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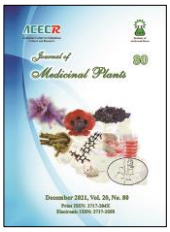

Research Article

Phytochemical investigation among different populations of *Papaver bracteatum* Lindl. using ultrasonic - assisted extraction method followed by HPLC analysis

Ali Reza Habibzade[1], Nasrin Mostaghi[2,*], Saeed Malekzade Shafaroudi[2]

[1] *PhD student in Agricultural Biotechnology, Ferdowsi University of Mashhad, Mashhad, Iran*

[2] *Department of Biotechnology and Plant Breeding, Ferdowsi University of Mashhad, Mashhad, Iran*

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ABSTRACT

Background: *Papaver bracteatum* Lindl. (Persian poppy) has a very long history as the major source of natural alkaloids. Among *Papaver* species, Persian poppy can be considered as a rich source of thebaine. **Objective:** The purpose of this study was to evaluate the thebaine content in different organs and different populations of Persian poppy. **Methods:** Seven populations of Persian poppy were collected from five different geographical regions of Iran including Damavand (Rineh, Abask), Chalous (Youssh, Pol-e Zanguleh), Mahabad, Meshgin Shahr and Zanjan (Taham) and were screened for its thebaine content. Extraction of thebaine was performed using the ultrasonic-assisted extraction (UAE) method. The parameters including pH, amount of solvent, extraction temperature and time, the repetition of extraction cycles and also the particle size were investigated and optimal conditions were determined to reach the maximum efficiency. Also, the correlation between phenology of the plant and thebaine content was studied during the developmental stages of poppy from pre-flowering to lancing. The developed UAE method followed by high performance liquid chromatography (HPLC) was used for quantitative analysis. **Results:** Thebaine content was significantly different in various organs and populations grew at different regions. The thebaine content was in the range of 0.9-1.4 % and 0.1-0.45 % in capsules and stems, respectively. The highest thebaine content was obtained in the capsules of Mahabad population. **Conclusion:** Extraction led to a high purity thebaine from Persian poppy. Eventually, a comprehensive profile of thebaine in different Persian poppy populations from different geographical regions in Iran was released.

1. Introduction

Medicinal plants have obtained a lot of attention due to their important roles in human life and health [1]. According to the World Health Organization (WHO) report, herbal

Abbreviations: CE, Capillary Electrophoresis; EME, Electrode Membrane Extraction; GC, Gas Chromatography; HPLC, High Performance Liquid Chromatography; RSD, Relative Standard Deviation; SPE, Solid Phase Extraction; SFE, Super Critical Fluid Extraction; TFA, Triflouroacetic Acid; UAE, Ultrasonic-Assisted Extraction; UV, Ultraviolet Spectrophotometry

* Corresponding author: moshtaghi@um.ac.ir

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medicines are so popular and they have been used in the industry [2].

Papaver bracteatum Lindl. (Persian poppy, great scarlet poppy) is an endemic plant in a region between the Caspian and the Black Sea. This plant has grown in many climates [3-5]. Usually, Poppy grows in mountains at high altitudes (1500-2500 m). This species is widely distributed in the north and northwest of Iran (Alborz mountains), northwest of Iran (Zagros Mountains, Caucasus slpoes) and Turkey (northeast) [6]. Lindly recognized this plant for the first time in 1821. Poppy has been used as a natural painkiller since the last century, and pharmaceutical industries have been interested in its natural alkaloids [7]. Persian poppy can be considered as a rich source of thebaine [8].

Quantitative analysis of thebaine can be carried out by high performance liquid chromatography (HPLC) [9], gas chromatography (GC) [10], capillary electrophoresis (CE) [11], ion mobility spectroscopy (IMS) [12] and ultraviolet spectrophotometry (UV) [13]. HPLC is the common method for quantitative analysis of alkaloids [14]. Because of complicated compounds of plant species, a sample preparation step is necessary before instrumental analysis [15]. Solvent extraction [16], solid phase extraction (SPE) [17], supercritical fluid extraction (SFE) [18], electrode membrane extraction (EME) [19] and ultrasonic-assisted extraction (UAE) [20] have been applied to extract alkaloids [21].

Here is a comprehensive study on the thebaine content of seven populations of Persian poppy collected from the west and center of Iran. The aerial parts of this plant have been studied in two developmental stages. Our aim was to study the phytochemical profile as the most important indicator in selecting the top populations of Persian poppy, to introduce it to breeding programs. The ultrasonic-assisted extraction (UAE) method was applied for extraction and all effective parameters which affect the extraction efficiency, were investigated. The best conditions for achieving the maximum extraction efficiency were determined.

2. Materials and Methods

2.1. Reagent and solvents

Pure powder of thebaine was provided from Sigma-Aldrich (Milwaukee, WI, USA) and dissolved in HPLC grade methanol to prepare the stock solution (1000 mg/L). The stock solution was stored at 4 ºC in a refrigerator.

Common organic solvents including methanol in HPLC grade was achieved from Caledon (George-Town, Ontario, Canada), Trifluoroacetic acid (TFA) was purchased from Merck Company (Darmstadt, Germany) and a milli-Q system from millipore (Bedford, MA, USA) was applied to prepare ultrahigh pure water (UPW).

2.2. Plant materials

In this study, seven populations of Persian poppy (available in seed bank of TEMAD Company) were collected from five eco-geographical regions of Iran including Rineh (PBR-129), Abask (PBP-124), Youssh (PBY-128/1), Pol-e Zanguleh (PBC-128), Mahabad (PBK-133), Meshgin Shahr (PBA-122) and Taham (PBZ-125). These samples were approved by the development of narcotic plant department of TEMAD Company. Capsule and stem were collected at two developmental stages including pre-flowering (when the terminal of stem loses its plasticity and flower buds become erect) and lancing (at which the petals fall and the ripe capsule becomes evident). Six samples were selected from each of seven populations and an analysis was performed.

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2.3. Phytochemical analysis of thebaine by HPLC

Samples were analyzed using high performance liquid chromatography (HPLC) (Agilent Company, Germany) with the following specifications: G1314B-1260 VWD diode array detector (DAD), G1311C-1260 quat pump VL, and A G1328C-1260 Man. The YMC-Triart C_{18} column (250 × 4.6 mm D, 5 µm, 12 nm) was applied for the analysis of alkaloids. The injection was done using a HPLC syringe (100 µl Hamilton). The extraction was done using ultrasonic system model TECNO-GAZ S.P.A at 50-60 HZ, and the phase separation was performed using a centrifuge model BHG 1100 (Germany). All phytochemical analysis were performed as follows: flow rate, 0.8 ml/min; wavenumber, 280 nm; injection volume, 20 µl; mobile phase, acetate buffer pH = 4: acetonitrile: methanol with gradient program as follow, 90:5:5 for 12.5 min, 50:25:25 for 11.5 min and 90:5:5 for 10 min. The percentage of thebaine in each sample was calculated by considering the calibration curve of thebaine standard (R^2 = 0.999, RSD % = 2.1 %, 6 points ranging from 10 - 500 mg/L).

2.4. Extraction procedure

To evaluate the thebaine content in capsules and stems of poppy plants, six samples were selected from each of seven populations and the analysis was performed. Therefore, 42 samples were analyzed. The samples were dried at 60 ºC for one day and crushed by an electric grinder and particles were produced in the range of 68-150 µm. The extraction of thebaine was done using UAE method [20]. For extraction, in each step, 10 ml of diluent, containing HPLC grade methanol and water in ratio of 75:25 with pH 2 (adjusted by TFA), was added to 300 mg of poppy powder, and the extraction was performed using an ultrasonic bath with the power of 50-60 HZ at 50 ºC for 22 min. The extraction solvent was separated from sample using a centrifuge with 1000 rpm. This step was repeated three times and extraction solvents were collected and filtered using a 0.42 µm filter paper. 20 µl of the extract was injected into HPLC instrument. The extraction condition was comprehensively evaluated, and the best condition to obtain the maximum extraction efficiency was determined. The effective parameters on the extraction procedure are included extraction solvent pH (2-12), temperature (25-60 ºC), volume (5-25 ml), extraction time and the repetition of extraction cycles. Also, the particle sample size (68-150 µm) which affects the extraction efficiency was evaluated. The effect of all parameters on the extraction efficiency was evaluated and the optimal condition was determined. Each extraction was repeated three times and relative standard deviation (RSD %) was calculated for each extraction.

2.5. Statistical analysis

Cluster analysis was performed based on Euclidean distance for thebaine content of samples at different regions, samples with different sizes and samples at different developmental stages. Clustering was done using SPSS 12 (SPSS Inc., Chicago) by one-way analysis of variance and mean comparisons were done based on the least significant difference test (LSD).

3. Results

3.1. Optimization of effective parameters on the extraction procedure

Extraction solvent pH, temperature, volume and the extraction time, sample particle size and the repetition of extraction cycles affected the extraction efficiency.

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The extraction solvent pH is an important parameter which determines the electrical charge of thebaine. Initially, the effect of pH was studied in the range of 2-12. The highest extraction efficiency was achieved in acidic solution (pH = 2) (Fig. 1A). Here, due to the protonation of functional groups of thebaine, the solubility in polar solvent increased so the extraction efficiency improved. Therefore, all the following extractions were done using extraction solvent with pH = 2.

Then, the effect of ultrasonic power and time was studied and according to the results shown in Fig. 1B, 22 min ultrasonic at 50-60 KHz was sufficient to achieve the maximum extraction efficiency.

The extraction temperature in the range of 25-60 ºC was studied. The results showed that 50 ºC was the optimal temperatures to extract thebaine (Fig. 1C). At higher temperatures than 50 ºC, the extraction efficiency didn't improve. Also, at higher temperature, decomposition of thebaine is possible [22].

The volume of extraction solvent was selected in the range of 5-25 ml for about 300 mg of sample and based on the results, 10 ml of extraction solvent was sufficient for extraction and by increasing the volume of extraction solvent, the extraction efficiency didn't improve (Fig. 1D).

Also, the extraction procedure was repeated 1 to 5 times. Results showed that the extraction efficiency reached maximum after 4 cycles and thereafter didn't improve (Fig. 1E).

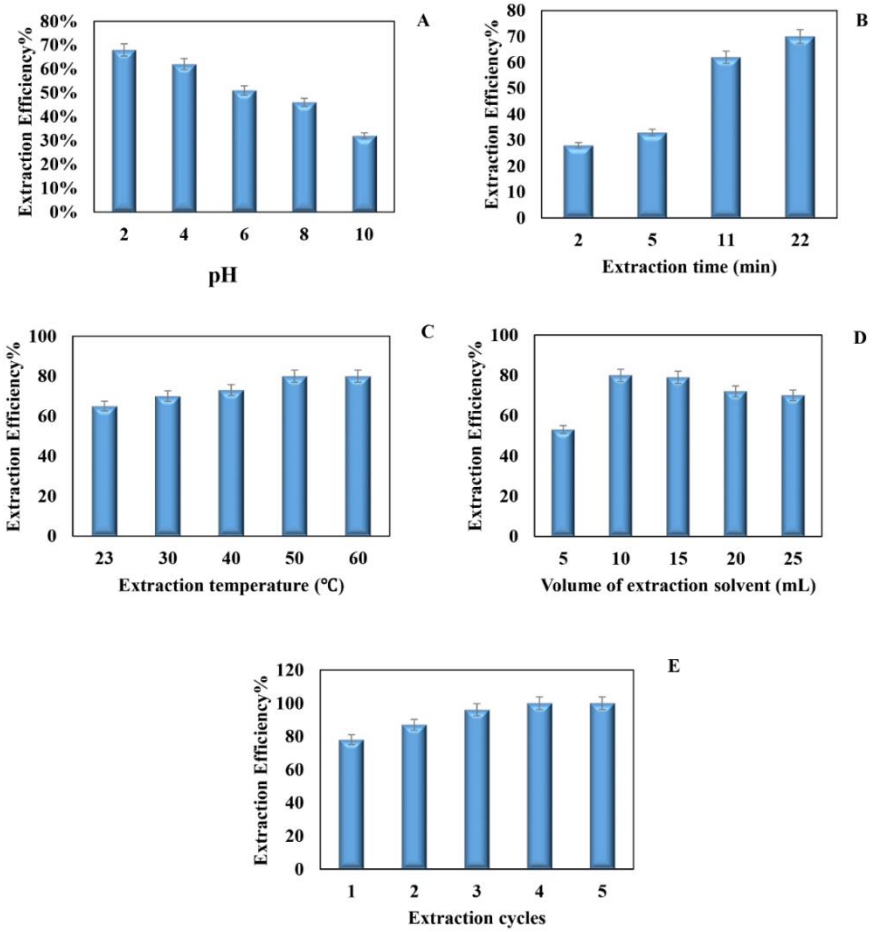

Fig. 1. The effect of (A) pH of extraction solvent, (B) extraction time, (C) extraction temperature, (D) volume of extraction solvent, (E) extraction cycles on the extraction efficiency.

The size of particles was also crucial in thebaine extraction. Samples were milled with a crusher in the range 68-150 µm. Increasing the milling time produced the finer samples. Here, the capsule and stem samples for one region with different sizes were selected as a model, and the effect of particle size on the percentage of extracted thebaine was studied. Results revealed that finer samples have more thebaine content (Table 1). This higher percentage can be due to higher surface area for extraction by solvent. So, the particle size is an effective parameter and has a significant effect on the thebaine yield.

3.2. Thebaine content during developmental stages

The thebaine content of samples can change at different developmental stages [23, 26]. So, it was evaluated during two developmental stages including pre-flowering (when the terminal of stem loses its plasticity and flower buds become erect) and lancing (at which the petals fall and the ripe capsule becomes evident). The stems and capsules were collected and analyzed. The thebaine content of capsules and stems in poppy was significantly different at various developmental stages. According to Table 2, the thebaine content of plant at the lancing stage was more than the preflowering stage in stems and capsules.

3.3. Phytochemical assay of thebaine

The quantitative analysis of the Poppy population was performed using HPLC, and the quantitative results of selected populations were summarized in Table 3. The thebaine contents in capsules and stems of seven populations were in the range of 0.4-0.5 % and 1.0-1.3 %, respectively. Among seven populations, Mahabad population has the highest amount of thebaine. The HPLC chromatograms for 2 populations containing Mahabad and Taham regions (capsule and stem of plant) and a standard of thebaine at concentration level of 250 mg/L were shown in Fig. 2. As the results revealed, in all populations, the thebaine content of capsules was higher than stem and the Persian poppy grown or collected in Mahabad has the highest thebaine content.

Table 1. The effect of particle size of Poppy samples on the thebaine contents.

Milling time (min)	Mesh size (µm)	Sieve weight (%)	Thebaine percentage (%)	RSD [a] (%)
1	≤ 68	5%	0.72	1.0
	68-125	11%		
	125-150	32%		
	≥ 150	53%		
2	≤ 68	10%	0.80	1.1
	68-125	22%		
	125-150	35%		
	≥ 150	43%		
3	≤68	15%	0.86	1.0
	68-125	36%		
	125-150	20%		
	≥150	29%		
4	≤68	31%	0.89	1.0
	68-125	38%		
	125-150	11%		
	≥150	20%		
5	≤68	38%	0.92	1.1
	68-125	41%		
	125-150	8%		
	≥150	13%		

[a]Relative standard deviation

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Table 2. The average of thebaine contents in aerial parts of Persian poppy during two developmental stages.

Area	Organ	RSD[a] %	Developmental stages	
			Pre-flowering (Thebaine content %)	Lancing (Thebaine content %)
Rine	Stem	1.0	0.31	0.36
	Capsule	1.1	0.89	1.0
Abask	Stem	1.0	0.21	0.26
	Capsule	1.2	0.83	0.90
Mahabad	Stem	1.1	0.35	0.42
	Capsule	1.1	1.10	1.4
Meshgin Shahr	Stem	1.1	0.43	0.45
	Capsule	1.3	1.08	1.3
Taham	Stem	0.9	0.08	0.1
	Capsule	1.0	0.38	0.51
Yoush	Stem	1.0	0.11	0.19
	Capsule	1.2	0.92	0.89
Pol-e Zanguleh	Stem	1.1	0.21	0.21
	Capsule	1.3	1.01	0.95
	Stem	1.2	0.23	0.24

[a]Relative standard deviation

Table 3. Evaluation of thebaine contents in aerial organ of poppy collected from different regions of Iran.

Accession	Aerial organ	Thebaine content %	RSD[a] %
Rine	Capsule	1.0	1.1
	Stem	0.35	1.0
Abask	Capsule	0.9	1.0
	Stem	0.26	1.0
Mahabad	Capsule	1.4	1.2
	Stem	0.42	1.1
Meshgin Shahr	Capsule	1.3	1.3
	Stem	0.45	1.0
Taham	Capsule	0.5	1.1
	Stem	0.1	0.8
Yoush	Capsule	1.0	1.1
	Stem	0.28	1.0
Pol-e Zanguleh	Capsule	1.1	1.2
	Stem	0.24	0.9

[a]Relative standard deviation

3.4. Cluster analysis

Cluster analysis was done based on Euclidean distance from thebaine content data for seven populations in three main groups. The first group consists of two subgroups. The first subgroup includes the groups of Rineh, Youish and Pol-e Zanguleh. The second subgroup includes the Abask group. The second group includes Mahabad and Meshgin Shahr groups and the third group includes Taham (Fig. 3, Table 4).

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There was a significant difference between two developmental stages including pre-flowering and lancing, and the thebaine content was higher at lancing than pre-flowering (Fig. 3, Table 4). The effect of particle size and surface area on the extraction efficiency was studied and based on the results, the quantitative content of thebaine in finer particles (about 0.05 %) was significantly higher. The highest thebaine quantity was at the samples which crushed 5 min (the size of majority of particles was in the range of 68-125 µm), and the lowest thebaine quantity was in samples which crushed 1 min (the size of particles was higher than 150 µm) (Table 4B).

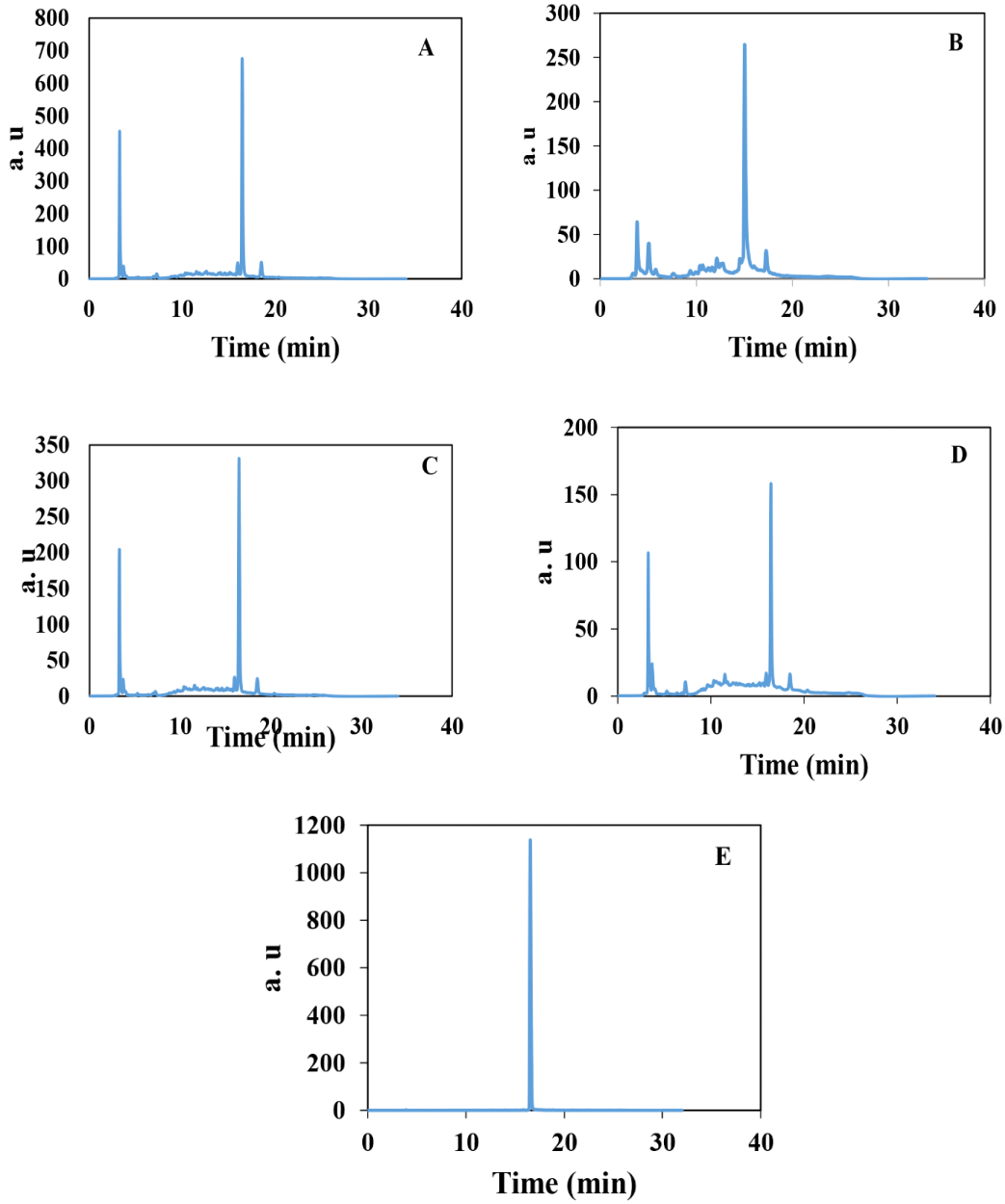

Fig. 2. HPLC chromatograms for (A) Capsule, (B) Stem of samples from Mahabad, (C) Capsule, (D) Stem samples from Taham, and (E) Standard of thebaine at concentration level of 250 mg/L.

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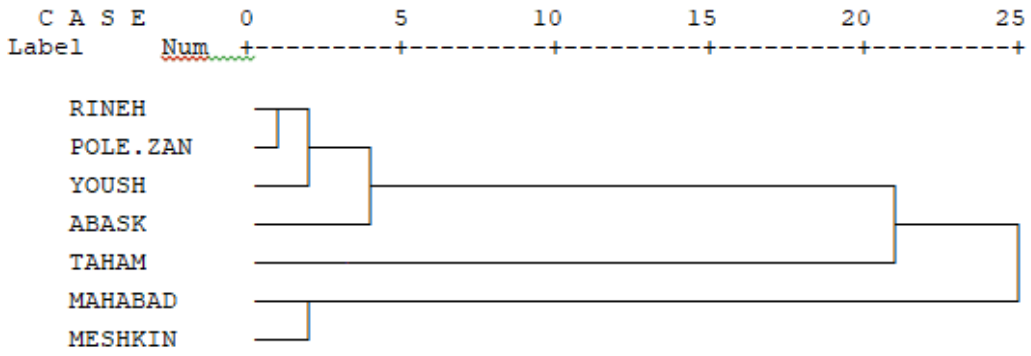

Fig. 3. Cluster of Persian poppy based on the Euclidean distance using Ward Method

Table 4A. Analysis of variance (ANOVA) in thebaine content of poppy populations

Yield	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1.519	6	.253	506	.000
Within Groups	.007	14	.0005		
Total	1.526	20			

Table 4B. Analysis of variance mesh size (ANOVA) milling powder in extracting thebaine

Yield	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.120	4	.030	300	.000
Within Groups	.001	10	.0001		
Total	.121	14			

4. Discussion

Secondary metabolites in plants can act as defense factors at various environmental conditions. Alkaloids as one of these metabolites have such interaction by environmental stresses. In the presented study, a comprehensive investigation has been done on the thebaine content of Persian poppy. Seven populations from west, north western and center of Iran were collected. The results of HPLC analysis revealed that geographical conditions affected the concentration of thebaine as a secondary metabolite. Here, the effect of geographical conditions on the population is quite evident. The creation of three different groups that differ in terms of altitude, longitude and latitude can be a reason for the impact of the environment along with other factors in changing the thebaine content.

These results were confirmed by Qaderi et al. in 2019. They mentioned that geographical condition could be an effective parameter on the concentration of thebaine as a secondary metabolite [24]. They have done a study on the molecular diversity and phytochemical variability in Persian poppy. Alkaloid contents of 6 populations (Anjomaneh, Eil-Teymoor, Mamhimeh, Ploor, Siah Bisheh and Taham) had been studied, and thebaine content in capsule of this species was reported in the range of 0.1-1.3 %.

In this study, the highest thebaine content was at western area which was divided into an independent sub-group. Lalezari et al. in 1974

confirmed the higher thebaine content of the Persian poppy population at western area and named them as Arya II [25]. This issue is obvious in the evaluation of cluster analysis. The populations in the first group (Abask, Rineh, Youish and Pol-e Zanguleh) were relatively similar but in comparison with other groups were different. These results can be due to the similarity of geographical conditions and climate between two regions.

Here, we studied the thebaine content in aerial parts (stem and capsule) during two developmental stages including pre-flowering and lancing. Thebaine content was higher at the lancing stage. A study investigated the alkaloid content in different parts of Persian poppy (root, stem, capsule, leaf and petal) during different developmental stages (annual rosette, perennial rosette, bud initiation, pendulous bud, pre-flowering, flowering and lancing). They introduced Persian poppy as a rich source for thebaine. Root and capsule were considered as the most potent organs for extraction of thebaine, also lancing was introduced as the best developmental stage [26]. In another study, they studied the developmental accumulation of thebaine and some gene transcripts in different organs of this plant [23]. Our results are in good agreement with the reported results. We studied seven populations of different regions of Iran (west, north western and center region) at different developmental stages which was a comprehensive study compared with similar studies.

The predominant advantages of the presented study was introducing a simple, facile and effective method for extraction of alkaloids from the poppy plants with considering the all effective parameters to improve extraction efficiency.

5. Conclusion

In conclusion, the extraction of thebaine was performed using a UAE method which was simple, fast and effective. The effective parameters on extraction efficiency were studied and at the optimum condition (extraction solvent; 40 ml of methanol: water (75:25) pH = 2, extraction temperature; 50 ºC, extraction time; 22 min, the number of extraction cycles, 4 times). Thebaine content of seven populations from five different regions of Iran with different climates was evaluated. Based on the obtained results, samples were gathered from Mahabad region have the highest thebaine content, and can be considered as a suitable population in breeding programs and also industrial plants.

The advantages of the method introduced for the extraction of thebaine include high efficiency and speed of extraction, simplicity and no need for extraction equipment.

Author contributions

N. M and S. M designed research and managed the project; A.R H performed experiments and analyzed data and wrote the manuscript with contributions from all other authors.

Conflict of interest

Authors declare that there is no conflict of interest.

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References

1. Karimi M and Raofie F. Micronization of vincristine extracted from *Catharanthus roseus* by expansion of supercritical fluid solution. *J. Supercrit Fluid.* 2019; 146: 172-179. doi: 10.1016/j.supflu.2019.01.021.

2. Ma Y, Cheng J, Inanaga S and Shui J. Induction and inhibition of *Striga hermonthica* (Del.) Benth. germination by extracts of traditional Chinese medicinal herbs. *J. Agron.* 2004; 96: 1349-1356. doi: 10.2134/agronj2004. 1349.

3. Lavie D, Rotman J, Levy A and Palevitch D. A rapid quantitative method for the determination of thebaine in *Papaver bracteatum*. *Phytochem*. 1979; 18: 2011-2013. doi: 10.1016/S0031-9422(00)82722-3.

4. Foroozandeh-Shahraki A, Mehrafarin A, Akhondzadeh S, Naghdi Badi H, Qaderi A, Hajiaghaee R, Khalighi-Sigaroodi F and Ghorbani Nahooji M. Analytical Review on Persian Poppy (*Papaver bracteatum* Lindl.) as a Native and Economical Medicinal Plant. *J. M. Plants*. 2013; 12: 1-12.

5. Qavami N, Azizi M, Yazdian F, Qaderi A and Nemati H. Effective Induction of Hairy Roots in Persian Poppy (*Papaver bracteatum* Lindl.) Using Sonication Method. *J. M. Plants* 2018; 17: 73-82.

6. Goldblatt P. Biosystematic studies in Papaver section Oxytona. *Pascal and Francis Bibliographic Database* 1974; 61: 264-294.

7. Meshulam H and Lavie D. The alkaloidal constituents of *Papaver bracteatum* Arya II. *Phytochem*. 1980; 19: 2633-2635. doi: 10.1016/ S0031-9422(00)83934-5.

8. Sharghi N and Lalezari I. *Papaver bracteatum* Lindl., a highly rich source of thebaine. *Nature*. 1967; 213: 1244-1244. doi: 10.1038/2131244a.

9. Jovanov P, Petrin-Miličević M, Radosavljević-Stevanović N, Vraneš M, Belić S, Sakač M, Nikolov J and Gadžurić S. Rapid Determination of the Primary Alkaloids in Illicit Heroin by High-Performance Liquid Chromatography with Tandem Mass Spectrometry (HPLC–MS/MS). *Anal. Lett.*. 2020; 54: 1224-1232. doi: 10.1080/00032719. 2020.1798454.

10. Paul B. D, Dreka C, Knight E. S and Smith M. L. Gas chromatographic/mass spectrometric detection of narcotine, papaverine, and thebaine in seeds of *Papaver somniferum*. *Planta Med.* 1996; 62: 544-547. doi: 10.1055/s-2006-957966.

11. Barnett N, Hindson B, Lewis S and Purcell S. Determination of codeine, 6-methoxycodeine and thebaine using capillary electrophoresis with tris (2, 2′-bipyridyl) ruthenium (II) chemiluminescence detection. *Anal Commun.* 1988; 35: 321-324. doi: 10.1039/A805955A.

12. Valadbeigi Y, Ilbeigi V and Mirsharifi M. S. Mechanism of atmospheric pressure chemical ionization of morphine, codeine, and thebaine in corona discharge -ion mobility spectrometry: Protonation, ammonium attachment, and carbocation formation. *J. Mass Spectrom.* 2020; 55: 4586. doi: 10.1002/jms.4586.

13. Agnihotri A, Tewari S. C, Khatod P, Banerjee S and Balasubramanian M. Determination of thebaine in crude thebaine samples by infrared and ultraviolet spectrophotometric methods. *Analyst*. 1984; 109: 1413-1416. doi: 10.1039/AN9840901413.

14. Williams RD and Ellis B. E. Age and tissue distribution of alkaloids in *Papaver somniferum. Phytochem*. 1989; 28: 2085-2088. doi: 10.1016/ S0031-9422(00)97924-X.

15. Bar C. Coffman C and Gentner W. Growth, Development, and thebaine Content of *Papaver*

bracteatum Lindl. in Relation to Spacing1. *J. Agron.* 1987; 79: 935-937. doi: 10.2134/agronj1987.00021962007900050036x.

16. Fang H, Zeng Z, Liu L and Pang D. On-line back-extraction field-amplified sample injection method for directly analyzing cocaine and thebaine in the extractants by solvent microextraction. *Anal. Chem.*. 2006; 78: 1257-1263. doi: 10.1021/ac0516537.

17. Yoshimatsu K. Kiuchi F. Shimomura K and Makino Y. A rapid and reliable solid-phase extraction method for high-performance liquid chromatographic analysis of opium alkaloids from *Papaver* plants. *Chem. Pharm. Bull.* 2005; 53: 1446-1450. doi: 10.1248/cpb.53.1446.

18. Janicot J, Caude M, Rosset R and Veuthey J. Extraction of major alkaloids from poppy straw with near-critical mixtures of carbon dioxide and polar modifiers. *J. Chromatogr. A.* 1990; 505: 247-256. doi: 10.1016/S0021-9673(01)93083-3.

19. Seidi S, Yamini Y, Heydari A, Moradi M, Esrafili A and Rezazadeh M. Determination of thebaine in water samples, biological fluids, poppy capsule, and narcotic drugs, using electromembrane extraction followed by high-performance liquid chromatography analysis. *Anal. Chim. Acta.*. 2011; 701: 181-188. doi: 10.1016/j.aca.2011.05.042.

20. Fakhari A. R. Nojavan S. Ebrahimi S. N and Evenhuis C. J. Optimized ultrasound-assisted extraction procedure for the analysis of opium alkaloids in *papaver* plants by cyclodextrin-modified capillary electrophoresis. *J. Sep. Sci.* 2010; 33: 2153-2159. doi: 10.1002/jssc.201000135.

21. Yazdani D, Rezazadeh Sh and Shahnazi S. Review of poppy (*Papaver somniferum* L.). *J. M. Plants* 2003; 2: 1-12.

22. Hofman P and Menary R. Alkaloid losses from the capsules of *Papaver somniferum* L. during kiln drying and storage under commercial conditions in Tasmania. *J. Stored Prod. Res.*. 1985; 21: 135-139. doi: 10.1016/0022-474X(85)90007-4.

23. Rezaei M. Naghavi M. R. Hoseinzade A. H and Abbasi A. Developmental accumulation of thebaine and some gene transcripts in different organs of *Papaver bracteatum*. *Ind. Crops Prod*. 2016; 80: 262-268. doi: 10.1016/j.indcrop.2015.11.009.

24. Qaderi A. Omidi M. Pour-Aboughadareh A. Poczai P. Shaghaghi J. Mehrafarin A and Etminan A. Molecular diversity and phytochemical variability in the Iranian poppy (*Papaver bracteatum* Lindl.): A baseline for conservation and utilization in future breeding programmers. *Ind Crops Prod*. 2019; 130: 237-247. doi: 10.1016/j.indcrop.2018.12.079.

25. Lalezari I. Nasseri P and Asgharian R. *Papaver bracteatum* lindl: Population arya II. *J. Pharm. Sci.* 1974; 63: 1331. doi: 10.1002/jps.2600630844.

26. Rezaei M, Naghavi MR, Hosseinzadeh AH and Abbasi A. Measurement of some benzylisoquinoline alkaloids in different organs of Persian poppy during onto genetical stages. *Chem. Biodivers.* 2006; 135: 539-543. doi: 10.1002/cbdv.201500172.

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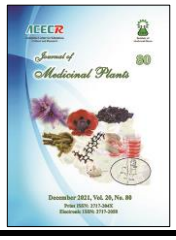

مقاله تحقیقاتی

بررسی فیتوشیمیایی جمعیت‌های مختلف خشخاش کبیر با استفاده از روش استخراج با امواج فراصوت و آنالیز کروماتوگرافی مایع با کارایی بالا

علیرضا حبیب‌زاده[۱]، نسرین مشتاقی[۲،*]، سعید ملک‌زاده شفارودی[۲]

[۱] *دانشجوی دکتری بیوتکنولوژی کشاورزی، دانشگاه فردوسی مشهد، مشهد، ایران*

[۲] *عضو هیات علمی گروه بیوتکنولوژی و به نژادی گیاهی، دانشکده کشاورزی، دانشگاه فردوسی مشهد، مشهد، ایران*

اطلاعات مقاله

گل‌واژگان:
خشخاش کبیر
فنولوژی
تبائین
استخراج
استخراج با امواج فراصوت

چکیده

مقدمه: گیاه خشخاش کبیر پیشینه طولانی به عنوان منبع آلکالوئیدهای طبیعی دارد. در میان گونه‌های خشخاش، این گونه می‌تواند به عنوان منبع غنی تبائین در نظر گرفته شود. **هدف:** هدف از این مطالعه بررسی میزان تبائین در اندام های مختلف جمعیت‌های متفاوت خشخاش کبیر بود. **روش بررسی:** هفت جمعیت از خشخاش کبیر از پنج ناحیه مختلف جغرافیایی شامل دماوند (رینه، آب اسک)، چالوس (یوش، پل زنگوله)، مهاباد، مشگین شهر و زنجان (تهم) انتخاب و میزان تبائین در آنها بررسی شد. استخراج تبائین با استفاده از روش امواج فراصوتی انجام پذیرفت. پارامترهای تاثیرگذار مانند pH، حجم حلال استخراجی، دمای استخراج و زمان آن، تکرار چرخه‌های استخراج و همچنین سایز ذرات، مورد بررسی قرار گرفت و شرایط بهینه برای رسیدن به بیشترین کارایی تعیین شد. همچنین ارتباط فنولوژی گیاه و میزان تبائین در دوره رشدی قبل از گلدهی و گلدهی مورد بررسی قرار گرفت. روش بهبود یافته استخراج با امواج فراصوت در این مطالعه به همراه آنالیز با دستگاه کروماتوگرافی مایع با کارایی بالا برای آنالیز کمی به کار برده شد. **نتایج:** میزان تبائین در اندام‌های مختلف و جمعیت‌های مختلف متفاوت بود. میزان تبائین در کپسول در محدوده ۰/۹- ۱/۴ درصد (بر اساس ماده خشک) و در ساقه این گیاه ۰/۱- ۰/۴۵ درصد بود. بیشترین میزان تبائین در کپسول‌های جمع‌آوری شده از مهاباد بود. **نتیجه‌گیری:** استخراج در این روش منجر به حصول تبائین با خلوص بالا از گیاه خشخاش کبیر می‌شود. در نهایت یک پروفایل جامع از میزان تبائین در جمعیت‌های مختلف خشخاش کبیر در ایران به دست آمد.

مخففها: CE، الکتروفورز مویینه؛ EME، استخراج غشای الکترود؛ GC، کروماتوگرافی گازی؛ HPLC، کروماتوگرافی مایع با کارایی بالا؛ RSD، انحراف استاندارد نسبی؛ SPE، استخراج فاز جامد؛ SFE، استخراج سیال فوق بحرانی؛ TFA، تری فلورواستیک اسید؛ UAE، استخراج با امواج فراصوت؛ UV، اسپکتروفتومتری ماورای بنفش

* نویسنده مسؤول: moshtaghi@um.ac.ir

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