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ORIGINAL ARTICLE

Profiling of Antiviral and Antioxidant Phytochemicals of *Pterocephalus frutescens* Hochist. using High-Resolution Ultra-Performance Liquid Chromatography/Quadrupole Time-of-Flight Mass Spectrometer

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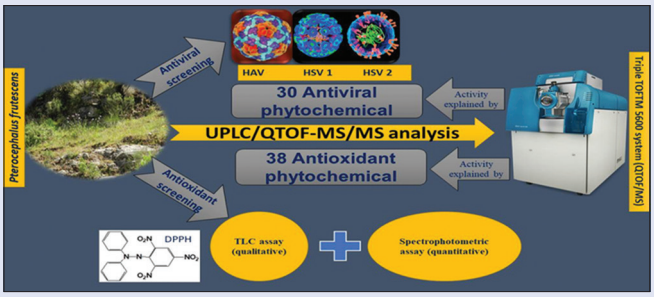
ABSTRACT

Background: Medicinal plant extracts and herbal preparations are complex mixtures of active and ballast substances, which may contain in frequently up to several hundreds of different constituents, with no exactly defined structures at which chromatography undoubtedly is a fundamental tool to overcome the challenges of these phytoanalytics. **Aim of the Study:** To investigate *Pterocephalus frutescens* aerial part extracts biologically and chemically and the correlation of biologically active constituents with their corresponding activities. **Materials and Methods:** Ultra-performance liquid chromatography/quadrupole time-of-flight mass spectrometer with principal component statistical analysis was used for the identification of phytoconstituents, while spectrophotometer was used for quantitative analysis and both qualitative and quantitative antioxidant effects using stable radical diphenylpicrylhydrazyl, and the cytopathic effect inhibition assay was used for antiviral screening. **Results and Conclusion:** *P. frutescens* extracts exhibited antioxidant and antiviral activities along with tentative identifying of 46 compounds from which 38 compounds were correlated with either antiviral and/or antioxidant activities, besides the quantification of phenolic, flavonoid, phenylethanoid, and iridoid contents.

Key words: Antioxidant, antiviral, mass spectrometry, principal component analysis, ultra-performance liquid chromatography

SUMMARY

- Biological investigation of *Pterocephalus frutescens* growing in Yemen showed both antiviral and antioxidant activities at which the responsible secondary metabolites for these activities were identified using rapid and accurate chromatographic analysis method as well. Quantification of the most important chemical classes was done for further confirmation and explaining of these activities.



Abbreviations used: BHT: Butylated hydroxy toluene; DPPH: Diphenylpicrylhydrazyl; ESI: Electrospray ionization; IC_{50} value: Inhibition concentration of sample at 50% fall in absorbance; HAV: Hepatitis virus type A; HSV-I: Herpes simplex virus type I; HSV-II: Herpes simplex virus type II; PCA: Principal component analysis; RCMB: Regional Center for Mycology and Biotechnology; RT: Retention time; UPLC/QTOF-MS/MS: Ultra-performance liquid chromatography/quadrupole time-of-flight mass spectrometer.

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INTRODUCTION

Quadrupole time-of-flight mass spectrometer attached to ultra-performance liquid chromatography (UPLC/QTOF-MS/MS) is nearly standard equipment in a modern analytical laboratory and is indispensable for scientific research on a high level as screening and identifying compounds in different medicinal plants in a fast and accurate manner.[1] The honeysuckle family or *Caprifoliaceae* (*Dipsacaceae*) family is consisting of about 860 species and 42 genera, which distributed in Europe, Asia, and Africa. Some species of this family have been naturalized in other places.[2] *Pterocephalus*, comprising 25 species, ranges from the Mediterranean, to Central Asia, the Himalayas, Western China, and tropical Africa.[3] Plants of the genus *Pterocephalus* are widely used in folkloric medicines, around the entire world, as anti-inflammatory, analgesic, antihepatotoxic, antioxidant, antibacterial, spasmolytic, hemostatic, and astringent properties.[3-6] Several active constituents have been isolated from genus *Pterocephalus* including iridoid, hydroxycinnamic acid esters, phenolic glycosides, lignans, triterpenoid saponins, and flavonoid C-glycosides.[7-10] Surprisingly, *Pterocephalus frutescens* was not evaluated phytochemically and/or biologically, up to this date. In the present work, it is worthy to give an idea about the evaluation of antiviral and antioxidant activities and illustrating secondary metabolites responsible for both activities using reliable, fast, accurate, simple, and reproducible technique UPLC/

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QTOF-MS/MS in association with principal component analysis (PCA) as a qualitative technique. Quantitative measurements of polyphenolic content are of interest as a lot of findings have been reported earlier, suggesting a relationship between total polyphenols content and health effects,[11] mainly antioxidant activity which tested in our work.

MATERIALS AND METHODS

Plant materials

The aerial parts of *P. frutescens* Hochist. were collected in June 2017 at flowering stage from Jabal An-Nabi Shu'ahyb, Sanaa, Al Yemen. The plant was collected, identified, and authenticated by Dr. Abdo H. Marey, Professor of Botany and Plant Taxonomy, Faculty of Science, Al-Azhar University, Cairo, Egypt. A voucher sample was kept in the Herbarium of Faculty of Pharmacy, Al-Azhar University, Cairo, Egypt.

Extraction and isolation

Air-dried powdered aerial part plant material of *P. frutescens* (250 g) was extracted by Soxhlet apparatus with n-hexane (2 × 2 L) and then with methanol (3 × 3 L) to afford (7.5 and 35 g), respectively, after evaporation under vacuum by a rotatory evaporator (BUCHI Rotavapor® R-210/R-215, Germany). From methanol extract, 0.5 g was subjected to the quantitative determination of polyphenolics and UPLC/QTOF-MS/MS analysis; 1 g reserved for biological investigations and the remaining residue were dissolved in water and then partitioned successively with ethyl acetate (2 × 200 mL) and n-butanol saturated with water (3 × 200 mL) to afford light brown masses (8.5 and 11 g), respectively.

Quantitative determination of polyphenolics

Quantitative determination of phenolic, flavonoid, phenylethanoid, and iridoid contents using selective method for each phytochemical class[12] was done using Genesys Spectrophotometer (Milton Roy, INC., Rochester, NY, USA). The reagents used for each were Folin-Ciocalteu (Sigma Chemical Co., St. Louis, MO, USA) and gallic acid (Merck, Darmstadt, Germany) for phenolic content quantification; quercetin (Merck Co. Darmstadt, Germany) and aluminum chloride (Merck, Darmstadt, Germany) for flavonoid content quantification, Arnow's reagent and verbascoside (Sigma Chemical Co., St. Louis, MO, USA) for phenylethanoid content quantification; and Trim and Hill reagent and Herbagoside (Sigma Chemical Co., St. Louis, MO, USA) for iridoid content quantification.

Antiviral assay

Screening of antiviral activity was achieved using cytopathic effect (CPE) inhibition assay against hepatitis virus type A (HAV) and herpes simplex virus type I (HSV-I) and II (HSV-II) at the Regional Center for Mycology and Biotechnology, Al-Azhar University.[13] Incubation was done at 37°C for 5 days and observation was made every 24 h to record the CPE. Antiviral activity was determined by the inhibition of CPE compared with control. The results were expressed as:

–ve: No inhibition, +: 25% inhibition, ++: 50 inhibition, +++: 75% inhibition, and ++++: 100% inhibition of CPE.

Antioxidant potentials

Determination of the antioxidant effect of the different extracts of *P. frutescens* was done using stable radical diphenylpicrylhydrazyl (DPPH) (Sigma Chemical Co., St. Louis, MO, USA). Both qualitative and quantitative antioxidant effects were experimented.

TLC assay (qualitative method)

20 µL aliquot of each extract was spotted on silica gel plates and developed using butanol:acetic acid:water (4:1:5) as a mobile phase; after development, the dried TLC plates were sprayed with 0.2% DPPH solution (Sigma Chemical Co., St. Louis, MO, USA) in methanol and examined after 30 min, where active extracts as antioxidants appeared as yellow spots against purple background.[14]

Spectrophotometric assay (quantitative method)

The test was carried out by adding 160 µL (0.1 mM) DPPH solution in methanol to 40 µL of each sample solution in methanol. After 30 min, the absorbance was measured at 517 nm. Lower absorbance of the reaction mixture indicates higher free radical scavenging activity according to recent published method.[15] Butylated hydroxy toluene (BHT) (Sigma Chemical Co., St. Louis, MO, USA) in methanol was used as standard. The capability of scavenging the DPPH radical was calculated using the following equation:

$$\text{DPPH scavenging effect (\%)} = [(A_{control} - A_{sample})/_{Acontrol}] - 100.$$

The the inhibition concentration of sample at 50% fall in absorbance of DPPH (IC_{50}) value was used to compare the DPPH scavenging activity of the highest free radical scavenging activity extract with two standard controls, BHT and quercetin using various concentrations in methanol according to previously discussed method.[14]

Ultra-performance liquid chromatography/quadrupole time-of-flight mass spectrometer analysis

The lyophilized plant extracted sample injected in the positive mode at which separation of small molecules was carried out on a Axion AC system (Kyoto, Japan) connected with an auto sampler system, an In-Line filter disks precolumn (0.5 µm × 3.0 mm, Phenomenex, USA), and a Xbridge C_{18} (3.5 µm, 2.1 mm × 50 mm) column (Waters Corporation, Milford, MA, USA) maintained at 40°C and a flow rate of 300 µL/min. The mobile phase composition and the gradient elution were performed according to the method discussed in Fayek *et al.*[16] LC-MS grade acetonitrile and gradient solvents including isopropanol, methanol, dichloromethane, and ethyl acetate were provided by Thermo-Fisher (Thermo Fisher Scientific, USA). Formic acid 98%, ammonium hydroxide, ammonium formate, and ammonium acetate were purchased from Sigma-Aldrich (Sigma-Aldrich Co., Louis St., MO, USA). MS was performed on a TripleTOF™ 5600 System QTOF/MS with a Duo-Spray™ source operating in the electrospray ionization mode (AB SCIEX, Concord, Canada). The sprayer capillary and declustering potential voltages were 4500 and 80 V. The source temperature, collision energy, ion tolerance, and Triple TOF 5600 operation parameters were reported in Fayek *et al.*[16] The method consisted of high-resolution survey spectra from 50 to 1100 *m/z*, and the mass spectrometer was operated in a pattern where a 50-ms survey scan was detected. Subsequently, the top 15 intense ions were selected for acquiring MS/MS fragmentation spectra after each scan.[16]

Data were processed using MS-DIAL 3.96 software for nontargeting small molecule comprehensive analysis of the sample. According to the

Table 1: Quantitative determination of total phenolic, flavonoid, phenylethanoid, and iridoid contents of the methanol extract of *Pterocephalus frutescens*

Extract yield (%)	Phenolic mg (%)	Flavonoids µg (%)	Phenylethanoids µg (%)	Iridoids µg (%)
17	98.93±1.35	75.93±0.80	212±4.4	40.56±2

acquisition mode, ReSpect Positive (2737 records), MassBank Positive (8068 records), and GNPS Positive (8782 records) databases were used as reference databases. The search parameters were set according to Tsugawa *et al.*[17] The MS-DIAL output was used to make manual annotation and confirmation of the identified peaks using PCA, which is an unsupervised clustering method requiring little prior knowledge of the data set.[18] PCA scores' plot analysis was performed in Excel using a macro written by Tsugawa *et al.*[17] and is available for free.

RESULTS AND DISCUSSION

Quantitative determination of polyphenolics

The results of this study provide evidence that the methanol extract has phenolic, flavonoid, phenylethanoid, and iridoid compounds in relatively high amounts [Table 1]; therefore, this species may have a great relevance in the prevention and therapies of diseases in which oxidants or free radicals are implicated.

Antiviral assay

The results of antiviral screening of different extracts of *P. frutescens* [Table 2] showed that the observed activity of both hexane and ethyl acetate extracts on (HAV, HSV-I, and HSV-II) was attributed to flavonoids identified in the plant extract by UPLC/QTOF-MS/MS, due to inhibitory structure–function relationship between flavonoids and various enzymes associated with the life cycle of viruses.[19] The chromatographic UPLC/QTOF-MS/MS method revealed the presence of 30 compounds with antiviral potentials which were reported previously as shown in Table 6. For example, amentoflavone has activity against HSV-I and HSV-II with EC_{50} values of 17.9 and 48.0 mg/ml, respectively, as well apigenin which reported to have the highest inhibition against HSV-I with the widest therapeutic range (0.4–1.6 µg/mL) and loliolide which has both anti-HSV and anti-hepatitis virus type C (HCV).[30,53,57] Phenolic and iridoid compounds also play an important role as antiviral agents; the presence of 98 mg% of phenolic compounds along with 40 µg% of iridoid compounds explains both hexane and ethyl acetate antiviral activities.[31]

Table 2: Antiviral screening of different extracts of *Pterocephalus frutescens*

Sample	Antiviral effect on		
	HAV	HSV-I	HSV-II
Hexane	+++	+	++
Ethyl acetate	+	+++	+
n-Butanol	+	−ve	−ve

HAV: Hepatitis virus type A; HSV-I: Herbs simplex virus type I; HSV-II: Herbs simplex virus type II

Table 3: Calculated percent free radical scavenging activity of the different extracts of *Pterocephalus frutescens* and butylated hydroxy toluene

Tested material	Concentration (mg/ml)	DPPH scavenging activity (%)
Hexane extract	0.1	29.93±0.23
Ethyl acetate	0.1	89.93±1.23
n-Butanol	0.1	44.12±o. 88
BHT	0.4	93.00±0.50

The reported values are mean±SD of three different readings. SD: Standard deviation; BHT: Butylated hydroxy toluene; DPPH: Diphenylpicrylhydrazyl

Table 4: Diphenylpicrylhydrazyl free radical scavenging activity IC_{50} (mg/ml) of *Pterocephalus frutescens* ethyl acetate extract, butylated hydroxy toluene, and quercetin

Tested material	IC_{50} (mg/ml)
Ethyl acetate extract	0.050
BHT	0.054
Querectin	0.010

Each value in the table is the mean of three results. BHT: Butylated hydroxy toluene

Antioxidant potentials

Qualitative TLC-DPPH assay of the tested extracts showed that they are active as DPPH scavengers appearing as zones with different R_f values in the chromatogram; then, quantitative estimation of their antioxidant potentials at dose level of 0.1 mg/ml revealed that they possess significant DPPH free radical scavenging activity which is proven comparable to the reference synthetic antioxidant (BHT). This activity may be explained by high polyphenolic compound contents[11] [Table 1] and further confirmed by tentatively identification of 38 antioxidant compounds [Table 6], which are distributed in different plant extracts. For example, flavonoid aglycons (apigenin, kaempferol, and quercetin) concentrated in hexane extract;[64] flavonoid glycosides and other phenolic compounds concentrated in both ethyl acetate and n-butanol extracts. The most promising extract was ethyl acetate with 89.93% DPPH scavenging activity [Table 3] and an IC_{50} of 0.05 mg/mL nearly similar to that of BHT [Table 4].

Interpretation of ultra-performance liquid chromatography/quadrupole time-of-flight mass spectrometer results

A total of 46 compounds were tentatively identified in Table 5; whereas the data were obtained from PCA scores' plot analysis from MS-DIAL exhibited a pseudomolecular ion peaks with different adducts. The compounds include amino acids (3), coumarins (4), simple phenolic compounds (9), and flavonoids of different subclasses (23) and of other different chemical classes (7) [Table 6].

Compound identification based on the data of MS-DIAL includes assigning of the compounds with the highest total score above 70%, dissociating the link between precursors and their fragment ions, compromising the molecular identification process, and expressing the fragment presence by %. These two criteria were achieved by DIA methods; reducing false-negative results in contrast to traditional data-dependent MS/MS acquisitions.[17] The further confirmation of the compounds was done by comparison with METLIN (http://metlin.scripps.edu), a freely accessible web-based.[65] In addition to traditional methods of confirmation utilizing retention time, comparison of fragmentation pattern of each chemical class with literature reviews and high-resolution mass error values calculations using the following formula:

Error = (Experimental mass − Calculated mass)/Experimental mass.[66]

To improve mass accuracy, no error exceeds 14 ppm.[18] For comparison with literatures of amino acids, the characteristic *m/z* was due to the loss of 18 and 46 amu appears to be prevalent so that the $[(M + H)-H_2O]+$ and $[(M + H)-CO_2H_2]+$, respectively.[67] The losses of hydroxyl, methyl, or carboxylic groups were helpful in the identification of the specific phenolic compounds to form fragments at *m/z* [M + H-16]+, [M + H-14]+, or [M + H-44]+, respectively.[68] Coumarin derivatives common fragment ions attributed to loss of CO from the pyrone ring to form a benzofuran ring at *m/z* [M + H-28]+ which is further fragmented by the loss of H_2O moiety to form a fragment at *m/z* [M + H-18]+. Another fragment ions have been attributed to generate a ketene ion at *m/z* [M + H-72]+. The hydroxy-substituted coumarins showed fragmentation process with the loss of C_2H_2O to generate a signal at *m/z* [M + H-42]+.[69] The fragmentation patterns of

Table 5: Metabolites identified from methanol extract of *Pterocephalus frutescens* using ultra-performance liquid chromatography/quadrupole time-of-flight mass spectrometer analysis in positive ionization mode

Tentatively identified compound and its adduct ion	RT (min)	Adduct (*m/z*)	Error	Adduct molecular formula	Fragment presence (%)	Total score (%)	MSMS data
Choline $[M]^+$	0.4960	104.107	−9	$C_5H_{14}NO^+$	100	93	58.06512:9562
							60.07928:7532
L-(-)-Phenylalanine $[M+H]^+$	0.5212	166.087	−1	$C_9H_{12}NO_2$	100	87	104.10532:4101 59.05287:107 120.0795:322
L-Tyrosine $[M+H]^+$	0.5212	182.08	−8	$C_9H_{12}NO_3$	100	78	166.07953:251 96.0414:914
Daidzein $[M+H]^+$	0.8677	255.063	−10	$C_{15}H_{11}O_4$	50	76	182.0784:888 81.03855:36
Esculin $[M+H]^+$	0.8810	341.088	−2	$C_{15}H_{17}O_9$	50	78	255.09662:72 179.03537:537
3-Formylindole $[M+H]^+$	1.0360	146.059	−8	C_9H_8NO	100	88	118.06079:107
Chlorogenic acid $[M+H]^+$	1.0525	355.103	−1	$C_{16}H_{19}O_9$	60	86	146.06093:692 163.03654:31858
Daphnetin $[M+H]^+$	1.4576	179.033	−6	$C_9H_7O_4$	71	91	77.03682:251
							123.04043:541
Esculetin $[M+H]^+$	1.4576	179.035	5	$C_9H_7O_4$	55	92	133.02432:394 179.02765:685 123.04058:358
Trigonelline $[M+H]^+$	1.6823	138.054	−9	$C_7H_8NO_2$	50	77	179.0316:765 94.654:378
Orientin $[M+H]^+$	2.8272	449.107	−3	$C_{21}H_{21}O_{11}$	100	93	78.034:215 299.05695:29478
							329.06662:33877
Isoorientin $[M+H]^+$	2.8272	449.107	−3	$C_{21}H_{21}O_{11}$	90	95	449.10669:10246 299.05695:29478
							329.06662:33877
							353.06964:15811
Scopoletin $[M+H]^+$	3.0741	193.048	−9	$C_{10}H_9O_4$	100	96	449.10669:10246 122.03386:14002
							133.0276:31412
							150.02983:13292
							178.02394:25020
Methyl chlorogenate $[M+H]^+$	3.2349	369.117	−4	$C_{17}H_{21}O_9$	46	86	193.04594:23932 135.04518:1063
							145.02919:1355
Vitexin $[M+H]^+$	3.5812	433.109	−11	$C_{21}H_{21}O_{10}$	67	84	163.0368:11366 283.06787:577
							313.06775:478
Quercetin $[M+H]^+$	3.7014	303.051	1	$C_{15}H_{11}O_7$	75	81	433.11127:614 137.02473:251
							229.03818:107
							303.06058:322
Hyperoside $[M+H]^+$	3.7014	465.101	−5	$C_{21}H_{21}O_{12}$	64	85	303.05075:17539
Delphinidin-3-O-beta-glucopyranoside $[M]^+$	3.7014	465.101	−5	$C_{21}H_{21}O_{12}^+$	100	90	303.05075:17539
Loliolide $[M+H]^+$	3.8109	197.117	−6	$C_{11}H_{17}O_3$	80	92	91.05166:6749
							105.0701:6377
							133.09926:15385
							135.1138:6256
							161.09511:6840
							179.10701:11231
							197.11652:8264

Contd...

Table 5: Contd...

Tentatively identified compound and its adduct ion	RT (min)	Adduct (*m/z*)	Error	Adduct molecular formula	Fragment presence (%)	Total score (%)	MSMS data
Swertisin $[M+H]^+$	3.8230	447.126	−8	$C_{22}H_{23}O_{10}$	94	88	297.07745:1430
							327.09222:683
							351.08789:706
							381.09201:507
							393.09308:470
							447.12817:509
Luteolin-4'-O-Glucoside $[M+H]^+$	3.8354	449.108	−2	$C_{21}H_{21}O_{11}$	60	89	287.05365:34161
							449.10693:2446
Cyanidin-3-glucoside $[M]^+$	3.8354	449.108	−2	$C_{21}H_{21}O_{11}$	100	93	287.05365:34161
							449.10693:2446
Riboflavin monophosphate $[M+H]^+$	3.8965	457.118	11	$C_{17}H_{22}N_4O_9P$	50	84	457.09589:179
S-Adenosyl-L-homocysteine $[M+H]^+$	3.9950	385.125	−11	$C_{14}H_{21}N_6O_5S$	50	85	384.94974:73
							385.13263:402
Kaempferol-3-O-rutinoside $[M+H]^+$	4.2938	595.168	2	$C_{27}H_{31}O_{15}$	50	84	287.05399:107
							595.0423:401
							595.18005:465
Kaempferol $[M+H]^+$	4.3060	287.057	4	$C_{15}H_{11}O_6$	100	84	287.04935:322
Apigenin-7-O-glucoside (Cosmosiin) $[M+H]^+$	4.5548	433.112	−4	$C_{21}H_{21}O_{10}$	100	92	271.06265:7842
							433.11679:1154
Apigenin-4`-O-glucoside $[M+H]^+$	4.5548	433.112	−4	$C_{21}H_{21}O_{10}$	60	83	271.06265:7842
							433.11679:1154
Rosmarinic acid $[M+H]^+$	4.5673	361.094	3	$C_{18}H_{17}O_8$	50	81	163.04037:251
Isochlorogenic acid $[M+H]^+$	4.6668	517.131	−7	$C_{25}H_{25}O_{12}$	83	88	145.02548:7914
							163.03645:104570
							499.12427:8671
Trans-cinnamic acid $[M+H]^+$	4.8528	149.06	−4	$C_9H_9O_2$	100	72	59.04857:179
							61.02662:179
							65.0372:251
							73.06177:215
							77.03946:215
							91.05447:179
							149.02849:287
							149.05263:322
3,5-Dicaffeoyl quinic acid $[M+H]^+$	5.4073	517.131	−6	$C_{25}H_{25}O_{12}$	60	73	163.04025:2259
Luteolin $[M+H]^+$	5.8146	287.054	−5	$C_{15}H_{11}O_6$	100	91	135.04178:465
							153.01608:994
							287.04898:7619
Ferulic acid $[M+H]^+$	6.0112	195.065	−6	$C_{10}H_{11}O_4$	50	81	109.03516:36
							195.17871:143
Tiliroside $[M+H]^+$	6.0607	595.146	2	$C_{30}H_{27}O_{13}$	100	91	147.04596:7941
							287.05377:1979
							291.08218:769
							309.09784:801
Genistein $[M+H]^+$	6.7017	271.063	9	$C_{15}H_{11}O_5$	44	83	253.08009:146
							271.04874:474
Apigenin $[M+H]^+$	6.7017	271.063	9	$C_{15}H_{11}O_5$	50	79	253.08009:146
							271.04874:474
Kaempferol-3-O-glucoside $[M+H]^+$	7.7500	449.117	14	$C_{21}H_{21}O_{11}$	50	79	287.05383:72
							449.11914:179
Amentoflavone $[M+H]^+$	8.0713	539.101	6	$C_{30}H_{19}O_{10}$	50	86	377.06702:430
							539.1004:6447
Quercetin -3-O-rutinoside (Rutin) $[M+H]^+$	8.1210	611.168	12	$C_{27}H_{31}O_{16}$	54	78	303.0516:252
Cyanidin-3, 5-di-O-glucoside $[M]^+$	8.6066	611.155	−11	$C_{27}H_{31}O_{16}^+$	61	74	449.1080:157
							287.0555:321
Hesperidin $[M+NH4]^+$	8.6314	628.23	9	$C_{28}H_{34}O_{15}NH_4$	72	87	611.15277:12205
Benzyl acuminose $[M+Na]^+$	10.5945	425.139	−8	$C_{18}H_{26}O_{10}Na$	50	85	425.13846:541

Contd...

Table 5: Contd...

Tentatively identified compound and its adduct ion	RT (min)	Adduct (*m/z*)	Error	Adduct molecular formula	Fragment presence (%)	Total score (%)	MSMS data
Butanone, 4-[3-(beta-D-glucopyranosyloxy)-4-hydroxy-2,6,6-trimethyl-1-cyclohexen-1-yl] $[M+Na]^+$	12.9701	411.196	−8	$C_{19}H_{32}O_8Na$	50	85	411.16925:107
2-acetoxy-4-pentadecylbenzoic acid $[M+Na]^+$	21.4543	413.266	−3	$C_{24}H_{38}O_4Na$	50	87	413.26782:1527
Betaine $[M+H]^+$	26.1427	118.085	−12	$C_5H_{12}NO_2$	100	95	58.06506:836 118.08548:442

RT: Retention time; MSMS: Mass spectrometry/mass spectrometry

Table 6: The suspected biological active metabolites and their chemical class

Compound	Chemical class	Antiviral reference	Antioxidant reference	Compound	Chemical class	Antiviral reference	Antioxidant reference
Choline	Amino alcohol	-	[20]	S-Adenosyl-L-homocysteine	Organic sulfide	-	-
L-(-)-phenylalanine	Amino acid	-	-	Kaempferol-3-O-rutinoside	Flavonoid	[21]	[22]
L-Tyrosine	Amino acid	-	-	Kaempferol	Flavonoid	[23]	[24]
Daidzein	Flavonoid	-	[25]	apigenin-7-O-glucoside (Cosmosiin)	Flavonoid	[26]	[26]
Esculin	Coumarin	-	[27]	apigenin-4`-O-glucoside	Flavonoid	-	[28]
3-formylindole	Simple indole	-	-	Rosmarinic acid	Phenolic compound	[29]	[29]
Chlorogenic acid	Phenolic compound	[30,31]	[29]	Isochlorogenic acid	Phenolic compound	[32]	[32]
Daphnetin	Coumarin	-	[33]	Trans-Cinnamic acid	Phenolic compound	[34]	[35]
Esculetin	Coumarin	[36]	[37]	3,5-Dicaffeoyl quinic acid	Phenolic compound	[29]	[29]
Trigonelline	Alkaloid	[38]	[39]	Luteolin	Flavonoid	[40]	[41]
Orientin	Flavonoid	[42,43]	[42,44]	Ferulic acid	Phenolic compound	-	[45]
Isoorientin	Flavonoid	[46]	[47]	Tiliroside	Flavonoid	[48]	[48]
Scopoletin	Coumarin	-	[49]	Genistein	Flavonoid	[30]	[24]
Methyl chlorogenate	Phenolic compound	-	[50]	Apigenin	Flavonoid	[30]	[24]
Vitexin	Flavonoid	[43]	[44,47]	Kaempferol-3-O-glucoside	Flavonoid	[51]	[22]
Quercetin	Flavonoid	[30,34,52]	[24,52]	Amentoflavone	Flavonoid	[53,54]	[54]
Hyperoside	Flavonoid	[55]	[56]	Quercetin -3-O-rutinoside (Rutin)	Flavonoid	[21]	[24]
Delphinidin-3-O-beta-glucopyranoside	Flavonoid	[52]	[52]	Cyanidin-3, 5-di-O-glucoside	Flavonoid	[52]	[52]
Loliolide	Monoterpen lactone	[57,58]	[59]	Hesperidin	Flavonoid	[60]	[60]
Swertisin	Flavonoid	[46]	[47]	Benzyl acuminose	Benzyl glycoside	-	-
Luteolin-4`-O-glucoside	Flavonoid	-	[41]	Butanone, 4-[3-(beta-D-glucopyranosyloxy)-4-hydroxy-2,6,6-trimethyl-1-cyclohexen-1-yl]	megastigmane glucoside	-	-
Cyanidin-3-glucoside	Flavonoid	[52]	[52]	2-acetoxy-4-pentadecylbenzoic acid	Phenolic compound	-	-
Riboflavin monophosphate	Flavin mononucleotide	[61]	-	Betaine	Amino acid	[62]	[63]

flavonoids were very diverse according to subclass (flavones, flavonols, flavanones, and/or isoflavones) and according to glycosylation pattern which previously published in details.[70-72]

CONCLUSION

Our results showed that *P. frutescens* has a high interesting value for pharmaceutical industries as it revealed antiviral and antioxidant activities. Tentative profiling of different secondary metabolites of *P. frutescens* aerial parts was done in a fast and accurate manner without tedious routine experiments, which could be applicable for chemical investigation of different plant materials.

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Conflicts of interest

There are no conflicts of interest.

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