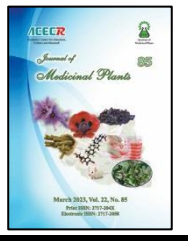
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Research Article

Phytochemical analysis and antimicrobial activities of *Scrophularia oblongifolia* Loisel. ethyl acetate extract

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ABSTRACT

Background: *Scrophularia oblongifolia* belongs to the Scrophulariaceae family, commonly known as the water figwort in Iran. It has been known as a traditional medicinal plant in Iran. **Objective:** This study was designed to investigate the phytochemicals and antimicrobial activity of ethyl acetate, methanol, MeOH: H_2O (50:50 v/v), and *n*-hexane extract of *Scrophularia oblongifolia* aerial parts, growing in the Kangavar region in the Kermanshah province of Iran. **Methods:** Compounds of ethyl acetate extract were isolated by chromatographic techniques (HPLC-PDA-MS/MS-Q-TOF) and their structures were elucidated by spectral methods (1D and 2D NMR). The antifungal and antibacterial activities of some new compounds and ethyl acetate, methanol, and *n*-hexane extract were evaluated. **Results:** Two new polyphenol iridoid glycosides with phenylpropanoid moiety namely scrophuloside A5 and scrophuloside A6; two known phenylpropanoid glycosides namely scrophuloside A3 and scrophuloside A4, and two known iridoid glycosides namely aucubin and harpagoside were isolated from *S. oblongifolia*. Pure compounds showed high antifungal activities with MIC values ranging from 0.35 to 0.74 µg/ml against *C. albicans* and high antibacterial activities with MIC values ranging from 0.70 to 1.48 µg/ml against *G. vajinalis*. *n*-Hexan extract showed moderate antibacterial and antifungal activity, while methanol and methanol:H_2O (50:50 v/v) extract showed no antimicrobial activities. **Conclusion:** Based on these findings, compounds isolated from *S. oblongifolia* have demonstrated potential antibacterial and antifungal activities.

Abbreviations: MPDRI, Medicinal Plants and Drug Research Institute; HPLC-MS-PDA, High-Performance Liquid Chromatography-Mass Spectrometry-Photodiode Array; TLC, Thin Layer Chromatography; EtOAc, Ethyl acetate; MeOH, Methanol; MIC, Minimum Inhibitory Concentration; UV, Ultraviolet; COSY, Correlated Spectroscopy; HMBC, Heteronuclear Multiple Bond Correlation; HSQC, Heteronuclear Single Quantum Coherence; CLSI, Clinical and Laboratory Standards Institute, BV; Bacterial Vaginosis

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1. Introduction

Recently, plants have been identified as a potential source of inexpensive, safe drugs [1]. According to the World Health Organization, 80 % of people use traditional medicines like plants to treat their illnesses [2]. Therefore, several studies have been conducted to support the effectiveness of herbal remedies [3]. One-third of the medicines used by humans are herbal medicines. The product and consumption of medicinal plants are increasing in industrialized and developed countries using different forms such as fresh, dried, and brewed [4].

About 300 species of the genus Scrophularia belong to the *Scrophulariaceae* family, which is widely distributed in temperate regions of the northern hemisphere. The use of some species in folk medicines dates back to ancient times for treating inflammation, itching, wounds, pain, dermatitis, fever, constipation, and tumours [5]. There have been several bioactive compounds isolated from these species, including iridoids, iridoid glycosides, phenylpropanoid glycosides, phenylethanoid glycosides, flavonoids, terpenoids, and saponins [6]. Compounds derived from these plants have shown antitumor, antioxidative, anticoagulant, antimicrobial, antipyretic, antiprotozoal, antifungal, antidiabetic, anti-inflammatory, hemodynamic, choleretic, immunomodulatory, hepatoprotective, and neuroprotective effects [7].

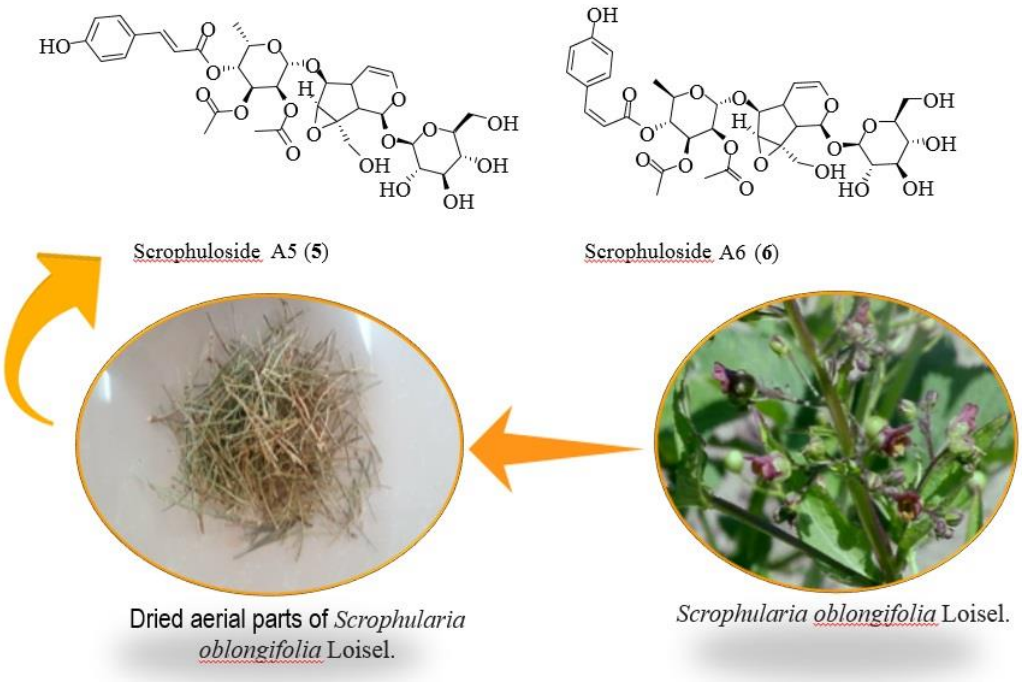


Fig. 1. Picture of dried and fresh aerial parts *Scrophularia oblongifolia* Loisel.

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Scrophularia oblongifolia Loisel. (syn: *Scrophularia umbrosa* Dumort.) commonly known as "water figwort," is one of the native species of Iran (Fig. 1). The species is mainly found in the west, northwest, north, central mountainous regions, northeast, and rarely in the south of Iran [8]. In previous studies, the content of iridoids is as high as 1.1 % in *S. oblongifolia* Dumortier; including catapol, aucubin, harpagide, and ajugol [9]. Inducements glandular-hairy are shiny, brown, yellow, black, and rarely white and can be found on the stem, leaves, peduncle, pedicel, calyx segments, and filament of stamens. Leaves are herbaceous with a thick or thin texture, can be lanceolate, obovate, or triangular, and grows mainly on riversides [10].

The chemical components of *S. striata* are cinnamic acid, flavonoids such as quercetin, isorhamnetin, and pethidine. Its essential oils contain short-chain terpenoids, for example, 1-octen-3-ol and phytol, that cause its odour [5]. During the research conducted by Man-Fei Han on *S. oblongifolia*, three new glycosylated and seven glycosylated phenylpropanoids were identified and isolated [11]. A pharmacological evaluation demonstrated that the iridoid and phenylethanol glycosides in Scrophularia exhibit more than 10 bioactivities, including anti-inflammatory, antioxidant, and antitumor activity [12].

Candida albicans is a diploid fungus and yeast that is commonly responsible for oral thrush and fungal vaginitis [13]. These microscopic fungi make up the body's flora, naturally live in a peaceful symbiosis with us, and do not cause disease [14]. *C. albicans* is the most common human pathogen among *Candida* species that causes genital, skin, and oral infections. *Gardnerella vaginalis* is a genus of anaerobic bacteria with variable warm staining [11].

A new approach to treating various malignant and inflammatory diseases is the inhibitory effects of *S. striata* aerial parts on metalloproteinase-1 Matrix. An investigation of the impact of this plant extract on two fungi, *Candida albicans*, and *Corynebacterium vaginale*, showed that it inhibited their growth [15]. In this study, seven iridoid glycosides were isolated and identified from *S. oblongifolia*. The antimicrobial activity of plant extracts and isolated compounds were evaluated against (*C. albicans*) and (*G. vajinalis*).

2. Materials and Methods

2.1. Chemical and materials

The solvents used in this experiment, including HPLC grade, were obtained from Merck (Germany). Analytical grade ethyl acetate (EtOAc), chloroform, formic acid, methanol (MeOH), and n-hexane were purchased from Neutron Pharmaceutical Co., Tehran, Iran. Deionized water was generated by a Millipore ultrapure water system (18 M, simplicity 185, Millipore, France). One filamentous fungus in this study was *Candida albicans* ATCC10231 and one bacterium *Gardnerella vajinalis* ATCC49145 was purchased from Taligene Pars company, Esfahan, Iran.

NMR spectra were recorded on a Bruker AVANCE III spectrometer operating at 500.13 MHz for ^{1}H-NMR and 125.77 MHz for ^{13}C-NMR. A 1 mm TXI microprobe with a z-gradient was used for 1H-detected experiments. HPLC bioassay-guided fractionation was done using a Knauer HPLC system by SunFire C_{18} column (250 mm × 4.6 mm) and Knauer photodiode array detector (PDA) (Fig. 2). The absorbance values were determined with a multi-well plate spectrophotometer (Elx 800 Microplate Reader, Bio-TEK, Winooski, Vermont, USA) at 570 nm. UHPLC-MS analyses

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were performed on a Waters Acquity UPLC system coupled to a Waters XevoTM quadrupole time-of-flight (QToF) mass spectrometer and equipped with an electrospray source with lock spray interface for accurate mass measurements. Acetylcholinesterase enzyme (AChE) from bovine erythrocyte, acetylthiocholine iodide (ATCI) and 5,5-dithiobis-(2-nitrobenzoic acid) (DTNB) were obtained from Sigma. Silica gel (70-230 mesh) was used for column chromatography and precoated silica gel F254 (20 × 20 cm) plates for TLC (both from Merck).

2.2. Plant Material

Aerial parts of *Scrophularia oblongifolia* (*S. oblongifolia*) (Fig. 1) were collected from the Kangavar region (34°30′15″N, 47°30′15″E), Kermanshah province, Iran, and identified and confirmed by Dr. Ali Sonboli, Medicinal Plants and Drug Research Institute (MPDRI), Shahid Beheshti University, Tehran, Iran. The plant was dried at 25 °C temperature for five days and gendered before extraction.

2.3. Extraction of dried aerial parts of S. oblongifolia

The maceration method was used for the extraction of the dried aerial parts of (*S. oblongifolia*) according to a previously developed methodology [16]. The grinder of dried aerial parts of (*S. oblongifolia*) (1 kg) was mixed with solvent in a ratio of 1:10 (sample: solvent). Extractions were carried out for 24 hours at room temperature with EtOAc, MeOH (95 %, analytical grade), MeOH:water (50:50 v/v) and *n*-Hexan as solvents separately. Afterward, the extract was filtered through filter paper and the solvent evaporated at 40 ºC using a rotary evaporator under a vacuum. Each extraction was done in triplicate. The yield of residues was obtained at 23.5 g for EtOAc, 6.4 g for *n*-hexane, and 107.19 g for methanol.

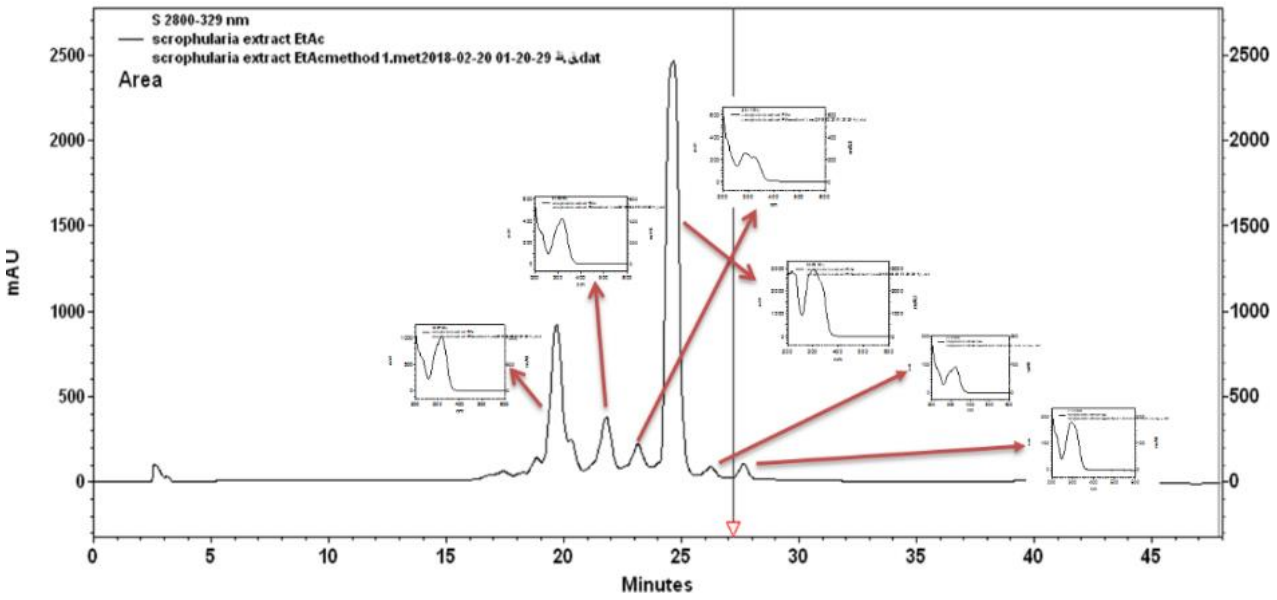


Fig. 2. HPLC-PDA chromatogram of EtOAc extracts recorded in 254 nm

2.4. Separation by column chromatography

A part of the EtOAc extract (16.6 g) was dissolved in a minimum amount of EtOAc:MeOH (50:50 v/v) and then mixed with 26 g of silica gel 60 (0.063-0.200 mm). The mixture was dried by rotary evaporation to obtain

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a fine powder and was charged on silica gel (70-230 mesh) in the column chromatography (80 × 6 cm) glass column. The fractionated was started by elution with solvent system *n*-hexane: EtOAc (50:50 v/v). The polarity of the mobile phase was increased by adding EtOAc (from 50 to 100 %), then the polarity of the mobile phase was increased by adding MeOH (from 0 % to 100 %). At the end of the fractionation process, 140 fractions were collected. Similar fractions with Rf (tested by TLC) values were combined into 17 (F1-F17) fractions and were dried by a rotary evaporator (Büchi, Flawil, Switzerland) at 40 °C under vacuum.

6 g of fraction F9 dissolved in 6 ml MeOH and were mixed with 8 g of silica gel (23-70 mesh) and dried by rotary evaporator (Büchi, Flawil, Switzerland) at 40 °C under vacuum and loaded on column (70 × 5 cm, with 200 g silica gel, 60, 70-230 mesh) glass column. The gradient eluted with chloroform followed by chloroform: MeOH with increasing polarity (from 100:0 % to 0:100 % MeOH). The fractionation process resulted in 107 fractions. A total of 19 fractions (S1-S19) were obtained by combining them based on similar fractions with R_f values (after screening by TLC). In sub-fractions of S3, 5 mg of compound (**3**), in sub-fractions of S4, 6.8 mg of compound (**2**), and in sub-fractions of S5, 5.4 mg of compound (**4**) was obtained. The Fraction S11 (20 mg) was subjected to the Semi-preparative HPLC method. 40 µl of fraction S11 with a concentration of 10 mg/ml was injected into the column. The elution was carried out with a water:acetonitrile (0-100 % to 100-0 %) gradient solvent system (flow rate of 4 ml/min at room temperature) containing formic acid (0.1 %) to afford compound (**1**) (1.5 mg), compound (**5**) (2 mg), compound (**6**) (1.8 mg) and compound (**7**) (2.5 mg).

2.5. Mass spectrometry

Mass spectrometry analysis Chromatography was performed on a Waters Acquity UPLC system coupled to a Waters XevoTM quadrupole time-of-flight (QTOF) mass spectrometer and equipped with a Z-spray electrospray ionization (E.S.I.) source with lock spray interface for accurate mass measurements. ESI MS data were acquired in positive ion mode over the 100-1000 m/z range using a survey scan method with lock correction allowing simultaneous acquisition of M.S. and MS/MS data for each sample. The structures of isolated compounds were elucidated by careful analysis of their spectroscopic as well as by comparison with those reported in the literature.

2.6. Antifungal and antibacterial activity assay

In the first screening step to finding the best antifungal and antibacterial compounds in *S. oblongifolia*, the minimum inhibitory concentration was performed (MIC) [17]. The EtOAc extracts of *S. oblongifolia* aerial part, *n*-hexane, MeOH, MeOH: water (50:50 v/v) and isolated and pure compounds (scrophuloside A2 **(3)**, A3 (**4**), A5 (**5**) and A6 **(6)**) were tested for their antifungal activity against one fungi pathogen namely: *Candida albicans* ATCC10231 (*C. albicans*) and antibacterial activity against one bacterium namely: *Gardnerella vajinalis* ATCC49145 *(G. vajinalis).* The broth micro-dilution susceptibility method for MIC was determined using 96-well microplate trays according to the standard protocol of the Clinical Laboratory and Standards Institute (CLSI).
A series of dilutions of the investigated material (0.015-32 mg/ml to 100 ml) for *C. albicans* were prepared in Mueller-Hinton culture. The inoculants of the fungi were adjusted to 0.5 McFarland standard turbidity sterile with normal

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saline and more diluted (1:100 for fungi) just before adding to the wells containing a favorite content of diluted samples in Mueller-Hinton broth medium. The results were recorded after incubating inoculated trays for 20 h at 37°C. The experiments were performed in duplicate. Nystatin (31.25 µg/ml) was used for *C. albicans* and clindamycin (500 µg/ml) was used for *G. vajinalis* as a positive control.

3. Results

The EtOAc extract of the aerial part of *S. oblongifolia* was partitioned to give four main fractions which were subjected to repeated column chromatography to yield seven compounds (**1**-**7**). Their structures (Fig. 3) were elucidated by careful analysis of their spectroscopic and spectrometric data as well and by comparison with some of those reported in the literature as aucubin (**1**) [18], angoroside C (**2**) [19], scrophuloside A2 (**3**), scrophuloside A3 (**4**), harpagoside (**7**) [20] and two new compounds including, scrophuloside A5 (**5**) and scrophuloside A6 (**6**). UHPLC-MS-QTOF chromatograms of scrophuloide A6 (6) were shown in Fig 4 at negative ion mode.

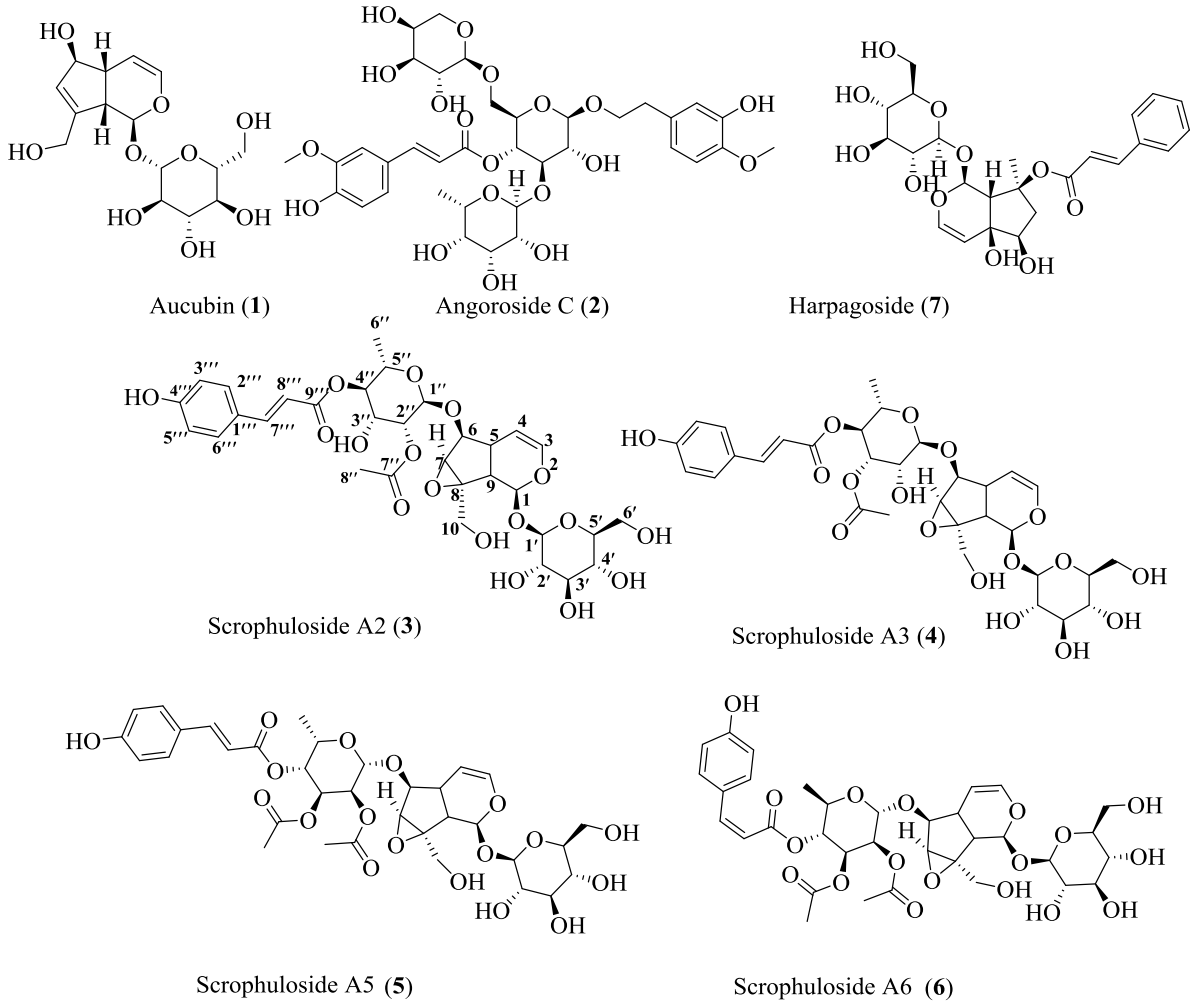


Fig. 3. Structures of compounds identified from EtOAc extract of *S. oblongifolia*

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Identified known compounds by UHPLC-MS/MS-QTOF and HPLC-PDA (Fig. 5) are listed in Table 1 with their ion mass in positive and negative modes. The structure of new compounds was confirmed using 1D (^{1}H- and ^{13}C-NMR) and 2D (HSQC, HMBC, and H-H COSY) NMR spectral data, in which ^{1}H- and ^{13}C-NMR data are given in Table 2. Their characterization was obtained using UHPLC-MS/MS-Q-TOF, HPLC-PDA, MS-QTOF techniques.

3.1. Identification of compounds Scrophuloside A2 *and* Scrophuloside A3

Scrophuloside A2 (**3**) (5 mg) and scrophuloside A3 (**4**) (5.4 mg) were obtained as a yellow powder with molecular weights of 696.22 g/mol and a molecular formula $C_{32}H_{40}O_{17}$ isolated. Scrophuloside A2 (3) was found to have a great similarity to scrophuloside A3 (4), which was identified as polyphenol iridoid glycoside with phenylpropanoid moiety. The COSY spectrum (Fig. 6) shows a splitting of H7''' by H8".

On the HMBC spectrum (Fig. 7), C9''' visible by 7''' and 8''' and 4" of rhamnose hydrogens. Equivalent hydrogens, H2''', H6"', and H3''', H5''' correlation with C7''', C4''' and C1''', respectively. The chemical shift of C4''' confirms the presence of the hydroxyl group on this carbon. H4" (δ 5.05 ppm) in H-H COSY correlates with H3" at 4.16 ppm and H5" at 4.02 ppm. In the HMBC spectrum, C5" is visible by methylene hydrogens, and H1" is apparent at 5.02 ppm. Also, correlations of this hydrogen with C5", C3", and C5 in 35.87 chemical shifts are seen. The signal at 35.87 ppm is related to a quaternary carbon of the iridoid structure of these compounds. Due to two electronegative groups attached to C1", C2" is not visible by H1". The connection of iridoid and phenylpropanoid groups is on C1" and C3" of rhamnose. The correlation between H3 and H4 and H1 and H9 were identified on the cozy spectrum, and H4, at 10.5 ppm on the HMBC spectrum, is in relation to C3, C5, and C9. Also, H1 correlation with C3, C5, C8, C9, and C1' at 98.50 ppm. C1 relates to section four of the anomeric carbon of glucose structure, and H9 connects to C1, C5, C6, C7, and C8. H5 correlation to C1, C3, C4, and C6 and H6 correlation to C4, C5, C7, and the anomeric carbon rhamnose at 96.40 ppm on the HMBC spectrum. H1' link to C1, C3' and C5' that seen as ^{3}J. The connection between H1' and H2' is seen on H-H-COSY; furthermore, H2' forms a cross-pick to H3'. On the COSY spectrum, H6' shows cross pick with H5' and diastereotopic H6' only correlation with C4'.

Table 1. The list of compounds identified in the EtOAc extract of *S. oblongifolia*

No	Compounds	RT	*m/z* [M-H]$^{-}$	m/z [M+H]$^{+}$
1	Aucubin **(1)**	8.06	391.12 [M+HCOOH-H^{-}]	364.16[M+NH_4^+]
2	Angoroside C **(2)**	25.77	-	802.31[M+NH_4^+]
3	Scrophuloside A2 **(3)**	29.39	695.22	697.22
4	Scrophuloside A3 **(4)**	29.39	695.22	697.22
5	Scrophuloside A5 **(5)**	29.76	737.23	739.23
6	Scrophuloside A6 **(6)**	29.76	737.23	739.23
7	Harpagoside **(7)**	40.04	493.17	495.17

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Table 2. The ^{1}H-NMR and ^{13}C-NMR chemical shifts of scrophuloside A2 (**3**) and scrophuloside A3 (**4**)

Position	Scrophuloside A2 (3)		Scrophuloside A3 (4)	
	δ_H	δ_C	δ_H	δ_C
1	5.08	93.96	5.08	93.96
2	-	-	-	-
3	6.40	141.00	6.40	141.00
4	5.10	102.03	5.10	102.03
5	2.46	35.87	2.46	35.87
6	4.03	83.18	4.03	83.18
7	3.68	58.05	3.68	58.05
8	-	65.30	-	65.30
9	2.59	42.04	2.59	42.04
10	3.86	60.13	3.86 4.16	60.13
1'	4.81	98.50	4.81	98.50
2'	3.31	73.51	3.31	73.51
3'	3.45	76.46	3.45	76.46
4'	3.33	70.43	3.33	70.43
5'	3.35	77.16	3.35	77.16
6'	3.68 3.93	61.59	3.68 3.93	61.59
1'''	-	125.95	-	126.30
2''', 6'''	7.64 (d, J = 8.6 Hz, 2H)	132.33	7.48 (d, J = 8.6 Hz, 2H)	130.02
3''', 5'''	6.78 (d, J = 8.6 Hz, 2H)	114.60	6.83 (d, J = 8.6 Hz, 2H)	115.62
4'''		159.95		159.95
7'''	7.68 (d, J = 15.9 Hz, 1H)	145.93	7.64 (d, J = 15.9 Hz, 1H)	146.28
8'''	6.38 (d, J = 15.9 Hz, 1H)	113.69	6.31 (d, J = 15.9 Hz, 1H)	113.20
9'''	-	167.45	-	166.34
1''	5.02	96.40	5.02	96.40
2''	5.15	71.93	4.09	67.04
3''	4.16	67.08	5.14	71.93
4''	5.05	73.61	5.00	73.61
5''	4.02	67.01	3.96	66.99
6''	1.20	16.56	1.18	16.56
7''	-	171.11	-	170.90
8''	2.17 (s)	19.61	2.02	19.55

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3.2. Identification of compound Scrophuloside A5 and Scrophuloside A6

Two new compounds scrophuloside A5 (**5**) (2 mg) and scrophuloside A6 (**6**) (1.8 mg) as a white powder, M; 738.23 g/mol, and the chemical formula $C_{34}H_{42}O_{18}$ were isolated. HPLC chromatogram of scrophuloside A6 (**6**), and UV absorption spectrum of the HPLC-UV were shown in Fig 8, 9, and 10, respectively. On the ^{1}H-NMR spectrum, H7‴ and H8‴ split each other, and on the HMBC spectrum C9‴ is visible by H7‴, H8‴ and H4" of rhamnose sugar. Equivalent hydrogens H2‴, H6‴ and H3‴, H5‴ have correlation with C7‴, C4‴ and C1‴, respectively. The chemical shift of C4‴ indicates the existence of hydrogen on this carbon. Most of the signals of the iridoid structure of the two new compounds (scrophuloside A5 and scrophuloside A6) are identical except for H4 and H6. On the HMBC spectrum, the connection between the rhamnose anomeric H (seen by HSQC) was found with C6 in the iridoid part. The chemical shifts of compounds have been shown in Table 3.

The assignments of protons (Fig 11) and carbons of Scrophuloside A5 and Scrophuloside A6 were made by detailed analysis H–H COSY (Fig 12), HMBC (Fig 13) and HSQC (Fig 14) spectra. H-H COSY spectrum displayed two coupled spin systems of H-1″/H-2″ and H-4″/H-5″. In ^{1}H-NMR two methyl proton signals at δH 2.18 and 2.03 indicated the presence of two acetyl moieties. HPLC chromatogram of Scrophuloside A5 (compound 5). ^{1}H-NMR and H-H-COSY of Scrophuloside A6 (compound 6) are shown in Fig. 15 & 16.

3.3. Antifungal and antibacterial activity assay

The disk diffusion and agar dilution method were used for testing the antibacterial activity of crude extracts and pure compounds. The *S. oblongifolia* EtOAc extracts showed moderate activity against *C. albicans* with a MIC value of 8 mg/ml and against *G. vajinalis* with a value of MIC = 2 mg/ml. Methanolic extraction showed no antifungal activity (MIC = 32 mg/ml) against *C. albicans* and moderate antibacterial activity against *G. vajinalis* (MIC = 8 mg/ml). *n*-Hexane extract showed activity at a lower concentration than methanolic and EtOAc extract. It showed moderate activity (MIC = 2 mg/ml) against *C. albicans* and *G. vajinalis*. In terms of MeOH: H_2O (50:50) extract, no activity was found against *C.albicans* and *G. vajinalis* (MIC = 32 µg/ml). In another study, the aerial part of *S. oblongifolia* dichloromethane extract (at concentrations > 2 mg/ml) revealed antimalarial activity [21].

In the same way, the antibacterial and antifungal activity of scrophuloside A2, A3, A5 and Scrophuloside A6 were determined against *C .albican* and *G. vajinalis.* Scrophuloside A2 and A3 showed very high activity (MIC = 0.35 mg/ml) against *C. albican* and good avtivity (MIC = 0.70 mg/ml) against G. *vajinalis*. While Scrophuloside A5 and A6 showed activity lower than Scrophuloside A2 and A3 against *C. albican* and *G. vajinalis.* Scrophuloside A5 and A6 showed good activity (MIC = 0.74 mg/ml) against *C. albican* and *G. vajinalis* MIC = 1.48 mg/ml. This indicates that purification boosted the biological activity of phytoconstituent comparing to the crude extract.

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Table 3. The ^{1}H-NMR and ^{13}C-NMR chemical shifts of compounds 5 and 6

Position	Scrophuloside A5 (5)		Scrophuloside A6 (6)	
	δ_H	δ_C	δ_H	δ_C
1	5.08	93.93	5.08	93.93
2	-	-	-	-
3	6.40	141.06	6.40	141.06
4	5.10	101.87	5.03	101.86
5	2.49	35.80	2.49	35.80
6	4.06	83.77	4.03	83.40
7	3.68	58.00	3.68	58.00
8	-	65.31	-	65.31
9	2.60	42.03	2.60	42.03
10	3.86 4.15	60.06	3.86 4.15	60.06
1′	4.80	98.50	4.80	98.50
2′	3.30	73.50	3.30	73.50
3′	3.44	76.45	3.44	76.45
4′	3.31	70.42	3.31	70.42
5′	3.34	77.17	3.34	77.17
6′	3.67 3.93	61.60	3.67 3.93	61.60
1″	5.08	96.43	5.06	96.23
2″	5.32	69.90	5.30	69.90
3″	5.38	69.33	5.28	69.44
4″	5.18	70.75	5.13	70.75
5″	4.09	67.00	3.97	66.81
6″	1.24	16.43	1.21	16.43
7″	-	170.50	-	170.39
8″	2.17 (s)	19.36	1.94 (s)	19.30
1‴	-	125.68	-	126.21
2‴, 6‴	7.48 (d, *J* = 8.6 Hz, 2H)	130.05	7.63 (d, J = 8.6 Hz, 2H)	132.31
3‴, 5‴	6.83 (d, *J* = 8.6 Hz, 2H)	115.61	6.79 (d, J = 8.6 Hz, 2H)	114.61
4‴	-	160.12	-	158.85
7‴	7.66 (d, *J* = 15.9 Hz, 1H)	146.50	6.96 (d, J = 12.7 Hz, 1H)	145.43
8‴	6.32 (d, *J* = 15.9 Hz, 1H)	112.84	5.77 (d, J = 12.7 Hz, 1H)	114.40
9‴	-	166.81	-	165.60

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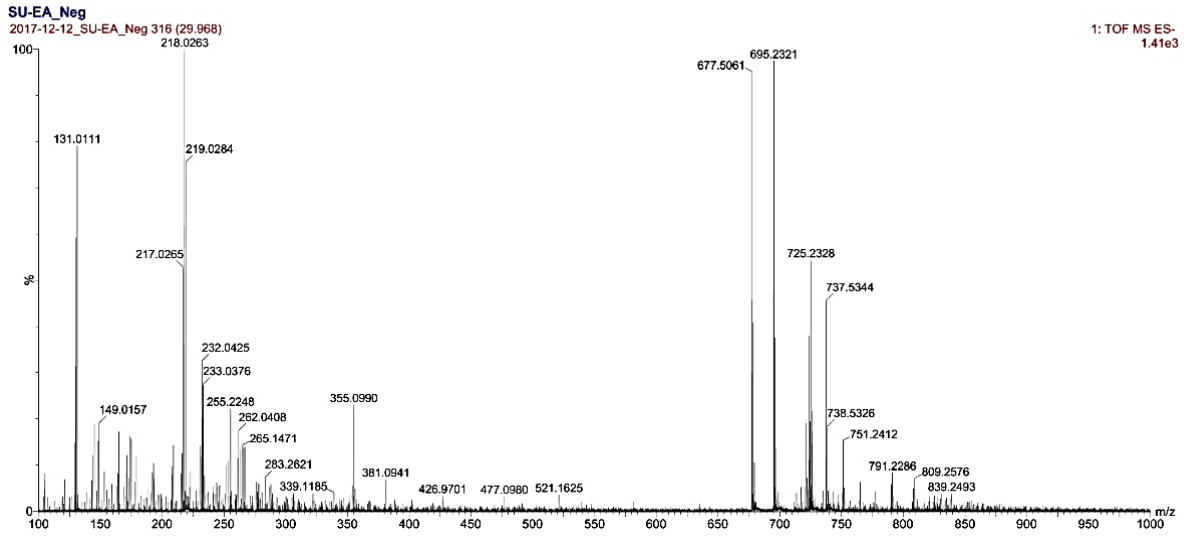


Fig. 4. UHPLC-MS-QTOF spectrum scrophuloide A6 (6) on negative mode

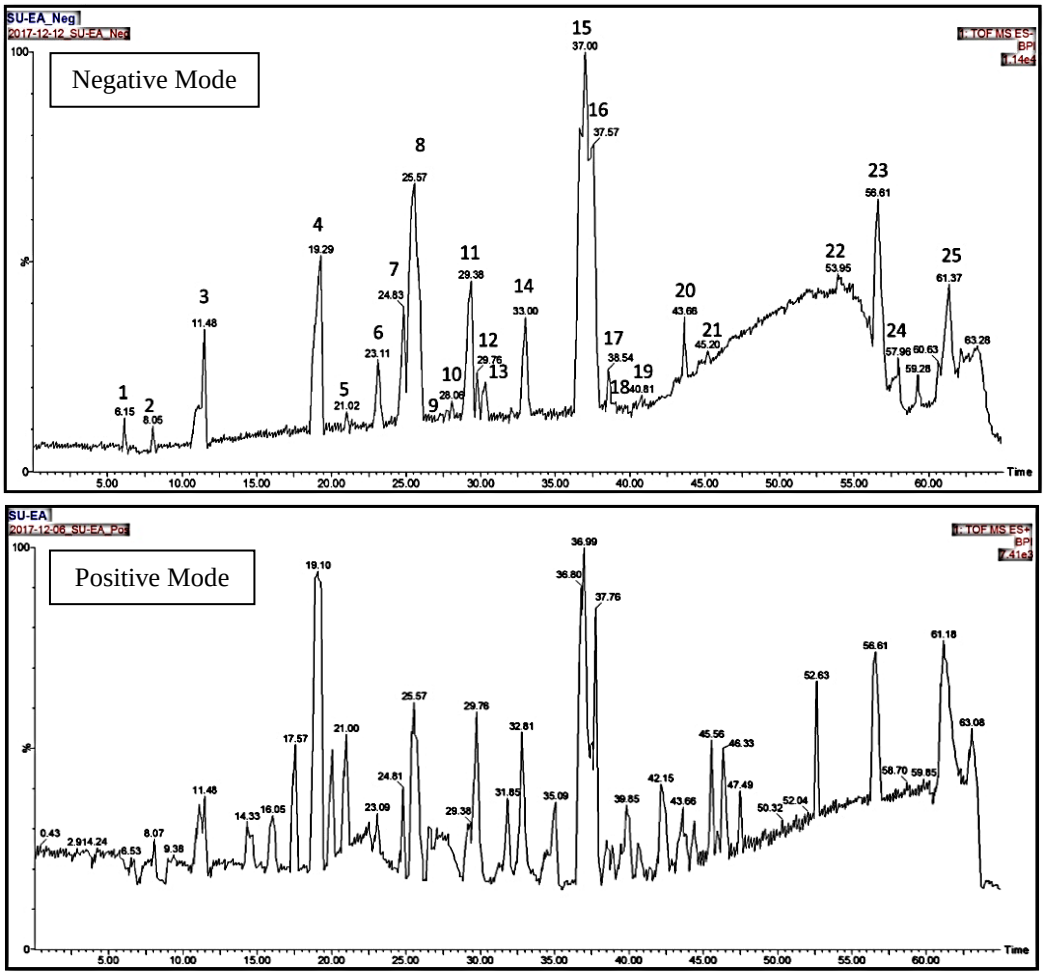


Fig. 5. UHPLC-MS/MS-Q-TOF of EtOAc extracts in negative and positive mode

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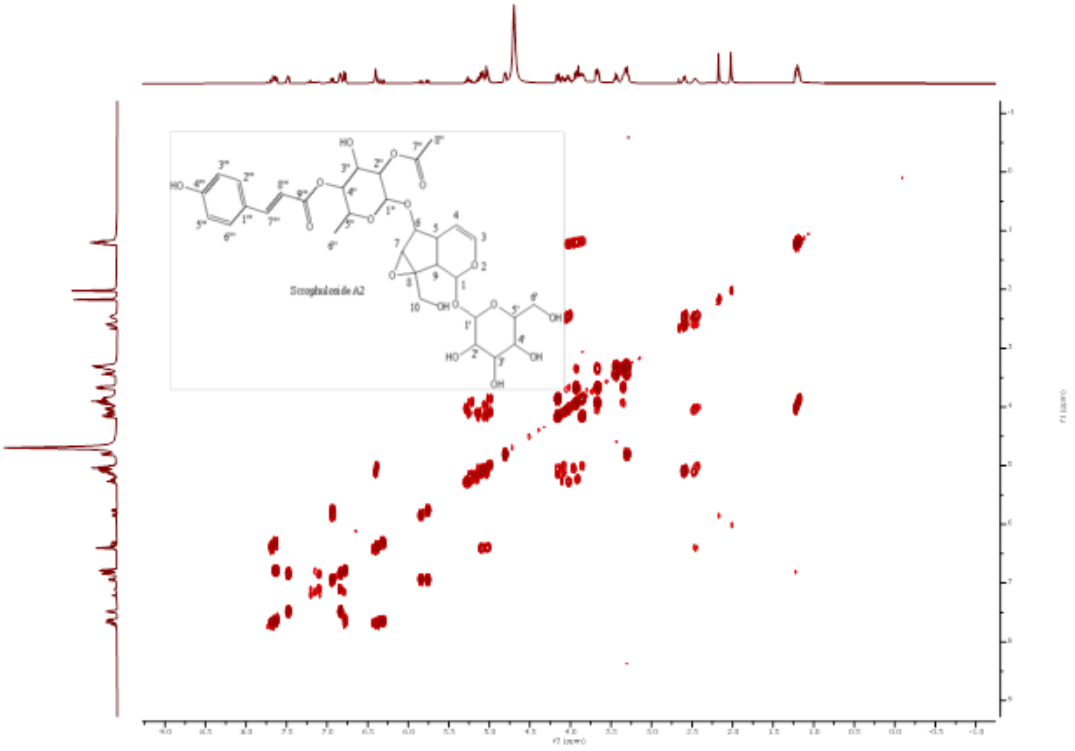


Fig. 6. H-H-COSY spectrum of scrophuloside A2, 500 MHz, DMSO-D6

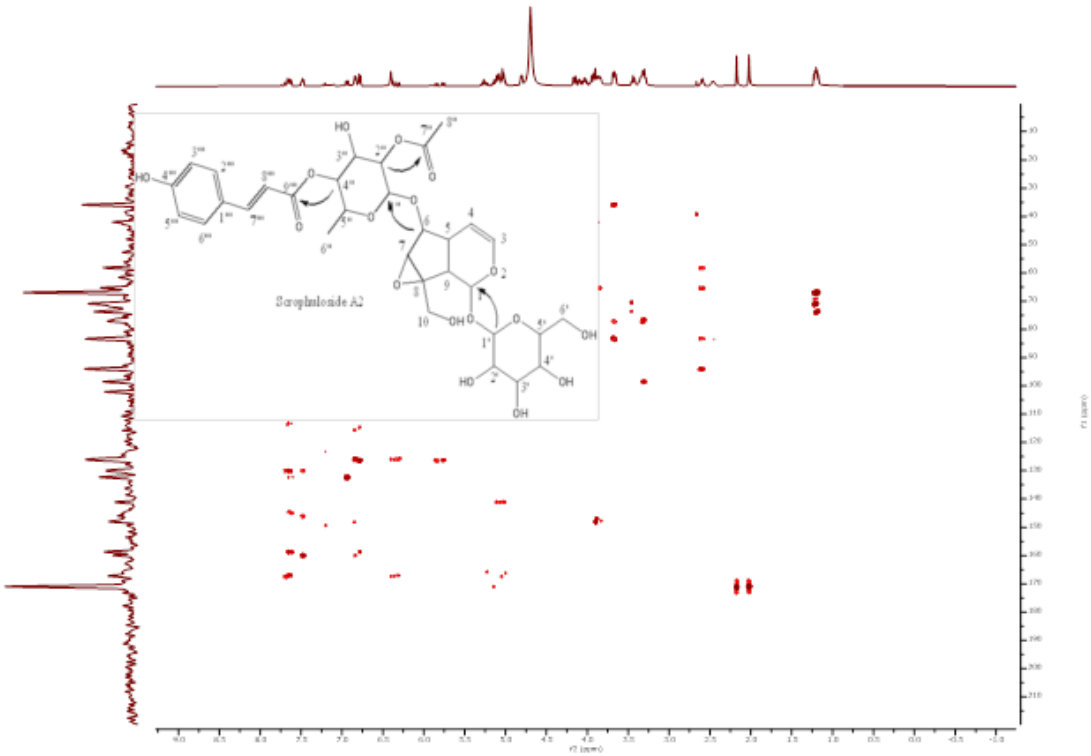


Fig. 7. HMBC spectrum of scrophuloside A2, 500 MHz, DMSO-D6

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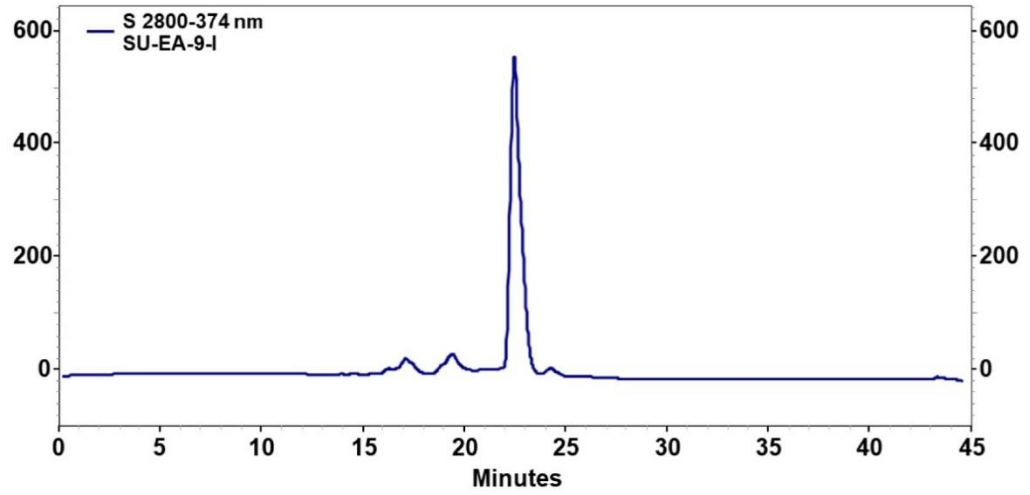


Fig. 8. HPLC chromatogram of scrophuloside A5 (compound 5)

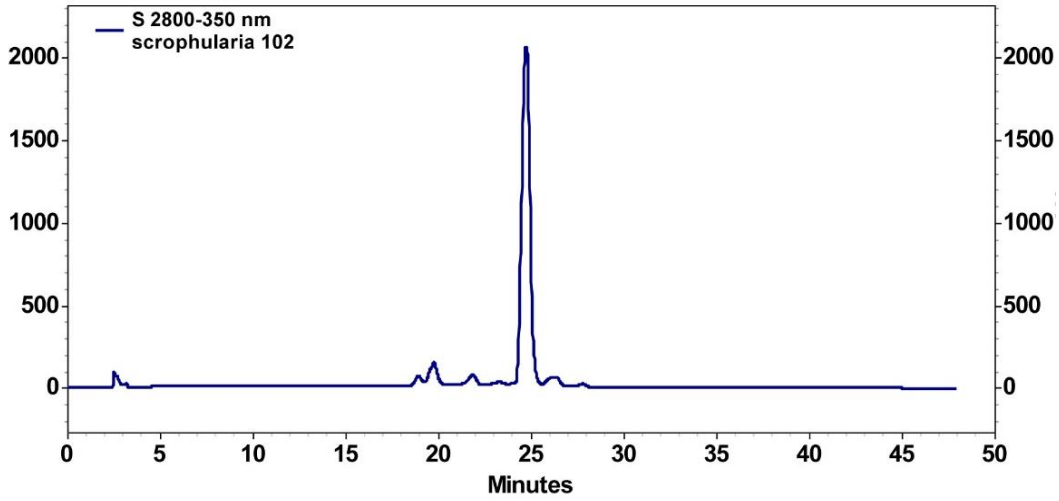


Fig. 9. HPLC chromatogram of scrophuloside A6 (compound 6)

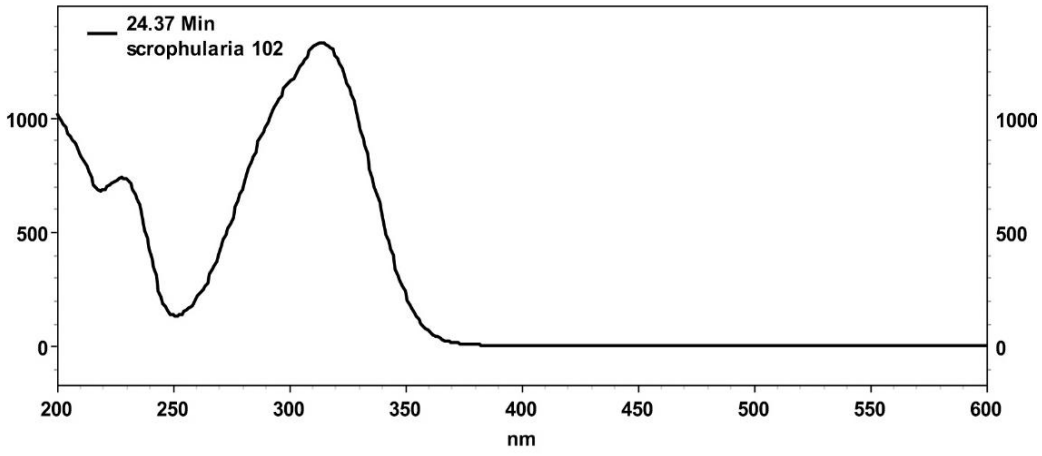


Fig. 10. UV absorption spectrum of the HPLC-UV

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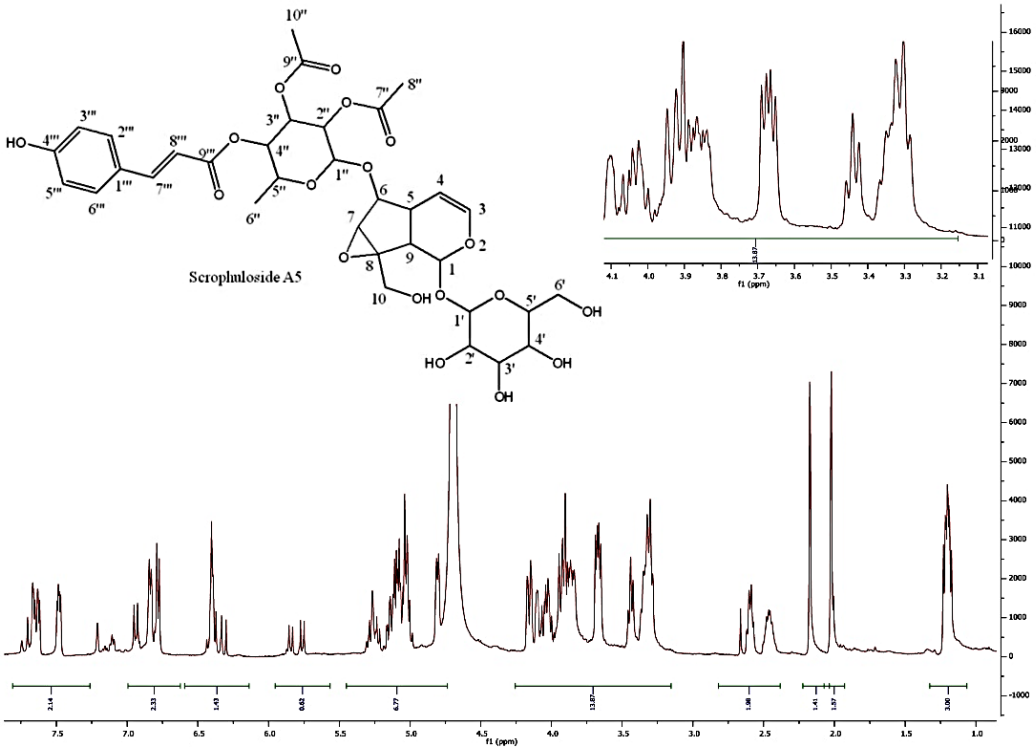


Fig. 11. [1]H-NMR spectrum of scrophuloside A5, 500 MHz, DMSO-D6

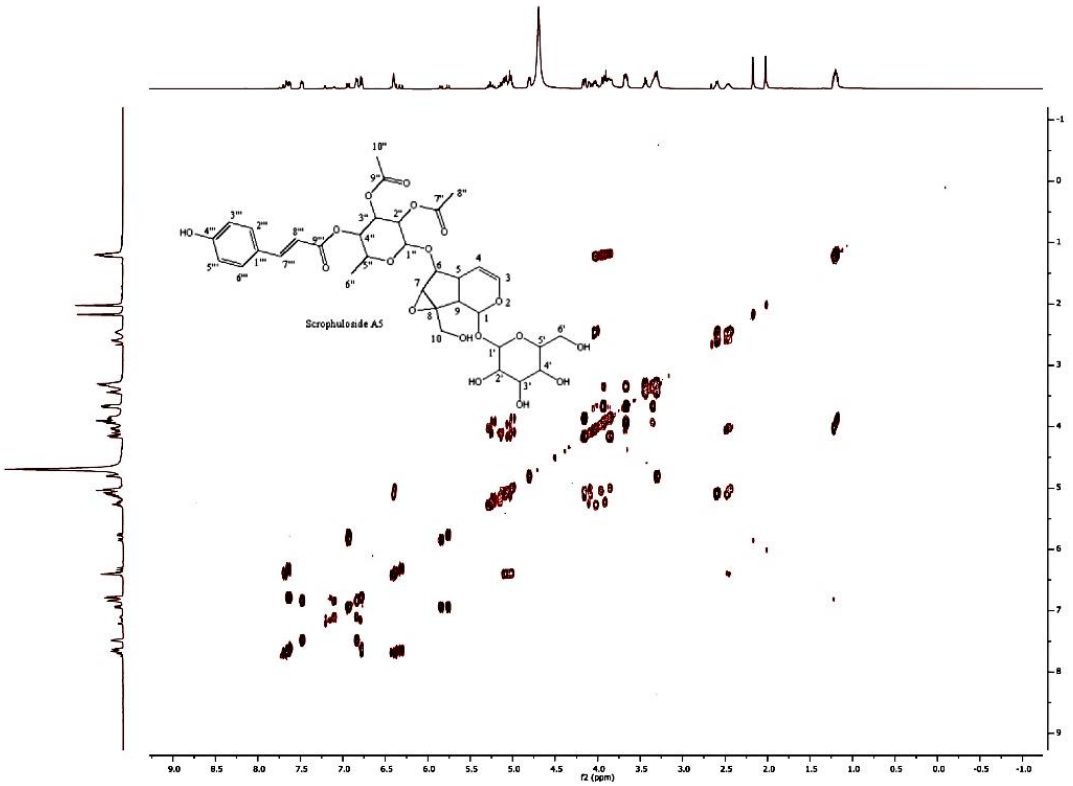


Fig. **12.** H-H-COSY spectrum of scrophuloside A5, 500 MHz, DMSO-D6

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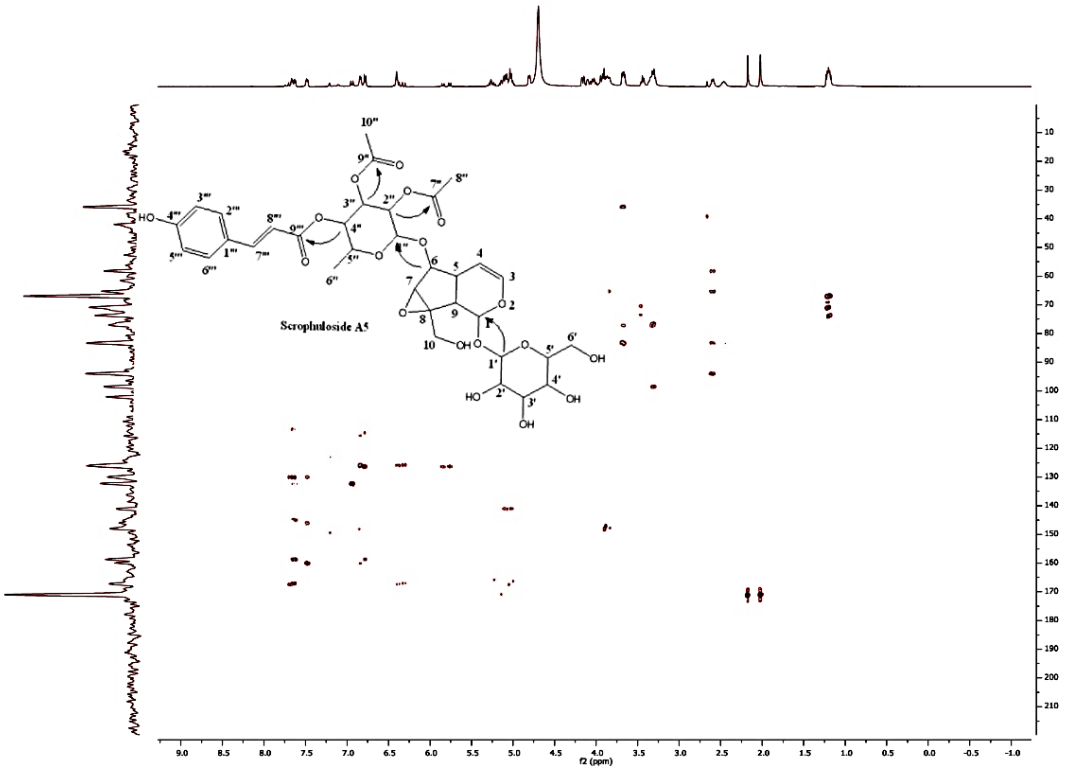


Fig. 13. HMBC spectrum of scrophloside A5, 500 MHz, DMSO-D6

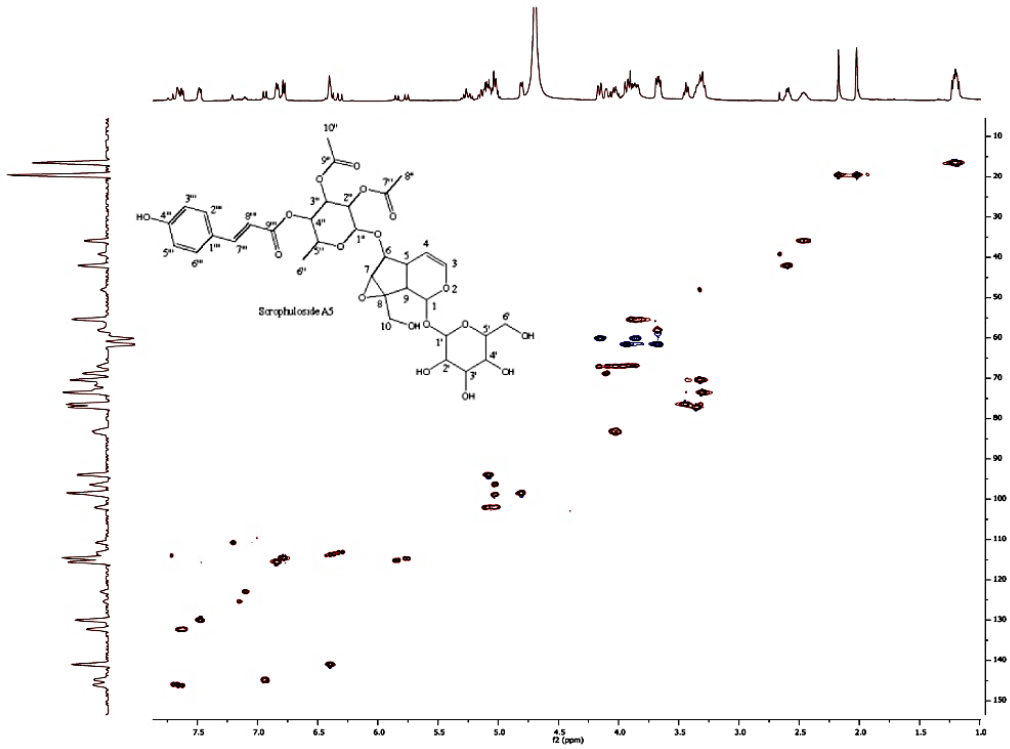


Fig. 14. HSQC spectrum of scrophloside A5, 500 MHz, DMSO-D6

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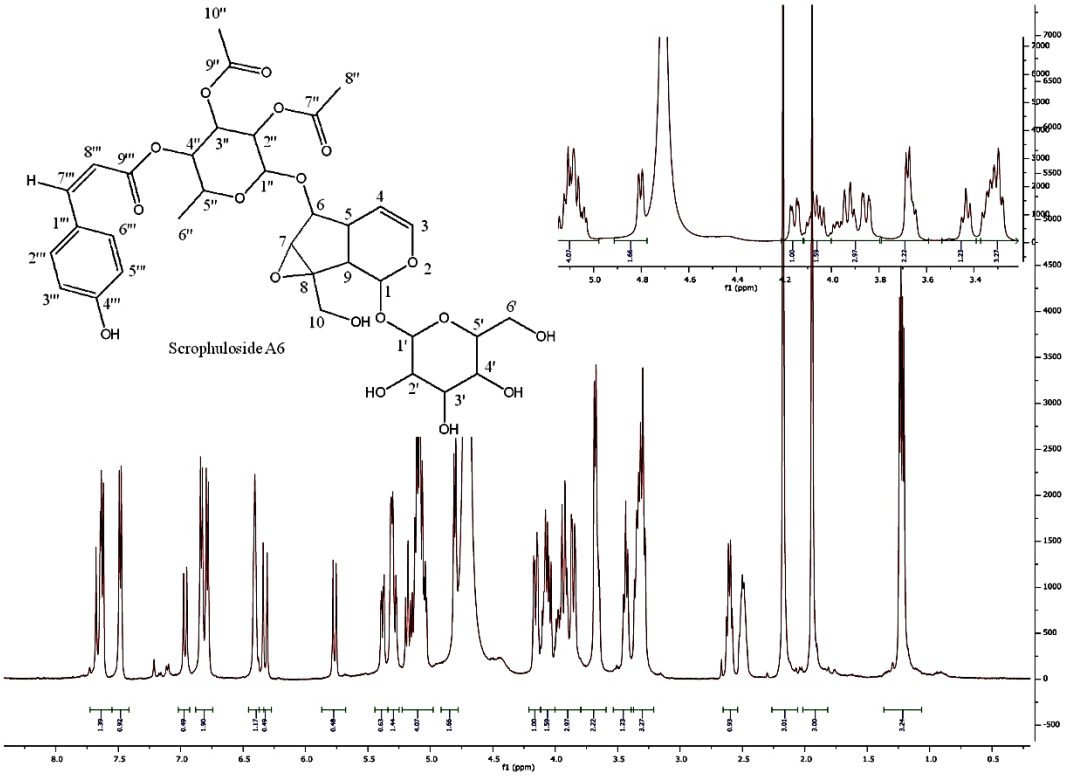


Fig. 15. ^{1}H-NMR spectrum of Scrophuloside A6, 500 MHz, DMSO-D6

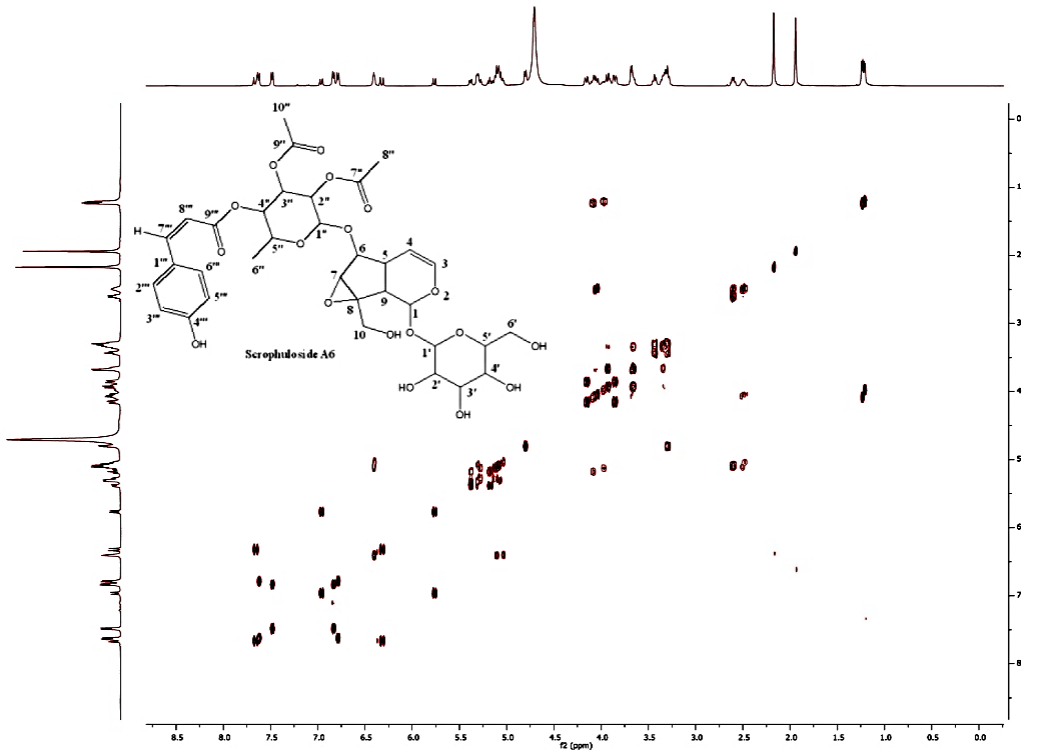


Fig. 16. H-H-COSY spectrum of Scrophuloside A6, 500 MHz, DMSO-D6

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4. Discussion

Plants are an important source of potentially useful structures for the development of new chemotherapeutic agents. The first step towards this goal is the *in vitro* antibacterial and antifungal activity assay [18]. However, there are few reports on the exploitation of antifungal or antibacterial properties of plants.

In this study, reversed-phase semi-preparative-HPLC extraction, and HPLC-PDA-MS/MS-Q-TOF analyses of the aerial part of *S. oblongifolia* EtOAc extract, led to the separation and characterization of seven compounds (**1**-**7**), including iridoid glycosides, and phenylethanoid glycosides Table 1. Two compounds, Scrophuloide A5 (**5**) and Scrophuloide A6 (**6**) are reported for the first time in *S. oblongifolia*. Mansouri et al. indicated that the *S. oblongifolia* methanolic extract contains an effective compound (s) for the treatment of breast cancer [22]. In another study, Han et al. identified and isolated three iridoid glycosides including umbrosides A, B, and C from ethanolic extract of the dried aerial of *S. oblongifolia* Dumortier, which are grown in China. They found that the isolated compounds inhibited the production of LPS-stimulated nitric oxide, with IC_{50} values ranging from 9.0 to 40.3 μM [12]. According to previous studies catapol, aucubin, harpagide, and ajugol have been isolated from *S. oblongifolia* Dumortier, with the largest amount of aucubin followed by harpagide and minor amounts of catalpol, methyl catalpol, ajugol and gluroside [23]. The present work indicates that *S. oblongifolia* can be an important source of antifungal and antibacterial compounds that may provide renewable sources of useful antifungal drugs against infections in humans. This justifies the use of many of these plants in traditional medicine to cure infections. However, further work is needed on the most active plants to develop new and more potent antifungal drugs from natural sources. Infectious diseases can be treated effectively with antifungals and antibacterial derived from plant sources.

The current findings showed that Scrophuloside A5 **(5)** and A6 (**6**) (new compounds), A2 (**3**) and A3 (**4**) have higher antimicrobial activity compared to the crude extracts. On the other hand, *n*-hexane extract had almost the same effects (moderate activity) against *C. albicans* and *G. vajinalis.* As shown in Table 4, EtOAc extract had moderate activity against *C. albicans* and *G. vajinalis* and MeOH:water extract showed no antibacterial and antifungal activities. In contrast, the antibacterial activities of the *S. oblongifolia* extracts (in terms of MeOH and EtOAc) were lower than the antifungal activities. The antifungal activities of pure compounds were greater than their antibacterial activities.

Table 4. For antimicrobial assays of extracts and pure compounds, the MIC values are in mg/ml

Compounds and extracts	MIC (*C. albicans*)	MIC (*G. vaginalis*)
n-Hexane	2.0	2.0
EtOAc	8.0	2.0
MeOH	32.0	8.0
MeOH: H_2O	32.0	32.0
Scrophuloside A2 **(3)** Scrophuloside A3 **(4)**	0.35	0.70
Scrophuloside A5 (**5**) Scrophuloside A6 (**6**)	0.74	1.48
Nystatin	0.03	-
Clindamycin	-	0.50

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5. Conclusion

In conclusion, the chemical study of *S. oblongifolia* led to the isolation and characterization of seven compounds. Among them, Scrophuloside A5 (compound **5**) and Scrophuloside A6 (compound **6**) are reported for the first time in *S. oblongifolia*. *n*-Hexane, MeOH, MeOH:water (50:50 v/v), as well as EtOAc extract and some of its isolated compounds were evaluated for their bacterial and fungal activity. The antibacterial and antifungal activity of EtOAc extract on *C. albicans* and *G. vaginalis* was lower than two pure compounds (**3**) and (**5**). *n*-Hexane showed moderate antimicrobial, which was higher than other *S. oblongifolia* extracts. It seems that *S. oblongifolia* extract is a potent inhibitor of the growth of bacterial and fungal pathogens in vitro. Thus, it can be assumed as a promising medicine for managing all types of vaginal infections. Further studies are needed to screen the phytochemicals of *S. oblongifolia* hexane extract due to the relatively higher biological activity than EtOAc extract.

Author Contribution

M.S.N. and H.K. prepared the draft of the manuscript and performed the experiment; S.N.E supervised the project and reviewed the Draft; AA contributed to the experimental biological part of the work and assisted in writing the draft of the manuscript.

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Conflicts of interest

The authors declare that there is no conflict of interest.

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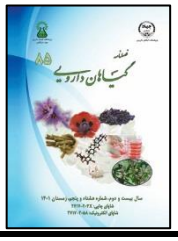
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مقاله تحقیقاتی

بررسی فیتوشیمیایی و فعالیت ضدمیکروبی عصاره اتیل استاتی گل میمونی جویباری

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اطلاعات مقاله

گل‌واژگان:
گل میمونی جویباری
گل میمونی سایه پسند
فعالیت بیولوژیکی
خاصیت ضدمیکروبی
گیاه تشنه داری
ایریدوئیدهای گلیکوزیدی

چکیده

مقدمه: گل میمونی جویباری از خانواده گل میمونی است که در ایران به تشنه‌داری نیز معروف است. در ایران به عنوان یک گیاه دارویی سنتی شناخته شده است. **هدف:** این مطالعه به منظور بررسی ترکیبات فیتوشیمیایی و فعالیت ضدمیکروبی عصاره‌های اتیل استات، متانول، متانول: آب (۵۰: ۵۰ حجمی/حجمی) و نرمال هگزانی اندام‌های هوایی گل میمونی جویباری از منطقه کنگاور در استان کرمانشاه ایران انجام شد. **روش بررسی:** ترکیبات عصاره اتیل استاتی با تکنیک‌های کروماتوگرافی (HPLC-PDA-MS/MS-Q-TOF) جداسازی و ساختار آنها با روش‌های طیف‌سنجی رزونانس مغناطیس هسته (تک بعدی و دو بعدی) مشخص شد. فعالیت ضد قارچی و ضدباکتریایی برخی از ترکیبات جدید و عصاره‌های اتیل استاتی، متانولی و نرمال هگزانی مورد بررسی قرار گرفت. **نتایج:** دو ترکیب جدید پلی فنل گلیکوزید ایریدوئیدی با یک بخش فنیل پروپانوئید، به نام اسکروفولوزید A5 و اسکروفولوزید A6؛ دو گلیکوزید فنیل پروپانوئیدی شناخته شده یعنی اسکروفولوزید A3 و اسکروفولوزید A4 و دو گلیکوزید ایریدوئیدی شناخته شده به نام‌های آکوبین و هارپاگوزید از گیاه گل میمونی جویباری جدا شدند. ترکیبات خالص جدا شده از عصاره اتیل استاتی گیاه، فعالیت ضدقارچی بالایی با مقادیر حداقل غلظت بازدارندگی ۰/۳۵ تا ۰/۷۴ میکروگرم در میلی‌لیتر در برابر قارچ کاندیدا آلبیکنس و فعالیت ضدباکتریایی قوی با مقادیر حداقل غلظت بازدارندگی ۰/۷۰ تا ۱/۴۸ میکروگرم در میلی‌لیتر در برابر گاردنرلا واژینالیس نشان دادند. عصاره نرمال هگزانی فعالیت ضدباکتریایی و ضدقارچی متوسطی را نشان داد، در حالی که عصاره متانولی و متانول: آب (۵۰: ۵۰ حجمی/حجمی) فعالیت ضدمیکروبی نشان نداد. **نتیجه گیری:** بر اساس این یافته‌ها، ترکیبات جدا شده از گیاه گل میمونی جویباری فعالیت ضدباکتریایی و ضدقارچی بالقوه‌ای را نشان داده‌اند.

مخففها: MPDRI، پژوهشکده گیاهان و مواد اولیه دارویی؛ HPLC-MS-PDA، کروماتوگرافی مایع با کارآیی بالا- طیف سنجی جرمی- با آرایه فتودیود؛ TLC، کروماتوگرافی لایه نازک؛ EtOAc، اتیل استات؛ MeOH، متانول؛ MIC، حداقل غلظت بازدارندگی؛ UV، فرابنفش؛ COSY، اسپکتروسکوپی ارتباطی؛ HMBC، طیف سنجی همبستگی چند پیوندی ناجور هسته‌ای؛ HSQC، طیف سنجی همبستگی تک کوانتومی هسته‌ای؛ CLSI، موسسه استانداردهای بالینی و آزمایشگاهی؛ BV، واژینوزیس باکتریایی

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