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Isolation of Anthraquinone from the Roots of North Western Himalayan Medicinal Plant *Eremurus himalaicus* Baker

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ABSTRACT: *Eremurus himalaicus* Baker, belonging to the Asphodelaceae family, is one of the least investigated valuable medicinal plant and is widely utilised in conventional and folk remedies as vegetable, for treating anaemia, and inducing lactation in post-partum period. In light of the pervasive use of *E. himalaicus* in traditional medicine and the paucity of research into its chemical constituents, the purpose of this study was to isolate and identify the characteristic secondary metabolite, anthraquinone. The air-dried underground part of the plant was investigated. Crude extract was obtained by successive hot extraction using Soxhlet apparatus. Using a wide range of chromatographic methods like Silica gel column chromatography and Thin Layer Chromatography, Aloechrysone was isolated from ethyl acetate and methanol fractions of the crude extract and structure elucidation was done by using spectroscopic techniques of NMR/MS. This identified phytoconstituent is the first report from *E. himalaicus*. Future research should be conducted to bridge the gaps in *in vivo* and *in vitro* study of this compound and pave the way for the clinical application of Aloechrysone.

Keywords: *Eremurus himalaicus*, Aloechrysone, NMR, MS, Compound isolation.

INTRODUCTION

The genus *Eremurus* belonging to the family *Asphodelaceae*, known as foxtail lilies or desert candles in the international horticulture trade used in landscaping and cut-flower markets as ornamental plants (Kamenetsky and Rabinowitch 1999) comprises some 62 species in the world (Bayrak and Ynardy 2021). From the eastern Mediterranean area eastward to the Himalayas, China, and Central Asia, the species grows in desert, semidesert, or moderate conditions (Beiranvand and Beiranvand 2022). About a wide range of applications of Eremurus in traditional Iranian medicine, and lack of research into its chemical constituents, this genus may prove to be a potential source of promising new cosmeceutical, nutraceutical and functional food formulations. In several regions of central and western Asia, the *Eremurus genus* is used in traditional folk medicine to treat and prevent liver and stomach diseases, haemorrhoids, diabetes, and hypertension (Karaman *et al*., 2011). Eremurus species have historically been used to cure a variety of illnesses e.g., *E. persicus* as an antidiabetic agent in Iranian folk medicine (Asgarpanah *et al*., 2011); medicinal supplement (Karaman *et al*., 2011); anti-inflammatory treatment in traditional Kurdish medicine (Gaggeri *et al*., 2013); gastrointestinal problems (Karaman *et al*., 2011), genitourinary infections (Mottaghipisheh *et al*., 2021), fungal skin diseases (Gaggeri *et al*., 2015) and arteriosclerosis. *E.spectibilis* have been used in treating rheumatism (Ozturk and Olcucu 2011; Cinar *et al*., 2017) , diabetes (Yesil and Akalin 2009; Tuzlacı and Dogan 2010), scabies (Karaman and Kocabas 2001); fungal skin diseases (Hashemi *et al*., 2014; Pourfarzad *et al*., 2014). Some people utilise the roots of *E. anisopterus* and *E. chinensis* Fedtsch to treat rheumatism and other bodily ailments (Li *et al*., 2000; Hu *et al*., 2011). In traditional Uygur medicine, stomach and intestinal problems are treated with a powder made from the dried roots of the Eremurus plant (Zhu *et al*., 2014). After being cooked, the plant's tender young leaves (*E. spectabilis* and *E. cappadocicus*) are eaten as food and medicine in Turkey for a wide variety of ailments, including rheumatism, physical weakness, scabies, liver and stomach disorders, eye inflammation, constipation, diabetes, relieving colon pain and inflammation, and snake and scorpion venom poisoning (Asgarpanah *et al*., 2011; Abubaker, 2015).

Eremurus himalaicus Baker, belonging to the Asphodelaceae family, is one of the least investigated valuable medicinal plant and is widely utilised in conventional and folk remedies as vegetable, for treating anaemia, and inducing lactation in post-partum period (Dhiraj and Anjna 2011; Kumari and Saggoo 2016; Shailja, 2011; Aziz *et al*., 2022; Haq *et al*., 2022). Tribal members treat fever, diarrhoea, and diabetes with powdered roots and cooked leaves (Shailja, 2011). *Eremurus himalaicus* Baker, often known as Himalayan Desert Candle, is a wild ornamental herb native to the North-western Himalayas and can be found only between the elevations of 2100 and 3300 metres, growing on stony slopes in arid regions from

Afghanistan to Himachal Pradesh (Wendelbo and Furse 1969). The compound hordenine is an alkaloid which is found in *Eremurus himalaicus* mostly in the roots. This compound has been found to possess various activities e.g., anti-asthmatic, uterine stimulant, anti-feedant, radioprotector, insect repellent, antibiotic, aphrodisiac and anti-obesity (Zargar, 2013). The plant *Eremurus himalaicus* has also been used in some herbal formulations. However, very little scientific research has been done on the therapeutic benefits of this plant, and much more has to be investigated.

In case of *E. himalaicus*, aerial parts have previously been demonstrated to have antioxidant activity (Mushtaq *et al.*, 2017; Amin and Bhat 2015), antibacterial activity (Mushtaq *et al.*, 2017), hepatoprotective activity (Amin and Bhat 2015) and hypoglycaemic activity (Mushtaq *et al*., 2014). There has been no report on secondary metabolite isolation from this plant till now. Considering the tribal use of powdered roots, the goal of this research was to identify and purify a secondary metabolite found in the underground parts of *E. himalaicus.*

MATERIALS AND METHODS

A. Plant material collection

The roots of *Eremurus himalaicus* Baker plants utilized in the study were collected in the month of July, 2021 in flowering stage from Chandanwari (75°23'52"E, 34°04'22" N) region of Anantnag District in Kashmir, India, at an elevation of 2888m above msl. Akhter Hussain Malik of the Centre for Biodiversity and Taxonomy (CBT), Department of Botany, University of Kashmir, authenticated the collected plant specimen as being genuine.

B. Extract preparation

After collecting the roots, they underwent a distilled water rinse after being cleaned with running water. Afterward, the material was shredded, dried for 25 days at room temperature in the shade, and then grounded in a lab electric grinder. A soxhlet extractor was used to extract the dried root powder (1.7 kg each) at a temperature below the boiling point of the methanol solvent. Until a colorless solvent was obtained, the extraction process was repeated with the same plant material. A rotary evaporator dried the filtrate at a decreased pressure after filtering it through Whatman No. 1 filter paper.

C. Silica Gel Column Chromatography

Hexane, ethylacetate, and methanol were used sequentially in order to extract the concentrated methanolic extract from the root. A hexane: EtOAc and EtOAc:MeOH gradient was used to chromatograph the methanol extract on silica gel (60-120 mesh), yielding 100 fractions (F1-F100). Based on their TLC profiles, fractions 12-24 were combined. Fractions 12-24 were chromatographed on silica gel (100-200mesh) columns and eluted with 25% methanol in ethyl acetate to provide the desired compound. Homogeneity across TLC confirmed the purity of the compound produced via re-crystallization from appropriate solvents.

D. Spectral analysis using NMR/MS

A Swiss-made BRUKER AVANCE II-NEO-500MHz FT-NMR spectrometer was used for the spectral investigation of recording NMR with DMSO-d6 as the solvent and TMS serving as an internal reference. The Waters Corporation, U.K.-made Alliance 2795, Q-TOF Micromass Mass Spectrometer, which has an ESI and lock spray ionisation source (HRMS), Ms/Ms, was used for the mass analysis. The mass spectrometer distinguishes the ions based on their mass to charge ratio and detects them according to their concentration. The mass spectrum of the molecule is thus produced. A graph displaying the result of ion abundance versus mass-to-charge ratio was presented. Information on the composition and structure of their parent molecule can be gleaned from ionic fragments. In the spectrum of a pure compound, the molecular ion, if it is present, is visible at the highest value of m/z and gives information on the molecular mass of the substance.

RESULTS

The ^{1}H NMR (Fig. 1) of the isolated compound showed one singlet peak at delta 1.21 which corresponds to three methyl protons present in the isolated molecule. Further, one singlet peak at 1.52 confirms the presence of one methoxy group integrating to three protons. The singlet peak at around 1.98 corresponds to two protons of one methylene carbon of one non-aromatic ring present in the isolated molecule. Similarly, singlet peak at around 2.01 corresponds to two protons of second methylene carbon of the non-aromatic ring of the isolated structure. The singlet peak at around 5.13 corresponds to the hydroxyl group proton attached to the chiral carbon atom of the non-aromatic ring. The four peaks at 7.51, 7.46, 7.68 and 8.26 are attributed to four aromatic protons present in two aromatic rings of the isolated molecule. In addition to this, the singlet peak at 10.48 corresponds to one hydroxyl group proton attached to the middle aromatic ring.

Further,^{13}C NMR (Fig. 2) shows one peak at 18.62 which corresponds to only methyl group carbon atom present in the isolated molecule. The peak around 21.81 corresponds to methoxyl group carbon atom and the four peaks at 25.29, 68.61, 69.33 and 76.73 are attributable to four carbon atoms of non-aromatic ring present in the isolated molecule. While the peaks at 77.04, 127.01, 127.42, 127.67, 127.80, 128.34, 130.87, 131.72, 136.74 and 162.50 corresponds to the ten carbon atoms of aromatic region of the target molecule hence establishing the structure of isolated molecule as Aloechrysone. In addition, the LCMS (Fig. 3) data shows the peak at M/Z =272.06 further confirming the structure of isolated molecule as Aloechrysone (Fig. 4).

DISCUSSION

The Aloechrysone was isolatedas transparent crystals. The 1D (^{1}H,^{13}C) NMR spectra obtained experimentally revealed the chemical structure to be $C_{16}H_{16}O_6$. By comparing the Aloechrysone's ^{1}H and ^{13}C NMR spectra to those found in the literature, its true identity was established (Dagne and Alemu 1991; Dagne *et al.*, 1992; Xiao *et al.*, 2014; Li, 2000; Saleen *et al.*, 1997).

These findings established the compound's identity as 3,9-dihydroxy-8-methoxy-3-methyl-2,4-dihydroanthracen-1-one.Aloechrysone's proton magnetic resonance spectra matched the published values for chemical shifts and coupling constants (Van Wyk *et al*., 1995; Garkava *et al*., 2019). Values for chemical shifts were reported in δ parts per million (ppm) relative to a TMS reference standard. Based on the analysis of MS data, the mass of Aloechrysone (3,9-dihydroxy-8-methoxy-3-methyl-2,4-dihydroanthracen-1-one) was determined. The study of molecular ions at high resolution revealed a peak for C, H at electronic mass 272.06. Both the CNMR and the MS values agreed perfectly with the structure (Fig. 4).

Pre-anthraquinones and anthraquinones are regarded as crucial chemotaxonomic indicators of Asphodelaceae plant species. The aglycon of compound 1, microcarpin, has been isolated from the genus Asphodelus, which are in the same family as Eremurus, and this is of chemotaxonomic relevance (Liliaceae). The preanthraquinone, Aloechrysone has previously been isolated from leaves of *Aloe barbadensis* (Saleen *et al*., 1997), *Aloe berhana, A. rivae* and *A. megacalantha* as oily greenish yellow pigment (Dagne and Alemu,1991), A. *berhana* (Dagne *et al*., 1992); *E. anisopterus* (Xiao *et al*., 2014) and *E. chinensis* (Li, 2000). It has been demonstrated that these compounds are usually found in the roots along with some anthraquinones (Chinchilla *et al*., 2013) which is in agreement with our results. Aleochrysone are known for their strong or mild laxative properties. They possess antioxidative, bacteriostatic, antiviral, cytotoxic and peristalsis stimulating effects. They are applied commercially as powder like laxatives (Salehi *et al*., 2018; Garkava *et al*., 2019). The presence of anthranoid derivatives is the characteristic of the genus Eremurus (Xiao *et al*., 2014).

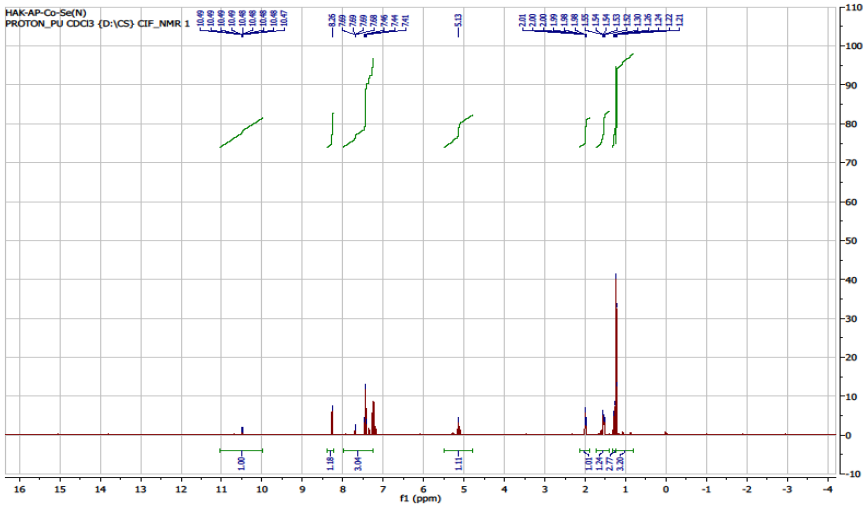

Fig. 1. [1]H NMR of isolated Aloechrysone from root of *E. himalaicus.*

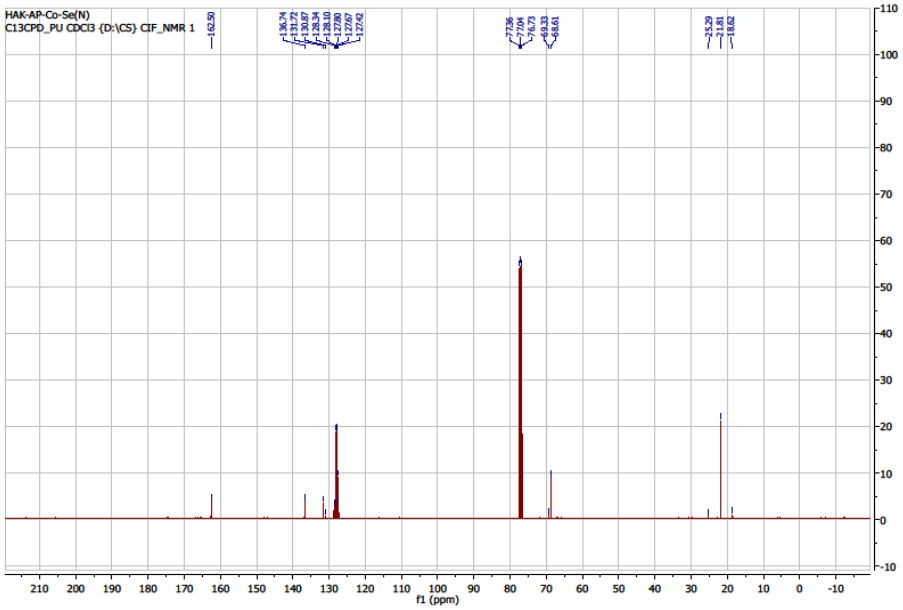

Fig. 2. [13]C NMR of isolated Aloechrysone from root of *E. himalaicus.*

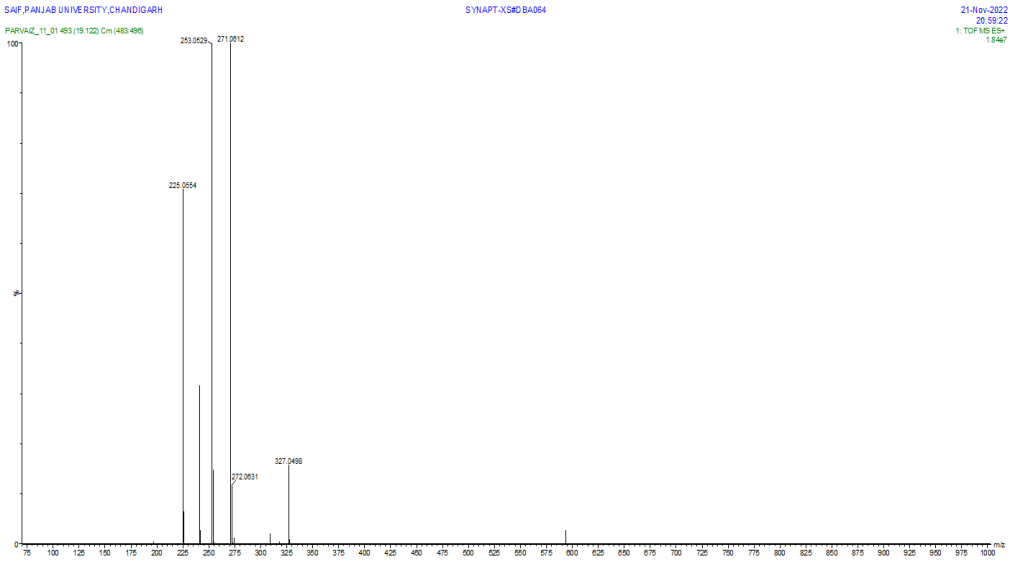

Fig. 3. M/z =272.06 of isolated Aloechrysone from root of *E.himalaicus.*

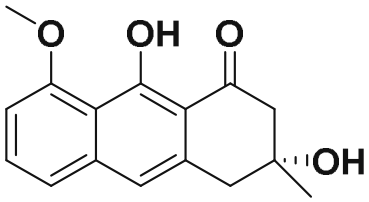

Fig. 4. Chemical Structure of isolated Aloechrysone.

CONCLUSIONS

This study is the first report on the isolation of Aloechrysone from the roots of *E. himalaicus*. Further research may be carried out for isolation of various other secondary metabolites from this under researched medicinal plant. Moreover, *in vitro* and *in vivo* studies of this compound can be carried out for establishing its pharmacological properties.

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Conflict of Interest. None.

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