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Original Article

Bioassay Screening of the Essential Oil and Various Extracts of Fruits of *Heracleum persicum* Desf. and Rhizomes of *Zingiber officinale* Rosc. using Brine Shrimp Cytotoxicity Assay

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Abstract

In the present work, the bioassay screening of the essential oil and various extracts of two plants including fruits of *Heracleum persicum* Desf. and rhizomes of *Zingiber officinale* Rosc. have been studied with brine shrimp test. There is only one report about cytotoxicity of *H. sphondylium* in literature and so *H. persicum* has been used as second selection. At first essentials oil and various extracts of two plants including petroleum ether, chloroform, methanol, ether and aqueous were provided. Then, different concentrations of them were prepared. These fractions were evaluated for toxicity using Brine Shrimp Lethality assay (BSL). Each of fractions was assessed by two methods of disk and solution. Survivors were counted after 24 h. These data were processed in Probit-analysis program to estimate LC_{50} values (the concentration at which 50% lethality was observed) with 95% confidence intervals for statistically significant comparisons of potencies. In disc method, methanol extract of *Z. officinale* (LC_{50}=28.3134 µg/ml) showed the most activity in comparison with positive standard of potassium dichromate (LC_{50}=23.2893 µg/ml); but in solution method, essential oil of *H. persicum* (LC_{50}=0.0071 µl/ml) was the most active fraction in comparison with potassium dichromate (LC_{50}=27.7528 µg/ml). Totally, among tested fractions, essential oil of the *H. persicum* has been exhibited the most cytotoxicity. The essential oil of *H. persicum* was analyzed by GC-MS. The major constituents were hexyl butyrate and octyl acetate.

Keywords: *Artemia salina*; *Zingiber officinale*; *Heracleum persicum*; Bioassay; Cytotoxicity.

Introduction

The importance of medicinal plants and traditional health systems in solving the health care problems of the world is gaining increasing attention. Because of this resurgence of interest, the research on plants of medicinal importance is growing phenomenally at the international level, often to the detriment of natural habitats and mother populations in the countries of origin. Bioactive compounds are almost always toxic in high doses. Pharmacology is simply toxicology at a lower dose, and toxicology is simply pharmacology at a higher dose. Thus, in vivo lethality in a simple zoo logic organism can

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be used as a convenient monitor for screening and fractionation in discovery and monitoring of bioactive natural products. In order to study the toxicity, we performed brine shrimp lethality bioassay which based on the ability to kill laboratory cultured brine shrimp *(Artemia nauplii)*. The shrimp lethality assay was proposed by Michael et al., and later developed by Vanhaecke et al., and Sleet and Brendel. The assay is considered a useful tool for preliminary assessment of toxicity, and it has been used for the detection of fungal toxins, plant extract toxicity, heavy metals, cyanobacteria toxins, pesticides, and cytotoxicity testing of dental materials (1).

Experimental

Brine shrimp

Brine shrimp, *Artemia salina* Leach , also known as sea monkeys, are marine invertebrates about 1 mm in size. Freeze-dried cysts are readily available at aquarium stores. The cysts last for several years and can be hatched without special equipment (2).

Plants material

1- Zingiber officinale Rosc. (zingiberaceae)

The dried rhizome of *Zingiber officinale* was purchased from a local medicinal plant store in Kerman. *In vitro* ant oxidative, anti tumor and immunomodulatory effects and is an effective antimicrobial and antiviral agent (4).

2- Heracleum persicum Desf. (Umbelliferae)

This plant is the endemic plant of Iran (5) and was collected from Lalezar region in June 2005, Kerman, Kerman province, Iran. Plant materials were authorized by Dr. Mirtajadini in Botany department of Bahonar university. A Voucher specimen (kf 1143) has been deposited at the Herbarium of the pharmacognosy department of faculty of pharmacy. Its fruits are used commonly in Iran as spices. In folk medicine, the fruits were administered because of their carminative activity (6).

Preparation of essentials oil and extracts

The air-dried fruits of *H. persicum* and the dried rhizome of *Z. officinale* were subjected to hydro distillation for 2 h using a Clevenger-type apparatus. Powdered plants materials were continuously extracted with petroleum ether, chloroform, methanol, ether and water, then filtered. Filtrates were concentrated, dried under vacuum and subjected for activity studies.

Brine shrimp cytotoxicity assay

The test was performed as described by Meyer *et al*. (8). Each extract or fraction solutions was tested at a concentration level of 10, 100 and 1000 µg/ml. Brine shrimp eggs (*A. salina*) were purchased in the locality and hatched in artificial sea water (solution of NaCl 3.8%) at room temperature. After 48 h, the larva (nauplii) were collected (7). This bioassay was done by disc (8) and solution (9) methods.

In the disc method, paper discs (d=0.5 cm) were used. The prepared concentrations were injected to discs in the test tubes and air-dried. Then artificial sea water and 10 nauplii were added and maintained at room temperature for 24 h under the light and surviving larvae were counted.

In the solution method without disc, assay was done. Solvent for extraction of petroleum ether, chloroform, ether and essential oil was DMSO (0.9%) but methanol and aqua extractions were solved in water. Both methods were repeated five times.

Potassium dichromate was used as positive control (9). Negative controls in disc and solution methods were air-dried discs with solvents petroleum ether, chloroform or methanol and DMSO (0.9%), respectively.

LC_{50} determination

Surviving larvae were counted after 24 h and the percent deaths at each dose and positive control were determined. LC50 values with 95% confidence intervals values were determined using the probit analysis method (Finney) (8).

Results

The results of GC/MS of essential oil are shown in Tables 1 and 2. The data obtained using brine shrimp cytotoxicity assay are also

Table 1. Components, their retention time and peak area (%) of *Z. officinale* essential oil.

No.	Compound	%	Retention times	Retention indices	Standard retention indices
1	*Alpha-pinene*	0.73	10.559	935	939
2	*Camphene*	2.4	11.21	953	953
3	*L-Limonene*	0.77	14.132	1029	1031
4	*beta-phellandrene*	3.48	14.22	1032	1031
5	*1,8-cineol*	1.59	14.277	1033	1033
6	*L-Linalool*	0.49	16.607	1093	1098
7	*1-Borneol*	0.85	19.292	1170	1165
8	*alpha-terpineol*	0.56	20.002	1189	1189
9	*2-Undecanone*	0.53	22.969	1280	1291
10	*Geranyl acetate*	0.78	25.478	1360	1383
11	*beta-Acoradiene*	1.12	28.482	1461	1466
12	*beta-Acoradiene*	3.97	28.568	1464	1466
13	*Zingiberen*	43.13	28.95	1477	1495
14	*Zingiberen*	16.16	28.951	1477	1495
15	*alpha-Farensene*	3.01	29.083	1480	1508
16	*beta-sesquiphellandrene*	5.65	29.288	1488	1524
17	*beta-sesquiphellandrene*	7.12	29.752	1506	1524

demonsterated in Tables 3-5.

Discussion

The brine shrimp lethality assay represents a rapid, inexpensive and simple bioassay for testing plant extracts bioactivity which in most cases correlates reasonably well with cytotoxicity and anti-tumor properties (10). In the present study the brine shrimp lethality of extracts of *Z. officinale* and *H. persicum* shrimp was determined using the procedure of Meyer et al (8). In disc method, methanol extract of *Z. officinale* (LC_{50}=28.3134 µg/ml) showed the

Table 2. Components, their retention time and peak area (%) of *H. persicum* essential oil.

No.	Compound	%	Retention times	Retention indices	Standard retention indices
1	*Hexanol*	1.07	8.066	867	867
2	*Butanoic acid butyl ester*	0.84	12.741	990	993
3	*octanal*	0.77	13.065	997.8	1001
4	*Hexyl acetate*	0.59	13.337	1005	1008
5	*Butyl isovalearate*	0.51	14.647	1042	
6	*1- octanol octilin*	1.36	15.505	1065	
7	*L-linalool*	1.73	16.602	1094	1098
8	*Hexyl iso butyrate*	4.58	18.214	1139	1150
9	*Hexyl butyrate*	38.99	19.774	1183	1186
10	*E4-dodecenyl acetate*	7.81	19.92	1187	
11	*Octyl acetate*	22.34	20.351	1198	
12	*Hexyl 2- methyl butyrate*	4.27	21.159	1223	1234
13	*Octyl isobutyrate*	2.07	24.466	1326	
14	*Hexyl caproate*	1.57	25.692	1366	
15	*Octyl butyrate*	0.9	25.787	1369	
16	*N-octyl 2- methyl butyrate*	2.25	27.051	1410	

Table 3. Brine shrimp cytotoxicity assay data of extracts of *Z. officinale* and *H. persicum* in disc method.

Plant	Fraction	LC_{50} (µg/ml)	Confidence interval (%)
Z. officinale	petroleum ether	32.7688	20.0278-52.8905
	chloroform	47.9604	29.2118-77.2529
	methanol	28.3134	16.5085-48.0061
	water	581.8463	336.1987-1012.3323
	ether	163.0376	107.6613-243.2662
H. persicum	petroleum ether	54.9333	34.2141-87.3450
	chloroform	103.3010	62.8054-168.3339
	methanol	233.4019	134.3310-405.3686
	water	966.4438	550.3395-1703.5518
	ether	230.3070	162.5628-319.5840
Potassium dichromate (Positve control)		23.2893	15.6576-34.0770

most cytotoxicity in comparison with positive standard of potassium dichromate (LC_{50}=23.2893 µg/ml); but the in the solution method, essential oil of *H. persicum* (LC_{50}=0.0071 µl/ml) was the most active fraction in comparison with positive standard of potassium dichromate (LC_{50}=27.7528 µg/ml). Totally, among tested fractions, essential oil of *H. persicum* has been exhibited the highest cytotoxicity activity. According to the results in Table 6 for known active natural, our plants have high cytotoxicity effect.

GC/MS analyses of the essential oil of *H. persicum* showed hexyl butyrate (38.99%) and octyl acetate (22.34%) were the main compounds. Octyl acetate is the major compound in *H.*

Table 4. Brine shrimp cytotoxicity assay data of the extracts and the essential oils of *Z. officinale* and *H. persicum* in the solution method.

Plant	Fraction	LC_{50} (µg/ml)	Confidence interval (%)
Z. officinale	petroleum ether	4.0361	2.3058-6.8789
	chloroform	8.8937	5.4395-14.2173
	methanol	7.9048	4.7398-12.8728
	water	121.7636	88.3362-165.5618
	ether	51.0471	35.1021-73.1523
	essential oil	0.0381	0.0257-0.0554
H. persicum	petroleum ether	38.3647	25.7746-56.2008
	chloroform	33.8133	22.8233 49.2650
	methanol	103.5435	73.4718-144.3479
	water	164.9033	121.2469-221.8643
	ether	93.9227	66.5782-131.0138
	essential oil	0.0071	0.0042-0.0116
Potassium dichromate (Positve control)		27.7528	18.2597-41.4450

Table 5. Brine shrimp bioassay results for known active natural products.

Natural compound	LC50 (µg/ml)
Podophyllotoxin	2.4
Berberine chloride	22.5
Strychnine sulfate	77.2
Digitalin	151
Quinidine sulfate	215
Ephedrine sulfate	215
Strophanthin	215
Arbutin	275
Caffeine	306
Thymol	514
Atropine sulfate	686
Santonin	<1000

sphondylium (11). This plant has cytotoxic and phototoxic effect (12, 13).

Thus octyl acetate may be cytotoxic agent in *H. persicum*. The major compounds of *Z. officinale* were zingiberen (59.29%) and β-sesquiphellandrene. There are reports about cytotoxicity of *Z. cassumunar* (14) and *Z. zerumbone* (3). Thus, both of these plants are cytotoxic and accordin to the obtained results and commonly using of *Z. officinale* and *H. persicum* in folk medicine, we suggested that using of them must reduce because high cytotoxic activity of them.

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