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PHYTOTOXICITY, CYTOTOXICITY, AND CHEMICAL COMPOSITION OF *PHLOMIDOSCHEMA PARVIFLORUM*

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Abstract

Aerial parts of *Phlomidoschema parviflorum* (Benth.) Vved. were investigated, which afforded eight known compounds. Compounds 1–5 were reported for the first time from *P. parviflorum*. Different spectroscopic techniques were employed to characterize the compounds' structures, and the structures were compared with literature data. In addition to isolation work, gas chromatography-mass spectrometry (GC-MS) was used to examine the *n*-hexane fraction. As a result, thirty-six known compounds were identified; notably, they were identified for the first time in this plant. Furthermore, phytotoxicity and cytotoxicity were performed on extract and fractions of *P. parviflorum.* DCM fraction showed significant phytotoxicity (100% growth regulation) and moderate cytotoxicity (LD_{50} = 160.09 μg/mL) at 100 and 1000 μg/mL doses.

Key words: Traditional uses, Isolation, Phytoconstituents, GC-MS, Biological activities, Herbicides.

Introduction

Phlomidoschema parviflorum (Benth.) Vved. is an herbaceous perennial plant, its synonym is *Stachys parviflora* (Benth.); its vernacular name is Baggibuti (WFO, http://www.worldfloraoe.org; Ahmad *et al*., 2006). This plant belongs to the genus *Phlomidoschema*., which has only one species (WFO, http://www.worldfloraonline.org.). It is found in three of the five provinces of Pakistan, such as Punjab (Stewart *et al*.,1972), Balochistan (Ahmad *et al*., 2006), and KPK (Farooq *et al*., 2015). In these provinces of Pakistan, *P. parviflorum* grows in very hot to moderate climate zones. Recently, it has been claimed that it is also native to Iran (Khorassan), India (Punjab), and Afghanistan (Sonboli *et al*., 2015; Shakeri *et al*., 2019).

Phlomidoschema parviflorum (Benth.) Vved has been used traditionally by the local people against various diseases such as common cold, high blood pressure, cramps, arthralgia, epilepsy, dracunculiasis, and as an analgesic (Ahmad *et al*., 2008; Shakeri *et al*., 2019; Tomou *et al*., 2020). Additionally, the ground stem of *P. parviflorum* has been used against abscesses caused by guinea worms, as a tonic, and for diuretic properties (Ahmad *et al*., 2007). The history of traditional uses related to this plant provoked researchers to isolate and characterize its chemical constituents. To date, most of the research conducted on this plant is of Pakistani origin.

So far, various types of secondary metabolites have been isolated from the aerial parts of *P. parviflorum* including triterpenoids (Ahmad *et al*., 2007; Ahmad *et al*., 2008), diterpenoids, sesquiterpenoids (Arshad, 2006; Farooq *et al*., 2015), flavonoids (Ahmad *et al*., 2007; Ahmad *et al*., 2008), steroids (Arshad, 2006), and phenethyl alcohol (Ahmad *et al*., 2006). Moreover, based on GC-MS analysis, some essential oils from this plant have also been identified (Shakeri *et al*., 2019).

To date, it has been confirmed by several researchers that extract of *P. parviflorum* possessed antimicrobial (Samin *et al*., 2015; Sonboli *et al*., 2015; Abdullah & Nadeem, 2016; Bashi *et al*., 2016; Shakeri *et al*., 2019), antioxidant (Majd *et al*., 2014; Bashi *et al*., 2016; Shakeri *et al*., 2019), antidiarrheal, anti-cancer (Rauf *et al*., 2017), anti-depressant, and muscle calmative activities (Farooq *et al*., 2015).

Many people around the world depend on medicinal plants for therapeutic cures and weed management, according to a report from the WHO on the ethnomedicinal agenda for 2014–2023. Nevertheless, ethno-pharmacological practices of medicinally important plants have shown that some of them are very toxic to human beings, and questions arise about their safety. A simple yet reliable bioassay, such as brine shrimp lethality assay, can primarily solve this problem. This assay is less expensive and simpler to use when analyzing the toxicity of plant substances such as extracts, fractions, and isolated chemicals (Nisar *et al*., 2009; Orumwensodia *et al*., 2021).

On the other hand, weed (a plant that is competing with a desired, cultivated plant and growing where it is not wanted) populations in agricultural areas are a major factor in the decline of crop yields (Marshall *et al*., 2003). For the last several decades, various synthetic herbicides (to prevent weed germination and spreading out) have been used, such as glyphosate, dicamba, and 2,4-dichlorophenoxyacetic acid (Green & Owen, 2011). However, due to the evolution of resistant weed genotypes, long-term usage (around 50 years) of synthetic herbicides approaches is unable to produce the desired results. Additionally, synthetic pesticides pollute the soil and water, which have several harmful consequences on the environment and living things, including human beings (Barros *et al*., 2021; Radosevich *et al*., 1997).

To address the above-cited problems, new ecologically friendly herbicides are highly desired. Natural sources, including plant extracts and its fractions are an appealing source of potential leads for new natural herbicides, because of the potential specificity of biological action and the significantly decreased likelihood of harmful bioaccumulation and/ or residues in soil and ground water. Numerous studies have shown that plant extracts and its fractions can successfully prevent weed seeds from sprouting and growing (Ghosheh, 2005; Cavalieri & Caporali, 2010; Motlagh, 2011; Harding & Raizada, 2015;

Dharsini *et al*., 2017). Thereby, in search of new natural herbicides, phytotoxicity has usually been performed on plant materials such as extracts and their fractions can easily be screened to discover the new herbicidal drugs (Carballo *et al*., 2002). Hence, the present study investigated the extract and its fractions of *P. parviflorum* for cytotoxicity and phytotoxicity. To support this, chemical composition analysis through the column chromatography (CC) and GC-MS of this plant were also conducted.

Experimental

Plant material: The plant material (aerial parts of *P. parviflorum*) was collected from Balochistan, and identified by Prof. Dr. Rasool Bakhsh Tareen, Dean of Basic Sciences, Department of Botany, University of Balochistan, Quetta. The voucher number of the specimen plant was deposited at the Botany Department for the future reference.

General experimental procedure: The UV-Vis. (ultraviolet-visible) spectrophotometer was used to record the spectra from Thermo Scientific Company. VECTOR 22 spectrophotometer was used for the measurement of IR (infra-red) spectra, and Finnigan Mat 312 Mass Spectrometer (MS), to record electron impact mass spectra (EI-MS), was used. Various types of NMR (nuclear magnetic resonance) techniques were carried out by using various types of Bruker-AV spectrometers such as 300, 400, 500, and 600 MHz, while employing TMS (Tetramethylsilane) as an internal reference. Silica gel 60 (mesh size 72-235 μm, Machery-Nagel) was used to load the columns for the column chromatography (CC). To detect the spots of phytoconstituents on the TLC (thin-layer chromatography) plates, a UV lamp having wavelengths of 254 and 365 nm was used. TLC cards were sprayed with a solution of Cs $(SO_4)_2$, and this solution was prepared by simply mixing 10% Cs (IV)SO_4 and 15% sulfuric acid.

Extraction and isolation: 2 kg of powdered plant material (aerial parts) was soaked in EtOH at ambient temperature (26°C) for two weeks, and the liquid extract was collected consequently. After evaporation of the solvent, under the reduced pressure, 200 g ethanolic extract was obtained. The liquid-liquid extraction was then carried out on the ethanolic crude extract by using *n*-hexane, DCM, ethyl acetate (EtOAc), and butanol (*n*-BuOH). As a result, five fractions were prepared.

DCM fraction was chromatographed on normal CC (silica gel; *n*-Hex-EtOAc.; 100:00–00:100%, v/v). Ten fractions (F1–F10) were thus acquired. A silica gel column by utilizing *n*-Hex-EtOAc (3:2, v/v) was used to purify F2, resulting in compound 6 (9.5 mg). Fraction (F8) was repeatedly chromatographed on CC. Finally, the targeted compound was purified with prep. TLC, and yielded **7** (5 mg). The EtOAc fraction was separated into seven sub-fractions (A-I) by normal CC. Fraction E1 was subjected to CC, yielding E1 to E9. Then E4 was loaded on prep. TLC (PE-EtOAc; 4:1, v/v) and resulted compound 8 (3 mg). E5 was also loaded on prep. TLC (PE-EtOAc; 3:2, v/v) and resulted compound was 1 (7 mg). Fraction D and F were also subjected to CC and fraction D yielded compounds 2 (9 mg), and 3 (5 mg), fraction F was purified using normal CC and yielded the compounds 4 (2 mg) and 5 (8 mg).

3-Methylbenzofuran-2-carboxylic acid (1), $C_{10}H_8O_3$: White powder; mp. 190–194ºC. EI-MS *m/z* 176.1 $[M]^+$. ^{1}H-NMR (300 MHz, $CDCl_3$): δ 7.65 (1H, d, J = 9.0, H-7); 7.55 (1H, d, J = 9.0, H-4), 7.47 (1H, t, J = 9.0, H-6), 7.30 (t, 1H, J = 9.0, H-5), 2.63 (3H, s, H-1′).

4-(2-Hydroxyethyl) phenol (2), $C_8H_{10}O_2$: White powder, mp. 88–92ºC. EI-MS *m/z* 138.1 $[M]^+$. ^{1}H-NMR (300 MHz, CD_3OD): δ 7.03 (2H, d, J = 9.0, H-3, H-5), 6.70 (2H, d, J = 9.0, H-2, 6), 3.69 (2H, t, J = 6.0, H-2′), 2.73 (t, 2H, J = 6.0, H-1′).

4-Hydroxybenzoic acid (3), $C_7H_6O_3$: White powder, mp. 213–215ºC. EI-MS *m/z* 138 $[M]^+$. ^{1}H-NMR (300 MHz, CD_3OD): δ 7.54 (2H, d, J = 8.0, H-2, 6), 6.70 (2H, d, J = 8.0, H-3, 5).

Vanillic acid (4), $C_8H_8O_4$: Brown powder, mp. 210–213ºC. EI-MS *m/z* 168 $[M]^+$. ^{1}H-NMR (300 MHz, $CDCl_3$): δ 7.58 (1H, d, J = 2.0, H-2), 7.54 (1H, dd, J = 8.5, 2.0, H-6), 6.82 (1H, d, J = 8.5, H-5), 3.89 (3H, s, H-1′).

Trans-triacontyl-4-hydroxy-3-methoxycinnamate (5), $C_{40}H_{70}O_4$: White crystals, mp. 73–74ºC. EI-MS *m/z* 614.3 $[M]^+$. ^{1}H-NMR (300 MHz, $CDCl_3$): δ 7.59 (1H, d, J = 15.6, H-3), 7.50 (1H, d, J = 8.0, H-8), 7.08 (1H, dd, J = 8.0, 2.0, H-9), 6.89 (1H, d, J = 2.0, H-5), 6.19 (1H, d, J = 15.7, H-2), 4.09 (2H, t, J = 6.3, H-1′), 3.89 (3H, s, H-31′), 1.26-1.30 (56 H, m, H-2′-29′), 0.90 (3H, t, J = 8.8, H-30′).

Stigmasterol (6), $C_{29}H_{48}O$: White powder, mp. 160–165ºC. EI-MS *m/z* 412.2 $[M]^+$.

β-Sitosterol (7), $C_{29}H_{50}O$: White powder, mp. 135–138 ºC. EI-MS *m/z* 414.1 $[M]^+$.

Ursolic acid (8), $C_{30}H_{48}O_3$: White powder, mp. 278–283ºC. EI-MS *m/z* 456.1 $[M]^+$.

Gas chromatography (GC): An OPTIMA®-5-Accent capillary column with a 0.25 m film thickness of 5% phenyl and 95% methylsilicone, and a 60 m × 0.32 mm internal diameter was employed as a flame ionization detector (FID) in GC. The FID was connected with Shimadzu GC-10 for GC-FID studies. Firstly, for the two mins, the primary temperature was kept at 50ºC; then, a 7ºC/min increase in temperature was made. 30 minutes of analysis resulted in a final temperature measurement of 260ºC. The injector had a splitting ratio of 1:8, and its temperature was 235 ºC while the FID's was 400ºC. Nitrogen gas, which serves as a carrier gas, was employed in this procedure at 12.334 mL/min flow rate under 9 kPa of a pressure.

Gas chromatography-mass spectrometry (GC-MS) analysis: A Jeol, JMS-600H mass spectrometer (MS) was coupled to an Agilent 6890 gas chromatograph with a column of the ZB-5MS (30 m 0.32 nm internal diameter and 0.25 m film thickness). The electron ionization (EI) mode was used to operate a Mass spectrometer. The temperature of the EI chamber was 250ºC and the energy was supplied as 70 eV. The nitrogen gas volume was fixed from a range of 1.0 to 5.0 μL, according to the detector response (Reddy *et al*., 2014). For chemical identification, total ion count (TIC) was utilized with a scan period of 200 ms, and the start and end masses of 20 amu and 650 amu, respectively. Using

AMDIS V 2.69, the spectra of the discovered compounds were matched to the Reference Spectra Library database of the NIST "National Institute of Standards and Technology", with a Match Factor (MF) of 700, "Automated mass spectral DE convolution and identification software". The relative percentage compositions of the constituents that were discovered were calculated using the GC peak area.

Phytotoxicity assay: Using the procedure (Orumwensodia *et al*., 2021), the extract and its fractions were tested for phytotoxicity against *L. minor*. To create the E-medium, several inorganic components were mixed in 1 L of distilled water. To change the medium's pH from 5.5 to 6.0, KOH pellets were added, and it was autoclaved at 121ºC for 15 minutes. In order to achieve the final concentrations of 500, 50, and 5 ppm, respectively; in sterilized conical flasks, ethanol extract and its fractions were added to methanol at various concentrations of 10, 100, and 1000 g/mL. After that, they were left to dry overnight. 10 *L. minor* plants with a rosette of three fronds, and each were introduced to each flask along with 20 mL of E medium. Finally, paraquat was employed as the reference inhibitor, and methanol was added to the flasks used as the negative controls. The flasks were cultivated for seven days at 30ºC in triplicate using the Fisons Fi-Totran 600H growth cabinet. The experimental conditions were established as 56 ± 10 rh, 12 hours of daylight, and 9000 lx light intensity. The number of fronds per dose was determined by counting in order to monitor the progress of *L. minor* within the flasks, in contrast to the negative control, growth inhibition in % was calculated as follows:

$$\%\ \text{Grouth regulation} = \left(\frac{\text{Number of the fronds in negative control} - \text{Number of fronds in a sample}}{\text{Number of the fronds in negative control}}\right) \times 100$$

Cytotoxicity assay: Brine shrimp lethality assay was used to test cytotoxicity. 3.8 g of sea salt is dissolved in 1000 mL of distilled water for the preparation of an artificial saltwater stock solution (pH 7.4). This solution was then filtered. This solution is placed now in an unequally divided hatching tray with a size of 22 × 32 cm. Brine shrimp eggs (50 mg) was spread on the larger half of the tray, this portion is darkened by covering it with aluminium foil in order to prevent light to enter inside. A lamp was placed on the tray's smaller side so that when the larvae grew, they would emerge through the holes and move toward the light. Allow shrimps to hatch and mature for forty-eight hours at room temperature (22–29°C). In the meanwhile, stock solutions (20 mg/2 mL) of extract and its fractions were prepared in DMSO, the final concentrations 10 μg/mL, 100 μg/mL, and 1000 μg/mL were obtained by dispensing 5 μg/mL, 50 μg/mL, and 500 μL from this stock solution in new vials in triplicates and let them dry overnight. After 2 days of hatching, by using a Pasteur pipette, transferred ten actively moving nauplii in different vial (30 shrimps/dilution), 5 mL of seawater was injected into each vial, and they were then incubated at 25–27°C for 1-day while being illuminated. Etoposide with LD_{50} value of 7.46 μg/mL was employed as a -ve control, while the solvent served as a +ve control. To find the percentage inhibition of larvae due to tested samples; after a day, the number of alive and dead larvae was calculated. (Albaayit *et al*., 2021).

$$\%\ \text{Inhibition} = \left(\frac{100 - \text{Number of larvae in the test sample}}{\text{Number of live larvae in untreated control}}\right) \times 100$$

Results and Discussion

Five new source compounds, including 3-methyl benzofuran-2-carboxylic acid (**1**) (Rao *et al*., 2020), 4-(2-hydroxyethyl) phenol (**2**) (Christophoridou *et al*., 2005; Liu *et al*., 2010), vanillic acid (**3**) (Ghareib *et al*., 2010; Youn *et al*., 2010), and 4-hydroxybenzoic acid (**4**) (Cho *et al*., 1998), trans-triacontyl-4-hydroxy-3-methoxycinnamate (**5**) (Boonyaratavej *et al*., 1992), and three know compounds, namely stigmasterol (**6**) (Shwe *et al*., 2019), *β*-sitosterol (**7**) (Chaturvedula & Prakash, 2012), and ursolic acid (**8**) (Samarakoon *et al*., 2018) were isolated as consequence of current work on *P. parviflorum* (Fig. 1). Moreover, GC and GC-MS analyses led to thirty-six new source compounds from the fraction of *n*-hexane. (Table 1) contains the results, and the main classes were identified as triterpenoids, diterpenoids, sesquiterpenoids, fatty acids, and their esters, steroids, ketones, and aldehydes.

Time is needed to develop new herbicides because those already in use are encountering weed resistance. As a result of their excessive use, which lowers crop yields and ultimately causes significant economic loss. The synthetic herbicides used today to prevent crop production have a detrimental effect on weeds' natural enemies and are a significant health and environmental risk. To solve this problem, researchers are now finding some new ways including isolation of secondary metabolites from natural products, i.e., plants. Plants have potential biological activities i.e., phytotoxic and antioxidant activities. These properties may be because of two types of secondary metabolites, such as flavonoids, phenols, etc. (De Martino *et al*., 2012). The herbicides identified from plant source may be a safer alternative to the synthetic agrochemicals because they are typically more efficient, biodegradable, and less harmful to the environment. In comparison to the reference drug, 'Paraquat'; the results of a phytotoxicity study of aerial parts of *P. parviflorum* against *Lemna minor* suggest that the maximal tested dose (1000 g/mL) of a phytotoxic constituent. DCM fraction of plant showed the significant phytotoxicity (100% growth inhibition) on fronds of *Lemna minor* at 100 and 1000 μg/mL of tested concentrations, while moderate activity (29% and 41%, respectively) was shown by EtOH extract and its n-BuOH fraction respectively at 1000 μg/mL, On the contrary, EtOAc and H_2O fractions showed no phytotoxic activities against all the tested concentrations. In table 2, the results of the tested extracts/fractions' phytotoxicity assay are displayed.

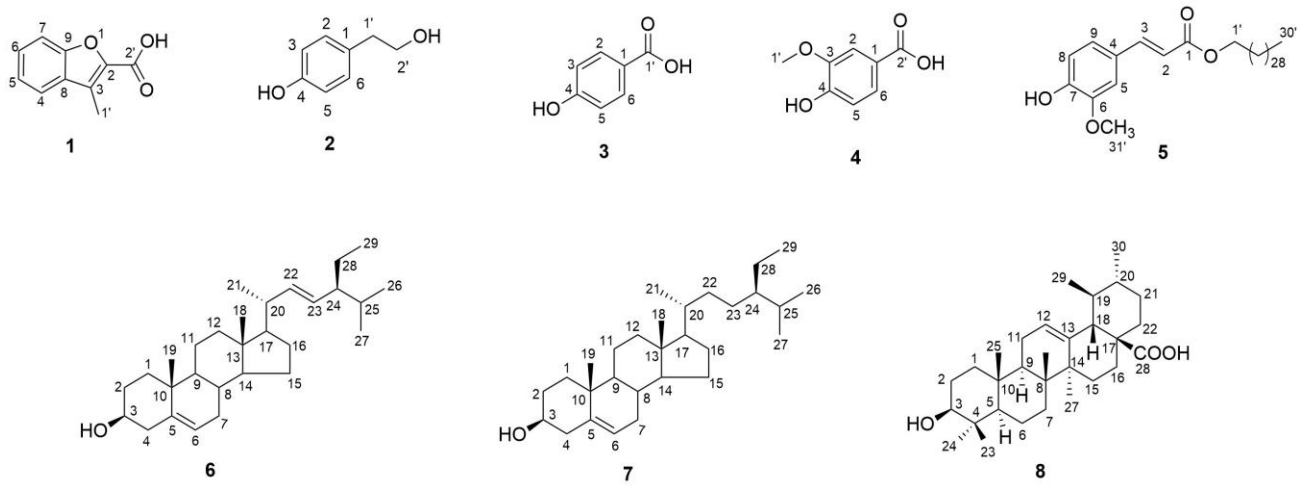

Fig. 1. Structures of compounds 1–8.

Table 1. Compounds identified through GC and GC-MS from *n*-hexane fraction.

Peak No.	RT (mins)	Name of compounds	Molecular formula	Molecular weight	MF values
1.	6.45	2-Pentylfuran	$C_9H_{14}O$	138	897
2.	9.92	Nonanal	$C_9H_{18}O$	142	858
3.	12.48	Octanoic acid ethyl ester	$C_{10}H_{20}O_2$	172	924
4.	13.78	2-sec-Butylcyclohexanone	$C_{10}H_{18}O$	154	759
5.	14.19	(*E*)-2-Decenal	$C_{10}H_{18}O$	154	862
6.	15.31	Undecanal	$C_{11}H_{22}O$	170	922
7.	16.64	2-Undecenal	$C_{11}H_{20}O$	168	900
8.	16.88	1-Undecanol	$C_{11}H_{24}O$	172	879
9.	17.69	Dodecanol	$C_{12}H_{24}O$	184	946
10.	18.13	9-oxo-Nonanoic acid methyl ester	$C_{10}H_{18}O_3$	186	919
11.	19.85	9-oxo-Nonanoic acid ethyl ester	$C_{11}H_{20}O_3$	200	915
12.	20.94	Dodecanoic acid	$C_{12}H_{24}O_2$	200	895
13.	21.84	Cedrol	$C_{15}H_{26}O$	222	876
14.	25.32	Tetradecanoic acid	$C_{14}H_{28}O_2$	228	799
15.	26.31	6,10,14-Trimethyl-2-pentadecanone	$C_{18}H_{36}O$	268	932
16.	28.03	Hexadecanoic acid methyl ester	$C_{17}H_{34}O_2$	270	866
17.	30.07	Hexadecanoic acid ethyl ester	$C_{18}H_{36}O_2$	284	901
18.	30.13	*n*-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	935
19.	32.89	Heptadecanoic acid	$C_{17}H_{34}O_2$	270	865
20.	36.95	13-Octadecenoic acid	$C_{18}H_{34}O_2$	282	909
21.	37.61	9,12-Octadecadienoic acid ethyl ester	$C_{20}H_{36}O_2$	308	912
22.	37.96	9-Octadecenoic acid ethyl ester	$C_{20}H_{38}O_2$	310	907
23.	39.15	Octadecanoic acid	$C_{18}H_{36}O_2$	284	890
24.	51.10	Eicosanoic acid	$C_{20}H_{40}O_2$	312	877
25.	56.71	Docosanoic acid	$C_{22}H_{44}O_2$	340	897
26.	58.59	Tricosanoic acid	$C_{23}H_{46}O_2$	354	799
27.	60.48	Tetracosanoic acid	$C_{24}H_{48}O_2$	368	822
28.	62.60	2-Heptacosanone	$C_{27}H_{54}O$	394	742
29.	65.58	2-Nonacosanone	$C_{29}H_{58}O$	422	783
30.	69.23	Stigmastan-7-one	$C_{29}H_{50}O$	414	757
31.	69.54	4,22-Stigmastadiene-3-one	$C_{29}H_{46}O$	410	800
32.	69.94	Lupeol	$C_{30}H_{50}O$	426	896
33.	69.96	Stigmasta-3,5-dien-7-one	$C_{29}H_{46}O$	410	833
34.	70.91	Stigmast-4-en-3-one	$C_{29}H_{48}O$	412	816
35.	71.92	(3β)-Lup-20(29)-en-3-ol acetate	$C_{32}H_{52}O_2$	468	884
36.	74.58	3,7,11,15-Tetramethyl-2-phytol	$C_{20}H_{40}O$	296	769

Table 2. Phytotoxicity of extract and its fractions.

Conc. of the sample (μg/mL)	No. of fronds				% Growth regulation		
	EtOH extract	DCM fraction	*n*-BuOH fraction	-ve Control	EtOH extract	DCM fraction	*n*-BuOH fraction
10	47	30	51		07.81	41.21	00
100	40	00	51	51	21.60	100	00
1000	36	00	30		29.44	100	41.24

Table 3. Cytotoxicity of extract and its fractions.

Sample names	Dose (μg/mL)	Number of shrimps	Number of survivors	LD_{50} (μg/mL)
EtOH extract	10	30	27	889.38
	100	30	26	
	1000	30	13	
DCM fraction	10	30	30	160.09
	100	30	20	
	1000	30	00	
EtOAc fraction	10	30	30	3869.19
	100	30	30	
	1000	30	22	
n-BuOH fraction	10	30	29	2304.80
	100	30	29	
	1000	30	18	
H_2O fraction	10	30	30	-
	100	30	30	
	1000	30	30	
Etoposide	-	-	-	7.46

In the brine shrimp lethality assay, the DCM fraction reduced brine shrimp survival by showing significant toxicity (100% lethality; LD_{50} 160 μg/mL). Delnavazi *et al*., (2018) found the aerial parts of *S. lavandulifolia* Vahl. contained a high concentration of O-polymethoxylated flavonoids including viscosine, chrysoeriol, hydroxygenkwanin, apigenin, chrysosplenetin, kumatakenin, velutin, penduletin, and chrysoeriol. Chrysosplenetin which has antiproliferative activity (Patel *et al*., 2022), while kumatakenin showed substantial cytotoxic action against ovarian cancer cell lines (SKOV3 and A2780) (Woo *et al*., 2017); also, apigenin was showed to be effective against prostate, liver, lung, colorectal, and breast cancers (Xu *et al*., 2016; Huang *et al*., 2016; Lee *et al*., 2016; Zhao *et al*., 2017; Gupta *et al*., 2002; Angulo *et al*., 2017). This is due to the fact that the presence of high flavonoid content in the DCM fraction exhibited high cytotoxic activity. EtOH extract showed moderate toxicity (43% lethality; LD_{50} 889 μg/mL) at the highest concentration of 1000 μg/mL. Thus, EtOAc, *n*-BuOH, and H_2O fractions were believed to be non-hazardous towards brine shrimp because the LD_{50} value is more than 1000 μg/mL *i.e*., 3869.19 (μg/mL) for EtOAc, 2304.80 (μg/mL) for *n*-BuOH and no result of LD_{50} for H_2O fraction as brine shrimp survival was 100% after exposure with fraction even at the highest concentration (1000 μg/mL) in comparison with etoposide (LD_{50} 7.46 μg/mL), which is used as a standard drug (Table 3). The findings demonstrated that, with the exception of the DCM fraction, all other fractions are non-cytotoxic, even at higher doses, suggesting that they could be employed in ethno-medicine with few side effects.

Conclusions

In this work, eight known compounds, including five compounds (**1**–**5)** for the first time, were isolated from *P. parviflorum*. Thirty-six new source compounds were identified from the *n*-hexane fraction of *P. parviflorum.* Moreover, in cytotoxicity assay, DCM fraction showed moderate cytotoxicity with LD_{50} = 160.09 μg/mL. Furthermore, DCM fraction showed significant phytotoxicity (100% growth regulation values at moderate and high doses). The phytotoxic property of DCM fraction may have the potential to be a defensive approach in agricultural applications (*i.e*., an effective natural agent against parasitic plants). Further study on DCM fraction is highly recommended.

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