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Importance of Opium Alkaloids of Papaveraceae Family in Iran

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ABSTRACT

The Papaveraceae family comprises more than 40 genera and 760 species. Papaver alkaloids have significant therapeutic importance in the treatment of various diseases and exhibit some antimicrobial properties. These alkaloids have been used in cancer medicine, cough, and hypertension. The main goal of this study is to determine the amounts of various alkaloids in samples collected from different regions of Iran. The findings of this research could provide valuable insights for the pharmaceutical industry. Two grams of dried and powdered plant material were extracted with 30 ml chloroform and 15 ml of 99% acetic acid for 30 minutes under sonication. The change in the pH of the sample was revealed by 10 through gradually adding ammonia. The mobile phases consisted of phosphoric acid and acetonitrile. The analysis of the Papaveraceae family in Iran was performed using high-performance liquid chromatography-ultraviolet detection. This paper focuses on the Papaver alkaloids (noscapine, morphine, thebaine, papaverine, and codeine) found in three species of Papaver and four species of *Glaucium*. The maximum contents of noscapine (21.02 mg/kg DW), morphine (34.51 mg/kg DW), thebaine (6.96 mg/kg DW), papaverine (103.76 mg/kg DW), and codeine (3.63 mg/kg DW) were reported in *Papaver rhoeas* L., *Papaver fugax* Poir and., *Glaucium oxylobum* Boiss. & Buhse, respectively.

Keywords: Noscapine, Morphine, Thebaine, Papaverine, Codeine, Pharmaceutical industries

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INTRODUCTION

The representatives of the *Papaver* genus have been extended with 170 species of alkaloids [1]. The Papaveraceae are used for markable medical and economic purposes. This significance stems from their ability to produce a variety of alkaloids and from the fact that this family of alkaloids includes several important opiate alkaloids used in the pharmaceutical industry, such as codeine and morphine, as well as several other alkaloids that have been developed and are essential for treating a wide range of illnesses [2]. *Papaver* alkaloids have been employed as antiarrhythmic, cough suppressant, anticancer, antimalarial, and antihypertensive [3].

Global demand for papaver plants, which contain high levels of certain poppy alkaloids, is increasing [4,5]. The phytochemical types of different poppy species have been explained in [6,7,8]. Investigated 300 obtainments of opium poppy and highlighted valuable variations in the number of alkaloids among tetraploid and diploid [9]. The number of 122 poppy genotypes investigated concern genotypic and phenotypic. In *P. bracteatum* obtained from two regions in Iran, the thebaine collected in dry ripe capsules was reported to be 3.5 to 5.8 percent [10]. Recently, global trends revealed an increase in the application of opium alkaloids. Valuable conclusions were obtained in screening accession applications, like high levels of particular antioxidants and alkaloids in breeding plans and pharmaceutical industries. The primary purpose of this research is to determine the alkaloids of the Papaveraceae family.

MATERIALS AND METHODS

Plant Materials

In addition to the distribution and structural aspects of alkaloids, determining their stereochemistry is also another meaningful issue, which is of great importance not only for structural clarification but also for elucidating reaction mechanisms. Planting species including *P. rhoeas*, *P. fugax*, *P. orientale*, *G. oxylobum*, *G. grandiflorum*, *G. flavum*, and *G. cornicalatum* were collected during the spring (May) in 2017 from various origins in Iran (Fig. 1).

Preparation of Plant Extracts

Whole *Papaver* and *Glaucium* species were dried under natural open air, and powdered. The extraction of plant material for alkaloid researchers instructed by ultrasonic extraction.

2000 mg of ground plant material of each sample was mixed with 25 ml of MeOH, 70 ml of chloroform, and 5 ml of ammonia and then placed in an ultrasonic bath.
Then, the samples were extracted by stirring for 2 hours, and the obtained samples were filtered with filter paper while the filter was washed with chloroform.

P. rhoeas

G. grandiflorum

G. oxylobum

P. orientale

G. flavum

G. cornicalatum

P. fugax

Fig. 1 Seven species of Papaveraceae family collected from different regions of Iran.

The extracts were concentrated utilizing a rotary evaporator to collect the solvent. As a result, *Papaver* alkaloid was extracted with 30 ml of chloroform and 15 ml of 99% acetic acid for 30 minutes under sonication.
The aqueous phase was made alkaline finally, extracted three times with chloroform, and the sample pH was set to 10 by increasing ammonia. The extraction was conducted according to Bulduk and Taktak [11] with minor alterations. Acetonitrile, water, and phosphoric acid were all HPLC grade, and others were analytical grade.

HPLC Tests of Alkaloids

Analysis was performed under conditions: HPLC System (Model: YL9100).
Column:
C18 (5µm, 4.6mmX 250 mm)
Mobile phase:
A (60%): acetonitrile
B (40%): phosphoric acid 10%
Flow rate: 1.0 ml/min
Temperature: 25 °C

Detection System

The analytical essay presents the results of the study conducted on the Papaver alkaloids, namely noscapine, morphine, thebaine, papaverine, and codeine. The study confirms the presence of these blends by the calculation of the UV spectrum at 254 nm, as shown in Figure 2. The isocratic system facilitated the easy separation of the aforementioned compounds, which were dissolved in acetonitrile and phosphoric acid at varying concentrations. To accuracy and consistency of results, the solution was filtered, and the cartridge was washed, before injection.
The residual time of each compound was compared with the corresponding standard to confine the identification of the blend. Reference compounds provided by Salehan Chemi, Temad, and Alborz Darou Co (Fig. 3). The standard calibration curve was generated for them, and the R2 values were calculated. The gradation graph was drawn with three repetitions for concentrations of 1, 3, 5, 7, and 10 ppm of the alkaloid mixture to determine the concentration of alkaloids in each sampling (Fig. 4). The calibration curve ranged from 1-10 (µg/ml) for each of them, such as noscapine, morphine, thebaine, papaverine, and codeine. The representative concentration was determined from the direct calibration graph, and the outcomes were analyzed, accordingly. The ultra-pure water, which was described with the calibration accuracy by the R2, was obtained from the University of Zanjan, Zanjan-Iran. The regression analysis of the determination value of noscapine, morphine, thebaine, papaverine, and codeine standards was higher than 0.99 (Table 1). While calculations of calibration curves were performed with Microsoft Excel, version Office Professional Plus 2016 quantification of unknowns was reached by the external standard method. Research publications were limited to the English language.

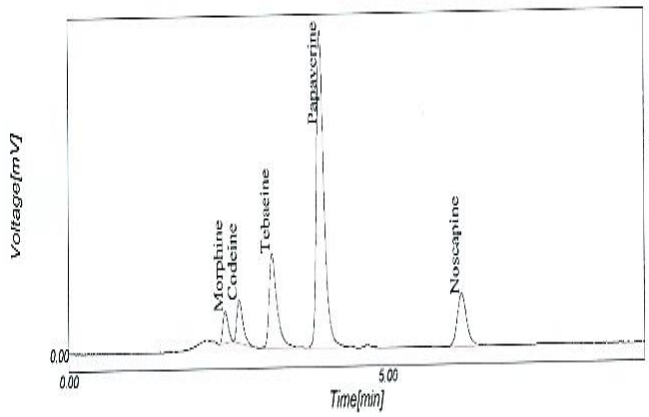


Fig. 2 HPLC-UV chromatograms of alkaloid standards. From *Papaver* in 254 nm

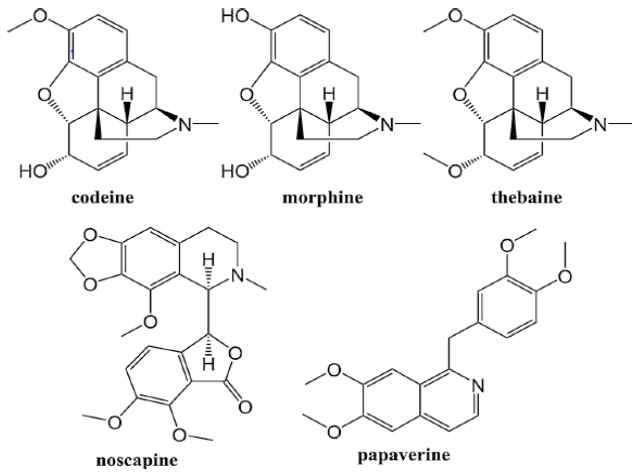
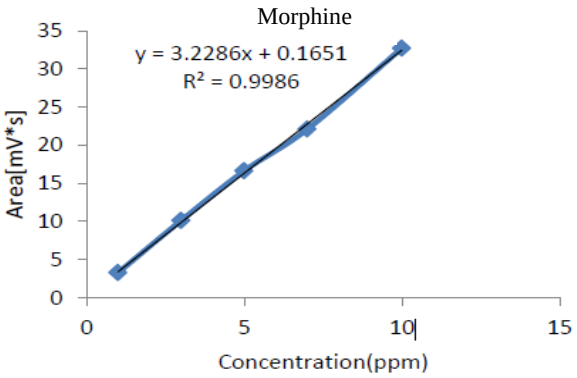
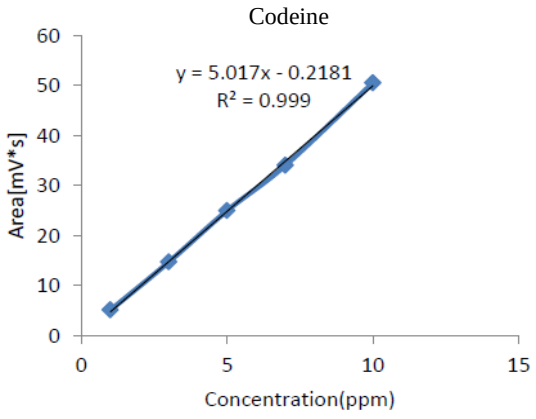
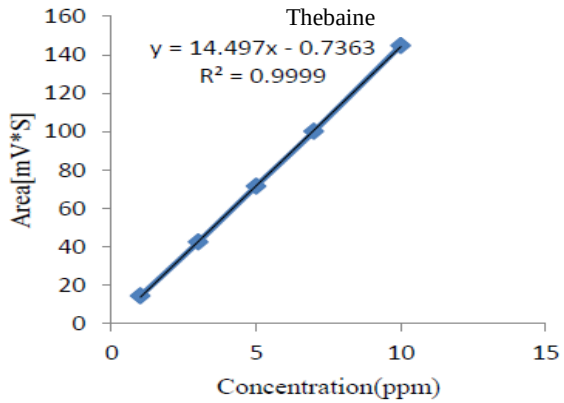
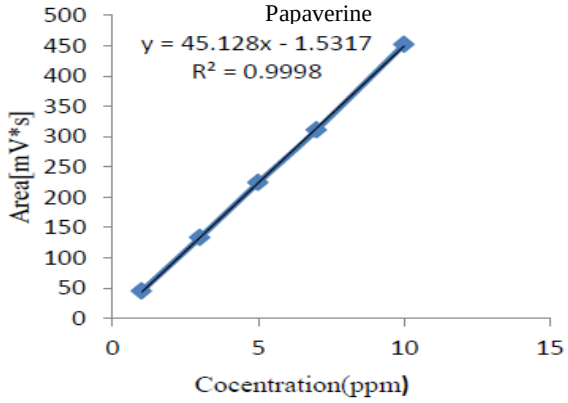


Fig. 3 Chemical structures of the Papaver alkaloids calculated in poppy









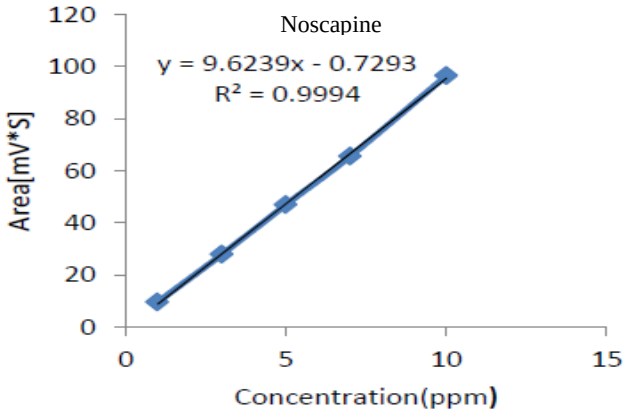


Fig.4 The linear calibration curve of the standards.

RESULTS

Extraction Weight

The extraction weight of alkaloids of the genus Papaver species demonstrated that the maximum value was 44.71 mg/kg DW in species P. *fugax*, and the minimum value was 8.98 mg/kg DW in P. *orientale*, While the genus *Glaucium* species demonstrated that the maximum value was 131.83 g in types G. *oxylobum* and the minimum value was 0.72 g in types G. grandiflorum (Table 2).

HPLC Analysis

Noscapine, morphine, thebaine, papaverine, and codeine were white crystalline solids. The five major opium alkaloids were represented for the Papaveraceae family in 3 species from the genera *Papaver* and 4 types from the genera *Glaucium* using HPLC-UV.

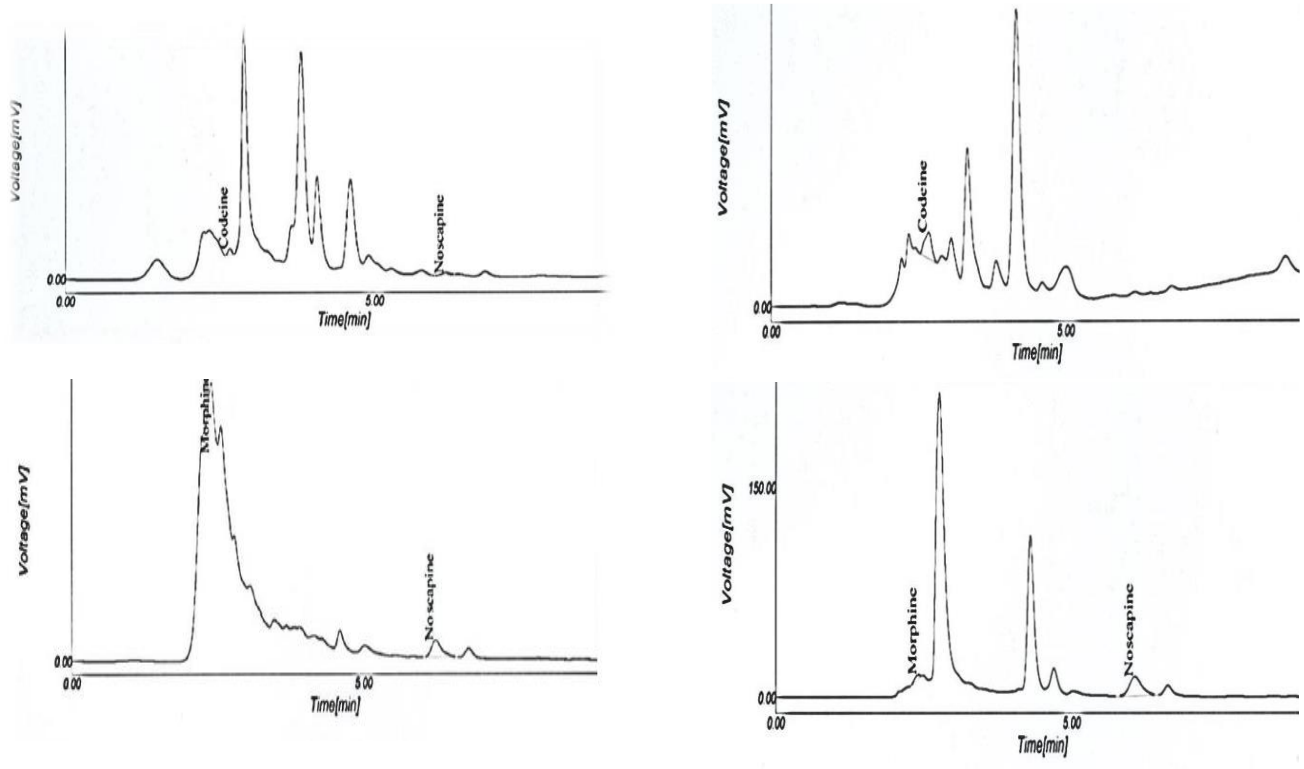


Fig. 5 The chromatograms of collected plant samples

The measurement was performed in all species by exploiting the wavelength 254 nm. Figure 2 reveals the HPLC-UV chromatogram of collected plant samples (Fig. 5).

According to Figure 4, the HPLC analysis of poppy shows that the alkaloids were separated, and separating was done by the column (C18 column, 5µm, 4.6mmX 250mm) was utilized. Finally, the regression analysis values ranged from 0.9986 to 0.9999. Furthermore, Noscapine, morphine, thebaine, papaverine, and codeine exhibited well-defined chromatograms with retention times of 6.15 min, 2.45 min, 3.19 min, 3.95 min, and 2.67 min, respectively in the selected conditions. The results reveal that the proportion of the five major opium alkaloids, including Noscapine, Morphine, Thebaine, Papaverine, and Codeine was variable among the Papaveraceae family. The amounts mg/kg DW of each alkaloid in different poppy plants of all seven populations are summarized in Table 3.

As the results show, Noscapine existed in most species (Fig. 6) with a maximum amount of 21.02 mg/kg/DW in *P. rhoeas*, and the minimum amount of 0.25 mg/kg/DW in G. grandiflorum was noted.

Moreover, the current study has investigated the presence of morphine in some Iran species, which under the influence of factors such as environmental factors, the maximum amount of 34.51 mg/kg in DW in P. fugax and the minimum amount of 1.45 mg/kg in DW in G. flavum reported (Fig. 7).

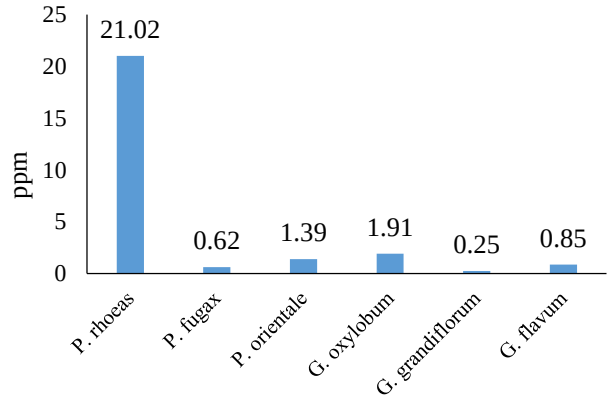


Fig. 6 Alterations in noscapine concentration for species.

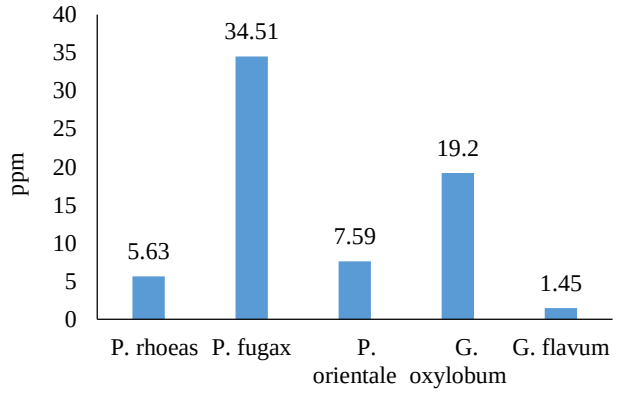


Fig. 7 Alterations in morphine concentration for species

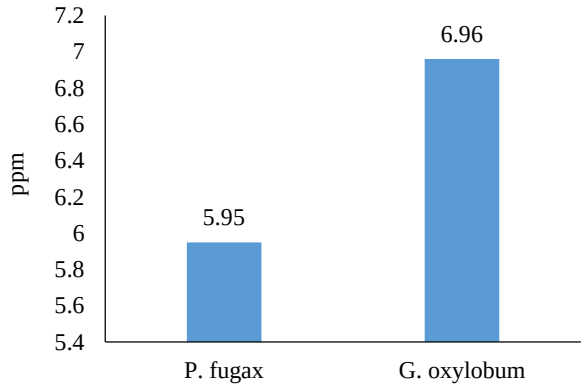


Fig. 8 Alterations in thebaine concentration for species.

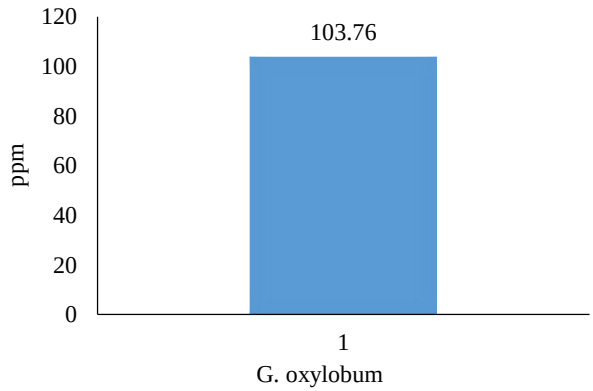


Fig. 9 Alterations in Papaverine concentration for species

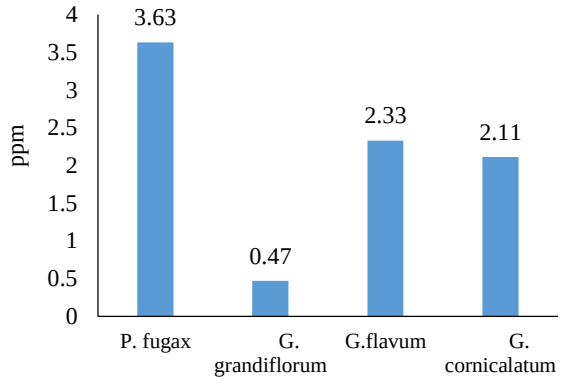


Fig. 10 Alterations in codeine concentration for species.

For example, in areas with a continental climate *G. corniculatum* creates an increasing number of alkaloids [15]. Rajat De-Sigh and Turkhede [21] evaluated the opium yield of the poppy and reported that a remarkable stage was floral initiation.

Also, Thebaine's maximum value was 6.96 mg/kg DW in *G. oxylobum*, and the minimum value was 5.95 mg/kg DW in *P. fugax* (Fig. 8).

In addition, Papaverine is presented at a high level just in *G. oxylobum* with a percentage of 103.76 mg/kg DW (Fig. 9).

Codeine was found in some species with a minimum value of 3.63 mg/kg DW in *P. fugax* and a minimum value of 0.47 mg/kg DW in *G. grandiflorum* and disappeared in *P. orientale* and *G. oxylobum*. *G. corniculatum* contains codeine only (Fig. 10).

DISCUSSION

Many isoquinoline alkaloids have been isolated from poppy by Serturner's isolation of morphine [13,14]. Different alkaloids have been reported to be influenced by various factors for *Glaucium coniculatum* [15], and factors such as environmental elements can regulate the expression of particular genes required in alkaloid biosynthesis [16]. In addition, temperature is a critical environmental factor that has the main benefit on the accumulation and combination of alkaloids [17,18] explained that high temperatures affect the biosynthesis of morphine in *P. somniferum* and also Nitrogen has the considerable value on the accumulation of alkaloid [19]. Consequently, nitrogen value differences in soils of various areas can affect the alkaloid concentration of poppy. Genetic and environmental factors contribute to the biosynthesis of Papaver alkaloids [20]. Alirezalu *et al.*, (2019) [22] showed that Papaver populations possessed striking antioxidant capacity, likely due to the polyphenolic and alkaloid components. Karajibani *et al.*, (2016) [23] explained that noscapine can appear as a resistance factors to oxidative stress. Secondary metabolites, environmental, and genetic background were involved in antioxidant capacity [24].

Table 1 Information of standard calibration curves for the species studied.

Compound	Range (mg/kg DW)	Equations	R^2
Noscapine	0.25 - 21.02	y = 9/62x – 0/73	0.9994
Morphine	1.45 - 34.51	y =3/23 x + 0/16	0.9986
Thebaine	5.95 - 6.96	y = 14/50x – 0/74	0.9999
Papaverine	0 - 103.76	y = 45/12x – 1/53	0.9998
Codeine	0.47 - 3.63	y = 5/02x – 0/22	0.999

Table 2 Extraction weight of alkaloids for the species studied

Species	Extraction weight (mg/kg DW)
P. rhoeas	26.65
P. fugax	44.71
P. orientale	8.98
G. oxylobum	131.83
G. grandiflorum	0.72
G. flavum	4.63
G. cornicalatum	2.11

Table 3 Contents of alkaloid in all seven samples summarized.

Noscapine	Morphine	Thebaine	Papaverine	Codeine
P. rhoeas	*P. rhoeas*	*P. fugax*	*G. oxylobum*	*P. fugax*
P. fugax	*P. fugax*	*G. oxylobum*		*G. grandiflorum*
P. orientale	*P. orientale*			*G. flavum*
G. oxylobum	*G. oxylobum*			*G. cornicalatum*
G. grandiflorum	*G. flavum*			
G. flavum				

Previous results explained the antimicrobial ability of some alkaloid components [25]. These benzylisoquinoline alkaloids are utilized as raw material by pharma industries [26]. The industrial structure of opiates from the cells' growth depends on the massive accumulation of many alkaloids in a cell growth medium. The cells' development of various explants of the poppy plant has been explained in the pieces of literature [27].

Although there has been considerable success in plant-cell growth depending on cells with extreme yield of isoquinolines, from a pharmaceutical and marketable viewpoint, the morphinans have stressed hard to produce in plant-cell cultures [28]. Regrettably, no correlations have been found between the extraction weight of alkaloids and the dry plants' yield. While conventional plant breeding has created a double in the number of alkaloids over the last two decades, nevertheless procedural rapid advances in morphinan yields by using conventional breeding will probably be limited [29].

The mutants have been proposed, often as parents in breeding programs [30]. Today, the use of new biotechnological solutions (in a mixture of classic breeding programs) to accurately manipulate the morphinane construction of poppy plants is necessary. A genetically modified opium poppy constructs a pharmaceutical precursor rather than codeine and morphine [31].

CONCLUSION

The main goal of the current study was to determine the amounts of the mentioned alkaloids in the samples collected from distinct regions of Iran, in which the maximum contents of noscapine, morphine, thebaine, papaverine, and codeine were indicated in *P. rhoeas*, *P. fugax*, *G. oxylobum*, *G. oxylobum*, and *P. fugax*, respectively.

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CRediT Authorship Contribution Statement

Fatemeh Torkian: Conceptualization, Investigation, Resources, Writing - review & editing, Methodology, Project administration. Fariba Saadati: Methodology, Project administration, Conceptualization, Methodology, Validation. Azizollah Kheiry: Investigation, Conceptualization, Validation. Abbasali Zamani: Investigation, Validation, Formal analysis.

Declaration of Competing Interest

There are no competing financial interests or known personal relationships to influence the work reported in this article, the authors declared.

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