spectra were baseline corrected and averaged using Omnic-v9 software (ThermoScientific) [24].

## 3. RESULTS AND DISCUSSION

### 3.1 GC-MS Analysis of the TMS Derivatized Extracts

To identify potential pharmaceutical candidates, the leaves of *BP* were extracted with $CH_2Cl_2$ and $CH_3OH$ and the extracts subsequently derivatized to make the compounds volatile for analysis. The $CH_2Cl_2$ extract of *BP* was analyzed by GC-MS as their TMS derivatives as shown in Fig. 1a and Table 1. The analysis revealed the presence of 34 prominent peaks, which represent 32 different compounds distributed among ten 'groups' of organic compounds. The different compounds have the following concentrations alkanes (99 mg $g^{-1}$), sugar (25 mg $g^{-1}$), alcohols (31.4 mg $g^{-1}$), fatty acids (404 mg $g^{-1}$), sterols (165 mg $g^{-1}$), alkenes (16 mg $g^{-1}$), monoarylphenolics (16 mg $g^{-1}$), vitamins (9 mg $g^{-1}$) and unknown steroid (8 mg $g^{-1}$).

Glycerol which is a product of hydrolysis of triglycerides was detected as one of the major compounds present in the extract (25 mg $g^{-1}$). Fatty acids are common occurrence in plant leaves and have been identified in leaves of *Viscus album* [25]. Free fatty acids as a group have the greatest concentration in the extract with linolenic acid, palmitic acid and linoleic acid having 102, 111 and 112 mg $g^{-1}$ respectively. This is consistent with the findings in the literature [21,26,27]. The next group of compounds identified is sterols with a concentration of 165 mg $g^{-1}$. Among the sterols present in the extract is stigmasterol, this is in agreement with the study carried out by other researchers [17,18], isolated stigmasterol and other steroidal derivatives from *BP*. Another

group is alkanes with 99 mg $g^{-1}$ concentration in the extract and has been previously observed [20]. Vitamins and alkenes have the least concentration in the extract. Those in between are monoarylphenolic and alcohols with concentration of 16 mg/g and 31 mg $g^{-1}$, respectively. The peaks at the retention times of 10.36, 33.48 and 55.14 min were unknowns.

The *BP* leave $CH_3OH$ extract was also analyzed by GC-MS as their TMS derivatives (Fig. 1b and Table 2). There were 31 prominent peaks identified in the analysis, which represent 27 different compounds distributed among ten 'groups' of organic compounds. The concentration of sugars in the extract is 503 mg $g^{-1}$ with disaccharides alone contributing 233 mg $g^{-1}$ of this concentration. The next class is sugar acids with the concentration of 189 mg/g of the extract of which 2-keto-gluconic acid has a concentration of 166 mg $g^{-1}$. Carboxylic acids are another group of compound that is contained in the extract with a total concentration of 144 mg $g^{-1}$. Monocarboxylic acid (4 mg $g^{-1}$), dicarboxylic acids (16 mg $g^{-1}$) and tricarboxylic acids (41 mg $g^{-1}$) were also detected and have been previously

**Table 1. GC-MS analysis of *B. pinnatum* $CH_2Cl_2$ extract TMS derivatives**

| Peak no. | RT (min) | Class | Compounds | Molecular formula | $M^+$ (m/z) | Conc. (mg $g^{-1}$) |
|---|---|---|---|---|---|---|
| 1 | 10.28 | ALC | Propan-1,2-diol $TMS_2$ ether | $C_9H_{24}O_2Si_2$ | 220 | 4.9 |
| 2 | 10.36 | | unknown | | 262 | 6.4 |
| 3 | 10.53 | ALC | Butane-2,3-diol $TMS_2$ ether | $C_{10}H_{26}O_2Si_2$ | 234 | 6.7 |
| 4 | 17.29 | SR | Glycerol $TMS_3$ | $C_{12}H_{32}O_3Si_3$ | 308 | 25 |
| | 29.23 | | Anthracene (IS) | $C_{14}H_{10}$ | 178 | |
| 5 | 30.09 | ALK | Eicosyne | $C_{20}H_{38}$ | 278 | 4.3 |
| 6 | 30.36 | FA | Myristic acid TMS ester | $C_{17}H_{36}O_2Si$ | 300 | 7.6 |
| 7 | 32.51 | ALC | Hexadecanol TMS ether | $C_{19}H_{42}OSi$ | 314 | 3.7 |
| 8 | 33.48 | | Unknown | | | 14.0 |
| 9 | 34.12 | FA | Palmitic acid, TMS ester | $C_{19}H_{40}O_2Si$ | 328 | 111 |
| 10 | 35.62 | ALKE | 9-Octadecenyl TMS ether | $C_{21}H_{44}OSi$ | 340 | 15.9 |
| 11 | 35.73 | ST | (17a)-3-methoxyestra-1,3,5(10)-triene-14,17-diyl $TMS_2$ ether | $C_{25}H_{42}O_3Si2$ | 446 | 4.3 |
| 12 | 36.46 | ALC | Phytol TMS ether | $C_{23}H_{48}OSi$ | 368 | 5.3 |
| 13 | 37.05 | FA | Linoleic acid TMS ester | $C_{21}H_{40}O_2Si$ | 352 | 112 |
| 14 | 37.17 | FA | Oleic + linolenic acid, TMS ester | $C_{21}H_{42}O_2Si$<br>$C_{21}H_{38}O_2Si$ | 354 + 350 | 102 |
| 15 | 37.58 | FA | Stearic acid, TMS ester | $C_{21}H_{44}O_2Si$ | 356 | 25.0 |
| 16 | 38.76 | MP | 3-Hydroxyanthranilic acid, $TMS_3$ | $C_{16}H_{31}NO_3Si_3$ | 369 | 15.9 |
| 17 | 40.78 | FA | Arachidic acid TMS ester | $C_{23}H_{48}O_2Si$ | 384 | 11.2 |
| 18 | 43.75 | FA | Docosanoic acid TMS ester | $C_{25}H_{52}O_2Si$ | 412 | 13.3 |
| 19 | 46.51 | FA | Tetracosonic acid TMS ester | $C_{27}H_{56}O_2Si$ | 440 | 12.2 |
| 20 | 47.27 | ALK | Triacontane | $C_{30}H_{62}$ | 422 | 10.3 |
| 21 | 47.48 | ST | Estra-1,3,5-(10)-triene-16,17-diacetate,2-Nitro-3-TMS ether | $C_{25}H_{35}NO_7Si$ | 489 | 12.3 |
| 22 | 47.89 | ALC | Hexacosanol, TMS ether | $C_{29}H_{62}OSi$ | 454 | 4.5 |
| 23 | 48.77 | VIT | Tocopherol TMS ether | $C_{31}H_{56}O_2Si$ | 488 | 9.1 |
| 24 | 49.82 | ALK | Dotriacontane | $C_{32}H_{66}$ | 450 | 20.6 |
| 25 | 50.38 | FA | Octacosanoic acid TMS ester | $C_{31}H_{66}OSi$ | 482 | 9.2 |
| 26 | 52.21 | ALK | Tetratriacontane | $C_{34}H_{70}$ | 478 | 56.8 |
| 27 | 52.63 | ST | 13,27-Cycloursan-3-one | $C_{30}H_{48}O$ | 424 | 11.4 |
| 28 | 52.71 | ALC | Triacontanol TMS ether | $C_{33}H_{70}OSi$ | 510 | 11.2 |
| 29 | 52.84 | ST | Stigmasterol TMS ether | $C_{32}H_{56}OSi$ | 484 | 19.0 |
| 30 | 53.03 | ST | Sitosterol TMS ether | $C_{32}H_{58}OSi$ | 486 | 46.3 |
| 31 | 54.45 | ST | Lup-20 (29) –en-3-ol, acetate | $C_{32}H_{52}O_2$ | 468 | 59.9 |
| 32 | 55.14 | ST | unknown steroid | | | 7.6 |
| 33 | 55.24 | ALC | Dotriacontanol TMS ether | $C_{35}H_{74}OSi$ | 538 | 6.7 |
| 34 | 55.66 | ST | Friedelan-3-one | $C_{30}H_{50}O$ | 426 | 12.0 |
| | | | **Total** | | | **798** |

*ALK: alkanes; SR: sugar; ALC: alcohols; FA: fatty acids; ST: sterols; ALKE: alkenes; MP: monoarylphenolics; VIT: vitamin; RT: retention time; MW: molecular weight; IS: internal standard.*

**Table 2. GC-MS analysis of *B. pinnatum* $CH_3OH$ extract TMS derivatives**

| Peak no. | RT (min) | Class | Compound | Molecular formula | $M^+$ (m/z) | Conc. (mg $g^{-1}$) |
|---|---|---|---|---|---|---|
| 1 | 10.28 | ALC | 1,2 propanediol$TMS_2$ ether | $C_9H_{24}O_2Si_2$ | 220 | 5.6 |
| 2 | 10.53 | ALC | 2,3 butanediol$TMS_2$ether | $C_{10}H_{26}O_2Si_2$ | 234 | 6.9 |
| 3 | 11.14 | CA | Lactic acid $TMS_2$ | $C_9H_{22}O_3Si_2$ | 234 | 3.8 |
| 4 | 15.29 | DCA | Malonic acid $TMS_2$ ester | $C_9H_{20}O_4Si_2$ | 248 | 4.72 |
| 5 | 17.30 | SR | Glycerol $TMS_3$ ether | $C_{12}H_{32}O_3Si_3$ | 308 | 68.4 |
| 6 | 18.20 | DCA | Succinic acid $TMS_2$ | $C_{10}H_{22}O_4Si_2$ | 262 | 11.1 |
| 7 | 18.82 | SA | Glyceric acid $TMS_3$ | $C_{12}H_{30}O_4Si_3$ | 322 | 6.27 |
| 8 | 22.81 | DCA | Malic acid$TMS_3$ | $C_{13}H_{30}O_5Si_3$ | 350 | 84.0 |
| 9 | 23.42 | SALC | Threitol/erythritol$TMS_4$ | $C_{16}H_{42}O_4Si_4$ | 410 | 9.09 |
| 10 | 24.27 | SA | Threonic acid $TMS_4$ | $C_{16}H_{40}O_5Si_4$ | 424 | 5.4 |
| 11 | 24.69 | SA | Erythronicacid$TMS_4$ | $C_{16}H_{40}O_5Si_4$ | 424 | 4.7 |
| 12 | 28.29 | SALC | Arabitol $TMS_5$ ether | $C_{20}H_{52}O_5Si_5$ | 512 | 26.3 |
| 13 | 28.53 | CA | unknown acid$TMS_2$ ester | $C_{15}H_{32}O_4Si_2$ | 322 | 7.0 |
| 14 | 28.63 | TCA | Aconitic acid $TMS_3$ ester | $C_{15}H_{30}O_6Si_3$ | 390 | 6.8 |
| | 29.22 | | Anthracene (IS) | $C_{14}H_{10}$ | 178 | |
| 15 | 30.06 | SA | 2-Keto-d-gluconic acid $TMS_5$ | $C_{21}H_{50}O_7Si_5$ | 554 | 166 |
| 16 | 30.22 | SR | Fructose $TMS_5$ ether | $C_{21}H_{52}O_6Si_5$ | 540 | 30.8 |
| 17 | 30.32 | TCA | Isocitric acid $TMS_4$ ester | $C_{18}H_{40}O_7Si_4$ | 480 | 33.8 |
| 18 | 31.79 | SR | Glucose$TMS_5$ | $C_{21}H_{52}O_6Si_5$ | 540 | 72.8 |
| 19 | 32.02 | SR | Hexose $TMS_5$ | $C_{21}H_{52}O_6Si_5$ | 540 | 13.3 |
| 20 | 32.55 | SALC | Hexitol$TMS_6$ | $C_{24}H_{62}O_6Si_6$ | 614 | 23.9 |
| 21 | 32.85 | MP | 3,4,5 Trihydroxybenzoic acid$TMS_4$ | $C_{19}H_{38}O_5Si_4$ | 458 | 25.4 |
| 22 | 33.62 | SR | Glucose $TMS_5$ | $C_{21}H_{52}O_6Si_5$ | 540 | 85.4 |
| 23 | 33.93 | SA | Gluconic acid$TMS_6$ | $C_{24}H_{60}O_7Si_6$ | 628 | 6.3 |
| 24 | 34.12 | FA | Palmitic acid TMS ester | $C_{19}H_{40}O_2Si$ | 328 | 13.1 |
| 25 | 34.47 | | Unknown | | | 32.7 |
| 26 | 35.46 | Cyc | Myo-Inositol$TMS_6$ | $C_{24}H_{60}O_6Si_6$ | 612 | 16.8 |
| 27 | 37.32 | SR | Disaccharide $TMS_8$ | $C_{36}H_{86}O_{11}Si_8$ | 918 | 6.2 |
| 28 | 37.60 | SR | Disaccharide $TMS_8$ | $C_{36}H_{86}O_{11}Si_8$ | 918 | 10.2 |
| 29 | 42.99 | SR | Disaccharide $TMS_8$ | $C_{36}H_{86}O_{11}Si_8$ | 918 | 14.9 |
| 30 | 44.58 | SR | Sucrose $TMS_8$ | $C_{36}H_{86}O_{11}Si_8$ | 918 | 181 |
| 31 | 46.01 | SR | Disaccharide $TMS_8$ | $C_{36}H_{86}O_{11}Si_8$ | 918 | 20.4 |
| | | | | **Total** | | **1003** |

*ALC: alcohols; CA: carboxylic acids; DCA: dicarboxylic acids; TCA: tricarboxylic acids; SR: sugars; SA: sugar acids; SALC: sugar alcohols; MP: monoarylphenolics; FA: fatty acids; Cyc: cyclic compound;*

observed [8,22]. Sugar alcohols are another constituent of the extract with 59 mg $g^{-1}$ and arabitol having the highest concentration (26 mg $g^{-1}$) in this group. Alcohols were a minor class of compounds detected (12 mg $g^{-1}$). Other groups of compounds in the extract include free fatty acids (13 mg $g^{-1}$), cyclitols (17 mg $g^{-1}$) and monoarylphenolics (25 mg $g^{-1}$). The peaks at retention times of 28.53 and 34.47 min were unidentified. The various types of sugars identified in the extract are consistent with the findings from flavonoid glycosides in *BP* [15], however, no flavonoids were detected.

A number of active compounds, including phenolics (3-hydroxyanthranillic acid), steroids (e.g. stigmasterol and sitosterol), and organic acids, have been identified in the *BP* extracts [28]. Other compounds (e.g. tocopherol) identified in the extracts are potentially responsible for its antimicrobial, antifungal, anticancer, antitumour and insecticidal activities [28]. *BP* anti-inflammatory activity can also be attributed to the presence steroids detected in the extracts [17].

## 3.2 FAME Analysis of the Extracts

All the fatty acids present in both extracts were converted to volatile FAME derivatives for GC-MS analysis (Table 3). The $CH_2Cl_2$ extract revealed 17 peaks in which 13 of them were identified for various fatty acids ranging from C14 (myristic acid) to C26 (hexacosanoic acid) (Fig. 2a). Palmitic acid (210 mg $g^{-1}$), linoleic acid (268 mg $g^{-1}$), linolenic + oleic acid (266 mg $g^{-1}$) and stearic acid (57 mg $g^{-1}$) were the major types detected. The minor acids identified in the $CH_2Cl_2$ extract are myristic acid, eicosanoic acid, behenic acid, and lignoceric acid. Other fatty acids with lesser concentrations in the extract include pentadecanoic, heptadecanoic,

tricosanoic acid, and hexacosanoic acid. The only class of compound identified in the $CH_2Cl_2$ extract apart from fatty acids were three alcohols, 3,5,11,15-tetramethyl-2-hexadecen-1-ol (7 mg $g^{-1}$), phytol (57 mg $g^{-1}$) and icosen-1-ol (25 mg $g^{-1}$). Fatty alcohols have been previously observed in *BP* [8].

In the $CH_3OH$ extract, 17 major peaks were revealed in the GC-MS analysis of the FAME derivatives (Fig. 2b, Table 3). There were 12 fatty acids identified in $CH_3OH$ extract from myristic acid to lignoceric acid. Palmitic acid (123 mg $g^{-1}$), linoleic acid (72 mg $g^{-1}$), linolenic + oleic acids (75 mg $g^{-1}$) and stearic acid (23 mg $g^{-1}$) were the major acids in the extract. The minor FAMEs and their concentrations were myristic acid, iecosanoic acid, behenic acid, tricosanoic acid, 2-hydroxy-docosanoic acid, lignoceric acid and 2-hydroxy-tetracosanoic acid. These fatty acids were also found in other naturally occurring plant product extracts [23,24,29]. Other non-fatty acids present in the extract were 3,5,11,15-tetramethyl-2-hexadecen-1-ol, phytol and icosen-1-ol with a total concentration of 21 mg $g^{-1}$. C14-C22 fatty acids were common to both extracts in varying concentrations. Pentadecanoic, heptadecanoic and hexacosanoic acids were present in the $CH_2Cl_2$ but absent in the $CH_3OH$ extract. On the other hand, 2-methoxyestra-1,3,5(10)-triene-3,16,17-triol, 2-hydroxy-docosanoic acid and 2-hydroxy-tetracosanoic acid were identified in $CH_3OH$ extract and not present in the $CH_2Cl_2$ extract.

This study is consistent with the results obtained by other researchers [13,18,19] which have identified steroids, organic acids, phenolics and sugars from the leaves of *BP*. Other compounds identified in the extracts were alkanes and alkanols, and this is in agreement with the work of others [20,30]. Several fatty acids such as palmitic, linoleic, linolenic, stearic, lignoceric and behenic acids were detected in the extracts. Linoleic acid has the highest concentration in the $CH_2Cl_2$ extract while linolenic + oleic acids have the highest concentration in the $CH_3OH$ extract. Almeida et al. [21] showed that fatty acids (palmitic, stearic, arachidic and behenic acids) from *BP* have an immunosuppressive effect *in-vivo*. Work by Agoramoorthy et al. [31] has shown that fatty acids have antifungal and antibacterial properties. Studies by other researchers have shown that fatty acids are ubiquitous in plant species [23,24,29,31].

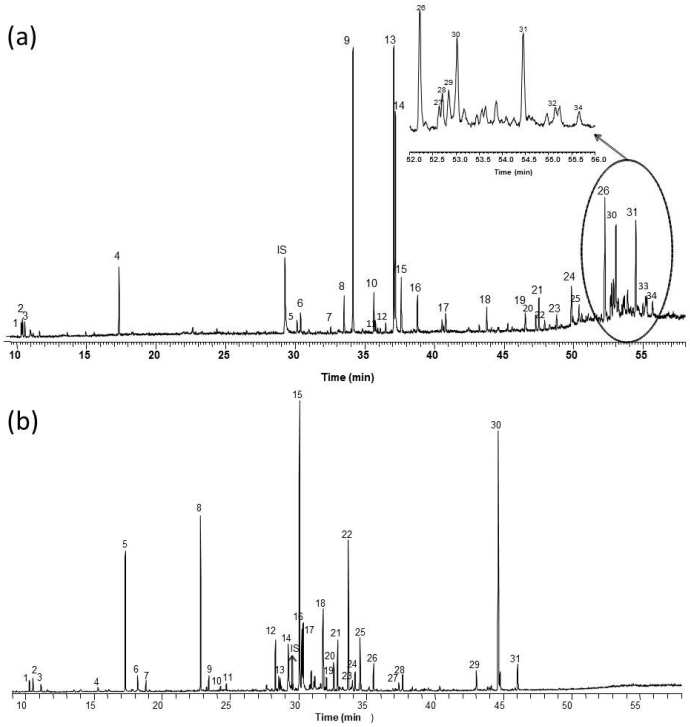


**Fig. 1. Total ion current chromatogram of *B. pinnatum* TMS derivatives of (a) $CH_2Cl_2$ extract and (b) $CH_3OH$ extract**

**Table 3. GC-MS analysis of FAME derivatives of *B. pinnatum* $CH_2Cl_2$ and $CH_3OH$ extracts**

| Peak no. | Compound | RT (min) | $M^+$ (m/z) | $CH_2Cl_2$ (mg $g^{-1}$) | $CH_3OH$ (mg $g^{-1}$) |
|---|---|---|---|---|---|
| | Naphthaleneacetic acid (internal standard) | 27.55 | 200 | | |
| 1 | Myristic acid (C14:0) | 27.88 | 242 | 32.5 | 6.99 |
| 2 | 3,5,11,15 tetramethyl-2-hexadecen-1-ol | 29.88 | 296 | 6.46 | 1.84 |
| 3 | Pentadecanoic acid (C15:0) | 29.99 | 256 | 2.0 | |
| 4 | cisphytol | 30.38 | 296 | 18.3 | 4.50 |
| 5 | trans phytol | 30.82 | 296 | 38.5 | 8.26 |
| 6 | Icosen-1-ol | 31.09 | 296 | 6.70 | 1.32 |
| 7 | Icosen-1-ol | 31.52 | 296 | 18.1 | 5.38 |
| 8 | Palmitic acid (C16:0) | 31.96 | 270 | 210 | 123 |
| 9 | heptadecanoic acid (C17:0) | 33.87 | 284 | 3.1 | |
| 10 | Linoleic acid (C18:2) | 35.11 | 294 | 268 | 72.3 |
| 11 | Linolenic acid (C18:3) + oleic acid (C18:1) | 35.24 | 292/296 | 266 | 75.2 |
| 12 | Stearic acid (C18:0) | 35.67 | 298 | 56.9 | 23.1 |
| 13 | 2-Methoxyestra-1,3,5(10) triene-3,16,17 triol | 36.83 | 318 | | 5.76 |
| 14 | Eicosanoic acid (C20:0) | 39.09 | 326 | 30.8 | 9.05 |
| 15 | Behenic acid (C22:0) | 42.23 | 354 | 39.0 | 14.9 |
| 16 | Tricosanoic acid (C23:0) | 43.77 | 368 | 2.6 | 1.93 |
| 17 | 2-Hydroxy-docosanoic acid (C22:0) | 44.30 | 370 | | 3.46 |
| 18 | Lignoceric acid (C24:0) | 45.58 | 382 | 32.6 | 6.09 |
| 19 | 2-Hydroxy-tetracosonic acid (C24:0) | 48.72 | 398 | | 7.20 |
| 20 | Hexacosanoic acid (C26:0) | 50.70 | 410 | 11.0 | |
| | **Total** | | | **1043** | **370** |

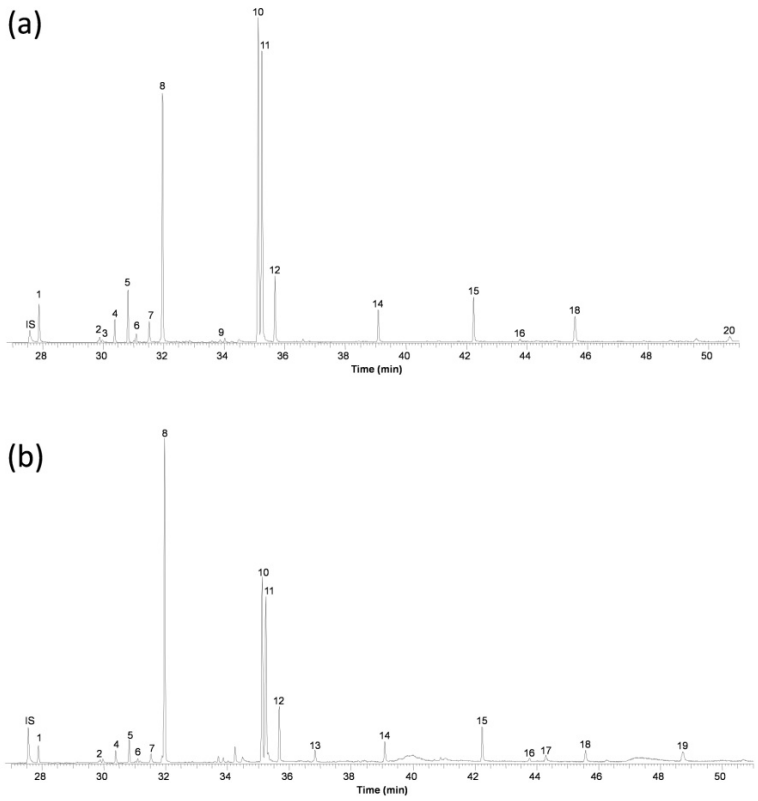


**Fig. 2. Total ion current chromatogram of B. *Pinnatum* FAME derivatives of (a) $CH_2Cl_2$ and (b) $CH_3OH$ extracts**

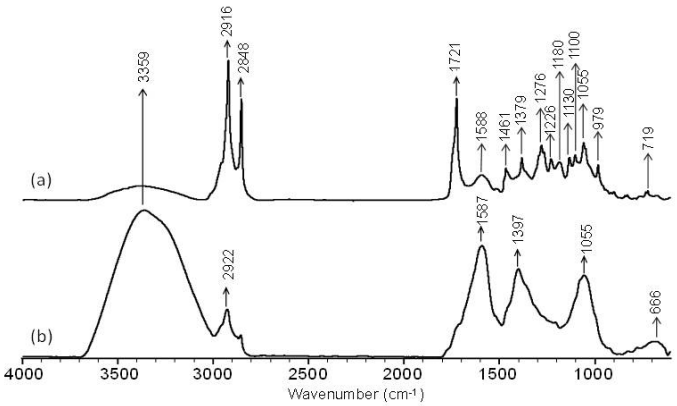


**Fig. 3. FTIR spectra of *B. pinnatum* (A) $CH_2Cl_2$ and (B) $CH_3OH$ extracts**

### 3.3 FTIR Spectroscopic Analysis of *B. pinnatum* Extracts

FTIR spectroscopy was used to investigate the functional groups in the extracts. The spectra are shown in Fig. 3 above and the assignments of bands are discussed as follows. The presence of strong O-H stretching is observed at 3359 $cm^{-1}$ corresponding to hydroxyl groups (Fig. 3B); however, carbonyl stretching of carboxylic acids was not seen at ~1700 $cm^{-1}$ [19,30]. Hence this O-H stretching band could be attributed to alcohols and sugars (see Table 2). The C-H stretching from methylene and methyl groups are shown at ~2922 and 2916 $cm^{-1}$, respectively, for both extracts and 2848 $cm^{-1}$ only in the $CH_2Cl_2$ extract [32]. The pronounced carbonyl group band at 1721 $cm^{-1}$ assigned to an ester was only observed in the $CH_2Cl_2$ extract. The intense bands at 1588, 1587 $cm^{-1}$ observed in both extracts were attributed to aromatic skeletal C=C vibrations of benzoic acid and other phenolics [32], however it is more pronounced in the $CH_3OH$ extract than in the $CH_2Cl_2$ extract (Fig. 3A and Table 3). The observed band at 1461 $cm^{-1}$ in the $CH_2Cl_2$ extract which was absent in the $CH_3OH$ extract, was attributed to methylene scissoring. Methyl asymmetrical bending absorptions of methyl symmetrical bending were observed at 1379 $cm^{-1}$ [32]. These spectral assignments are consistent with the FAME analysis results (Table 3). The intense band at 1068 $cm^{-1}$ was assigned to C-O bonded stretching vibration of an alcohol [32,33].

## 4. CONCLUSION

Characterization of dichloromethane and methanol extracts from *B. pinnatum* has been successfully carried out by means of GC-MS and FTIR analysis. The analysis revealed the presence of alkanes, alkene, carboxylic acids, dicarboxylic acids, tricarboxylic acids, sugars, sugar acids, alcohols, sugar alcohols, fatty acids, monoarylphenolics, steroids, vitamin and cyclic compounds. A number of active compounds, including phenols, steroids, and organic acids, have been identified in *BP* extracts. Compounds identified (e.g. tocopherol) in the extracts are most likely responsible for its antimicrobial, antifungal, anticancer, antitumour and insecticidal activities. *BP* anti-inflammatory activity can also be attributed to the presence of steroids. The present study shows the phytoconstituents of *BP*, which is very helpful to subsequent researchers to explore more into the potential benefits of this miracle plant.

## CONSENT

It is not applicable.

## ETHICAL APPROVAL

No animals or human test subjects were involved in this study.

## ACKNOWLEDGEMENTS

The first author would like to acknowledge Bowen University and Osun State Government, Nigeria for their financial support towards this work.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

## REFERENCES


1. Ogundipe S, Obinna C, Olawale G. Shortage of medical personnel: Tougher times ahead for Nigerians. Vanguard Newspaper; 2015. Accessed 01/07/2016. Available:http://www.vanguardngr.com/2015/01/shortage-medical-personnel-tougher-times-ahead-nigerians-1/.
2. Anon. 2016, Accessed 01/07/2016. Available: www.africapedia.com/DOCTOR-TO-PATIENT-RATIO-IN-AFRICA
3. Chopra RN, Nayal SL, Chopra IL, Glossary of Indian medicinal plants. Council Sci. Ind. Research, New Delhi. New Delhi. 1956;42.
4. Rastogi RP, Mehrotra BN. Compendium of Indian Med. Plants, Vol. 2. Central Drug Research Institute, Lucknow and National Institute of Science Communication. New Delhi; 1991.
5. Tatsimo SJN, Tamokou Jde-D. Havyarimana L, Csupor D, Forgo P, Hohmann J, et al. Antimicrobial and antioxidant activity of kaempferol rhamnoside derivatives from *Bryophyllum pinnatum*. BMC Research Notes. 2012;5:158-163.
6. Lans CA. Ethnomedicines used in Trinidad and Tobago for urinary problems and diabetes mellitus. J Ethnobiol Ethnomed. 2006;2:45-55.
7. Yadav NP, Dixit VK. Hepatoprotective activity of leaves of *Kalanchoe pinnata* Pers. J Ethnopharmacol. 2003;86:197-202.
8. Kamboj A, Saluja AK. *Bryophyllum pinnatum* (Lam.) Kurz. Phytochemical and pharmacological profile: A review. Pharmacognosy Rev. 2009;3:364-374.
9. Siddiqui S, Faizi S, Siddiqui BS, Sultana N. Triterpenoids and phenanthrenes from leaves of *Bryophyllum pinnatum*. Phytochemistry. 1989;28:2433-2438.
10. Dalziel JM. The useful plants of West Tropical Africa. Crown Agents for Overseas Governments and Administrations, London. 1955;28.
11. Okwu DE. Nigerian medicinal plants II. Medicinal and Aromatic Plant Science and Biotechnology. 2007;1(1):97-102.
12. Egereonu UU, Mokwe NR. Characterization of the extract from *Bryophyllum pinnatum* leaves. J. Chem. Soc. Nigeria. 2005;30:192-196.
13. Majaz QA, Nazim S, Asir Q, Shoeb Q, Bilal GM. Screening of in vitro anthelmentic activity of *Kalanchoe pinnata* roots. Int J Res Ayurveda Pharm. 2011;2:221-223.
14. Ghani A. Medicinal plants of Bangladesh. The Asiatic Society of Bangladesh, Dhaka, 2003;2:382.
15. Fürer K, Raith M, Brenneisen R, Mennet M, Simoes-Wüst AP, von Mandach U, et al. Two flavonol glycosides and a metabolite profile of *B. pinnatum*, a phytotherapeutic used in obstetrics and gynaecology. Planta Med. 2013;79:1565-1571.
16. Majaz QA, Tatiya AU, Khurshid M, Nazim S. The miracle plant (*Kalanchoe pinnata*): A phytochemical and pharmacological review. Int J Res Ayurveda Pharm. 2011;2:1478-1482.
17. Afzal M, Gupta G, Kazmi I, Rahman M, Afzal O, Alam J, et al. Anti-inflammatory and analgesic potential of a novel steroidal derivative from *Bryophyllum pinnatum*. Fitoterapia. 2012;83:853-858.
18. Kamboj A, Saluja AK. Isolation of stigmasterol from petroleum ether extract of aerial part of *Bryophyllum pinnatum* (Crassulaceae). Journal of Pharmacy Research. 2010;3(12):2802-2803.
19. Gaind KN, Gupta RL. Identification of waxes from the leaves of *Kalanchoe pinnata*. Plantamedica. 1974;23:193-197.
20. Gaind KN, Gupta RL. Alkanes, alkanols, triterpenes and sterols of *Kalanchoe pinnata*. Phytochemistry. 1972;11:1500-1502.
21. Almeida AP, Da Silva SAG, Souza MLM, Lima LMTR, Rossi-Bergmann B, Goncalves de Moraes VL, et al. Isolation and chemicals analysis of a fatty acid fraction of *Kalanchoe pinnata* with a potent lymphocyte suppressive activity. Planta Medica. 2000;66:134-137.
22. Marriage PB, Wilson DG. Analysis of Organic acids of *Bryophyllum pinnatum*. Can. J. Biochem. 1971;49:282-295.
23. Osman NB, McDonald AG, Laborie MPG. Analysis of DCM extractable components from hot-pressed hybrid poplar. Holzforschung. 2012;66:927-34.
24. Faboro E, Wei L, Liang S, McDonald AG, Obafemi GA. Characterization of dichloromethane and methanol extracts from the leaves of a medicinal plant: *Globimetula oreophila*. Industrial Crops and Products. 2016;83:391-399.
25. Orhan DD, Orhan I. Fatty acid composition of *Viscum album* subspecies from Turkey. Chemistry of Natural Compounds. 2006;42:641-44.

26. Rastogi RP, Mehrotra BN. Compendium of Indian Medicinal Plants Vol.5. Central Drug Research Institute, Lucknow and National Institute of Science Communication. New Delhi. 1994;141-143.
27. Yamagishi T, Yan X-Z, Wu R-Y, McPhail DR, McPhail AT, Lee K-H. Structure and stereochemistry of Bryophyllin A, A novel potent cytotoxic Bufadienolide orthoacetate from *Bryophyllum pinnatum*. Pharm Bull. 1988;36:1615-1617.
28. Kaur N, Bains R, Niazi J. A Review on *Bryophyllam pinnatum* - A medicinal herb. J Pharmceutical Med Innov. 2014;1(3):13-19.
29. Liang S, McDonald AG. Chemical and thermal characterization of potato peel waste and its fermentation residue as potential resources for biofuel and bioproducts production. J Agric Food Chem. 2014;62:8421-29.
30. Kannathasan K, Senthilkumar A, Venkatesalu V, Chandrasekaran M. Larvicidal activity of fatty acid methyl esters of Vitex species against *Culex quinquefasciatus*. Parasitol Res. 2008; 103:999-1018.
31. Agoramoorthy G, Chandrasekaran M, Venkatesalu V, Hsu MJ. Antibacterial and antifungal activities of fatty acid methyl esters of the blind-your-eye mangrove from India. Braz J Microbiol. 2007;38:739-742.
32. Mayo DW. Course notes on the interpretation of infrared and Raman spectra. John Wiley & Sons, New York; 2003.
33. Wang X, Sun R, Zhang J, Chen Y, Liu N. Structure and antioxidant activity of polysaccharide POJ-U1a extracted by ultrasound from *Ophiopogon japonicas*. Fitoterapia. 2012;83:1576-1584.



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