مجلة الدراسات التربوية والعلمية - كلية التربية - الجامعة العراقية
العدد الخامس والعشرون - المجلد الخامس - علوم الحياة - آذار 2025 م
doi.org/10.52866/esj.2025.05.25.19

Comparative study among Alkaloids extracted from some species belongs to Onosma L. (Boraginaceae) growing in northern of Iraq

Suhaib Muhammad Jasim Al-Hijazi [1] , Mohammad A. Hashem Al-Blesh [2]
mf230089ued@st.tu.edu.iq
Dept. of Biology – College of Education for Pure Sciences , University of Tikrit , Tikrit/Iraq.

Abstract:

The study showed a clear variation in the quality of alkaloid compounds, their concentrations and percentages using the High-Perforance Liquid Chromatography technique. Eleven alkaloid compounds were identified, namely 7-epi-echimiplatine, Indicine, Echimidine, Heliotridine, Intermidine, Lycopsamine, Onosmerctine, Retronecine, Supinidine, Uplandicine, Virdinatine N-oxide, in the leaves of the species, they were distributed in different concentrations and percentages, which made the species different from each other, and clear differences appeared in the studied species.

The current study of the alkaloid content showed that the two species *O.echioides* and *O.erecta* is the species that contains the most alkaloids with seven alkaloids for each of them and they shared the presence of four alkaloids, which are 7-epi-echimiplatine, Indicine, Intermidine and Virdinatine N-oxide, and they differed in the presence of three compounds for each of them, as the species *O.echioides* contained Echimidine, Lycopsamine and Retronecine, while the species *O.erecta* contained Heliotridine, Onosmerctine and Uplandicine, and the species that contained the least alkaloids was the species *O.stellulata*, which contained four alkaloids, which are Heliotridine, Lycopsamine, Retronecine and Supinidine, and the highest concentration among the compounds was for the alkaloid Intermidine, which reached 86.52 µg/ml in the species *O.erecta*, and the lowest concentration among the species was for the compound Indicine in the species *O.mutabilis*, which reached 11.60 µg/ml , while The compound Intermidine recorded the highest percentage, reaching 38.20%, in the species *O. tricerosperma*, and the lowest percentage was recorded by the species *O. shehbazii* for the compound Indicine, reaching 5.99%, compared to the rest of the studied species, indicating the importance of these compounds in separating these species from the rest of the studied species.

Keywords: Chemical study, Alkaloids , Boraginaceae .

دراسة مقارنة للقلويدات المستخلصة من بعض الأنواع التابعة للجنس *Onosma* L. (Boraginaceae) النامية في شمال العراق

صهيب محمد جاسم الحجازي , محمد عدنان هاشم ال ابليش

مستخلص:

أظهرت الدراسة الحالية تبايناً واضحاً في نوعية المركبات القلويدية وتراكيزها ونسبها المئوية إذ تم تشخيص أحد عشر مركباً في أوراق الأنواع المدروسة وهي Heliotridine ,Echimidine ,Indicine ,epi-echimiplatine Viridinatin , Uplandicine , Supinidine , Retronecine , Onosmoerctine , Lycopsamine , Intermidine , N-Oxide توزعت بتراكيز ونسب مختلفة جعلت الأنواع متباينة فيما بينها وظهرت فروقات واضحة في الأنواع المدروسة وبلغ أعلى تركيز للقلويد Intermidine حيث بلغ 86.52 مايكروغرام / مللتر في النوع O.erecta بينما أقل تركيز بلغ 11.60 للمركب epi-echimiplatine في النوع *O.mutabilis* أما من حيث النسبة المئوية بلغت أعلى نسبة مئوية 38.20 ٪ للمركب Lycopsamine في النوع *O.tricerosperma* أما أقل نسبة مئوية كانت للمركب Indicine في النوع *O.shehbazii* وبلغت 5.99٪ .

الكلمات المفتاحية : دراسة كيميائية ، القلويدات ، عائلة لسان الثور .

Introduction

The borage family Boraginaceae comprises 154 genera and 2,500 species distributed in different regions of the world (Boulus,2000), this family is globally widespread and widely distributed in tropical, subtropical and temperate regions and is found in the Mediterranean, Mediterranean, American and northern regions (Al- Shehbaz, 1991) and (APG,2016) The borage family Boraginaceae belongs to the order Lamiales(Conquist ,1981) and is no longer the same as before, as it has been divided into several families, although some of its genera have been adopted to the level of a family as in the case of the genus *Heliotropium* to Heliotropaceae and has medicinal importance and some of its plants are used in folk medicine (Abolhassani,2010) and the Boraginaceae family is one of the families around which wide controversy and taxonomic issues.

The study aimed to find out the variation in alkaloid content between the studied species in terms of identifying the compounds, their numbers and concentrations to separate between species using HPLC technology. It is important to separate between species on the basis of the ratio and concentration and current study aimed at the most important current and previous studies and trying to solve and the issues and controversies about the origin of this family because it is one of the least studied families in Iraq and there are few studies such as the study of (Abbas,1990) for the genus Heliotropium, the study of (Al-Mashhadani ,1991) for the genus *Onosma*, (Al-Balesh, 2012) and (Al-Hijazi, 2021) Due to the lack of Iraqi and Arabic studies, the current study aimed to identify the chemical aspect by diagnosing some compounds of secondary metabolism such as phenols, alkaloids, glycosides and flavonoids using HPLC technique and to know their concentrations and percentages after qualitative detection.

Material and methods

Chemical Study

The study was conducted on ten species belonging to the genus *Onosma*, namely *O.alborosea, O.arenaria, O.echioides, O.sinicum, O.shehbazii, O.erecta, O.tricerosperma, O.dichroantha, O.stellulata and O.mutabilis.*

Detection of Alkaloids:

To conduct this detection, the following method was used:

Marquis's test

This reagent was prepared by mixing 1 ml of formaldehyde with 10 ml of concentrated sulfuric acid. It was used to detect the presence of alkaloids. It was observed that the presence of alkaloids caused turbidity when the reagent was added to the plant extract (Harbone, 1984).

Alkaloids extraction method:

The alkaloids were isolated from pre-dried plants at room temperature in powder form, and (1) gram of leaf powder was taken for the species under study. Extraction was carried out using 50 ml of hexane, which removes fats, oils, terpenes, waxes, etc. The residue was filtered and the remaining material in alcohol was extracted using 20 ml of ethanol and the extract was evaporated to obtain 1 ml containing a mixture of crude alkaloids, and the alkaloids were isolated using a Shimadzu LC-HPLC high-performance liquid chromatograph 10A with a double pump, and the resulting sample volume was 20 ml using a rapid liquid chromatography column with a 3 µm column with a Reodyne 7125 injector with a 20 ml injection loop, which separates tropane-containing alkaloids (atropine, cocaine, scopalmine and hyoscyamine), but species belonging to the genus *Onosma* contained pyrrolizidine alkaloids that are extracted using acidified ethanol (Jone *et al*., 1995), 1995).

separation condition :

The alkaloids were separated from the mixture by reversed-phase chromatography using a C-18 separation column (50 x 2.6 radius, 3 micrometre particle size). The mobile phase consisted of 80:20 ml of distilled water and 70% ethanol, respectively, the flow rate was 1 ml/min at 25 °C, and the standard concentration was 50 mg/ml.

The separation peak was printed by UV detector at a wavelength of 254 nm and quantitatively analysed by comparing the areas of the samples and samples with the areas of the standard concentration under the same separation conditions. The concentration of alkaloid compounds in the species under study was measured using the following equation: Concentration of compound ml/mg = area of compound bundles in the sample / area of compound bundles in

the standard x standard concentration x number of dilutions, while the percentage was measured using the following equation: Percentage of compound = partial concentration / total concentration x 100.Table 1 , Figure 1 .

Table 1: Alkaloid compounds in standard analysis, retention time, area and concentration.

Compounds	Retention time	Area	Concentration
7-epi-echimiplatine	4.87	4512	8.7594
Indicine	6.32	6301	12.5007
Echimidine	8.56	4863	9.91
Heliotridine	9.19	3945	7.6686
Intermidine	10.27	3623	7.0625
Lycopsamine	12.47	7507	14.8094
Onosmerctine	17.83	5332	10.4438
Retronecine	18.76	3208	6.2007
Supinidine	19.22	2363	4.7459
Uplandicine	20.56	5172	10.322
Virdinatine N-oxide	21.085	3749	7.577
Total		47367	100

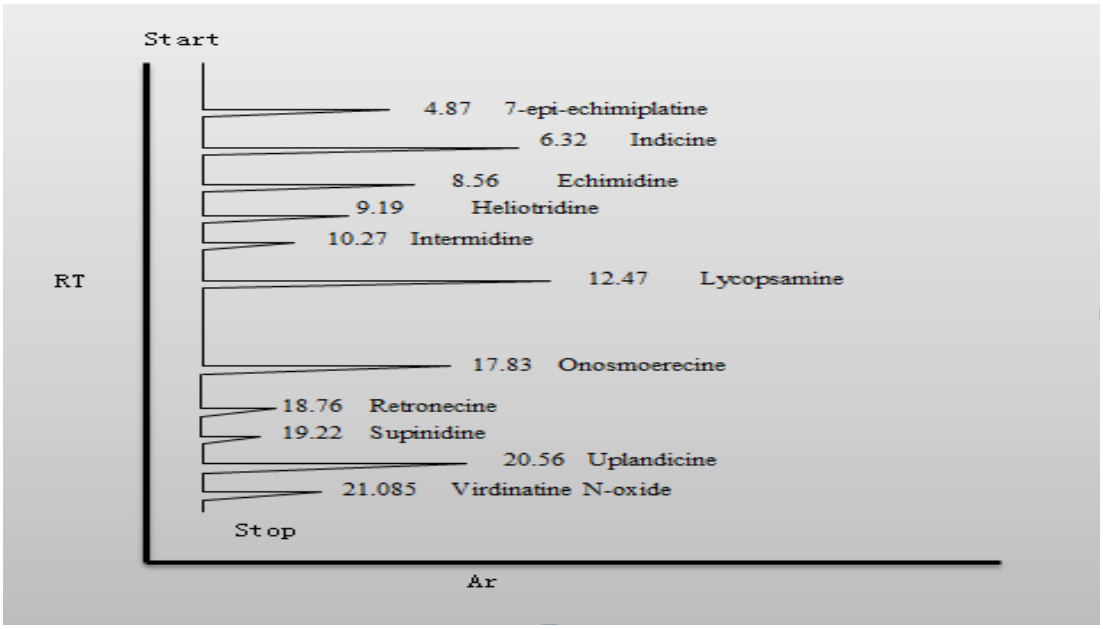

Figure 1: Alkaloid compounds of the species under study and retention time in standard analysis.

Results & Discussion

Chemical Study

Alkaloid Compound Study

The current study showed a clear variation in the quality of alkaloid compounds, their concentrations and percentages using the High-Perforance Liquid Chromatography technique, as eleven compounds were identified in the leaves of the studied species, distributed in different concentrations and percentages, which made the species different from each other, and clear differences appeared in the studied species. The current study of the alkaloid content of the studied species showed that the highest concentration of the compound 7-epi-echimiplatine was in the species *O. erecta* and reached 43.797 µg/ml, while the lowest concentration was in the species *O. mutabilis* and reached 11.60 µg/ml, while the alkaloid Indicine had its highest concentration in the species *O. dichroantha* and reached 35.59 *micrograms / milliliter*, while the lowest concentration was 12.24 µg/ml in the species *O. shehbazii*. It was also noted that the highest concentration of the alkaloid Echimidine was in the species *O.echioides* and reached 63.12 µg/ml, while the lowest concentration was in the species *O.shehbazii* and reached 19.20 µg/ml. As for the alkaloid Heliotridine, its highest concentration was in the species *O.erecta* and reached 45.92 µg/ml, while its lowest concentration was 24.27 µg/ml in the species *O.triceros-perma*. As for the alkaloid Intermidine, its highest concentration was noted in the species *O.erecta* and *O.triceros-perma*, reaching 86.52 - 85.24 µg/ml, respectively, while the lowest concentration of the alkaloid was 30.16 µg/ml in the species *O.dichroantha*.

The current study showed that the species *O. stellulata* had the highest concentration of the alkaloid Lycopsamine, which reached 36.77 µg/ml, while the lowest concentration was recorded by the species *O. echioides*, which reached 16.85 µg/ml. As for the alkaloid Onosmoerctine, the highest concentration was found in the species *O. mutabilis*, which reached 58.96 µg/ml, and the lowest concentration of the alkaloid, which reached 18.35 micrograms/ml, was in the species *O. tricerosperma*. The current study of *Onosma* species showed that the species *O.tricerosperma* had the highest

concentration of Retronecine alkaloid, reaching 32.47 µg/ml, while the lowest concentration of the alkaloid was recorded by *O.stellulata*, reaching 12.80 µg/ml. The current study of *Onosma* species showed that the highest concentration of Supinidine alkaloid reached 56.717 µg/ml in *O.arenaria*, while the lowest concentration of the alkaloid appeared in *O.sinicum* and *O.mutabilis*, reaching 14.63-15.55 µg/ml, respectively. As for the highest concentration of Uplandicine alkaloid, it was found in *O.arenaria* and *O.shehbazii*, ranging from 53.89-59.43 µg/ml, respectively, and the lowest concentration was 18.92 µg/ml in The species *O.mutabilis* and *O.erecta* recorded the highest concentration of the alkaloid Viridinatin N-Oxide, which reached 65.60 µg/ml, while the lowest concenstration of the alkaloid reached 18.84 µg/ml in the species *O.sinicum*

The current study of the alkaloid content showed a variation in the percentage of the studied species belonging to the genus *Onosma*, as the highest percentage of the alkaloid 7-epi-echimiplatine appeared in the species *O.sinicum* and reached 21.88%, while the lowest percentage of the alkaloid appeared in *O.mutabilis*, *O.dichroantha* and reached 6.14% - 8.90%. It was also noted that the highest percentage of Indicine was in the species *O.dichroantha* and reached 19.83%, while the lowest percentage of the alkaloid appeared in the species *O.erecta, O.shehbazii* and reached 6.62% and 5.99% respectively. The alkaloid Echimidine recorded the highest percentage in the species *O.echioides*, *O.dichroantha* and reached 26.32% - 30.25% respectively, while the lowest percentage of the alkaloid was recorded by the species *O.shehbazii* and reached 9.40%. The study showed that the alkaloid Heliotridine had the highest percentage of 24.24% in the species *O.stellulata* and the lowest percentage of Heliotridine alkaloid was 9.91% in the species *O.arenaria*, while the highest percentage of the alkaloid Intermidine was in the species O.triceros-perma and reached 38.20% while the lowest percentage was in the species *O.dichroantha* and reached 16.81%. It was noted that the species *O.stellulata* contained the highest percentage of the alkaloid Lycopsamine 30.38% while the lowest percentage of the alkaloid was 7% in the species *O.echioides*. It

was noted that the species *O.mutabilis* contained the highest percentage of the alkaloid Onosmerctine and reached 31.22% while the lowest percentage of the alkaloid was 7.71% in the species. *O.erecta*

The current study of the studied species belonging to the genus *Onosma* showed that the two species *O.mutabilis*, *O.tricerosperma* contained the highest percentage of the alkaloid Retronecine, and the percentages reached (16.59%, 14.55%) respectively, while the lowest percentage reached 10.57% and 7.41% respectively in the species *O.stellulata , O.echioides.* The study showed that the alkaloid Supinidine had the highest percentage in the species *O.stellulata* and reached 34.8%, while the lowest percentage reached 8.23% in the species *O.mutabilis*. As for the alkaloid Uplandicine, its highest percentage was in the species *O.sinicum* and reached 31.43%, while the lowest percentage of the alkaloid reached 8.47% in the species *O.alborosea*, and the highest percentage of the alkaloid Viridnatine N-Oxide 20.31% in *O. erecta* while the lowest percentage was in *O. sinicum* and reached 15.85., Table 3.

Table 3: Alkaloids in *Onosma* species, their concentrations and percentages

Compounds / Species	7-epi-echimi-platine		Indicine		Echimidine		Heliotridine		Intermidine		Lycopsamine		Onos-merctine		Retronecine		Supinidine		Uplandicine		Virdinatine N-oxide	
	%	Con	%	Con	%	Con	%	Con	%	Con	%	Con	%	Con	%	Con	%	Con	%	Con	%	Con
O.alborosea	-	-	-	-	16.72	40	14.47	34.62	34.38	82.26	13.86	33.18	12	28.90	-	-	-	-	8.47	20.27	-	-
O.arenaria	-	-	-	-	-	-	9.91	26	24.41	64	-	-	12.88	33.79	10.58	27.76	21.63	56.717	20.55	53.89	-	-
O.dichroantha	8.90	15.97	19.83	35.59	30.25	54.28	-	-	16.81	30.16	-	-	-	-	-	-	24.19	43.40	-	-	-	-
O.echioides	11.45	27.47	10.35	24.83	26.32	63.12	-	-	24	57.60	7	16.85	-	-	7.41	17.79	-	-	-	-	13.40	32.14
O.erecta	13.56	43.797	6.62	21.38	-	-	14.22	45.92	26.79	86.52	-	-	7.71	24.90	-	-	-	-	10.77	34.78	20.31	65.60
O.mutabilis	6.14	11.60	-	-	-	-	-	-	27.78	52.47	-	-	31.22	58.96	16.59	31.34	8.23	15.55	10	18.92	-	-
O.shehbazii	-	-	5.99	12.24	9.40	19.20	-	-	26.81	54.74	11.54	23.56	-	-	-	-	-	-	29.11	59.43	17.12	34.95
O.sinicum	21.88	26	-	-	-	-	-	-	-	-	18.5	22	-	-	-	-	12.31	14.63	31.43	37.35	15.85	18.84
O.stellulata	-	-	-	-	-	-	24.24	29.34	-	-	30.38	36.77	-	-	10.57	12.80	34.8	42.13	-	-	-	-
O.triceros-perma	-	-	-	-	-	-	10.87	24.27	38.20	85.24	-	-	8.22	18.35	14.55	32.47	11.20	25	16.95	37.82	-	-

indicates absence of the compound .(-)

The current study showed the alkaloid content of the leaves of the species belonging to the genus *Onosma*. The species differed among themselves in the number, type and concentration of alkaloid compounds and showed clear differences between the studied species. The species *O.alborosea* contained six alkaloid compounds: Echimidine, Heliotridine, Intermidine, Lycopsamine, Onosmerctine and Uplandicine. The alkaloid Intermidine appeared in the highest concentration, reaching 82.26 µg/ml, and the lowest concentration was 20.27 µg/ml in the Uplandicine species, while the highest percentage was recorded by the alkaloid Intermidine, reaching 34.38%, and the lowest percentage was 8.47 in the alkaloid Uplandicine,

The current study agreed with the study of (Ahmad *et al*(2018) because these alkaloid compounds are related to the rocky environment in which this species was found, as it often faces harsh conditions such as water scarcity, high temperatures and exposure to high levels of From ultraviolet rays, these alkaloids are alkaloids belonging to the pyrrolizidines that are part of the species' response to these conditions, as they play an important role in protecting the plant from insect pests, as they are toxic substances against microbes and fungi, and they also contribute as antioxidants under stress conditions such as drought and help reduce this damage by neutralizing free radicals or enhancing cellular defense mechanisms, as well as helping to regulate enzymatic activity by stimulating the production of anti-stress enzymes such as superoxide dismutase or glutathione peroxidase, as well as in protecting plant tissues by stimulating other defensive compounds, which enhances the plant's ability to resist environmental conditions. Figure 3 .

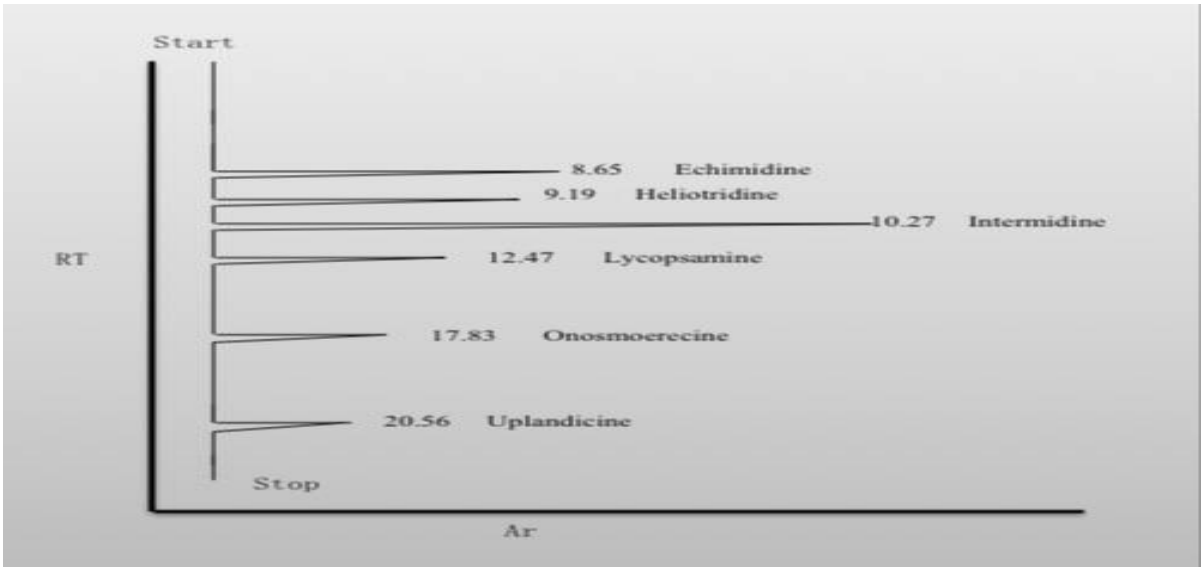

Figure 3: Chemical analysis of alkaloid compounds isolated from *O. alborosea* and retention time for each alkaloid.

The species *O.arenaria* contained six alkaloids, namely Heliotridine, Intermidine, Onosmerctine, Retronecine, Supinidine and Uplandicine. The alkaloid Intermidine showed the highest concentration, reaching 64 µg/ml, and the lowest concentration was 26 µg/ml in the compound Heliotridine, while the highest percentage appeared in the compound Intermidine, reaching 33%, and the lowest percentage was 9.91% in the compound Heliotridine. The study fully agreed with what was confirmed by the study of El-Shazly *et al* (2003) about the presence of pyrrolizidine compounds, as they are toxic and work to analyze liver cells (hepatotoxicity), stop mitosis in them, and dissolve cell membranes. They are also highly toxic to herbivores such as sheep. Among their traditional medical uses are as antibacterial, antimicrobial, and anti-insect, and anti-cancer effects. They have an important role in influencing environmental applications through their effect on the food chain when animals feed. Herbivorous plants contain pyrrolizidine alkaloids which can cause toxic effects in predators or humans who eat the meat of herbivores. F 4 .

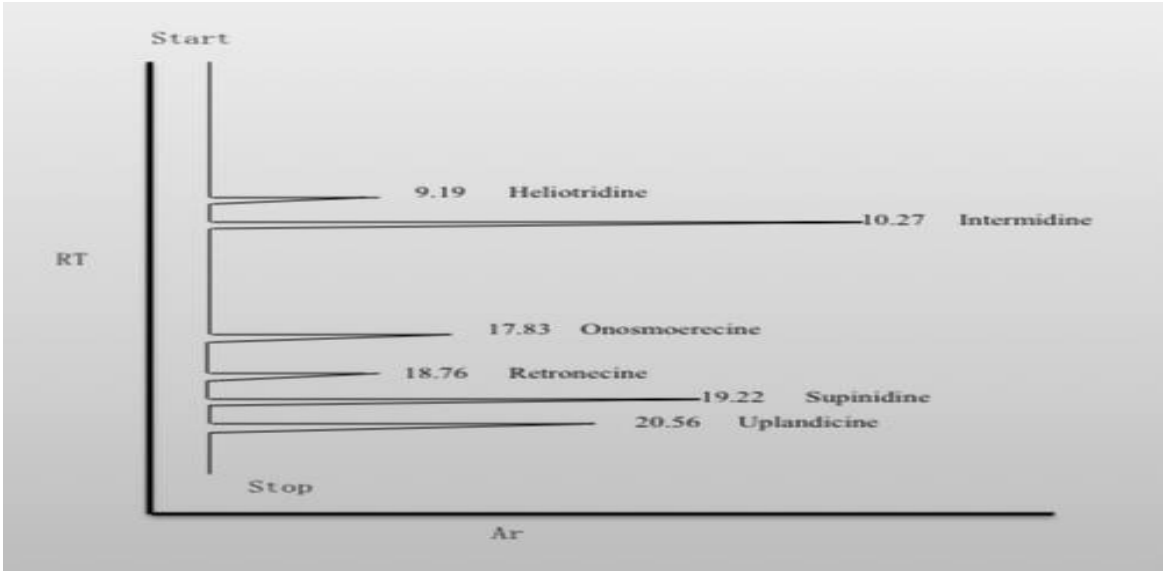

Figure 4: Chemical analysis of alkaloid compounds isolated from *O. arenaria* and retention time for each alkaloid.

The present study showed that *O. sinicum* species contains five alkaloids, namely 7-epi-echimiplatine, Lycopsamine, Supinidine, Uplandicine and Viridnatine N-Oxide. The highest concentration was 37.35 µg/ml for Uplandicine and the lowest concentration was 14.63 µg/ml for Supinidine. Uplandicine contained the highest percentage of 31.43%, while Supinidine had the lowest percentage of 12.31%, This variation in chemical composition may be related to genetic and environmental factors that control the biosynthetic pathways of these compounds, such as the availability of nutrients in the soil, temperatures, solar radiation levels, and environmental stresses that the plant faces in its natural habitat. Environmentally, these alkaloids play a role in resistance to herbivores and microorganisms, and may contribute to the plant's tolerance to environmental stresses such as drought or high temperatures by regulating metabolic and metabolic processes. Taxonomically, the variation in the proportion of alkaloids between species is an important chemical indicator used to differentiate between species within the genus *Onosma*, as this variation reflects genetic divergence and ecological adaptation specific to each species. The presence of specific compounds in high proportions in one species rather than another may be a distinctive taxonomic feature

that contributes to understanding the evolutionary relationships within the Boraginaceae family, making the study of these alkaloid compounds an important tool in the chemical classification and phylogenetic studies of plants belonging to this genus . Figure 5 .

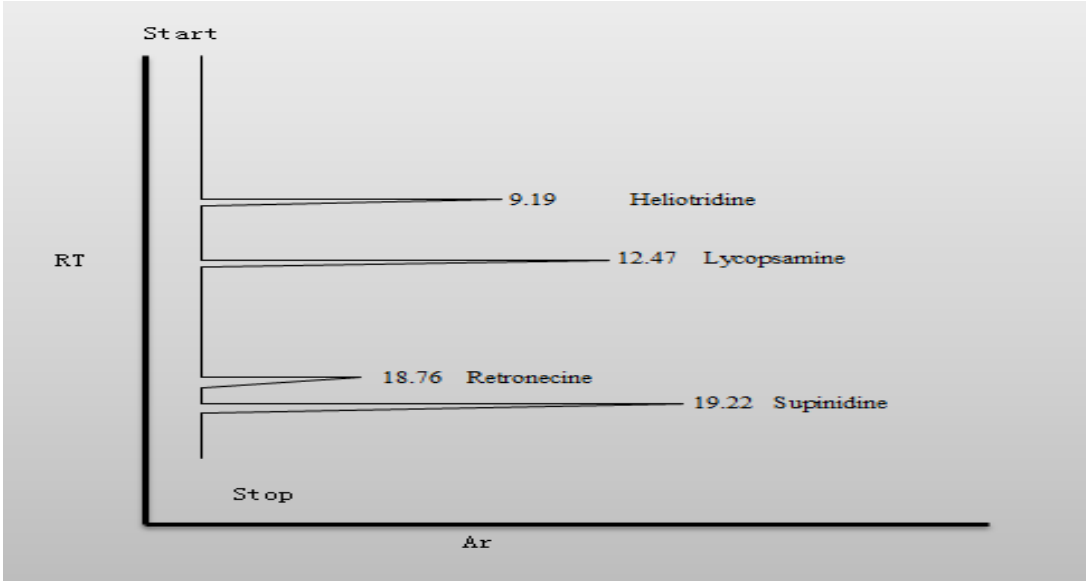

Figure 5: Chemical analysis of alkaloid compounds isolated from *O. sinicum* and retention time for each alkaloid.

The current study of the species belonging to the genus *O.dichroantha* showed that it contains five alkaloid compounds, namely 7-epi-echimiplatine, Indicine, Echimidine, Intermidine and Supinidine. The Echimidine compound showed the highest concentration among the other compounds, reaching 54.28 µg/ml, while the lowest concentration was 15.97 µg/ml, which was shown by the 7-epi-echimiplatine compound, while the highest percentage of the Echimidine compound was 30.25% and the lowest percentage was 8.90% in the 7-epi-echimiplatine compound. The current study differed from the study of (Aruna and Mamta ,2021) and the study of Safavi *et al* (2019) regarding the presence and absence of alkaloid compounds and their causes, such as genetic diversity, the surrounding environment, different levels of minerals, biological interactions, growth stages and even internal chemi-

cal diversity. Concentrations can differ within a single plant species, which is what the current study indicated. Figure 6 .

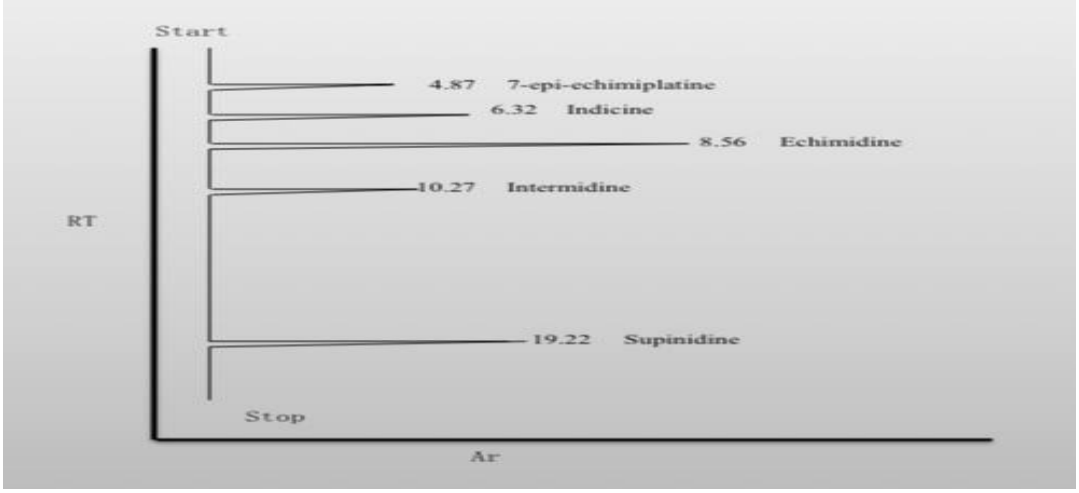

Figure 6: Chemical analysis of alkaloid compounds isolated from *O. dichroantha* and retention time for each alkaloid.

The species *O.echioides* showed seven alkaloid compounds, namely 7-epi-echimiplatine, Indicine, Echimidine, Intermidine, Lycopsamine, Retronecine and Viridnatine N-Oxide. The highest concentration reached 63.12 µg/ml in the compound Echimidine and the lowest concentration reached 16.85 µg/ml in the alkaloid Lycopsamine, while the highest percentage reached 26.32% and was recorded by the compound Echimidine and the lowest percentage was found in the compound Lycopsamine and reached 7%. The current study is consistent with the study of Nikita *et al* (2015), in which four compounds were isolated from the reproductive parts. This is an indication of the spread of alkaloids not only in the leaves of the studied species, but also in the reproductive parts, which included the study of isolating the alkaloids Intermidine, Echimidine, Indicine and Lycopsamine from the parts of the fruits, seeds and flowers, These results are consistent with the results of the study by(Nikita et al., 2015) which showed the prevalence of alkaloids in reproductive parts such as fruits, seeds and flowers, indicating that the production of alkaloids in this species is not limited to leaves only, but extends to include different parts of the plant. From a taxonomic perspective, these results

support the idea that alkaloids are not just random by-products, but express different genetic patterns between species, which can be adopted as a taxonomic tool in identifying species within the genus *Onosma*. The recurrence of the compounds Intermidine, Echimidine, Indicine, and Lycopsamine in the two studies indicates their stability as distinctive chemical characters, which enhances their importance in taxonomic distinction within the Boraginaceae family. In addition, the presence of alkaloids in reproductive parts may have an ecological role in protecting seeds from predators or in enhancing germination opportunities, which reflects the association of chemical adaptation with the taxonomic and evolutionary system of plants belonging to this genus , Figure 7 .

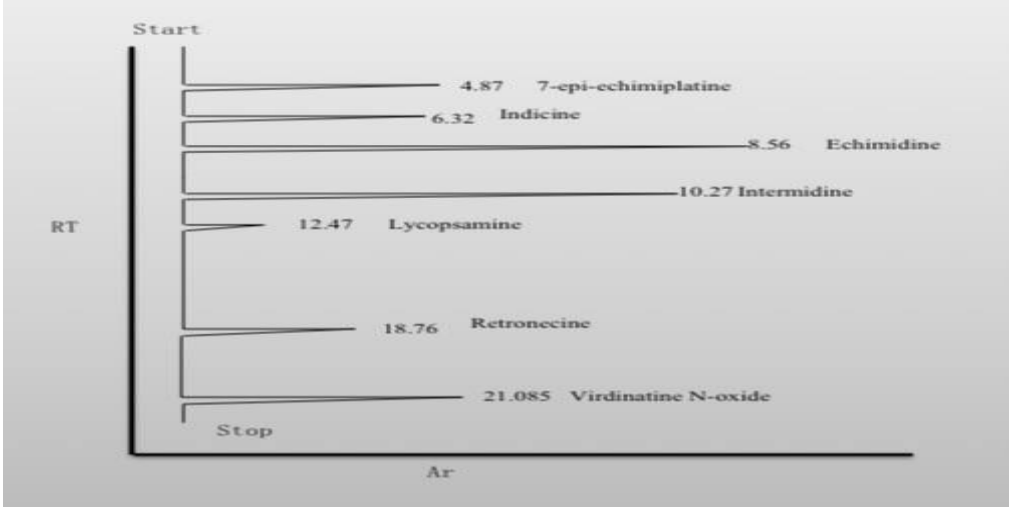

Figure 7: Chemical analysis of alkaloid compounds isolated from *O. echioides* and retention time for each alkaloid.

The present study showed that the species *O.erecta* contained seven alkaloids, namely 7-epi-echimiplatine, Indicine, Heliotridine, Intermidine, Onosmerctine, Uplandicine and Viridnatine N-Oxide. The species *O. erecta* contained the alkaloid compound Intermidine at the highest concentration, reaching µg/ml, while the lowest cone centration was 21.38 µg/ml in the compound Indicine. As for the percentage, the highest percentage was in the compound Intermidine, reaching 26.79%, while the lowest percentage was 6.6%

in the alkaloid compound Indicine. This was similar to the study of Harilaos *et al* (2013), where they isolated the pyrrolizidine alkaloids Viridnatine N-Oxide, 7-epi-echimiplatine, Onosmerctine from the leaves and fruits of the species *O. erecta* and the study of Damianakos *et al* (2013) . The results confirm the chemical diversity within the genus Onosma, as the difference in types and concentrations of alkaloids between species indicates their genetic and chemical differentiation, which can be adopted in their classification within the family Boraginaceae. The agreement with the results of Harilaos et al., 2013, who isolated the pyrrolizidine alkaloids Viridnatine N-Oxide, 7-epi-echimiplatine, and Onosmerctine from the leaves and fruits of *O. erecta*, reinforces the concept of the spread of alkaloids in different parts of the plant, indicating their chemical stability in this species. The presence of the compound Onosmerctine in both studies indicates the possibility of considering it a distinctive compound for this species, which supports the use of alkaloids as an accurate taxonomic tool in distinguishing between species within the same genus. In addition, the similarity in the composition of alkaloids between previous and current studies reflects the stability of the chemical pattern of this species, which enhances its taxonomic value and confirms its evolutionary relationships within the genus *Onosma* . Figure 8 .

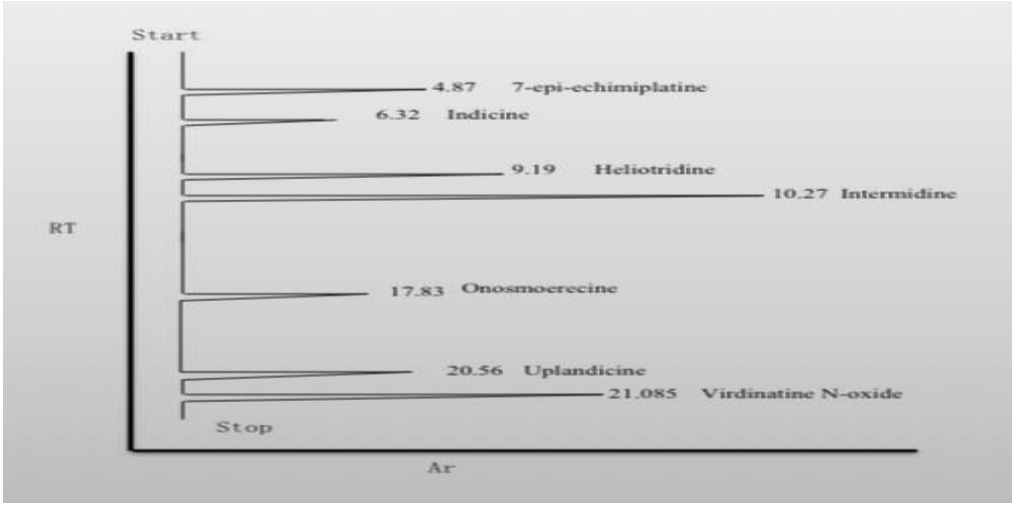

Figure 8: Chemical analysis of alkaloid compounds isolated from *O. erecta* and retention time for each alkaloid.

The study showed that the species *O.mutabilis* contains six compounds, namely 7-epi-echimiplatine, Intermidine, Onosmerctine, Retronecine Supinidine and Uplandicine. The compound Onosmerctine showed the highest concentration, reaching 58.96 micrograms / milliliter, while the lowest concentration was 11.60 µg/ml in the compound 7-epi-echimiplatine. The compound Onosmerctine showed the highest percentage, reaching 31.22%, and the lowest percentage, reaching 6.14% in the compound 7-epi-echimiplatine. The current study agreed with the study (Jabbar, 2021) on the isolation of compounds from the leaves and flowers of the species *O.mutabilis*. The discovery of compounds in *O. mutabilis* suggests their potential as taxonomic clues, especially when compared to other compounds isolated from closely related species within the same genus. The presence of compounds such as Intermidine and Uplandicine previously found in *O. alborosea* may indicate a taxonomic affinity between the two species within the genus *Onosma*, enhancing the value of chemical analysis in supporting traditional taxonomy. The variation in concentrations of the compounds detected, with Onosmerctine having the highest concentration and 7-epi-echimiplatine the lowest, may be related to ecological or evolutionary factors affecting metabolism within the same species. Confirmation of these findings by previous studies such as Jabbar (2021) enhances the reliability of chemical compounds as taxonomic clues, especially when compared to other Onosma species. Integrating chemical data with morphological and molecular studies contributes to a deeper understanding of taxonomic relationships, as alkaloids can help resolve taxonomic controversies when morphological characters are insufficient to distinguish closely related species. This study supports the importance of chemical analysis as a taxonomic support tool, as chemical compounds reflect evolutionary relationships and variation among species within the same genus, and emphasizes the need to compare chemical data with previous studies to enhance the validity of plant classification. Figure 9 .

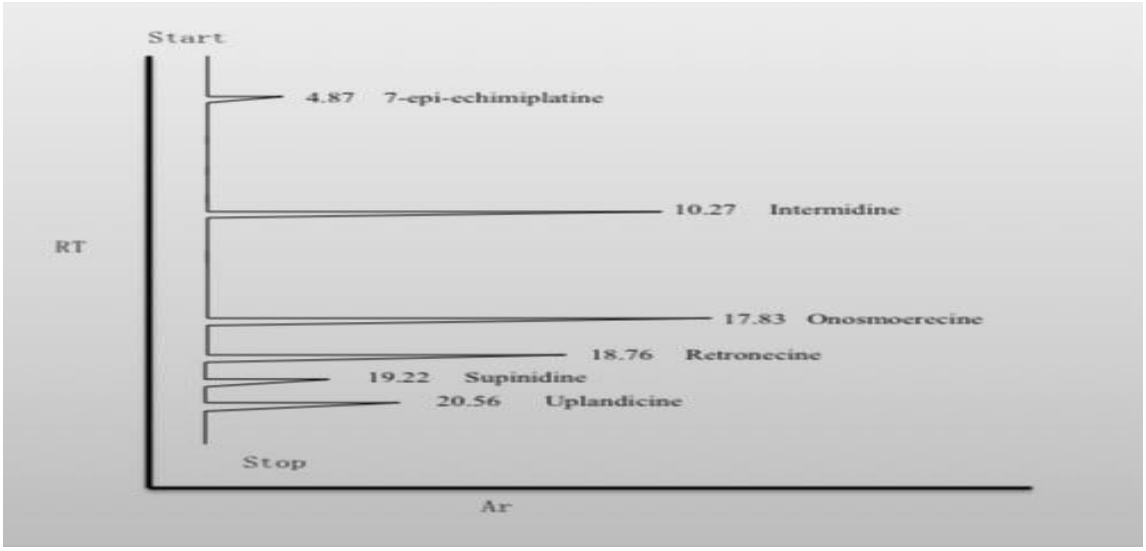

Figure 9: Chemical analysis of alkaloid compounds isolated from *O. mutabilis* and retention time for each alkaloid.

The present study showed that the leaves of *O.shehbazii* species contain six alkaloid compounds, namely Indicine, Echimiplatine, Intermidine, Lycopsamine, Uplandicine and Viridnatine N-Oxide. Uplandicine was the highest concentrated compound, reaching 59.43 µg/ml, while the lowest concentrated compound was Indicine, reaching 12.24 µg/ml. The highest percentage was recorded by Uplandicine, reaching 29.11%, while the lowest percentage was 5.99% in Indicine. This variation in the concentrations of compounds may reflect the influence of the environment on the production of secondary chemical compounds, which may be a response to specific environmental challenges such as climate and soil type. Taxonomically, this variation can be considered an indicator of support for plant classification, as chemical compounds provide additional information about the relationship between species and their environments, enhancing the chemical understanding of the taxonomic relationships between different species within the genus *Onosma*. Figure 10 .

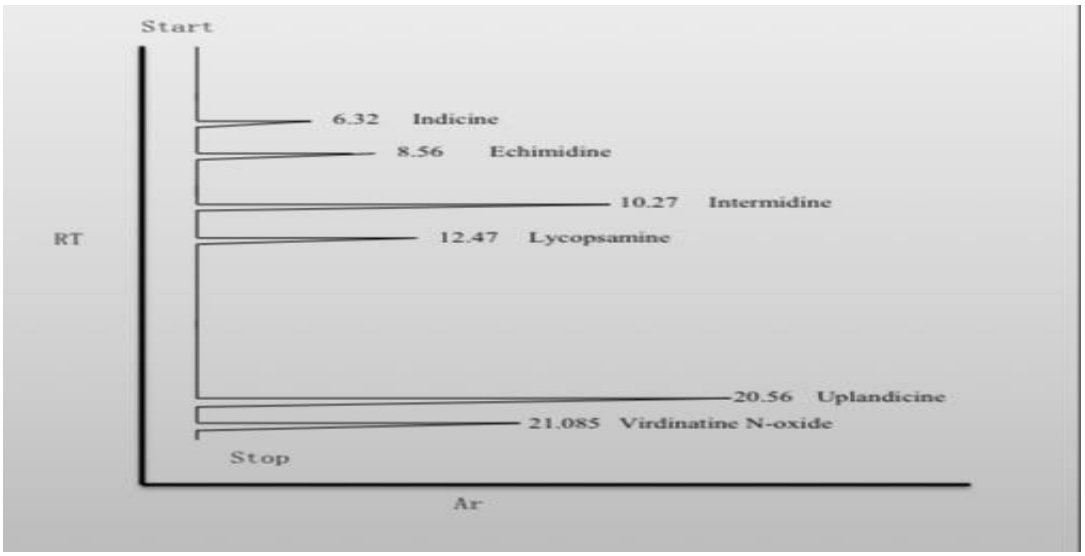

Figure 10: Chemical analysis of alkaloid compounds isolated from *O. shehbazii* and retention time for each alkaloid.

The study showed that *O.stellulata* contains four alkaloid compounds with different concentrations and percentages in the species, namely Heliotridine, Lycopsamine, Retronecine and Supinidine. The highest concentration was 42.13 µg/ml for Supinidine, while the lowest concentration was in Retronecine, which was 12.80 µg/ml. Supinidine showed the highest percentage, which was 34.8%, while the lowest percentage was 10.5% in Retronecine. The high concentration of Supinidine reflects the plant's adaptation to its environment, as the compound is associated with defense mechanisms against harsh environmental conditions or harmful organisms. Taxonomically, this variation in the concentrations of alkaloid compounds provides additional criteria for differentiating between species within the genus *Onosma*, as the chemical compounds serve as environmental indicators that help determine the relationship between species and their environmental conditions. .Figure 11 .

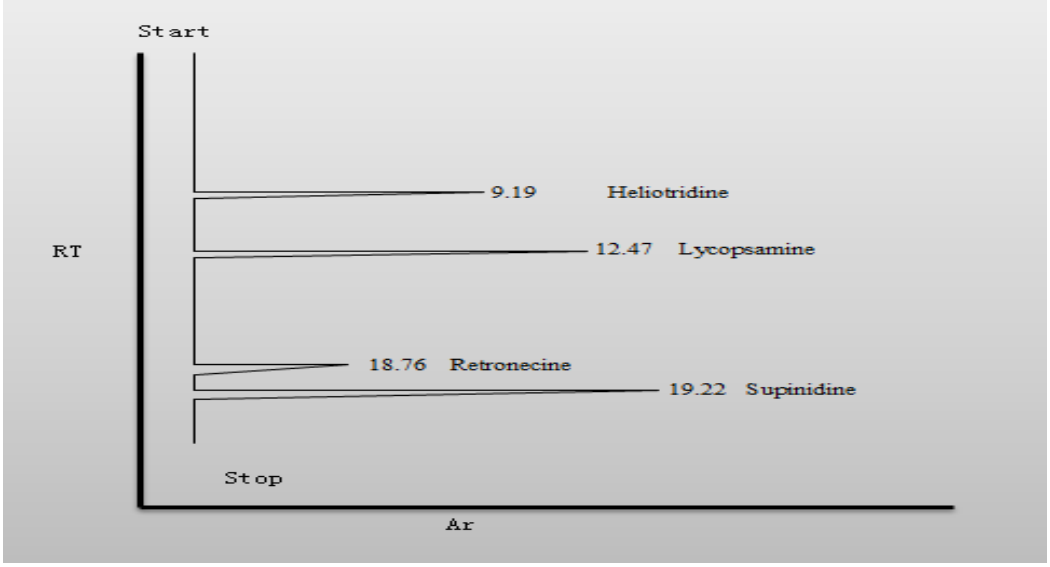

Figure 11: Chemical analysis of alkaloid compounds isolated from *O. stellulata* and retention time for each alkaloid.

The study showed the variation of alkaloid compounds in their concentrations and percentages for the species *O.tricerosperma*, which are six compounds: Heliotridine, Intermidine, Onosmerctine, Retronecine, Supinidine and Uplandicine. The highest concentration reached 85.24 µg/ml in the compound Intermidine, and the lowest concentration in the compound Onosmerctine, which reached 18.35 µg/ml. The highest percentage reached 38.20 in the compound Intermidine, and the lowest percentage was recorded by the compound Onosmerctine, which reached 8.22%. The high variability among compounds reveals differences in their chemical or biological activity. For example, the highest concentration was recorded for Intermidine, which may indicate that it is the most abundant compound in the plant and also the most biologically active. In contrast, Onosmerctine recorded the lowest concentration and lowest percentage, which may reflect a less important role in the plant or a lesser effect on organisms that may interact with it. These results open the way to study the relationship between high concentration of some alkaloids and increased therapeutic efficacy or toxicity. In addition, the variability in percentages suggests that the uneven distribution of these compounds may have different effects on plant interactions with

the surrounding environment or on the formation of active compounds that may be useful for medicinal purposes. Overall, this study provides a valuable database for understanding and evaluating the chemical properties of plants and their use in pharmaceutical applications. Figure 12 .

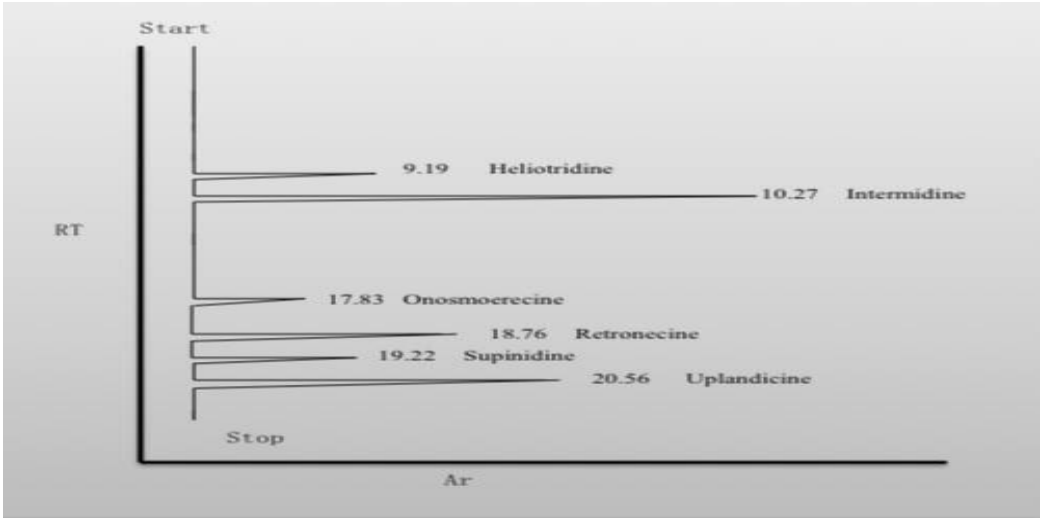

Figure 12: Chemical analysis of alkaloid compounds isolated from *O. tricerosperma* and retention time for each alkaloid.

Conclusions

Chemical study using HPLC technology is important for separating species based on ratio and concentration, and this has a distinct role in providing researchers with the nature of chemical metabolites that contribute to many important biological activities.

Despite the great diversity in the characteristics that a single species possesses, there are still characteristics that distinguish the species from the rest of the species and separate it completely, especially if the characteristic is stable in the face of environmental conditions. Therefore, one relies on chemical characteristics and HPLC technology.

Species within the same genus can be separated based on chemical characteristics.

Recommendations

Studying the possibility of using chemical compounds and their biological effectiveness found in species and their entry into the pharmaceutical industry as natural products effective

against microscopic pathogens and studying the effect of plant extracts in treating diseases. Use of GC-MAS technology to detect active compounds.

References

- Abbas, Yass Khadir (1990). A taxonomic study of the genus *Heliotropium* L. in Iraq. PhD thesis. College of Science. University of Baghdad

Abolhassani,M.(2010). Antivirial activity of borage (*Echium amoenum*). Arch med Sci :366 pp.

- Ahmad L, He Y, Semotiuk AJ, Liu QR, Hao JC. (2018). Survey of pyrrolizidine alkaloids in the tribe Lithospermeae (Boraginaceae) from Pan-Himalaya and their chemotaxonomic significance. Biochem Syst Ecol.;81:49-57. DOI: 10.1016/j.bse.2018.09.002

- Al-blesh, Mohammed Adnan Hashem (2012). A comparative taxonomic study of the species of the genus *Heliotropium* L. (Boraginaceae) in central and northern Iraq. PhD thesis. Tikrit University, College of Education.

- Al-Hijazi, Suhaib Muhammad Jasim (2021). Morphological, anatomical, chemical and pollen characteristics of some species of the Boraginaceae family growing in Salah al-Din Governorate. Tikrit University, College of Education for Pure Sciences.

- Al-Mashhadani, Adhiya Nahi Salman (1991). A comparative taxonomic study of the species of the genus *Onsoma* L. (Boraginaceae). PhD thesis. University of Baghdad, Ibn Al-Haytham College of Education: 253 pages

- Al-Shehbaz, I.A. (1991). The genera of Boraginaceae, the southeastern United States. Journal of the Arnold Arboretum 1 (Suppl. 1): 1–69.

- APG IV. (2016). An update of the Angiosperm Phylogeny Group classification for the orders and families of flowering plants: APG IV. Botanical Journal of the Linnean Society 181: 1–20.

- Aruna M. Rajapara, Mamta B. Shah (2021), THE GENUS *ONOSMA* L.: A COMPREHENSIVE REVIEW, 1Department of Pharmacognosy, L. M. College of Pharmacy, Ahmedabad, Gujarat, India.

- El-shazly A, Ghani AA, Wink M.(2003). Pyrrolizidine alkaloids from Onosma arenaria (Boraginaceae). Biochem Syst Ecol;31:477-85.

- Harilaos Damianakos, Georgios Sotiroudis, loanna Chinou.(2013). Pyrrolizidine Alkaloids from Onosma erecta, Journal of Natural Products, Cite this: J. Nat. Prod. 2013, 76, 10, 1829-1835

- Jabbar AA.(2021) *Onosma mutabilis*: phytochemical composition, antioxidant, cytotoxicity, and acute oral toxicity. Food Sci Nutr.;9:5755-64

-.**Jone , A.(1995).**Determintation and identification of Chemical Compounds by HPLC..A.J.B. 30(1): 15-20.

- Nikita G, Vivek P, Chhaya G. (2015) Wound-healing activity of an oligomer ofAlkaloids, isolated from Leaves parts of Onosma echioides. Nat Prod Res.;29:1584-8.

- Safavi F, Farimani MM, Golalipour M, Leung PC, Lau KM, Kwok HF, (2019),Investigations on the wound healing properties of *Onosma dichroantha* Boiss root extracts. S Afr J Bot.;125:344 52.

- Sagratini G, Cristalli G, Giardinà D, Gioventù G, Maggi F, Ricciutelli M, (2008). Flavonoids , Phenols ana Alkaloids mixture from roots and Flowers of *Onosma echioides* (L.) L.: extraction method study and quantification. J Sep Sci;31:945-52.

- Shoaib, A.; Siddiqui, H.H.; Badruddeen; Dixit, R.K. (2020). Evaluation of Noxious Consequence of Bark Extract of *Onosma echioides* Linn Root: Hematology, Biochemistry, and Histopathological Findings. J. Diet. Suppl., 17, 110–119.