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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; Fingerprint; Resveratrol; Proanthocyanidin; Isoflavones; Sapogenin; Lunamarine; Quality control

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 phytopharmaceuticals [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°C. The extracts were centrifuged and then evaporated to dryness in a vacuum evaporator at 40°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 (15 m × 250 um × 0.15 um) was used. The injector temperature was 280°C with split less injection of 2ul of sample and 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 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 2 nm, using a 10-mm cell at room temperature. The extract was examined under visible and UV light in the wavelength ranging from 200-1100 nm. 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.

Result

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	3.95	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	13.3	4918.608	385.135
Phytate	2.3335	Ug/ml	15.783	12794.38	919.8
Phenol	15.8944	ppm	19.516	12631.143	566.996
Flavonones	4.971	ppm	22.293	4749.758	372.508
Kaempferol	2.158	Ug/ml	26	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	4.605	Ug/ml	37.26	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	40.93	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	2.39	12389.68	359.89
Anthocyanin	8.7466	Ug/ml	4.12	6510.992	189.596
Naringenin	8.7462	Ug/ml	7.47	8262.225	243.618
Spartein	20.3999	Ug/ml	10.366	19604.33	566.984
Ribalinidine	22.4271	Ug/ml	12.97	6241.454	181.452
Phytate	0.9063	Ug/ml	15.46	4969.206	144.44
Phenol	16.0589	ppm	20.313	12761.9	364.58
Flavonones	10.0212	ppm	22.73	9575.297	277.523
Kaempferol	10.0212	Ug/ml	25.65	10075.52	292.502
Epicatechin	30.0678	Ug/g	27.536	11524.97	334.285
Flavone	7.7678	Ug/ml	29.86	5480.175	159.265
Rutin	20.2313	Ug/ml	32.996	14273.19	414.434
Oxalate	1.6266	Ug/ml	34.6	6001.661	175.391
Quinine	4.9342	Ug/ml	36.876	6991.776	202.71
Catechin	36.3548	Ug/ml	39.2	10235.9	296.639
Tannins	8.3093	Ug/ml	42.276	3507.926	101.976
Steroids	26.0077	ppm	44.17	10546.14	306.059
Isoflavones	3.1891	ppm	0.276	3662.177	146.97
Sapogenin	20.3999	Ug/ml	6.016	17997.12	524.988
Lunamarin	6.5659	Ug/ml	17.966	11342.56	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.

Wavelength (nm)	Absorbance	
	***M.s.* Peel**	***M.s.* Pulp**
200	1.189	0.782
240	1.457*	0.892*
320	1.078	0.473
400	2.289*	3*
450	1.103	2.284
500	0.348	1.447
550	0.084	0.187
700	0.106	0.1
800	0.04	0.11
Wavelength (nm)	**Absorbance**	
	***M. s.* whole-fruit**	
200	0.235	
220	0.344*	
260	0.365	
290	0.67*	
310	0.395	
330	0.359	
380	0.4	
400	0.421	
420	0.439	
450	0.5	
470	0.966	
500	1.289	
550	1.458*	
580	0.544	
600	1.11*	
620	0.44	
700	0.223	
750	0.111	
800	0.057	
850	0.032	
870	0.032	
900	0.054*	

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 %

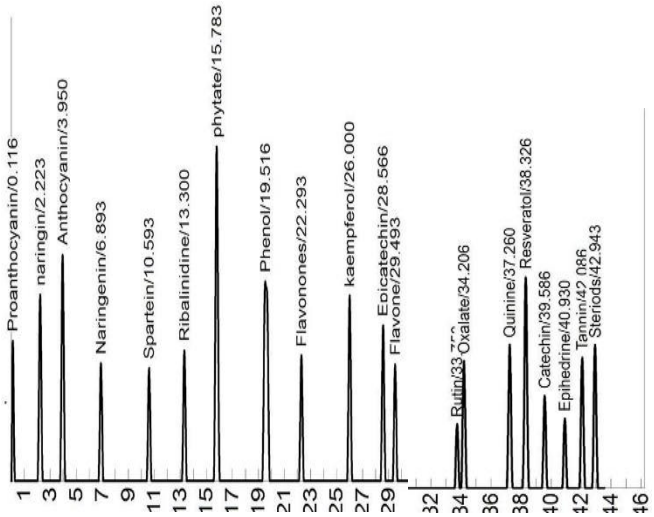

Figure 1: GC-FID chromatogram of the hydromethanolic extract of the banana pulp.

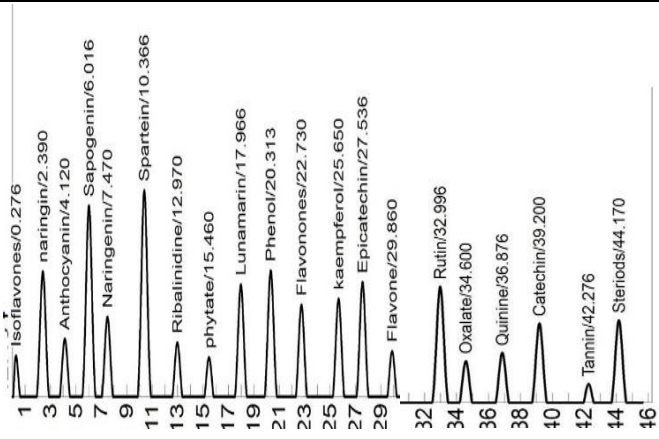

Figure 2: GC-FID chromatogram of the hydromethanolic extract of the banana peel.

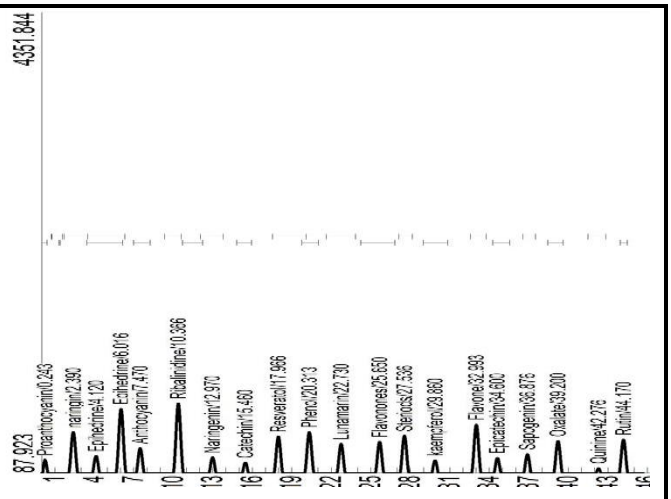

Figure 3: GC-FID chromatogram of the hydromethanolic extract of the banana whole fruit.

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 200-900 nm, 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, λ max 550 nm and 600 nm as represented in Figure 5 and Table 3. At a maximum wavelength of approximate 400 nm, the peak of absorption of the organic phytochemical compounds present in pulp (56.72%) was much higher than its peel (43.28%), Meanwhile the maximum peak of absorption for whole fruit was at λ max 550 nm [11-13].

Conclusion

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 phytopharmaceutical 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.

Conflict of Interest

The authors declare no potential conflict of interest.

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