



Extraction, Characterization And Comparison The Components Of *Eruca Sativa* Mill And *Eruca Vesicaria* L And Study Their Biological Activity

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ABSTRACT

In this work high performance liquid chromatography (HPLC) was used to isolate and study the components of plants *Eruca sativa* and *Eruca vesicaria*, both are belonging to the family Brassicaceae. The extractions have done using Soxhlet apparatus. However, the result shows that there are some different in the compounds for each. The *Eruca* extracts are a powerful antioxidant agent and the analysis of both plant samples using the HPLC chromatogram with standard sample shows that the extracts of both plants contains an antioxidant compounds. The antibacterial activities of the extracts were tested against *Escherichia coli*, *Staphylococcus aureus* and the activity was good towards the selected bacteria. In addition, the extracts show a good antifungal activity towards candida albicans).

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1. INTRODUCTION

Eruca sativa and *Eruca vesicaria* are an herb vegetables belonging to the Brassicaceae family (Pasini et al. 2011) (Bell and Wagstaff 2014) (Sharifi-Rad et al. 2020). It is the native to the coast of the Mediterranean region, but also widely grown all over the world. Both types considered as a medicinal plant. The study of identifying phenolic compounds that they can be used as a chemical classification study to Useful for pharmaceutical In future studies (Al-Taie et al. 2018). *Eruca* leaves contain good sources of vitamin C and beta-carotene in high density, so they can be considered a good source of micronutrient supplementation, especially for vulnerable groups such as infants and young children (Keyata et al. 2021). Phytonutrients are natural biologically active compounds obtained from plants that perform specific functions and have an effect in modifying physiological functions to improve human health (Niaz et al. 2020).

In fact, it was known for its diuretic, astringent, digestive, laxative, depurative, tonic, rubefacient, and stimulant effect (Bouacida et al. 2016) (Jaafar and Jaafar 2019). *Eruca sativa* mill, the results of the study recently showed that its leaves were of interest to scientific communities around the world due to its strong biological activity. It also confirmed that the metabolite of the ethanolic crude extract of the leaves using high-resolution liquid chromatography and mass spectrometry (HR-LC/MS), as well as antimicrobial potential. For bactericidal, antioxidant and anticancer activity against human colorectal cancer cell lines, the identified plant components

showed promising pharmacological properties, representing a valuable resource for drug or nutraceutical development (Awadelkareem, A. M., Al-Shammari, E., Elkhailifa, A. E. O., Adnan, M., Siddiqui, A. J., Snoussi, M., ... & Ashraf 2022).

It exhibits antiulcer effect, anticancer activity in preventing the melanoma growth and contains a broad range of phytochemicals such as vitamin C, flavonoids, carotenoids, fibers, and glucosinolates known by their health benefits (Bell, Oruna-Concha, and Wagstaff 2015) (Piślewska-Bednarek et al. 2018). Furthermore, numerous studies have been carried out on rocket salad demonstrating thus its health related properties on human being (Ameen et al. 2015) (Jaafar and Jaafar 2019) (Taffner et al. 2019). the phytochemical study, antioxidants and analgesic activities of the hydroalcoholic extract of the *Eruca vesicaria* plant used in traditional medicine. Phytochemical examination revealed the presence of tannins, flavonoids, sterols, flavonoid glucosides, saponins, phenols and alkaloids (Sihem et al. 2022), the extracts of *E. sativa* leaves and sprouts produced using different extraction methods, have a substantial beneficial antioxidant, antimicrobial and anticancer activities) (F Ahmad and A Shehta 2020).

Plant solvent extracts may exhibit antimicrobial, anti-inflammatory, fungicidal and insecticidal activities, proving thus their economical and environmentally safe bioactive compounds (Ehtaiwwesh and Qarimidah 2021) (Michael, Shafik, and Rasmy 2011). Besides these properties, several plant solvent extracts have been classified as natural antioxidants, substituting thereby the synthetic antioxidants restricted in many countries regarding their potential health related issue (Michael, Shafik, and Rasmy 2011) (Koubaa et al. 2015). All tested oils showed variable antimicrobial activities against the tested strains, *Eruca* oils were the most active oils (Bassyouni et al. 2022). The seed oil of the species *Eruca sativa* has an antimicrobial effect against antibiotic-resistant Gram-negative (*Escherichia coli*, *Pseudomonas aeruginosa* and *Shigella flexneri*) and antibiotic-resistant Gram-positive (*Staphylococcus aureus* and *Bacillus subtilis*) bacteria, are rich sources of nutrients and phytochemicals, but their high erucic acid content may preclude their use in food applications, due to the health risks of erucic acid (Rozan and Boriy 2022).

The results of the study indicated that the essential oil of *E. sativa* has higher resistance to self-oxidation, is of higher quality, has a longer shelf life, is suitable for use as salad or cooking oil and is edible, and has antimicrobial activities, including seven types of human pathogenic bacteria. Showed resistance to some antibiotics (Rayaes Kamel et al. 2022)

It is the study of the chemical composition and bioactivity of essential oils (EOs) of different parts (leaves, flowers, stems and roots) of the *Eruca vesicaria* plant. The essential oil of the roots had the highest antioxidant activity (Hichri et al. 2019) (Bouacidiaa et al. 2020) study indicated that marinating meat with fresh watercress leaves and ethanolic extract. These results showed that the presence of watercress leaves has an important role in improving meat quality (Afifi et al. 2023). On the other hand, the results of the study indicated that the ethanolic extract was more effective than fresh leaves in this improvement (Olvera-Aguirre et al. 2022). The results of microbiological analysis of cheese showed that *E. vesicaria* leaves have inhibitory activity against coliforms, epiphytes, psychoactive plants, staphylococci, yeast and molds, which may lead to the potential of the leaves as an antibacterial agent.

2. RESEARCH METHOD

2.1 Plant Material

Both *Eruca sativa* and *Eruca vesicaria* were obtained from valley regions in Diyala governorate Iraq

2.2 Experimental condition

The procedure of the extraction was done at the College of Education for Pure Science/ University of Diyala. The main compounds were separated under optimum condition; column phenomenonex C-18, 3 µm particle size (50 X 2.0 mm I.D) column, Mobile phase linear gradient of solvent A: ammonium acetate 10 mM, pH 4.5, B was acetonitrile, gradient from 0% B to 100% B for 16 minute flow rate 1.2 mL/min. photodiode array detector PAD at 270 nm.

The plants were collected from the local farm at Baqubah/ Diyala government, the leaves of the plants were washed with distilled water and dried and grinded using household blender. The *Eruca sativa* extracts was obtained by 20 g of fresh leaves of *E. sativa* were mixed with 100 mL

solvent (80 mL MeOH and 20 mL H₂O) in the Soxhlet for 5 hours. The solution was collected, solvent was evaporated, the yield was stored at 4 °C until used the next day. Using the same method, *Eruca vesicaria* extracts was obtained by adding 20 g of fresh leaves.

3. RESULTS AND DISCUSSIONS

Analytical HPLC was used to separate the components of both plants extract. These indicates the presence of some similar compounds in both type of plants:

3.1 *Eruca sativa*

Total 10 compounds were identified in *Eruca sativa* extract by HPLC see figure 2, and are listed in table 2. This type contained ten types of chemical compounds and concentrations. (20.03 - 254.52) µg/ml, as this hyperoside compound occupied the highest concentration of 254.52 µg/ml, while the compound occupied caffeoylquinic acid at the lowest concentration of 20.03 µg/ml, as shown in Table (1) as well as Figure (1,2) showing the results of antibiotics. type of oxidative stress.

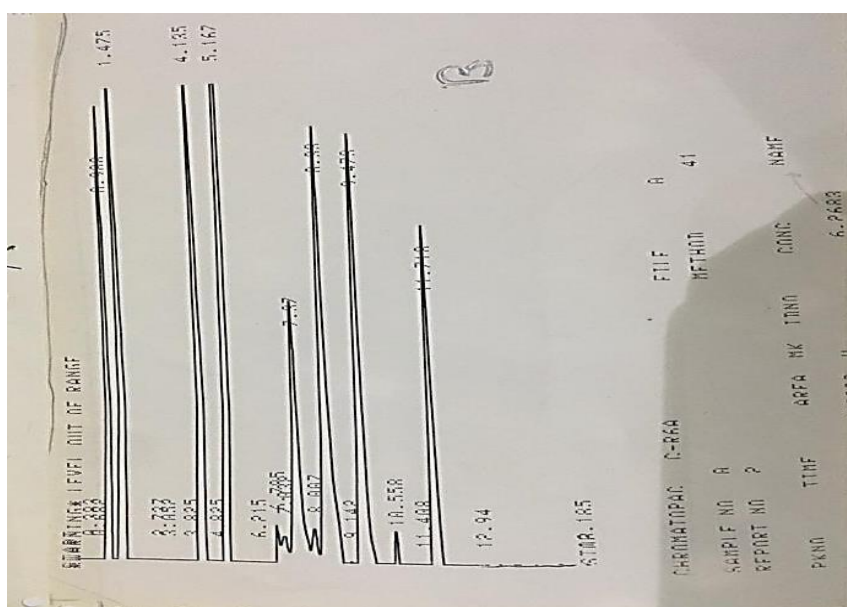


Figure 1. HPLC chromatogram of the species *Eruca sativa*.

Table 1. Compounds found of the species *Eruca sativa* extract

T	Name of the active compound	compound concentration µg/mL	Compound %
1	Caffeoyl quinic acid (chlorophenic acid)	226.17	20.03
2	hypercin	78.82	6.982
3	hyperforin	127.80	11.32
4	hyperoside	254.52	22.54
5	l3.1lβ, biapgenin	58.006	5.13
6	Iso Quercirtin	176.94	15.67
7	pseudohypercin	34.41	3.04
8	quercetin	24.88	2.20
9	Quercitrin, (quercetin-3-O-α-l-rhamnopyranoside)	73.80	6.53
10	rutin	73.54	6.51

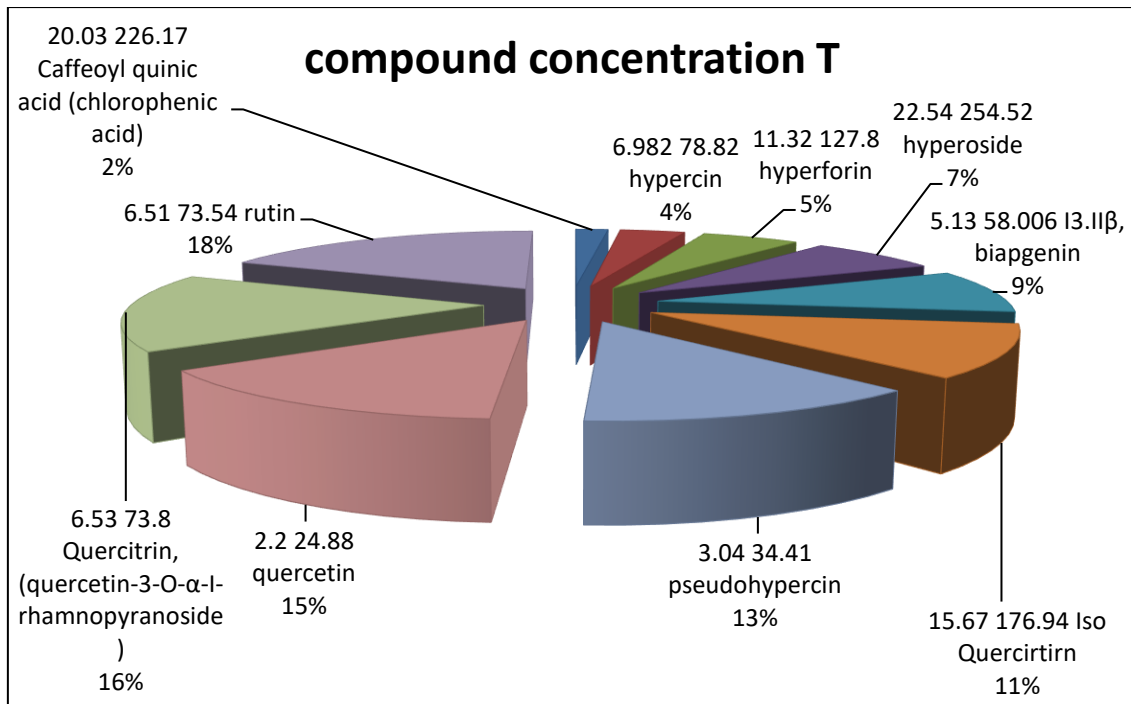


Figure 2. shows the concentrations and percentages of the species *Eruca sativa*.

3.2 *Eruca vesicaria*

Total 10 compounds were identified in *Eruca vesicaria* extract by HPLC see figure 1, and are listed in table 1. This type contained ten chemical compounds. The compound hyperoside occupied the highest concentration of 270.47 $\mu\text{g/ml}$, while the compound quercitrine was present in the lowest amount, as it occupied the lowest concentration of 35.25 $\mu\text{g/ml}$, as shown in Table (1) as well as Figure (2,3) showing an analysis of Antioxidant chemotype.

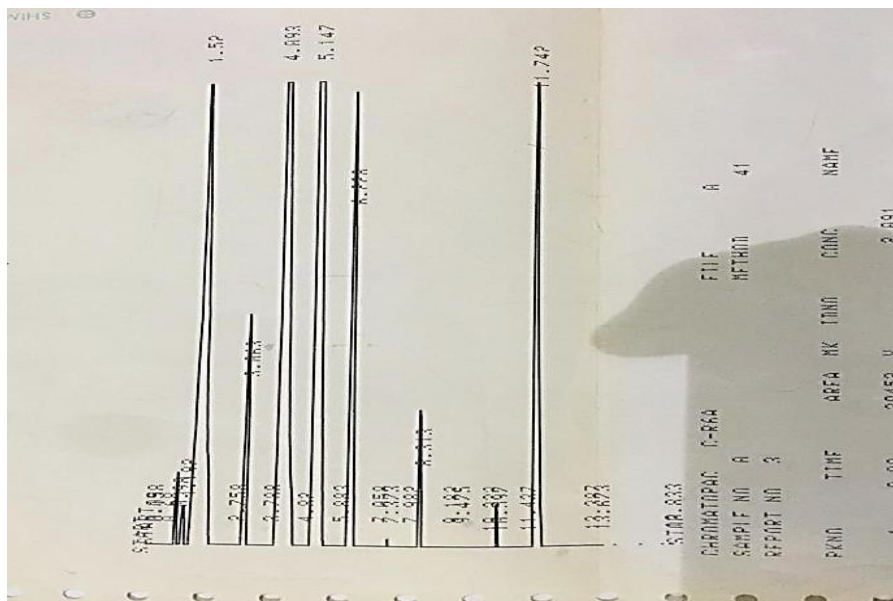
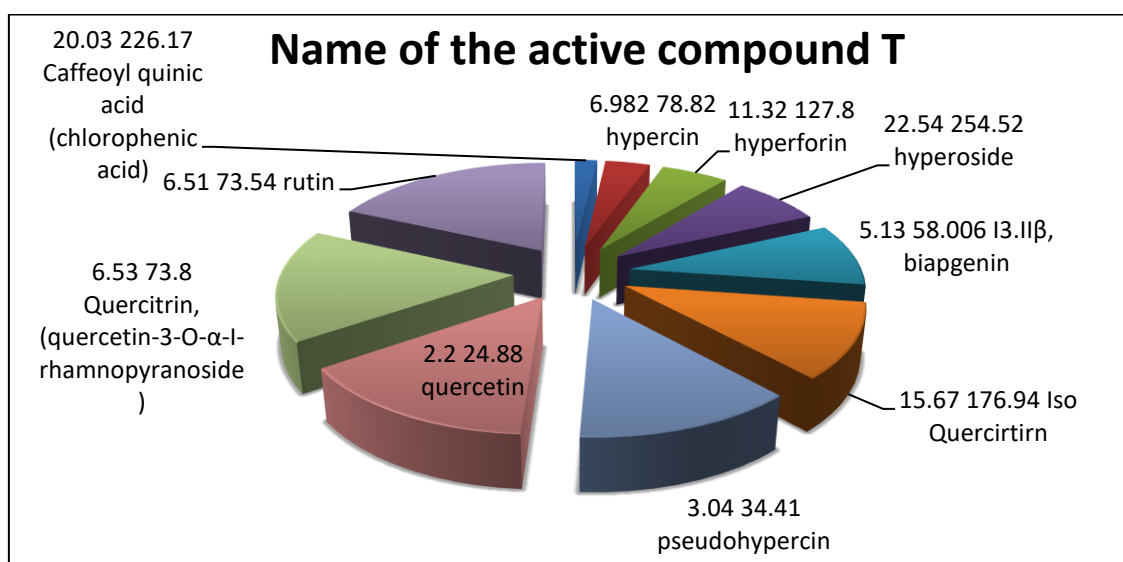


Figure 3. HPLC chromatogram of the species *Eruca vesicaria*.

Table 2. compounds found in *Eruca vesicaria* extract

T	Name of the active compound	compound concentration µg/mL	Compound %
1	Caffeoyl quinic acid (chlorophenic acid)	156.24	9.59
2	hypercin	134.94	8.28
3	hyperforin	169.52	10.41
4	hyperoside	270.47	16.61
5	I3.IIβ, biapgenin	250.494	15.38
6	Iso Quercirtirn	157.84	9.69
7	pseudohypercin	238.98	14.68
8	quercetin	161.56	9.92
9	Quercitrin, (quercetin-3-O-α-l-rhamnopyranoside)	35.25	2.16
10	rutin	52.33	3.21

**Figure 4.** shows the concentrations and percentages of the species *Eruca vesicaria*

The separated compounds are listed in table 1 and 2 including area, the retention time and concentration for each peak. From the information that obtained from the HPLC chromatogram and based on the retention time there are 10 compounds have the same time. *Eruca vesicaria* extract have 3 compounds which are different which and are not in the another type of species. *Eruca sativa* have 10 compounds and are not exist in the *Eruca vesicaria*. On the other hand, the concentration of similar compounds in both extract is different, the concentration of compounds and 10 in the *Eruca vesicaria* plant is higher than compounds caffeoylquinic acid (chlorophenic acid), hyperoside, Iso Quercertern and 10 in the *sativa* plant and compound number. While the concentration of other similar compounds in the *Eruca vesicaria* plant are less than in *Eruca sativa* plant.

3.3 Antioxidant Analysis

The *Eruca* extracts are a powerful antioxidant agent. The analysis of both plant samples using the HPLC chromatogram with standard sample shows that the extracts of both plants contains an antioxidant compounds which are listed with the names and retention time in table 3.

Table 3. shows standard compounds

T	Name of the active compound	detention time	compound space
1	caffeoylquinic acid (chlorophenic acid)	1.61	348439
2	hypercin	9.71	196597
3	hyperforin	10.89	198918
4	hyperoside	3.24	274350
5	I3.IIβ, biapgenin	7.56	242054
6	Iso Quercertern	4.21	304460
7	pseudohypercin	8.67	245173
8	quercetin	6.57	309205

9	Quercitrin, (quercetin-3-O- α -l-rhamnopyranoside)	5.37	340692
10	routine	2.77	256004

3.4 Antimicrobial activities

Eruca extracts have a potent antimicrobial activity, as a promising antibacterial agent. Its promising effect against bacteria confirmed by using them as a remedy in medicine and in meal. The antibacterial activities of the extracts were tested using pathogen bacterial strains. The species that used in this work were: *Escherichia coli*, *Staphylococcus aureus*. The plates were incubated overnight at 37°C for 24 hours. Antibacterial activities were measured as the diameter of the clear zone of growth inhibition (Bauer, PERRY, and KIRBY 1959). In addition, the extracts show a good antifungal activity towards *Candida albicans*.

Table 4. The antimicrobial activity

Extract	<i>E. coli</i>	<i>S. aureus</i>	<i>Candida Albicans</i>
<i>E. vesicaria</i>	20	24	22
<i>E. sativa</i>	22	27	21
DMSO	0	0	0

4. CONCLUSION

The extract of the *Eruca* plant of two types *E. sativa* and *E. vesicaria* were successfully carried out using Soxhlet with good yield. The HPLC chromatograph analysis shows that the two types of plants contain similar compounds in different concentrations and some new different compounds as well. The extracts of both plants gave good antibacterial activity against two types of bacteria, gram positive and gram negative. The extracts show a good activity towards *Candida albicans* as well.

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