CME LIVE OPEN ACCESS

Chemistry Research and Practice

Research Article

Tentative identification of polyphenols from persea americana stem bark using LC-MS/MS.

Amuda Mutiu Olasunkanmi

Ladoke Akintola University of Technology, Ogbomoso, Oyo state.

Corresponding Author: Amuda Mutiu Olasunkanmi, Ladoke Akintola University of Technology, Ogbomoso, Oyo state.

Received: 2024 Feb 26 **Accepted:** 2024 Mar 17 **Published:** 2024 Mar 25

Abstract

Natural products from medicinal plants provide unlimited opportunities for new drugs leads. This study identify the polyphenols present in methanol fraction of persea americana stem bark by ultra-high performance liquid chromatography coupled with tandem mass spectrometer (LC-MS/MS), the Polyphenols were extracted using EtOH/H2O (80:20 v/v) and the extract obtained was subjected to successive maceration using three different solvents leading to hexane, ethyl-acetate, and methanol fraction which were labeled as PAHF, PAEF and PAMF respectively. Four polyphenols were identified in PEAF by LC-MS/MS analysis: Silybin B, Delphinidin, and Hersperetin. The major peaks observed from isolated fragmentation of silybin B were 481, 391, 167, and 149 m/z which were assigned to (M-H)-, (M-+C2H2O4), (+C8H8O4), (+C6H4O3) fragmented ions respectively. The total phenolic and flavonoid contents was determined. Phytochemical screening of the crude extract was also carried out.

Keywords: Polyphenols, Extraction, Phytochemicals. LC-MS/MS, Persea Americana.

1. Introduction

Polyphenols are plant secondary metabolites that are found in a wide variety of plants [1]. These natural compounds constitute a group of molecules that are divided according to their chemical structure, although they can also be classified by their source of origin, natural distribution or biological function. In particular, according to their chemical structure, they can be classified into different groups, as function of the number of phenol rings contained and the structural elements that bind these rings [2]. The most common classification of polyphenols includes five main classes, namely phenolic acids, stilbenes, flavonoids, lignans, and others [3].

In recent years, numerous studies have shown that the consumption of polyphenols in the diet provides numerous health benefit [4]. This is largely due to the antioxidant properties that help to prevent various diseases associated with oxidative stress [5]. Studies like those of Scalbert et al. And Seo et al. demonstrated that the antioxidant activity of plant polyphenols can retard the development of diseases such as cancer and cardiovascular and neurodegenerative diseases [6, 7]. Extraction and characterization of polyphenol compounds in plant matrices are complex, since the compounds can be found in simple or highly polymerized structures, which can also form complexes with various other plant-matrix components. In this regard, many polyphenols are often associated with sugar moieties [2]. Thus, the use of different methods of extraction combined with proper solvents characterized by different polarities are strongly required to recover them, the extraction of polyhenol compounds in plants is influenced by several factors. For example, some phenolic compounds are very photosensitive, as a result, rapid extraction methods are necessary to avoid the degradation of them [8, 9]. The most used technique for the quantification of polyphenols is UV spectroscopy, due to its simplicity and low cost. However, this technique only gives an estimation of the total phenolic content and it does not separate the compounds individually, a vaccum these study fill [5].

1.1. Persia Americana Plant

Persea americana is a native plant of central Mexico, classified in the flowering plant family Lauraceae. They are commonly valuable and are cultivated in tropical and Mediterranean climates throughout the world. The tree grows to 20m, with alternately arranged leaves 12-25cm long, the flowers are inconspicuous, greenish-yellow 5-10mm wide. The plant is remarkably versatile as to soil adaptability, doing well on such diverse types as red clay sand, volcalnic loam, or lateritic soil.it has been found healthier on nearly neutral or slightly alkaline soil than on moderately or highly acid soil.

1.2. Taxonomy

Synonyms(s): Laurus persea L, Persea drymifolia Schlecht, and cham, Persea gatissima Gaertn.f.,

Common names:

- **Amharic:** Avocado

- **English:** Alligator pear, Avocado-pear, Butter fruit
- **Filipino:** Avocado
- **French:** Avocet, Avocatier, zabelbok, zaboka
- **Current name:** Persea americana
- **Authority:** Miller
- **Filipino:** Avocado

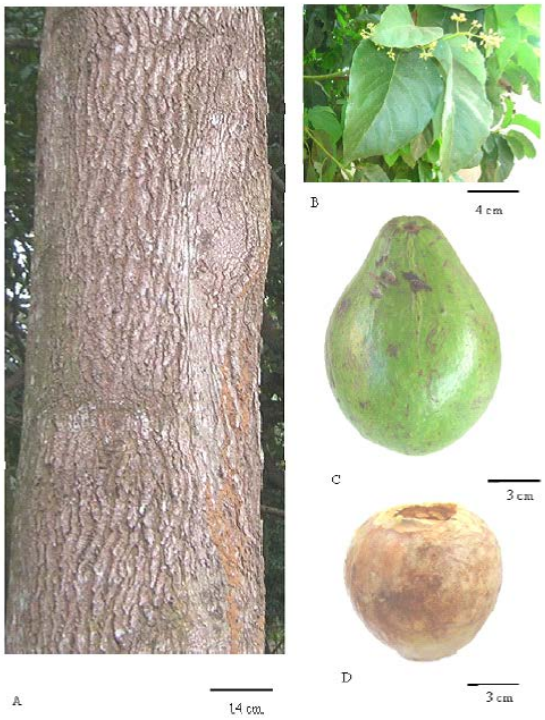


Plate 1: showing the various part of the Peasia americana.

1.3. Reagents and chemicals

TLC silica gel plate (60 F254), n-hexane, methanol, ethyl-acetate and other solvents used were procured from Sigma Aldrich agent in Nigeria and were used without further purification.

1.4. Sample collection and preparation

Fresh stem back of Persia americana was collected from an uncultivated farm land in Aroje, Ogbomosho in Oyo State (Latitude 8o 07' 60.00" N, Longitude 4o 14' 60.00" E). The stem back was taken to a botanist in the Dept. of Pure and Applied Biology, Ladoke Akintola University of Technology, Ogbomoso, for proper identification and classification with voucher number (Voucher No. LHO 656). The stem was then taken to the laboratory and air-dried at room temperature for three weeks. The stems were pulverized to increase its surface area and allow a better contact of the extracting solvents with the sample for effective extraction.

1.5. Extraction

Extraction was achieved using cold extraction method. Powdered sample (1000g) was soaked with n-hexane (non-polar solvent) in a glass jar. The solvent was decanted and filtered off after 24 hours. The process was repeated three times to remove chlorophyll and other non-polar compounds. The defatted sample was then soaked with 80:20 EtoH/H2O for 24 hours. The supernatant was carefully decanted, and the residue re-soaked with 80:20 EtoH/H2O until 5 L of supernatant was obtained. The clear supernatant was thereafter concentrated in vacuo at 40oC using a rotary evaporator to obtain a crude extract which was kept airtight in readiness for maceration. Crude extract (63.92g) was macerated successively with n-hexane, ethyl acetate and methanol.

1.6. Phytochemical Screening

The extract from of Persea Americana stem bark was analysed in order to determine the phytochemicals present.

1.7. Test for Flavonoids

1ml of 10% NaOH was added to 1ml of the extract. A yellow colouration indicates the presence of flavonoids.

1.8. Test for Steroids (Salkovoski test)

5 drops of concentrated sulphuric acid (H2SO4) was added to 1ml of the crude extract. Red coloration indicates the presence of steroids.

1.9. Test for Alkaloids

5 ml of 1% HCl solution was added to 2mls of the extract in a test tube, the mixture was heated and filtered. The filtrate was used for the following tests;

- To 1ml of the filtrate, 2 drops of Mayer's reagent was added. A creamy precipitate indicates the presence of alkaloids in the extract.
- To 1ml of the filtrate, add 2 drops of Wagner's reagent, a reddish-brown precipitate indicates the presence of alkaloids.

1.10. Test for Saponins (frothing test)

2 ml of the extract in a test tube was vigorously shaken for about 2 minutes to observe if there would be frothing. Frothing indicates the presence of saponins.

1.11 Test for Tannins

1 ml of freshly prepared 10% KOH was added to the plant extract. A dirty white precipitate indicates the presence of tannins in the extract. Alternatively, 2 drops of 5% FeCl3 was added to 1ml of the extract. A greenish precipitate indicates the presence of tannins.

1.12. Test for Glycosides

To 1 ml of the extract in a test tube, 10 ml of 50% H2SO4 was added. The mixture was heated in boiling water for 15 minutes, 10 mls of Fehling's solutions (I & II) was added and the mixture was boiled. A brick-red precipitate. indicates the presence of glycosides in the extract.

1.13. Total Flavonoid contents

The AlCl3 method was used for determination of the total flavonoid contents of the sample. The methanolic extract was (1.5 ml) was added to 10 ml volumetric flask filled with 5 ml distilled water and 0.3 of 5% NaNO2 and mixed [10]. A reagent blank was using distilled water. After 5 mins, 1.5 ml of 2% methanolic AlCl3 was added. Two ml of 1 mol dm-3 NaOH was added 5 mins later and then the volume was made up to 10 ml with distilled water. The mixture was shaken for 5 mins and after 10 mins the absorbance was taken at 420 nm. Flavonoids contents were calculated using a standard calibration curve, prepared from quecetin. The flavonoids contents were expressed as mg quecetin g-1 of extract.

1.14. Total Phenolic contents

Total phenolic were determine using Folin-Ciocalteu method of Jagadish et al., [10]. The methanolic extract (0.5 ml) were added to a 25 ml volumetric flask filled with 10ml distilled water and 2.5 ml of 0.2 Folin-Ciocalteu phenol reagent. A reagent blank using distilled water instead of sample was prepared. After 5 mins., 2 ml of 2% NaCO3 solution were added with mixing. The solution was dilluted to the volume (25 ml) with distilled water and then allowed to stand for 90 mins., and the absorbance was measured at 725 nm. Total phenolic contents were calculated as mg quecetin g-1 dry weight of sample.

1.15. LC-MS Analysis

A UHPLC–ESI-QTOF-MS method was set up for the screening of compounds in MEBCS, with typical injection volumes of 50 µl extract. Separation was performed on a Dionex Ultimate 3000 UHPLC system (Thermo Scientific, Dionex, Sunnyvale, California, USA) equipped with a RaptorTM ARC-18 2.7 um 100 x2.1 mm column, held at a temperature of 25 °C, and using a gradient system composed of A: 0.1 % formic acid in water, and B: 0.1 % formic acid in methanol. The flow was maintained at 0.3 ml min−1 throughout the run. The developed gradient program was 70% to 50% A in 3 min - hold 2 min, 50% to 20% A in 2 min - hold for 2 min, 20% to 95% A in 2 min- hold for 3 min.

Time-of-flight detection was performed using a compact QTOF orthogonal mass spectrometer (Bruker Daltonics, Bremen, Germany) operated at a resolving power of ~23000 full widths at half maximum (FWHM). The instrument was equipped with an orthogonal electrospray ionization source, operated at positive mode, and auto MSMS spectra were recorded in the range m/z 50–1300 with five scans per second. For calibration, 1 µl 10 mmol L^{-1} sodium formate was injected at the beginning of each chromatographic run, using the divert valve (0.3– 0.4 min). ESI +\- capillary voltage was maintained at 5000 V, the gas flow to the nebulizer was set to 1.8 bar, the drying temperature was 220 °C, and the drying gas flow was 9.0 L min^{-1}.

Identification of the compounds in fractions was achieved by using the full mass spectrum and its unique mass fragmentation spectrum. This was achieved via comparison of the observed MS spectra with those found in the literature and Databases, such as MassBank (http://www.massbank.jp/), PubChem (https://pubchem.ncbi.nlm.nih.gov), mz Cloud Database (www.mzcloud.org).

2. Results

2.1. Percentage yield of various fractions

The Percentage yield of hexane, ethylacetate and methanol fraction from Persea americana stem bark is shown below as table 1. Plate 1: showing the various part of the Peasia americana.

Table 1: Percentage yield of various fractions.

S/N	EXTRACT	WEIGHT (g)	%YEILD
1	Hexane	0.995	0.10
2	Ethylacetate	25.4	2.54
3	Methanol	37.5	3.75

Table 2: Phytochemical screening of methanolic crude extract.

SOLVENT EXTRACT	STEROID	FLAVONOID	SAPONIN	TANNIN	ALKALOID	GLYCOSIDE
n-hexane	-ve	-ve	+ve	-ve	+ve	-ve
Chloroform	-ve	+ve	+ve	-ve	+ve	-ve
Ethylacetate	-ve	+ve	+ve	+ve	+ve	+ve
n-butanol	+ve	+ve	+ve	-ve	-ve	+ve

The table below shows the result of the phytochemical test carried out on the plant extract of Persea Americana stem bark in organic solvent.
+ve = present -ve = absent

Table 3: Total phenolic and flavonoid contents of methanolic extract of Persea americana stem bark.

FRACTION	PHENOLIC CONTENT (µg/g)	FLAVONOID CONTENT (µg/g)
Methanolic extract	500	250

Table 4: Constituents identified in PEAF by LC-MS/MS in positive ion mode.

COMPOUND IDENTIFY	FORMULAR	M/Z (M-H)	TIME (mins)	#	FRAGMENTS
Silybin	$C_{25}H_{22}O_{10}$	481.1135	7.51	5085	391.2125, 331.0815, 167.0357, 149.0233, 123.0440, 105.0332.
Delphinidin	$C_{15}H_{11}O_7$	471.0906	4.63	3121	303.0501, 285.1418, 275.0503, 167.0346, 153.0183, 1230463, 81.0346.
Hesperetin	$C_{16}H_{14}O_6$	417.0445	4.60	3125	303.0502, 150.0322, 125.0229, 112.9855, 96.9555, 68.9946.

PEAF represent Persea americana ethyl-acetate fraction.

2.2. Structure of compounds tentatively identified from PAEF extract

Figure 1: Structure of Silybin B

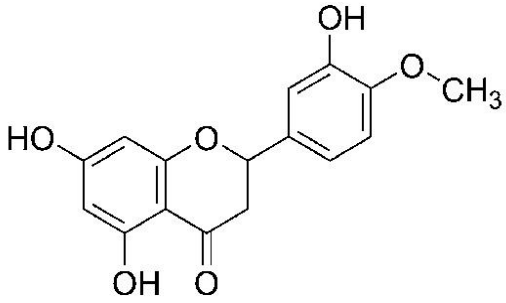


Figure 2: Structure of Hesperetin

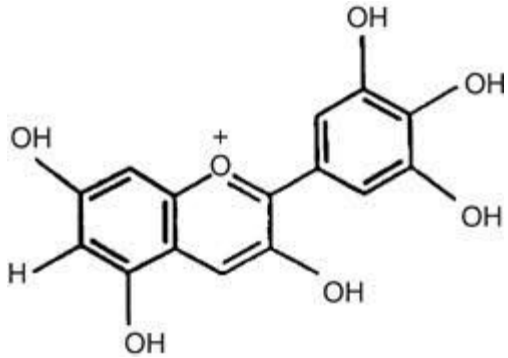


Figure 3: Structure of Delphinidin.

3. Discussion

The preliminary phytochemical test was carried out on each of the stem bark extract, the result for the hexane fraction shows the present of saponin and alkaloid, while flavonoids, steroids, tannins and glycosides were observed to be absent. Chloroform fraction showed present of flavonoids, alkaloids and saponins, while steroids, tannins and glycosides were absent. Furthermore, the ethyl acetate fraction showed the present of flavonoids, tannins, saponins, glycosides and alkaloids, only steroids were absent. The butanol fraction showed present of steroids, flavonoids, saponins, glycosides but the absent of tannins and alkaloids. However, the results show the leave is rich in saponins, flavonoids and alkaloids. They are known to show medicinal activity as well as exhibiting physiological activity [11].

Total phenolic contents were determined using Folin-ciocalteu method of Jagadish et al., while the total flavonoid contents were determined using AlCl3 method of Jagadish et al., The result shows that the plant leave contains a high level of phenol and flavonoids [10, 10].

Chromatographic and spectrophotometric methods were employed in this study and modified where necessary in the study. LC--MS/MS was used for metabolite profiling of methanol fraction of P. americana stem-bark. Four polyphenols were identified in PEAF by LC-MS/MS analysis: Silybin B, Delphinidin, and Hersperetin [12].

Full chromatogram and mass spectra of the extract are presented in appendix, the structure of identified compounds is Silybin B, Hesperetin, and Delphinidin. The fragmentation details of all the identified compounds with their typical fragments (m/z) is shown in Table .4.

Proposed fragmentation pattern of silybin B as fig. 1, Silybin B appeared at retention time (tR) 7.51 mins having molecular ion peak [M-H] – at 481 m/z and its characteristic fragment ions were observed at 391 m/z, [M-H-+C2H2O4]; 167 m/z, [+C8H8O4]; and 149 m/z [+C6H4O3].

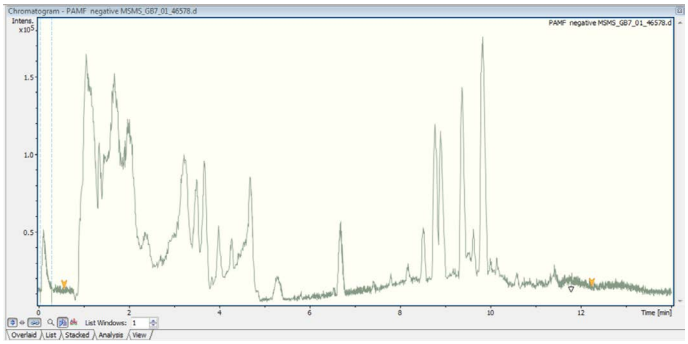
Hesperetin as fig. 2, appeared at a retention time (tR) 4.60 mins with molecular ion peak [M-H]- at 417 m/z and its characteristic fragment ions were observed 124 m/z, [M-H-$^{+}C_7H_7O_2$]; 96 m/z [+$C_5H_5O_2$]; and 303 m/z as result in loss of H_2O.

Delphinidin as fig. 3. appeared at a retention time (tR) 4.63 mins with molecular ion peak at 471 m/z, with characteristics fragments ions at 124 m/z, [M-H-+ $C_6H_5O_3$].

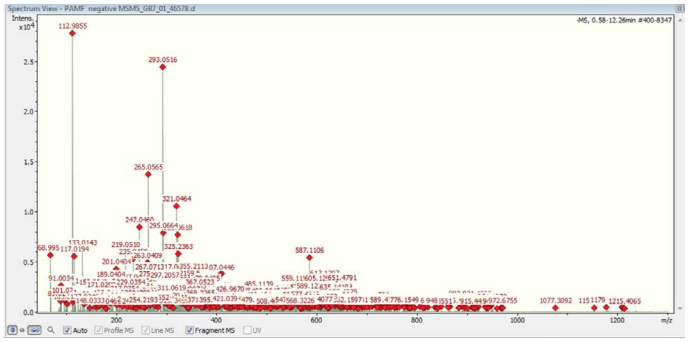
4. Conclusion

Compounds tentatively identified from the methanolic fraction of the extract from stem bark of Persea americana include, silybin B, hesperetin, delphinidin. Therefore, this research work is an addition to the world data on natural product.

Appendix.



Chromatogram of LC-MS/MS of the extract



Mass spectrum of the extract

References

1. McMaster, M. C. (2007). HPLC: a practical user's guide. John Wiley & Sons.
2. Rajauria, G. (2018). Optimization and validation of reverse phase HPLC method for qualitative and quantitative assessment of polyphenols in seaweed. Journal of pharmaceutical and biomedical analysis, 148, 230-237.
3. Belščak-Cvitanović, A., Durgo, K., Huđek, A., Bačun-Družina, V., & Komes, D. (2018). Overview of polyphenols and their properties. In Polyphenols: Properties, recovery, and applications (pp. 3-44). Woodhead Publishing.
4. López-Fernández, O., Domínguez, R., Pateiro, M., Munekata, P. E., Rocchetti, G., & Lorenzo, J. M. (2020). Determination of polyphenols using liquid chromatography–tandem mass spectrometry technique (LC–MS/MS): A review. Antioxidants, 9(6), 479.
5. Amuda M O and. Oderinlo O.O. (2023). Anti-oxidant analysis of Persea americana leaf. Int. J. Chem. Res. Vol. 7, Issues 3.
6. Scalbert, A., Manach, C., Morand, C., Rémésy, C., & Jiménez, L. (2005). Dietary polyphenols and the prevention of diseases. Critical reviews in food science and nutrition, 45(4), 287-306.
7. Seo, O. N., Kim, G. S., Kim, Y. H., Park, S., Jeong, S. W., Lee, S. J., ... & Shin, S. C. (2013). Determination of polyphenol components of Korean Scutellaria baicalensis Georgi using liquid chromatography–tandem mass spectrometry: Contribution to overall antioxidant activity. Journal of Functional Foods, 5(4), 1741-1750.
8. Naczk, M., & Shahidi, F. (2004). Extraction and analysis of phenolics in food. Journal of chromatography A, 1054(1-2), 95-111.
9. Pires, F. B., Dolwitsch, C. B., Dal Prá, V., Faccin, H., Monego, D. L., Carvalho, L. M. D., ... & Rosa, M. B. D. (2017). Qualitative and quantitative analysis of the phenolic content of Connarus var. angustifolius, Cecropia obtusa, Cecropia palmata and Mansoa alliacea based on HPLC-DAD and UHPLC-ESI-MS/MS. Revista Brasileira de Farmacognosia, 27, 426-433.
10. Jagadish, L. K., Krishnan, V. V., Shenbhagaraman, R., & Kaviyarasan, V. (2009). Comparitive study on the antioxidant, anticancer and antimicrobial property of Agaricus bisporus (JE Lange) Imbach before and after boiling. African Journal of Biotechnology, 8(4).
11. Edeoga, H. O., Okwu, D. E., & Mbaebie, B. O. (2005). Phytochemical constituents of some Nigerian medicinal plants. African journal of biotechnology, 4(7), 685-688.
12. McMaster, M. C. (2007). HPLC: a practical user's guide. John Wiley & Sons.