Trends Phytochem. Res. 2(2) 2018 65-74

Trends in Phytochemical Research (TPR)

Journal Homepage: http://tpr.iau-shahrood.ac.ir

*Original Research Article*

# Phytoconstituents profiling of *Cissus rotundifolia* (Forssk.) Vahl. by HPLC-MS/MS, and evaluation of its free radical scavenging activity (DPPH) and cytotoxicity

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**ABSTRACT**

Characterization of phytoconstituents in the methanolic extract (70%) of *Cissus rotundifolia* (Forssk.) Vahl. was performed using high-performance liquid chromatography (HPLC) coupled with electrospray ionization mass spectrometry (EIMS). Furthermore, tandem mass spectrometry (MS/MS) was performed to assist the structural elucidation of compounds. Accordingly, twenty-seven compounds were identified involving four acids, sixteen phenolics, two steroidal saponins, two coumarins, two stilbenoids and one triterpene. Astragalin **1**, β-amyrin **2** and 1β-hydroxy-kryptogenin-1-*O*-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranoside **3** were isolated by column chromatography and their structures were identified by spectral analysis including 1D and 2D NMR. The plant extract and the isolated compounds **1**-**3** were evaluated for their radical scavenging activity. Compounds **1** and **2** showed significant activity relative to that of ascorbic acid as a standard. The cytotoxicity of the plant extract, the isolated compounds and the standard doxorubicin were evaluated using the three human tumor cell lines HT116, MCF-7 and PC-3. In this regard, the plant extract exhibited significant cytotoxicity against MCF-7 while no activity was observed against the other cell lines. The isolated compounds **1**-**3** showed no activity against the used cell lines, as well.



**ARTICLE HISTORY**

Received: 01 January 2018
Revised: 19 March 2018
Accepted: 25 May 2018
ePublished: 05 June 2018

**KEYWORDS**

*Cissus rotundifolia* (Forssk.) Vahl.
HPLC-ESI-MS
Tandem mass
Free radical scavenging activity
Cytotoxicity

## 1. Introduction

Medicinal plants rich in secondary metabolites are widely used in many aspects such as cosmetics along with drug, food and beverage industries (Aidi Wannes et al., 2017; Camilo et al., 2017). In fact, a large number of medicinal plants continue to provide valuable therapeutic agents for the treatment of a broad spectrum of diseases throughout the world (Mohammadhosseini et al., 2017a, 2017b; Nunes and Miguel, 2017). In many developing and underdeveloped countries, the traditional use of medicinal plants is the answer to many health ailments (Ganesan and Xu, 2017; Pavunraj et al., 2017). Since synthetic compounds are known to have undesirable side effects, there is a growing interest to investigate the chemical constituents of medicinal plants and their biological effects (Mohammadhosseini et al., 2016; Mohammadhosseini, 2017a, 2017b).

*Cissus rotundifolia* (Forssk.) Vahl. (Family: Vitaceae) is a woody climbing vine indigenous to Africa and cultivated in Egypt for ornamental purposes. In recent years, plants of the genus *Cissus* were reported to contain sterols, triterpenoids, phenolics, flavonoids, stilbene derivatives, coumarin glycosides and iridoids (Beltrame et al., 2002a; Quílez et al., 2004; Singh et al., 2007; Kumar et al., 2010; Pan et al., 2013). Many *Cissus* species were reported to possess antimicrobial, antiosteoporotic, hypoglycemic, antioxidant, antitumor, analgesic, anti-inflammatory, gastroprotective, hepatoprotective, immunomodulatory and anti-allergic activities (Al-Mamary, 2002; Alzoreky and Nakahara, 2003; Al-Fatimi et al., 2007).

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*C. rotundifolia* (Forssk.) Vahl. is used traditionally to treat fever and digestive disorders in Yemen (Al-Fatimi et al., 2007). This herbal plant represents remarkable antioxidant, antibacterial, analgesic, anti-inflammatory and anti-ulcerative activities (Al-Mamary, 2002; Alzoreky and Nakahara, 2003; Said et al., 2015a). In our previous study, phytochemical screening of *C. rotundifolia* (Forssk.) Vahl. extract showed the presence of triterpenes, carbohydrates and/or glycosides, tannins, flavonoids, coumarins and saponins (Said et al., 2015a). However, up to present, no phytochemical studies have been reported concerning the isolation and identification of the phytoconstituents of *C. rotundifolia* (Forssk.) Vahl..

As a part of our investigations on plants cultivated in Egypt, we previously screened *C. rotundifolia* (Forssk.) Vahl. for promising bioactivities (Said et al., 2015a). The findings of that study drew our interest to fully investigate the phytoconstituents profile of the plant. In the current study, *C. rotundifolia* (Forssk.) Vahl. phytoconstituents were qualitatively analysed using HPLC-MS/MS and three of the phytoconstituents were isolated by column chromatography and characterized using spectral analysis. Moreover, the isolates and the plant extract were evaluated for their potential antioxidant activity and cytotoxicity against three human cancer cell lines.

## 2. Experimental

### 2.1. General

UV measurements were carried out using UV-Visible Jasco spectrophotometer. EI-MS and High-Resolution ESI-Mass Data (HRESIMS) were measured using Jeol JMS-Ax500 spectrometer and Bruker micrOTOF-QII MS (Amherst, MA), respectively. NMR spectra were measured using Bruker High-Performance Digital FT-NMR Spectrometer Avance III operating at 400 MHz for $^{1}H$ and 100 MHz for $^{13}C$ nuclei. Diaion® HP-20 for column chromatography (CC) (Sigma-Aldrich Chemie GmbH, Germany), Silica gel 60 (Merck) and Sephadex LH-20 (Pharmacia, Uppsala, Sweden) were used for column chromatography (CC). Pre-coated silica gel plates G 60 ($F_{254}$-Merck) were used for thin layer chromatography (TLC). TLC plates were observed under UV (254 and 366 nm), then visualized by heating after spraying with vanillin-$H_2SO_4$. Sheets of Whatmann No.1 filter paper (Whatmann Ltd., Maidstone, Kent, England) were used for paper chromatography (PC), while the Whatmann sheets (3MM) filter paper were employed for preparative paper chromatography (PPC). In our investigation, paper chromatography (PC) was observed under UV (366 nm), then visualized by heating after spraying with $AlCl_3$.

**Fig. 1.** Photos of *Cissus rotundifolia* (Forssk.) Vahl. unflowering aerial parts.

### 2.2. Plant material

The unflowering aerial parts of *C. rotundifolia* (Forssk.) Vahl. were collected from Orman botanical garden, Giza, Egypt in June 2009, kindly authenticated by Dr. Mohammed El-Gebaly, Department of Botany, National Research Centre. Voucher specimen (2014-13) was deposited in the Herbarium of Pharmacognosy Department, Faculty of Pharmacy, Cairo University (Fig. 1).

### 2.3. Chemicals and reagents

Acetonitrile and formic acid (HPLC grade) were obtained from Sigma-Aldrich (St. Louis, MO, USA). Water was purified using Milli-Q water purification system (Millipore, USA). Methanol (analytical grade) was obtained from Sigma-Aldrich (St. Louis, MO, USA). 1,1-Diphenyl-2-picryl-hydrazil (DPPH) and sulphorhodamine B (SRB) were provided by Sigma-Aldrich. Doxorubicin was provided by Pharmacia (Belgium).

### 2.4. HPLC-MS/MS analysis

#### 2.4.1. Sample preparation

One mg of methanolic extract (70%) of the plant was dissolved in 1 mL of MeOH (100%). After sonication for 2 min., the sample was centrifuged at 13000 rpm for 3 min. to remove insoluble materials and the supernatant was then injected into the HPLC system.

#### 2.4.2. Apparatus and conditions

An Agilent MSD SL Trap mass spectrometer was connected to an Agilent Technologies Series 1100 HPLC system via an ESI interface, consisting of an automatic sample injector, a binary pump, a continuous vacuum degasser and a column heater-cooler (Agilent Technologies, USA). All the operations as well as the acquiring and analysis of data were controlled by MSD Trap Control Version 4.2 software. The chromatographic separation was performed on a Kromasil RP-$C_{18}$ (4.6

mm×150 mm, 5 µm) column at a temperature of 30 ºC. The mobile phase consisted of water containing 0.1% formic acid (A) and acetonitrile (B). All the solvents used were filtered through a 0.45 µm nylon filter prior to use. The mobile phase flow-rate was adjusted at 1 mL/min. The outflows were divided into two shares, and only one share entered the MS detector at an average flow-rate of about 0.3 mL/min. A gradient program was used according to the following profile: 0-3 min, 5-26% B; 3-9 min 26% B; 9-10 min 26-30% B; 10-15 min, 30-44% B; 15-35 min, 44-55% B; 35-40 min, 55-60% B; 40-45 min, and 60-100% B. The sample injection volume was 10 µL. The elution order followed a sequence of decreasing polarity, whereby diglycosides eluted first, followed by monoglycosides, free aglycones and highly oxygenated terpenes.

2.4.3. Tandem mass spectrometry (MS-MS)

Precursor ions were selected and fragmented in the collision cell applying collision energies over the range 10-30 eV. Argon was used as collision gas. Product ions were detected using the following parameter settings: pulser frequency, 10 kHz; spectral rate, 1.5 Hz. For CID of in-source fragment ions, in-source CID energy was increased from 0 to 100 V. MS/MS spectra were obtained on Thermo Orbitrap Fusion instrument with Thermo Ultimate 3000 RSLC system and an Agilent ZobaxSB $C_{18}$ column (ThermoElectron, San Jose, USA), using the same elution gradient as in HPLC-MS. The system equipped with an ESI source under an electrospray voltage of 4.0 kV, having nitrogen as the sheath gas and a capillary temperature of 275 ºC in negative ionization mode.

2.5. Extraction and isolation

The air-dried powdered unflowering aerial parts (1 Kg) of *Cissus rotundifolia* were extracted twice by maceration with 70% aqueous methanol. The combined methanolic extract was evaporated under vacuum to dryness. The dark brown residue (410 g) was applied on a chromatographic column packed with Diaion HP-20 (1 kg) and eluted with gradient solvent system $H_2O$-MeOH (1:0-0:1), then MeOH-$CH_2Cl_2$ (7:3) to give 35 fractions (1 L) of each. Fractions were detected by silica TLC using two different solvent systems MeOH-$CH_2Cl_2$ (2:8) and EtOAc-*n*-hexane (4:6). The spots were visualized by spraying with vanillin-$H_2SO_4$ reagent and heating at 100 ºC. Similar fractions were pooled to give 14 main fractions. Fraction 3 (3 g) eluted with $H_2O$-MeOH (7:3) was chromatographed on Sephadex LH-20 CC (50 g) eluted with $H_2O$-MeOH (7:3) to give 15 fractions 50 mL of each. Fractions (8-10) were combined (2 g) and rechromatographed on Sephadex LH-20 CC (50 g) and eluted with $H_2O$-MeOH (9:1) to afford 30 fractions 10 mL of each. The material from fractions (21-23) that showed one major spot on PC with solvent systems *n*-BuOH-$CH_3COOH$-$H_2O$ (BAW, upper layer) (4:1:5) and $CH_3COOH$-$H_2O$ (15:85), was rechromatographed using PPC and eluted with BAW to afford compound **1** (17 mg). Compound **1** was finally purified on Sephadex LH-20 CC (25 g) and eluted with MeOH-$CH_2Cl_2$ (7:3) before spectroscopic analysis. Fraction 6 (15 g) eluted from the main column with $H_2O$-MeOH (4:6), was dried and partitioned between *n*-hexane and water. The hexane layer was dried and subjected to silica gel CC (50 g) eluted with gradient *n*-hexane:EtOAc (9:1,1:9) to give 15 fractions 20 mL of each. Fraction 7 (25 mg) eluted with *n*-hexane-EtOAc (9:1) exhibiting one major spot on silica gel TLC ($CH_2Cl_2$-MeOH, 99:1), was rechromatographed using silica gel CC (10 g) and eluted with *n*-hexane-EtOAc: (9:1) for elution to afford compound **2** (12 mg). The aqueous layer was dried and subjected to silica gel CC (30 g), eluted with gradient MeOH-$CH_2Cl_2$ (0:1, 1:0) to give 25 fractions 20 mL of each. The material from fractions 9 and 10 eluted with MeOH-$CH_2Cl_2$ (8:2), exhibiting one major spot on silica gel TLC ($CH_2Cl_2$-MeOH, 2:8), was combined and purified using Sephadex LH-20 CC to afford compound **3** (20 mg).

2.6. Identification of compounds

The isolated compounds were identified conclusively by comprehensive spectroscopic data analysis, e.g., UV, MS and 1D and 2D NMR, and by comparison with respective published data.

Astragalin **1**, also known as kaempferol-3-*O*-β-D-glucopyranoside, was obtained as a yellow amorphous solid (17 mg). UV $\lambda_{max}$ (log ε): (MeOH) 260, 320 sh., 355 nm, (NaOMe) 275, 325 sh., 390 nm, (NaOAc) 270, 385 nm, (NaOAc/$H_3BO_3$) 270, 350 nm, ($AlCl_3$) 270, 305 sh., 335, 395 nm, ($AlCl_3$/HCl) 265, 305 sh., 350, 395 nm. $^1$H NMR (400MHz, DMSO): 8.02 (2H, d, *J*=8.8 Hz, H-2`, 6`),6.86 (2H, d, *J*=8.8 Hz, H-3`,5`), 6.27 (1H, d, *J*=1.4 Hz, H-8), 6.17 (1H, d, *J*=1.4 Hz, H-6), 5.40 (1H, d, *J*=6.6 Hz, H-1``), 3.21-3.90 (6H, m, H-2``, 3``, 4``, 5``, $6_a$``, $6_b$``) (Han et al., 2004).

β-Amyrin **2**: obtained as white amorphous crystals (12 mg). $^1$H NMR (400 MHz, $CDCl_3$): δ 5.21 (1H, t, *J*=3.7, H-12), 3.24 (1H, d, *J*=5.0, H-3), 1.70 (2H, H-2), 0.82, 0.86, 0.88, 0.89, 0.96, 0.99, 1.02, 1.16 (each 3H, s, H3-23, 24, 25, 26, 27, 28, 29, 30). $^{13}$C-NMR (100 MHz, $CDCl_3$): δ 38.59, 27.24, 79.04, 38.75, 55.18, 18.38, 32.66, 38.79, 47.64, 37.15, 23.54, 121.73, 145.21, 41.73, 26.94, 26.16, 32.50, 47.36, 46.84, 31.09, 34.74, 36.95, 36.95, 27.24, 15.37, 15.59, 16.81, 26.16, 28.00, 33.34, 23.70 (C-1-30, respectively) (Ara et al., 2009; Sunil et al., 2014).

1β-Hydroxy-kryptogenin-1-*O*-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranoside **3**: obtained as colourless amorphous solid. $[\alpha]_D^{20}$ -6.97 (*c* 0.1, MeOH). IR (KBr) $\nu_{max}$($cm^{-1}$): 3406, 2929, 1725, 1646, 1452, 1375, 1257, 1047. HRESIMS (positive ion mode) *m/z*: 763.3666 $[M+K]^+$ (calcd for $C_{38}H_{60}O_{13}K$ 763.3671), 747.3926 $[M+Na]^+$ (calcd for $C_{38}H_{60}O_{13}Na$ 747.3932), 707.4001

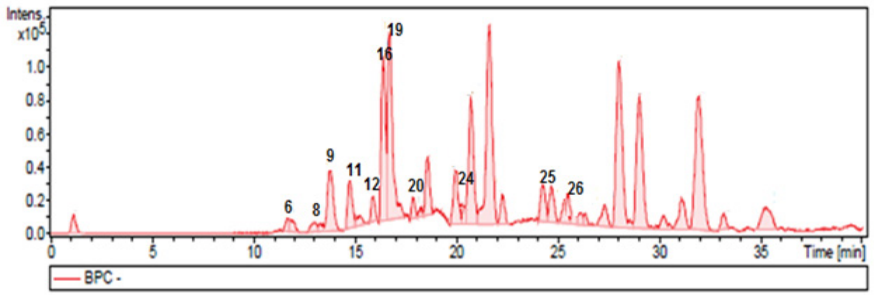


**Fig. 2.** HPLC/ MS chromatogram of 70% methanolic extract of *Cissus rotundifolia* (Forssk.) Vahl. unflowering aerial parts.

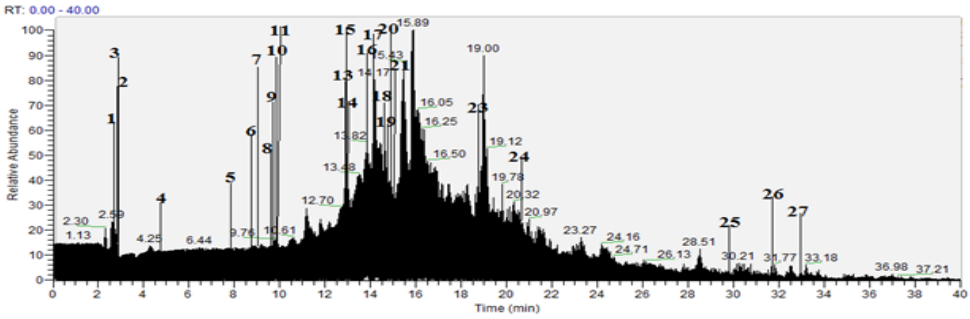


**Fig. 3.** Total MS/ MS chromatogram of 70% methanolic extract of *Cissus rotundifolia* (Forssk.) Vahl. unflowering aerial parts.

$[M-H_2O+H]^+$ (calcd for $C_{38}H_{59}O_{12}$ 707.4007). $^1H$ NMR (400 MHz, $CD_3OD$): δ 3.42 (1H, H-1), 1.70 and 2.16 (each 1H, $H_2$-2), 3.78 (1H, H-3), 2.20 and 2.19 (each 1H, $H_2$-4), 5.58 (1H, br d, *J*=5.2 Hz, H-6), 1.96 and 1.59 (each 1H, H-7), 1.55 (1H, H-8), 1.42 (1H, H-9), 2.63 and 1.52 (each 1H, $H_2$-11), 2.00 and 1.60 (each 1H, $H_2$-12), 1.63 (1H, H-14), 2.12 and 1.70 (1H, $H_2$-15), 2.57 (1H, d, *J*=12.0 Hz, H-17), 0.85 (3H, s, H-18), 1.15 (3H, s, H-19), 2.74 (1H, H-20), 1.07 (3H, d, *J*=6.8 Hz, $H_2$-21), 2.72 and 1.70 (each 1H, $H_2$-23), 1.79 and 1.37 (each 1H, $H_2$-24), 1.63 (1H, H-25), 3.43 and 3.36 (each 1H, $H_2$-26), 0.93 (3H, d, *J*=6.8 Hz, H-27), 4.31 (1H,d, *J*=6.8 Hz, H-1`), 3.72 (1H, H-2`), 3.48 (1H, H-3`), 3.88 (1H, H-4`), 3.84 and 3.54 (each 1H, d, *J*=12.0 Hz, H-5`), 5.31 (1H, br s, H-1``), 3.92 (1H, H-2``), 3.67 (1H, H-3``), 3.40 (1H, H-4``), 4.12 (1H, H-5``), 1.28 (3H, d, *J*=6.9 Hz, H-6``). $^{13}C$ NMR (100 MHz, $CD_3OD$): δ 83.14, 36.93, 69.42, 41.30, 138.36, 124.21, 31.01, 32.08, 49.75, 41.97, 23.14, 39.30, 41.97, 51.40, 36.93, 215.39, 66.50, 12.34, 14.61, 43.34, 13.84, 218.93, 39.30, 26.43, 34.95, 66.80, 15.61 (C-1-27, respectively), 99.62, 74.24, 74.24, 67.74, 66.12 (C-1`-5`), 100.23, 70.96, 70.72, 72.74, 68.25, 17.00 (C-1``-6``) (Said et al., 2015b).

### 2.7. Evaluation of antioxidant and cytotoxic activities

#### 2.7.1. Antioxidant activity

A stock solution (1.0 mg/mL) of each tested sample was prepared in methanol. Sample concentrations of 20, 50, 100, 150, 200 and 250 mg/mL were prepared in methanol. The free radical scavenging activity of the plant extract and the isolated compounds (**1**-**3**) were evaluated according to the method described by Braca et al. (2001). Accordingly, the test sample (0.1 mL) was added to 3 mL of a methanol solution (0.004%) of DPPH (1,1-diphenyl-2-picrylhydrazyl). In the final step, the absorbance of each solution was determined at 517 nm after 30 min, and the percentage inhibition activity was calculated from $[(A_0-A_1)/A_0]x100$, where $A_0$ is the absorbance of the control, and $A_1$ is the absorbance of the test sample or standard (Ascorbic acid).

#### 2.7.2. Cytotoxicity assay

The cytotoxicity of the 70% methanolic extract and the isolated compounds **1**-**3** were tested against HCT116 (Colon Cancer), MCF-7 (Breast Cancer) and PC-3 (Prostate Cancer) cell lines using Sulphorhodamine-B (SRB) method as previously described by Skehan et al. (1990), using doxorubicin as a standard drug. The cells were obtained from American Type Culture Collection (ATTC) (University Boulevard, Manassas, Virginia, USA). Following 72 h treatment, the cells were stained. The absorbance (reference wavelength) was measured at 490 nm with an ELISA microplate reader. The $IC_{50}$ values were calculated using sigmoidal concentration-response curve fitting models (SigmaPlot software).

## 3. Results and Discussion

### 3.1. HPLC-MS/MS analysis

Being sensitive and selective, the coupling of mass spectrometry with high-performance liquid chromatography (HPLC/MS) has revealed new aspects of chemical research and quality control of natural products. In particular, electrospray ionization mass spectrometry (ESI-MS), as a soft ionization technique

forms mainly molecular ion peaks, and the data from multi-stage tandem mass spectrometry (MS) with collision-induced dissociation (CID) reactions on the molecular ions can grant richer structural information. The MS/MS spectrum is of precise importance in the metabolite identification, as it grants a characteristic fingerprint for different metabolites even if these metabolites have the same elemental formula (Zhang et al., 2010). In this study, HPLC/MS analysis suggests a fingerprint profile for the aerial parts of *C. rotundifolia* (Forssk.) Vahl. (Figs. 2-3). To the best of our knowledge, this is the first study on the phytoconstituents profile of this plant using HPLC/MS. The phytoconstituents were assigned by comparing their retention times, accurate mass spectral fragmentation patterns in negative ion mode with reported literature and on-line database (Mass Bank database) (Horai et al., 2010). In this study, twenty-seven compounds were identified in *C. rotundifolia* (Forssk.) Vahl. extract. Eight of the identified compounds were previously isolated from the genus *Cissus*, while the rest 19 compounds were identified in this work for the first time. The identities, retention time, observed molecular and fragment ions for each compound are compiled in Table 1. The identified compounds were four acids, sixteen phenolics, two steroidal saponins, two coumarins, two stilbenoids and one triterpene. The identified acids were malic acid, aconitic acid, quinic acid and kynurenic acid. The identified phenolic compounds included two phenolic acids, namely gallic acid and vanillic acid, phenolic acid glucoside (vanillic acid hexoside), a xanthoid (mangiferin), four flavones, five flavanols, an isoflavone, a lignan, and a phenylethanoid glucoside. Gallic acid was isolated previously from stem and roots of *Cissus pteroclada* (Lin et al., 2012). The molecular ion at *m/z* 329, with the molecular formula of $C_{14}H_{17}O_9$, has been assigned to vanillic acid hexoside as shown by the presence of a fragment ion at *m/z* 167, which indicates vanillic acid (Abu-Reidah et al., 2013). The molecular ion at *m/z* 421, with formula $C_{19}H_{18}O_{11}$, was produced from mangiferin (2-*C*-β-D-glucopyranosyl-1,3,6,7-tetrahydroxyxanthone), a xanthone *C*-glycoside occurring in a variety of plants (Schieber et al., 2003). The molecular ions at *m/z* 431, 447, 343 and 447 were generated from the flavones, isovitexin (apigenin-6-*C*-glucoside), isoorientin (luteolin-6-*C*-glucoside), 3`,5-dihydroxy-4`,6,7-trimethoxyflavone and orientin (luteolin-8-*C*-glucoside), respectively. *C*-glucosides such as isoorientin, orientin and mangiferin showed a fragmentation pattern different from those of the *O*-glucosides. Using a triple-quadrupole mass spectrometer, the tandem mass spectra of *C*-glycosides did not reveal abundant $[M-H]^-$ ions, but showed characteristic ions due to fragmentation in the *C*-glycosidic unit. The observed losses of 120 and 90 mu were produced from cross-ring cleavages in the sugar unit (Sánchez-Rabaneda et al., 2003). The two produced ions at *m/z* 357 (loss of 90 mu) and *m/z* 327 (loss of 120 mu) obtained from isoorientin and orientin differ in their relative abundance. Isovitexin showed ions at *m/z* 431 (deprotonated molecule), 341 (loss of 90 mu) and 311 (loss of 120 mu) as characteristic ions in the MS/MS mode (Sánchez-Rabaneda et al., 2003). The molecular ions at *m/z* 343 was assigned for 3`,5-dihydroxy-4`,6,7-trimethoxyflavone (Reed, 2009).

Peaks at the retention times of 12.96, 13.04, 13.27, 13.28 and 20.56 were due to flavonols. The molecular ions at *m/z* 447 and 431 were produced from kampferol-3-*O*-glucoside (astragalin) and kaempferol-3-*O*-rhamnoside, respectively, with the characteristic ion of kaempferol at *m/z* 285. Kaempferol-3-*O*-rhamnoside was previously isolated from aerial parts of *Cissus sicyoides* (Beltrame et al., 2002a). The molecular ions at *m/z* 463 and 447 were produced from isoquercitrin and quercitrin, respectively, with the characteristic ion of quercetin at *m/z* 301. Quercitrin was previously isolated from *Cissus quadrangularis* (Singh et al., 2007). The molecular ion at *m/z* 271 was assigned to pinobanksin (3,5,7-trihydroxyflavanone), a common constituent of different types of propolis (Castro et al., 2014). The molecular ion at *m/z* 267 was produced from formononetin, *O*-methylated isoflavone found in a number of plants as the red clover *Trifolium pratense* (Wu et al., 2003).

The molecular ion at *m/z* 521 with formula $C_{26}H_{34}O_{11}$ was assigned to cyclolariciresinol hexoside, a lignan derivative identified previously in pomegranate (*Punica granatum* L.) juice by UHPLC-MS (Mena et al., 2012). Isolation of lignan derivatives from aerial parts of *Cissus repens* was previously reported (Wang et al., 2006). The molecular ion at *m/z* 623 with the molecular formula of $C_{29}H_{36}O_{15}$ was attributed to acteoside, a phenylethanoid glycoside found as major constituent of *Euphrasia rostkoviana* (Blazics et al., 2011).

Two steroidal saponins, 1β-hydroxy-kryptogenin-1-*O*-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranoside and alliospiroside A (25(S)-ruscogenin-1-*O*-α-L-rhamnopyranosyl-(1→2)-arabinopyranoside) were identified by their characteristic fragmentation pattern. To the best of our knowledge, the presence of steroidal saponins in the genus *Cissus* is reported here for the first time.

Two coumarins were identified as bergenin and 5,6,7,8-tetrahydroxycoumarin-5β-xylopyranoside. Both these compounds were previously isolated from *Cissus sicyoide* and *Cissus pteroclada* (Beltrame et al., 2002a; Lin et al., 2012).

Two stilbenoids, pallidol (a resveratrol dimer) and resveratrol were identified by the characteristic mass fragment at *m/z* 227 which were previously isolated from *Cissus pallida* and *Cissus sicyoides* (Quílez et al., 2004; Xue et al., 2014). Stibenoids are responsible for many health benefits of grapes, the famous member of family Vitaceae (Xiao et al., 2008). The molecular ion at *m/z* 425 was attributed to β-amyrin. Triterpenes were previously isolated from many *Cissus* species; particulary β-amyrin isolated from *Cissus quadrangularis* (Beltrame

**Table 1**
Compounds identified by HPLC/ MS and MS/MS of 70% methanolic extract of *Cissus rotundifolia* (Forssk.) Vahl. unflowering aerial parts.

| No. | Rt. (min.) | [M-H]$^-$ | Formula | Identification | Main fragments | References |
|---|---|---|---|---|---|---|
| 1 | 2.62 | 191 | $C_7H_{12}O_6$ | Quinic acid | 173 [M-H-$H_2O$]$^-$<br>127 [M-H-$CH_4O_3$]$^-$<br>111 [M-H-$CH_4O_4$]$^-$<br>85 [M-H-$C_3H_6O_4$]$^-$ | (Han et al., 2004). |
| 2 | 2.68 | 133 | $C_4H_6O_5$ | Malic acid | 115 [M-H-$H_2O$]$^-$ | (Abu-Reidah et al., 2013) |
| 3 | 2.71 | 173 | $C_6H_6O_6$ | Aconitic acid | 85 [M-H-$C_2HO_4$]$^-$ | (Horai et al., 2010) |
| 4 | 4.65 | 169 | $C_7H_6O_5$ | Gallic acid | 125 [M-H-$CO_2$]$^-$ | (Sánchez-Rabaneda et al., 2003) |
| 5 | 7.77 | 167 | $C_8H_8O_4$ | Vanillic acid | 152 [M-H-$CH_3$]$^-$<br>123 [M-H-$CO_2$]$^-$<br>108 [M-H-$CH_3$-$CO_2$]$^-$ | (Gómez-Romero et al., 2011) |
| 6 | 8.87 | 421 | $C_{19}H_{18}O_{11}$ | Mangiferin (1,3,6,7-tetrahydroxy-xanthone-2-*C*-glucoside) | 403 [M-H-$H_2O$]$^-$<br>331 [M-H-$C_3H_6O_3$]$^-$<br>301 [M-H-$C_4H_8O_4$]$^-$ | (Schieber et al., 2003) |
| 7 | 9.00 | 188 | $C_{10}H_7NO_3$ | Kynurenic acid | 144 [M-H-$CO_2$]$^-$ | (Horai et al., 2010) |
| 8 | 9.72 | 431 | $C_{21}H_{20}O_{10}$ | Isovitexin (apigenin-6-*C*-glucoside) | 413 [M-H-$H_2O$]$^-$<br>341 [M-H-$C_3H_6O_3$]$^-$<br>311 [M-H-$C_4H_8O_4$]$^-$ | (Sánchez-Rabaneda et al., 2003) |
| 9 | 9.74 | 447 | $C_{21}H_{20}O_{11}$ | Isoorientin (luteolin-6-*C*-glucoside) | 357 [M-H-$C_3H_6O_3$]$^-$<br>327 [M-H-$C_4H_8O_4$]$^-$ | (Sánchez-Rabaneda et al., 2003) |
| 10 | 9.88 | 521 | $C_{26}H_{34}O_{11}$ | Cyclolariciresinol hexoside | 566 [M+HCOO$^-$]$^-$<br>359 [M-H-hexosyl]$^-$<br>344 [M-H-hexosyl-$CH_3$]$^-$<br>313 [M-H-$C_7H_{12}O_7$]$^-$<br>255 [M-H-$C_3H_6O_2$]$^-$ | (Mena et al., 2003) |
| 11 | 9.94 | 329 | $C_{14}H_{17}O_9$ | Vanillic acid-hexoside | 167 [M-H-hexosyl]$^-$ vanillic acid<br>123 [M-H-$C_7H_{10}O_7$]$^-$ | (Abu-Reidah et al., 2013) |
| 12 | 12.96 | 447 | $C_{21}H_{20}O_{11}$ | Astragalin (kaempferol-3-*O*-glucoside) | 285 [M-H-glucosyl]$^-$<br>255 [M-H-glucosyl-$C_2H_6$]$^-$ | (Sánchez-Rabaneda et al., 2003) |
| 13 | 13.04 | 463 | $C_{21}H_{20}O_{12}$ | Isoquercitrin (quercitin-3-*O*-glucoside) | 301 [M-H-glucosyl]$^-$<br>271 [M-H-glucosyl-$CH_2O$]$^-$ | (Sánchez-Rabaneda et al., 2003) |
| 14 | 13.12 | 453 | $C_{28}H_{22}O_6$ | Pallidol (resveratrol dimer) | 341 [M-H-$C_6H_8O_2$]$^-$<br>227 [Resveratrol]$^-$ | (Weber et al., 2008) |
| 15 | 13.27 | 447 | $C_{21}H_{20}O_{11}$ | Quercitrin (quercetin-3-*O*-rhamnoside) | 895 [2M-H]$^-$<br>301 [M-H-rhamnosyl]$^-$ | (Simirgiotis et al., 2013) |
| 16 | 13.82 | 431 | $C_{21}H_{20}O_{10}$ | Kaempferol-3-*O*-α-L-rhamnoside | 285 [M-H-rhamnosyl]$^-$<br>255 [M-H-rhamnosyl-$C_2H_6$]$^-$ | (Beltrame et al., 2002a; Horai et al., 2010) |
| 17 | 14.14 | 343 | $C_{18}H_{16}O_7$ | 3`,5-dihydroxy-4`,6,7-trimethoxyflavone | 325 [M-H-$H_20$]$^-$<br>229 [M-H-$C_5H_6O_3$]$^-$ | (Reed et al., 2009) |
| 18 | 14.64 | 327 | $C_{14}H_{16}O_9$ | Bergenin | 283 [M-H-$C_2H_4O$]$^-$<br>225 [M-H-$C_4H_6O_3$]$^-$ | (Lin et al., 2012; Horai et al., 2010) |
| 19 | 14.76 | 723 | $C_{38}H_{60}O_{13}$ | 1β-hydroxy-kryptogenin-1-*O*-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranoside | 769 [M+HCOO$^-$]$^-$<br>577 [M-H-rhamnosyl]$^-$<br>445 [M-H- rhamnosyl-arabinosyl]$^-$ | (Said et al., 2015b) |
| 20 | 14.77 | 447 | $C_{21}H_{20}O_{11}$ | Orientin (luteolin-8-*C*-glucoside) | 357 [M-H-$C_3H_6O_3$]$^-$<br>327 [M-H-$C_4H_8O_4$]$^-$ | (Sánchez-Rabaneda et al., 2003) |
| 21 | 14.96 | 341 | $C_{14}H_{14}O_{10}$ | 5,6,7,8-tetrahydroxycoumarin-5β-xylopyranoside | 209 [M-H-xylosyl]$^-$ | (Beltrame et al., 2002a) |
| 22 | 16.00 | 227 | $C_{14}H_{12}O_3$ | Resveratrol | 183 [M-H-$C_2H_4O$]$^-$ | (Kıvrak et al., 2013) |
| 23 | 18.54 | 707 | $C_{38}H_{60}O_{12}$ | Alliospiroside A<br>(25(S)-ruscogenin-1-*O*-α-L-rhamnopyranosyl-(1→2)-arabinopyranoside) | 753 [M+HCOO-]$^-$<br>561 [M-H-rhamnosyl]$^-$<br>429[M-H-rhamnosyl-arabinosyl]$^-$<br>ruscogenin aglycon | (Said et al., 2015b) |
| 24 | 20.56 | 271 | $C_{15}H_{12}O_5$ | Pinobanksin | 253 [M-H-$H_2O$]$^-$<br>225 [M-H-$CH_2O_2$]$^-$ | (Castro et al., 2014) |
| 25 | 29.79 | 623 | $C_{29}H_{36}O_{15}$ | Acteoside | 461 [M-H-glucosyl]$^-$<br>443 [M-H-glucosy-$H_2O$]$^-$<br>179 [$C_9H_7O_4$]$^-$ | (Blazics et al., 2011) |
| 26 | 31.73 | 267 | $C_{16}H_{12}O_4$ | Formononetin | 313 [M+HCOO-]$^-$<br>251 [M-H-$CH_4$]$^-$ | (Kang et al., 2007) |
| 27 | 32.91 | 425 | $C_{30}H_{50}O$ | β-amyrin | 257 [M-H-$C_{11}H_{20}O$]$^-$<br>185 | (Fingolo et al., 2013) |

et al., 2002b).

### 3.2. Isolation and characterization

Three known compounds were isolated, purified and identified as: astragalin (kaempferol-3-*O*-β-D-glucopyranoside) **1**, β-amyrin **2** and 1β-hydroxy-kryptogenin-1-*O*-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranoside **3**. The structures of compounds **1**, **2** and **3** were identified by comparing their $^1$H- NMR, $^{13}$C-NMR, EI-MS, HRESIMS and UV spectral data with those reported in the literature (Han et al., 2004; Ara et al., 2009; Sunil et al., 2014; Said et al., 2015b). Both astragalin **1** and β-amyrin **2** are important bioactive phytoconstituents. Astragalin is a bioactive flavonoid with promising anti-inflammatory, antioxidant, anti-allergic, antimicrobial and anti-trypanocidal activity (Marín et al., 2011; Choi et al., 2013; Cho et al., 2015).

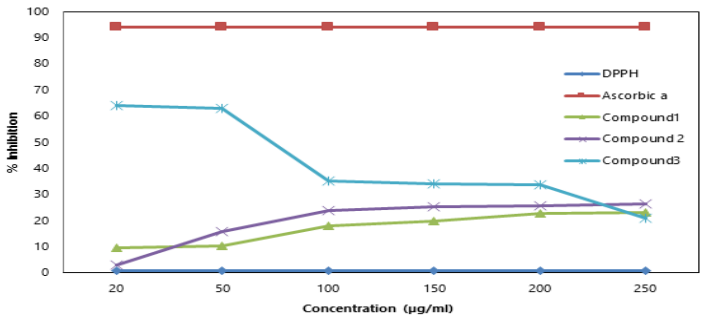


**Fig. 4.** Inhibition (%) of DPPH free radicals by standard antioxidant (ascorbic acid) and the isolated compounds.

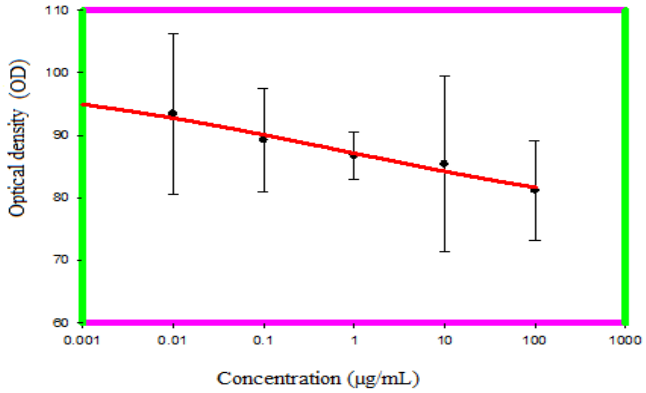


**Fig. 5.** Concentration-response curve for the plant extract on MCF-7 cell line.

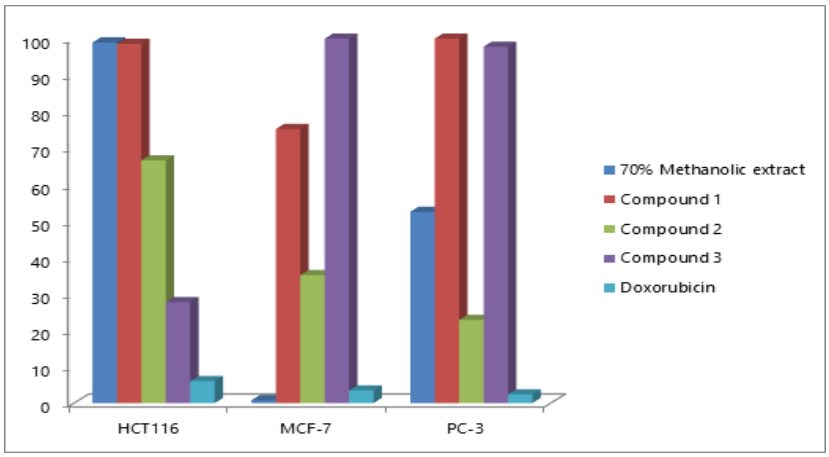


**Fig. 6.** $IC_{50}$ values of the plant extract and the isolated compounds compared with doxorubicin on different tumor cell lines.

β-Amyrin is a pentacyclic triterpene with various bioactivities such as anti-inflammatory, antiviral, antifungal and antiulcer behaviors (Vázquez et al., 2012). This is the first reported occurrence of the three compounds in *C. rotundifolia* (Forssk.) Vahl. as we believe. Moreover, the steroidal saponin **3** had never been reported before from any species of the genus *Cissus*.

### 3.3. Evaluation of antioxidant and cytotoxic activities

#### 3.3.1. Antioxidant

The methanolic (70%) extract of the plant and the isolated compounds **1**, **2**, **3** were evaluated for their radical scavenging activity by DPPH assay with ascorbic acid as standard. The plant extract showed no activity. The $IC_{50}$ values of the isolates **1**, **2**, **3** were 5.77, 5.79, 67.7 µg/mL, respectively. The $IC_{50}$ value of ascorbic acid was 33.3 µg/mL. The results revealed that isolated compounds **1** and **2** exhibited significant activity relative to that of ascorbic acid (Fig. 4). β-Amyrin isolated from *Symplocos cochinchinensis* Moore. leaves showed a significant radical scavenging effects on DPPH previously (Sunil et al., 2014). *Morus alba* L. leaves extract astragalin possessed antioxidant activity against free radical-induced oxidative hemolysis of human red blood cells (Choi et al., 2013).

3.3.2. Cytotoxicity

The methanolic (70%) extract of the plant and the isolated compounds **1**-**3** were tested for cytotoxicity against HCT116 (Colon Cancer), MCF-7 (Breast Cancer) and PC-3 (Prostate Cancer) cell lines by SRB method using doxorubicin as a standard drug. The $IC_{50}$ values are represented in Table 2. The plant extract exhibited significant cytotoxicity against MCF-7 (Figs. 5-6). However, the isolated compounds **1**-**3** showed no activity against the used cell lines. This significant cytotoxicity of the plant extract may be due to another phytoconstituents other than the isolated ones. According to the HPLC/MS data, the main phytoconstituents of the plant extract were mainly of phenolics, steroidal saponins, coumarins, stilbenoids and triterpene types which were reported previously to possess cytotoxicity activities (Lopez-Lazaro et al., 2002; Kawase et al., 2005; Wu et al., 2008; Podolak et al., 2010; Walczak et al., 2011; Gold-Smith et al., 2016).

**Table 2**

$IC_{50}$ values of the plant extract and the isolated compounds compared with doxorubicin on different tumor cell lines.

| Tested sample | $IC_{50}$ (µg/mL) | | |
|---|---|---|---|
| | HCT116 | MCF-7 | PC-3 |
| 70% methanolic extract | 98.96 | 0.77 | 52.48 |
| 1 | 98.57 | 75.20 | >100 |
| 2 | 66.59 | 35.09 | 22.80 |
| 3 | 27.70 | >100 | 97.78 |
| Doxorubicin | 6.00 | 3.45 | 2.36 |

## 4. Concluding remarks

This is the first study on the phytoconstituents profile of *C. rotundifolia* (Forssk.) Vahl. using the HPLC/MS approach. Out of the identified 27 compounds, only 8 of them were previously isolated from the genus *Cissus*, while the rest 19 compounds were identified in this work for the first time. The HPLC phytoconstituents profile gives a better understanding of the chemistry of *C. rotundifolia* (Forssk.) Vahl. and justifies its bioactivities and ethnomedicinal uses. Astragalin (kaempferol-3-*O*-β-D-glucopyranoside) **1**, β-amyrin **2** and 1β-hydroxy-kryptogenin-1-*O*-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranoside **3** were isolated from the plant for the first time. Thus, *C. rotundifolia* (Forssk.) Vahl. is a new source for bioactive phytoconstituents with various health benefits. Moreover, this is the first report on the presence of steroidal saponins in the genus *Cissus*. The isolated compounds **1** and **2** exhibited significant free radical scavenging activity relative to that of ascorbic acid. The plant extract exhibited a significant cytotoxicity against MCF-7, indicating that *Cissus rotundifolia* can be a potential source of selective cytotoxic agents and further investigations should be undertaken in this case in future.

## Conflict of interest

The authors declare that there is no conflict of interest.

## Acknowledgments

The authors wish to acknowledge the National Research Centre for financial support and Dr Stephen J. Eyles, Director of UMass-Amherst Mass Spectrometry Center, University of Massachusetts, Maryland, USA, for helping in HPLC-MS analysis.

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