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Original article

New flavane gallates from the aerial part of an African/Arabian medicinal plant *Plicosepalus curviflorus* by LC–MS and NMR based molecular characterization

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ABSTRACT

Plicosepalus curviflorus is distributed in Africa, Yemen and Saudi Arabia and applied to treat cancer, tonsillitis, otitis media and for curing diabetes. The objective of this study is to isolate chemical constituents from this medicinally important plant and it yielded one undescribed gallocatechin derivative **1** along with nine known compounds **2**–**10**. In this study, the metabolite profiling of *n*-butanol fraction of *P. curviflorus* was also measured by using liquid chromatography-quadrupole time-of-flight mass spectrometry (LC-QTOf-MS). Our research provided the facts for the theory that this plant is a good source of flavane and flavonoids and can be used as medicine. This application allowed to unambiguously identify eight compounds **11**–**18** from *n*-butanol soluble fraction of *P. curviflorus*. We describe here complete characterization of one new compound isolated from the *n*-butanol soluble fraction of *P. curviflorus* by NMR and ESI-MS. To the best of our good grasp, compounds **12**–**15**, represents the example of substituted catechin derivatives identified by LC-QTOf-MS method.

1. Introduction

Traditional medicinal systems have relied upon the presence of herbs and spices and these systems have been set up from many centuries, and it supplied protected and powerful remedies and cures to humankind (Ameenah, 2006). At present, the significance of various plants for human health has increased like, *Cynanchum paniculatum* (Xirong et al., 2020), *Ganoderma lucidum* (Faruque, 2020); *Thalictrum foliolosum* (Nitin et al., 2020); Tagetes Patula (Muhammad et al., 2020); *Cissus rotundifolia* (Jawaher et al., 2020) and many types of research have been done and proved herbal effects on health for curing incurable diseases. Kingdom of Saudi Arabia (KSA) is distinguished by the diversity of vegetation, and it confirmed that KSA flora has all over 837 genera include over 2253 *spp*., which distributed around 134 families, about 20% of these are novel and scarce plants (Collenette, 1998); (Hanan et al., 2019). The parasitic flora of KSA includes at least 13 genera with eight different families. Loranthaceae family, popularly known as mistletoe and it is one of the famous parasitic family which comprises about seventy genera and thousand of *sp*. (Calvin and Wilson, 2006). Species of Loranthaceae family consists on hemi parasitic and epiphytic plants of the southwestern mountains of the KSA. Many species of Loranthaceae have ethnobotanical importance and used as medicinal plants in various regions of the world. *Plicosepalus* is a salient genus of the Loranthaceae family and it comprises about 11 species distributed throughout Middle East, Africa and Arabian Peninsula. In KSA, it is denoted by two species, *P. acacia* and *P. curviflorus* (Anthony et al., 1996). This plant used worldwide as traditional medicine, like in Yemen the stem used for curing cancer, tonsillitis and otitis media (Al-Shanawani, 1996), while in KSA plant leaves consumed for curing diabetes (Mossa, 1985). Phytochemical screening of *P. curviflorus* leaves extracts proved the presence of sterols, terpenoids and flavane

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gallates as well flavonoids (Badr et al., 2015). Previous investigations on genus *Plicosepalus* showed interesting biological activities such as antiviral, antimicrobial, cytotoxic, anti-diabetic and anti-hepatotoxic activities (Areej et al., 2012). Previously, our group carried out a research study on the isolation and structure elucidation of Saudi *P. curviflorus* extract, revealed to the isolation of two new flavane gallates from the polar fractions, namely; pentahydroxy flavane–5–*O*–gallate and hexahydroxy flavane 5–di–*O*–gallate. Consequently, to explore the pharmacological activity of *P. curviflorus* and to verify its traditional use, the previous isolated compounds were investigated for their hypoglycemic (Areej et al., 2012), cytotoxic (Ghada et al., 2014), peroxisome proliferator-activated receptors agnostic, antimicrobial and anti-inflammatory activities (Raha et al., 2019). Results of previous studies on the same plant showed potent hypoglycemic activity in Albino mice with the isolation of new flavane gallates from the polar fraction, which possessed the highest anticancer effect against colon and liver cancer cell lines (Ghada et al., 2014). On the other hand, catechin proved dual PPAR activation effect and quercetin showed promising anti-inflammatory activity. Interestingly, both *P. curviflorus* polar extract and the pure compounds showed strong antioxidant potential while weak antimicrobial outcome against tested microbes (Raha et al., 2019). Thus, polar fractions of this plant contain interesting biological active compounds which encourage us to continue the work on *n*-butanol fraction to identify more interesting compounds that could be used as starting materials for producing medicinally active and safe drugs for treating contemporary diseases like the same fraction of other medicinal plants (Shagufta et al., 2019a, 2019b).

From last two decades LC–MS technique has been commonly used for the chemical characterization of various medicinal plants extracts (Chunlian et al., 2020; Marco and Deirdre, 2020; Rachel et al., 2018). ESI-MS (Electrospray ionization mass spectrometry) method has proven to be a robust technique that ease the detection of polar, thermally labile and stable plants constituents. Several high-resolution mass instruments have been used for chemical characterization of plant secondary metabolites. Hybrid high resolution mass spectrometry instruments such as LC-ESI-MS, IT (ion-trap mass), FT (Fourier trasformation) and QTOF (quadrupole-time-of-flight) can provide high quality mass and MS/MS spectra for the identification of compounds. As far as we know, many other recent studies on this medicinal plant *P. curviflorus* have only described the isolation of few compounds from it without providing full information about it. The aim of our research is to report the proper analysis on the characterization of pure chemical constituents of the *n*-butanol fraction of this plant by using 1D and 2D NMR (for isolated compounds) and LC–MS/MS instruments-based structures elucidation by their fragmentation motif. These metabolomic approaches were concentrated on mass inspection in negative (−ve) ion mode, as this mode is more delicate than positive (+ve) mode for establishing the variance between fragments connectivity.

2. Materials and methods

2.1. General

Optical rotations were measured in LC grade methanol (CH_3OH) using a polarimeter (JASCO P-2000, 2967-5, Japan). The one-dimensional (1D) and two-dimensional (2D) NMR spectra were recorded by using a Bruker AVANCE spectrometer (Bruker, MA, USA) (700 and 175 MHz for ^1H, ^{13}C; respectively). Chemical shift δ calculated in ppm, tetramethyl silane TMS used as standard, were calculated basing on the residual solvent signal, and *J* coupling constants are reported in Hertz (Hz). The ESI-MS experiments for the pure constituents (**1**–**10**) were measured on an Triple Quadrupole 6410 QQQ LC/MS mass spectrometer (Agilent, Santa Clara, CA, USA) with ESI ion source (gas temperature was 350 °C, nebulizer pressure was 60 psi, and gas flow rate was 10 L/min), operating in the −ve/+ve ions scan modes of ionization through direct infusion method using CH_3OHnH_2O (1:1 v/v) at a flow rate of 0.6 ml/min. Column chromatography (CC) procedures were performed using silica gel (230–400 mesh, Merck, Germany), RP-18 (LiChroprep 25–40 μm, Merck, Germany), Sephadex LH-20 (25–100 μm, Merck, Germany). TLC method was performed by using aluminum precoated silica gel 60 F_{254} and RP-18 (Merck, Germany) TLC plates, and spots were visualized on exposure under UV light (short 254/long 365 nm) and by spraying with ceric sulphate and thymol spraying reagents. Analytical grade solvents and reagents were purchased from Sigma-Aldrich (St. Louis, USA). Deuterated dimethyl sulfoxide (DMSO d_6) was purxhased from Cambridge Isotope Laboratories (Tewksbury, USA). Methanol, dichloromethane, ethanol and *n*-butanol was purchased from Thermo Scientific (Rockford, USA). Formic acid, catechin, epicatechin and gallic acid was purchased from Sigma-Aldrich (USA). Millipore water (30 ml) was obtained from Milli-Q Direct Water Purification System (Merck, Germany).

2.2. Plant material

The aerial parts of *P. curviflorus* (900 gm) were collected in February 2019 from the South of Hijaz region of Saudi Arabia and identified by Dr. M. Atiqur Rahman (plant taxonomist) College of Pharmacy, King Saud University. A voucher specimen (No. #127) is kept in the herbarium of College of Pharmacy, King Saud University.

2.3. Extraction and isolation

Finely chopped dried aerial plant parts (0.9 kg) were extracted with ethanol:water (8:2) mixture at room temperature 28 °C (3 × 2.5 L). The hydroalcoholic extract was evaporated by using Buchi (Flawil, Switzerland) rotary evaporator system. Concentrated extract (70 g) was dissolved in half liter of millipore H_2O and then successively extracted with dichloromethane and *n*-butanol, remaining water-soluble fraction was freeze dried in powder form. The *n*-butanol fraction (20 g) was then subjected to an open silica (Si) gel column chromatography (CC 400 mm × 50 mm) by using dichloromethane and methanol as a gradient solvent system (v/v, 100: 50–50: 100) to give seven (1–7) major sub-fractions. Sub-fraction 2 (200 mg) was loaded to an open Si gel CC which developed with dichloromethane (DCM) and methanol ((v/v, 8:2) to give **1** (6 mg) and **2** (10 mg). Fraction 3 (100 mg) was further subjected to C-18 Si gel CC, eluted with a gradient mixture of water:methanol (8.0:2.0 → 3.0:7.0), which gave in compounds **8** (10 mg) and **9** (12 mg). Fraction 4 (100 mg) was loaded to C-18 Si gel CC using solvent mixture water:methanol (8.0:2.0 → 1.0:1.0) to provided compound **5** (12 mg). Fraction 5 (200 mg) was loaded to Si gel CC eluting with DCM and methanol (v/v, 8: 2), and further purified by using HPLC (RP-18, 9.4 × 250 mm, 7 μm, 1.5 ml/min) eluted with H_2O-MeOH (7: 3) to obtain pure isolate **4** (10 mg) and **6** (15 mg). Fraction 6 (100 mg) was separated on LH-20 Sephadex CC by using mixture of water:methanol mixture (9.0:1.0 → 6.0:4.0), to give in compound **3** (25 mg). Fraction 7 (300 mg) was loaded to C-18 Si gel CC using mixture of water:methanol (8.0:2.0 → 3.0:7.0) to give in compounds **7** (20 mg) and **10** (25 mg) from top and tail fraction, respectively.

2.3.1. Compound ***1***

Red amorphous solid; $[\alpha]_D^{27}$ −15 (*c* 0.10, analytical grade methanol); ^1H and ^{13}C NMR data: see Table 1. HR-ESIMS: *m*/*z* 457.1855

Table 1
^{1}H- & ^{13}C NMR data for Compound **1**.

No.	1 δ_H	δ_C
2	4.76 d 7.0	81.1
3	3.99 brs	66.2
4a	2.56 dd, *J* = 8.4, 16.8	28.1
4b	2.80 dd, *J* = 4.9, 16.8	
5	–	150.2
6	6.23 d, *J* = 2.1	101.3
7	–	156.6
8	6.18 d, *J* = 2.1	100.9
9	–	156
10	–	106.1
1′	–	130.6
2′& 6′	7.05 s	109.4
3′& 5′	–	146.1
4′	–	139.4
1″	–	164.9
2″		118.9
3″& 7″	7.09 s	109.6
4″& 6″	–	146.2
5″	–	139.6

(DMSO, *J* in Hz, δ in ppm).

$[M-H]^-$, 457.3574 calcd. for $C_{22}H_{17}O_{11}$, *m/z* 459.2036 $[M+H]^+$, 459.3730 calcd. for $C_{22}H_{19}O_{11}$.

2.4. LC–MS metabolomic analysis

Powdered *n*-butanol fraction of *P. curviflorus* were dissolved in water:methanol mixture (3:7, 10 ml) at 5 mg/mL of final concentration and then filtered by a 0.45 μm cellulose filter (Millipore, MA) and then subjected to Agilent 1260 LC analysis. Analyses were performed on Agilent 1260 Infinity HPLC chromatographic system (Agilent, Germany) hyphenated with Agilent 6530 Quadrupole Time of Flight (Agilent QTOf, Singapore), fitted with degasser, an automatic injector, a column compartment (40 °C) and a quaternary pump, incorporated with Quadrupole and Time of Flight mass spectrometer along with an orthogonal ESI source with Jet Stream thermo focusing technique. 2 μl of the plant sample was injected into the Agilent Zorbax SB-Carbon 18 column (4.6 mm × 150 mm, 1.8 μm) at 40 °C temperature. Compounds separation was accomplished by using following elution gradient; 0–5 min, 10% B; 5–25 min, 10–100% B; 26–30 min, 100% B; 30–31 min, 100–5% B; 31–35 min, 5% B using mobile phase A (0.1% HCOOH in Millipore water) and mobile phase B (0.1% Formic acid HCOOH in Acetonitrile, CH_3CN) and the flow rate was as 250 μl/min. MS1 (full scan) was used as acquisition mode and the mass was ranged from 100 to 1000 *m/z* with scan rate 1 (Spectra/sec). The mass spectrometer frameworks were set as following: gas temperature 300 °C, gas flow l/min, sheath gas temperature 350 °C, nebulizer 35 psi and sheath gas flow was 11 L/min. Mass chromatograms were obtained in negative ion (-ve) mode.

3. Results and discussion

Previous investigations on *P. curviflorus* leaves selected the methanol soluble fraction as the richest in polar flavane gallates and phenolic derivatives. In continuation of our work on this medicinal plant, the chromatographic separation was achieved on its *n*-butanol fraction by using a sequence of Sephadex LH-20, Si gel and RP-18 column chromatography (CC), which yielded one undescribed compound **1** and nine previously reported constituents **2**–**10**. The structures of pure compounds (Fig. 1) were established by 1D (one dimensional) and 2D (two dimensional) NMR and ESIMS. Compounds **2**–**10** were identified as 3,3′,4′,5,7-pentahydroxyflavane-5-*O*-gallate **2**, catechin **3** (Areej et al., 2012), caffeic acid **4**, caffeoyl-β-D-glucose **5**, gallic acid **6**, chlorogenic acid **7** (Shagufta et al., 2020), querecetin-3-*O*-glucoside **8**, kaempferol-3-*O*-glucoside **9**, and protocatechuic acid **10** (Nawal et al., 2011) by a collation of their spectroscopic facts with those published earlier. To the best of our expertise compounds **4**–**10** are first time purified from *P. curviflorus*.

n-Butanol extract of aerial part of *P. curviflorus* were analyzed by LC-ESI mass instrument to detect its chemical composition based on mass fragmentation patterns. In general, we observed -ve ion mode deprotonated molecule $[M-H]^-$ and its typical fragment ions by MS/MS experimental analysis. Eight compounds are listed in Table 2 along with their retention time, molecular formula and experimental mass and the theoretical mass of each investigated compound. Thus, owing to the importance of catechin based compounds as chemo taxonomical markers, an unambiguous chemical characterization of *P. curviflorus* was obtained.

3.1. Structure elucidation of isolated new compound *1*

Compound **1** was segregated as red amorphous talc with the formula $C_{22}H_{18}O_{11}$ (458), determined by positive/negative ion mode ESIMS (*m/z* 459.2036 $[M+H]^+$, *m/z* 457.1855 $[M-H]^-$) (Figs. 2 and 3).

The ^{1}H NMR spectrum of **1** showed the existence of two meta coupled doublets at δ 6.23 (1H, d, *J* = 2.1) and 6.18 (1H, d, *J* = 2.1) which were congruous with a 5,7-dioxygenated ring A of flavonoid class of compounds. One singlet with two protons integration at δ 7.05 (2H, s) was evident for 1′,3′,4′,5′-tetrasubstituted aromatic ring B. The ^{1}H NMR spectrum further showed resonances in conjunction with δ 2.56 (1H, dd, *J* = 16.8, 8.4 Hz), 2.80 (1H, dd, *J* = 16.8, 4.9 Hz), 3.99 (1H, *brs*) and 4.76 (1H, d, *J* = 7.0 Hz) revealed that **1** was hexahydroxy flavane. Further exploration of the ^{1}H NMR spectrum of **1** manifested one more deshielded signal integrated for two protons at δ_H 7.09 (2H, s) and one downfield shifted carbonyl carbon signal at δc 164.9 in ^{13}C NMR attesting the existence of galloyl group in **1**. It was validated by upfield C-5 carbon shift at δ 150.0 for and downfield shift at δ 101.3 for C-6, δ 100.9 for C-8 and δ 106.1 for C-10 signals, in conformation of the presence of galloyl moiety at C-5 carbon atom. All of the explained experimental data were firmly related to the formerly isolated compound pentahydroxy flavane-5-*O*-gallate (Areej et al., 2012) excluding the occupancy of additional hydroxyl moiety at C-5′ carbon atom at ring B. The point of attachment of hydroxy moiety at C-5′ was firm by the HMBC long range intercorrelations. The oxygen carriage carbons of the rings A and B revealed signals at δC 156.0, 150.0, 146.1 × 2, and 139.4 indicating the presence of five hydroxy groups in compound **1**. The flavane gallate frame of **1** was deduced on the strong proofs of NMR and mass spectroscopy. Acid hydrolysis of compound **1** yielded gallic acid and (−)-gallocatechin (Areej et al., 2012), which were identified by keen comparison of their TLC, optical rotations and ^{1}H NMR with standard sample. A considerable analysis of the 1D and 2D NMR spectrum supported the structural assignment of compound **1** as gallocatechin 5-*O*-gallate.

3.2. Acid hydrolysis of compound *1*

Compound **1** (2.0 mg) was fully liquefy in 0.5 ml of 1 N methanol-HCl (1/1) solution. Soluble material was warmed up at 60 °C for 1 h and evaporated under Buchi, then water was poured and the mixture was treated with EtOAc (ethyl acetate). The EtOAc soluble part was filtered and the filtrate was concentrated by using Buchi rotavapor, and chromatographed over C-18 silica by using methanol:water which afforded (−)-gallocatechin and gallic acid from top and tail fraction, respectively. The experimental data

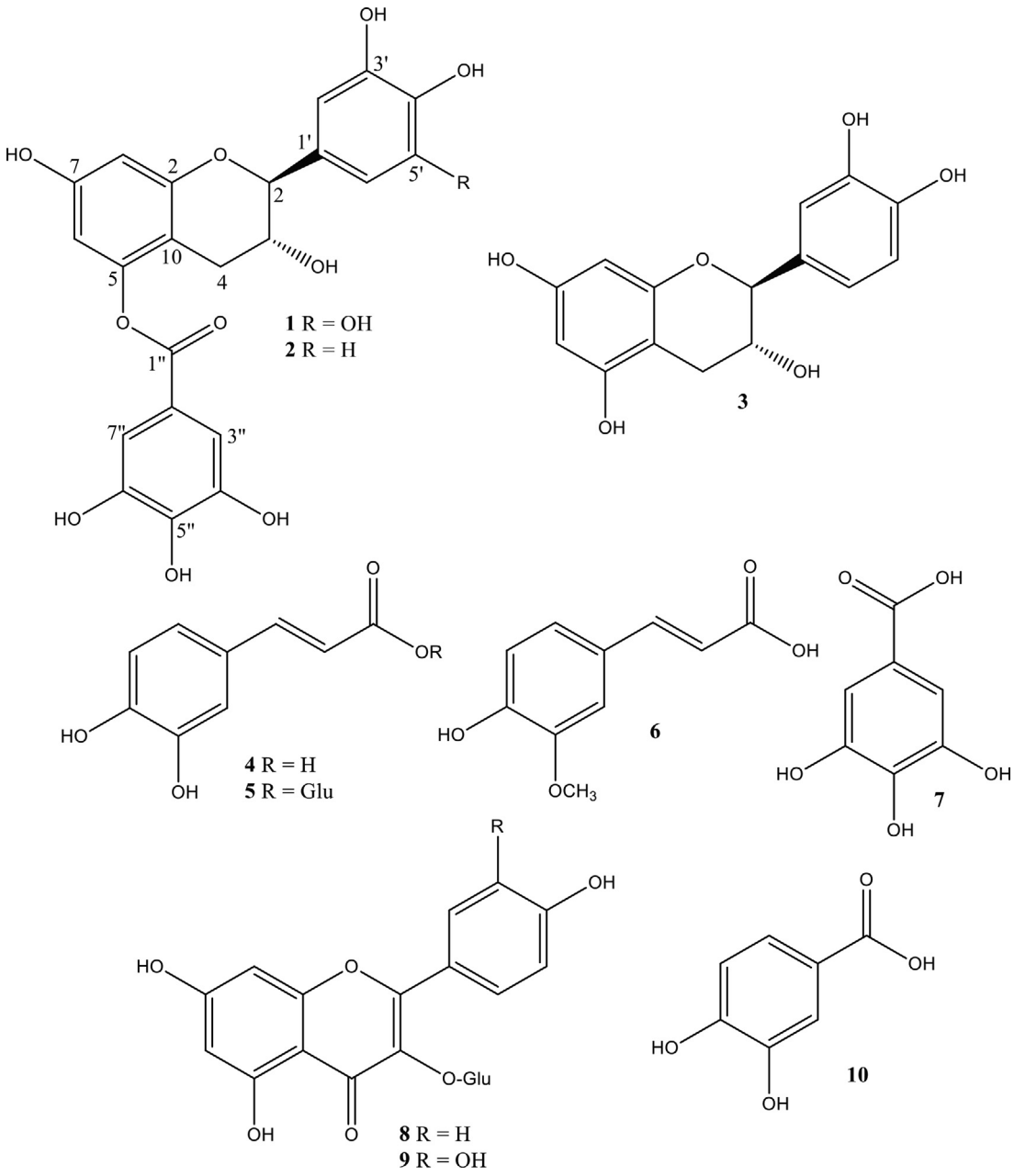

Fig. 1. Structures of isolated compounds **1**–**10.**

Table 2
Identification of chemical constituents from the *n*-butanol extract of *P. curviflorus*.

Signal	R_t min	Assigned identity	Mol. ion $[M-H]^-$	Fragment *m/z*	Elemental composition	Theoretical mass	Ref.
1	0.55	Procyanidin trimer **11**	865.2010	577	$C_{45}H_{37}O_{18}$	865.2715	(Jawaher et al., 2020)
2	1.03	Pentahydroxy flavane di-*O*-gallate-mono protocatechuate **12**	729.1609	593, 441, 289, 245, 197, 125	$C_{36}H_{25}O_{17}$	729.1786	(Marco and Deirdre, , 2020)
3	1.60	Catechin mono gallate caffeic/cinnamic acid derivative **13**	725.4118	793 [M + 3Na], 441, 289	$C_{38}H_{29}O_{15}$	725.6185	(Collenette, 1998)
4	2.03	Catechin monogallate di-*O*-methyl inositol **14**	631[b]	699 [M + 3Na], 571, 767 [M + 6Na]$^-$, 441, 289	$C_{30}H_{31}O_{15}$	631.5485	(Mohammad, 1996)
5	2.23	Catechin monogallate amide derivative **15**	540.3432	608 [M + 3Na], 441, 289, 197	$C_{27}H_{26}NO_{11}$	540.4880	(Collenette, 1998)
6	3.12	Diosmetin glucoside **16**	461.2809	529 [M + 3Na]	$C_{22}H_{21}O_{11}$	461.3887	(Mossa, 1985)
7	5.01	Catechin **17**	289.0735	245	$C_{15}H_{13}O_6$	289.2622	(Collenette, 1998)
8	8.25	Gallic acid **18**	169.0970		$C_7H_5O_5$	169.1090	(Hanan et al., 2019)

[a]Elemental composition refers to the deprotonated ion of molecules observed by mass spectrometry.
[b] decimal numbers not appeared

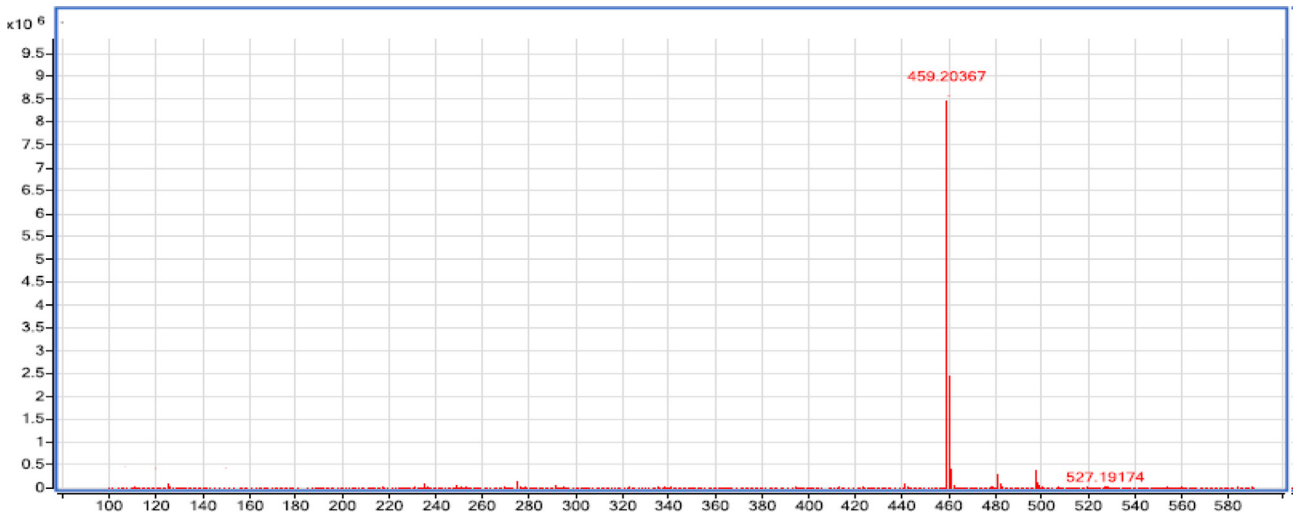

Fig. 2. The MS product positive ion mode scan of compound **1**.

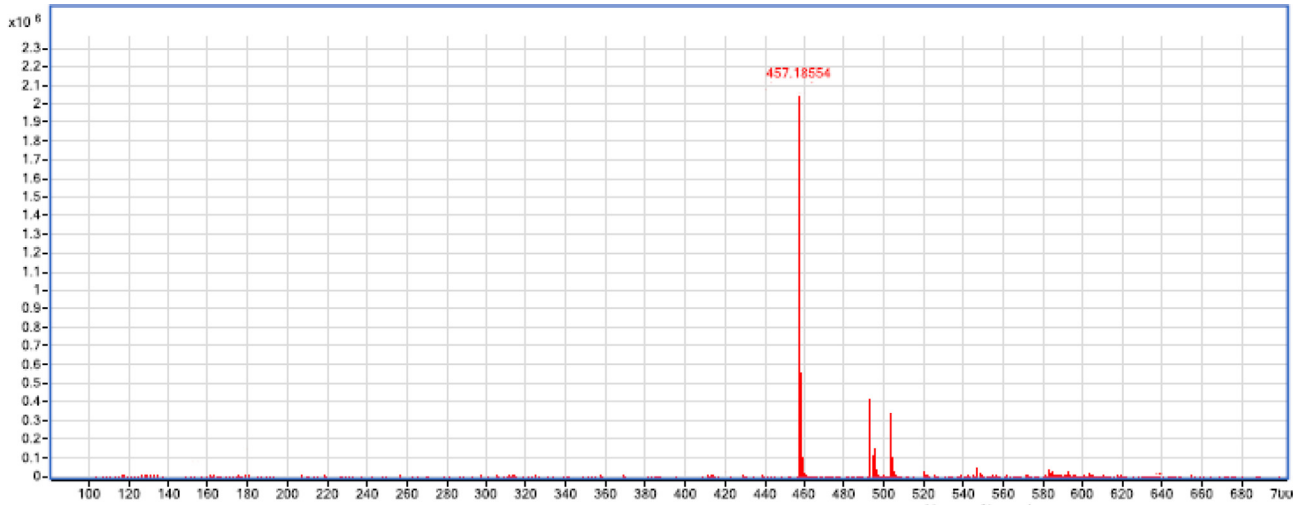

Fig. 3. The MS product negative ion mode scan of compound **1**.

were recognized by contrast of optical rotations and NMR resemblances with standard samples.

3.3. Structural identification of compounds by LC-ESI-MS/MS method

The goal of metabolomic study is to acquire a chemical constituent fingerprint by detecting all secondary metabolites exist in the *n*-butanol fraction of *P. curviflorus* (Fig. 4). Total eight compounds were identified in the *n*-butanol fraction of aerial parts of *P. curviflorus* by LC-ESI-MS, which were mainly characterized as catechin and its derivatives and flavonoid and its glycosides. Identification of metabolites was achieved by comparison to the available mass spectral (MS) data in available literature presented in Table 1. Most importantly, this method detected almost all catechin derivatives present in *n*-butanol fraction of this plant. In addition, our analysis revealed the presence of compounds **11**–**18** for the first time in *P. curviflorus*. New compounds detected by this approach are complexed catechin derivatives, which were described for the first time as natural products.

Compound **11** displayed a molecular ion signal at *m*/*z* 865.2010 ($[M-H]^-$, $[C_{45}H_{37}O_{18}]^-$) for procyanidin trimer and it further showed precursor ion at *m*/*z* 577 indicative of type B procyanidin dimers, so these two important signals confirmed compound **11** as catechin trimer (Emily et al., 2018).

The ESI-MS spectrum of *P. curviflorus n*-butanol extract displayed compound **12** molecular ion signals in negative ion mode at *m*/*z* 729.1609 ($[M-H]^-$, $[C_{36}H_{25}O_{17}]^-$) and MS/MS spectrum of its precursor ions at *m*/*z* 593.1070 $[C_{29}H_{21}O_{14}]^- \rightarrow$ *m*/*z* 441.0941 $[C_{22}H_{17}O_{10}]^- \rightarrow$ *m*/*z* 289.0811 $[C_{15}H_{13}O_6]^-$ allowed us to identify **12** as pentahydroxy flavane-di-*O*-gallate-mono protocatechuate (Fig. 5). In addition, further detected ions in MS/MS spectrum showed more $[M-H]^-$ fragmentations at *m*/*z* 245.0905 $[C_{14}H_{13}O_4]^-$, *m*/*z* 197.0534 $[C_9H_9O_5]^-$ and 125.0306 $[C_6H_5O_3]^-$, which were ascribed to catechin derivatives (Rosa, 2014). On completion of full literature survey, we tentatively identified compound **12** as the new compound which was fixed on the evidences of MS fragmentation sequences listed in Table 2.

Compound **13** has $[M-H]^-$ molecular ion at *m*/*z* 725.4118 and $[M-H+3Na]^-$ at *m*/*z* 793 with the fragment pattern *m*/*z* 441 → *m*/*z* 289 and → *m*/*z* 197. The major fragment was at *m*/*z* 441 and 289 indicated that this compound is a derivative of catechin gallate which has phenolic constituent attached at free hydroxyl groups. It indicating that carboxylation process has occurred at the hydroxy group which provide enough space for occupying

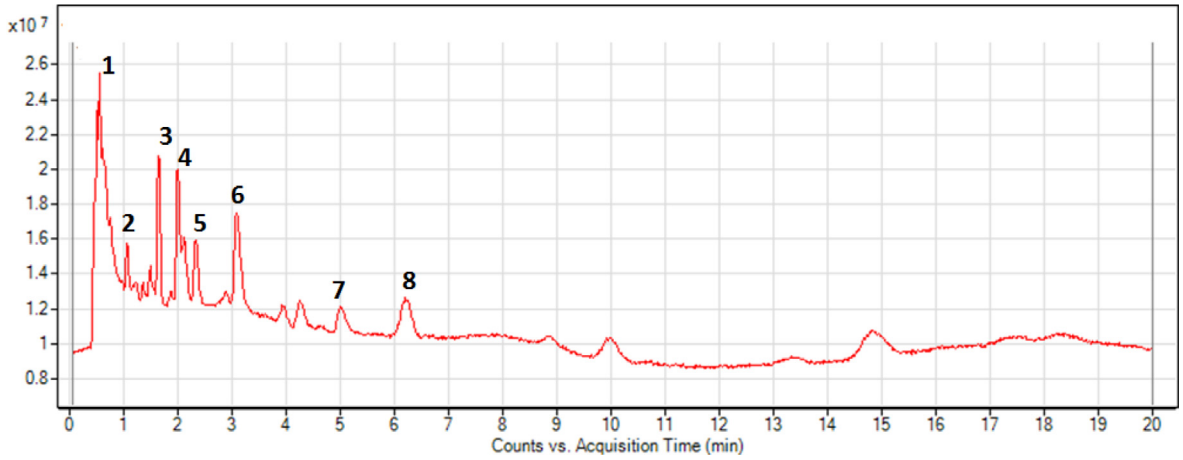

Fig. 4. Typical HPLC-ESI-MS chromatogram from of *P. curviflorus* *n*-butanol fraction analyzed in negative ion mode.

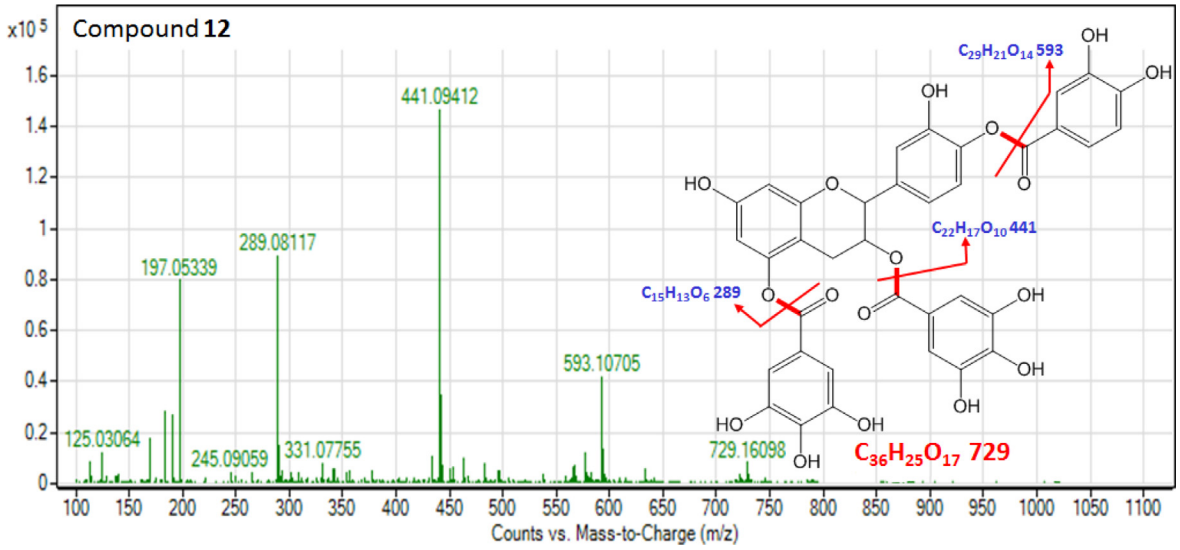

Fig. 5. The MS product negative ion mode scan of compound **12**.

other phenolics. On the basis of mass spectral data and previously published data (Areej et al., 2012), compound **13** was tentatively identified as catechin mono gallate caffeic/cinnamic acid derivative. (Fig. 6).

Compound **14** displayed the deprotonated molecular ion signal at *m*/*z* 631 and exhibited fragment ions at 571 (after loss of two methoxy groups), 441 (catechin gallate) and, 289 (catechin) and 699 [631 + 3Na-H = 699]. This fragmentation pattern is consistent

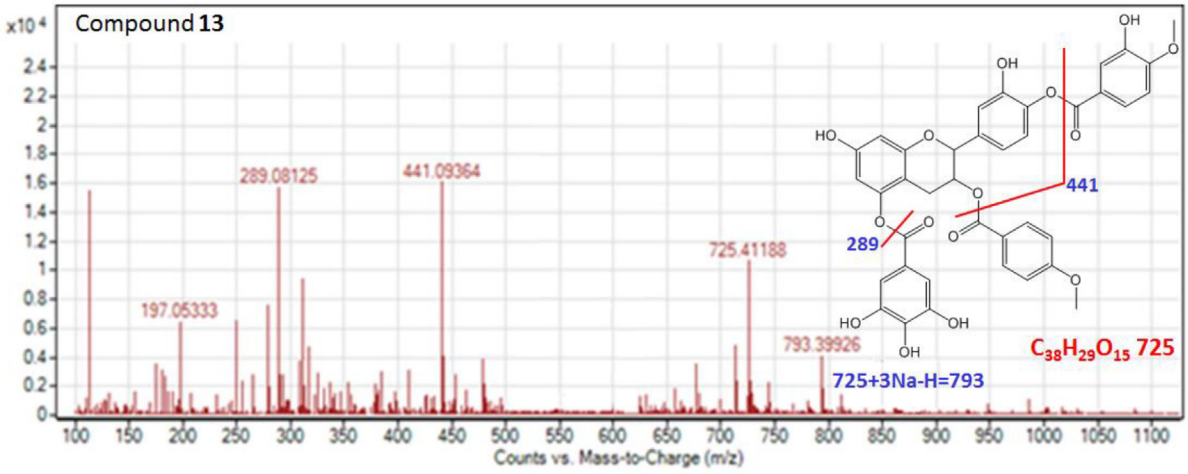

Fig. 6. The MS product negative ion mode scan of compound **13**.

with a catechin gallate derivative, condensed product of catechin with methyl inositol. From these evidences, compound **14** was found a catechin monogallate di-*O*-methyl inositol derivative, recently isolated from the Saudi *P. curviflorus* in 2016 (Giuseppe et al., 2016). The structural assignment of compound **14** was performed on the basis of the ESI-MS/MS fragment ions (Fig. 7). The described HPLC-ESI-MS course of actions offer the capability to obtain different flavane gallates and flavonoids from *P. curviflorus* *n*-butanol extract in an expeditious, inexpensive and effectual manner. Moreover, the use of the proposed HPLC-ESI-MS and negative ion MS/MS profiling allows the identification of its almost all metabolites in mixture with high assurance.

One minor signal of compound **15** showed $[M^{-}H]^{-}$ signal at *m*/*z* 540.3432 with the fragment ions at *m*/*z* 441, 289 and 197. The presence of nitrogen atoms in the molecule was proved by presence of even molecular weight at *m*/*z* 540. The *m*/*z* 441 ion and its conjugate ion at 289 were produced after cleavage of the inter flavan bond. On the basis of these evidences, compound **15** conditionally identified as amine derivative of catechin gallate which is a new natural product and to our best knowledge, it is reported here for the first time (Fig. 8).

Compound **16** was identified as diosmetin glucoside **16** and it showed $[M{-}H]^{-}$ ion at *m*/*z* 461.2809. It further showed the signal at 529 after the addition of three sodium ions. It is a known compound diosmetin glucoside and previously isolated from many plants specially from *Caucasian vetch* (Andreeva et al., 1998). Compound **17** and **18** was characterized as catechin and gallic acid with the molecular ion signals at 289 and 169, respectively. If liquid chromatography coupled with ESI/MS, positive ion mode is generally more favorable as many compounds are expected to ionized by using this mode. Although, the major dominance of negative ion mode is the lesser background noise. Up to now, positive ion mode ESI technique has generally been preferred among researchers; though, it can be seen in many instances that, negative ion mode ESI is much better choice owing to its finer sensitivity and potential for lower limits detection. Our results showed that the negative ion mode was more efficient and sensitive for the analyzed compounds present in this plant (Piia et al., 2017). The described LC-ESI-MS course of actions offered the potential to acquire different flavane gallates from *P. curviflorus* *n*-butanol extract in an expeditious, inexpensive and effectual manner. Moreover, the use of the proposed LC-ESI-MS and negative ion MS/MS profiling allows the

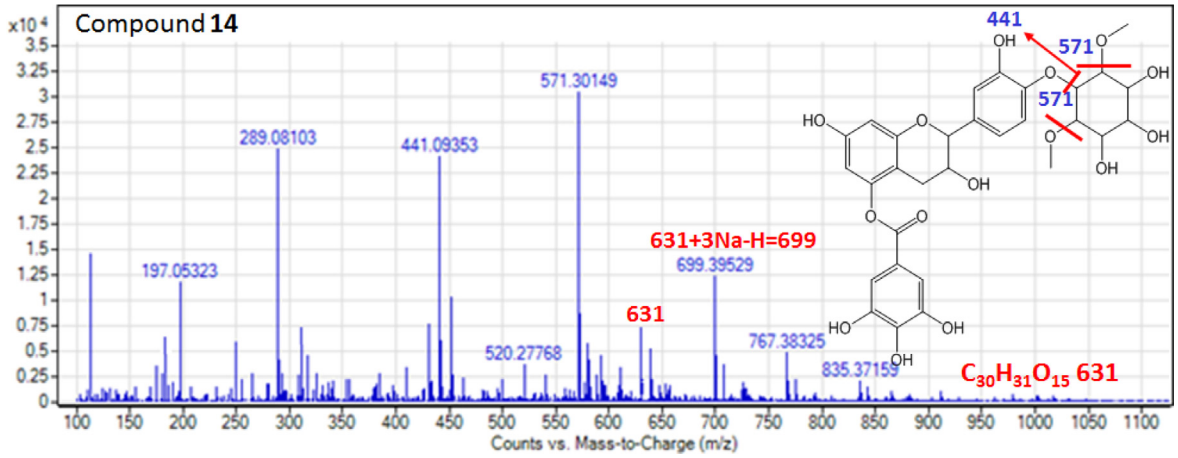

Fig. 7. The MS product negative ion mode scan of compound **14**.

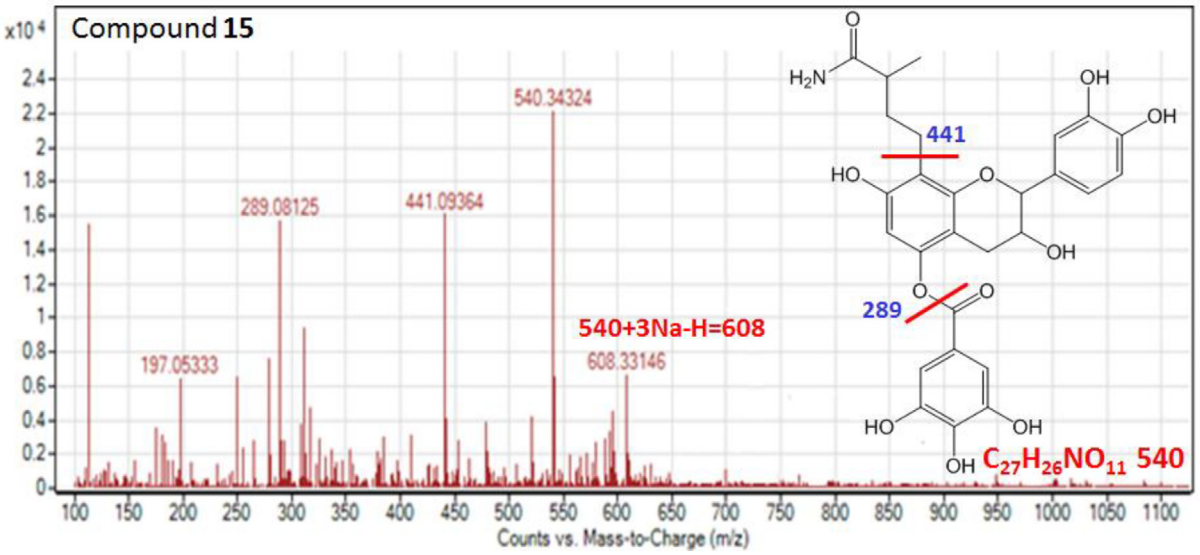

Fig. 8. The MS product negative ion mode scan of compound **15**.

identification of almost all metabolites in mixture with high assurance/confirmation

4. Conclusions

This phytochemical investigation on the Arabian/Africa medicinal plant *P. curviflorus* yielded one new **1** and nine **2**–**10** known secondary metabolite, elucidated by 1D and 2D NMR techniques. The potential of an untargeted metabolomic experimental procedure was illustrated for the first time as a meaningful tool for evaluating *P. curviflorus n*-butanol fraction, which provided the data of eight compounds **11**–**18** related to flavane gallets and flavonoid glycosides. These compounds were identified by ESI-MS spectra acquired by using LC-ESI-MS. QTOf-MS coupled with LC provides a swift and tactful method for the identification of flavane gallets, by using this method, new structures were deduced for the first time. The diversity of the structures of catechin gallate derivatives was divulged by MS chromatograms. Arabian folk medicinal plants have not yet achieved the popularity it deserves in global arena; therefore, this study distinctly provided the potential evidences of *P. curviflorus* to bestow a long list of polyphenols of the Saudi Arabian flora to enhance the worldwide phytochemical attempts. The facts obtained will reinforce the exploitation of this plant as a potential source of natural antioxidant.

5. Declarations

5.1. Ethical approval and consent to participate

Not applicable

5.2. Consent for publication

Not applicable

5.3. Availability of data and materials

The datasets used during the current study are available from the corresponding author on reasonable request.

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CRediT authorship contribution statement

Areej Mohammad Al-Taweel: Investigation, Writing - original draft, Writing - review & editing, Funding acquisition. **Shagufta Perveen:** Investigation, Writing - original draft, Writing - review & editing, Funding acquisition. **Saleh Ibrahim Alqasoumi:** Writing - review & editing. **Raha Orfali:** Investigation. **Hanan Y. Aati:** Investigation. **Shahnaz:** . **Ebtesam Nasser Alsultan:** . **Bandar Alghanem:** Writing - original draft. **Hayat Shaibah:** Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jksus.2020.101289.

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