

Original Research Article

Isolation, phytochemical and antioxidant screening of roemerine obtained from *Papaver dubium* and *Papaver macrostomum* grown in Iraq

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Abstract

Purpose: To isolate roemerine from *Papaver dubium* and *Papaver macrostomum*, two plants widely grown in Northern Iraq and to evaluate its antioxidant properties.

Methods: Aerial parts of the plants were harvested and extracted using methanol. Roemerine (alkaloid) was isolated from the methanol extracts, followed by identification and quantification using Gas Chromatography-Mass Spectrometry (GC-MS), Thin-layer Chromatography (TLC) and High-Performance Liquid Chromatography (HPLC) analysis. Determination of antioxidant potential of varying concentrations of roemerine (50, 100, 200, 300, and 400 µg/mL) in dimethyl sulphoxide (DMSO) was performed using 2,2-diphenyl-1-picrylhydrazyl radical (DPPH) and ferric reducing antioxidant power (FRAP) assays and results were compared against those of standard ascorbic acid (vitamin C).

Results: Roemerine was present in the extracts of both *Papaver* species. However, a higher concentration of the alkaloid was found in *P. dubium* extract (2.67 mg/g) compared to *P. macrostomum* extract (1.92 mg/g; $p < 0.05$). The free radical scavenging activity of DPPH revealed that roemerine possessed weak antioxidant activity with an IC_{50} value almost two times higher than that of ascorbic acid. Furthermore, the scavenging activity of roemerine at the highest concentration was 50 % lower than that of ascorbic acid (33 vs 67 %, respectively). There was also no significant increase ($p > 0.05$) in the reducing power of roemerine at all concentrations assayed compared to vitamin C.

Conclusion: Roemerine has been isolated from *P. dubium* and *P. macrostomum*. A higher concentration of the alkaloid is seen in *P. dubium* compared to *P. macrostomum*. This report presents the first evidence of roemerine availability in *Papaver dubium* and *Papaver macrostomum* in Iraq.

Keywords: Roemerine, *Papaver dubium*, *Papaver macrostomum*, HPLC, GC-MS, DPPH, FRAP

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INTRODUCTION

Ancient civilizations have long recognized plants as safe and therapeutic options, compiling the plants they used in their environments [1]. The Papaveraceae family is an important source of several phytochemicals and it includes 780 species under 44 genera, comprising both

annual and biannual plants. They are well-distributed in the north temperate regions of Iraq, South Africa, Turkey, Iran, Syria, and Iraq, and the plants have been used for many medicinal applications with economic benefits [2]. In Iraq, one of the most important genera of this family is *Papaver* L, which comprises 15 species spread across most parts of Iraq from the south to the

north (including cities like Erbil, Sulaymaniyah and Jabal Sinjar) and in the eastern areas [3,4]. This genus consists of about 170 alkaloids sharing different features, making them important medicinal source for cancer therapy [5].

Alkaloids isolated from *Papaver* species have several uses in traditional medicine, such as analgesic, anxiolytic, cough suppressive, antiviral and gastrointestinal effects, as well as for anesthesia [6,7]. They are also used in the treatment of depression, myocardial infarction, angina and pulmonary embolism [7,8].

Roemerine is an important alkaloid isolated previously from *Papaver decaisnei* Hochst, which is grown in northern Iraq [5]. This alkaloid has activity against *Candida albicans* and methicillin-resistant *Staphylococcus aureus*. It also possesses antidepressant effects, as well as improved vinblastine cytotoxic activity [7,9]. *Papaver macrostomum* Boiss. & Huet is widely distributed in Turkey and is used as an antitussive and sedative agent in combination with *Papaver rhoeas* L. in traditional Turkish medicine [10]. Previous investigations on the alkaloids of this species revealed the presence of the benzylisoquinolines, aporphine, protopine, and rhoeadine [11]. In this study, *P. dubium* and *P. macrostomum* were investigated for the presence of roemerine using different methods, including gas chromatography-mass spectrometry (GC-MS), thin-layer chromatography (TLC) and high-performance liquid chromatography (HPLC). Furthermore, the isolated alkaloid was evaluated for its free radical scavenging activity by 2,2-diphenyl-1-picrylhydrazyl radical (DPPH) and Ferric reducing antioxidant power (FRAP) assays.

EXPERIMENTAL

Collection of plant materials

Aerial parts of the plants (*P. macrostomum* and *P. dubium*) were collected in May 2022 from different zones (Sulaymaniyah-Dukan road, Halabja-Byara road and Dukan-Rania Road, respectively) in Sulaymaniyah City (35 ° North latitude and 45 ° East longitude) in the north of Iraq. Plant samples were taxonomically classified based on the flora of Iraq [4]. They were allowed to dry in shade in a well-ventilated area at room temperature and then packaged into labeled containers.

Extraction of alkaloids from plant samples

Extraction of the samples involved two main sequential procedures [12,13]. First, 20 g of dried

plant samples of each species were macerated in 300 mL of 80 % methanol for 48 h. The solution was continuously stirred with a magnetic stirrer, followed by filtration with filter paper. Isolation of alkaloids was done by first concentrating the filtrates using a rotary evaporator, followed by the addition of 100 mL HCl (5 % v/v) to dissolve the concentrate. Thereafter, 100 mL of petroleum ether was added to the solution in a separatory funnel. The separated acidic aqueous part was made alkaline by slowly adding 32 % ammonia solution. The aqueous alkaline solution was then mixed with 100 mL of chloroform in a separatory funnel, shaken well and allowed to separate. The organic layer was subsequently separated from the aqueous solution and further dried by adding anhydrous sodium sulphate.

Determination of alkaloid content

Presence of alkaloids in the purified samples of *P. macrostomum* and *P. dubium* was qualitatively assayed using methods described previously [14,15].

Gas chromatography-Mass spectrometry (GC-MS)

The methanolic extracts of the plant samples were screened qualitatively for alkaloid content using GC-MS. The GC was equipped with HP-5 MS (5 % phenylmethyl siloxane), a capillary column (30 m length, 0.25 mm internal diameter, 0.25 µm thickness), temperature set from 100 to 300 °C for 10 min at a rate of 20 °C/min. Helium gas was used as carrier at a flow rate of 1 mL/minute. The ionization energy used was 70 eV [5,12].

Thin-layer chromatography (TLC)

Pre-coated aluminum silica plates measuring 200 x 200 mm and 60 Å pore size were carefully cut into fragments measuring 100 x 50 mm and used with different mobile phase systems as shown in Table 1. The final extracts of the two plant species were dissolved in chloroform and compared to standard roemerine, which was also prepared using chloroform as solvent. Determination of roemerine concentration was done at wavelengths between 254 and 365 nm [16,17].

High Performance Liquid Chromatography (HPLC) for estimation of roemerine

The final isolates of the purification procedure were dissolved in chloroform and analyzed using HPLC for the qualitative and quantitative

estimation of roemerine. Analysis was carried out using simple and rapid reverse-phase high-performance liquid chromatography (RP-HPLC) system (Shimadzu, Japan) at the Ministry of Science and Technology, Baghdad. The mobile phase was composed of a gradient of methanol and distilled water (78:22) and 20 mL of the extract was injected into a C-18 absorption-type column (4.6 mm x 25 cm, 5 micron), with a flow rate of 1.0 mL/min and at room temperature. Detection using a photo diode array PDA detector was done at a wavelength of 288 nm [18].

Estimation of DPPH free radical scavenging activity

Free radical scavenging activity for roemerine was evaluated using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) technique against ascorbic acid as standard. Five concentrations of roemerine (50, 100, 200, 300, and 400 µg/mL) were dissolved in dimethyl sulphoxide (DMSO). In addition, the same concentrations of ascorbic acid were prepared in distilled water and used as standard. To each concentration, 1 mL of 0.8 nm/mL DPPH was added and the sample was allowed to stand for 30 minutes at room temperature.

Thereafter, absorbance was determined at 517 nm. Scavenging activity (SA) of roemerine was calculated according to Eq 1.

$$SA = \{(A_o - A_s)/A_o\}100 \dots\dots (1)$$

Where A_o is the absorbance of negative control (solvent), and A_s is the absorbance of sample. The IC_{50} values were also calculated for the samples [19,20].

Ferric reducing antioxidant power

The ferric reducing antioxidant power assay of roemerine was done by preparing four concentrations of roemerine and ascorbic acid (50, 200, 300 and 400 µg/mL) in DMSO and

H₂O, respectively. Each sample (1 mL) was added to 2.5 mL of phosphate buffer (pH 6.6) and 2.5 mL of potassium ferricyanide (1 %) solution. The solution was mixed and incubated at 50 °C for 20 min, then filtered by centrifuging at 3000 rpm for 10 min. Thereafter, 2.5 mL of the supernatant was added to a solution containing 2.5 mL of distilled water and 0.5 mL of freshly prepared ferric chloride (1 %). Absorbance was read at 700 nm using a UV double-beam spectrophotometer [21].

Statistical analysis

Statistical analysis was done using SPSS software (version 21). This analysis was done for DPPH and FRAP results. Average values, standard deviations (SD) and analysis of variance (ANOVA) were computed from triplicate determinations. P -value < 0.05 was considered significant.

RESULTS

Presence of alkaloids

Table 2 shows the presence of alkaloids in the methanol extracts of *P. dubium* and *P. macrostomum*. Wagners', Dragndroff's and Mayer's methods revealed the presence of alkaloids in both plant samples.

Identification of roemerine using GC-MS

The GC-MS chromatograms for the methanolic extract of *P. dubium* and *P. macrostomum* are shown in Figure 1 and Figure 2. Roemerine was detected in both plants; however, it was less in *P. macrostomum*, as shown in Table 3.

Identification of roemerine using TLC

Analysis of the purified extracts by TLC showed the presence of roemerine in *P. dubium* and *P. macrostomum*.

Table 1: Mobile phase for thin-layer chromatography (TLC)

System A	Volume (mL)	System B	Volume (mL)	System C	Volume (mL)
Toluene	45	Ethyl acetate	85	Methanol	100
Acetone	45	Methanol	10	Ammonia	1.5
Ethanol	7	Ammonia	5		
Ammonia	3				

Table 2: Presence of alkaloids in the methanol extracts of *P. dubium* and *P. macrostomum*

Species/Detection	Wagner's	Dragndroff's	Mayer's
<i>P. dubium</i>	+	+	+
<i>P. macrostomum</i>	+	+	+

show the presence of roemerine with mild differences in the Area under the curve, resulting in different concentrations in the two samples as shown in Table 4.

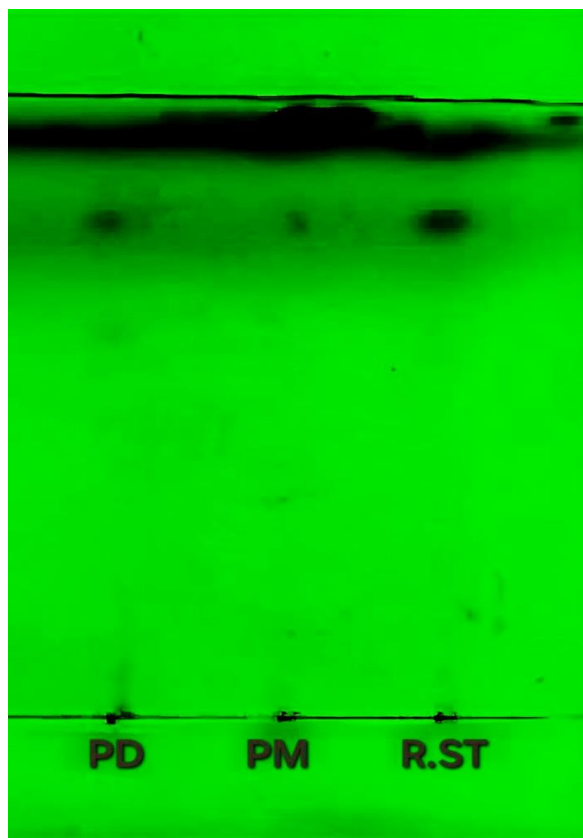


Figure 3: Chromatogram showing separated bands of roemerine in purified extracts of *P. dubium* (PD) and *P. macrostomum* (PM). Roemerine standard (R.ST) was used as a control

Free radical scavenging activity of roemerine

The result of the DPPH free radical scavenging activity of standard roemerine is shown in Figure 6. At all concentrations tested, vitamin C (used

as standard) showed a significantly higher ($p < 0.05$) scavenging activity compared to roemerine. The IC_{50} of ascorbic acid (positive control) was found to be 303.0 $\mu\text{g/mL}$, while that of roemerine was $> 400 \mu\text{g/mL}$. Scavenging activity of roemerine at 400 $\mu\text{g/mL}$, which was the highest concentration used in the test and gave 33 % free radical scavenging activity compared to ascorbic acid which showed 67 % free radical scavenging activity at the same concentration.

Determination of antioxidant reducing activity of roemerine

The reducing power of standard roemerine and vitamin C was determined using FRAP assay and results are presented in Figure 7. There was no significant increase ($p > 0.05$) in the reducing power of roemerine at all concentrations assayed compared to vitamin C.

DISCUSSION

Roemerine, an aporphine alkaloid, was isolated from aerial parts of *P. dubium* and *P. macrostomum*, which were harvested from Sulaymaniyah City, Iraq. The extracted alkaloid gave positive results with Wagner's, Mayers', and Dragendorff's tests, which are indicators of alkaloid availability. The GC-MS analysis also showed that roemerine is present in both plants, although higher in *P. dubium* (27.43 %) compared to *P. macrostomum* (0.369 %), which agrees with the finding of Ajabduljabbar *et al* for *P. decaiseni* (70 %) from Iraq [5].

The TLC outcomes show clearly separated bands for roemerine in both species' samples, which confirms its isolation and availability gave an improvement for the findings in *P. rhoeas* by Çoban *et al.* and *P. decaiseni* by Jafaar *et al.*,

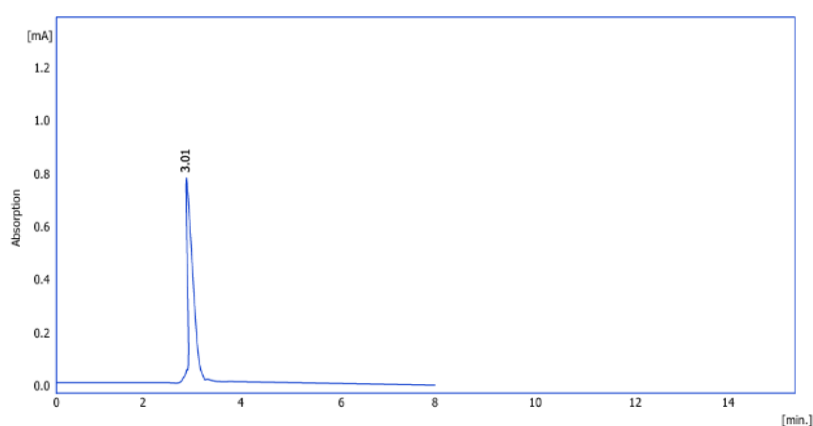


Figure 4: HPLC chromatogram of roemerine standard

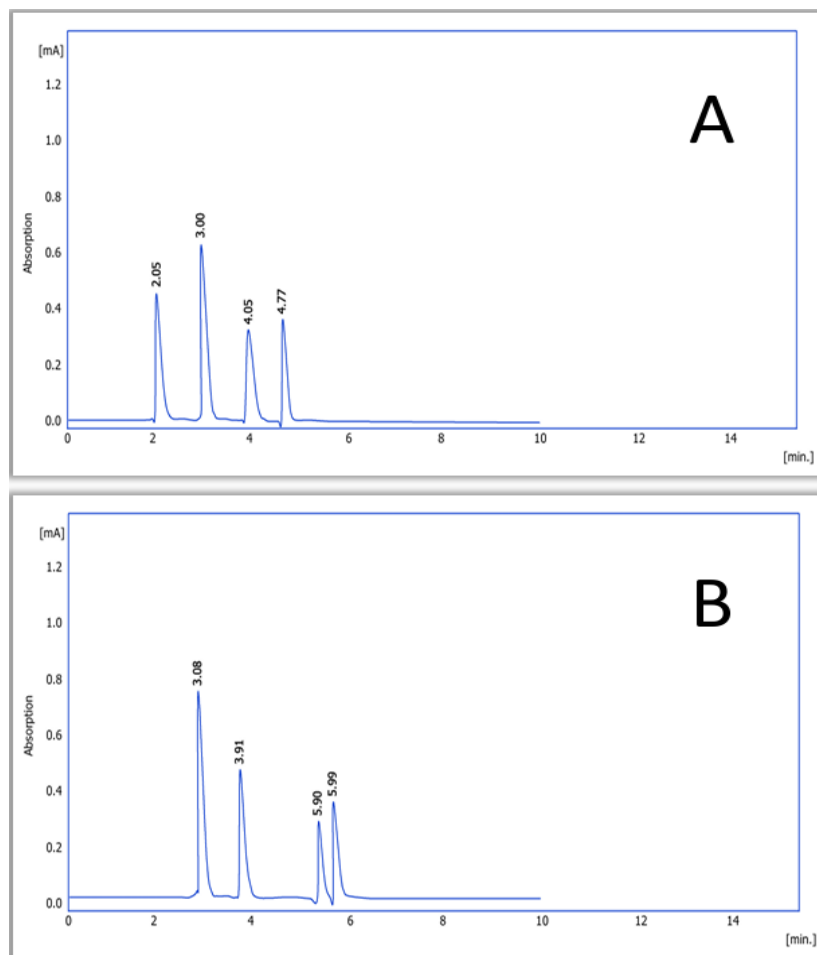


Figure 5: HPLC chromatogram of *P. dubium* (A), *P. macrostomum* (B)

Table 4: HPLC analysis of roemerine in extracts of *P. dubium* and *P. macrostomum*

Sample	Retention time (min)	Area (mAU.s)	Height (mAU)	Area (%)	Height (%)	ppm
Romerine St.	3.01	2411.14	798.00	100	100	10.00
<i>P. dubium</i>	3.04	135654.70	630.66	40	40	2672.58
<i>P. macrostomum</i>	3.08	97452.14	785.45	40	40	1919.94

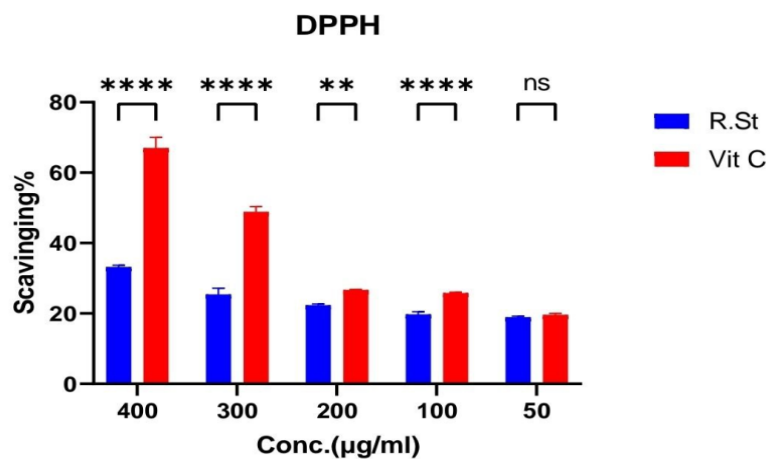


Figure 6: DPPH scavenging percentage of standard roemerine (R.St) compared with ascorbic acid (Vit C). **** $P < 0.01$, ** $P < 0.05$ vs Vit C within group, ns = not significant

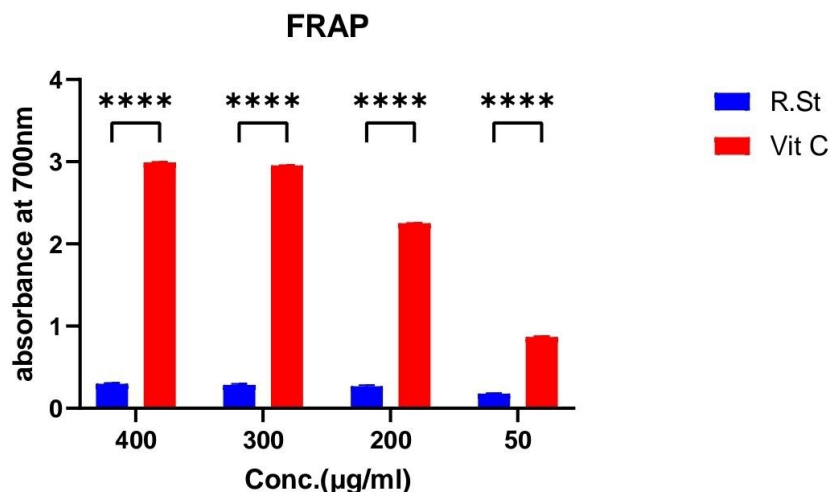


Figure 7: FRAP assay showing the antioxidant reducing activity of standard roemerine compared to ascorbic acid (vit C). **** $P < 0.05$ vs Vit C within group

while HPLC matches the findings in other species, *P. rhoeas* findings by Çoban *et al*, with higher concentration in *P. dubium* compared to *P. macrostomum*, which is the first evidence of roemerine availability in these two species [13,16,23].

The free radical scavenging assay for roemerine by DPPH showed weak antioxidant activity compared to ascorbic acid as positive control, which was significantly higher ($p < 0.05$) with increasing doses and almost half the free radical scavenging effect at the highest dose of the extract at 400 µg/mL. This antioxidant effect of roemerine may be related to the lone pair on nitrogen of the tertiary amine that is shared with aporphine alkaloids skeleton, hence leading to a weak antioxidant effect as previously shown [24,25]. The FRAP assay also shows significant differences in the outcomes of roemerine to ascorbic acid as positive control for all doses ($P < 0.05$), showing roemerine have very weak antioxidant effect that agrees with the findings of Liu *et al* [26].

CONCLUSION

Roemerine has been isolated from *Papaver dubium* and *Papaver macrostomum* grown in Iraq and quantified using GC-MS, TLC, and HPLC, with a higher concentration seen in *P. dubium* compared to *P. macrostomum*. In comparison with ascorbic acid, roemerine shows weak free radical scavenging and reducing activities. This report presents the first evidence of roemerine availability in *P. dubium* and *P. macrostomum* in Iraq. Further studies to evaluate other biological activities such as antimicrobial,

antifungal and cytotoxic effects would be required.

DECLARATIONS

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Conflict of interest

No conflict of interest is associated with this work.

Contribution of authors

We declare that this work was done by the authors named in this article, and all liabilities pertaining to claims relating to the content of this article will be borne by the authors. All authors read and approved the final manuscript.

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