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BANANA (*MUSA SAPIENTUM)* PSEUDO-STEM SAP AND ITS PHYTOCHEMICALS, PHYSIO-CHEMICALS AND POLY-AROMATIC HYDROCARBON(PAHs) CONTENTS.

BY

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ABSTRACT

Investigations were carried out on the resin from banana (*Musa-sapientum)* pseudo-stem sap to identify its chemical compositions and their possible industrial applications. The proximate analysis carried on the bio-resin gave the pH value of 6.17, high conductivity of value of 9140, salinity of 5.10, etc. Colorimetric platinum cobalt method was used to determine the apparent color with H183300 Hanna bench photometer. The percentage yield of poly-aromatic hydrocarbons(PAH) were fluorene (12.47 %), flouranthene (16.53 %), phenanthrene (14.51 %), anthracene (9.801 %), xylene (21.89%), etc. The analysis for phytochemical compounds were performed using BUCK M910 Gas chromatography equipped with a flame ionization detector. Proanthocynin, naringin, quinine, flavan-3-ol, anthocyanin, ribalinidine, naringenin, spartein, sapogenin, phenol, steroids, flavones, epicatechin, kaempferol, phytate, flavones, oxalate, catechin, resveratol and tannin were identified as the phytochemicals from the pseudo-stem resin. Most of the compounds from the banana pseudo-stem sap had been evaluated as vital source of raw materials/chemicals for most pharmaceutical, food, and other industrial applications.

Keywords: Banana, Pseudo-stem, Poly-aromatic, Phytochemicals, Bio-resin

1.1 INTRODUCTION

Banana plant is normally tall and fairly sturdy and is often mistaken for a tree. However, the trunk of a banana plant is actually a false stem or pseudo-stem, which is about 5 to 7.6 meters tall, and varies from species to species (Nelson et al., 2006). The pseudo-stem consists of a tender core and several outer sheaths carries the immature inflorescence until it emerges at the top. Most of the nutrients of the banana pseudo-stem are present in the tender core. Each banana produces a single bunch once before dying and then is replaced by new pseudo-stem (Anhwange et al., 2009). Since each plant produces only one bunch of bananas and cannot be used for the next harvest, this agricultural activity generates a large amount of waste, (Cordeiro et al., 2004). It has been reported that banana is the second largest produced fruit in terms of quantity, contributing about 16 % of the world's total fruit production (Mohapatra et al., 2010). Therefore, every year after harvesting, a large amount of bare pseudo-stem is cut and left behind as waste worldwide, which ultimately causes contamination of water sources as well as affecting the environment and health of living microorganisms (Aziz et al., 2011, Hossain et al., 2011).

However, utilization of the banana waste—pseudo-stems has gained more attention in recent years. It has been reported that banana pseudo-stem could be used in animal feeds formulation, clothing, paper making, furniture making, etc. Buragohain et al., (2010) claimed that banana pseudo-stem could be used as an important stable food for pigs, in banana producing areas. Starch extracted from banana pseudo-stem could also be used to produce glue, which is applicable in the manufacture of cardboard papers, wood work, etc. Banana pseudo-stem fibers had been studied as a good reinforcement material, which is biodegradable, eco-friendly, sustainable and cheap. It could also be used for epoxy resin formulation according to Tuan and Nguyen,(2021); Umaz et al., (2005). Banana sap resin was prepared and reacted with styrene as an initiator, cured at room temperature and compared to the petroleum resin according to Vim and Kanny (2013).

Fig.1:- Banana pseudo-stem Fig. 2: Inner structure of banana pseudo-stem

The inner structure of banana pseudo-stem is displayed in Figures 1 and 2 above.

Moreover, it has been reported that these banana waste materials are rich in minerals and nutrients, especially dietary fiber (Aziz et al., 2011). However, little is known on the composition of the pseudo-stem; its drying properties; effects of drying on composition; quality of dried product or its utilization in food manufacture and the characterization of fibre components. The banana pseudo-stem could potentially be used more in food rather than in other industrial applications. Furthermore, the replacement of commercial wheat flour with 10% of banana pseudo-stem flour in the preparation of bread, revealed that the composite bread had greater content of insoluble fiber, total dietary fiber, total phenolics and antioxidant properties than the control bread (Ho et al. 2013). The exploitation of waste banana pseudo-stems into food products could significantly benefit the environment and increase its economic value. Our focused is based on the efficient utilization of banana pseudo-stem sap, its compositions and possibility of transformation to bio-resin.

3.0 MATERIALS AND METHOD

3.1 Sample collection and preparations

The banana plant was felled, the inner part isolated using a trowel, squeezed to get liquid sap, filtered, and the filtrate stored in an air tight container for further analysis.

3.2 Acidity/ Basicity Determination

50 ml of the sap was measured into a beaker, the electrode of the pH meter (OHAUS STARTER 2100) inserted in and the record noted, when the readings stabilized.

3.3 Conductivity, Salinity and Total Dissolved Solids (TDS)

50 ml of the sap was measured into a dry beaker, and mode of the machine (OHAUS STARTER 310 ^{0}C) selected. The conductivity, salinity or TDS, parameters were determined accordingly.

3.4 Color (colorimetric platinum cobalt method)

The color (apparent) was determined using H183300 Hanna multi-parameter bench photometer. The machine was switched on and method for color of sample was selected. 10 ml cuvette was filled with distilled water and used as blank to zero the machine, then 10 ml of the banana sap was poured into another cuvette. The read button was pressed and the value of the apparent colour was noted in pcu (platinum cobalt unit).

3.5 Sulfate

Sulfate was determined using cadmium reduction method using H183300 Hanna multi parameter bench photometer. 10 ml of the sample was poured into two cuvettes each , one was used as blank to zero the machine, to the other was poured one sachet of sulfate reagent powder, shake for 90 seconds and inserted into cell compartment and the timer button selected, after the time, the value was noted as displayed in milligram per liter.

3.6 Chloride

20 ml of the sample was transferred into a clean 250 ml conical flask.. 1 ml of Potassium Chromate indicator was added to get a light yellow color. The burette was rinsed with silver nitrate solution of 0.0282 N. The sample was titrated against silver nitrate solution until the color changes from yellow to brick red. The volume of Silver nitrate noted as (A). The reaction was repeat to get a concordant values.

3.6.1 Blank Titration

20 ml of distilled water was added to a clean 250 ml conical flask, 1 ml of Potassium Chromate indicator was added to get light yellow color.

The sample(sap) was titrated against silver nitrate solution until the color changes from yellow to brick red.

The volume of silver nitrate in water added is represented as (B)

Where Va = Volume of Silver Nitrate of the sample (ml)

3.7 Preparation of Samples for Gas Chromatography (GC).

3.7.1 Soxhlet Extraction Method

20 g of homogenized banana sap sample, was mixed with 60 g of anhydrous sodium sulphate in a mortar to agetate. The homogenate was placed in a 500 ml beaker to soak for 24 hrs, 300 ml of n-hexane was added to extract. Crude extract obtained was evaporated using a rotary vacuum evaporator at 40 ^{0}C, to dryness. The residue was transferred with n – hexane into a 5 ml florisil column for clean up.

3.7.2 Florisil Clean Up

Florisil is heated in the oven at 130 ^{0}C for 15 hrs, transferred to a 250 ml size beaker and placed in a desiccator. 0.5 g anhydrous Na_2SO_4 was added to 1.0 g of activated flosiril (Magnesium Silicate) at 60 – 100 nm mesh, in a 8 ml column blocked with glass wool. Packed column was filled with 5 ml of n – hexane for conditioning. Open stopcock was used to allow n– hexane run out until, it reaches the top of Sodium Sulphate into a receiving vessel, while tapping the top of the column gently, till the florisil settles well in the column. The extract was transferred into the column with disposable Pasteur pipette from the evaporating flask, rinsed twice with 1 ml portions of n – hexane and add to the column.

The elute collected from the evaporating flask of the rotary evaporator was heated to dryness, dissolved in 1 ml n–hexane and kept ready for PAH Chromatographic analysis.

3.7.3 Gas Chromatography Process

The gas flow was adjusted towards the columns, the injectors, the detectors, and the split ratio. The detectors were generally held at the end of the oven with higher temperature range to minimize the risk of analyte precipitation. Buck 530 gas chromatograph equipped with an "on–column", automatic injector, mass spectroscopy, HP 88 capillary column (100 m x 0.25 μm film thickness,) was used. The detector temperature was 250 ^{0}C, injector temperature was 22 ^{0}C, while, integrator chart speed was 2 cm per min. The oven was set at 180 ^{0}C to warm and activate the gas chromatography, while warming, the operational temperature were set as shown in the table below:

Table 1.0 : Operational Temperature ranges

Initial Temp. (^{0}C)	Hold(min)	Ramp (min)	Final Temp (^{0}C)
70	5 min	10 min	220
220	2 min	5 min	280

When the instrument is ready, the ''NOT READY'' light turned off, and the analysis was carried out, by injecting 1 microliter of the sample into the column A using proper injection technique according to AOAC, 2009 Standard.

3.7.4 Extraction of Phytochemicals

1 g of sample was weighed and transferred in a test tube and 15 ml of ethanol was added. The test tube was placed in a water bath at 60 ^{0}C for 60 mins. The product was transferred to a separation funnel, washed with 20 ml of ethanol, 10 ml of cold water, 10 ml of hot water and finally 3 ml of hexane, all transferred to the funnel. This extracts were combined and washed three times with 10 ml of 10 % v/v ethanol aqueous solution. The solution was dried with anhydrous sodium sulfate and the solvent allowed to evaporate. The sample was solubilized in 1000 µl of hexane of which 200 µl was transferred for further qualitative analysis.

3.7.5 Quantification by GC-FID

The analysis of phytochemicals were performed on a BUCK M910 Gas chromatography equipped with a flame ionization detector. A RESTEK 15 meter MXT-1 column (15 m x 250 µm x 0.15 µm) was used. The injector temperature was 280 ^{o}C with split-less injection of 2 ul of sample and at a linear velocity of 30 cms^{-1}, Helium 5.0 pa.s was the carrier gas with a flow rate of 40 ml min^{-1}. The oven was initially operated at 200 ^{0}C, heated to 330 ^{0}C at the rate of 3 ^{0}C min^{-1} and was kept at this temperature for 5 min, while, the detector was operated at 320 ^{0}C.

The phytochemicals were determined by the ratio between the area, mass of internal standard and the area of the identified phytochemicals. The concentration of the different phytochemicals was express in µg/g, according to, Kelly and Nelson (2014).

4.0 RESULTS AND DISCUSSIONS

4.1 Results on proximate analysis of banana pseudo-stem sap

Table 4.1 displays the result of proximate values of the resin from banana pseudo-stem. It was observed that the banana resin is coloured and has light absorbency at the wavelength of 728 pcu(platinum cobalt unit). This shows that natural dyes could be isolated from the banana resin. Researches has shown that natural dyes from plants, may have other components or properties and could be useful in cultural, ritual, craft, fabrics, or weather control. Shyam, et al(2015) had suggested however that, dye yielding plants have not received significant attention and suggest more researches in this area. The sap has a pH of 6.17 which indicates that it is slightly acidic. Its conductivity is high at 9140 µs/cm, which shows the presence of some metallic ions. This confirms that the sap has some components that are good electric conductors, which could be essential or heavy metals. Our findings could be related to that of Zhang (1993), who observed that, most liquid foods contain more ions, that can conduct electricity as well as heat. Our results also conform with that of Rulman, et al (2000), who reported that total dissolved solid at 4570 mg/lt., is high, indicating the presence of other materials (solid).

The salinity value of the resin is 5.10, showing high concentration of salt. The concentrations of sulphate and chloride in milligram per liter as shown in Table 4.1 are 23.00 mg/lt. and 1584.51 mg/lt. respectively. The presence of these inorganic minerals show that banana pseudo-stem resin could be a good raw material for some industrial formulations. Some researchers had incorporated banana pseudo-stem sap into formulation of organic manure to improve strawberry yields, Binaya, et al,(2021). They observed an increased performance in the growth, development and yield of the strawberry. They attributed this to the presence of nitrate, chloride or sulphate compounds in the pseudo-stem-sap, which, chemically combined to improve the manure, which, consequently contributed to enriching the soil manure, resulting into an improved crop yield. However, they suggested that, some of the components identified could be used in diets or nutrition.

Table 4.1: Results on the Proximate Analysis of Banana Sap

Parameter	Value
pH	6.17
Conductivity (μs/cm)	9140
Total dissolved solids (mg/l)	4570
Salinity (psu)	5.10
Colour (pcu)	728
Sulphate (mg/l)	23.00
Chloride (mg/l)	1584.51

4.2 Results of Poly-aromatic Hydrocarbons(PAHs)

Polycyclic aromatic hydrocarbons (PAHs) which are commonly hydrocarbons consisting of more than one benzene rings were identified as stated on Table 4.2. The percentage composition of xylene was 21.89 %. The presence of xylene (C_8H_{10}), as one of the poly-aromatic hydrocarbon shows that, the banana sap could be used in medicinal, pharmaceutical or food industries and as an organic solvent too. Reena, et al(2010), observed that xylene is a good solvent in printing, rubber, paint, and leather industries. Our findings also suggested that xylene as a flammable solvent could be isolated from banana pseudo-stem as a raw material for airplane fuel, gasoline, etc.

Benzofluranthene($C_{20}H_{12}$) content is 16.83 %, and is a compound with four benzene rings around five ringed carbon atom. Studies had shown that high concetration of Benzofluranthene compound could be toxic when inhaled, and could exhibit carcinogenic effect in different bioassays in experimental animals, Garcia-Suastequi, et al(2011). Benzo(ghi)perylene($C_{22}H_{12}$) is one of the polycyclic aromatic hydrocarbons, that is 13.168 % in the banana pseudo-stem sap, as shown in Table 4.2. It can also occur in crude oil, coal tar, incomplete combustion of tobacco, edible oil, automobile exhaust, etc , according to, EFSA(2008). They estimated the maximum daily dietary intake of ($C_{22}H_{12}$) to be approximately 6-8 µg for person weighing 70 kg, or one faces the risk of long term adverse health effect. We also determined the percentage composition of other PAHs as shown in Table 4.2; Fluoranthene (16.35 %); phenanthrene(14.51 %); fluorine (12.471 %), and Anthracene (9.801 %). Garcia-Suastequi, et al(2011) reported that, long term exposure to PAHs had been suspected to be the cause of cell damage, or gene mutation and cardiopulmonary mortality. Jorn A., et al (2019), concluded that no health benefits had been reported of any PAHs and therefore calls for more investigations. The results the gas chromatographic analysis of the pseudo stem sap is shown in Figure 3.

Table 4.2: Showing the Percentage Composition of Poly-aromatic hydrocarbons (PAHs)

Poly-aromatic hydrocarbon	**Percentage composition (%)**
Fluorene	12.47
Flouranthene	16.35
Phenanthrene	14.51
Anthracene	9.80
Benzo(k)fluranthene	16.83
Benzofluranthene	25.033
Xylene	21.89
Benzo(g-h-i)pexylene	13.17

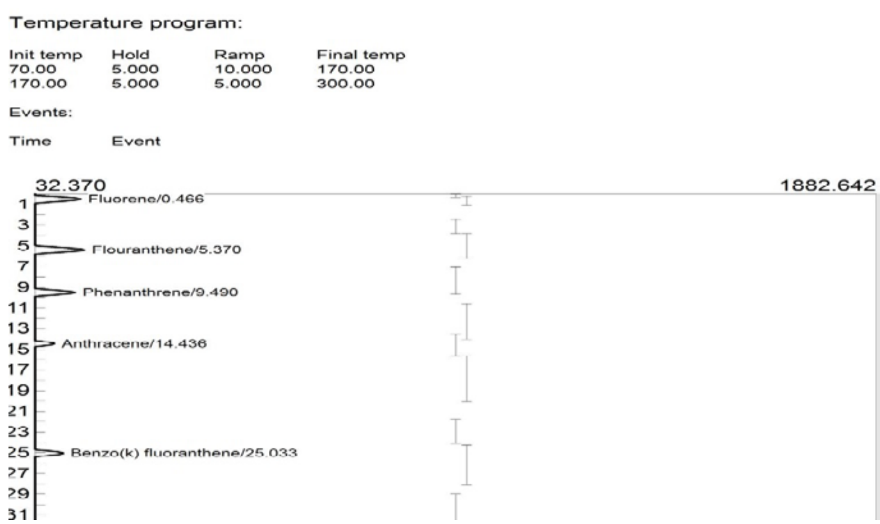

Fig.3: Showing results of Gas Chromatographic (GC) analysis of the Polycyclic Aromatic Hydrocarbons, PAHs.

4.3: Results of Phytochemical Compositions of the Pseudo-stem Resin.

Table 4.3 displays different phytochemicals in the banana pseudo-stem resin. We observed that Ribalindine is one of the highest value with 10 % content. Polyphenol compounds, such as Proanthocyanin(1.389 %) was also identified as shown in Table 4.3. This phytochemical had been reported to be one of the components of red wine that influences its aroma, mouth feel and reduces heart disease, Vivas, et al (2006). Naringin, which is also a flavonoid glycoside is also identified from the banana sap. Researches had shown that, they are the source of bitter tastes in most fruits and could be neutralized by hydrolyzing with an enzyme known as naringinase.

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identified includes, quinine, phenol, catechin, flavonones etc. These are good raw materials in drug productions, cosmetics, adhesive, or bio-resins and life support products.

Barhanpurkar, et al (2015) extracted dyes used as mordants for dying fabrics from banana pseudo-stem resin. They achieved this by forming a complex with the dye extract. They concluded that Tannin which is about 5.611 % of the pseudo stem sap is responsible for the coloring in the fabrics matrix. Banana pseudostem sap was also used in making thermally stable cellulosic substrate, according to Basak, et al,(2016).They treated some cotton fabrics with the sap to lower flammability property. Banana pseudo-stem fibres had been extracted and used as bio-composites in blending of plastics, for automobile parts with remarkable tensile strength elongation at break properties (Pothan and Thomas, 2003). The pseudo-sap consist of quality dye for silk fabrics, (Ammayappan et al, 2004). There is presence of polyphenols, such as tannin,

Table 4.3: Percentage composition of phytochemicals in the Sap

Phytochemical	Percentage composition (%)
Proanthocyanin	1.389
Naringin	6.380
Quinine	3.321
Flan-3-ol	9.677
Anthocyanin	4.388
Ribalinidine	10.361
Naringenin	3.252
Spartein	2.576
Sapogenin	5.875
Phenol	6.588
Flavonones	4.994
Steroids	5.303
Epicatechin	6.017
Kaempferol	2.824
Phytate	7.595
Flavone	3.094

Oxalate	3.557
Catechin	5.341
Resveratol	1.856
Tannin	5.611

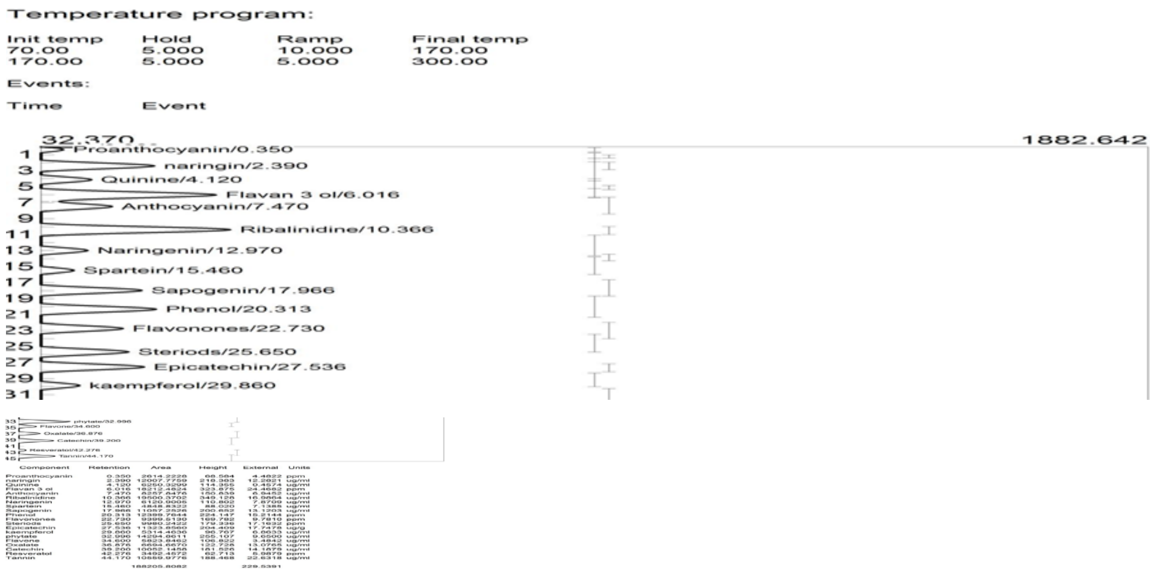

Fig.4: Showing results of Gas Chromatography (GC) analysis of the Percentage composition of the phytochemicals in the banana pseudo-stem resin.

Conclusion

This work shows the presence of sulphate, chloride, high salt concentration, and is colored. It shows the presence and levels of some phytochemicals like proanthocynin, oxalate, catechin, resveratol and tannin in banana resin from Chukwuemeka Odumegwu Ojukwu University, Uli campus..

2.6 Sap Phytochemical Composition Of Some Banana

In Thailand Banana sap has some special properties relating to various phenomena such as browning of fruits after harvesting, permanent staining of cloth and fibers, and antioxidant and antibleeding properties.

Analysis of banana sap using high-performance liquid chromatography-electrospray ionization mass spectrometry (HPLC-ESI-MS) indicated the presence of phenolic and aromatic amino compounds of interest due to their special properties. With the online positive electrospray ionization mode (ESI), the possible structures of specific compounds were determined from the fragmentation patterns of each particular ion appearing in the mass spectra. Moreover, the identities of these phytochemical compositions may be used as markers for banana diet, the assessment of physiochemical status, or the classification of banana clones.

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