Since 1987
REVIEW OF CLINICAL PHARMACOLOGY AND PHARMACOKINETICS, INTERNATIONAL EDITION |38 (Sup2):101-104 (2024) Rcpp

Received: 14 February 2024 | **Accepted**: 28 February 2024 | **Published**: 5 May 2024

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Isolation and characterization of a tetrahydroprotoberberine alkaloid from *Crassula ovata*

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Abstract

The presence of alkaloids in *Crassula ovata* is a topic that is still unexplored, as there are no published studies on the matter. This study demonstrates the presence of an alkaloid compound (and its class) for the first time in *Crassula ovata*. The plant material was defatted with n-hexane, and a Soxhlet apparatus was used for the extraction process, while the acid-base method was used for the isolation of alkaloids from the chloroform fractions. The quaternary alkaloid was precipitated from the aqueous layer spontaneously, in high quantity. By using standard spectroscopic methods (including liquid chromatography - mass spectroscopy) we were able to clarify the structure of the precipitated compound as a tetrahydroprotoberberine alkaloid based on the general fragmentation pattern of this class of alkaloids and the retro-Diels-Alder reaction; a characteristic fragmentation pathway of tetrahydroprotoberberine alkaloids.

KEYWORDS
protoberberine, *Crassula ovata*, alkaloid, solvent extraction

How to cite: Al-yassery H. K., Kadhim E. J. Isolation and characterization of a tetrahydroprotoberberine alkaloid from *Crassula ovata*. *Rev. Clin. Pharmacol. Pharmacokinet. Int. Ed.* 38 (Sup2): 101-104 (2024). https://doi.org/10.61873/IJZC4054

Publisher note: PHARMAKON-Press stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.

1. INTRODUCTION

Recently, developed nations are increasingly turning to the employment of traditional medical practices that use herbal medications and remedies, leading to an increased reliance on these types of medicines. Several medications and other products have been developed and extracted from plants in industrialized countries [1]. *Crassula ovata* is a plant that belongs to the Crassulaceae family, and has formerly been utilized as a medicinal plant in South Africa and in other countries. In China, it is used in order to treat wounds and diarrhoea [2], while in North-East India (particularly in Manipur) *Crassula ovata* is primarily used for the treatment of diabetes (by consuming the plant's leaf juice) and various infections [3]. There is no previous study indicating the presence of alkaloids in *Crassula* plants, except for one study demonstrating a positive Mayer's test for *Crassula ovata*

[4]. However, the distribution of alkaloids has been studied in the Crassulaceae family, and only *Echeveria venezuelensis* has been found to contain piperidine alkaloids, while *Crassula multicava* does not [5]. This study focuses on the extraction of alkaloids from chloroform fractions of *Crassula ovata* by acid-base methods.

2. MATERIALS AND METHODS

Materials: The sample consisted of whole plants (leaves, steam, and root) of *Crassula ovata* collected from the Babylon Nursery Plantation in March 2023. The plant was identified and authenticated by Dr Esraa Abdel-Al Razzaq Majeed (2023-1-29) at the Department of Biology, College of Sciences, University of Baghdad.

Extraction and isolation: About 200 gr of the powdered plant were defatted for 48 h in n-hexane so as to remove any wax and fatty material. The plant material was then filtered and dried at room temperature, while 3 h after spraying the plant with ammonia, the extraction process began by using a Soxhlet apparatus for 9 h. A total of 300 mL of 85% aqueous ethanol was used as an extractor solvent, through simple filtration so as to remove any boiling chips and other residues, and to obtain the crud extract by rotary evaporation under reduced pressure. The crude extract (37.421 gr) was dissolved in 300 mL of water that was sequentially partitioned first with chloroform, then with ethyl acetate, and lastly with n-butanol (3 x 100 mL for each fraction). The chloroform fraction (crude extract) entered isolation and further purification through the acid-base method. It was first dissolved in acidified water and portioned with ethyl acetate, then after removing the aqueous layer, it was made basic by the addition of ammonia, and was further portioned in a separator funnel with a lipophilic organic solvent (chloroform) [6]. The compound precipitated from the aqueous layer as white crystals spontaneously, and this provided a mark that our alkaloid was a quaternary compound. The structure was clarified by liquid chromatography - mass spectroscopy (LC-MS).

Structural analysis: The standard spectroscopic methods for investigating the structure of natural products comprise of infrared spectroscopy (IR) and ultraviolet (UV) spectroscopy, which are both carried out in the Environmental and Water Research Department of the Ministry of Sciences and Technology; these methods are often combined with MS, including LC-MS (AB SCIEX 3200 QTRAP; Mashhad University of Medical Sciences).

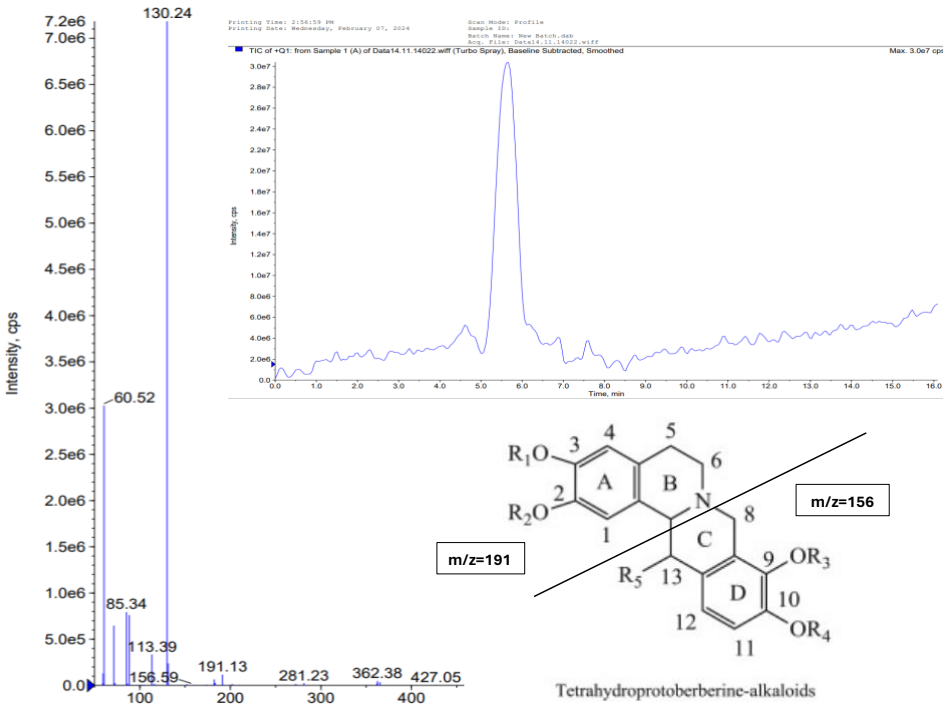

Figure 1. LC-MS chart of the isolated alkaloid compound (m/z of 362 $[M+H]^+$), where m/z=130 corresponds to the base peak result from the isoquinoline nucleus fragment, m/z=191 and m/z=156 correspond to the retro-Diels-Alder reaction, while m/z=113, m/z=85, and m/z=60 correspond to the isoquinoline nucleus fragmentation by loss (NH_3, CH_2=CH_2, and CH_2=CH_2, respectively).

3. RESULTS

As shown in Figure 1, LC-MS has clarified the structure of the isolated alkaloid compound as a tetrahydroprotoberberine compound, based on the general MS fragmentation pathways of protoberberine-type alkaloids [7], and a UV / visible spectrophotometer λ_{max} of 202.5. Moreover, the obtained Fourier transform IR (FT-IR) revealed a 3400 N-H bending, a 3119 C-H bending of CH_2=CH_2, a 2800 C-H bending of CH_2, a 1669 C=C bending, a 1387 C-N bending, and a 1042 C-O bending. The isolated compound started to melt at 180°C, then underwent decomposition at 220°C.

4. DISCUSSION

An alkaloid is a basic substance. When adding acidified water, it turns into salts and is present in the aqueous layer because it is dissolved in it. It is portioned with ethyl acetate so s to remove other substances and obtain the aqueous layer. Subsequently, ammonia is added and the alkaloid compound becomes free because the strong base releases the weak base from its salt and becomes dissolved in any lipophilic solvent (like chloroform), except if the alkaloid is quaternary; in the case of the latter, it remains in the aqueous layer. In this experiment, the compound precipitated from the aqueous layer automatically, and it was collected in high quantity from this plant. The most important spectroscopic method for investigating the structure of natural products is MS. The mass spectrum of a tetrahydroprotoberberine alkaloid like tetrahydropalmatine or corydaline shows the predominant ions at m/z of 192 and 165 or 150 after the loss of a methyl group from the retro-Diels-Alder reaction, which is a characteristic fragmentation pathway of tetrahydroprotoberberine, resulting in a C-ring opening so as to form tetrahydroisoquinoline fragment ions (m/z=130). It is significant that this tetrahydroisoquinoline fragment ion is not only a characteristic feature, but also gives structural information about this class of alkaloids. It is always the most abundant fragment in the mass spectra of these alkaloids, and most likely represents the substitution pattern in the A ring in Figure 1. For instance, the most prevalent ion in both corydaline and tetrahydropalmatine, the product ion with m/z of 192, is suggestive of two methoxy substituents in the A ring [8].

5. CONCLUSION

In conclusion, this study indicates the presence of alkaloids in *Crassula ovata*. The mass spectrum of the isolated crystals revealed that the possible compound is a tetrahydroprotoberberine alkaloid like corydaline and tetrahydropalmatine. Quaternary protoberberine alkaloids represent a very interesting and significant group of natural products with a broad range of biological activities, including antiparasitic, antitrypanosomal, and antileishmanial activities.

ACKNOWLEDGEMENTS

The authors are deeply grateful to the College of Pharmacy of the University of Baghdad, for giving us the opportunity and the facilities to accomplish this work.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

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PMID: **26579385**