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The significance of opium alkaloids in the classification of Papaveraceae in Iraq

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Abstract
The Papaveraceae family includes over 40 genera and 760 species in the world, of that 5 genera, namely *Papaver* L. with 15 species, *Glaucium* Mill with 5 species, *Hypecoum* L. with 3 species, *Roemeria* Medic with 2 species and *Eschscholzia* Cham with one cultivated species occur in Iraq. Opium alkaloids of 14 species of Papaveraceae in addition to two species of *Fumaria* L. were chemically studied using High Performance liquid Chromatography (HPLC) analysis. Five opium alkaloids, Morphine, Codeine, Thebaine, Papaverine and Noscapine were significantly detected and variably distributed in the species. The highest concentration of opium alkaloids was Noscapine with 814.624 ppm in *P. somniferum* while the lowest concentration was codeine with 66.978 ppm in the same species. Our results support maintaining *Fumaria* in distinct family Fumariaceae.

Keywords: significance, opium alkaloids, classification, Papaveraceae, Iraq

1. Introduction
The Papaveraceae, known as a poppy family, are economically important. It includes over 40 genera and 760 species in the world, as mentioned by Cullen (1980) [2] 5 genera present in Iraq for Papaveraceae and 2 genera for Fumariaceae.
The Papaveraceae family is characterized by its economic and medical importance from the ancient times. This importance associated with their rich production of different types of alkaloids, and because of the presence of opiate alkaloids that have importance in the pharmaceutical industry, such as morphine, codeine and many others alkaloids were discovered in this family, which they have unique therapeutic value in the treatment of many diseases (Yu *et.al.* 2014) [10].
The systematic position and the relationship between the three families Papaveraceae, Fumariaceae and Hypocoaceae are still a matter of discussion (Taia, 2009) [9]. Many alkaloids have been used for a long time in medicine and some are still usable drugs today. Alkaloids have many pharmacological effect including antihypertensive effects (many indole alkaloids), antimalarial activity (quinine), antiarrhythmic effect (quinidine, spareien), and anticancer actions (dimeric indoles, vincristine, vinblastine) (Roberts & Wink, 1998) [7]. The Papaveraceae and Fumariaceae are famous as taxonomic groups, and both are very wealthy in alkaloids some of them are valuable in medicine and some of the others can be considered as promising for producing many drugs (Brossi, 1986) [1].
In view of the lack of chemical study for the family in general and genus *Papaver* in particular, this study was carried out for the determination and quantification alkaloids compounds of some species under study and find out how much convergence and divergence between species.

2. Materials and Methods
2.1 Samples collection
An intensive survey was carried out during the spring (February to May) in 2013 and 2014 for collocation and identification of plants.
2.2 Cultivation and harvesting raw Opium
In October 2013 and 2014 a suitable field in Saraji date palm orchards was selected for planting *Papaver* species (*P. acrochaetum*, *P. argemone*, *P. armeniacum*, *P. bornmuelleri*, *P. dubium*, *P. fugax*, *P. glaucum*, *P. rhoeas*, *P. macrostomum*) seeds of two species were obtained from market (*P. somniferum* and *P. rhoeas*), weeds and stones were tossed aside and the ground was leveled off. The soil was turned and then break up the large clumps of its, manures and fertilizers were added at the time of soil preparation. After that, seeds were sown by hand. Before maturity of the capsule when the flower petals were fall off.

The surface of the capsule was cut or scored with a knife, and this process was done in the afternoon. The opium latex was oozes out through these cuts, in the next day the capsules latex was scrubbed with a flat tool then collected the latex and dried it for several days.

2.3 Chemical study
The Chemical study was carried out in the department of Agronomy and Plant Breeding, and the Central Laboratory of College of Agriculture and Natural Resources, University of Tehran, Karaj-Iran.

2.4 Preparing materials
After plant collection, cleaned the plants and dried it under natural open air, then grinded and placed in plastic bags and labeled.

2.5 Alkaloids extraction
Alkaloids were extracted according to (Harbone, 1984):

1. The dried and powdered materials (5gm) were extracted with (100ml) of (1 M HCL in methanol), stirring for 24h.
2. The solution was filtered by filter papers, the filtrate was taken and the fiber was neglected.
3. The extract was concentrated to one-quarter of the original volume.
4. The alkaloids were precipitated by addition of Conc. NH4OH until the pH became (10-11).
5. Alkaloids were collected by centrifugation.

2.6 Alkaloid reagent
2.6.1 Dragendorffs reagent
Extract Solution + Dragendroffs reagent ⟶ reddish-brown precipitate (Harbone, 1984).
2.6.2 Wagner reagent
Extract solution + few drops of Wagner reagent ⟶ Turbidity of the resulting precipitate with white milky color (Harbone, 1984).
2.6.3 Hagers reagent (Picric acid)
Extract solution + few drops of Hagers reagent ⟶ Turbidity of the resulting precipitate (Hultin, 1965) [5].
2.6.4 Marquis reagent
Extract solution + few drops of reagent ⟶ violet – red – green color (Sim, 1970) [8].
2.6.5 Meconic acid reagent
It was prepared by warming alkaloids powder in 2-3 ml of water for a few minutes, and then filtered. Thereafter added a few drops of 5% ferric chloride solution to the filtrate ⟶ purplish (dirty blood) red color (Sim, 1970) [8].
2.6.6 HPLC analysis of alkaloids
Analysis was performed on the following conditions:
HPLC System (Model: Platinblue, Knauer), Autosampler: Platinblue, Knauer
2.6.7 Column: NUCLEODUR C18 (250 x 4 mm x 5 µm)
2.6.8 Mobile phase:
Eluent A (25%): acetonitrile
Eluent B (75%): 20 mM ammonium phosphate - pH 2.5
Flow rate: 1.0 mL/min
Pump: Dual Pump (Platinblue, Knauer)
Temperature: 25 °C
Detection system: PDA (Photodiode array detector) at 254 and 280 nm
Injection volume: 10µL
Run time: 60 min

In ethanol, the standards were dissolved at different concentration thereafter were filtered by 0.22 µm sterile filters before the injection. The UV absorbance at 254 nm and 283 nm was read in the eluate. Then comparing the compounds by identified it with standards of each compound through used the retention time and the spectrum profile absorption from the peak heights of the internal standard and every compound in the samples, concentrations were calculated by comparing peak areas of standard compounds with those in the samples run under the same conditions. The retention time and concentration of alkaloids was identified by using HPLC software.

3. Results & Discussion
3.1 Extraction weight
The extraction of alkaloids for the species of genus *Papaver* showed that the maximum value was 0.3401 g in species *P. somniferum* and the minimum value was 0.0113 g in *P. argemone*, while in other genera the maximum value was 0.061g in *F. bracteosa* and the minimum value was 0.011 in *H. gesilinii* as shown in (table 1).

Table 1: Extraction weight and detection of alkaloids by different reagents for the species studied

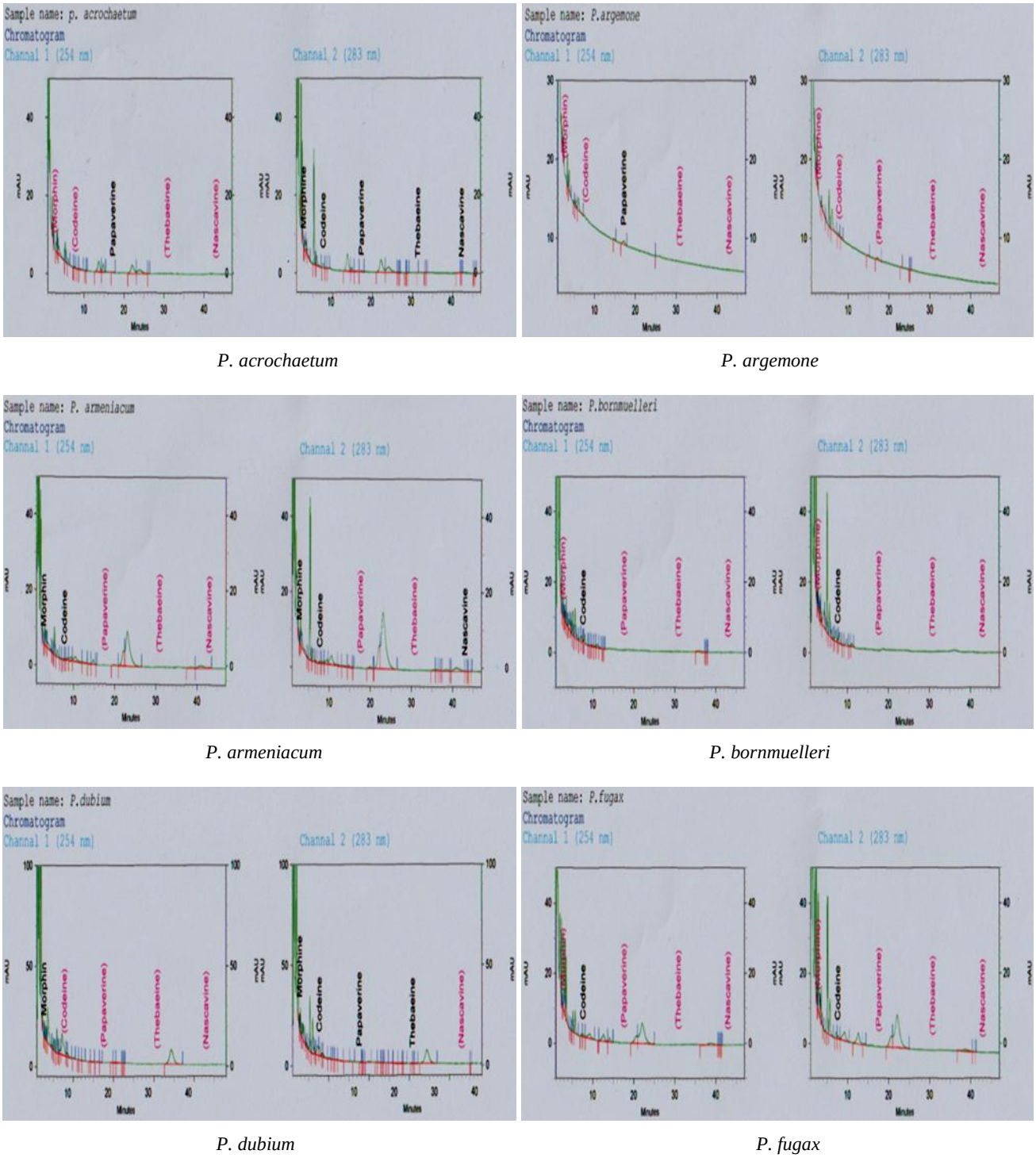
Species	Extraction weight / g	Dragen-dorffs	Wagner	Hagers	Marquis	Meconic acid
Papaver acrochaetum	0.017	+	+	+	+	+
Papaver argemone	0.0113	+	--	±	+	+
Papaver armeniacum	0.029	+	+	+	+	+
Papaver bornmuelleri	0.0763	--	--	+	+	+
Papaver dubium	0.043	+	+	+	+	+
Papaver fugax	0.0845	+	+	--	+	+
Papaver glaucum	0.0478	+	+	±	--	+
Papaver rhoeas / wild	0.0350	--	+	+	--	+
Papaver rhoeas / cultivated	0.0124	+	+	±	--	+
Papaver macrostomum	0.026	+	+	+	+	+
Papaver somniferum	0.3401	+	+	+	+	+
Papaver somniferum / capsul	0.1118	+	+	+	+	+
Papaver somniferum / opium	0.00115	+	+	+	+	+
Hypecoum gesilinii	0.011	--	+	+	+	+
Hypecoum pundulum	0.0196	--	+	+	+	+
Roemeria refracta	0.0176	--	+	+	+	+
Fumaria bracteosa	0.061	±	+	+	+	--
Fumaria parviflora	0.054	+	+	±	+	--

3.2 Alkaloid reagent

The results of chemical reagents showed that some species give disparity in its results which gave a positive result in all reagents while in other species gave a positive result in some of them and negative in the others, as in *P. acrochaetum*, *P. argemone*, *P. armeniacum*, *P. dubium*, *P. fugax*, *P. macrostomum* and *Papaver somniferum* which have positive results for all reagents. Some reagents were shown more ability than others in the detection of alkaloids, as in wagner and meconic acid as shown in table 1. This is due to the change in the ratios of alkaloid present in the species as well as the variation in the chemical ingredients found in each species. For this, we note that some species was responded to certain reagents without other (Roberts and Wink, 1998) [7].

3.3 HPLC analysis

The opium alkaloids were measured for Papaveraceae family in 15 species that belong to different genera of *Papaver*, *Roemeria*, *Hypecoum* and *Fumaria* by using HPLC technique. The measurement was done in all species by using the wavelength 254 nm for channel 1 and 283 nm for channel 2. When the name of the alkaloids appears at black colour in the curve, this means that this kind of alkaloids was diagnosed in the plant by the system, but when it appears in pink colour in the curve, this that means the alkaloids is not detected in this species by the system, fig (1-3).

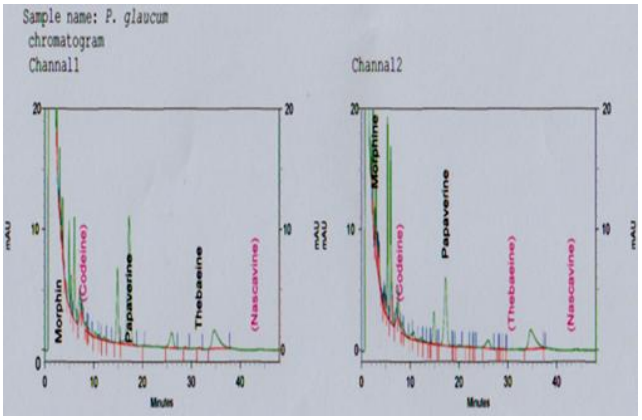


P. acrochaetum *P. argemone*

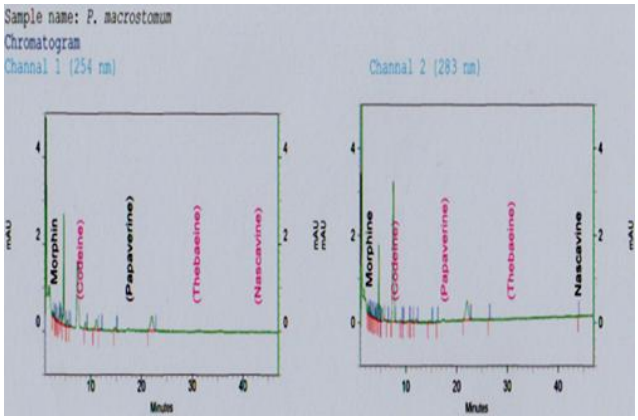
P. armeniacum *P. bornmuelleri*

P. dubium *P. fugax*

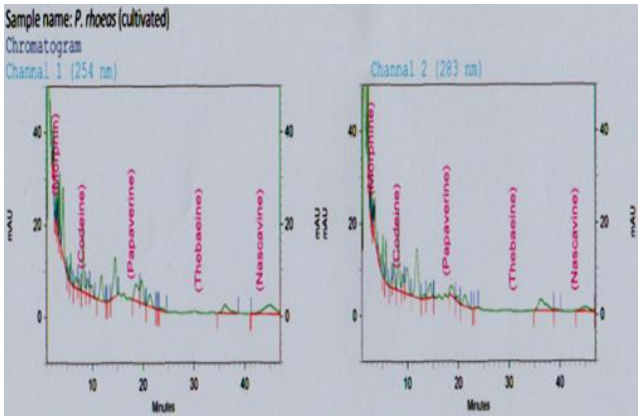
Fig 1: HPLC analysis of opium alkaloids in Papaveraceae



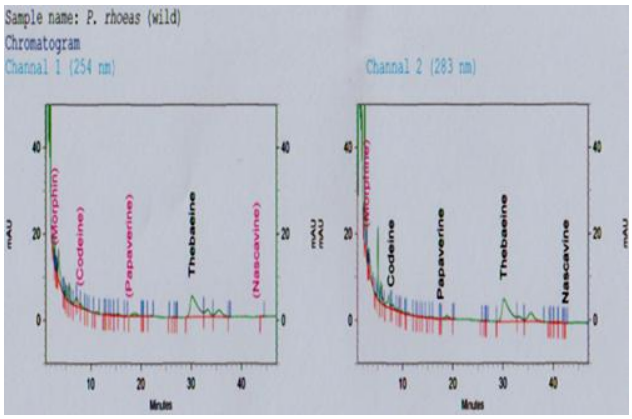
P. glaucum



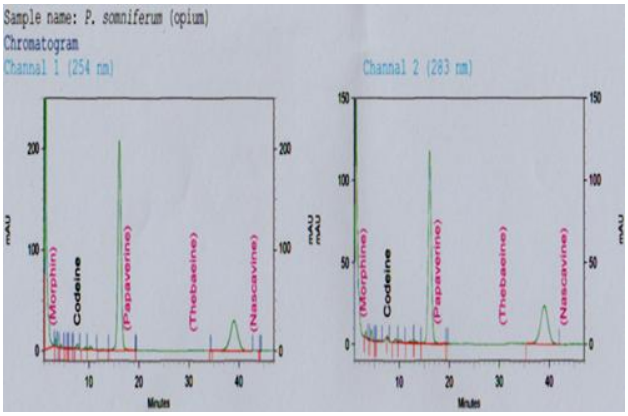
P. macrostomum



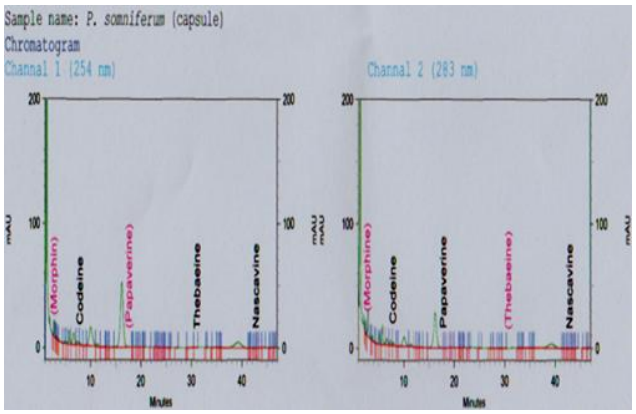
P. rhoeas cultivated



P. rhoeas wild

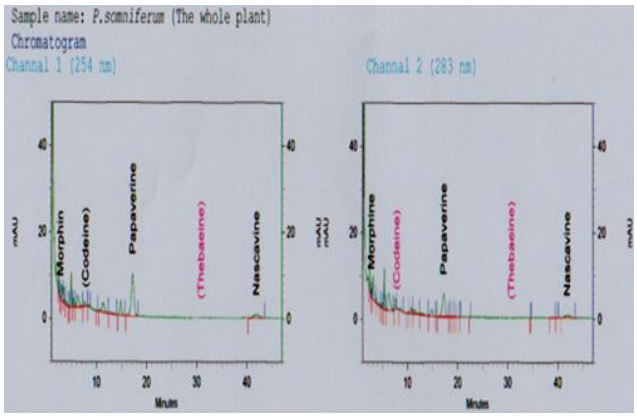


P. somniferum (opium)

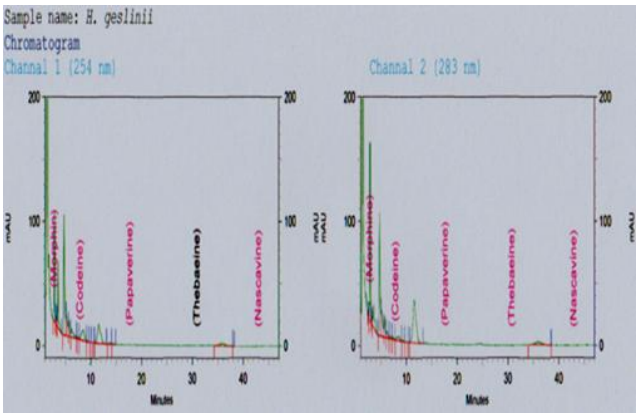


P. somniferum (capsule)

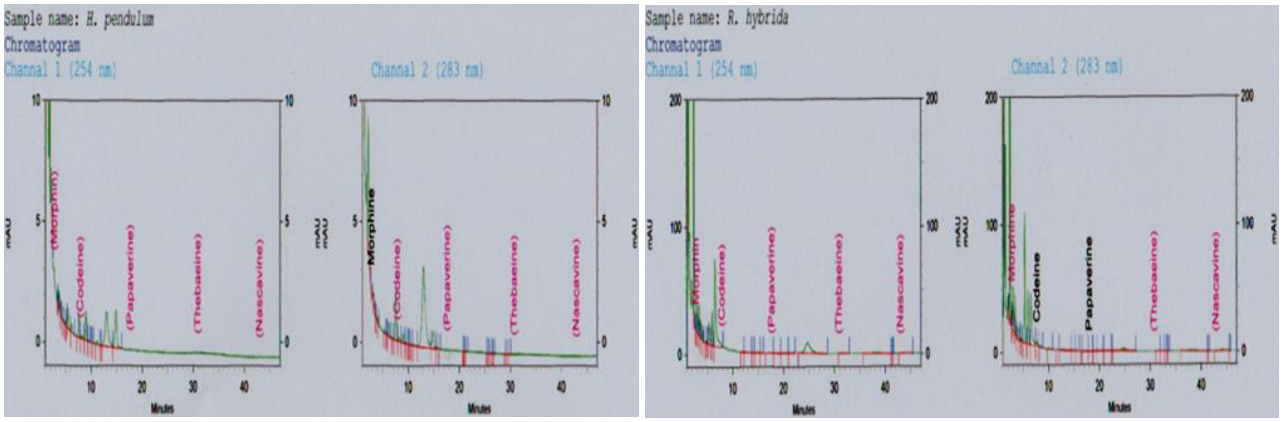
Fig 2: HPLC analysis of opium alkaloids in Papaveraceae



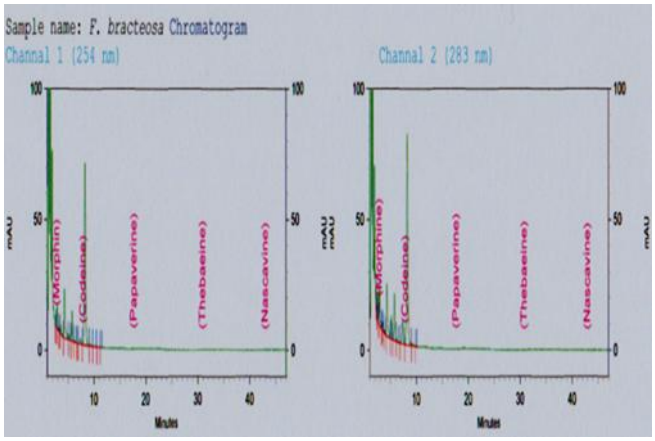
P. somniferum (Whole plant)



H. geslinii



H. pendulum *R. hybrid*



F. bracteosa

Fig 3: HPLC analysis of opium alkaloids in *F. bracteosa*

Results have shown by using the HPLC technique that genus *Papaver* contain the five opium alkaloids. Presently, the ratio of these alkaloids was variable between species. Some species contained all the five alkaloids such as *P. somniferum* and *P. acrochaetum*, while some species contained some of these alkaloids and the another included one type of alkaloids like *P. argemone* which contain papaverine only, despite the fact that some of the ancient literature mentioned that it contains thebaine (Duke, 1973) [3], but we did not find any presence of thebaine in this study.

Also it appears from HPLC analysis the presence of other alkaloids in species under study of the genus *Papaver*. Some of these alkaloids at high rates as are evident in the figures, and because of the difficulty of obtaining other standard alkaloids we do not determine these alkaloids. Montgomer *et al*., (1983) mentioned that rhoeadines and papaverrubines have only been observed with the Papaveraceae and all the sections of *Papaver* contain rhoeadines and or papaverrubines, but the plenty and distribution of the alkaloids vary from one section to another. Table 1 gives us an opinion about the presence of opium alkaloids in the sections of genus *Papaver*. These alkaloids present can be reflected a significant characteristic of the genus *Papaver*.

In genus *Roemeria* the results have shown that genus contains two types of opium alkaloids codeine and papaverine. Although they contain trace amounts of opium alkaloids, but it contains a high peaks of another alkaloids in high concentrations. Genus *Hypecoum* contain two types of opium alkaloids which were thebaine in *H. geslinii* and morphine in *H. pendulum*. In the genus *Fumaria*, the results appeared that they have not contain any of the five opium alkaloids, but they contain other alkaloids as it is cleared from the figures of HPLC. For the other species in genera *Glaucium*, *Eschscholzia* and *Corydalis* we didn't have enough materials for chemical analysis.

In this study we confirm the presence of morphine in some Iraqi species. The maximum value was 284.92 ppm in *P. somniferum* and minimum value was 1.452 ppm in *H. pendulum* as shown in fig.4. Although some species contains opium alkaloids in trace amounts. This may be due to the nature of the soil and the habitat and weather in Iraq. This was confirmed by the study of Doncheva *et al*., (2014), who studied *G. corniculatum* in more than one region and noted a variation in the chemical contents, the authors explain these observations by referring to the biosynthesis of alkaloids in the species *G. corniculatum* depends on the geographical region whose plant grow in and could be used as a chemotaxonomic sign to assist for recognizing the source of the plants, and they mention that the Iraqi type of this species is characterized by the presence of an alkaloid different from the other species that grow in other areas that have different climate. We observed also the presence of morphine in wild - type of *P. rhoeas* and absence in cultivated-type and this may be due to the nature of the environment, temperature and the richness of the soil in minerals and nutritious. Lošák *et al*., (2009) [6] mention that morphine content is affected by a number of factors like weather during harvest and level of mineral nutrition.

Codeine was available in most species of genus *Papaver*, but in a low percentage. While the morphine presence in high percentage but in limited species. The maximum value was 66.978 ppm in *P. somniferum* whole plant and minimum

value was 3.488 ppm in *R. hybrida*, fig. 5.
The baine found in some species and the maximum value was 70.792 ppm in *P. rhoeas* (wild type) and minimum value was 0.057 ppm in *P. acrochaetum*, fig. 6. Papaverine found in some species the maximum value was 613.78 ppm in *P. somniferum* and minimum value was 0.015 ppm in *P. macrostomum*, fig.7, and disappear in *P. armeniacum* and *P. fugax.* Noscapine is present at high level just in *P. somniferum* with percentage 814.62 ppm and and the minimum percentage was 0.31 ppm in *P. macrostomum*, fig 8.

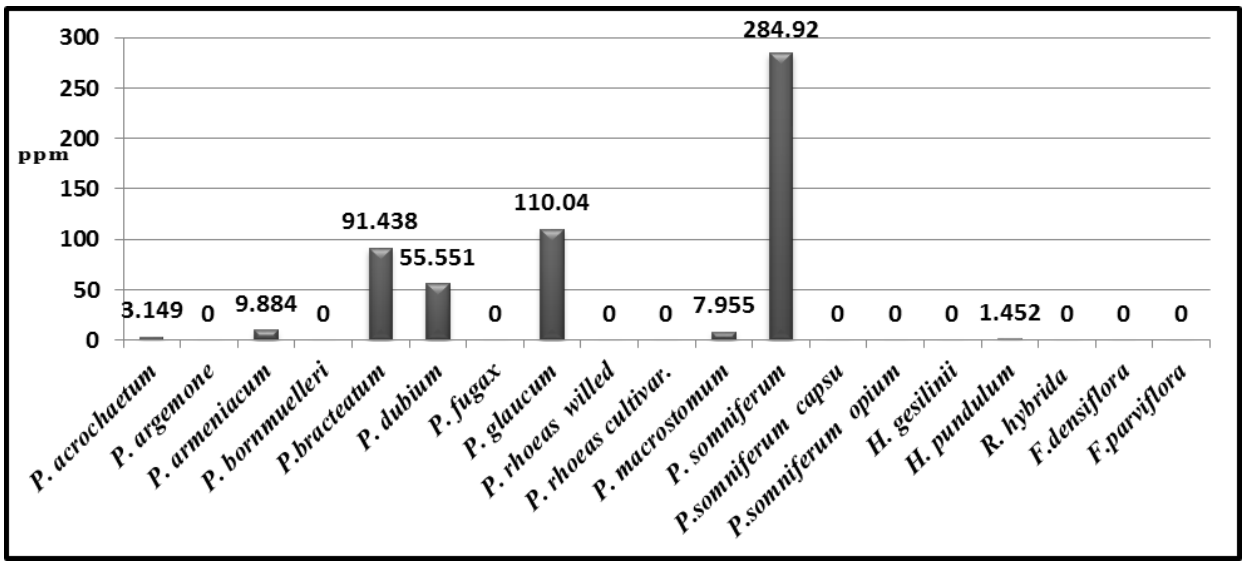


Fig 4: Variation in morphine concentration for species under studied

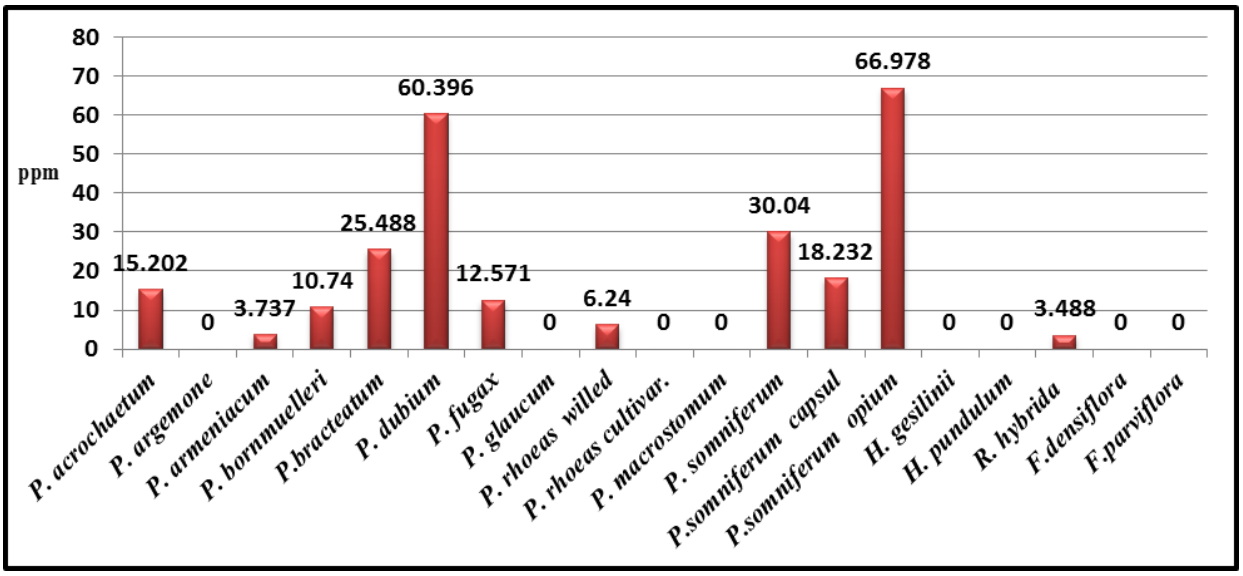


Fig 5: Variation in codeine concentration for species under studied

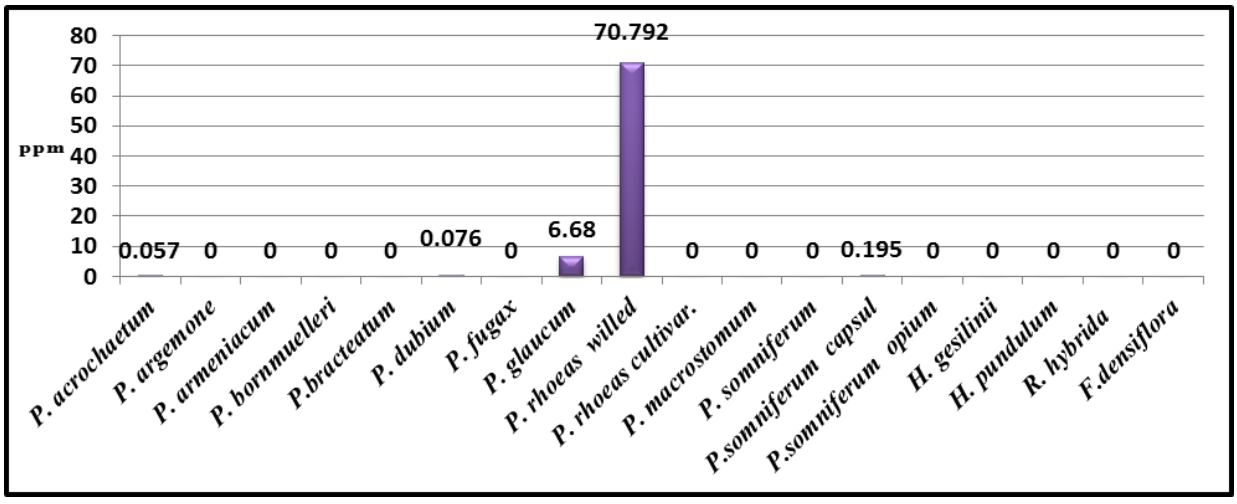


Fig 6: Variation in thebaine concentration for species under studied

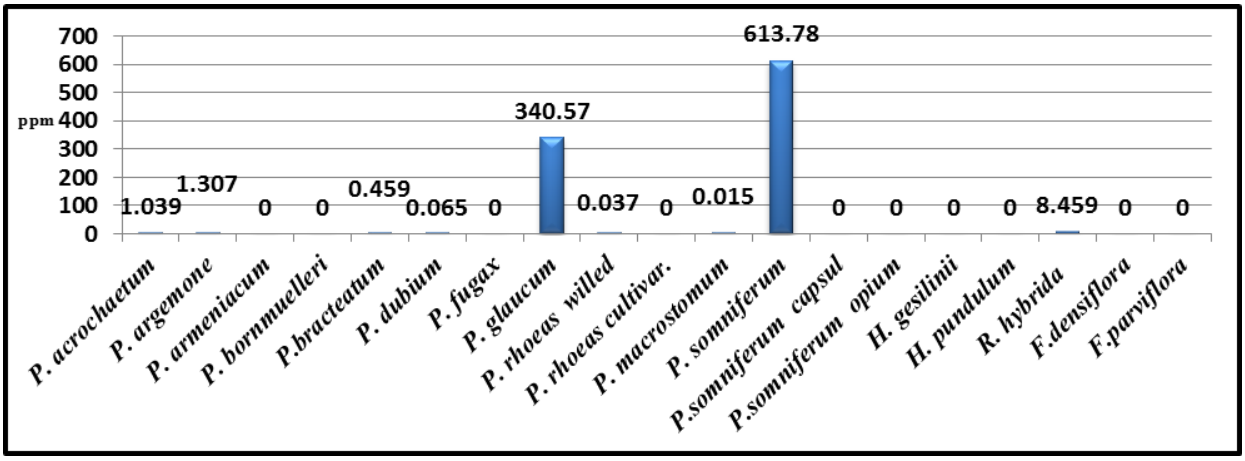


Fig 7: Variation in papaverine concentration for species under studied

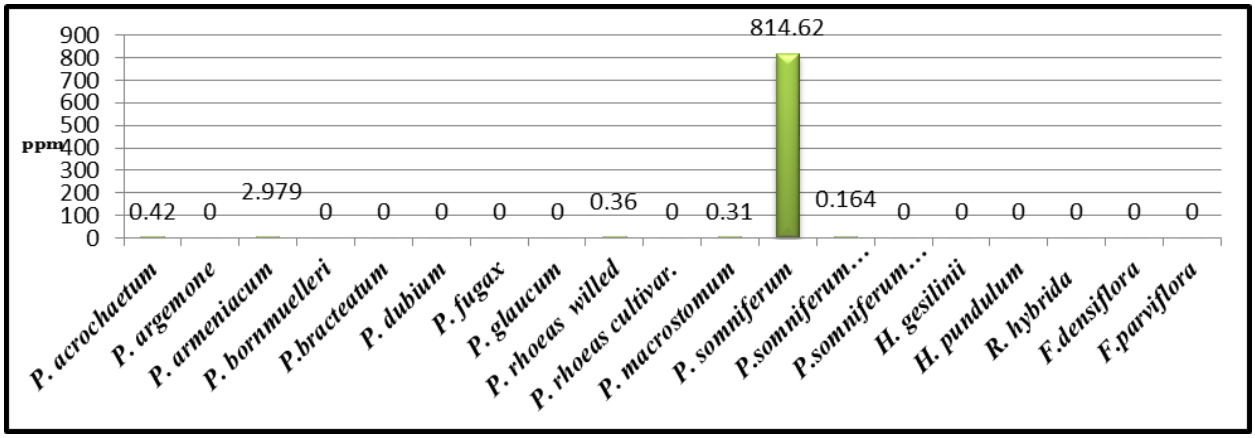


Fig 8: Variation in noscapine concentration for species under studied

From all of the above it was illustrated that the possibility of knowing the degree of affiliation and the spacing between the genera and that depends on the presence of opium alkaloid compounds which distinguish the presence of this alkaloid in genus *Papaver* where all species contain at least one type of these alkaloids and this confirms the degree of relationship between species.

It is worth mentioning that the chemical characteristics provide additional support when gathered with other studies. This study gives us an idea of the diversity in the chemical content in the Iraqi species on which we can classify the Papaveraceae species depending on its opium alkaloids as shown in table 2.

Table 2: Alkaloids present in species under studied

Morphine	Codine	Papaverine	Thebaine	Noscapine
P. acrochaetum	*P. acrochaetum*	*P. acrochaetum*	*P. acrochaetum*	*P. acrochaetum*
P. armeniacum	*P. armeniacum*	*P. argemone*	*P. glaucum*	*P. armeniacum*
P. bracteatum	*P. bracteatum*	*P. bracteatum*	*P. rhoeas* willed	*P. macrostomum*
P. glaucum	*P. bornmuelleri*	*P. glaucum*	*P. somniferum*	*P. rhoeas* willed
P. macrostomum	*P. dubium*	*P. macrostomum*	*H. geslinii*	*P. somniferum*
P. somniferum	*P. fugax*	*P. rhoeas* willed		
H. pendulum	*P. rhoeas* willed	*P. somniferum*		
	P. somniferum	*R. hybrida*		
	R. hybrida			

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