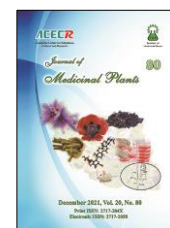




Institute of  
Medicinal Plants

## Journal of Medicinal Plants

Journal homepage: [www.jmp.ir](http://www.jmp.ir)



### Research Article

## Phytochemical analysis and biological activities of essential oil and extract of *Phlomis rigida* Labill.

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### ARTICLE INFO

#### Keywords:

*Phlomis rigida* Labill.

Antibacterial

Antioxidant

Cytotoxic

Luteolin

Luteolin-7-O-glucoside

Rosmarinic acid

### ABSTRACT

**Background:** The genus *Phlomis* is a member of Lamiaceae (Labiatae) family. About 10 species of this genus, among more than 100 species, are endemic to Iran. *Phlomis rigida* Labill. can be used as antimicrobial, anti-inflammatory, wound healing and antioxidant in drug investigations, based on literature. **Objective:** The aim of this study is to evaluate the composition, antioxidant, antibacterial and cytotoxic activity of essential oils and extract from leaves and flowers of *P. rigida* from Iran. The present study is the first research on *P. rigida* from Iran. **Methods:** Essential oils of leaves and flowers of *P. rigida* were studied by GC-MS and GC-FID to evaluate the chemical compositions. DPPH free radical scavenging method was used to evaluate the antioxidant effect of leaves and flowers extracts. Antimicrobial properties of the essential oils and extracts were investigated against various microorganisms in brain heart infusion agar to evaluate the minimum inhibitory concentration. A brine shrimp test (BST) was done to study the cytotoxicity of methanol extracts and essential oils from leaves and flowers in different concentrations. The growth inhibitory effects of the methanol extract from leaves and flowers were assessed against four cancer cell lines including MCF-7, MDBK, HT-29 and A-549. **Results:** Essential oils analysis showed 34 compounds and the main compounds were (*Z*)- $\beta$ -ocimene (25.6 %), isobornyl acetate (16.6 %), *trans*-verbenol (12.6 %) and  $\alpha$ -pinene (7.6 %). As a result of analysis of methanol extracts from leaves, luteolin, luteolin-7-O-glucoside and rosmarinic acid were isolated and identification for the first time from *P. rigida* growing in Iran. Investigation of antibacterial properties of the essential oil of flowers also showed 16 mm diameters of inhibition zone against *Proteus vulgaris*. **Conclusion:** The results of this study showed that the antibacterial effect of *P. rigida* essential oil could be due to the presence of its monoterpenes.

### 1. Introduction

Medicinal plants are important in discovering new drug because of their biologically active

compounds [1]. The genus *Phlomis* (Lamiaceae) comprises 100 species indigenous to the Mediterranean region and spread across central

**Abbreviations:** NMR, Nuclear Magnetic Resonance; GC-MS, Gas Chromatography-Mass Spectrometry; DPPH, 2,2-Diphenyl-1-picrylhydrazyl

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doi: 10.52547/jmp.20.80.13

Received 17 June 2021; Received in revised form 19 October 2021; Accepted 19 October 2021

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Asia to China. This genus is consisted of 17 species in Iran, 10 of which are endemic such as *P. olivieri*, *P. bruguieri*, *P. rigida* and *P. kurdica* [2, 3]. In folk medicine several species of *Phlomis* are used as tonics, stimulants, diuretics and for the treatment of hemorrhoid and respiratory tract diseases [4-7]. In addition, many studies demonstrate numerous biological and pharmacological activities for some plants of the genus such as anti-malarial [8], antimicrobial [9-11], anti-allergic [12] and antifebrine effects [13]. *P. rigida* has been used in Anatolian traditional medicine as a wound healer. *P. rigida* is a perennial plant that can grow as high as 125 cm with glandular hairs. Its leaves are large, oblong, elliptic or cuneate and are 5-30 x 2-10 cm in size with pale greenish colour and tomentose hairs. It is verticillate, has 5-8- 18 flowers, numerous bracteoles, subulate 20 - 25 mm, the calyx is 15 - 23 mm and dense hispid stellate-tomentose hairs, the corolla is pink-purple [14]. In Iran this plant has been seen in parts of Azerbaijan, Kurdistan, Hamedan and Kermanshah. The studies have demonstrated that the main compounds of essential oil from aerial parts of *P. rigida* are (E)-2-Hexenal,  $\beta$ -Caryophyllene, Germacrene D, n-Hexanal,  $\beta$ -selinene [15, 16]. Investigation of volatile components from *P. rigida* aerial part has showed the major compounds were hexadecanoic acid, neophytadiene isomer, myrcene, pentacosane, methyl hexadecanoate, and tricosane, respectively. Analysis of phytochemical compositions of the *P. rigida* extracts led to the identification of different compounds. In recent studies in Turkey, the major components of *P. rigida* aerial part methanol extract were luteolin and apigenin [17]. From pharmacological point, the studies have demonstrated that *P. rigida* can be utilized as

antimicrobial, antibacterial, anti-inflammatory, wound healing, antioxidant agents [17, 18].

## 2. Materials and Methods

### 2.1. Plant material

In the July of 2018, *P. rigida* was collected from Sarab Gian, near Nahavand in Hamadan Province. The plant was identified and authenticated by Dr. Majid Agha Ahmadi in Research Institute of Forests and Rangelands, Tehran (TARI) herbarium (TARI-106632).

### 2.2. Extraction and fractionation

The 400 g of dried leaves of *P. rigida* were grounded and powdered parts of the plant were macerated with methanol: water (4: 1) solvent for 48 hours (3 times). The extract was then shaken, filtered and then the solvent evaporated in a rotating evaporator under reduced pressure at approximately 40 °C until dryness to obtain total extraction. The methanol extract was dissolved in methanol and poured to a silica gel column chromatography (normal phase, 8 × 50 cm, 400–230 mesh) with a gradient of chloroform: EtOAc (100:0 to 0:100 V/V) as eluent, followed by increasing concentrations of MeOH (up to 5 %) in EtOAc. Fraction number 10 (1.4 g) was submitted to Sephadex LH-20 CC (1 × 90 cm) MeOH was used as the mobile phase to result a compound we marked as M1. Fraction 11 (86 mg) was chromatographed on Sephadex LH-20 CC (1 × 90 cm) using MeOH as mobile phase to give compound M2. Fraction 8 (0.5 g) was purified with Sephadex LH-20 CC (2 × 80 cm) using MeOH as eluent to obtain compound M3. All the solvents and silica gel (230 - 400 mesh) used in this study were purchased from Merck (Germany). The identification was done through <sup>1</sup>H-NMR and <sup>13</sup>C-NMR.

### 2.3. Essential oils

Using a Clevenger apparatus, the shade-dried and comminuted aerial parts of the plant (100 g) were subjected to hydrodistillation for 4 hours. The resulted essential oils were dried over anhydrous sodium sulphate and kept at 4 °C until analyses.

### 2.4. GC-MS and GC-FID analysis

Essential oils of the leaves and flowers of *P. rigida* Labill. were analyzed using a HP-5973 gas chromatograph with a HP-INNOWAX column (60 m × 0.320 mm id, 0.50 µm film thickness), equipped with HP-5973 mass detector (Ionization energy: 70 eV). The oven temperature was kept at 80 °C for 10 min and programmed to 230 °C at a rate of 4°C/min, then increased to 250 °C and kept constant for 10 min. The injector temperature and detector temperature were set at 250 °C. Injection volume was 0.1 µl with a split ratio: 1:90 and carrier gas: helium (Flow rate: 1.5 ml min<sup>-1</sup>). The Kovats retention indices (KIs) were calculated for all identified compounds using a homologous series of *n*-alkanes injected under the same conditions described to samples identification of the compounds done based on computer matching with the Wiley275.L and Wiley7n.L libraries, as well as by comparison of KIs and mass fragmentation patterns with those published for standard compounds [19]. The percentage compositions of the individual components were obtained from the gas chromatograph with a HP-INNOWAX column fitted with FID detector in conditions were equal to GC-MS analysis [20].

### 2.5. DPPH free radical scavenging

A 0.1 mM DPPH in methanol solution was prepared, and 2.4 ml of this solution was mixed with 1 ml of extract from leaves and flowers at different concentrations (800, 500, 250, 100,

50, 5, 0.5 µg/ml). The reaction mixtures were vortexed thoroughly and left in the dark at RT for 30 min. The absorbance of the mixture was measured spectrophotometrically at 517 nm. BHT was used as a reference [12]. The following equation calculated the percentage of DPPH radical scavenging activity:

$$I \% = \left[ \frac{A_0 - A_1}{A_0} \right] \times 100$$

Where  $A_0$  is the absorbance of the control, and  $A_1$  is the absorbance of the extractives/standard. Then the percentage of inhibition was calculated against various concentrations (0.5, 0.25, 0.125, 0.0625 and 0.03125 mg/ml) of essential oils from leaves and flowers.

### 2.6. Minimum Inhibitory Concentration (MIC)

The suspensions of bacterial strains that was provided from Iranian Research Organization for Science and Technology (IROST) including *Proteus vulgaris* (PTCC 1182), *Bacillus subtilis* (ATCC 6633), *Klebsiella pneumoniae* (ATCC 10031), *Salmonella paratyphi-A serotype* (ATCC 5702), *Staphylococcus aureus* (ATCC 29737), *Shigella dysenteriae* (PTCC 1188), *Escherichia coli* (ATCC 10536), *Aspergillus brasiliensis* (ATCC 1015), *Aspergillus niger* (ATCC 16404), *Staphylococcus epidermidis* (ATCC 12228). Microorganisms were prepared in standard turbidity (0.5 McFarland) from their 12 h broth cultures. A concentration range of extracts and essential oils (8 to 500 µg/ml) were made in sterile test tubes containing brain heart infusion (BHI) broth. 95 µl of the media, 5 µl of the inoculum and 100 µl from sample dilutions were dispensed into each well of the 96-well plates. A well containing 195 µl of the medium and as negative control 5 µl of the inoculum without the test sample was used. The content of

plates were mixed on a plate shaker at 300 rpm for 20 s and then incubated at appropriate temperatures for 24 h. 30 mg/ml using DMSO as a solvent and filtered through 0.45 µm Millipore filters for sterilization. 100 µl of suspension containing 108 CFU/ml of bacteria was spread onto the nutrient agar (NA). The impregnated discs (6 mm in diameter) with 10 µl of the volatile oils or the extracts solutions (300 µg/disc) and DMSO (as negative control) were placed on the inoculated agar. All plates were incubated at 37 °C for 24 h and the diameters of inhibition zones (mm) were measured. Gentamicin (10 µg/disc) and rifampin (5 µg/disc) were also used as positive controls [21].

### 2.7. Brine shrimp lethality test (BST)

The toxicity of different extractions was examined by brine Shrimp lethality test. BST has been applied as an alternative bioassay technique to screen the toxicity of plant extracts. The *Artemia salina* eggs were hatched under direct light in an aerated tank containing 35 % artificial sea water with 28 – 30 °C temperature for 24 hours. Blank test tubes contained 50 µl of dimethyl sulfoxide and were diluted with brine water. After 48 h, 15 nauplii were added to tubes with different concentrations of each extraction (10, 100, 300, 500, 700 and 1000 µg/ml), and incubated at the same previous condition. Each test was performed in triplicate. After 24 h, survivals were evaluated by counting dead larvae and mortality percentage was calculated for each concentration and negative control group (vehicle without any fraction) by the following formula:

$$\text{Mortality percentage} = \left[ \frac{(D_{\text{test}}/N_{\text{test}} - D_{\text{control}}/N_{\text{control}})}{(A_{\text{control}}/N_{\text{control}})} \right] \times 100$$

D, A and N indicated the number of dead, alive, and total larvae of *A. salina*, respectively.

The results were reported for potential toxic extracts [22].

### 2.8. MTT assay

Four cancer cell lines, MCF-7, MDBK, HT-29 and A-549 using the MTT method (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) were used to assess the growth inhibitory effects of the methanol extracts from leaf and flower. These cells were prepared at Pasteur Institute, Iran. In 96-well plates containing RPMI medium cells were incubated for 24 h at 37 °C. Then the cells were treated with a fresh medium containing different concentrations of extract and compounds in triplicates. After 24 h the medium was replaced by phosphate-buffered saline (PBS) medium containing 10 µl of MTT (5 mg/ml) and incubated for an additional 3 h. After that, the formazan crystals produced from MTT were completely dissolved in DMSO, and the absorbance of samples was recorded at 630 nm using a microplate reader. IC<sub>50</sub> was described as the concentration of extracts (in µg/ml) and pure compounds (in mM) which caused a 50 % reduction in the number of viable cells relative to the negative control [23]. The %viability is calculated based on the following equation:

$$\% \text{ Viability} = \frac{\text{Mean OD sample}}{\text{Mean OD blank}} \times 100$$

Where the OD is optical density unit (OD = 590 nm) and shows the amount of absorbance.

## 3. Results

12 main fractions (1-12) were obtained in the extraction process. Phytochemical investigation of the methanol extracts resulted from the leaves of *P. rigida* Labill. Sephadex LH-20 columns led to the isolation of three compounds: luteolin, luteolin-7-O-glucoside and rosmarinic acid (Fig. 1).

GC-MS and GC-FID analysis of the essential oils obtained from *P. rigida* leaves and flowers showed 34 compounds, in which (*Z*)- $\beta$ -ocimene (25.6 %), isobornyl acetate (16.6 %), *trans*-verbenol (12.6 %) and  $\alpha$ -pinene (7.6 %) were the main compounds (Table 1). DPPH free radical scavenging investigation in various extract concentrations demonstrated relatively fair antioxidant activity (Table 2). The results reported mild antibacterial effects for the extracts and essential oils against *Proteus vulgaris*, *Staphylococcus aureus* and *Escherichia coli* (Table 3). Brine shrimp test of methanol extractions and essential oils from flowers and leaves corroborated low cytotoxic effects of evaluated samples against brine shrimp larvae (Table 4). Weak cytotoxic activity of methanol extracts confirmed against MCF-7 (> 100), MDBK (> 100), HT-29 (> 100) and A-549 (0) cell line (Table 5).

### 3.1. Spectral Data

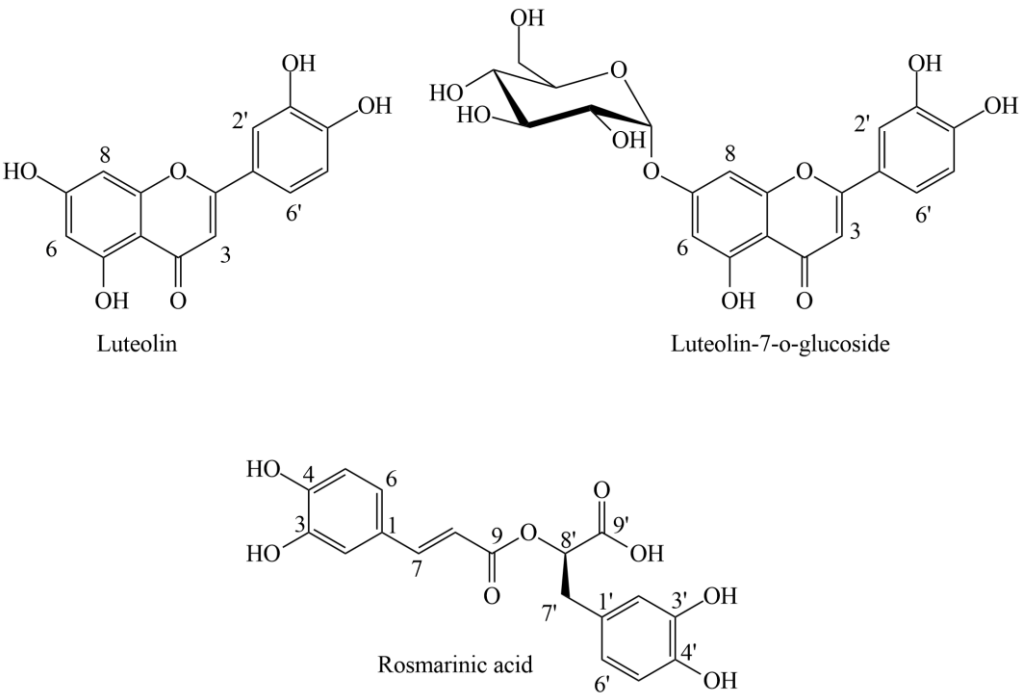
The isolated and purified compounds from methanol extraction were identified as below [24, 25]:

**Luteolin (M1)**,  $^1\text{H-NMR}$  (200 MHz, DMSO- $d_6$ ):  $\delta_{\text{H}}$  7.38 (2H, m, H2',6'), 6.87 (1H, d,  $J$ = 10.7 Hz, H5'), 6.64 (1H, s, H3), 6.43 (1H, s, H8), 6.19 (1H, s, H6);  $^{13}\text{C-NMR}$  (125 MHz, DMSO- $d_6$ ):  $\delta$  182.66 (C-4), 164.91 (C-7), 164.01 (C-2), 161.94 (C-5), 158.15 (C-9), 149.88 (C-4'), 144.84 (C-

3'), 122.42 (C-1'), 119.01 (C-6'), 115.53 (C-5'), 112.91 (C-2'), 103.87 (C-10), 102.82 (C-3), 98.84 (C-6), 93.71 (C-8).

**Luteolin -7-O-glucoside (M2)**,  $^1\text{H-NMR}$  (500 MHz, DMSO- $d_6$ ):  $\delta_{\text{H}}$  7.53 (2H, H2',6'), 7.26 (1H, d,  $J$  = 7.9 Hz, H5'), 6.81 (1H, s, H8), 6.50 (1H, s, H3), 6.21 (1H, s, H6), 4.90 (1H, d,  $J$  = 6.5 Hz, H1"), 3.1-4.2 (5H, m, H2",3",4",5" and 6");  $^{13}\text{C-NMR}$  (125 MHz, DMSO- $d_6$ ):  $\delta$  181.66 (C-4), 165.04 (C-2), 163.14 (C-7), 161.53 (C-5), 157.13 (C-9), 148.60 (C-4'), 147.09 (C-3'), 124.81 (C-1'), 118.42 (C-6'), 116.03 (C-5'), 113.49 (C-2'), 103.91 (C-10), 103.56 (C-3), 101.26 (C-1"), 99.10 (C-6), 94.13 (C-8), 77.33 (C-5"), 75.95 (C-3"), 73.31 (C-2"), 69.82 (C-4"), 60.77 (C-6").

**Rosmarinic acid (M3)**,  $^1\text{H-NMR}$  (500 MHz, DMSO- $d_6$ ):  $\delta_{\text{H}}$  7.35 (1H, d,  $J$  = 16.00 Hz, H7), 7.07 (1H, br s, H2), 6.90 (1H, br d,  $J$  = 6.5, H6), 6.75 (1H, d,  $J$  = 7.4 Hz, H5), 6.70 (1H, br s, H2'), 6.67 (1H, d,  $J$  = 7.8 Hz, H5'), 6.54 (1H, br d,  $J$  = 7.8 Hz, H6'), 6.24 (1H, d,  $J$  = 16.00 Hz, H8), 4.90 (1H, d,  $J$  = 8.4, H8'), 3.09 (1H, br d,  $J$  = 13.57 Hz, H7'b), 2.82 (1H, dd,  $J$  = 13.57, 11.02 Hz, H7'a);  $^{13}\text{C-NMR}$  (125 MHz, DMSO- $d_6$ ):  $\delta$  173.06 (C-9'), 166.48 (C-9), 149.21 (C-4), 146.27 (C-7), 145.02 (C-3), 144.47 (C-3'), 143.63 (C-4'), 130.14 (C-1'), 125.72 (C-1), 120.84 (C-6), 118.79 (C-6'), 116.48 (C-2'), 116.08 (C-5), 115.29 (C-5'), 115.02 (C-2), 114.24 (C-8), 75.80 (C-8'), 37.19 (C-7')



**Fig. 1.** The isolation of three compounds from leaves extract of *Phlomis rigida* Labill.

**Table 1.** Chemical compositions of the essential oils of *P. rigida* (flowers and leaves).

No.	Compounds <sup>a</sup>	KI <sup>b</sup>	KI <sup>c</sup>	Plant Part	
				Leaves (%)	Flowers (%)
1	$\alpha$ -Pinene	935	936	9.2	7.6
2	Camphene	952	954	3.7	1.3
3	Verbenene	957	968	1.1	1.0
4	Sabinene	976	975	0.2	0.6
5	$\beta$ -Pinene	981	979	0.6	0.9
6	Myrcene	992	991	1.1	1.5
7	$\alpha$ -Phellandrene	1010	1003	0.0	0.4
8	<i>o</i> -Cymene	1031	1026	1.9	3.4
9	Limonene	1034	1029	3.3	2.3
10	$\beta$ -Phellandrene	1036	1030	0.8	0.2
11	( <i>Z</i> )- $\beta$ -Ocimene	1041	1037	22.5	25.6
12	( <i>E</i> )- $\beta$ -Ocimene	1050	1050	2.1	0.8
13	$\gamma$ -Terpinene	1063	1060	0.8	2.0
14	Terpinolene	1090	1089	0.7	4.1
15	<i>p</i> -Cymenene	1098	1091	2.2	0.2
16	Linalool	1106	1097	1.0	0.3
17	allo-Ocimene	1134	1132	3.2	2.2
18	( <i>Z</i> )-Myroxide	1137	1135	0.0	0.2
19	<i>cis</i> -Verbenol	1152	1141	1.3	2.9
20	<i>trans</i> -Verbenol	1156	1145	9.3	12.6
21	<i>p</i> -Mentha-1,5-dien-8-ol	1183	1170	0.7	2.5
22	Terpinen-4-ol	1190	1177	0.0	0.3
23	<i>m</i> -Cymen-8-ol	1199	1180	0.4	1.3
24	Verbenone	1205	1205	0.8	0.3
25	<i>cis</i> -Chrysanthenyl acetate	1288	1265	0.0	0.4

**Table 1.** Chemical compositions of the flowers and leaves essential oils of *P. rigida*. (Continued)

No.	Compound <sup>a</sup>	Plant Part			
		KI <sup>b</sup>	KI <sup>c</sup>	Leaves (%)	Flowers (%)
26	Isobornyl acetate	1292	1286	18.1	16.6
27	<i>E</i> -Anethole	1300	1285	0.2	0.9
28	Myrtenyl acetate	1332	1327	1.2	0.2
29	$\alpha$ -Copaene	1381	1377	1.0	0.3
30	$\beta$ -Elemene	1395	1391	2.8	0.2
31	$\beta$ -Cedrene	1425	1421	0.9	0.2
32	$\gamma$ -Curcumene	1483	1483	1.6	0.3
33	Germacrene D	1490	1485	2.4	1.0
34	$\delta$ -Cadinene	1526	1523	1.9	0.7
	Monoterpene Hydrocarbons			52.5	54.1
	Oxygenated Monoterpenes			33.0	38.4
	Sesquiterpene Hydrocarbons			10.6	2.6
	Total Identified			96.1	95.1

Note: <sup>a</sup>Compounds listed in order of elution from HP-5MS column. <sup>b</sup>Retention indices in literature. <sup>c</sup>Retention indices to C8–C24 *n*- alkanes on HP-5MS column.

**Table 2.** Antioxidant activity of leaves and flowers extracts from *P. rigida*.

Sample	DPPH free radical scavenging assay IC <sub>50</sub> (μg/ml)
Leaves extract	201.1 ± 9.08
Flowers extract	206.8 ± 8.16
BHT	21.2 ± 2.6

**Table 3.** Antibacterial activity of the extracts and essential oils from leaves and flowers of *P. rigida*.

Microorganism <sup>a</sup>	Total extract of Leaves	Total extract of flowers	Essential oil of leaves	Essential oil of flowers	Rifampin	Gentamicin
<i>P. v</i> IZ <sup>b</sup> (MIC <sup>c</sup> )	9 (> 500)	11 (> 500)	14 (> 500)	16 (= 500)	-	20 (= 125)
<i>B. s</i> IZ (MIC)	-	-	-	-	13 (15)	21 (= 500)
<i>K. p</i> IZ (MIC)	9 (> 500)	10 (> 500)	-	-	7 (= 250)	22 (= 250)
<i>S. p</i> IZ (MIC)	-	-	11 (> 500)	12 (> 500)	-	21 (= 500)
<i>S. a</i> IZ (MIC)	-	-	15 (> 500)	14 (= 500)	10 (= 250)	21 (= 500)
<i>S. d</i> IZ (MIC)	12 (> 500)	11 (> 500)	9 (> 500)	12 (= 500)	40 (= 250)	35 (= 500)
<i>E. c</i> IZ (MIC)	-	-	14 (> 500)	13 (> 500)	11 (= 500)	20 (= 500)
<i>A. b</i> IZ (MIC)	-	-	9 (> 500)	10 (= 500)	-	-
<i>A. n</i> IZ (MIC)	-	-	10 (> 500)	10 (> 500)	-	-
<i>S. e</i> IZ (MIC)	11 (> 500)	-	-	-	8 (= 250)	18 (= 500)

A dash (-) indicates no antimicrobial activity. <sup>a</sup> Microorganism: *P. v* (*Proteus vulgaris*), *B. s* (*Bacillus subtilis*), *K. p* (*Klebsiella pneumoniae*), *S. p* (*Salmonella paratyphi-A serotype*), *S. a* (*Staphylococcus aureus*), *S. d* (*Shigella dysenteriae*), *E. c* (*Escherichia coli*), *A. b* (*Aspergillus brasiliensis*), *A. n* (*Aspergillus niger*), *S. e* (*Staphylococcus epidermidis*). <sup>b</sup> Inhibition zone in diameter (mm) around the impregnated discs including diameter of the disc (6 mm) [weak activity (< 10 mm), moderate activity (10 - 15 mm), strong activity (15 - 20 mm), very strong activity (> 20 mm)]; <sup>c</sup>Minimal inhibition concentrations (as mg/ml).



**Table 4.** BST mortality percentage and LC<sub>50</sub> for extract and essential oils from leaves and flowers of *P. rigida*.

Mortality percentage of different concentrations (µg/ml)							
Concentrations (µg/ml)	10	100	300	500	700	1000	LC <sub>50</sub> (µg/ml)
Flowers extract	0	0	3.33	6.66	30.0	56.66	930 ± 9
Leaves extract	0	0	3.33	6.66	36.66	60.0	953 ± 7
Essential oils of leaves	0	0	6.66	10.0	13.33	23.33	> 1000
Essential oils of flowers	0	16.66	20	23.33	75.5	89.66	561 ± 6

**Table 5.** IC<sub>50</sub> of MTT test (µg/ml) of methanol extract for *P. rigida* leaves and flowers.

Cell lines	Methanol extract of flowers (IC <sub>50</sub> : µg/ml)	Methanol extract of leaves (IC <sub>50</sub> : µg/ml)
MCF-7	> 100	> 100
MDBK	> 100	> 100
HT-29	> 100	> 100
A-549	> 100	> 100

#### 4. Discussion

Based on previous studies, the main compounds of essential oil from aerial parts of *Phlomis rigida* Labill. were (E)-2-hexenal, β-caryophyllene, germacrene D, n-hexanal, β-selinene (15, 16). The results of this study show that the main compounds of essential oils are (Z)-β-ocimene (25.6 %), isobornyl acetate (16.64 %), trans-verbenol (12.60 %) and α-pinene (7.65 %). Analysis of methanol extracts from leaves led to the identification of luteolin, luteolin-7-O-glucoside and rosmarinic acid. There is only one report from Turkey about the identification of these compounds by the LC-MS. According to the pharmacological results of this study, *Phlomis rigida* Labill. is a plant that is rich in bioactive compounds. Noticeable antioxidant and antibacterial activities from different extracts and essential oils of *P. rigida* make it an appropriate leading plant for developing new antioxidant and antibiotic agents to prevent oxidative stress-related diseases and fight against infections caused by the increasing number of antibiotic-resistant microorganisms. Moreover, the relatively mild toxicity of extracts and essential oils against cell lines represents *P. rigida* as a potential candidate for cytotoxic drug development research.

#### 5. Conclusion

In conclusion, based on the pharmacological potential and phytochemical content, *P. rigida* is suggested as an antibacterial, antioxidant and cytotoxic plant. Considering these antibacterial, antioxidant and cytotoxic effects of extract and essential oils of *P. rigida* new investigations are necessary to detect more therapeutic effects of the plant.

#### Author contributions

F.H., R. Gh., R. H., S. T.: Experiment performing and data gathering, M. K.: GC/Mass operator, SV. Gh.: HPLC operator, M. M: writing, S. T: Editing

#### Conflict of interest

Authors declare that there is no conflict of interest.

#### Funding

This work was financially supported by Medicinal Plants Research Center, Institute of Medicinal Plants, ACECR, Karaj, Iran.

#### Acknowledgment

We thank Ms. Rose Tavakoli for her cooperation in this project.

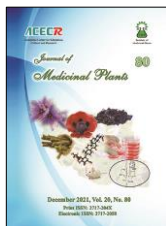


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How to cite this article: Heydari F, Ghafarzadegan R, Mofasseri M, Ghasemi SV, Kashefi M, Hajiaghaee R, Tavakoli S. Phytochemical analysis and biological activities of essential oil and extract of *Phlomis rigida* Labill. *Journal of Medicinal Plants* 2021; 20(80): 13-22. doi: 10.52547/jmp.20.80.13



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## مقاله تحقیقاتی

## آنالیز فیتوشیمیایی و بررسی فعالیت‌های بیولوژیکی اسانس و عصاره گیاه گوش بره طناز

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اطلاعات مقاله	چکیده
گل‌واژگان:	مقدمه: جنس گوش بره متعلق به خانواده نعنائیان (Lamiaceae) است. حدود ۱۰ گونه از این جنس در بین بیش از ۱۰۰ گونه آن بومی ایران هستند. <i>Phlomis rigida</i> Labill، بر اساس مطالعات می‌تواند به عنوان عامل ضد میکروبی، ضد التهاب، التیام‌دهنده زخم و آنتی‌اکسیدان در تحقیقات دارویی استفاده شود. هدف: هدف از این مطالعه بررسی ترکیبات موجود در اسانس و عصاره برگ و گل و همچنین بررسی فعالیت ضد اکسیدانی، ضد باکتریایی و سمیت سلولی این گیاه در ایران می‌باشد. روش بررسی: با استفاده از کروماتوگرافی گازی - اسپکتروسکوپی جرمی اسانس برگ و گل به منظور ارزیابی ترکیبات موجود در اسانس ارزیابی شد. خاصیت ضد اکسیدانی عصاره و اسانس برگ و گل گیاه با استفاده از روش DPPH مورد مطالعه قرار گرفت. خاصیت ضد باکتریایی عصاره و اسانس برگ و گل گیاه با استفاده از روش غلظت بازدارنده حداقل بر روی چند نوع ضد باکتریایی مختلف ارزیابی شد. خاصیت سمیت سلولی عصاره و اسانس برگ و گل گیاه توسط آزمون میگوی آب شور مورد سنجش قرار گرفت. همچنین اثرات بازدارنده رشد سلولی عصاره‌های متانولی برگ و گل گیاه با غلظت‌های مختلف بر روی سلول‌های MCF-7، MDBK، HT-29 و A-549 انجام گرفت. نتایج: تجزیه و تحلیل اسانس گیاه منجر به شناسایی ۳۴ ترکیب شده که ترکیبات عمده آن به ترتیب بتا-اوسیمین (۲۵/۶٪)، ایزوبورنیل استات (۱۶/۶٪)، ترانس-وربنول (۱۲/۶٪) و آلفا-پینن (۷/۶٪) بودند. بررسی عصاره متانولی برگ گیاه منجر به جداسازی و شناسایی ترکیبات لوتئولین، لوتئولین ۷-او-گلیکوزید و رزمارینیک اسید شد. همچنین بررسی‌های انجام گرفته بر روی اسانس گل گیاه نشان داد که گیاه خواص ضد باکتریایی با هاله عدم رشد ۱۶ میلی‌متر بر روی باکتری پروتئوس ولگاریس دارد. نتیجه‌گیری: نتایج این مطالعه نشان داد که اثر ضد باکتریایی اسانس <i>P. rigida</i> می‌تواند به دلیل وجود مونوترپن‌های آن باشد.

مخفف‌ها: NMR، رزونانس مغناطیس هسته‌ای؛ GC-MS، کروماتوگرافی گازی متصل به طیف‌سنج جرمی؛ DPPH، ۲،۲-دی فنیل-۱-پیکریل هیدرازیل

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تاریخ دریافت: ۲۷ خرداد ۱۴۰۰؛ تاریخ دریافت اصلاحات: ۲۷ مهر ۱۴۰۰؛ تاریخ پذیرش: ۲۷ مهر ۱۴۰۰

doi: 10.52547/jmp.20.80.13

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