MORPHOLOGICAL AND CHEMICAL CHARACTERISTICS OF TWO SPECIES BELONG TO ALYSSEAE AND LEPIDIEAE TRIBES SPREAD IN NORTHERN IRAQ

N. M. Al-Abide
Assist. Prof.
Depart. of Biology, Coll. of Edu. for Pure Sci., University of Tikrit, Salahaddin, Iraq.
naglaa.mustafa@tu.edu.iq

ABSTRACT

This research was aimed to study three species which are prevalent in northern Iraq: *Alyssium strigosum* Banks and Sol., *Clypeola jonthlaspi* L, and *Isatis tinctoria* L. belonging to the *Alysseae* and *Lepidieae* tribes. The general characteristics of the roots, stems, leaves, fruits and seeds are studied and it turned out that and the two species *A. strigosum* and *C. jonthlaspi* are similar due to their belonging to the Alysseae tribe, and the species *I. tinctoria* differs since it belongs to the Lepidieae tribe. In addition, 6 secondary metabolites are diagnosed using the qualitative tests: alkalis, phenols, tannins, flavonoids, glycosides, and sapindales. The presence of terpenoids was not observed, and the alcohol extract is superior to the aqueous extract regarding the accuracy of the results. The phenols are detected using HPLC technology and four compounds are found: Rutin, Quercetin, Kaempferol and P-Coumarin. The importance of studying the chemical content comes from its use in subsequent studies and knowledge of its uses in the medical fields.

Keywords: Brassicaceae, phenolic compounds, alysseae, lepidieae.

مجلة العلوم الزراعية العراقية -2022 :53(4):911-921 العبيد

الخصائص المظهرية والكيميائية لأنواع من عشيرتي Alysseae وLepidieae المنتشرة في شمال العراق

نجلاء مصطفى العبيد

استاذ مساعد

قسم علوم الحياة ، كلية التربية للعلوم الصرفة ،جامعة تكريت ، صلاح الدين ، العراق

المستخلص

يهدف البحث لتحديد وتشخيص الأنواع المختلفة في الحقل مباشرةً ودعمها بالخصائص النوعية للمركبات الكيميائية المتواجدة في الانواع النباتية، ودرست ثلاثة انواع هي: *Alyssium strigosum* Banks and Sol. و *Clypeola jonthlaspi* L و *Isatis tinctoria* L . التي تنتمي لعشيرتي Alysseae و Lepidieae. ودرست الخصائص العامة للجذور والسيقان والأوراق والثمار والبذور وتبين تشابه النوعين *A. strigosum* و *C. jonthlaspi* وذلك لانتمائهم إلى عشيرة Alysseae و اختلف النوع *I. tinctoria* لكونه ينتمي الى عشيرة Lepidieae . كما تم تشخيص 6 من مركبات الأيض الثانوية بالاستعانة بالاختبارات النوعية وهي: القلويات، الفينولات، التانينات، الفلافونويدات، الكلايكوسيدات، الصابونيات، ولم يلاحظ وجود التربينات وتفوق المستخلص الكحولي على المستخلص المائي بدقة النتائج . بينما تم الكشف عن الفينولات باستخدام تقنية HPLC وتبين وجود أربعة مركبات: روتين وكويرستين وكايمبفيرول و.P–coumarin واشارت الدراسة لاختلاف تراكيز هذه المركبات والنسبة المئوية للأنواع التي درست، على الرغم من أنها جمعت من مقاطعة واحدة، تأتي أهمية دراسة المحتوى الكيميائي لاستخدامها في الدراسات اللاحقة ومعرفة استخداماته في المجالات الطبية.

الكلمات المفتاحية : العائلة الكرنبية، مركبات فنوليه، نباتات برية، دراسة مظهرية.

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INTRODUCTION

The mustard family (Brassicaceae or Crucifera) is considered as the fourth largest flower family. It is easily diagnosed based on the actinomorphic flowers, the Cruciform corolla, and the type of fruit whether Silicula or Silique (7,9) . The Brassicaceae includes about 338 -380 genera and 3000-4060 species, which are spread globally in the temperate regions of the northern hemisphere and are found worldwide(6,11,16). In Iraq, this family includes 5 tribes, 80 genera, and 177 species (10,20,39). In the Turkish Botanical Encyclopedia, it has 91 genera, 555 species, 51 subspecies, 22 varieties and 621 orders (13,14,17). Also, there are 120 genera and 358 species in Iran (22). The significance of the Brassicaceae is that it is one of the 10 most important families in economic terms, and includes many economic crops (10). There are many studies focus on the exact morphological traits, including Orcan and Binzet (28). study of *Alyssum obtusifolium* species (21). Also studied the exact morphological properties of *Clypeola* L. genus pollen in Iran, (33) studied the morphological and anatomical features of stems and leaves for the *Ricotia L.* genus grown in Turkey. Whereas Abbasian & Keshavarzi (1) studied the morphological and exact morphological traits of the *Clypeola* genus in Iran. Al-abide and Al-Shamary(4) study the indumentum ,crystals and stomata in 14 species from the tribe Brassiceae for the Brassicaceae in Iraq, as well as Al-abide (7) studied the morphological properties of four species of the Lepidieae tribe: *Aethionema cordifolium* DC., *Biscutella ciliate* L., *Thlaspi perfoliatum* L., and *Calepina irregularis* Asso. that spread in Iraq, specifically in Erbil Governorate. In addition, a taxonomic, morphological and anatomical study has been conducted on the reproductive parts (fruits and seeds) for different species of the Brassicaceae in Iraq by (5). There are also few chemical studies of some different types, including the study by Al-abide *et al*. (8)and the study of Obeid and Jaber (27) of *Pelargonium graveolens* species. which focuses on discovering the effective chemical compounds of some species of the Brassicaceae, its biological efficacy and the effect on the growth of some Candida fungi. Also, a study by Saeed (31) of four species related to the *Arabis* L. genus and the diagnosis of some phenolic compounds. The current research aims to collect the largest amount of information concerning the morphological differences and classification of the studied species using chemical evidence in order to facilitate their field diagnosis in addition to knowing and diagnosing the effective chemical compounds of wild plants and the extent of their future use as an alternative to the manufactured chemical compounds.

MATERIALS AND METHODS

Specimens' collection

The plant samples of the Brassicaceae are collected through several field trips to northern Iraq, specifically in the mountainous areas of Erbil Governorate, in the spring season for the three years between 2018-2020. The collected samples are diagnosed based on the available botanical encyclopedias (Iraqi, Turkish, Iranian and Saudi Arabian (13,23,37) and compared with the dry samples deposited in the Iraqi National Herbarium at Abu Ghraib. The morphologically fresh samples are studied and differences are determined for the fresh parts represented by roots, stems and leaves using the scaled ruler. The reproductive parts (flowers, fruits and seeds) are also studied under an anatomical microscope and by using the scaled ophthalmic lens under the powers 4x, 10x and 40x, measurements are taken and tabulated in tables and photographed using the Nikon camera using the method of Al-abide(7).

Preparation of aqueous and alcoholic extracts: The hot aqueous extract is prepared by mixing 5 gm of plant sample powder (Aerial parts) with 25ml of hot distilled water, put into the vibrator for 10 minutes, then left for 24 hours.(30)The mixture is filtered through Whatman No.1 filter paper, and the filtrate is collected and put in opaque bottles and kept in the refrigerator until the qualitative (inferential) checks are made. The same previous method is adopted and the water is replaced with ethyl alcohol with a concentration of only 96%, after which several inductive tests are performed to detect the presence of alkaloids using Mayer's and Wagner's test, phenols, flavonoids, glycosides,

sapindale, terpenoids and tannins by following the method mentioned in (2, 30, 38).

Extraction

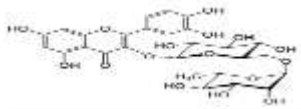
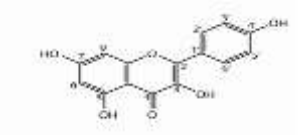
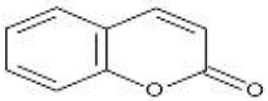
10 gm of plant powder were dissolved in 200 ml hexane to remove fat, resin, then 200 ml of 80:20 (methanol: water), The extract was subjected to ultra-sonication (Branson sonifier, USA) at 60% duty cycle for 25 min at 25C, followed by centrifugation at 7,500 rpm for 15 min . The clear supernatant of each sample was decantation and filtered through filter paper Watman no 1, then the aqueous extraction was evaporated under vacuum (Buchi Rowas evaporated Re type). Dried samples were re-suspended in 1.0ml HPLC grade methanol by vortexing, the mixture was passed through 2.5 mμ disposable filter, and stored at 4℃ for further analysis, then 20 µl of the sample injected into the HPLC system according to the optimum condition.(24 , 36).

Separation condition

The main compound was separated on FLC (Fast Liquid Chromatographic) column under the optimum condition . Column: phenomenex C-18,3µm particle size (50 X 2.0 mm I.D),Column Mobile phase:linear gradient of solvent A0.1% formic acid, gradient program form 0% B to 100% B for 10 minutes .Flow rate 1.2ml\ min . Detection :UV280 nm. The separation occurred on liquid chromatography Shimadzu 10AV-LC equipped with binary delivery pump model LC-10A Shimadzu, the eluted peaks were monitored by UV-Vis 10A-SPD spectrophotometer. Chemical compounds in the species under study were estimated based on (35). As shown in (Table 1) and (Figure 1) Chemical structure and Retention time of standard Rutin, Quercetin, Kaempferol and P-coumarin. The concentration of the chemical compounds and the percentage of the ratio were calculated based on the equation below .

Concentration of sample mg\ml = $\frac{\text{area of sample}}{\text{Area of standard}}$ **× conc. of standard × dilution factor**

Table 1. Retention time, area, and structural formula for standard phenolic compounds using HPLC technology

seq	subjects	Retention time min	Area µ volt	structural formula	concentration
1	Rutin	2.10	271358		25 mg\ ml for all
2	Quercetin	3.45	233447		
3	Kaempferol	4.19	207707		
4	p-coumarin	5.09	196874		

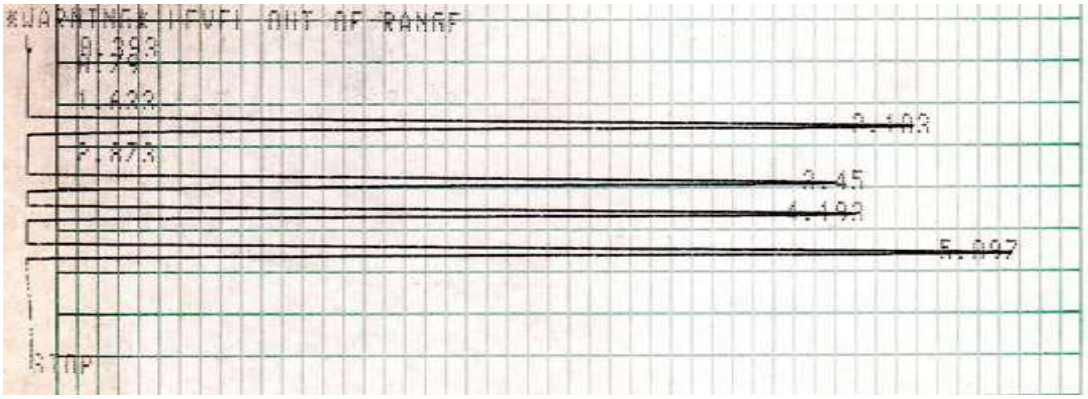


Figure 2.Area and retention time of standard phenolic compounds using HPLC.

RESULTS AND DISCUSSION

Morphological properties: The field study indicates that all the species under study are annual herbals, growing wild between February to March of 2018-2020. (Plate 1). The roots are tap roots of the conical type, not branching in *A. strigosum, and C. jonthlaspi* species. Except for the *I. tinctoria* species, the roots are wedge-branched at the base of the root. Its length ranged from 2-13 mm, with the lowest root length in *A. strigosum* species and highest in *I. tinctoria* species. (Table 2 and Plate 1). The roots are similar in all the studied species for the reason of their growth in the mountainous region, the lack of surface water and the nature of the rocky soil. Since the roots are affected by environmental factors faster than other plant parts, changes appear faster,whereas *C. jonthlaspi* and *I. tinctoria* were similar to the apical polymorphic erect stem, while the stem of *A. strigosum* is un branched (Plate 1), while the differences of the stems are due to the impact of this trait by many environmental and genetic factors. Examination of the leaves show that all are simple, undivided, and cordate in *I. tinctoria* and elongated in *A. strigosum and C. jonthlaspi*. The leaf apex varies in shape, with acute in *I. tinctoria* and obtuse in *A. strigosum and C. jonthlaspi*. Cordate base in *I. tinctoria*, truncate plane in *A. strigosum, C. jonthlaspi* and undulate margin in *I. tinctoria* and entire smoothness in the other species under study, while leaf size ranged between 10-40 mm and average width between 2-20 mm. (Table 2 and Plate 1). The results of the current study show the similarity of the flowers of all the species under study. They are bisexual with white petals in *A. strigosum and C. jonthlaspi* except for *I. tinctoria* which appears with yellow petals while the fruits are of the silicula type. Here it should be noted that there is a difference in the shape of the fruits and they are ovate. Sides compressed in *A. strigosum* and *C. jonthlaspi* and oblong in *I. tinctoria*. The size and shape of the fruit vary between 6-8 mm, the beak between 2-3 mm, and the petiolate size between 10-15 mm. The fruit contains 2 seeds and trichomes of the glandular and eglandular stellate cell-multicellular type in *A. strigosum* while the size of *C. jonthlaspi* fruit ranges from 4-6 mm. The beak ranges between 1-2 mm, the petiolate between 8-10 mm, its ovate shape containing a single seed, and the hairs covering of the glandular type. while the size of the beak ranges between 5-8 mm and the petiolate between 4-6 mm and fruit 15-20 mm which is smooth superficial containing a single seed in *I. tinctoria*. The results of the present study show a difference in seed shape among the species under study. They are oval in *A. strigosum* and *C. jonthlaspi* and oblong in *I. tinctoria*. In addition to the emergence of variation in seed color, they are dark brown in *A. strigosum* and *I. tinctoria*, and light brown in *C. jonthlaspi*, and the surface trimmings are smooth in *C. jonthlaspi* and *I. tinctoria* and granular in *A. strigosum* (Table 3 and Plate 2). The results of the present study are in agreement with the results of (3,18,24,25), which indicate the similarity of many appearance properties (shape, habitat, stem branching and leaf shape, apex, base and margin, fruit type and Indumentum surface of the seed) for both *A. strigosum* and *C. jonthlaspi*. The reason for this is the common origin of the two species and their affiliation to the same Alysseae tribe, while the different properties of *I. tinctoria* are due to the difference of its Isatideae tribe. De Candolle (15) and Al-Shehbaz et al. (10) classified the studied species and are placed within different families while Hayek (19) classified *I. tinctoria* within the Arabideae tribe, the sub-clan Isatidinae, while Schulz (32) placed it within Lepidieae subtribe Isatidinae. The difference in the dimensions and shapes of the fruits helps in the diagnosis of the studied species in the field.

Table 2. Morphological features in some Brassicaceae species. (Mean ± SE).

Taxa / Characters	Plant length (Cm)	Root length (mm)	Leaf shape	Leaf size Rate (mm)	
				Length	width
***A. strigosum* Banks and sol.**	**15-20 (17 ± 2.5)**	**1-3(2 ±1)**	**oblong**	**10 ± 4**	**2 ± 1**
***C. jonthlaspi* L.**	**10-20 (7 ± 5)**	**2-4(2.5 ± 1)**	**oblong**	**12 ± 4**	**3 ± 1**
***I. tinctoria* L.**	**40-60(50 ± 10)**	**10-15(13±2.5)**	**cordate**	**40 ± 4**	**20 ±2.5**

Table 3. Morphological features of seeds in some Brassicaceae species. (Mean ± SE).

Characters / Taxa	Shape	Colors	Indumentum	Size Rate (mm)	
				Length	width
A. strigosum	ovate	Dark Brown	granular	1-1.5 (1.25±0.2)	0.5-1 (0.7±0.2)
C. jonthlaspi	ovate	Light brown	smooth	2-2.5 (1.5 ±0.5)	1-1.5 (1.25±0.25)
I. tinctoria	oblong	Dark Brown	smooth	4-5 (4.5±0.5)	1-1.5 (0.8±2.5)

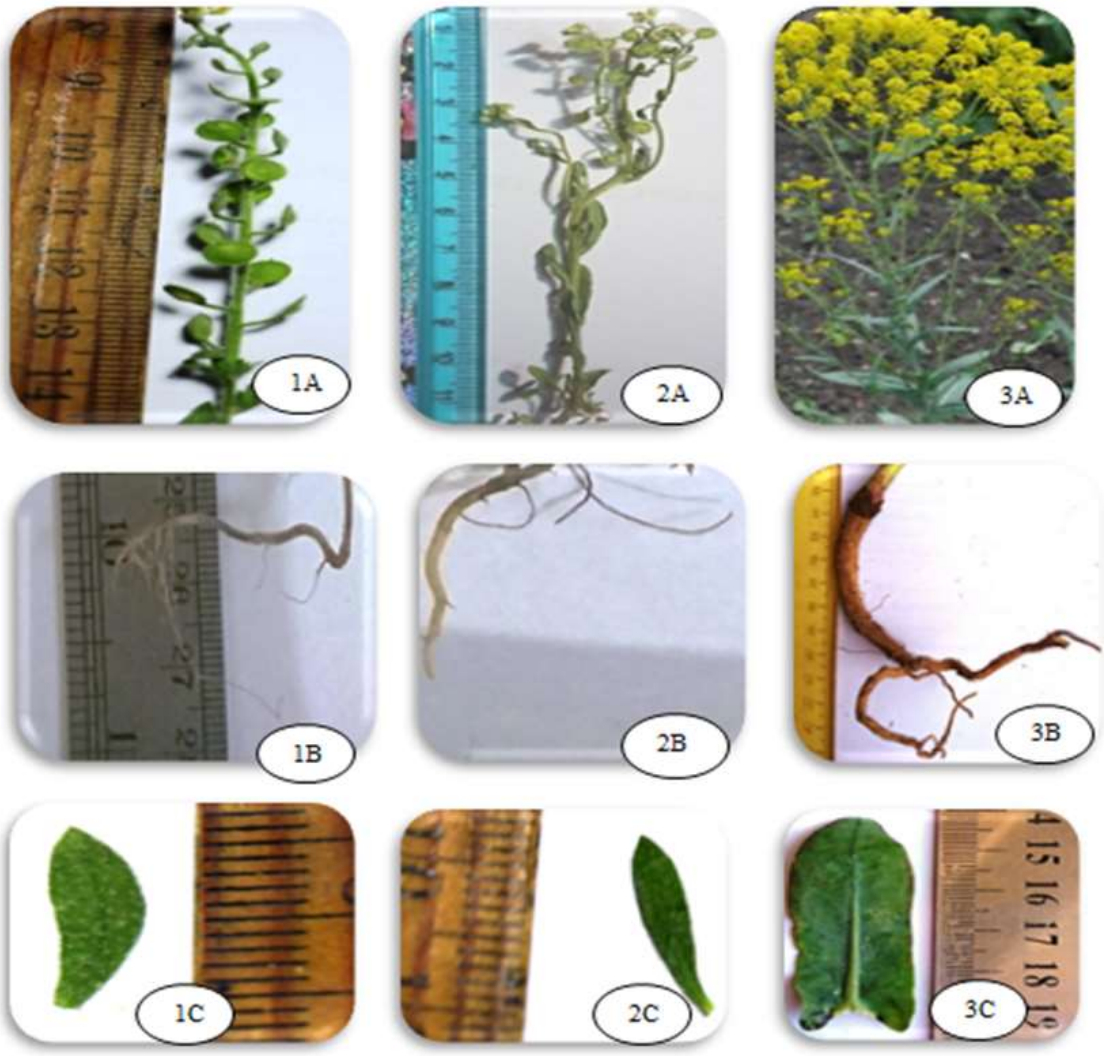


Plate 1. Morphological structure of vegetative parts in species under studies 1-*A. strigosum* 2- *C. jonthlaspi* 3- *I. tinctoria* .(A- plants, B- root , C-leaf). 0.5mm

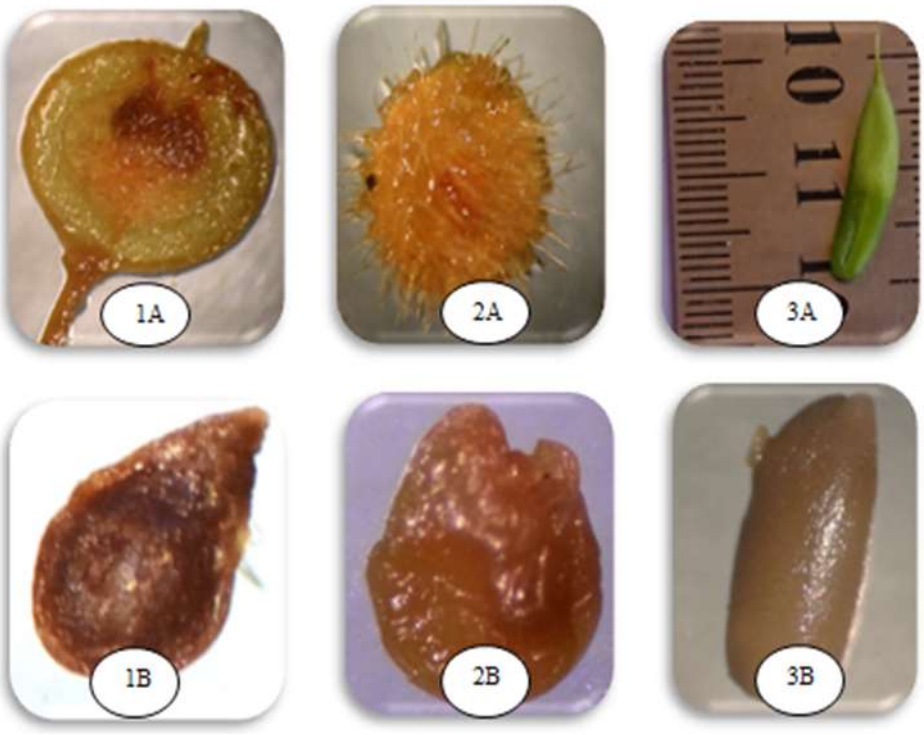


Plate 2. Morphological structure of Reproductive parts in species under studies.

1. ***Alyssium strigosum*** **2.*Clypeola jonthlaspi*** **3. *Isatis tinctoria* . (A- fruit, B-seed).**

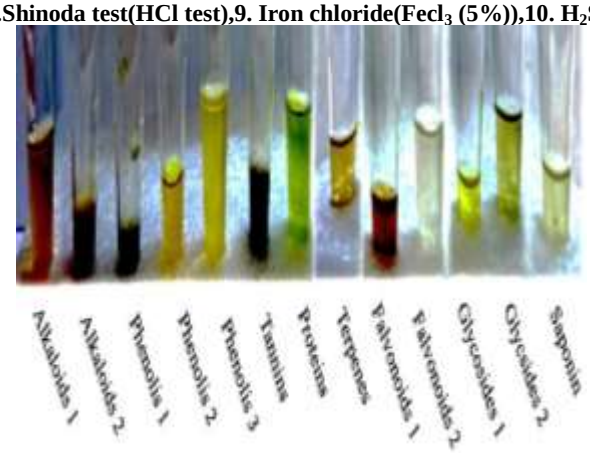
Diagnosing chemical compounds: The presence of alkaloids is detected in *C. jonthlaspi* and *I. tinctoria* and does not appear in *A. strigosum* using Mayer's and Wagner's test. The presence of flavonoids, phenols, glycoside glycosides, tannins, sapindales, and terpenoids are not detected in all studies. (Table 4 and Plate 3). The HPLC results show the diagnosis and the presence of four phenolic compounds in the species under study, the compounds are Rutin, Quercetin, Kaempferol and P-coumarin, and the concentrations of the compounds differ in different species. The highest concentration of rutin appears to be 377.3, 518.9 and 296. 3mg / ml in the species *A. strigosum, C. jonthlaspi* and *I. tinctoria* respectively. From the results above, it is found that the concentration of rutin is higher in the *C. jonthlaspi*, while the lowest concentration of quercetin is 97.1 mg / ml in the *A. strigosum* and reaches the lowest concentration of 246.4 mg / ml of p-coumarin reported in *C. jonthlaspi*, while the lowest concentration of kaempferol is 45.5 mg / ml in *I. tinctoria* (Figure 2,3 and Table 5). The results of Table .4 indicate the richness of the studied plants with phenolic compounds, flavonoids and glycosides. Tannins, Sapindales and lack of terpenoids. This indicates the exposure of plants to environmental stresses, which helps in building secondary metabolites and chemical compounds forming a defense system against bacteria, viruses ,fungi. and insects. In addition, the plant produces secondary metabolites as a means of confronting environmental stresses, and the diagnosis of these compounds may contribute to solving the classification problems through the use of indicators of Phytotaxonomy. From the results of Figuer 2,3 and Table .5 , it is evident that the alcoholic extract is superior in detecting compounds compared to the aqueous extract. The current study with the results of the (21). indicated the presence of many phenolic compounds within the cruciferous family plant. It also agrees with the results of Abbasian and Kishavarzi (1) in the detection of secondary metabolic compounds and their biological effectiveness against pathogens, as they state that the chemical compounds that are isolated from the leaves of the Brassicaceae using HPLC technology have an effective role against bacterial pathogens, as well as that phenolic compounds provide evidence that helps in classification of plants that contributes and enhances morphological properties (29), Shankar *et al*.(34) study indicates the use of Brassicaceae plants as main food plants for some of the world's

population, and the oil extracted from their plants constitutes 14% of the vegetable oils suitable for human consumption. Its plants are an important source for medicinal, agricultural and economic purposes because they contain nutritional health features beneficial to the human body and possess antioxidants, including carotenoids, ascorbic acid and phenols that are used in the treatment of heart diseases and cancers (34). In addition, the concentration of phenolic compounds can be calculated to help choose the best types that can be used.Finally, it should be noted that the concentrations of phenolic compounds are important depending on many variables, such as the study method, environmental factors, time of collection, and place of collection (12). Therefore, this research mainly focuses on the morphological properties of some species of the Brassicaceae , in addition to the differences in the concentrations of phenolic compounds.

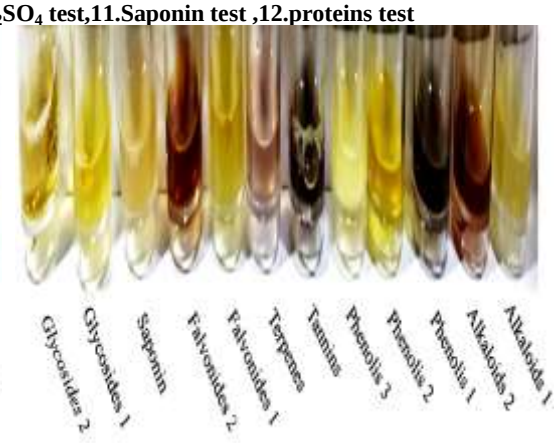
Table 4 . Phytochemical test of species in the Aqueous & alcoholic extracts

Chemical compounds / Taxa	Alkaloids		Phenols		Tannins	Falvonoides		Glycoside	Saponin	Proteins
	1	2	3	4	5	6	7	8	9	10	11	12
A. strigosum	-	-	+	+	+	+	+	+	+	+	+	+
C. jonthlaspi	+	+	+	+	+	+	+	+	+	+	+	+
I. tinctoria	+	+	+	+	+	+	+	+	+	+	+	+

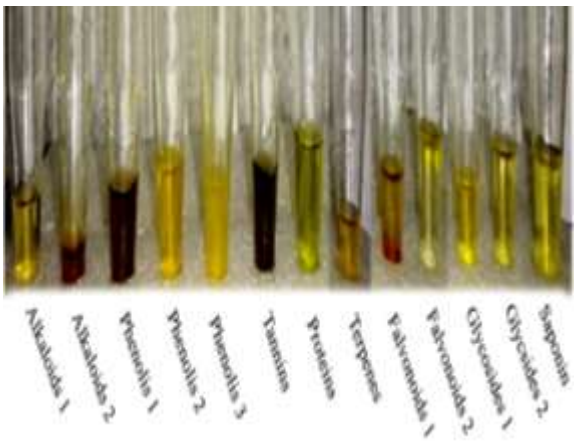
Mayer's test, 2. Wagner's test,3.Iron chloride($Fecl_3$(7.5%)),4.Di chromium potassium,5. Lead acetate, 6.Tannins test 7.H_2SO_4 test ,8.Shinoda test(HCl test),9. Iron chloride($Fecl_3$ (5%)),10. H_2SO_4 test,11.Saponin test ,12.proteins test



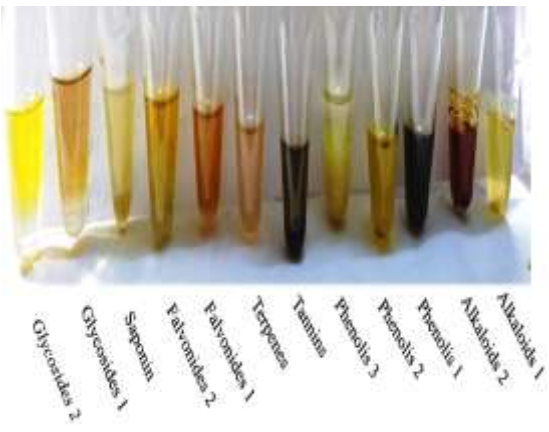
1.Alcohol extracts of *A. strigosum*



2.Aqueous extracts of *A. strigosum*



3.Alcohol extracts of *C. jonthlaspi*



4.Aqueous extracts of *C. jonthlaspi*

Plate 3. Phytochemical studies in Aqueous and alcohol extracts of some species

Table 5.Concentration and Percentage in the Phenolic compounds in the species

	Type of compound	Area of sample (µvolt)	Number of dilutions	Concentration mg/ml	Percentage %
A. strigosum	Rutin	341242	12	377.3	49.79
	Quercetin	61515		79.1	10.34
	Kaempferol	117372		169.5	22.3
	p-coumarin	86488		131.8	17.39
	total			757.6	99.9
C. jonthlaspi	Rutin	468988	10	518.9	34.17
	Quercetin	283793		364.7	24.01
	Kaempferol	234105		388.1	25.56
	p-coumarin	161867		246.7	16.24
	total			1518.4	99.98
I. tinctoria	Rutin	267987	12	296.3	49.014
	Quercetin	129948		167	27.62
	Kaempferol	31511		45.5	7.52
	p-coumarin	62789		95.7	15.82
	total			604.457	99.9

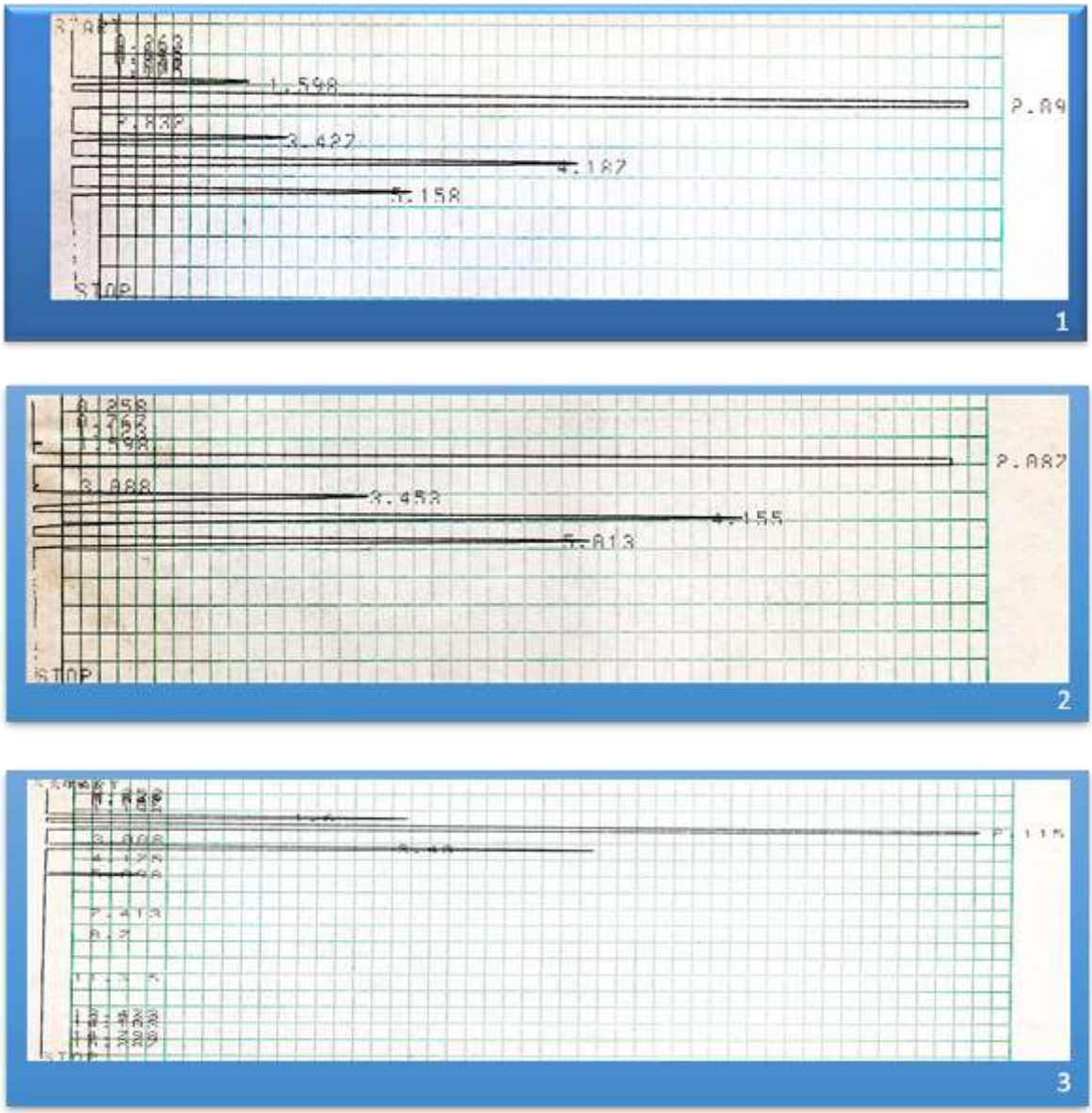


Figure 2. Difference area of sample and Retention time of Phenols compounds in the species. 1.*A. strigosum* 2. *C. jonthlaspi* 3. *I. tinctoria*.

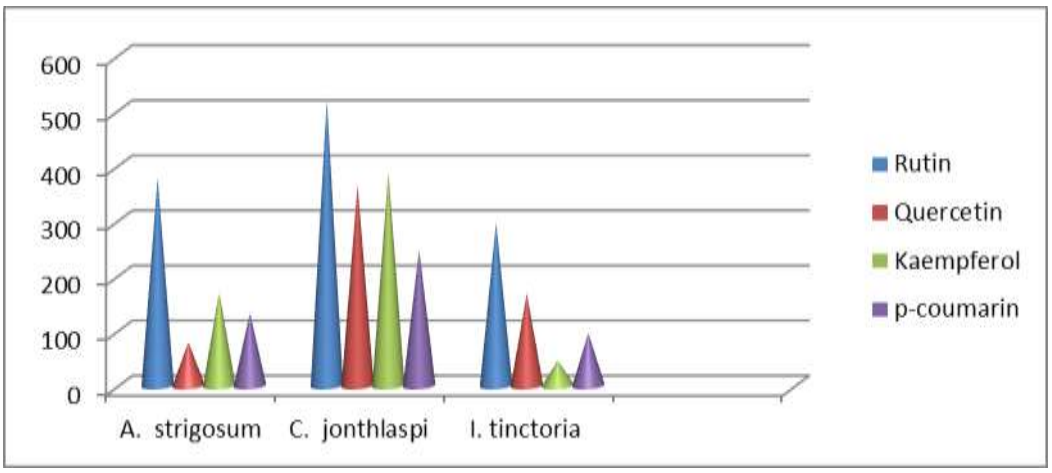


Figure 3. Difference concentration in the Phenolic compounds in the species

CONCLUSION

• The results showed a similarity in the morphological properties of the *A. strigosum* and *C. jonthlaspi* on the one hand (Apex, base and margin), while the *I. tinctoria* differed in its properties, and a difference appeared in the dimensions of leaves for the three studied species.

• It is found that the fruits of the two species *A. strigosum* and *C. jonthlaspi* are of the species silicle, while the fruit of the species *I. tinctoria* is of the type Silique.

• The emergence of a difference in the shapes and colors of the seeds in addition to the Indumentum dimensions and surface inscriptions of the three studied species.

• Qualitative chemical tests indicate the presence of 6 secondary metabolites, which are alkaloids, phenols, tannins, Falvonoides, glycosides, sapindales, and the absence of terpenoids and the superiority of the alcoholic extract in detecting compounds compared to the aqueous extract.

• HPLC analysis shows the presence of 4 phenolic compounds which are Rutin, Quercetin, Kaempferol and P-coumarin in all studied species and their concentration and ratios differ due to the difference in biological activity.

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