

**Proximate, Selected Elements and Bioactive Compounds in *Jatropha tanjorensis* and *Bryophyllum pinnatum* Leaves**

**ABSTRACT**

A comparative research was performed on two medicinal leaves: *Jatropha tanjorensis* and *Bryophyllum pinnatum* collected from Angalabiri community, Nigeria, to ascertain the proximate content, mineral composition, and secondary metabolite profiling in the leaves. The proximate analyses of the leaves of *J. tanjorensis* and *B. pinnatum* revealed: moisture (84.87 %, 92.66 %), ash (7.91 %, 1.11 %), protein (6.20 %, 1.30 %), fat (14.54 %, 8.40 %), fibre (14.67 %, 21.31 %) and carbohydrate (54.28 %, 24.78 %) respectively. Mineral analyses showed: potassium (0.018 %, 0.060 %), iron (0.134 %, 0.003 %), manganese (0.001 %, 0.0002 %), calcium (1.47 %, 1.30 %) and phosphorus (1.47 %, 0.170 %) separately. Examining methanolic extracts obtained from *J. tanjorensis* and *B. pinnatum* leaves through gas chromatography-mass spectrometry (GC-MS) uncovered fifteen and six bioactive constituents, respectively. Fascinatingly, GC-MS findings unveiled the existence of distinct active biological compounds in both leaves: benzyl 1-[(1-hydroxy-4-oxo-1-phenyltetrahydroquinolin-3-yl)carbamoyl]cyclohexane-1-carboxylate, cycloheptanehexol, and ginsenoside Rh2, at different percentage concentrations. All identified bioactives are stated to demonstrate anti-tumorigenic properties, antibacterial, anticancer and anti-irritant properties. Undoubtedly, the bioactive compounds in *Jatropha tanjorensis* and *Bryophyllum pinnatum* leaves, affirm their use in ethnomedicine.

**Keywords:** *Jatropha tanjorensis*, *Bryophyllum pinnatum*, ethnomedicine

**1. INTRODUCTION**

Medicines sourced from plants have significantly contributed to enhancing human well-being. They could serve as the foundation for the creation of a medicinal product, acting as a natural blueprint for oral phytomedicine advancement, aimed at addressing various diseases through treatment. Medicinal plants have the potential to consistently provide crucial elements, notably metals, which are essential for the vital metabolic processes of organisms [1]. Minerals in plants include: vitamins, iron, water, calcium, etc., which are essential for cell growth and prevention

against foreign substances [2]. The bioactive components found in plants possess distinct physiological effects, giving rise to definite active compounds. These compounds are valuable not only for the pharmaceutical, cosmetic, and food sectors but also, more recently, for pest management in agriculture [3]. Bioactive constituents in plants can be extracted for the treatment of ailments as reports show they have little or no side effects.

The increased public interest and widespread adoption of herbal medicine have sparked a renewed focus and motivation in the field of medicinal plant research. *Jatropha tanjorensis* and *Bryophyllum pinnatum* leaves among others have been reported traditionally to play key responsibilities in managing diverse illnesses, encompassing bacterial and fungi infections.

Figure 1 illustrates *Jatropha tanjorensis*, a member of the flowering plant genus found within the *Euphorbiaceae* family and the *Acalyphoideae* subfamily. It is prevalent as a ubiquitous weed in agricultural fields, areas of bush re-growth, roadsides, and disrupted environments across regions of elevated rainfall, such as India, Nigeria, and Canada [4].



**Figure 1:** *Jatropha tanjorensis* leaves

The local name for the plant is “Hospital too far”. *Jatropha tanjorensis* leaves are employed in ethnomedicine to enhance blood vitality. Studies have proven that *J. tanjorensis* leaves are edible and medicinal. Olayiwola *et al.*<sup>5</sup> conducted a study examining the potential hypoglycemic effects and anti-diabetic properties of an ethanol-water leaf extract derived from *J. tanjorensis* leaves. This research was carried out on rats that were either fasted or given a glucose load. Based on their research, the extract demonstrated significant effects in reducing glucose levels in rats loaded with glucose when administered at 2 g/kg. However, its in-vitro insulin secretion capacity was observed to be restricted.

*Plasmodium berghei* infected mice were treated with *J. tanjorensis* leaf extract and *Hippocratea africana* root bark extract. *Jatropha tanjorensis* leaf extract was reported to enhance hematopoietic capabilities, thereby boosting red blood cell counts and increasing hemoglobin level [6]. Administration of *J. tanjorensis* leaf extracts resulted in the reduction of renal and hepatic index disturbances induced by Benzoic acid sodium salt in rats [7]. Phenolic compounds extracted from *J. tanjorensis* leaves demonstrated a significant inhibitory effect (statistically significant at  $P < 0.05$ ) on oxidative deterioration of lipids in the brains and rat livers [8].

Although it has been recorded that the leaves of *J. tanjorensis* possess medicinal properties, however, the leaves can be poisonous if eaten raw. The leaves release cyanogenic glycosides as cyanides which function as defense chemicals that are highly toxic to most living organisms, because of its ability to inhibit the electron transport chain system by binding to cytochrome.<sup>5</sup>



**Figure 2:** *Bryophyllum pinnatum* leaves

*Bryophyllum pinnatum* is of the family Crassulaceae native of Madagascar [9]. It is commonly recognized by its popular names: Africa never die, life plant, and air plant. The leaves of this species are thick, fleshy and elliptical in shape, curved with a scalloped or serrated margin, often reddish as shown in Figure 2.

*Bryophyllum pinnatum* plant holds significant popularity in traditional medicine tales. Alcoholic and aqueous extracts of the leaves are used medicinally as anti-diabetes, antitumor and antimicrobial by indigenous communities.

Research has demonstrated that the *B. pinnatum* plant is abundant in essential minerals such as calcium (Ca), magnesium (Mg), potassium (K), zinc (Zn), sodium (Na), iron (Fe) and phosphorus (P) [10]. The botanical specimen also harbors an extensive assortment of dynamic phytochemicals, encompassing alkaloids, triterpenes, glycosides, flavonoids, steroids, bufadienolides, lipids, and organic acids [11]. These compounds have been examined for their role in confirming the plant's traditional applications, which encompass a range of pharmacological effects such as

immunomodulation, depression of the central nervous system, pain relief, reduction of inflammation, inhibition of microbial growth, anticancer potential, ulcer prevention, insecticidal action, diabetes management, seizure control, antioxidation, and management of high blood pressure [12,13,14].

Ogidi *et al.* [15] documented the presence of plant alkaloids, glycosides, polyphenols, phenolic compounds, saponins, terpenoids, and flavonoids in the roots, leaves, and stems of *B. pinnatum*. Kalu *et al.* [16] conducted an assessment of the nutritional elements including proximate contents, vitamins, amino acids, phytochemicals, and mineral composition found within both the leaf and root components of *B. pinnatum*. The possible anthelmintic effects against the Indian earthworm, *Pheretima posthuma*, using ligroin, trichloromethane, aqueous and methanol extracts obtained from dried leaves of *B. pinnatum* were investigated with promising results [17].

Apparently, reports on the phytochemical composition of *Jatropha tanjorensis* and *Bryophyllum pinnatum* leaves abound in the literature. Nevertheless, the present research is to compare the proximate, minerals and secondary metabolites in *J. tanjorensis* and *B. pinnatum* leaves obtained from Anglabiri Community, Bayelsa State, Nigeria. This research is thought to contribute into the bioactive components and medicinal significance of the leaves of *J. tanjorensis* and *B. pinnatum*.

## **2. MATERIALS AND METHODS**

### **2.1 Chemicals and Reagents**

Analytical grade chemicals, sourced from BDH and Labtech chemicals, were employed without requiring supplementary purification.

### **2.2 Plant Collection and Identification**

Fresh leaf samples of *J. tanjorensis* and *B. pinnatum* were obtained from the Angalabiri Community in the Sagbama L.G.A., Bayelsa State, Nigeria. Samples were identified in the Biotechnology laboratory, University of Africa, Toru-Orua, Nigeria. Identified samples underwent a twenty-one-day air-drying period **under shade**, after which they were electronically pulverized. The resulting pulverized samples were then stored in a desiccator for subsequent analyses.

## **2.3 Proximate Investigation**

### **2.3.1 Amount of Moisture (water)**

The amount of moisture (water) was evaluated in accordance with the A.O.A.C [18] guidelines. A quantity of 5 grams from the sample was measured and placed into pre-weighed (W1) Petri dish. The weight of the Petri dish and the sample was recorded (W2). The sample in the petri dish was heated in an oven at 105°C for 3 hrs, to reduce its moisture (water) content. The sample was allowed to **cool in a desiccator for 30 minutes** and re-weighed (W3). The percentage moisture content was calculated using Equation 1

$$\% \text{ Moisture Content} = \frac{W2 - W3}{W1} \times 100 \quad \text{Equation 1}$$

### **2.3.2 Amount of Fat**

A.O.A.C [18] outlined procedure was followed in determining the amount of fat in the samples. For each sample 5 g was measured and placed in a pre-weighed filter paper. After weighing, the sample was oven dried and secured with a thread. The sample in the filter paper was introduced into a soxhlet apparatus receiver. A 500 mL round bottom flask was filled to 3/4 capacity with the extraction solvent, n-hexane (bp 68 °C). The flask, equipped with a reflux condenser, was attached to the soxhlet apparatus and placed in an electro mantle heater. Extraction commenced as the solvent refluxed for 4 hrs. Subsequently, the defatted sample in the filter paper was extracted and oven dried to a constant weight **50 °C for 48 hours**. The weight difference of the sample before

and after extraction was recorded to determine the extracted amount of fat. Percentage extraction was determined using Equation 2:

$$\% \text{ Fat content} = \frac{\text{Weight of fat extracted}}{\text{Weight of sample}} \times 100 \quad \text{Equation 2}$$

#### 2.3.4 Amount of Ash

The amount of ash in each sample was determined using the A.O.A.C [18] procedure. 2 g of the sample was weighed in a porcelain crucible and positioned in a muffle furnace at 500 °C for 3 hrs. The resulting ash was cooled in a desiccator for 24 hours and weighed.

#### 2.3.5 Amount of Crude Fibre

The amount of crude fiber was determined following the procedure outlined in A.O.A.C [18]. 2 g defatted sample (W<sub>1</sub>) was placed in a 500 mL conical flask and 200 mL of 1.25 % H<sub>2</sub>SO<sub>4</sub> was transferred into the flask. The sample was boiled for 30 minutes with cooling fingers employed to maintain a consistent temperature. The resulting mixture was transferred to a filter cloth in a butch funnel and washed with hot distilled water, after which it was transferred to a conical flask containing 200 mL 1.25 % NaOH and boiled for another 30 minutes with constant shaking. The sample solution was filtered, and washed with hot distilled water and 1% hydrochloric acid. To eliminate any remaining fat, the sample was washed twice with ethanol and washed three times with petroleum ether. The residue, in a porcelain crucible was oven-dried at 50 °C for 12 hours, cooled in a desiccator for 12 hours and weighed (W<sub>2</sub>). The crucible was later placed in a muffle furnace for 2 hrs at 450 °C, cooled in a desiccator for 12 hours and re-weighed (W<sub>3</sub>). Amount of crude fibre was determined using Equation 3

$$\text{Amount of crude fibre} = \frac{W_2 - W_3}{W_1} \quad \text{Equation 3}$$

Where W<sub>1</sub> is the weight of the sample

W<sub>2</sub> is the weight of the sample plus the weight of the crucible after oven drying

W<sub>3</sub> is the weight of the sample plus the weight of the crucible after ashing

Percentage ash extracted was calculated using Equation 4:

$$\% \text{ crude fibre} = \frac{\text{Weight of crude fibre}}{\text{Weight of sample}} \times 100 \quad \text{Equation 4}$$

### 2.3.6 Protein content

The process for assessing protein adhered to the A.O.A.C [18] protocol and involved three primary stages:

- (1) Digestion stage: 10 g of each sample was transferred into a Micro-Kjeldahl digestion flask, 10 mL of H<sub>2</sub>SO<sub>4</sub> was added and 0.5 g of selenium was added as a catalyst. The mixture was heated using an electro-thermal heater to obtain a clear solution. The flask was allowed to cool and the digested solution (digest) was diluted with distilled water and transferred to the distillation unit.
- (2) Steam Distillation Unit: 10 mL 40 % NaOH solution was added to the digest to liberate ammonia. To indicate this process, 3 drops of mixed indicator were introduced into a receiving flask holding 10 mL of 2 % boric acid solution, resulting in a pink solution. Distillation continued until about 30 mL of the distillate was collected in the receiving flask. A colour change from red wine to green was observed indicating the presence of ammonia.
- (3) Titration: The resulting solution, 30 mL (green distillate) was titrated against 0.1 M hydrochloric acid solution, until the colour changed to red wine, indicating the end point.

Percentage Nitrogen was calculated using Equation 5

$$\% \text{ Nitrogen} = \frac{\text{true value of acid used} \times 0.014 \times \text{dillution factor} \times 100}{\text{Weight of the sample}} \quad \text{Equation 5}$$

Percentage crude protein was calculated using Equation 6:

$$\text{Crude protein (\%)} = \% \text{ Nitrogen} \times 6.25 \quad \text{Equation 6}$$

Where 6.25 is the conversion factor

### 2.3.7 Amount of carbohydrate present

The amount of carbohydrate present was determined by difference using Equation 7:

$$\% \text{ carbohydrate} = 100 - (M + P + F1 + A + F2) \quad \text{Equation 7}$$

Where M represents the percentage of moisture, P denotes the percentage of protein, F1 stands for the percentage of fat, A represents the percentage of ash, and F2 indicates the percentage of crude fiber.

### 2.4 Elemental Analysis

A.O.A.C [18] outlined procedure was employed in determining the mineral composition in the samples. 1g of each sample in a porcelain crucible was ashed in a muffle furnace for 5 hrs at 550 °C. The ashed sample was cooled in a desiccator for 12 hours and dissolved in a solution of 1mL of HNO<sub>3</sub> and 1mL of HCl, and diluted to 100 mL. The solution was used to analyze the presence of manganese, iron, phosphorus, calcium and potassium.

### 2.5 Statistical Analysis

Data from GC-MS, proximate and elemental analyses were subjected to one-way analysis of variance (ANOVA) using SPSS version 21.0. Significant values were those with  $p \leq 0.05$ . All analyses were performed in triplicate, and the results are expressed as mean values  $\pm$  standard deviation

### 2.6 Bioactive Chemicals

Analyzing the sample extracts involved utilizing an Agilent 6890 gas chromatograph (GC) connected with an Agilent 5973N Mass Spectrometer (MS), (Agilent Technology in Palo Alto, CA, USA). The GC-MS setup included an Automatic Liquid Sampler injector (Agilent 7683

Series) and a META X5 coated fused capillary column that was 30 m long and had a diameter of 0.25 mm, featuring a film thickness of 0.25  $\mu\text{m}$  and capable of reaching up to 325  $^{\circ}\text{C}$ . Helium, with a purity of 99.99 %, served as the carrier gas, maintained at a steady flow rate of 1.0 mL/min. To carry out the analysis, a 1 $\mu\text{L}$  sample was introduced in a split mode of 20:1, while maintaining the temperatures of the MS source and MS Quad at 230 $^{\circ}\text{C}$  and 150 $^{\circ}\text{C}$ , respectively. The injection, transfer line, and ion source temperatures were uniformly set at 280 $^{\circ}\text{C}$ . Mass spectra were recorded within a scan range of 50 – 550 amu, utilizing an ionizing energy of 70 eV, and the electron multiplier voltage was determined through autotune.

The identification and characterization of bioactive compounds in the sample extracts were dependent on the analysis of GC retention times. The mass spectra underwent computational comparison with a spectral library. Analysis of the GC-MS mass spectrum involved utilizing the National Institute of Standards and Technology (NIST) database, which encompasses more than 590,000 patterns for interpretation. Specifically, the mass spectra of the unidentified components were aligned with archived spectra in the NIST library (2014 edition), facilitating the identification of the name, molecular weight, molecular formula, structure, and mass fragmentation of the components detected in the sample.

### **3. RESULTS AND DISCUSSION**

Tables 1 and 2 contain the detailed outcomes of the proximate and elemental analyses conducted on the air-dried leaves of *Jatropha tanjorensis* (commonly called "hospital too far") and *Bryophyllum pinnatum* (commonly called "Never die"). Furthermore, Tables 3 and 4 showcase the results obtained through Gas Chromatography-Mass Spectrometry (GC-MS), complemented by visual representations in Figures 3 to 6. These investigations offer valuable insights into the chemical composition of bioactive compounds found in the examined plant species.

The following sections outline the findings from the examination conducted on the leaves of *J. tanjorensis* and *B. pinnatum*:

### 3.1 Proximate composition

Table 1 displays the proximate composition outcomes for the leaves of *J. tanjorensis* and *B. pinnatum*. Results showed statistically significant difference on all the proximate parameters for the plant species. Moisture content was higher in *B. pinnatum* leaves, 92.66 %. High moisture content signifies lower shelf life and more susceptibility to microbial attack [19,20,21]. Similar value, 91.03 %, was reported by Nwali *et al.* [22]. The ash value is higher in *J. tanjorensis* leaves, 7.91 %, indicating that the leaves contain higher organic matter. Lower value, 1.15 %, was reported in the roots of *J. tanjorensis* [23]. Protein content was higher in *J. tanjorensis* leaves, 6.20 %. A lower protein value was reported by Egbon *et al.* [24]. Crude fat composition of the leaves of *J. tanjorensis* and *B. pinnatum* were 14.54 % and 8.40 %. Lower value, 11.730 % of crude fat in *J. tanjorensis* was reported by Chigozie *et al.* [25].

**Table 1:** Proximate analysis (Mean % ± S.D) of *J. tanjorensis* and *B. pinnatum* leaves

Plant species	Moisture	Ash	Protein	Fat	Fiber	Carbohydrate
<i>J. tanjorensis</i>	84.87 ± 0.02 <sup>a</sup>	7.91 ± 0.01 <sup>b</sup>	6.20 ± 0.01 <sup>b</sup>	14.54 ± 0.01 <sup>b</sup>	14.67 ± 0.02 <sup>a</sup>	54.28 ± 0.03 <sup>b</sup>
<i>B. pinnatum</i>	92.66 ± 0.02 <sup>b</sup>	1.11 ± 0.01 <sup>a</sup>	1.30 ± 0.02 <sup>a</sup>	8.40 ± 0.01 <sup>a</sup>	21.31 ± 0.02 <sup>b</sup>	24.78 ± 0.02 <sup>a</sup>

Values are presented as mean ± standard deviation (n=3): values on same column with different superscripts differ significantly (p<0.05)

The leaves of *J. tanjorensis* and *B. pinnatum* exhibited fiber contents of 14.67 % and 21.31 %, respectively. The leaves of *B. pinnatum* contain significantly higher fiber content than the leaves of *J. tanjorensis*; indicating that *B. pinnatum* leaves are abundant in dietary fiber, making them a more substantial reservoir of this essential nutrient. The carbohydrate content of *J. tanjorensis* leaves was higher, 54.28 %. The leaves of *J. tanjorensis* offer a more potent and efficient source

of energy [20]. Carbohydrate content of *J. tanjorensis* leaves was lower than the values observed by Egbon *et al.* [24] 58.7 %. Latif *et al.* [26] reported lower carbohydrate content in *B. pinnatum* leaves.

### 3.2 Elemental screening

In Table 2, the elemental analysis outcomes for the leaves of *J. tanjorensis* and *B. pinnatum* are presented. Results show that the values of potassium, iron, phosphorous are significantly the same for the two plants while, there is statistically significant difference on the values of manganese and calcium for the two plant species. The potassium (K) content in the leaves of *J. tanjorensis* and *B. pinnatum* was found to be 0.018 % and 0.060 %, respectively. This suggests a higher concentration of potassium in the leaves of *B. pinnatum*. Potassium plays an essential role in ensuring proper kidney and heart functioning as well as facilitating muscle contractions and the transmission of nerve signals [27].

The concentration of Iron in the leaves of *J. tanjorensis* and *B. pinnatum* were 0.134 % and 0.003 % respectively. Lower Fe content in the leaves of *J. tanjorensis* was documented by Anhwange *et al.* [28]. The leaf content of Manganese in *J. tanjorensis* and *B. pinnatum* were 0.001 % and 0.0002 % respectively. Chigozie *et al.* [25] reported a lower manganese concentration in *J. tanjorensis*, 0.000415 %. The leaves of *J. tanjorensis*, 1.47 %, contained more calcium than *B. pinnatum* leaves, 1.30 %. Calcium serves as a storage reservoir within the bones, ensuring the maintenance of appropriate calcium levels in the bloodstream. These levels are crucial for the proper functioning of nerves and muscles, promoting their healthy and functionality [29,30].

**Table 2:** Elemental analysis (Mean %  $\pm$  S.D) of *J. tanjorensis* and *B. pinnatum* leaves

Plant species	(K)	(Fe)	(Mn)	(Ca)	(P)
<i>J. tanjorensis</i>	0.018 $\pm$ 0.01 <sup>a</sup>	0.134 $\pm$ 0.01 <sup>b</sup>	0.001 $\pm$ 0.01 <sup>a</sup>	1.470 $\pm$ 0.02 <sup>a</sup>	1.470 $\pm$ 0.02 <sup>b</sup>
<i>B. pinnatum</i>	0.060 $\pm$ 0.02 <sup>b</sup>	0.003 $\pm$ 0.02 <sup>a</sup>	0.002 $\pm$ 0.03 <sup>a</sup>	1.300 $\pm$ 0.02 <sup>a</sup>	0.170 $\pm$ 0.02 <sup>a</sup>

Values are presented as mean  $\pm$  standard deviation (n=3); values on same column with different superscripts differ significantly (p<0.05)

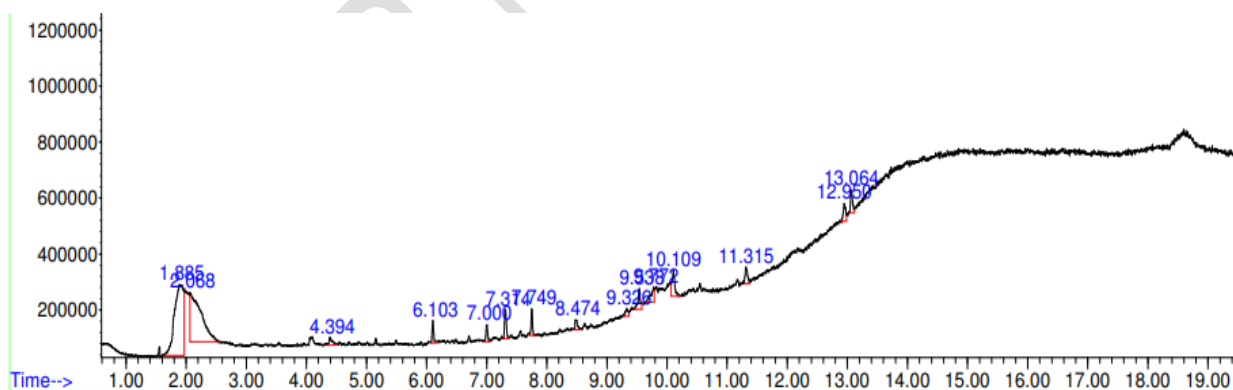
Phosphorus content in *J. tanjorensis* and *B.pinnatum* leaves were 1.47 % and 0.170 % respectively, indicating that more phosphorus is contained in the leaves of *J. tanjorensis* than in the leaves of *B. pinnatum*. Phosphorus holds significance as it contributes to the production of adenosine triphosphate (ATP) in the body. ATP plays a crucial role in trapping the chemical energy released from the metabolism of food molecules, which aids many cellular processes [29]. The current study confirms that *J. tanjorensis* and *B. pinnatum* leaves contain essential minerals for body functions.

### 3.3 Analysis of bioactive compounds present in *Jatropha tanjorensis*

Figure 3 illustrates the chromatogram derived from GC-MS spectrometry analysis of the methanol extract obtained from *J. tanjorensis* leaves. The corresponding data is presented in Table 3 and the structures of the metabolites are shown in Figure 4. The analysis disclosed the existence of fifteen bioactives as indicated by the number of peaks on the chromatograms; while the amount of compounds in the extract is proportional to the % peak heights. These include: benzyl 1-[(1-hydroxy-4-oxo-1-phenyltetrahydroquinolin-3-yl) carbamoyl] cyclohexane-1-carboxylate, **1a** (28.7 %); diphenylpyrrolopyridazine **3a** (48.0 %); 2-hexadecanol, **4a** (17.5%);  $\beta$ -acrenol, **5a** (19.5 %); propanoic acid, 2-(3-acetoxy-4,4,14-trimethylandro-8-en-17-yl, **9** (17.1 %); 7,8-epoxylanostan-11-ol, 3-acetoxy, **11** (18.6 %); and ginsenoside Rh 2, **13** (66.0 %). The highest peak, at 66.0%, was attributed to ginsenoside Rh2, while the lowest peak, at 6.11 %, was associated with 2-nonadecanone 2,4-dinitrophenylhydrazine. Bioactive constituents with peak area percentages below 5 % were considered inconsequential.

Benzyl 1-[(1-hydroxy-4-oxo-1-phenyltetrahydroquinolin-3-yl)carbamoyl]cyclohexane-1-carboxylate, (**1a and 4b**) or N-(1-Hydroxy-4-oxo-1-phenylperhydroquinolizin-3-yl)carbamic acid, benzyl ester is a potent HIV-1 protease inhibitor [31].

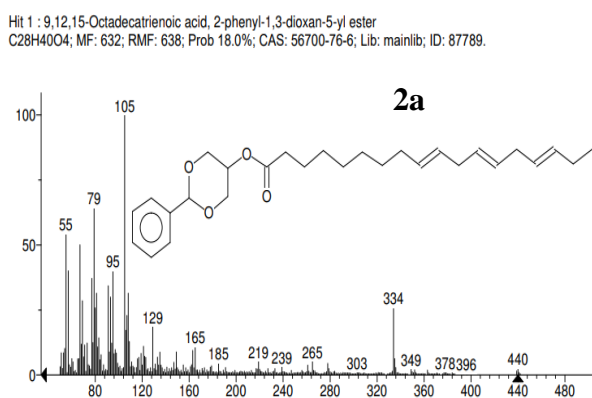
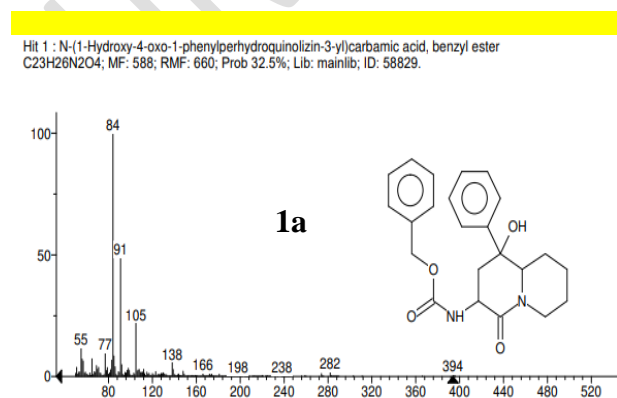
Sosa *et al.*, [32] reported propanoic acid, 2-(3-acetoxy-4,4,14-trimethylandrosta-8-en-17-yl), (**9**, and **4b**) a steroidal ester, as exhibiting anticancer and antimicrobial properties. Ginsenoside Rh2, (**13**) or olean-12-ene-3,15,16,21,22,28-hexol, (3 $\beta$ ,15 $\alpha$ ,16 $\alpha$ ,21 $\beta$ ,22 $\alpha$ )- is a key biologically active ginsenoside that has been reported to show anti-tumorigenic properties [33] while 9,12,15-octadecatrienoic acid, 2-phenyl-1,3-dioxan-5-yl ester, (**2a**) has antiobesity and antiviral activities [34]. Research has revealed that diphenylpyrrolopyridazines such as 2,7-Diphenyl-1,6-dioxopyridazino[4,5:2',3']pyrrolo[4',5'-d]pyridazine, (**3a**) demonstrated powerful antibacterial effects against both Gram +ve and Gram -ve bacterial species [35].  $\beta$ -Acorenol, (**5a**) has previously been reported as an antioxidant and antitumor agent against MCF-7 cells, human breast cancer cell [36]. Hassan Mohammad and his co-workers [37] reported the anti-inflammatory and antimicrobial properties of 7,8-Epoxyolanostan-11-ol, 3-acetoxy, (**11**). Diosgenin or spirost-8-en-11-one, 3-hydroxy-, (3 $\beta$ ,5 $\alpha$ ,14 $\beta$ ,20 $\beta$ ,22 $\beta$ ,25R)- (**15**), shown to possess anti-inflammatory and anticancer effects also improves the management of oxidative diseases such as diabetes[38].



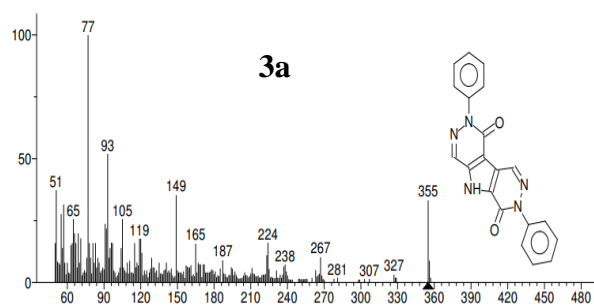
**Figure 3:** GC-MS chromatogram of *J. tanjorensis* leaf extract

**Table 3:** GC-MS examination of the bioactive compounds present in *J. tanjorensis*

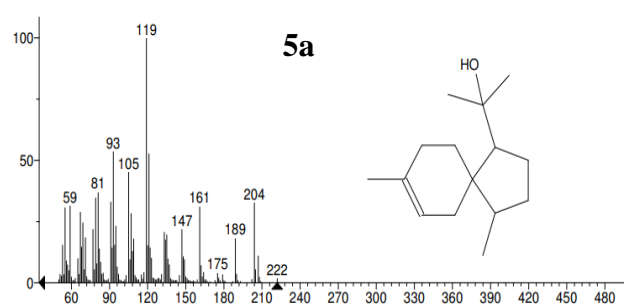
S/N	Retention Time	Name(s) of compounds	Chemical formula	Molar mass	% Peak height
1a.	1.885	Benzyl 1-[(1-hydroxy-4-oxo-1-phenyltetrahydroquinolin-3-yl)carbamoyl]cyclohexane-1-carboxylate	C <sub>23</sub> H <sub>26</sub> N <sub>2</sub> O <sub>4</sub>	394	28.7
2a.	2.068	9,12,15-Octadecatrienoic acid, 2-phenyl-1,3-dioxan-5-yl ester	C <sub>28</sub> H <sub>40</sub> O <sub>4</sub>	440	18.0
3a.	4.394	2,7-Diphenyl-1,6-dioxopyridazino[4,5:2',3']pyrrolo[4',5'-d]pyridazine	C <sub>20</sub> H <sub>13</sub> N <sub>5</sub> O <sub>2</sub>	355	48.0
4a.	6.103	2-Hexadecanol	C <sub>16</sub> H <sub>34</sub> O	242	17.5
5a.	7.000	β-Acorenol	C <sub>15</sub> H <sub>26</sub> O	222	19.5
6a.	7.314	7-Epi-cis-sesquisabinene hydrate	C <sub>15</sub> H <sub>26</sub> O	222	8.26
7.	7.749	Octadecanal,2-bromo	C <sub>18</sub> H <sub>35</sub> BrO	347	17.9
8.	8.474	Stearic acid, 3-(octadecyloxy)propyl ester	C <sub>39</sub> H <sub>78</sub> O <sub>3</sub>	584	10.8
9.	9.326	Propanoic acid, 2-(3-acetoxy-4,4,14-trimethylandro-8-en-17-yl	C <sub>27</sub> H <sub>42</sub> O <sub>4</sub>	430	17.1
10.	9.538	2-Nonadecanone dinitrophenylhydrazine	2,4- C <sub>25</sub> H <sub>42</sub> N <sub>4</sub> O <sub>4</sub>	462	6.11
11.	9.772	7,8-Epoxyanostan-11-ol, 3-acetoxy	C <sub>32</sub> H <sub>54</sub> O <sub>4</sub>	502	18.6
12.	10.109	Cycloheptanehexol	C <sub>30</sub> H <sub>44</sub> O <sub>11</sub>	580	11.9
13.	11.315	Ginsenoside Rh2	C <sub>30</sub> H <sub>50</sub> O <sub>6</sub>	506	66.0
14.	12.950	Cyclobutane, 1,3-bis[2-(2-isopropyl-3,3-dimethyloxiran-2-yl)ethenyl]-2,4-diacetyl	C <sub>26</sub> H <sub>40</sub> O <sub>4</sub>	416	8.95
15.	13.064	Diosgenin	C <sub>27</sub> H <sub>40</sub> O <sub>4</sub>	404	48.3



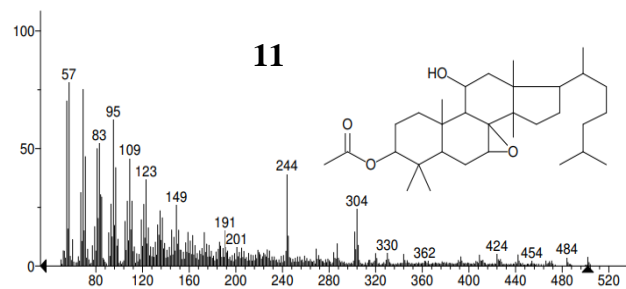
Hit 1 : 2,7-Diphenyl-1,6-dioxopyridazino[4,5:2',3']pyrrolo[4',5'-d]pyridazine  
C20H13N5O2; MF: 611; RMF: 667; Prob 48.0%; CAS: 91757-06-1; Lib: mainlib; ID: 51189.



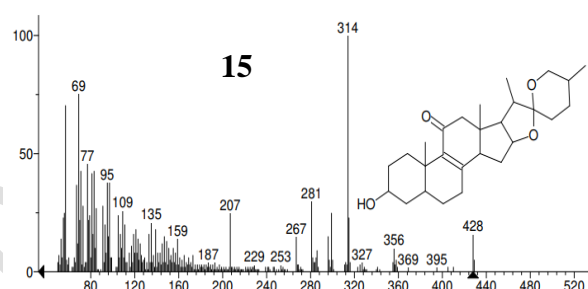
Hit 1 :  $\beta$ -Acorenol  
C15H26O; MF: 623; RMF: 779; Prob 19.5%; CAS: 28400-11-5; Lib: mainlib; ID: 109011.



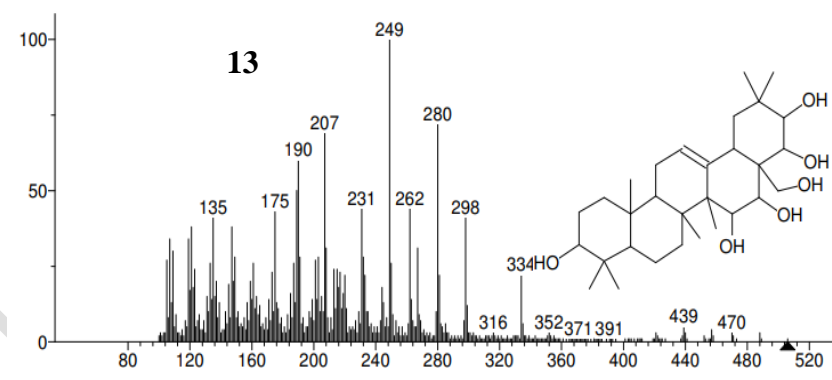
Hit 1 : 7,8-Epoxyolanostan-11-ol, 3-acetoxy-  
C32H54O4; MF: 704; RMF: 708; Prob 18.6%; Lib: mainlib; ID: 16568.



Hit 1 : Spirost-8-en-11-one, 3-hydroxy-, (3 $\beta$ ,5 $\alpha$ ,14 $\beta$ ,20 $\beta$ ,22 $\beta$ ,25R)-  
C27H40O4; MF: 686; RMF: 746; Prob 25.9%; CAS: 58072-54-1; Lib: mainlib; ID: 250995.



Hit 1 : Olean-12-ene-3,15,16,21,22,28-hexol, (3 $\beta$ ,15 $\alpha$ ,16 $\alpha$ ,21 $\beta$ ,22 $\alpha$ )-  
C30H50O6; MF: 666; RMF: 683; Prob 20.2%; CAS: 15399-43-6; Lib: mainlib; ID: 230349.



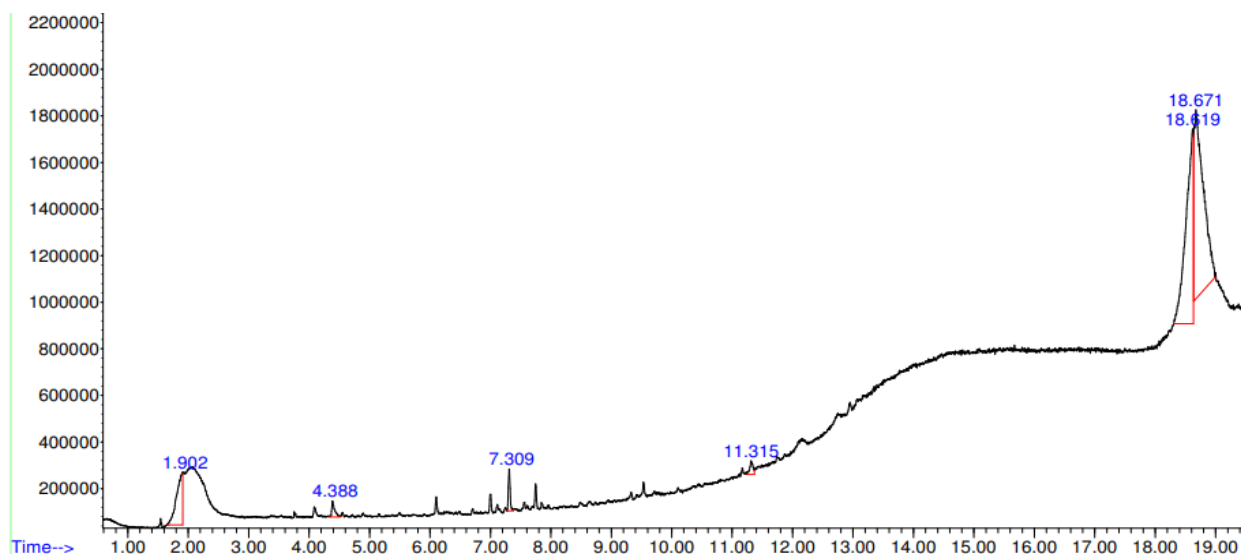
**Figure 4:** Chemical structures of the major bioactive compounds present in *J. tanjorensis*

Benzyl 1-[(1-hydroxy-4-oxo-1-phenyltetrahydroquinolin-3-yl) carbamoyl] cyclohexane-1-carboxylate, (**1a**); 9,12,15-octadecatrienoic acid, 2-phenyl-1,3-dioxan-5-yl ester, (**2a**); 2,7-Diphenyl-1,6-dioxopyridazino[4,5:2',3']pyrrolo[4',5'-d]pyridazine (**3a**);  $\beta$ -Acorenol (**5a**); 7,8-Epoxyolanostan-11-ol, 3-acetoxy (**11**); Ginsenoside Rh2(**13**); Diosgenin (**15**).

### 3.4 Analysis of bioactive compounds present in *Bryophyllum pinnatum*

Methanolic extract of *B. pinnatum* leaves showed six bioactive compounds, as shown in Figures 5 and 6. The corresponding data has been organized in Table 4. These compounds include: benzyl 1-[(1-hydroxy-4-oxo-1-phenyltetrahydroquinolin-3-yl)carbamoyl]cyclohexane-1-carboxylate, **1b**; aurin, **2b**; propanoic acid, 2-(3-acetoxy-4,4,14-trimethylandrosta-8-en-17-yl), **4b** (45.1 %); cycloheptanehexol, **6b**, 10.4 %. Maximum peak (32.5 %) was shown by benzyl 1-[(1-hydroxy-4-oxo-1-phenyltetrahydroquinolin-3-yl)carbamoyl]cyclohexane-1-carboxylate and minimum peak (9.85 %) was shown by humulene, **3b**.

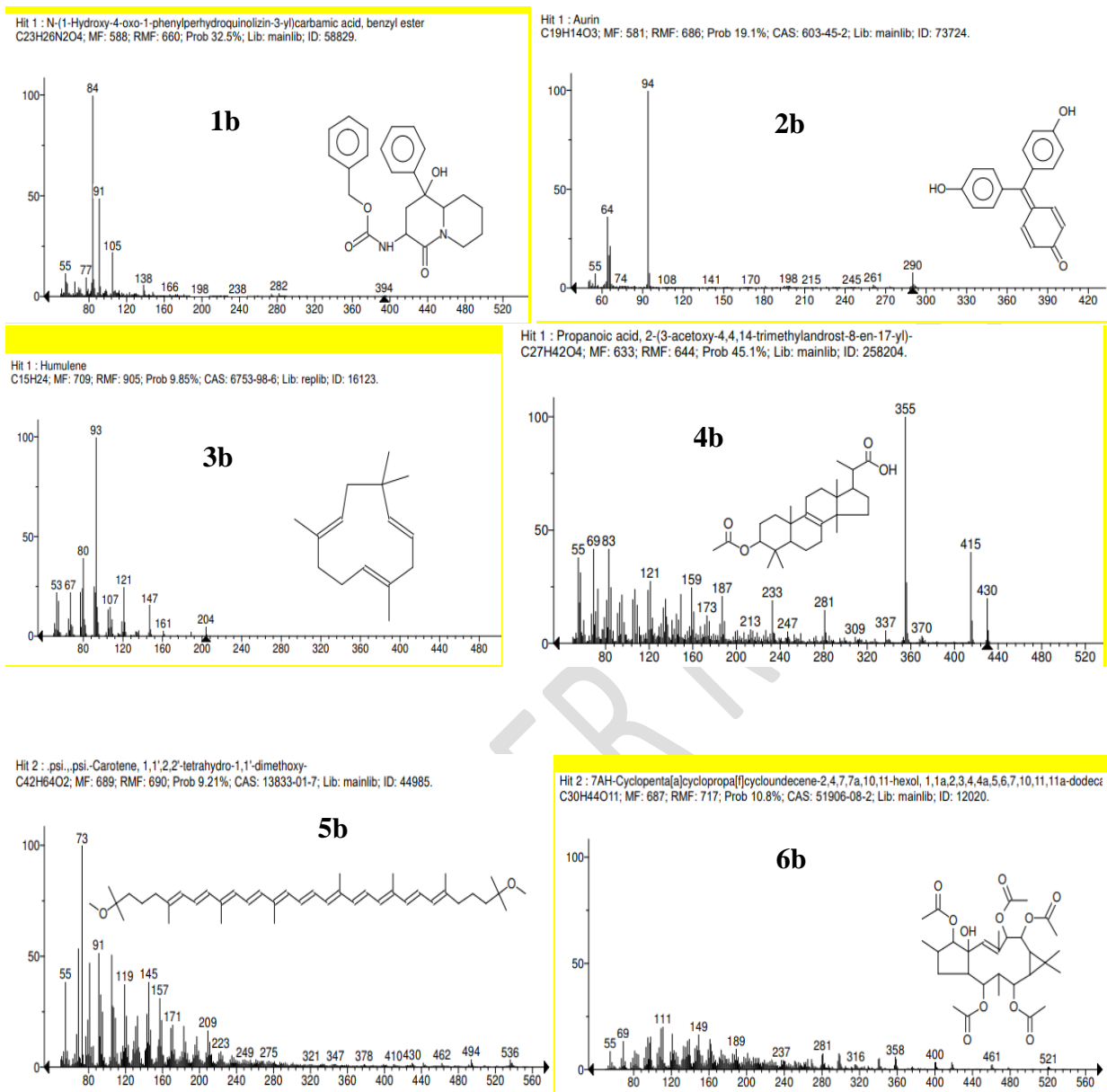
Aurin (**2b**) derivatives such as aurine tricarboxylic acid that impairs exopolysaccharides protein-eDNA has been demonstrated to effectively eradicate biofilms generated by *Pseudomonas aeruginosa* [39]. Humulene (**3b**) is a cyclic sesquiterpene that asserts both sedative and analgesic effects. It is also known to exhibit strong antibacterial and anti-inflammatory properties [40]. Tetrahydrospirilloxanthin (**5b**) also known as psi.psi.-carotene, 1,1',2,2'-tetrahydro-1,1'-dimethoxy- is a carotenoid ether which possesses antiasthmatic, antioxidant and anti-cancer, properties. It is also utilized for the prevention of atherosclerosis [41]. Cycloheptanehexol, (**6b and 12**) or 7AH-cyclopenta[a]cyclopropa[f]cycloundecene-2,4,7,7a,10,11-hexol, 1,1a,2,3,4,4a,5,6,7,10,11,11a-dodecane is a sesquiterpene lactone that exhibits a wide-ranging biological activities such as anti-inflammatory, phytotoxic, antimicrobial and anticancer [42].



**Figure 5:** GC-MS chromatogram of *B. pinnatum* leaf extract

**Table 4:** Bioactive components in *B. pinnatum* leaves

S/N	RT	Name(s) of compounds	Chemical formula	Molecular mass	% Peak height
1b.	1.902	Benzyl1-[(1-hydroxy-4-oxo-1-phenyltetrahydroquinolin-3-yl)carbamoyl]cyclohexane-1-carboxylate	C <sub>23</sub> H <sub>26</sub> N <sub>2</sub> O <sub>4</sub>	394	32.5
2b.	4.388	Aurin	C <sub>19</sub> H <sub>14</sub> O <sub>3</sub>	290	19.1
3b.	7.309	Humulene	C <sub>15</sub> H <sub>24</sub>	204	9.85
4b.	11.315	Propanoic acid, 2-(3-acetoxy-4,4,14-trimethylandrosta-8-en-17-yl	C <sub>27</sub> H <sub>42</sub> O <sub>4</sub>	430	45.1
5b.	18.619	Tetrahydrospirilloxanthin	C <sub>42</sub> H <sub>64</sub> O <sub>2</sub>	600	13.7
6b.	18.671	Cycloheptanehexol	C <sub>30</sub> H <sub>44</sub> O <sub>11</sub>	580	10.4



**Figure 6:** Chemical structures of the major bioactive compounds present in *B. pinnatum*

Benzyl 1-[(1-hydroxy-4-oxo-1-phenyltetrahydroquinolin-3-yl)carbamoyl]cyclohexane-1-carboxylate (**1b**); Aurin (**2b**); Humulene (**3b**); Propanoic acid, 2-(3-acetoxy-4,4,14-trimethylandroster-8-en-17-yl) (**4b**); Tetrahydrospirilloxanthin (**5b**); Cycloheptanehexol (**6b**)

Interestingly, the GC-MS analyses showed that both plants contain benzyl 1-[(1-hydroxy-4-oxo-1-phenyltetrahydroquinolin-3-yl)carbamoyl]cyclohexane-1-carboxylate, propanoic acid, 2-(3-

acetoxy-4,4,14-trimethylandro-8-en-17-yl and cycloheptanehexol at varying percentage concentrations.

#### **4. Conclusion**

The analyses obtained in this research have provided scientific information and an in-depth knowledge of the proximate, mineral content and secondary metabolites in *J. tanzorensis* and *B. pinnatum* leaves. Proximate values showed that both leaves have high protein, fat, fibre and carbohydrate contents. In terms of medicinal value, *J. tanzorensis* leaves stand out as the most promising option due to their notably elevated levels of proximate and elemental components. Benzyl-1-[(1-hydroxy-4-oxo-1-phenyltetrahydroquinolin-3-yl)carbamoyl]cyclohexane-1-carboxylate, ginsenoside Rh2 and cycloheptanehexol present in both plant samples have been noted to display anti-tumorigenic, bactericidal, hypoglycemic and anti-inflammatory properties. Thus, bioactive compounds in *Jatropha tanzorensis* and *Bryophyllum pinnatum* leaves affirm their use in ethno-medicine.

#### **Disclaimer (Artificial intelligence)**

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

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