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Gas chromatography–flame ionization detector, phytochemical and quality control of unripe *Musa sapientum* through UV-VIS spectrophotometric analysis

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Abstract
Musa sapientum (banana) is widely consumed for its health benefits. Its pulp, peel, leaves, bark as well as whole fruit is used in reducing the risk of chronic diseases of clinical interests. This study aims to find out whether or not there are any distinctive phytochemical constituents present in unripe *Musa sapientum* peel, pulp and whole fruit hydromethanolic extract using GC-FID techniques and to demonstrate the importance of spectral data in contribution to quality control of its medicinal properties. The UV-VIS profile showed different peaks ranging from 200-900 nm with different absorption respectively. UV-VIS profile showed 9 peaks with three distinct peaks at 240 nm, 400 nm, and 700 nm for both pulp and its peel while 22 peaks with four at 220 nm, 290 nm, 550 nm and 600nm for whole fruit. GC-FID analysis provided characteristic peaks determining the presence and concentration of phytochemical compounds in peel, pulp and whole fruit. Three major phytoconstituents were found almost exclusively in peel, including, Isoflavones, lunamarine and sapogenin while proanthocyanidin and resveratrol were exclusively in its pulp. Spartein, phytates, tannins and isoflavones were absent in whole fruit. The concentration of flavone was minimal. In conclusion, the study justifies the nutritional and medicinal properties of the plant and also represents an additional support to the quality control of their fruit drugs. The presence of the distinctive phytochemicals may be mechanistic link for the specific-efficacy of their physio-pharmacologic and therapeutic activities. Ingested together, these study data suggests that peel, pulp or whole fruit supplementation may be a potential alternative to conventional treatment for various types of infirmities and may confer other potential industrial, nutritional and medicinal advantages.

Keywords: GC-FID, UV-Vis spectrophotometric analysis, Resveratrol, proanthocyanidin, isoflavones

Introduction

Musa sapientum (banana), has worldwide consumption, and most importantly, there is Interesting evidence indicating that unripe *Musa sapientum* peel, pulp as well as whole fruit are exceptionally helpful as a natural medicine for various purposes, including, in a lower risk of degenerative diseases such as cancer, high blood pressure, diabetes, and heart disease among others, resulting from biologically active phyto pharmaceuticals [1]. In their gas chromatography-mass spectrometry (GC-MS) analysis, it has been established the presence phytochemicals with *Musa sapientum* (banana) peels and pulps respectively [2-8]. Furthermore, it has been suggested indeed that UV-Vis spectrophotometric chemical techniques contribute as an additional tool as a support to the quality control of plant-based drugs, allowing information to be obtained without the need for previously isolation of chemical constituents [1, 9, 10]. The literature survey discloses that information on GC-FID analysis as well as spectroscopic analysis of UV–visible of the plant extracts is limited. Hence this study aims to determine the presence and concentration of bioactive compounds distinct to unripe banana peel, pulp together with the whole fruit with the aid of GC-FID, combined with spectral data which would provide understanding of the chemicals present in a sample as well as concentration through peak length in quality control analysis of its drugs.

Materials and Methods

Material and Preparation of Samples

Nigeria variety of unripe banana peel, pulp and whole fruit were prepared as previously described [7] Briefly, Peels were separated from pulps and both parts as well as whole fruit were dipped in 0.5% citric acid to prevent enzymatic degradation. They were shade dried for 96 hours. Dried peels, pulps and whole fruit were ground to paste. The ground unripe banana peels, pulps and whole fruit were extracted with hydromethanolic solvent (1:4 *v/v*) [11, 12]. The extraction was carried out in sealed test tubes placed in water bath for 120 min at 25^{0}C. The extracts were centrifuged and then evaporated to dryness in a vacuum evaporator at 40^o C. The final residues of unripe peels, pulps and whole fruit obtained were subjected to gas chromatography-flame ionization detector (GC-FID) and UV-VIS Spectroscopic analysis.

Gas Chromatography-Flame Ionization Detector

The analysis of phytochemical was performed on a BUCK M910 Gas chromatography equipped with a flame ionization detector.

A RESTEK 15 meter MXT-1 column (15m x 250um x 0.15um) was used. The injector temperature was 280oC with split less injection of 2ul of sample and a linear velocity of 30cms-1, Helium 5.0pa.s was the carrier gas with a flow rate of 40 ml min-1. The oven operated initially at 2000c, it was heated to 3300c at a rate of 30c min-1 and was kept at this temperature for 5min. the detector operated at a temperature of 3200c. Phytochemical were determined by the ratio between the area and mass of internal standard and the area of the identified phytochemicals. The concentration of the different phytochemicals express in ug/g.

UV-VIS Spectroscopic analysis

UV-visible spectrophotometric analysis was conducted on the sample using a UV-visible spectrophotometer (Apel 3000UV) with a slit width of 2nm, using a 10-mm cell at room temperature. The extract was examined under visible and UV light in the wavelength ranging from 200-1100nm. This facilitated to understanding the various peaks arising due to multiple components present in the extract as well as to find out the wavelength at which maximum absorbance was observed. Thus the wavelength maximum of the unripe banana peel, pulp as well as whole fruit from hydromethanolic extract is not known.

Conflicts of Interest

Both authors declare no conflicts of interest.

Results

The following are the results of this study;

Table 1: Area of percentages, heights and retention time of bioactive compounds of the hydromethanolic extract of banana pulp obtained from GS-FID

	Pulp				
Phytoconstituents	**Concentration**		**Retention time**	**Area**	**Height**
Proanthocyanidin	3.882	ppm	0.116	3681.8254	411.025
Naringin	8.588	Ug/ml	2.223	6793.221	528.999
Anthocyanin	10.988	Ug/ml	3.950	8180.043	637.037
Naringenin	4.754	Ug/ml	6.893	4491.191	350.845
Spartein	4.515	Ug/ml	10.593	4339.038	337.681
Ribalinidine	17.6738	Ug/ml	13.300	4918.608	385.135
Phytate	2.3335	Ug/ml	15.783	12794.38	919.800
Phenol	15.8944	ppm	19.516	12631.143	566.996
Flavonones	4.9710	ppm	22.293	4749.758	372.508
Kaempferol	2.1580	Ug/ml	26.000	6833.379	529.584
Epicatechin	14.988	Ug/g	28.566	5744.947	450.313
Flavone	6.3209	Ug/ml	29.493	4459.3978	349.793
Rutin	4.5461	Ug/ml	33.753	3207.273	252.912
Oxalate	1.6079	Ug/ml	34.206	5932.528	458.609
Quinine	4.6050	Ug/ml	37.260	6525.253	508.857
Resveratrol	12.8283	ppm	38.326	9393.732	732.257
Catechin	15.6715	Ug/ml	39.586	4412.402	345.673
Epihedrine	8.5119	Ug/ml	40.930	3451.568	270.978
Tannins	14.212	Ug/ml	42.086	6000.131	470.143
Steroids	16.089	ppm	4.943	6524.263	510.968
Total	175.2409 (26.56%)		487.836 (36.02%)	125064.0922 (24.51%)	9390.113 (42.62%)

Table 2: Area of percentages, heights and retention time of bioactive compounds of the hydromethanolic extract of banana peels obtained from GS-FID

	Peel				
Phytoconstituent	**Phytochemical**		**Retention time**	**Area**	**Height**
Naringin	15.6633	Ug/ml	2.390	12389.6768	359.890
Anthocyanin	8.7466	Ug/ml	4.120	6510.9915	189.596
Naringenin	8.7462	Ug/ml	7.470	8262.2252	243.618
Spartein	20.3999	Ug/ml	10.366	19604.3322	566.984
Ribalinidine	22.4271	Ug/ml	12.970	6241.4544	181.452
Phytate	0.9063	Ug/ml	15.460	4969.2060	144.440
Phenol	16.0589	ppm	20.313	12761.9008	364.580
Flavonones	10.0212	ppm	22.730	9575.2969	277.523
Kaempferol	10.0212	Ug/ml	25.650	10075.5224	292.502
Epicatechin	30.0678	Ug/g	27.536	11524.9702	334.285
Flavone	7.7678	Ug/ml	29.860	5480.1748	159.265
Rutin	20.2313	Ug/ml	32.996	14273.1864	414.434
Oxalate	1.6266	Ug/ml	34.600	6001.6606	175.391
Quinine	4.9342	Ug/ml	36.876	6991.7764	202.710

Catechin	36.3548	Ug/ml	39.200	10235.9017	296.639
Tannins	8.3093	Ug/ml	42.276	3507.9260	101.976
Steroids	26.0077	ppm	44.170	10546.1352	306.059
Isoflavones	3.1891	ppm	0.276	3662.1774	146.970
Sapogenin	20.3999	Ug/ml	6.016	17997.1178	524.988
Lunamarin	6.5659	Ug/ml	17.966	11342.5578	329.088
Total	272.041(41.2%)		433.231 (31.99%)	191954.1905 (37.62%)	5612.388 (25.47%)

Table 3: Wavelength of the absorption peaks of various organic compounds in banana

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Total	272.041(41.2%)		433.231 (31.99%)	191954.1905 (37.62%)	5612.388 (25.47%)

Table 4: Overall percent distribution of phytochemical parameters

Unripe banana components	% Phytochemical compounds	Percent area	Retention time	Percent Height
Peel	41.23%,	37.62%	31.99%	25.47%,
Pulp	26.56%,	24.51%,	36.02%	36.02%
Whole fruit	32,21%,	37.87%,	31.99%	31.91%

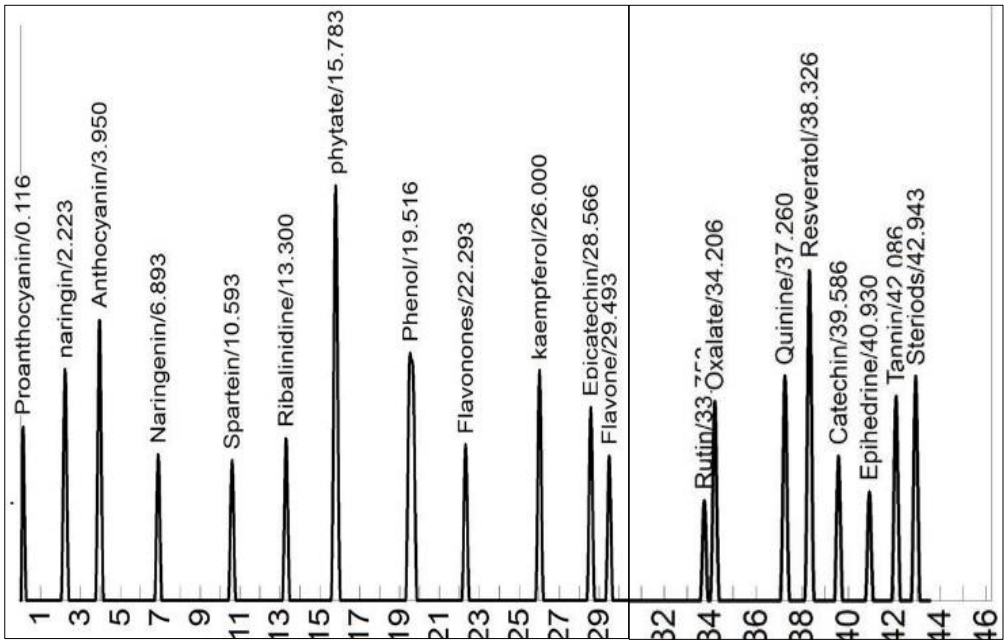

Fig 1: GC-FID chromatogram of the hydromethanolic extract of the banana pulp

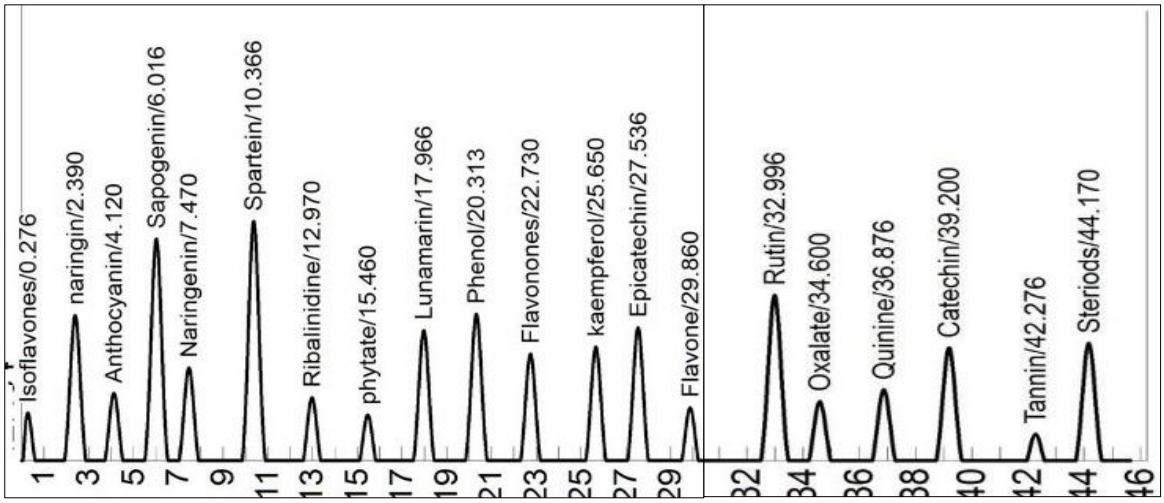

Fig 2: GC-FID chromatogram of the hydromethanolic extract of the banana peel

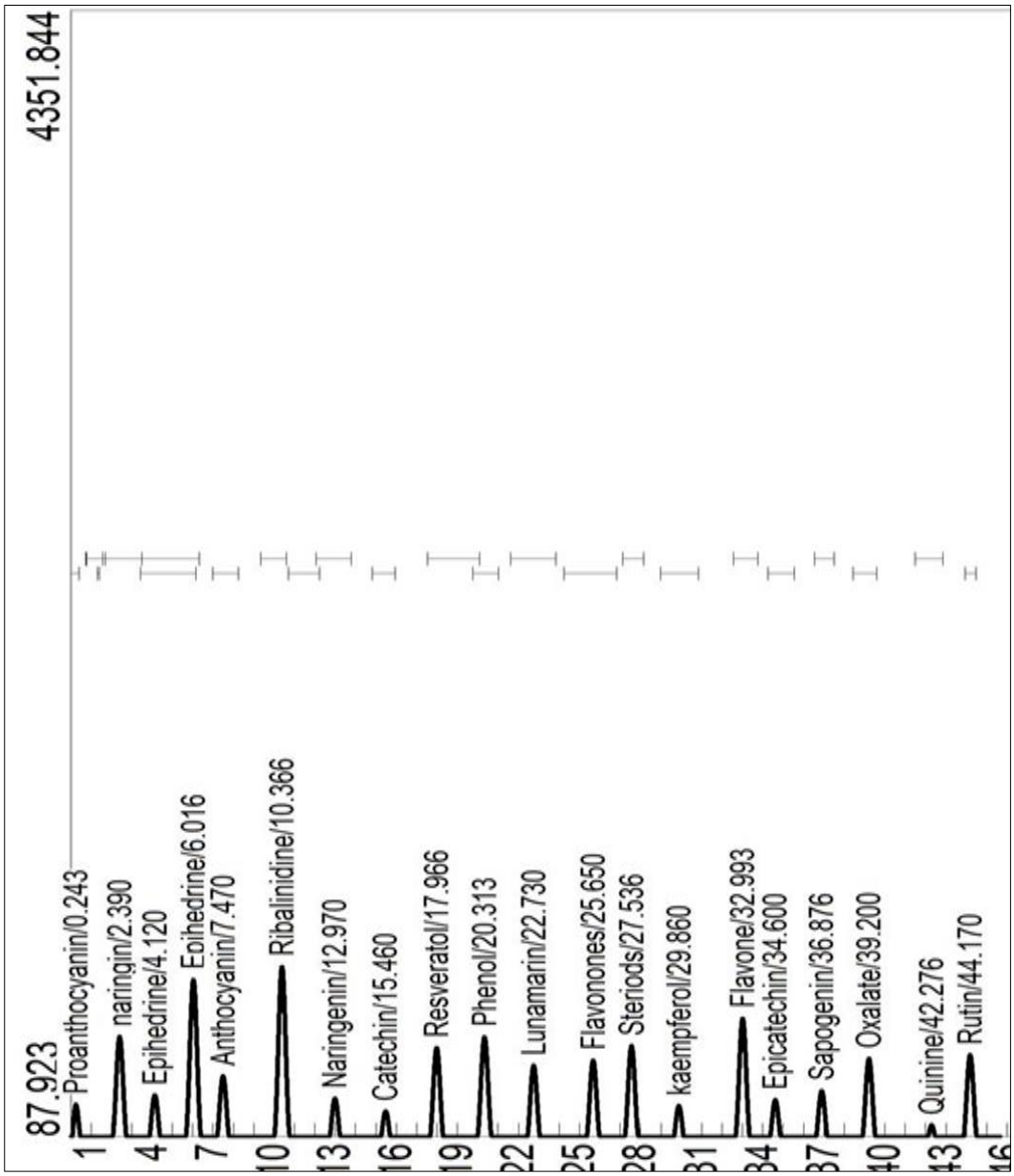

Fig 3: GC-FID chromatogram of the hydromethanolic extract of the banana whole fruit.

Discussion

The chromatogram of the gas chromatography – flame ionization detector (GC-FID) comparative analysis of phytochemicals from hydromethanolic extracts of unripe banana peel, pulp and whole fruit are shown in figures 1, 2 and 3. GC-FID analysis identified twenty characteristic types of possible phytochemicals in unripe banana peel, pulp and whole fruit as depicted in Tables 1, 2 and 3. Table 4 depicts in general, the typical percent phytochemical compounds, area, retention time and height of phytoconstituents in peel, pulp and whole fruit respectively, which is a clear demonstration of the broad efficacy spectrum of pharmacological and therapeutic activities. Indeed, the unripe banana peel is rich in phytochemical compounds than its pulps [4, 6] as well as whole fruit. Interestingly, GC-FID also showed three major phytoconstituents with known specific-efficacy therapeutic properties and whose levels are apparently nontoxic to humans almost exclusively in peels namely, Isoflavones (1.17%), lunamarine (2.41%) and sapogenin (7.66%) while proanthocyanidin (2.22%), and resveratrol (7.38%) were identified in its pulp. On the other hand, spartein, phytates, tannins and isoflavones were absent in the whole fruit. The concentrations of flavones were minimal in the whole fruit. These identified specific phytochemicals perhaps might validate their diverse health benefit aspects or plausible mechanistic link for the wide variety of its physio-pharmacological and therapeutic potentials. In the present study, the spectral profile obtained with application of UV–vis spectrophotometry data ranged from 200-900nm, revealed different absorptions, respectively, indicating the presence of specific chemicals for pharmacological activities present in a sample as well as concentrations through peak length thus providing an additional tool for quality control of their drug properties (1, 9, 10). The profile for both peel and pulp showed 9 peaks with three distinct absorption bands at λ_{max} 240 nm, λ_{max} 400 nm, and λ_{max} 700 nm (Table 3 and figures 3 and 4). On the other hand, the spectral data observed for whole fruit showed 22 peaks with four distinct absorption peaks at approximately λ_{max} 220 nm, λ_{max} 290 nm, λmax 550 nm and 600nm as represented in figure 5 and Table 3. At a maximum wavelength of approximate 400nm, the peak of absorption of the organic phytochemical compounds present in pulp (56.72%) was much higher than its peel (43.28%), Mean while the maximum peak of absorption for whole fruit was at λ_{max} 550nm

In conclusion, this investigation has given scientific information to determine the chemical compositions of unripe banana peel, pulp as well as whole fruit using UV-VIS, and GC-FID techniques. The presence of these distinct bioactive compounds thus lends credence to its local use and also represents an additional support to the quality control of their fruit drugs. It also holds that the phytochemicals may be mechanistic link for the specific-efficacy for the Phyto pharmaceutical activities as well as production of novel drugs with isolation of specific compounds. It could be concluded that unripe banana peel, pulp and whole fruit when combined contains efficacious bioactive compounds which may be a potential alternative to conventional treatment for various types of infirmities and may confer other potential industrial, nutritional and medicinal advantages.

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