



Research Article

Impact of *Erodium moschatum* (L.) L.'Hér. extracts against some bacteria and fungi species

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Abstract: The effectiveness of *Erodium moschatum* extracts (ethanol and methanol, water) were tested against pathogenic bacteria (two gram positive: *Staphylococcus aureus* and *Streptococcus lactis* and two gram negative: *Escherichia coli* and *Proteus mirabilis*) and four pathogenic fungi (*Microsprum canis*, *Trichophyton mentagrophytes*, *Penicillium chrysogenum* and *Fusarium oxysporum*). Tannins, flavonoid and glycoside contents were studied by using HPLC technique and their antimicrobial activities were examined. The results showed the extracts efficacy on the tested microbes in which the ethanol with 11.11mm methanol with 11.22mm diameter were the most effective against the studied bacteria compared with the rest of the studied extracts, either in the fungi it was found that the studied extracts did not have significant differences in their effectiveness against the tested fungi, the results seemed that there are a difference in the concentration of studied active compounds according to the environment (geographical locations) and the difference in the plant parts, rutin showed the highest concentration in the entire plant (Sulaymaniyah) at concentration 22.92ppm compared to the rest of the compounds and Saladin locality and the studied plant part.

Keywords: Phytochemical, Bioactivity, *Erodium moschatum* (L.) L.'Hér. extracts

Introduction

The genus *Erodium* belongs to the family Geraniaceae which consist of 11 genus with 850 species, it include 75 species (Wagh *et al.*, 2015), widely distributed in the temperate and subtropical regions of the northern and southern hemispheres, in Iraq the *Erodium* has 15 species spread throughout the country (Al-Halfi, 2000). Medically some of *Erodium* species uses in treatment of diseases such as influenza, inflammation, fever, bowel disorders, liver disease, gas repellent and effective anti-oxidation (Bautista *et al.*, 2015). Extracts of some species are also used to inhibit the growth of different microbes (Proestos *et al.*, 2013 and Radulovic *et al.*, 2011) and contain aromatic oils and many effective chemical compounds (Leyton *et al.*, 2015 and Igwenyi, 2016). Thus this study aimed to:

- Identify the antimicrobial effectiveness of water and alcohol extracts against pathogenic fungi and bacteria.
- Identification of the active chemical content of flavonoid and tannin using HPLC technique.
- Separation of some effective compounds and study their antimicrobial efficacy.

Materials and Methods

Plant sampling: Plant specimens of the *E. glaucophyllum* were collected in different localities of Tikrit (Saladin) and Sulaimania governorate (middle and northeast Iraq) during the flowering period from March-May of 2015 and 2016. Specimens

were dried at room temperature away from the light (banning optical oxidation) thereafter grinded and placed in dark-airtight cans and kept in frozen until use.

Aqueous Extraction:

Hot water extracted was done according to Grand, 1988 with some modification done by mixing 40gm of plant powder (aerial plant parts) with 160ml of distilled water (1: 4 weight: volume) then heated to boiling point 100C° and add it to the plant powder, shaking the mixture and leave to cool down, placed in the refrigerator for 24 hours, then filtrated through several layers of medical gauze and filtrated again through the Buchner funnel by using Whatmann papers No. 1 to get rid of non-powdered parts and fibers to obtain raw liquid extract, mixture put in the rotary evaporator at a temperature not exceeding 40C° which working on the basis of evaporation under rarefied pressure to obtain a thick layer of extract, the extract has been dried at temperature (30-35C°) and then placed in airtight lid bottles and kept freeze until use. Cold water extraction was done in accordance to the previous steps by using cold water rather than hot water.

Ethanol Extraction:

Ethanol and methanol extract was prepared according to (Grand, 1988) method, as follows: 20gm of plant powder (aerial plant parts) as

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dissolved in 200ml of ethanol alcohol (80%) (1:10 weight: volume), the mixture was left in the refrigerator for 24 hours to soak, filtration was done through several layers of gauze and filtered again by using Whatmann papers No. 1 to get rid of non-powdered parts and fibers, after evaporation it was placed at a temperature under (40C°), alcohol was evaporated in the mixture, thick layer of extract was obtained, extract was dried at the temperature (30-35C°) then put the it in airtight bottles and kept frozen until use. Methanol extraction was followed according to previous steps were followed using of methanol rather than ethanol.

Glycosides Extraction: According to the method of Ukida, 2006, 10gm of plant powder was added to 100ml of ethyl alcohol (80%), mixture leaves for 24 hours in the refrigerator at 4-7C° and filtrated for getting ethanol extract, the extract was concentrated by rotary evaporator to one-third of its volume, 50ml of ether and 5ml of lead acetate solution (0.3 molarity) were added to the mixture by separation funnel with shaking and pulled the water layer, repeating the process three times and dried water drawn layer at a temperature of 30C° until full drying and then preserved in bottles with airtight lid and kept freeze.

Tannin extraction: Methods of (Ahmad and Nazil, 1989) was followed, 0.5gm of plant powder was mixed with 50ml of distilled water and boiled for 30 minutes, mixture filtrated and centrifuging (2000r/m) for 20 minutes, the floating part was transferred into volumetric flask (100ml) then 20ml of lead acetate solution (4%) was added to it and complete the volume with distilled water to 100ml, the extract was filtrated and dried by oven (60C°) then preserved in airtight bottles and kept freeze until use.

Sample preparation for estimation of flavonoid and tannin content: 1mg of dry plant sample was dissolved in 150ml of chloroform and placed it on stirrer hotplate for 10 hours and 0.5gm of tartaric acid was added to it, it was transferred to ultrasonic for 2 hours and filtrated through filter paper (0.45mm) and put in rotary evaporator to remove solvent and take the remaining, methanol was added to it to injection into the HPLC (Seal, 2016).

Separation conditions:

HPLC column: C18 (25 x 4.6mm I.D. 5µm).

Mobile phase

A = MeOH: Acetic Acid: D.W (10: 2: 88) ml

B = MeOH: Acetic Acid: D.W (90: 3: 7) ml

Wavelength 348 nanometers

Flow ratio: 1 ml/min.

Temperature 28C°

Standard concentration 3mg/m

$$\text{Unknown sample concentration } (\mu\text{g}) = \frac{\text{Standard solution concentration} \times \text{Specimen area}}{\text{Standard solution area}} \times \text{Number of dilutions}$$

Antimicrobial activity:

Preparation and sterilization of the stock solution was done according to (Egorove, 1985), in which 1gm of dry plant extract was dissolved in 10ml of distilled water to get a stock solution of 100mg/ml, the solution was sterilized to get rid of the bacterial contaminants, the solution was used as a source of the preparation of the dilutions 25, 50 and 100 mg/ml. Two species of gram positive bacteria (*S. aureus* and *St. lactis*) and two gram negative (*E. coli* and *P. mirabilis*) were activated directly prior to use, agar diffusion method was used by drilling to observe the microorganisms sensitivity to the studied plant extracts at concentrations 25, 50 and 100mg/ml, Mueller Hinton Agar media was used to grow the tested fungi.

Three control coefficients (negative, positive for bacteria and positive for fungi) were prepared by:

1. 3 pits was done and taking (0.5µl/hole) of distilled water and it was placed in the pit assigned to it by three dishes (Three replicates per treatment) (negative control).
2. Tetracycline tablet (30mg) was placed in the dish with three replicates of one treatment (positive control of the bacteria).
3. The first method was repeated except 0.5µl/hole of Nystatin (100mg/ml) was took, which acts as an antifungal and antibacterial (positive control of fungi and bacteria)

Results and Discussion

Antibacterial efficacy of extract concentrations:

The results showed the effectiveness of different extracts (ethanol, methanol, glycoside and tannin, hot water, cold water) at concentrations 25, 50 and 100 mg/ml in inhibition of *S. aureus*, *St. lactis*, *E. coli* and *P. mirabilis*, the diameter of the inhibition area is by (mm) compared to the control 1, 2 and 3 (table 1). The concentrations 50 and 100 mg/ml of ethanol extract with 11 and 11.5mm inhibitor diameter and concentrations 25, 50 and 100mg/ml of methanol with 11, 11 and 11.66mm diameter are the most effective in inhibiting bacteria compared to control 1, 3 and all concentrations of extracts, moreover control 2 is the most effective in inhibiting bacteria compared to controls 1 and 3 and all extract concentrations. The concentration of 100 mg/ml of hot water extract with 10mm diameter, cold water extract with 10mm diameter, concentrations of 25, 50 and 100mg/ml of ethanol and methanol with 10, 10 and 10mm diameter and concentrations of glycosides 10.67, 10.67 with 10mm diameter are the most effective in inhibiting species *S. aureus* compared to control 1, 3 and all concentrations. Control 2 with 20mm diameter is most effective in inhibition of *S. aureus*, the

concentrations of 25, 50 and 100mg/ml of all extraction methods (hot water, cold water and ethanol and methanol, glycoside and tannin) were more effective in inhibiting the species *St. lactis* compared to control 1 and 2. No significant difference among the concentrations of all extraction methods and control 3. The concentrations 25, 50 and 100 mg/ml of ethanol with 11.33, 10.67 and 12mm diameter and methanol with 10, 10 and 11.33mm diameter and concentrations of 50 and 100mg/ml of the glycoside with 10 and 10.67mm diameter were the most effective in inhibiting the species *E. coli* compared to control 1 and all concentrations. The concentrations 50 and 100mg/ml of ethanol with 13.33 and 14mm diameter and concentration of 100mg/ml of methanol with 13.33mm.

Diameter were most effective in inhibition the species *P. mirabilis* compared to control 1 and 3 and all extracts concentrations, furthermore the control 2 is the most effective in inhibiting the bacteria with a diameter of 19mm. The results revealed that the ethanol and methanol was the most effective in inhibiting bacteria compared to control and all the studied extracts with average 11.11 and 11.22mm. the species *St. lactis* and *P. mirabilis* were most affected by extract concentrations compared to *S. aureus* and *E.coli* with an average of 10.52 and 9.38mm diameter.

Antifungal efficacy of extract concentrations:

The results showed the efficacy of different extracts (hot water, cold water, ethane, methanol, glycoside, tannin) with concentrations of 25, 50 and 100mg/ml in inhibition of *M. canis*, *T. mentagrophytes*, *P. chrysogenum* and *F. oxysporum*, the diameter of the inhibition area is by (mm) compared to the control 1, 2 (table 2).

Table 1: Impact of *Erodium moschatum* extracts on the studied bacterial growth (mm)

Extract	Concentration mg/ml	Bacteria species				Average concentration	Average extract
		<i>P. mirabilis</i>	<i>E. coli</i>	<i>St. lactis</i>	<i>S. aureus</i>		
Hot water	25	A10	C3.33	A10	B8	7.83	bc 8.443
		cd	e	a	bcd	de	
	50	A10	B6	A10	B7.33	8.33	
		cd	cd	a	cde	cde	
	100	A10	B6.67	A10	A10	9.168	
		cd	cd	a	bc	bcd	
Cool water	25	B7.33	C2.67	A10.67	C4	6.17	c 7.667
		ef	ef	a	e	e	
	50	A10	C3.33	A10.67	B5.33	7.33	
		cd	e	a	e	de	
	100	A10	B7.33	A10.67	A10	9.500	
		cd	c	a	bc	bc	
Ethanol	25	A12	AB11.33	B10	B10	10.833	a 11.111
		bc	b	a	bc	bc	
	50	A13.33	B10.67	B10	B10	11.000	
		b	b	a	bc	b	
	100	A14	B12	C10	C10	11.500	
		b	b	a	bc	b	
Methanol	25	A12	B10	A12	B10	11.000	a 11.222
		bc	b	a	bc	b	
	50	A12	B10	A12	B10	11.000	
		bc	b	a	bc	b	
	100	A13.33	BC11.33	AB12	C10	11.000	
		b	b	a	bc	b	
Glycoside	25	C3.33	B6	A12	A10.67	8.00	ab 9.722
		h	cd	a	bc	cde	
	50	B9.33	B10	A12	AB10.67	10.500	
		de	b	a	bc	bc	
	100	B10	AB10.67	A12	B10	10.668	
		cd	b	a	bc	bc	
Tannin	25	B5.33	C2.67	A12	B5.33	6.33	c 7
		fg	ef	a	e	e	
	50	B6	C2.67	A12	B6.67	6.84	
		fg	ef	a	de	e	
	100	C5.33	C4.67	A12	B9.33	7.83	
		fg	ef	a	bcd	de	
control	Distilled water 1	A 0	A 0	A 0	A 0	0.000	d 0
	Tetracycline 2	A 19	B 16.67	C 7	A 20	15.67	a 12.17
	Nystatin 3	B4.67	A 10	A 10	A 10	8.67	
Average bacteria		9.38 A	7.52 B	10.52 A	8.92 AB		

* the numbers represent the average of three replicates

- similar large horizontal letters mean no significant differences between them at the probability (0.05).
- similar small vertical letters mean no significant differences between them at the of probability (0.05).

Table 2: Impact of *Erodium moschatum* extracts on the studied Fungal growth (mm)

Extract	Concentration mg/ml	Fungal species				Average concentration	Average extract
		<i>F. oxysporum</i>	<i>P. chrysogenum</i>	<i>T. mentagrophytes</i>	<i>M. canis</i>		
Hot water	25	B 10 c	AB 11.67 b	A 13 bc	C 7.33 bcd	10.50 b	b 7.472
	50	B 10 c	A 12.67 b	AB 11.33 bc	C 7.33 bcd	10.33 b	
	100	B 10.33 c	A 12.33 b	A 12.33 bc	C 8 bc	10.75 b	
Cool water	25	A 10 c	A 10 b	A 11 bc	B 4.67 d	8.92 b	b 6.602
	50	B 10 c	A 12.67 b	AB 11 bc	C 4.67 d	9.59 b	
	100	B 1 c	A 12 b	AB 11 bc	C 6.67 bc	9.92 b	
Ethanol	25	A 7 d	A 9.33 b	A 10 c	A 8.33 bc	8.665 b	b 6.292
	50	A 10 c	A 9.33 b	A 10 c	A 8 bc	9.332 b	
	100	A 10 c	A 9.33 b	A 10 c	A 9.33 b	9.665 b	
Methanol	25	A 10 c	A 10.67 b	A 10.33 c	B 4.67 d	8.92 b	b 5.877
	50	A 10 c	A 10.67 b	A 10.67 c	B 5 cd	9.09 b	
	100	A 10 c	A 11 b	A 10.67 c	C 5 cd	9.17 b	
Glycoside	25	A 12.33 bc	AB 10.33 b	A 13 bc	B9.33 b	11.247 b	b 6.737
	50	A 13.67 b	AB 9.33 b	AB 11.67 bc	B 8.67 bc	10.84 b	
	100	A 12.33 bc	AB 9.67 b	A 12 bc	B 8.33 b	11.50 b	
Tannin	25	AB 11.33 bc	B 10 b	A 14.33 b	C 5.67 cd	10.33 b	b 7.223
	50	A 11.33 bc	A 11 b	A 12 bc	B 9.33 b	10.915 bbc	
	100	A 11.67 bc	A 11.33 b	A 14 d	B 9 b	11.50 b	
control	Distilled water 1	A 0 e	A 0 c	A 0 d	A 0 e	0.000 c	c 0
	Nystatin 2	A 32 a	A 33.33 a	A 32.67 a	A 33 a	33.000 a	a 33
Average fungi		11.099A	11.333 A	12.05 A	B 8.116		

* the numbers represent the average of three replicates

- similar large horizontal letters mean no significant differences between them at the probability(0.05).

- similar small vertical letters mean no significant differences between them at the of probability (0.05).

There is no significant variations among the extract concentrations in inhibition of the studied fungi compared to control 1. The control 2 is the most effective in inhibiting fungi compared to the extract concentrations and control 1 with the average of 33mm. Concentration 100mg/ml of ethylene with 9.33mm diameter, 2mg/ml of glycoside with 9.33mm diameter and concentration 50 and 100mg/ml of tannin with 9.33 mm and 9mm diameter are the most effective in inhibition of *M. can* compared to control 1 and the and all concentrations. The concentrations 25 and 100mg/ml of tannin with 14.33mm and 14mm diameter were the most effective inhibition of *T. mentagrophytes* compared to control 1 and all extract concentrations. However there was no significant difference among the extract concentrations in their effectiveness in inhibition of *P. chrysogenum* compared to control 1, while the concentration 50mg/ml with 13.67mm is the most effective in inhibiting the species *F. oxysporum* compared to the control.

Table 3: Active chemical content of the *E. moschatum*

<i>E. moschatum</i>		Active compounds (ppm.)				
Locality	Plant part	Tannins	Quercetin	Catechin	Rutin	Kaempferol
Saladin	Whole plant	+	0.092	0.002	1.68	0.17
	Vegetative part	0.007	0.043	0.002	0.22	0.002
	flowers	0.003	0.010	0.013	0.157	+
	fruit	0.012	0.011	0.049	0.604	0.002
Sulaimanya	Entire plant	3.836	2.870	4.015	22.92	0.058

(+) means the compound with low concentrations

Results confirmed there is no significant difference among the six extracts of studied plants in their effect in inhibiting the examined fungi and no significant difference among *M. canis*, *T. mentagrophytes*, *P. chrysogenum* and *F. oxysporum* affected by concentrations of plant extracts.

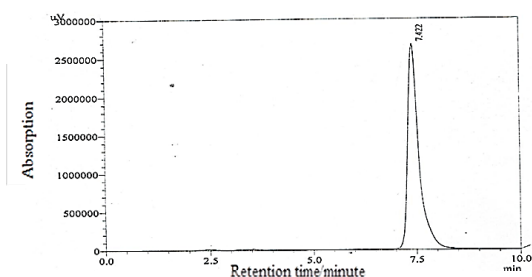


Figure 1. Tannin standard curve by HPLC of studied

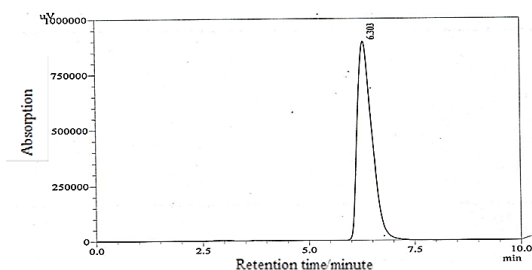


Figure 2. Quercetin standard curve by HPLC of

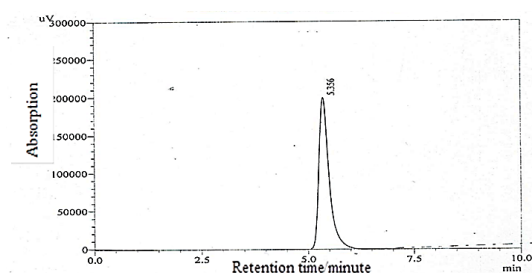


Figure 3. Catechin standard curve by HPLC of

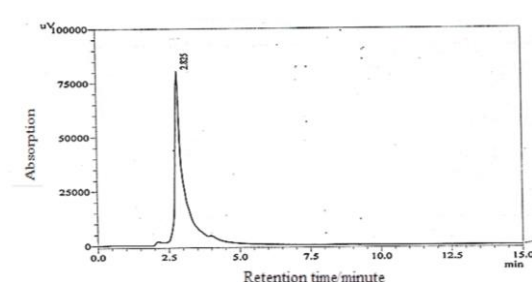


Figure 4. Rutin standard curve by HPLC of studied

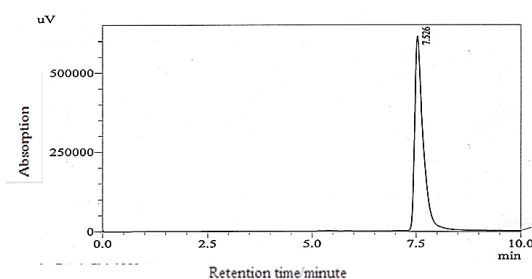


Figure 5. Kaempferol standard curve by HPLC of

Erodium species have been found to contain several types of flavonoids and glycosides that are effective in their anti-microbial activity, which are water-soluble (Öhretoulu, 2011 and Leyton, 2015), as for the hot water extract, the effectiveness against bacteria and fungi may be due to Gallic acid which is found in the family Orobanchaceae that dissolves in hot water 60°C and has the effectiveness of anti-bacteria and fungi or may return to tannins due to

the hot water is the best solvent used to isolate it (Ahmad, 1989 and Pantev *et al.*, 2006). The inhibitory effect of the hot or cold water extract may be due to interference between the active compound or the interaction between the active compounds therefore it may reduce or increase the inhibitory activity against the microorganisms, the inhibitory activity may be due to some volatile oil dissolved in water. The inhibitory activity of ethane and methanol extracts is mainly due to the existence of active soluble compounds in alcohol, especially phenols such as flavonoids (quercetin and kaempferol), tannins and rutin or interaction with each other or with other active compounds dissolved in alcohol. Pantev *et al.*, (2006) was indicated to that the anti-bacterial and fungal efficacy is due to the flavonoid content of the ethanol extract of tannins, rutin, quercetin and kaempferol. The different effect of one plant extract on microorganisms activity varies according to the extraction method (Nostro, 2000), in water glycosides extract may be due to the role of glycoside, especially flavonoids (rutin) or non-diabetic part of the glycoside structure (Nostro, 2000 and Cowan, 1999). The scarcity of studies on glycoside content and its bioactivities of studied plants which requires further research and investigation. The anti-microbial activity of tannins is due to its ability to inhibit proteins transfer enzymes (transferase) existent in cell membrane, tannins are described as a group of phenol compounds and are classified into two groups: biodegradable tannins and intensive tannins, both of which have antimicrobial efficacy, it is highly effective in inhibiting the peroxidase enzyme and can interfere with the function of the cell wall (Scalbert, 1991 and Ukida *et al.*, 2006).

Active chemical content of the *E. moschatum*:

The aerial plant parts of *E. moschatum* growing in Saladin and Sulaymaniyah contains all effective studied active compounds (table 3), the table showed that the whole plant growing in Saladin contains rutin at a higher concentration than the other studied active compounds at 1.68ppm followed by kaempferl 0.17ppm, quercetin at 0.092ppm and catechin at 0.002ppm, moreover vegetative parts of the plant contain rutin at a higher concentration than other active compounds at 0.22ppm followed by quercetin at 0.043ppm, tannins 0.007ppm, catechin 0.002ppm and kaempferl 0.002ppm. In the flowers the rutin was higher than the rest of the active compounds at 0.157ppm followed by catechin 0.013ppm, quercetin 0.010ppm and tannins 0.003ppm. In the fruits the rutin with a higher concentration than others at a concentration of 0.604ppm followed by catechin at 0.049ppm, quercetin, tannins and kaempferl at ppm 0.011, 0.012 and 0.002 respectively. In the entire plant growing in Sulaymaniyah the rutin has a higher concentration than others at ppm 22.92 followed by catechin at 4.015ppm, tannins at

3.836ppm, quercetin at 2.870ppm and kaempferl at 0.058ppm. The whole plant growing in Sulaymaniyah contains tannins with higher concentration compared to the rest of the plant parts at a concentration of 3.836ppm followed by fruits at a 0.012ppm, vegetative plant parts at 0.007ppm and flowers at 0.003. Quercetin, Catechin and rutin are found at a higher concentration in the entire plant growing in Sulaymaniyah compared with the rest of the plant parts at concentration of ppm 2.870, 4.015, 22.92 and kaempferl with a higher concentration in the entire plant growing in Saladin at a concentration of 0.17ppm. The whole plant growing in Sulaymaniyah has a higher concentration of tannins, quercetin, catechin, and rutin than other growing Saladin, the whole plant in Saladin has a higher concentration of kaempferl than of Sulaymaniyah.

Some of plant specimens contained different concentrations of flavonoids (quercetin, catechin, rutin and kaempferol) that is agreed with the study (Lin JH and Lin MF, 1997), which showed that *E. moschatum* contains several compounds (protocatechuic acid, Gallic acid, methyl Gallate, caffeic acid, brevifolincarboxylic acid and flavonoids (kaempferol, quercetin). Regarding the impact of the environment (locality) the study agreed with the results of (Dyubeni *et al.*, 2012), which showed that the environment affected the quality and quantity of active compounds (volatile oils) in *Pelargonium capitatum* and *Pelargonium radens* as well as with the study (Abdul-Wahab, 2016) on *Pelargonium graveolens* which showed that the effective content varies according to the plant environment.


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