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Some Fungal Species

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Abstract

This study was carried out to show the effect of extracts (aqueous, alkaloid and glycosides) of *Eruca sativa* Mill. on the fungal growth, the results revealed the inhibitory effectiveness of the aqueous, alcoholic and glycosides extracts of *E. sativa* by four different concentrations (10%, 20%, 40% and 100% mg/ml) on the growth of three fungal species in three different genera (*Aspergillus niger*, *Fusarium oxysporium* and *Pencellium sp.*). The results showed that these concentrations of the aqueous extracts have an impact on inhibiting the growth of two fungal species (*Pencellium sp.* and *F. oxysporium*) variously, while the same concentrations of aqueous extracts did not affect on the growth of *A. niger* as well as it showed the ineffectiveness of various concentrations of alcoholic extracts in the inhibition of these fungi, whereas it was appeared the effectiveness of the same concentrations of glycoside extracts in the inhibition of two fungi *F. oxysporium* and *Penceillium sp* differently, whilst the same concentrations did not inhibit the growth of *A. nigar*

Key words: Phytochemical, extracts effectiveness, *Eruca sativa*

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تأثير مستخلصات نبات الجرجير *Eruca sativa* Mill. في تثبيط نمو بعض الفطريات

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الخلاصة

هدفت الدراسة الحالية الى معرفة الفعالية التثبيطية للمستخلصات المائية و الكحولية و الكلايكوسيدية لنبات الجرجير *Eruca sativa* Mill. وبتراكيز مختلفة (10, 20, 40 و 100 ملغم/مل) تجاه ثلاث انواع من الفطريات هي *Pencillium sp*, *Fusarium oxysporium* و *Aspergillus niger*. وبينت نتائج الدراسة فعالية المستخلص المائي و الكحولي و الكلايكوسيدي بتراكيزه المختلفة في تثبيط الانواع الفطرية المدروسة، حيث اثر المستخلص المائي في تثبيط الفطرين *Pencillium sp* و *F. oxysporium* بصورة متباينة، في حين لم يؤثر المستخلص المائي وبتراكيزه الاربعة في الفطر *A. nigare*. كما بينت نتائج الدراسة عدم فعالية المستخلصات الكحولية لهذا النبات وبتراكيزه (10, 20, 40 و 100 ملغم / مل) في تثبيط الفطريات المدروسة بينما اثرت المستخلصات الكلايكوسيدية للنبات وبتراكيزها الاربعة في تثبيط نمو الفطرين *Pencillium sp* و *F. oxysporium* بينما لم تؤثر نفس التراكيز في نمو النوع *A. nigare*

الكلمات المفتاحية: كيمياء النبات، فعالية المستخلصات، نبات الجرجير *Eruca sativa*

Introduction

The species *Eruca sativa* (Arugula) is an herbaceous [annual plant](#) of the family [Brassicaceae](#) which is a large family comprises about 365 genera and 3200 species all over the world (APG III, 2009). With widespread be cultivated or wild, plant seeds contain glycosides, alkaloids, volatile oils, vitamin C, Iodine, Iron sulphate and Calcium (Evans, 1999; Hussein, 1985 and Kochhar, 1981). This plant has therapeutic and medical importance that uses in Dermatitis treatment, boils, burns, indigestion, tuberculosis, gingivitis and alopecia (Roiha,

Effect of Extracts of *Eruca sativa* Mill. on the Growth Inhibition of Some Fungal Species

Sirwan Hassan Salih and Mostafa Qahtan Mosataf

1983). Jamba oil was extracted from the seeds that is useful for humans, which is used in many industries including industry of soaps , shampoos for hair loss, cosmetics, food appetizers, massage oil and the production of bio-fuel (Mohammed and Rafiq, 2009). Arugulas oil has medical effectiveness include improving liver function and a reduced rate of blood sugar (El-Gengaihi et al., 2004), improve the effectiveness of sex hormones (Merza et al., 2000) and an anti-bacterial and fungi (Abdou et al., 2005) also the oil used in a Biological control against of many pests (Yaniv, 1997). Some of the extracted oils are rich in sulphate compounds that characterized by its ability to kill microbes also the existence of glucosinolates compounds which has biological activities against microorganisms (Holt et al, 1994).

Due to the importance of the chemical compounds in this species, this study aimed to know the inhibitory effectiveness of *E. sativa* extracts in the inhibition of some fungal species.

Materials and methods

Plant specimens were collected from cultivated orchards during spring season. Some specimens were pressed and dried for diagnosis, tagged and deposited in the herbarium of the Biology department. Other selected specimens were cleaned from the dust and impurities and then rinsed with the water then dried at room temperature away from the light to avoid oxidation. After drying the plant specimens has been grinding by using laboratory electric mill and then placed in opaque plastic cans finally stored refrigerated until extraction.

Hot Aqueous Extraction:

According to (Al- Joboory and Al-Rawi , 1994) the hot water extraction was conducted by mixing 40mg of specimen with the 160 ml of distilled water (rate 1:4 weight: size) (overheating 95-100 C °) twice with shaking the mixture to break down plant cell walls and leave it in the refrigerator for 24 hours for the soaking. Mixture was filtrated through several layers of medical gauze, and filtrated once again by Buchner funnel using watman papers No.1 to get rid of the non-crushed parts and fibers to obtain plant extract raw liquid which put them in a Rotary vacuum evaporator at the temperature of not more than 40° as it works on the basis evaporation under rarefied pressure. Extracted output was placed in Shaker incubator at a temperature of 30-35°. Dried extract was saved freeze in airtight containers and labeled until in use.

Effect of Extracts of *Eruca sativa* Mill. on the Growth Inhibition of Some Fungal Species

Sirwan Hassan Salih and Mostafa Qahtan Mosataf

Ethanollic Extraction:

The ethanolic extraction was prepared by mixing 20 gm of specimen powder with the 200 ml of absolute ethanol (rate 1:4 weight: size), shaking the mixture to break down plant cell walls and leave it in the refrigerator for 24 hours for the soaking. It was filtrated by several layers of medical gauze and repeated again by Buchner funnel using whatman papers No.1 to get rid of the non-crushed parts and fibers to obtain plant extract raw liquid which put them in a Rotary vacuum evaporator at the temperature of not more than 40° as it works on the basis evaporation under rarefied pressure. Extracted output was placed in Shaker incubator at a temperature of 25-30°. Dried extract was saved freeze in airtight containers and labeled till in use (Grand et al,1988).

Glycoside Extraction:

Glycosides extracted by adding 10 gm of plant powder to 100 ml of ethanol (80%) move the mixture and leave it in the refrigerator for 24 hours for the soaking. The extraction was concentrated to 1/3 it sizes by Rotary vacuum evaporator then added 50 ml of Ether and 5 ml of Lead acetate (0.3 molarity), in separating funnel with shaking and has withdrawn aquifer. This step was repeated three times and dried the drawn water layer at a temperature of 30° until fully dry. (Ukida, et al, 2006)

Alkaloids Detection:

The method of (Fahmy, 1933) was followed by boiling 10 gm of plant powder with 50ml of distilled water acidified hydrochloric acid 4%, then filtrated after the solution was cooled, the amount 0.5ml of filtrate was tested in a test tube with each of the following reagents:

- 1- Meyer's reagents (white sediment)
- 2 - Wagner's reagents (brown sediment)

Saponins Detection:

The aqueous solution of plant specimens strongly shacked in the tube test, evidenced by the presence of saponins emergence of dense foam remain for a long time (Al-Ani, 1998).

Effect of Extracts of *Eruca sativa* Mill. on the Growth Inhibition of Some Fungal Species

Sirwan Hassan Salih and Mostafa Qahtan Mosataf

Resins Detection:

Mixing 10ml of plant extract with the 20 ml of distilled water acidified hydrochloric acid HCl 4%, evidenced by the existence of resins emergence of turbidity in it. (Newall et al., 1996).

Phenol Detection:

Adding 3ml of aqueous plants extract to 2ml of ferric chloride solution FeCl₃, the appearance of a bluish-green color evidence of the presence of phenolic compounds (Harborne, 1973).

Tannins Detection:

This step was conducted by boiling 10 gm of plant powder in 50 ml of distilled water, then filtrated the solution and leave to cool, the solution was divided into two equal parts, few drops of lead acetate 1% solution was added to one part, as evidenced by the existence of tannins the emergence of gelatinous precipitate and two drops of ferric chloride solution 1% was added to the other part, as it indicates the emergence of bluish green color to the presence of tannins (Al-Dalali and Hakim, 1987).

Glycosides Detection:

The detection was carried out by mixing two equal parts of the Fehling's reagent with aqueous plant extracts, placed it in boiling water bath for 10 minutes, and evidenced by a positive test by the appearance of red sediment and is evidence of the presence of sugars, to insure of this result has been added 1 ml of water plant extracts to 5ml of Benedict's reagent as it confirms the emergence of red sediment on the presence of sugars, while the blue color appearance indicates to absence of sugars (Al-Shaikhli et al., 1993).

Table (1) Results of the chemical detection for effective materials of *E. sativa*

Effective Material	Alkaloids	Saponins	Tannins	Glycosides	Resins	Phenols
Detection results	+	+	+	+	+	+

(+) positive detection

Effect of Extracts of *Eruca sativa* Mill. on the Growth Inhibition of Some Fungal Species

Sirwan Hassan Salih and Mostafa Qahtan Mosataf

Effectiveness of anti-fungal

The effective of the aqueous and alcoholic extracts of the studied species on the growth of the three fungal species (*A. nigar*, *F. oxysporium* and *Pencillium sp.*) was investigated by using culture media (Sabroud dextrose agar) that prepared in accordance to the manufacturer instructions, to prepare the petri dishes for effectiveness testing the agar diffusion method was used by drilling for observing the fungus sensitivity towards plant extracts at the concentrations of (10, 20, 40 and 100 mg/ml) as it drilled by piercing sterile cork with diameter of 3 mm to drilling in the sabroud dextrose agar and the dishes was inoculated by fungal species, the extracts concentrations was placed in the drills with three replicates for each concentration and the dishes incubated at a temperature of 25° for 7 days, the results was recorded by measuring the diameter of inhibition area (mm).

Results and Discussion

The results indicated to positive detection whereas the studied plant was contained on all the detected materials (phenols, resins, tannins, saponin, alkaloids and glycosides) table (1). It seemed the effectiveness of the aqueous extracts of the plant with concentrations (10, 20, 40 and 100 mg/ml) in the inhibition of two fungi species *Pencillium* and *F. oxysporium*, compared to the control differently table (2), as it showed the concentration of 100 mg/ml with highest effective inhibition compared to the others and then the concentration of 20 mg/ml, while concentrations of 10 and 40 mg/ml are the least effective in the inhibition of *Pencillium*. As well as the aqueous extract with concentrations of 20, 40 and 100 mg/ml had its inhibitory activity against the *F. oxysporium* than controls and the concentrations of 40 and 100 mg/ml have shown the higher inhibitory effectiveness in the inhibition of fungus compared to concentration of 10 mg/ml, which was less effective than concentrations above, as it turns out aqueous extracts showed no inhibitory effective against *A. nigar* than controls. Figure (1)

The results of the current study also revealed that alcoholic extracts of this plant was ineffective in the inhibition of studied fungus spp. compared to the control (Table 3). The glycosides extracts with concentrations of (10, 20, 40 and 100 mg/ml) has been shown effective in the

Effect of Extracts of *Eruca sativa* Mill. on the Growth Inhibition of Some Fungal Species

Sirwan Hassan Salih and Mostafa Qahtan Mosataf

inhibition of both of *Pencillium* sp. and *F. oxysporium* variously compared to the control (table 4), the concentration of 20 mg/ml seemed inhibitory effective against the species *Pencillium* that higher than the concentration 10 mg/ml.

Table (2) inhibitory effectiveness of aqueous extracts against studied fungal species

fungi concentration (mg/ml)	Diameter of inhibition area (mm)		
	<i>Pencillium</i>	<i>A. nigar</i>	<i>F. oxysporium</i>
10	3	0	0
20	4	0	1.66
40	3.33	0	2.33
100	6	0	2.33
Control	0	0	0

Table (3) inhibitory effectiveness of alcoholic extracts against studied fungal species

fungi concentration (mg/ml)	Diameter of inhibition area (mm)		
	<i>Pencillium</i>	<i>A. nigar</i>	<i>F. oxysporium</i>
10	0	0	0
20	0	0	0
40	0	0	0
100	0	0	0
Control	0	0	0

Table (4) inhibitory effectiveness of glycosides extracts against studied fungal species

fungi concentration (mg/ml)	Diameter of inhibition area (mm)		
	<i>Pencillium</i>	<i>A. nigar</i>	<i>F. oxysporium</i>
10	1.33	0	1.33
20	4	0	0
40	0	0	0
100	0	0	0
Control	0	0	0

Effect of Extracts of *Eruca sativa* Mill. on the Growth Inhibition of Some Fungal Species

Sirwan Hassan Salih and Mostafa Qahtan Mosataf

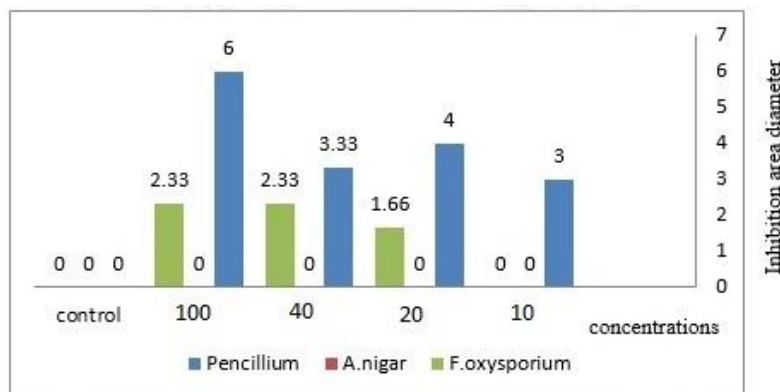


Figure 1: inhibitory effectiveness of aqueous extracts of the *E. sativa* against studied fungal species

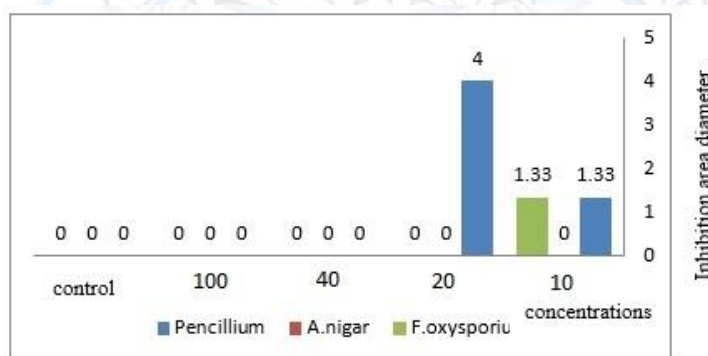


Figure 2: inhibitory effectiveness of glycosides extracts of the *E. sativa* against studied fungal species

The results also showed that the concentration of 10 mg/ml was only effective in the inhibition of *F. oxysporium* compared to control and to other concentrations, as it is shown in table (4), the ineffectiveness of glycosides extracts with concentration of (10, 20, 40 and 100 mg/ml) for the inhibition of *A. nigar* than controls figure (2). The leaves of this plant contain glycosides and sulfur glycosides which affect the cells proteins and thus inhibit the cell activity inside the cell (Kyung and Lee, 2001) in addition to the alkaloids, that stop manufacture of nucleic acids in living cell through the inhibiting DNA Gyrase enzyme (Al-Palani, 2003). The saponine also affect the removal of living cells membranes (Qutib,1979) and the tannins are working on the inhibition of enzymes and carrier proteins in the cell membrane (Cowon, 2000), the mechanical

Effect of Extracts of *Eruca sativa* Mill. on the Growth Inhibition of Some Fungal Species

Sirwan Hassan Salih and Mostafa Qahtan Mosataf

action of glycosides and sulfur glycosides include the interaction with a group (-SH) in cells, which presence in cells proteins' that interact with amino acid cysteine and likewise make bi-sulfur bond (S-S) and the sulfur group (SH-) has a special stimulators multiply the cells where the sulfur compounds are working on break down this group and consequently will discourage biological activity and replication in the cell (Kyung and Lee, 2001)

Conclusions

This study concluded that the aqueous extracts of the *Eruca sativa* with concentrations (10, 20, 40 and 100 mg/ml) has effectiveness in the inhibition of two fungal species *Pencillium* and *F. oxysporium*, which the concentration of 20, 100 mg/ml with highest effective inhibition compared to the others, while the concentrations of 10 and 40 mg/ml are the least effective in the inhibition of *Pencillium*, the alcoholic extracts was ineffective in the inhibition of studied fungus species, whilst the concentration of 20 mg/ml of glycosides extracts seemed inhibitory effective against the species *Pencillium* that higher than the concentration of 10 mg/ml. and the concentration of 10 mg/ml was only effective in the inhibition of *F. oxysporium*, however the growth of the species *A. niger* was not affected by all concentrations of all extracts.

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References

1. Abdou, I. A.; Abou-Zeid A. A.; El-Sherbeeney, M. R. and Abo-El-Gheat, Z. H. (2005). Antimicrobial activities of *Allium sativum* L. , *Allium cepa* L., *Raphanus sativus* L., *Capsicum frutescens* L., *Eruca sativa* Mill. and *Allium kurrat* L. on bacteria, Plant Foods Human Nutrition, 22 (1): pp: 22-29.
2. Al-Ani, Hilal; A. J. (1998). Study of local black bean components *Nigella sativa* extracts and the effect of its extracts on some of Microorganisms. Master Thesis, college of Science, University of Mustansiriya, Iraq

Effect of Extracts of *Eruca sativa* Mill. on the Growth Inhibition of Some Fungal Species

Sirwan Hassan Salih and Mostafa Qahtan Mosataf

3. Al-Dalali, B. K. and Al-Hakim, S. H. (1987). Food analysis, dar al-kutib, Mosul University. Iraq.
4. Al-Joboory, A. and Al-Rawi, M. (1994). Natural pharmacology. 1st ed. Baghdad, dar Al-Huriah.
5. Al-Palani and Rashid, M. (2003). Effect of the crude plant extracts and vasisin alkaloid of shrubby *Antirrhinum majus* L. on some of pathogenic prions.
6. Al-Shaikhly, M. A. S. et al. (1993). Practical Biochemistry, Mustansiriya University. Iraq.
7. Angiosperm Phylogeny Group (2009). "An update of the Angiosperm Phylogeny Group classification for the orders and families of flowering plants: APG III". Botanical Journal of the Linnaean Society 161 (2): pp: 105–121.
8. Cowon, M. M. (2000). Plant product as antimicrobial agents. Clinical Microbiology Reviews, 15: pp: 100-110.
9. El-Gengaihi, S. E.; Salem, A.; Bashandi, S. A.; Ibrahim, N. A. and El-Hamid, S. R. (2004). Hyperlipidemic effect of some vegetable oils in rats. Food Agri. and Env., 2 (2): pp: 88-93.
10. Evans, W. C. (1999). Tease and Evans Pharmacology. 14th ed WB Saunders company Ltd; London.
11. Fahmy, I. R. (1933). Constituents of plant crude drugs. 1st ed., Poul Barbey. Cairo.
12. Grand, A., Verpoort, R., Wonder gem, P.A. and Pousset, J.L. (1988). Anti-infection
13. Harborne, J.B., and Baxter, H. (1993). Phytochemical Dictionary. A hand book of Bioactive compound from plants. Taylor and Francis, London.
14. Holt, J. G.; Krieg, N. R.; Sneath, P. H. A.; Staley, J. T. and Williams, S. T. (1994). Gram-negative aerobic microaerophilic rods and cocci. In Bergeys manual of determinative bacteriology. 9th ed. Williams and Wilkins, U.S.A. pp: 71-100.
15. Hussein, F. T. K. (1985). Medicinal plants in Libya. Al-Fatih university. Arab Encyclopedia House, Libya. 1st print. Libya.
16. Kochhar, S. L. (1981). Economic Botany in the tropics. Elegant printers, New Delhi-India. pp: 153-158.

Effect of Extracts of *Eruca sativa* Mill. on the Growth Inhibition of Some Fungal Species

Sirwan Hassan Salih and Mostafa Qahtan Mosataf

17. Kyung, K. H. and Lee, Y. C. (2001). Antimicrobial activity of sulfur compound derived from some S-Alkyl-cysteine sulfoxides and Allium and Brassica food Rev. Int. 17(2): pp: 190-198.
18. Merza, H. H.; Hussein, H. H.; Tarawneh, K. A. and Shakhanbe, hJ. M. (2000). Effects of applications of some medicinal plant extracts used in Jordan on social aggression as well as testicular and prenuptial gland structures in male mice, Pakistan J. Biol. Sci., 3 (3): pp: 398-402.
19. Mohammed, H .C. and A. Rafiq. (2009). Investigating possibility of using least desirable edible oil of *Eruca sativa* Mill. in bio diesel production, Pakistan J. Bot., 41 (1): pp: 481-487.
20. Newall, C. A.; Anderson, L. A. and Phillip son, J. D. (1996). Herbal Medicines . A guide for Health care Professionals . The Pharmaceutical Press. London . pp:69-70.
21. phytotherapies of tree-Sarannah Senegal (west-Africa). 11-Antimicrobial activity of 33 species. J. Ethanopharmacol., 22: pp. 25-31.
22. Qutib and Fawzi, H. (1979). Medicinal plants cultivation and their components, Dar Al Arabia for the book Libya - Tunisia.
23. Roaiha and Amin, (1983). Herbal medicine in a scientific way include modern and ancient medicine, 7th edition. Dar al-qalam. Beirut. Lebanon .
24. Ukida, M.; Akihisa, T.; Yasukawa, K.; Tokuda, H.; Suzuki, T. and Kimura, Y. 2006. Anti-inflammatory, anti-tumor promoting and cytotoxic activities of constituents of Marigold (*Calendula aofficinallis*) flowers. J. Nat. Prod., 96: pp: 1692- 1696.
25. Yaniv, Z. (1997). Traditions Uses and Research on Rocket in Palestine. In Rocket: A Mediterranean crop for the world. Report of a workshop, 13-14. December 1996, Legnaro, Italy; Pignone, D., Padulosi, S., Eds.; IPGRI: Rome. pp: 76-80.