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Metabolites Profiling of *Pouteria Campechiana* (Kunth) Baehni Different Organs Using UPLC-PDA-MS and its Biological Activities

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Abstract
Pouteria campechiana (Kunth) Baehni is native to America and is cultivated in Egypt for its edible fruit. UPLC-PDA-qTOF-MS technique was performed to detect the metabolites profile of three organs of the Egyptian plant, namely, leaves, seeds, and pericarps. UPLC-MS spectra was analyzed using Principal Component Analysis to explore the relative variability within organs. The antioxidant and antimicrobial activities of the ethanol extracts were assessed by free radical 1,1-diphenyl-2-picrylhydrazyl scavenging activity and agar well diffusion assay methods, respectively. The IC50 values of the leaves, seeds and pericarps ethanol extracts were 73.68±8.12, 235.615±10.3 and 858.952±18.16 µg/mL, respectively. They showed antimicrobial activity against three bacterial and three fungal strains out of the eight tested microorganisms. Sixty-three chromatographic peaks were detected among organs. Two main principle components (PC1 and PC2) were responsible for the discrimination of the three organs. Flavonol glycosides were found highest in the leaves. Taxifolin and ursolic acid were predominant in the seeds. Unsaturated fatty acids contributed the most in the discrimination of pericarps from leaves and seeds.
Keywords: *Pouteria campechiana*; UPLC-MS; antioxidant; antimicrobial; principle component analysis.

1. Introduction

Ultra-performance liquid chromatography (UPLC) coupled with PDA and high resolution qTOF-MS achieves accurate and rapid metabolite analysis with better peak separation. *Pouteria campechiana* (Kunth) Baehni is a member of family Sapotaceae native to America and its edible fruit is commonly known as Canistel. Traditionally, it used to treat inflammation, skin eruptions, ulcers, and back pain [1]. In addition to its reported protective effect against neurological disorders [2], antioxidant [3], immunostimulatory [4], anti-inflammatory [5] and mosquitocidal [6] activities. However, up till now, there has not been systematic detailed characterization of secondary metabolites profiling of *P. campechiana* (Kunth) Baehni family Sapotaceae organs. This study attempts to compare the phytochemical composition of Egyptian *P. campechiana* different organs via UPLC-MS in addition to assessing their antioxidant and antimicrobial activities.

2. Experimental

2.1. Plant material and extracts

Plant material was collected in December 2010 from El–Mansouria area, Giza governorate, Egypt and was kindly identified by Shahina A. Ghazanfar, Head of Temperate Regional Team, Kew Royal Botanic Gardens, UK and Dr. Mohammed El–Gibali, former senior botanist at the National Research Center, Cairo, Egypt. A voucher specimen (No. 19.4.2015) was deposited in the Herbarium of the Pharmacognosy Department, Faculty of Pharmacy, Cairo University.

Twenty g of the dried plant organs (leaves, seeds) and fresh pericarps were extracted with ethanol (50 ml×3) at room temperature. The combined ethanol extracts were separately filtered and concentrated using a rotator evaporator under reduced pressure to yield 2.23 g, 1.50 g and 1.25 g of the leaves, seeds, and pericarps ethanolic extracts', respectively.

2.2. Chemicals and reagents

DPPH (1,1-diphenyl-2-picrylhydrazyl), (sigma,

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USA), was purchased and prepared in a concentration of 0.004% w/v in methanol. Agar, nutrient broth (Oxoid laboratories, UK), ampicillin, gentamicin, and amphotericin B (Sigma –Aldrich, Germany) were used for the antimicrobial activity.

2.3. Analysis of plant extracts using UPLC-MS

Dried plant organs (leaves, seeds) and fresh pericarps were ground with a pestle in a mortar using liquid nitrogen. The powder (30 mg) was then homogenized with 2.5 mL 70% MeOH containing 5mg/mL umbelliferone (an internal standard for relative quantification using a Turrax mixer (11,000 RPM) for five 20 s periods. Extracts were then vortexed vigorously and centrifuged at 3000 g for 30 min to remove plant debris [7]. Chromatographic separation was performed on an Acquity UPLC system (Waters) equipped with a HSS T3 column (100£1.0 mm, particle size 1.8 mm; Waters). The analysis was carried out according to procedures described previously [7].

2.4. Antioxidant activity

The antioxidant activity was measured using 1,1-diphenyl-2-picrylhydrazyl (DPPH) reagent [8]. Ascorbic acid was used as a positive control at a concentration of 1-50 µg/ml. Antioxidant activity was expressed as IC50. Percentage inhibition was calculated, and each measurement was performed in triplicate.

2.5. Antimicrobial activity

Antimicrobial activity was determined using the agar well diffusion assay [9]. Eight pathogenic microbes were supplied from Regional Center for Mycology and Biotechnology (RCMB), Al-Azhar university, Cairo, Egypt. The tested microbes were *Streptococcus pneumoniae* (RCMB 010010), *Bacillis subtilis* (RCMB 010067). *Pseudomonas aeruginosa* (RCMB 010043), *Escherichia coli* (RCMB 010052), *Aspergillus fumigatus* (RCMB 02568), *Syncephalastrum racemosum* (RCMB 05922), *Geotricum candidum* (RCMB 05097) and *Candida albicans* (RCMB 05036). Amphotericin B, ampicillin and gentamicin were used as positive controls. Antimicrobial activity was determined as mentioned by measuring the zones of inhibition [10].

2.6. Determination of Minimum Inhibitory Concentrations

The minimum inhibitory concentrations (MIC) of the samples were estimated for each of the tested organisms in triplicates [11].

3. Results and Discussion

3.1. Identification of metabolites in Pouteria campechiana organs

A non-targeted metabolite profiling of extracts prepared from the leaves, seeds, and pericarps (Figure S1) were conducted. Sixty-three chromatographic peaks belonging to various metabolite classes e.g., flavonoids, phenolic acids, triterpenes and fatty acids were detected (Table 1 and Figure S2). Metabolite's identification was made by comparing retention times, UV spectra and MS data (accurate deprotonated mass, isotopic distribution, and fragmentation pattern in negative ionization mode) of the detected compounds with those previously reported. The UPLC chromatograms of *P. campechiana* different organs were characterized by two main regions; the first region with retention time from 30s until 300s (phenolic acids and flavonoidal derivatives) while the second region starts from 300 s until 690s (triterpenes and fatty acids).

3.1.1. Identification of flavonoids and phenolic acids

The study was able to identify seventeen phenolic compounds, eight of which were reported for the first time in *P. campechiana* (peaks 4, 12, 15, 17, 21, 23, 24 and 26).

The characteristic UV absorption of different flavonoidal subclasses was helpful in the determination of the aglycone moiety.

Peaks 15, 17 and 19 were identified as myricetin-3-*O*-glycosides. Product ions at *m/z* 317 $[M-H]^-$, *m/z* 271 $[M-H-HCOOH]^-$ and Retro Dial Alder (RDA) fragments characteristic for myricetin at *m/z* 179 ($^{1,2}A^-$) and *m/z* 151 ($^{1,3}A^-$) suggested the presence of myricetin derivatives [12]. The glycosylation position was suggested by the relative higher abundance of the radical aglycone ion $[Y_0–H]^-$ at *m/z* 316 than the aglycone $(Y_0)^-$ at *m/z* 317 [13]. Peak 15 at *m/z* 479.08 $[C_{21}H_{19}O_{13}]^-$ produced the fragment ion at *m/z* 316 $[M–162–2H]^-$ due to loss of hexose moiety (162 amu). Peak 17 at *m/z* 449.072 $[C_{20}H_{17}O_{12}]^-$ exhibited product ion at *m/z* 316 $[M–132–2H]^-$ and was identified as myricetin-3-*O*-pentoside. The sugar moiety was assigned to be pentose due to the loss of (132 amu). The ESI-MS spectrum of peak 19 [*m/z* 463.088 $(C_{21}H_{19}O_{12})^-$] exhibited product ion at *m/z* 316 $[M–146–2H]^-$ due to loss of deoxyhexose (146 amu), and was assigned as myricetin 3-*O*-rhamnoside; which was previously isolated from *P. campechiana* leaves [14].

Peak 22 showed parent ion at *m/z* 447.09 $[C_{21}H_{19}O_{11}^-]$ and produced aglycone ion at *m/z* 301 due to homolytic loss of a rhamnose moiety (146 amu), as well as fragments at *m/z* 179 ($^{1,2}A^-$) and *m/z* 151 ($^{1,3}A^-$) due to RDA. Such fragmentation suggested the presence

of quercetin-3-*O*-rhamnoside, which was previously isolated from *P. campechiana* leaves [14].

Two flavanonol (peaks 16 and 20) at *m/z* 435.094 $[C_{20}H_{19}O_{11}]^-$ were detected showing a fragment at *m/z* 303 derived from the loss of a pentose moiety (132 amu) and RDA fragments represented by *m/z* 177 ($^{1,4}B^-$) and *m/z* 151 ($^{1,3}A^-$) characteristic for taxifolin aglycone. Taxifolin-3-O-pentoside and its isomer were suggested. The presence of taxifolin-3-*O*-pentoside as two isomers suggested the presence of taxifolin-3-*O*-arabinofuranoside and taxifolin-3-*O*-arabinopyranoside which were previously isolated from *P. campechiana* leaves [14]. Peak 21 showed $[M–H]^-$ at *m/z* 303.052 $[C_{15}H_{11}O_7^-]$ which was in accord to the presence of the taxifolin aglycone.

Peaks 4, 5, 7 and 12 exhibited UV absorption characteristic for flavanol derivatives at 270-280 nm (Band II). Peak 7 showed parent ion at *m/z* 289.072 $[C_{15}H_{13}O_6^-]$, produced ($^{1,3}A^-$) at *m/z* 137 due to loss of (152 amu), ($^{1,4}B^-$) at *m/z* 163 by RDA and was assigned as (epi)catechin [15]. (Epi)gallocatechin, peak 5, revealed a molecular ion at *m/z* 305.066 $[C_{15}H_{13}O_7^-]$ was confirmed by the detection of a gallic acid moiety at *m/z* 169 and intense ion at *m/z* 261 $[M–44–H]^-$. Peak 12 has parent ion at *m/z* 457.007 $[C_{22}H_{17}O_{11}^-]$ and exhibited facile loss of galloyl moiety (152 amu) to give *m/z* 305 (gallocatechin unit). Another fragments were noted at *m/z* 289 (catehin unit) and at *m/z* 169 (gallic acid). Accordingly, the compound was identified as (epi)gallocatechin gallate. Peak 4 has $[M–H]^-$at *m/z* 609.12 $[C_{30}H_{25}O_{14}^-]$ and was assigned as (epi)gallocatechin dimer. The parent ion fragmented to give *m/z* 451 and *m/z* 305 (gallocatechin unit). This compound was not reported before in *P. campechiana*.

Two hydroxybenzoic acids were detected in this study. MS signal for gallic acid, peak 2, was identified with the molecular ion at *m/z* 169.013 and fragmentation product at *m/z* 125 (–44 amu, CO_2) [15].

Similarly, protocatechuic acid, peak 3, was identified by the parent ion at *m/z* 153.0190 $[C_7H_5O_4^-]$ and fragmentation product at *m/z* 109 $[M–44–H]^-$.

3.1.2. Identification of fatty acids and triterpenes

Several saturated, unsaturated, and oxygenated fatty acids were detected in the UPLC chromatogram in the range of 300s–690s. Peaks 59 and 63 were assigned as palmitic acid and stearic acid. Both compounds were fragmented to yield $[M–18–H]^-$ typically for saturated fatty acids [16]. ESI spectrum of oleic acid (peak 60) revealed fragments at *m/z* 183 and *m/z* 127 due to cleavage of the carbon-carbon bond β to the double bond. Similar fragmentation patterns were observed for the other unsaturated fatty acids detected; linolenic acid (peak 54), palmitoleic acid (peak 56) and linoleic acid (peak 57). ESI-MS spectra revealed several hydroxy fatty acids (peaks 28, 31, 33, 39, 41, 43, 45, 46, 48, 50, 53 and 61). This is the first report for the presence of oxygenated fatty acids in *P. campechiana.* Peak 43 [*m/z* 309.208, $(C_{18}H_{29}O_4)^-$], peak 39 [307.19 $(C_{18}H_{27}O_4)^-$] and peak 41 [305.17 $(C_{18}H_{25}O_4)^-$] exhibited product ions due to loss of H_2O molecules $[M–18–H]^-$ which is characteristic in these types of compounds. A mass difference of 2 amu suggested an extra double bond [17]. Accordingly, Peaks 43, 39 and 41 were tentatively identified as hydroxyl-oxo-octadecadienoic acid, hydroxyl-oxo-octadecatrienanoic acid and hydroxyl-oxo-octadecatetranoic acid, respectively. Similarly, a mass difference of 2 amu between peaks (28 and 31), (45 and 46) and (48 and 50) indicated an extra double bond in the carbon chain.

The ESI-MS spectrum of peak 55 at *m/z* 455.35 $[C_{30}H_{47}O_3^-]$ produced fragments at *m/z* 407, *m/z* 391, *m/z* 377 and *m/z* 363 in the negative ion mode and was assigned as ursolic (or oleanoic) acid [18]. Peaks 34, 38, 40, 42 and 44 were suggested to be triterpene acids derivatives. MS signals at *m/z* 487.34 $[C_{30}H_{47}O_5]^-$, peaks 42 and 44, were tentatively identified as trihydroxy ursenoic acids while peaks 34, 38 and 40 at *m/z* 503.33 $[C_{30}H_{47}O_6]^-$ were assigned as tetrahydroxy ursenoic acids. Both compounds were previously isolated from the genus *Pouteria* [19]. However, tandem spectrometry is required to confirm the identification of those compounds.

3.2. Multivariate data analysis of UPLC/MS data

UPLC–MS spectra was analysed using principal component analysis (PCA) to explore the relative variability within the different organs. The main principal component (PC) to differentiate between organs is PC1 which account for 76% of variance. The PC1/PC2 scores plot (Figure 1) showed that three major distinct clusters are formed, corresponding to the three organs studied. The seeds sample is positioned on the right side of the plot (positive PC1 values), whereas on the left side, leaves and pericarps are located (negative PC1 values). Discrimination of pericarps sample from leaves and seeds is also possible along PC2. The PC1/PC2 loading plot (Figure 1) explained the separation observed in PCA in terms of the identified compounds through PC1 which had the most discriminatory signals. MS signals for flavonol glycosides quercetin-3-*O*-rhamnoside, myricetin-3-*O*-rhamnoside and myricetin-3-*O*- hexoside (peak 22, 19 and 15, respectively) showed a negative effect on PC1 and were found highest more enriched in the leaves sample. MS signals for taxifolin (peak 21) and ursolic acid (peak 55) showed a positive effect on PC1 and were found more in the seeds sample. Along PC2 loading plots, MS signals assigned for unsaturated fatty acids; linolenic acid (peak 54) and oleic acid (peak 60), contributed the most in the discrimination of pericarps from leaves and seeds samples.

Table 1. Identified metabolites in *P. campechiana* organs; leaves (L), seeds (S) and pericarps (P) using UPLC-PDA-MS in negative ionization mode:

Peak No.	R_t (sec)	UV	Mol.Ion $[M-H]^-$	Elemental Composition	Error	MS^n	Identification	L	S	P
1	38.6	263	191.019	$C_6H_7O_7^-$	2	179, 96	(iso)citric acid	-	+	-
2	96.2	253/292	169.013	$C_7H_5O_5^-$	3	125	gallic acid	-	+	-
3	114.4	259/294	153.020	$C_7H_5O_4^-$	4.6	109	protocatechuic acid	-	+	-
4	139.6	272	609.120	$C_{30}H_{25}O_{14}^-$	0.9	593, 451, 305	(epi)gallocatechin dimer	+	-	-
5	164.2	273	305.066	$C_{15}H_{13}O_7^-$	1.9	261, 233, 169	(epi)gallocatechin	+	-	-
6	168.9	274	451.210	$C_{20}H_{35}O_{11}^-$	1.3	357, 293	unidentified	+	-	+
7	174.6	275	289.072	$C_{15}H_{13}O_6^-$	2	245, 137, 121	(epi)catechin	+	-	-
8	185.8	285	451.088	$C_{20}H_{19}O_{12}^-$	0.5	325, 299, 289	galloylated hexose derivative	+	-	-
9	186.1	277/320	411.224	$C_{17}H_{31}O_{11}^-$	5.5	248,161	isopentyl dihexoside	-	-	+
10	194.2	274	445.170	$C_{20}H_{29}O_{11}^-$	3.2	285, 199, 161	phenolic acid derivative	-	-	+
11	196	272	281.137	$C_{15}H_{21}O_5^-$	5.6	112	octyl gallate	-	+	-
12	196.2	275	457.007	$C_{22}H_{17}O_{11}^-$	1.3	289, 169	gallocatechin gallate	+	-	-
13	198	272	269.100	$C_{13}H_{17}O_6^-$	4.5	230, 174	benzyl hexoside	-	+	-
14	203.3	277/320	469.191	$C_{19}H_{33}O_{13}^-$	4.2	371, 161	p-coumarouly hexose derivative	-	-	+
15	210.3	298/355	479.080	$C_{21}H_{19}O_{13}^-$	4.7	317, 271, 179, 151	myricetin-3-*O*-hexoside	+	-	-
16	212.4	283/350	435.094	$C_{20}H_{19}O_{11}^-$	2.8	303, 285	taxifolin-3-*O*- pentoside	+	-	-
17	216.4	275/344	449.072	$C_{20}H_{17}O_{12}^-$	1.5	317, 271, 179, 151	myricetin-3-*O*- pentoside	+	-	-
18	219.4	280/307	425.202	$C_{18}H_{33}O_{11}^-$	0.4	249, 174	phenolic acid derivatives	-	-	+
19	224.8	260/344	463.088	$C_{21}H_{19}O_{12}^-$	1.3	317, 271, 179, 151	myricetin-3-*O*-rhamnoside	+	-	-
20	232.6	280	435.090	$C_{20}H_{19}O_{11}^-$	0.7	303, 285	taxifolin-3-*O*-pentoside isomer	+	-	-
21	233.9	289/333	303.052	$C_{15}H_{11}O_7^-$	2.4	285, 177	taxifolin	-	+	-
22	243.4	268/351	447.090	$C_{21}H_{19}O_{11}^-$	2.2	301, 179, 151	quercetin- 3-*O*-rhamnoside	+	-	+
23	250.1	274	419.090	$C_{20}H_{19}O_{10}^-$	0.4	287,331,229	eriodictyol -*O*-pentoside	+	-	-

Table 1. Identified metabolites in *P. campechiana* organs; leaves (L), seeds (S) and pericarps (P) using UPLC-PDA-MS in negative ionization mode (continued):

Peak No.	R_t (sec)	UV	Mol. Ion $[M-H]^-$	Elemental Composition	Error	MS^n	Identification	L	S	P
24	258.9	286	287.050	$C_{15}H_{11}O_6^-$	2.8	259,161	eriodictyol	-	+	-
25	282.8	288	493.290	$C_{24}H_{45}O_{10}^-$	4.3	334, 259	unidentified	-	+	-
26	288.5	287/367	301.035	$C_{15}H_9O_7^-$	0.1	179,151	quercetin	-	+	-
27	298.2	274	495.240	$C_{22}H_{39}O_{12}^-$	1.6	481, 225	unidentified	+	-	-
28	313.7	274	327.210	$C_{18}H_{31}O_5^-$	0.9	309, 281	trihydroxyoctadecadienoic acid ($C_{18:2}$)	+	-	-
29	316.4	277/288	353.219	$C_{16}H_{33}O_8^-$	4.4	327,285	unidentified	-	+	-
30	324.9	274	517.300	$C_{30}H_{45}O_7^-$	0.3	499,363,248	unknown triterpene	+	-	+
31	329.2	287	329.231	$C_{18}H_{33}O_5^-$	4.4	311, 283	trihydroxyoctadecenoic acid ($C_{18:1}$)	-	+	-
32	329.6	275	565.330	$C_{31}H_{49}O_9^-$	2.2	465, 397	unidentified	+	-	+
33	334.3	275	213.140	$C_{12}H_{21}O_3^-$	5.1	195,175	oxododecanoic acid($C_{18:0}$)	+	-	-
34	341.7	273	503.330	$C_{30}H_{47}O_6^-$	1	485,457,431, 363, 248	tetra hydroxy ursenoic acid	+	-	+
35	349.4	272	501.322	$C_{30}H_{45}O_6^-$	0.1	461, 377, 248	unknown triterpene	+	-	+
36	350.4	268/280	485.290	$C_{29}H_{41}O_6^-$	0	325, 309	unidentified	-	+	+
37	351	284	553.133	$C_{28}H_{25}O_{12}^-$	2.8	531, 303	unidentified	-	+	-
38	357.9	275	503.330	$C_{30}H_{47}O_6^-$	0.8	377, 248	tetra hydroxy ursenoic acid isomer	+	-	+
39	368	276	307.190	$C_{18}H_{27}O_4^-$	2.7	289, 261	hydroxy-oxo-octadecatrienoic acid ($C_{18:3}$)	+	-	-
40	386.5	Nd	503.000	$C_{30}H_{47}O_6^-$	2	485, 377	tetra hydroxy ursenoic acid isomer	+	-	+
41	391.9	275	305.170	$C_{18}H_{25}O_4^-$	2.1	287, 259	hydroxy-oxo-octadecatetranoic acid ($C_{18:4}$)	+	-	-
42	399	272	487.340	$C_{30}H_{47}O_5^-$	1.1	469, 377,248	trihydroxyursenoic acid	+	-	+
43	411.4	Nd	309.208	$C_{18}H_{29}O_4^-$	3.2	291, 263	hydroxy-oxo-octadecadienoic ($C_{18:2}$)	-	-	+
44	419.2	272	487.300	$C_{30}H_{47}O_5^-$	1.2	469, 248	trihydroxyursenoic acid isomer	+	+	+
45	428.3	Nd	313.237	$C_{18}H_{33}O_4^-$	2.2	295, 267	dihydroxyoctadecenoic acid ($C_{18:1}$)	-	+	-
46	436.7	Nd	311.220	$C_{18}H_{31}O_4^-$	2.3	293, 265	dihydroxyoctadecadienoic acid ($C_{18:2}$)	+	-	-
47	440.4	Nd	491.260	$C_{27}H_{39}O_8^-$	1.7	325, 277	unidentified	-	+	-

Table 1. Identified metabolites in *P. campechiana* organs; leaves (L), seeds (S) and pericarps (P) using UPLC-PDA-MS in negative ionization mode (continued):

Peak No.	R_t (sec)	UV	Mol.Ion $[M-H]^-$	Elemental Composition	Error	MS^n	Identification	L	S	P
48	461.9	Nd	293.210	$C_{18}H_{29}O_3^-$	1.3	275, 247	hydroxyoctadecatrienoic acid ($C_{18:3}$)	+	-	+
49	471	Nd	505.210	$C_{37}H_{29}O_2^-$	10.8	311, 286	unidentified	-	+	-
50	485.5	225	295.220	$C_{18}H_{31}O_3^-$	2.9	277, 249	hydroxyoctadecadienoic acid ($C_{18:2}$)	+	+	+
51	490.5	Nd	311.160	$C_{13}H_{27}O_8^-$	4.9	243	unidentified	+	+	+
52	506.7	Nd	471.348	$C_{30}H_{47}O_4^-$		433,407,248	unknow triterpene	+	+	+
53	572.7	Nd	271.220	$C_{16}H_{31}O_3^-$	0.1	253,225,197	hydroxy palmitic acid ($C_{16:0}$)	-	+	+
54	578.1	Nd	277.218	$C_{18}H_{29}O_2^-$	3.1	233, 183	linolenic acid isomer ($C_{18:3}$)	-	+	+
55	587.6	Nd	455.350	$C_{30}H_{47}O_3^-$	2.8	407,363, 248	ursolic acid	+	+	+
56	593.6	Nd	253.216	$C_{16}H_{29}O_2^-$	2.2	183	palmitoleic acid ($C_{16:1n-9}$)	-	-	+
57	610.1	Nd	279.230	$C_{18}H_{31}O_2^-$	2.2	261, 183	linoleic acid ($C_{18:2\ n-6}$)	-	+	+
58	623.6	Nd	453.330	$C_{30}H_{45}O_3^-$	0.5	441, 311, 248	unkwon triterpene	-	+	-
59	639.1	Nd	255.234	$C_{16}H_{31}O_2^-$	4.2	237	palmitic acid ($C_{16:0}$)	+	+	+
60	648.2	Nd	281.240	$C_{18}H_{33}O_2^-$	2	263,183, 127	oleic acid ($C_{18:1\ n-9}$)	+	+	+
61	681.5	Nd	327.289	$C_{20}H_{39}O_3^-$	2.5	309, 281,253	hydroxyeicosanoic acid ($C_{20:0}$)	-	-	+
62	685.2	Nd	383.350	$C_{24}H_{47}O_3^-$	2.5	365, 283	hydroxy fatty acid	-	+	-
63	688.3	Nd	283.260	$C_{18}H_{35}O_2^-$	0.2	265	stearic acid ($C_{18:0}$)	+	-	-

Note: (+) and (-) indicate presence and absence of a metabolite, respectively; L, leaves; S, seeds; P, pericarps; Nd, not detected; Rt, retention time.

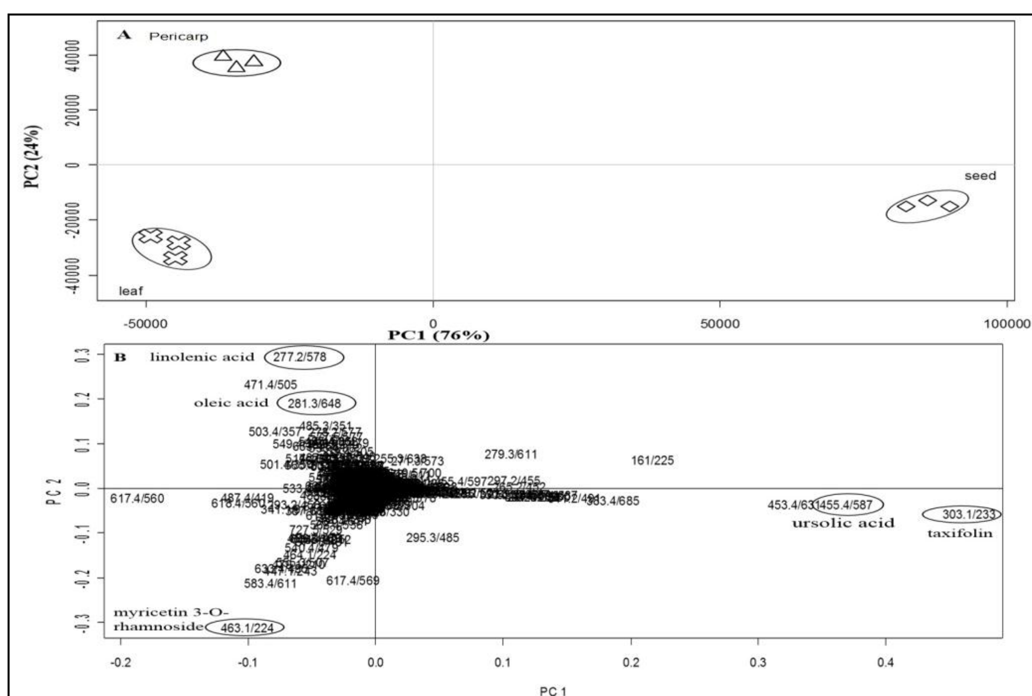

Figure 1. Principal component analysis (PCA) of *P. campechiana* (Kunth) Baehni leaves, seeds and pericarps analysed by UPLC-qTOF-MS; A: scoring plot, B: loading plot.

3.3. Antioxidant activity

The IC_{50} values of ethanol extracts of ascorbic acid and *P. campechiana* leaves, seeds and pericarps were 19.37±4.53, 73.68±8.12, 235.615±10.3 and 858.952±18.16 µg/mL respectively. Consequently, the ethanol extract of the leaves had higher antioxidant activity than that of the seeds and pericarps.

3.4. Antimicrobial activity

P. campechiana extracts (Table 2) showed moderate to strong antimicrobial activity against three bacterial strains; *S. pneumonia, B. subtili and E. coli* and three fungal strains; A. *fumigatus*, *S. racemosum and G. candidum* strains out of the eight tested microorganisms (at the concentration of 5mg/ml) and all tested samples were inactive against *P. aeruginosa* and *C. Albicans.* Therefore these two strains were either resistant to the tested extracts or needed higher concentrations to be assessed. The minimum inhibitory concentration (MIC) values varied from 1.95 to 15.63 µg/ml, respectively for the three extracts (Table 3).

The lowest MIC value (1.95 µg/ml) was obtained with leaves ethanol extract against *B.subtilis.* The pericarps ethanol extract MIC was the same as that of the reference drug amphotericin B for *S.racemosum* fungi which was 3.9 µg/ml.

Antimicrobial and antioxidant activities of flavonoids and phenolic acids were previously established [20, 21]. Quercetin (peak 26) and myricetrin-3-*O*-rhamnoside (peak 19) were found to be active against *B.subtilis* and *E.coli* [22]. Furthermore, pentacyclic triterpenes and their derivatives had been proved to have antibacterial and antioxidant activities [23, 24] like ursolic acid (peak 55)[25]. Hydroxyl fatty acids derivatives were also reported to have an antimicrobial activity [26, 27]. Moreover, the higher antioxidant activity of leaves correlates well with PCA results of having flavonol glycosides as the discriminant metabolites in contrast to fatty acids and steroids present in seeds and pericarp.

Table 2. Antimicrobial activity of P. campechiana leaves, seeds and pericarps ethanol extracts.

Microorganisms	Diameter of zone of inhibition* (mm) ± Standard deviation			
	Seeds	Leaves	Pericarps	Standard
Fungi:				Amphotericin B
A. fumigatus	15.6± 0.44	17.6 ± 0.58	16.3± 0.44	23.7± 0.1
S.racemosum	16.2 ± 0.58	18.2 ± 0.25	18.6± 0.58	19.7± 0.2
G.candidum	17.9 ± 0.37	20.3 ± 0.38	19.8 ± 0.25	28.7± 0.2
C.albicans	NA	NA	NA	25.4± 0.1
G^+ Bacteria:				Ampicillin
S.pneumoniae	16.9 ± 0.44	20.3 ± 0.43	17.3± 0.63	23.8± 0.2
B.subtilis	19.3± 0.25	21.4 ± 0.53	20.2± 0.44	32.4± 0.3
G^- Bacteria:				Gentamicin
P.aeruginosa	NA	NA	NA	17.3± 0.1
E.coli	14.9± 0.44	16.9 ± 0.25	15.9± 0.37	19.9± 0.3

* Mean zone of inhibition in mm using a concentration of 5 mg/mL of tested samples. G^+ (gram positive), G^- (gram negative), *A. fumigatus (Aspergillus fumigatus), S.racemosum (Syncephalastrum racemosum), G.candidum (Geotricum candidum),C. Albicans (Candida albicans), S.pneumoniae J (Streptococcus pneumoniae), B.subtilis (Bacillis subtilis), P. aeruginosa (Pseudomonas aeruginosa), E.coli (Escherichia coli),* NA, no activity

Table 3. Minimum inhibitory concentrations (µg /ml) of P. campechiana leaves, seeds and pericarps ethanol extracts.

Microorganisms	Minimum inhibition concentration (MIC) (µg/mL)			
	Seeds	Leaves	Pericarps	Standard
Fungi				Amphotericin B
A.fumigatus	15.63	7.81	15.63	0.98
S.racemosum	15.63	7.81	3.9	3.9
G.candidum	7.81	3.9	3.9	0.49
G^+ Bacteria				Ampicillin
S.pneumoniae	15.63	3.9	15.63	0.98
B.subtilis	3.9	1.95	3.9	0.24
G^- Bacteria				Gentamicin
E.coli	15.63	15.63	15.63	3.9

4. Conclusions

This study is the first report of *P. campechiana* leaves, seeds and periacrps metabolites profiling using UPLC-MS. Sixty-three peaks were characterized, of which 17 phenolic compounds, 18 fatty acids and 6 triterpenes were identified. Flavonol glycosides were found highest in the leaves sample and contributed the most to the discrimination between organs. All organs extract of *P. campechiana* under investigation exhibited significant antioxidant activity against DPPH radical scavenging activity. The plant extracts showed moderate to strong antimicrobial activity against 3 bacterial and 3 fungal strains.

5. Conflicts of interest

The authors declare no conflict of interest.

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7. Supplementary Material

Figures of *P. campechiana* organs and the UPLC-ESI-MS total ion chromatogram were provided as supplementary file.

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