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# Evaluation of Pharmacognostic, Nutraceutic and Phytotherapeutic Constituents of Unripe *Musa sapientum* Hydromethanolic Extracts

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## ABSTRACT

Despite the recent surge in demand for unripe banana products, there is a scarcity of literature regarding their potential health tendencies. This study investigated the various organic biomolecules, minerals and phytotherapeutic constituents in unripe *M. sapientum* pulp and peel extracts and their associated medicinal relevance. Hydromethanolic solvent (1:4 *v/v*) was used for extraction. Biochemical analysis of the various plant samples was done using standard laboratory protocols and separate detectors; GCMS and GCFID. Proximate and mineral analysis of unripe *M. sapientum* samples was compared to the corresponding ripe samples. The results showed the presence of functionally distinct predominant constituents in both studied unripe *M. sapientum* peel and pulp extracts, in which some of the constituents have several medicinal benefits. The study revealed the nutritional relevance of the unripe peel and pulp extract of *M. sapientum*.

**Keywords:** *Musa sapientum*, Banana, Nutraceutic, Pulp, Peel.

## INTRODUCTION

Medicinal plants are commonly used in folk medicine to manage a variety of diseases in different biogeographic regions, globally. This indigenous knowledge through oral and encrypted tradition, passed down from generation to generation, has contributed significantly to various innovations of different traditional orientations of medicine as well as initiated investigations of different plants to find the biomedical basis of their traditional uses [1]. This exploration of biologically active natural organic and inorganic constituents has played an essential role in revealing new chemical entities (NCEs) of interests [1, 2], knowledge indispensable to pharmacology, pharmacognosy, phytotherapeutics and applied sciences [3]. Banana, botanically called *Musa sapientum* [1, 4], is a familiar tropical fruit [5]. From its South-western Pacific nativity, the banana plant spread to India by 600 BC and later on, it spread all over the tropics [6]. It is possibly the world's most ancient cultivated crop [7]. It even spread into the areas of Islands of the Pacific and also to the West Coast of Africa as early as 200-300 BC [8]. *Musa sapientum* is a perennial tree-like herbaceous plant that grows 5 - 9 m in height, with tuberous rhizome, hard, long pseudostem and hard peel [8, 9]. *M. sapientum* is used, traditionally, in dysentery [2], diarrhoea [8] (unripe), intestinal trauma in ulcerative colitis, diabetes (unripe), in sprue, nephritis, uremia, gout and cardiovascular diseases [9, 10]. *M. sapientum* is also used in the treatment of menstrual defects [11]. Asians ingest *M. sapientum* because they believe it enhances neuromuscular performance. People do not usually consume the fresh unripe *M. sapientum*, which may be due to its characteristic hardness and high astringency, caused by the presence of soluble phenolic compounds as tannins [4, 5]. Therefore, studies have employed different unripe *M. sapientum* products like flour, and green banana biomass (GBB) [8]. Despite the growing worldwide demand for unripe *M. sapientum* products, there is no literature regarding the potential health benefits of unripe *M. sapientum* and its derivatives. This study determined the bio-active, phytotherapeutic and nutraceutical constituents in Unripe *M. Sapientum* fruit using different scientific protocols.

## MATERIALS AND METHODS

### Study protocol

This study followed the basic guidelines of the European Food Safety Authority (EFSA) [4]. Experiments that evaluate the health benefits of fruit consumption were included without any restriction.

### Plant, Chemicals and Reagents

Unripe banana fruit was obtained from a farm in Anambra state, Nigeria, in November, 2019. Some were left for some days to ripe. The unripe peels were separated from pulp and both parts washed with

filtered water and sodium chloride (NaCl). The peels and pulp were dipped in 0.5% citric acid to prevent enzymatic blackening. Both parts were shade dried for 96 hours. Dried peels and pulp were ground to make coarse powder and stored. All chemicals and Reagents used for this study are branded by Sigma-Aldrich.

**Plant authentication**

The plant samples were identified and authenticated in the Herbarium for Department of Plant Science and Biotechnology, University of Port Harcourt (UPH) with reference UPH/PSB/2020/014.

**Figure 1:** A *Musa sapientum* plant

**Taxonomical classification**

Kingdom: Plantae
Division: Magnoliophyta
Class: Liliopsida
Order: Zingiberales
Family: Musaceae
Genus: *Musa*
Species: *Musa sapientum*

**Preparation of unripe and ripe banana extract**

The unripe and ripe banana peel and pulp powder were extracted with hydromethanolic solvent (1:4 *v/v*). The extraction was carried out in a sealed water bath tub for 120 min at 250C. The extracts were centrifuged. The different extracts obtained were evaporated to dryness in a vacuum evaporator at 400 C. The final residue obtained was subjected to proximate, mineral, GC-FID and GC-MS analysis.

**Proximate analysis**

Proximate analysis was performed using standard analytic methods [5].

**Moisture content**

Using air-oven moisture content was measured following official protocols of Association of Official Analytical Chemists (AOAC). An M720 material test chamber (Binder GmbH, Tuttlingen, Germany) was used to dry the samples till constant weight. The percentage of moisture content was calculated as:

$$\% \text{ moisture} = (1 - \text{weight}_{\text{dry sample}} / \text{weight}_{\text{wet sample}}) \times 100$$

**Lipid content**

Determination of lipid content was performed following Soxtec method previously described by Noureddini and Byun, using a SoxtecTM 2050 automated analyzer (FOSS Analytical, Hillerød, Denmark). Petroleum ether was used for the extraction, whereas percentage of lipid was obtained following equation below:

$$\% \text{ lipid} = \text{Weight}_{(\text{extraction cup+residue})} - \text{weight}_{(\text{extraction cup})} / \text{weight}_{\text{sample}} \times 100$$

**Protein content**

Using an Auto Distillation Unit; Kjeltec® 2200 (FOSS Tecator, Höganäs, Sweden). A nitrogen-to-protein conversion factor, 4.4, was utilized for assay of protein present in the samples.

**Ash Content**

A dry ash method was used to assay the ash composition. The samples were incinerated in a Barnstead/Thermolyne Furnace 62700, Dubuque, IA, USA, at 550 °C. The residue of inorganic material was cooled, weighed and used further for assay of mineral constituents. An ash solution was well prepared by dissolving the ash in 100 mL of 1 M hydrochloric acid (HCl).

**Carbohydrate content**

The total content of carbohydrates (%) in the samples was calculated using difference method. The caloric value was calculated by sum of the various percentages of proteins and of carbohydrates which was multiplied by a factor of 4 (kcal/g) and total lipids multiplied by a factor of 9 (kcal/g).

**Mineral Analysis**

A NOVA 400 atomic absorption spectrometer (Analytik Jena AG, Jena, Germany) with an air/acetylene flame and respective hollow-cathode lamps was used for absorbance measurements. The results for mineral contents were expressed as mg/100 g DW.

**Gas Chromatography Mass Spectrophotometry (GCMS)**

Gas Chromatography-Mass Spectrometry GC-MS analysis was carried out on a GC 7890 (Agilent) comprising automatic liquid sampler and gas chromatograph interfaced to mass spectrophotometer (GC-MS). Helium was used as a carrier gas and the injector temperature was kept at $350^0$C. The oven temperature was programmed from $100^0$C held for 5 minutes to $375^0$C at $20^0$C/min. The name, molecular weight and structure of the component were ascertained.

**Gas Chromatography Coupled to flame ionization detector (GC-FID)**

The determination of phytochemicals present in samples was performed on BUCK M910 Gas chromatography coupled with a flame ionization detector (GC-FID). A RESTEK 15 meter MXT-1 column-type (15m x 250um x 0.15um) was used.

**Statistical analysis**

Microsoft Excel 2010 was used for basic statistical illustrations. Percentage difference was calculated using standard formular according to Chuemere, *et al*., 2019 [12].

## RESULTS

**Table 1:** Proximate analysis of unripe and ripe *M. sapientum* peel extract

| Parameters | Quantity (%) | | |
|---|---|---|---|
| | Unripe peel | Ripe peel | % difference |
| Moisture content | 23.63 | 47.53 | 67.17 |
| Ash content | 1.00 | 5.30 | 136.50 |
| Fat content | 5.00 | 1.70 | 98.50 |
| Crude fiber | 1.80 | 2.81 | 43.81 |
| Protein content | 2.15 | 4.59 | 72.40 |
| Carbohydrate content | 47.10 | 24.40 | 63.49 |

From table 1, the moisture (23.63%), ash (1.00%), crude fiber (1.80%) and protein content (2.15%) of unripe *M. sapientum* peel was less than that present in the ripe peel. The ash content had the highest percentage difference (136.50%) with the least percentage difference in crude fiber (43.81%).

**Table 2:** Proximate analysis of unripe and ripe *M. sapientum* pulp extract

| Parameters | Quantity (%) | | |
|---|---|---|---|
| | Unripe pulp | Ripe pulp | % difference |
| Moisture content | 29.20 | 56.38 | 63.51 |
| Ash content | 1.30 | 2.70 | 70.00 |
| Fat content | 5.50 | 1.60 | 109.85 |
| Crude fiber | 2.20 | 2.56 | 15.12 |
| Protein content | 4.62 | 2.41 | 62.87 |
| Carbohydrate content | 43.42 | 28.37 | 41.92 |

From table 2, the least contents in unripe pulp when compared to the ripe pulp of *M. sapientum* include Moisture (29.20%), ash (1.30%) and crude fiber (2.20%). the fat content had the highest percentage difference (109.85%), with the least percentage difference for crude fiber (15.12%).

**Table 3:** Mineral concentration of unripe and ripe *M. sapientum* peel extract

| Minerals | Concentration (mg/g) | | |
|---|---|---|---|
| | Unripe peel | Ripe peel | % difference |
| Fe | 88.40 | 72.00 | 20.44 |
| Ca | 186.46 | 156.40 | 17.53 |
| Mg | 36.20 | 37.65 | 3.92 |
| Mn | 4.42 | 3.75 | 16.40 |
| Zn | 3.12 | 2.90 | 7.30 |

From table 3; mineral analysis of unripe *M. sapientum* peel revealed the presence of iron (88.40 mg/g), calcium (186.46 mg/g), manganese (4.42 mg/g) and zinc (3.12 mg/g) higher than the concentration in ripe peel. The highest percentage difference was in iron (20.44%) and the least in magnesium (3.92%)

**Table 4:** Mineral concentration of unripe and ripe *M. sapientum* pulp extract

| Minerals | Concentration (mg/g) | | |
|---|---|---|---|
| | Unripe pulp | Ripe pulp | % difference |
| Fe | 28.67 | 12.10 | 81.28 |
| Ca | 130.33 | 138.40 | 6.00 |
| Mg | 28.20 | 28.25 | 0.17 |
| Mn | 2.00 | 0.60 | 107.69 |
| Zn | 12.35 | 4.48 | 93.52 |

From table 4, mineral screening of unripe *M. sapientum* pulp revealed iron (28.67 mg/g), manganese (2.00 mg/g) and zinc (12.35 mg/g) that is higher than the concentration in ripe pulp. The highest percentage difference was in manganese (107.69%) while the least was in magnesium (0.17%).

**Table 5:** GCMS analysis of unripe *M. sapientum* peel extract

| | Component ID | RT | Area % |
|---|---|---|---|
| **1** | trans-13-Octadecenoic acid | 16.614 | 63.97 |
| **2** | n-Hexadecanoic acid | 13.818 | 15.95 |
| **3** | 9,17-Octadecadienoic acid | 17.682 | 3.12 |
| **4** | 9-Octadecenoic acid, methyl ester | 15.425 | 2.74 |
| **5** | Erucic acid | 27.057 | 2.54 |
| **6** | 2-Heptadecanone | 12.280 | 1.36 |
| **7** | Cyclopropaneoctanal | 21.127 | 1.27 |
| **8** | Oleic acid | 30.256 | 1.06 |

Table 5 shows that trans-13-octadecenoic acid had a retention time of 16.614 and the largest area % of 63.97.

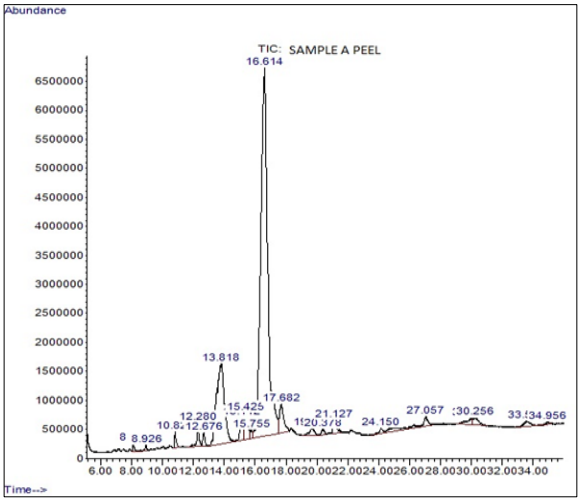


**Figure 2:** Spectrophotometric representation of unripe *M. sapientum* peel extract

**Table 6:** GCMS analysis of unripe *M. sapientum* pulp extract

| | Component ID | RT | Area % |
|---|---|---|---|
| **1** | 5-Hydroxy-4-methyl-3-heptanone | 8.361 | 7.35 |
| **2** | Oleic acid | 27.405 | 7.02 |
| **3** | Stigmasterol | 34.566 | 5.32 |
| **4** | n-Hexadecanoic acid | 13.931 | 4.90 |
| **5** | Dodecane | 10.588 | 4.83 |
| **6** | trans-13-Octadecenoic acid | 16.413 | 4.34 |
| **7** | Ethyl 2-acetamido-3,3,3-trifluoro-2 -(4-fluoranilino) propionate | 29.263 | 3.36 |
| **8** | Thymol | 30.685 | 2.52 |

Table 6 shows retention time 8.361 and 7.35 in area % for 5-Hydroxy-4-methyl-3-heptanone. Stigmaterol and thymol were present with retention time 34.566 and 30.685, respectively.

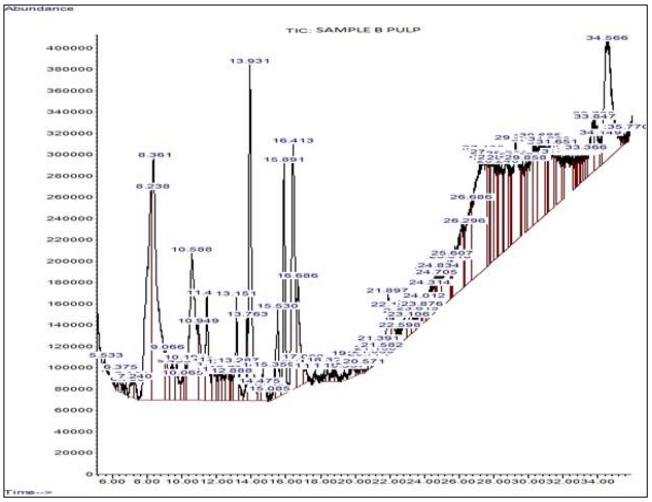


**Figure 3:** Spectrophotometric representation of unripe *M. sapientum* pulp extract

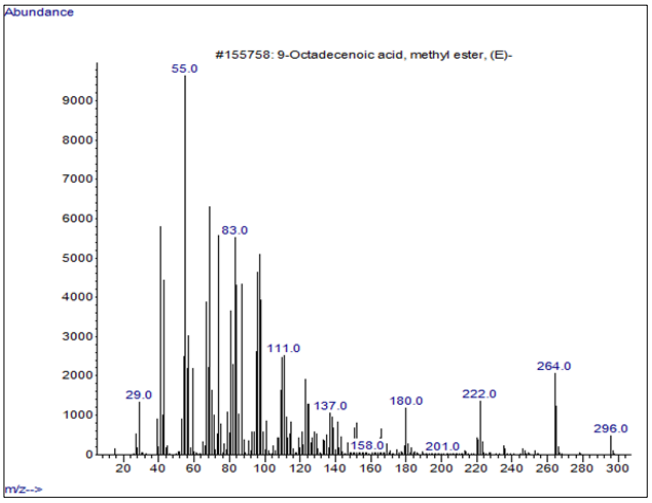


**Figure 4:** 9-octadecenoic acid methyl ester component detected

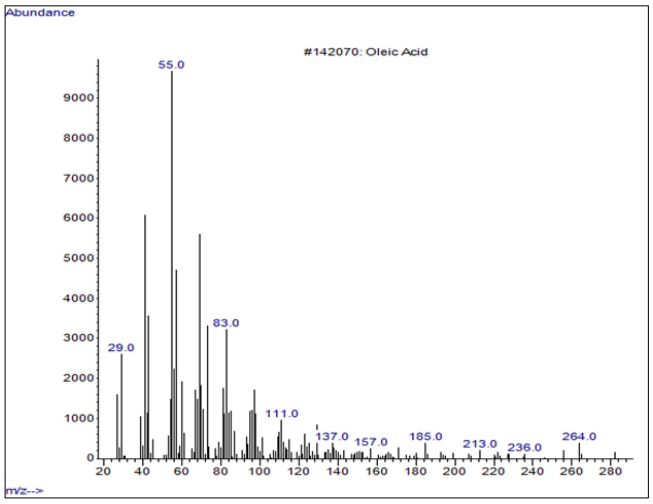


**Figure 5:** Oleic acid component detected

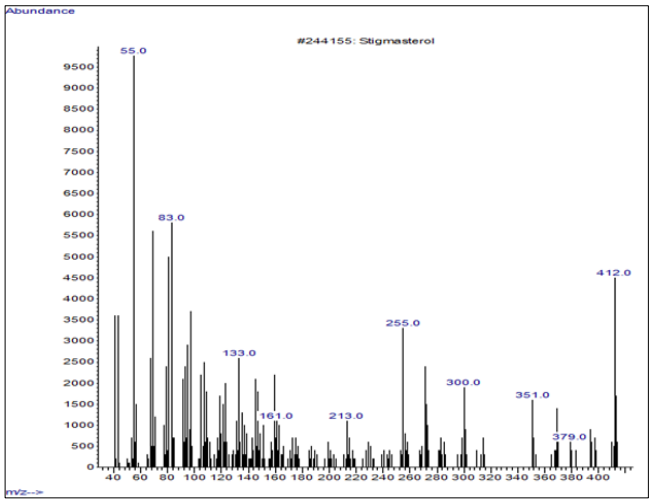


**Figure 6:** Stigmasterol component detected

**Table 7:** Compound nature and natural occurrence of some bioactive constituents in Unripe *M. sapientum* pulp and peel extracts

| Molecule | Compound nature | Natural occurrences |
|---|---|---|
| 9 – Octadecenoic acid, methyl ester (E)- | Fatty acid methylated ester | *Salvia officinalis* (garden sage) oil |
| Methyl stearate | Fatty acid methylated ester | Cloves, cassava, garri, raw beef. |
| Oleic acid | Monounsaturated Fatty acid | Cell membrane phospholipids, pecan oil, canola oil, peanut oil, sunflower oil, sesame oil, poppyseed oil, olive oil. |
| trans-13-Octadecenoic acid | Monounsaturated Fatty acid | *Psidium guajava* (Guava), *Terminalia catappa* (Tropical almond), *Anacardium occidentale* (Cashew). |
| Erucic acid | Monounsaturated Fatty acid | Rapeseed, wallflower seed, mustard oil. |
| Stigmasterol | Phytosterol | Soybean, rape seed, *Physostigma venenosum* (Ordeal bean) Chinese herbs *Ophiopogon japonicus*, |

**Table 8:** Phytochemical composition of unripe *M. sapientum* peel and pulp hydromethanolic extract.

| Parameters | Peel | Unit | Pulp | Unit |
|---|---|---|---|---|
| Proanthocyanidin | | | 3.8821 | ppm |
| Naringin | 15.6633 | Ug/ml | 8.5881 | Ug/ml |
| Anthocyanin | 8.7466 | Ug/ml | 10.9888 | Ug/ml |
| Naringenin | 8.7462 | Ug/ml | 4.7543 | Ug/ml |
| Spartein | 20.3999 | Ug/ml | 4.5151 | Ug/ml |
| Ribalinidine | 22.4271 | Ug/ml | 17.6738 | Ug/ml |
| Phytate | 0.9063 | Ug/ml | 2.3335 | Ug/ml |
| Phenol | 16.0589 | ppm | 15.8944 | ppm |
| Flavonones | 10.0212 | ppm | 4.9710 | ppm |
| Kaempferol | 10.0212 | Ug/ml | 2.1580 | Ug/ml |
| Epicatechin | 30.0678 | Ug/g | 14.9881 | Ug/g |
| Flavone | 7.7678 | Ug/ml | 6.3209 | Ug/ml |
| Rutin | 20.2313 | Ug/ml | 4.5461 | Ug/ml |
| Oxalate | 1.6266 | Ug/ml | 1.6079 | Ug/ml |
| Quinine | 4.9342 | Ug/ml | 4.6050 | Ug/ml |
| Resveratrol | | | 12.8283 | ppm |
| Catechin | 36.3548 | Ug/ml | 15.6715 | Ug/ml |
| Epihedrine | | | 8.5119 | Ug/ml |
| Tannins | 8.3093 | Ug/ml | 14.2127 | Ug/ml |
| Steroids | 26.0077 | ppm | 16.0894 | ppm |
| Isoflavones | 3.1891 | ppm | | |
| Sapogenin | 20.3999 | Ug/ml | | |
| Lunamarin | 6.5659 | Ug/ml | | |
| TOTAL | **272.0421** | | **175.2409** | |

## DISCUSSION

Fruits are indispensible components of a healthy diet for humans due to their composition of vitamins and minerals, fiber, and beneficial non-nutrient, phytochemical substances as bioactive compounds [13]. This study investigated the pharmacologic, nutraceutic and phytotherapeutic constituents of unripe *M. sapientum* hydromethanolic peel and pulp extracts. Proximate analysis compared the unripe samples to ripe samples of corresponding plant parts. Fats and carbohydrates are the only organic constituents present in greater quantities in the unripe peel of *M. sapientum* compared to its ripe peel. Fats, proteins and carbohydrates are more concentrated in unripe *M. sapientum* pulp compared to ripe pulp. This study revealed that iron (Fe), calcium (Ca), Manganese (Mn) and Zinc (Zn) are minerals more abundant in unripe peel compared to ripe peel of *M. sapientum*, while the unripe pulp has more iron (Fe), Manganese (Mn) and Zinc (Zn) than the ripe pulp. Calcium is an important component of intracellular biologic processes that occur within insulin receptive tissues like skeletal muscle and adipose tissue [14]. Alteration in calcium ion flux can have undesired effects on secretion of insulin which is a calcium ion-dependent process [14, 15]. Thus the considerable amounts of calcium in the peel of unripe *M. sapientum* as observed in this study, suggests the pharmacognostic importance of *M. sapientum* unripe peel to diabetics. There are only few literatures concerning the anti-diabetogenic effect of unripe *M. sapientum* peel, an effect that may need to be investigated. Furthermore, the study outcome also showed a significant loss of calcium as banana peel ripens. The relationship is inverse in *M. sapientum* pulp extract. The ripening of *M. sapientum* pulp is accompanied by a marked increase in calcium ion concentration. Magnesium is an ubiquitous cofactor of the glycolytic enzymes; hexokinase and pyruvate kinase and it also modulates glucose transport across cell membranes [14, 15]. Iron is an essential constituent or precursor for hemoglobin synthesis and it is critical to the proper function of the human immune system which includes immune response and immune tolerance and also the production of energy currencies [15]. Zinc plays a key role in the biophysical regulation of anti-diabetogenic hormone production by pancreatic tissues and glucose utilization by myocytes and adipocytes [15, 16]. GCMS analysis detected *M. sapientum* peel hydromethanolic extract has the highest percentage area of trans-13-Octadecenoic acid with the least as oleic acid. For the pulp extract, 5-Hydroxy-4-methyl-3-heptanone and thymol were detected to have the highest and least percentage area, respectively. Interestingly, GCFID revealed that unripe *M. sapientum* peel and pulp extract differ in 6 phytoconstituents. The unripe peel extract is deficient in proanthocyanidin, resveratrol and epihedrine, while the unripe pulp extract is deficient in isoflavones, sapogenin and lunamarin. In recent years, catechins have been used as antioxidants in oils and fats [16], in lipid peroxidation and also as an agent in foodstuffs and essential ingredients in various dietary supplements [12, 14]. Catechin has anti-uveal melanoma activity, antitoxic and anti-inflammatory activities [12]. Epicatechin appears to be helpful in protecting pancreatic islets against adverse effect of streptozotocin *in vitro* and *in vivo* systems [17, 18]. Other phytotherapeutic constituents detected through GCFID include ribalinidine, rutin, steroids and sapogenin for *M. sapientum* peel extract and ribalinidin, phenols, steroids and resveratrol for *M. sapientum* pulp extract. Ripe *M. sapientum* pulp is the most ingested form and part of the fruit, especially in the tropics, although the result of this study may sensitize the public on the better potential therapeutic tendency of its unripe samples.

## CONCLUSION

This study presented the proximate, mineral, phytochemical composition and potential medicinal equivalent of unripe *M. sapientum* hyromethanolic peel and pulp extracts. The importance of the extracts can be traced to the various bioactive components in both parts of the plant.

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