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CHEMICAL AND MICROBIAL STUDIES ON *Reaumuria hirtella* JAUB and SPASH

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ABSTRACT

The main objectives of the current investigation were to study the chemical constituents as well as the anti-microbial effect of Egypt *Reaumuria hirtella* growing naturally in the Western Mediterranean Coastal region (Ageba region) in different seasons. Phyto-chemical screening of the plant revealed the presence of sterols, tannins, flavonoid, alkaloids, carbohydrates, sulphates and chlorides. It is clear that the total flavonoids and tannins reached their maximum values (2.08%, 8.63%, respectively) during winter while the total phenolics and alkaloids reached their maximum values (1.52%,0.38% respectively) during summer. We also indicated that, glucose, maltose, ribose, arabinose and sucrose are present as free sugars while glucose, ribose, arabinose and rhamnose are present as combined sugars. The amino acid analysis showed that, the plant contains 12 free amino acids and 15 protein amino acids. In addition, the chemical constants of lipids of *Reaumuria hirtella* were determined. The unsaponifiable matter of the lipid contains 10 hydrocarbons and *β*-sitosterol as well as the fatty acid content showed that, stearic acid was found as major content followed by nine fatty acids. Preliminary screening of the anti –microbial activity of different successive extracts (petroleum ether, ether, chloroform, ethyl acetate, acetone, ethyl alcohol 70% and water) of *R. Hirtella* against some pathogenic microorganisms respectively three species of bacteria and two species of fungi were performed using the method described by Zahra (1990). All extracts were not effective in inhibiting the growth of the organisms except with the high concentration (2000 ppm) of ethyl acetate. From these data, it is suggested that under stress *Reaumuria hirtella* plants tend to accumulate secondary metabolic products, which may be a part of adaptation to unfavourable conditions.

Key words: *alkaloids, chemical, flavonoids, microbial, phyto-chemical screening, tannins and phenolic compounds.*

1. INTRODUCTION

Reaumuria hirtella belongs to family Tamaricaceae which is a limited family, it contains only 5 genera and 79 species distributed all over the world. The family is native to dry areas, many grown on saline soils, tolerating up to 15,000 ppm soluble salt and can also tolerate alkaline conditions. Many plants of this family are used as medicinal plants (Mabbereley, 2008). Genus *Reaumuria* contains four species (*Reaumuria hirtella*, *Reaumuria mucronata*, *Reaumuria vermiculata* and *Reaumuria alternifolia).* Stems and often also the leaves of *Reaumuria hirtella* are covered during daytime by salt crystals, which absorb water during the night when the shrublet is bading in water drops. Flowers white or pink with purple anthers (Tackholm, 1974).

Yoshida *et al.* (1991) investigated the tannins of the leaf extract of *Reaumuria hirtella* and found that, it contains two new monomeric hydrolysable tannins, remurin A (12), remurin A (13) and a new dimer hirtellin A (7). Four new dimeric hydrolysable tannins, hirtellins C, D, E, F besides previously hirtellins A and B have been isolated from the leaf extract of *Reaumuria hirtella* (Yoshida *et al.*, 1993). New dimeric, trimeric and tetrameric ellagitannins, hirtellin T2 (1), hirtellin T3 (25), hirtellin T (5), hirtellin G (1) and hirtellin Q (6) have been isolated from the leaf extract of *Reaumuria hirtella* (Ahmed *et al.*, 1994 a&b). Nawwar *et al.* (2012) isolated three ellagitannins and one disulphated flavonol from the aerial parts of *Reaumuria vermiculata*.

The medicinal values of certain members of *Reaumuria* which have been in literature, stimulated us to carry out the present study.

2. MATERIALS AND METHODS

The aerial parts of *Reaumuria hirtella* Jaub.

& Spach. were collected during winter, spring, summer and autumn seasons of 2013 from Ageba region (Northern Coastal Region Egypt). The collected plants were cleaned, dried in an oven at 60 ºC for 48 hours, ground to fine powder and presented for the following chemical investigation.

2.1. Preliminary phytochemical screening

2.1.1. Steam distillation

About 50g of fresh plant material were extracted by steam distillation according to British Pharmacopoeia (1980) method for volatile oil content.

2.1.2. Screening of the active constituents

About 50 g of air dried powdered plant material were refluxed with about 50 ml of 80 % ethyl alcohol for 6 hours, then filtered. The residual powder was then washed several times with hot alcohol. The combined alcohol filtrates were concentrated under reduced pressure at 50ºC, then used for determination of tannins and saponins (Balbaa 1986), sterols and terpens (Balbaa *et al.,* 1981), flavonoids (Wall *et al.* 1954), alkaloids (Woo *et al.,* 1977), carbohydrates and/or glycosides (Harper, 1975), chlorides and sulphates (A.O.A.C. 1970), coumarins and anthraquinones (Fransworth, 1966).

2.2. Extraction using organic solvents

2.2.1. Successive extraction technique

Powdered air-dried plant material were subjected to extraction with successive selected organic solvents using soxhlet apparatus, in order of increasing polarity including petroleum ether (B.p. 40-60 ^{o}C), diethyl ether, chloroform, acetone , ethyl acetate, 96%, ethyl alcohol and 70% ethyl alcohol. The obtained residue from each solvent was dried and weighed.

2.2.2. Selective extraction technique

The powdered air dried plant material were extracted completely once using soxhlet apparatus with the same solvents. The obtained residue from each solvent was calculated by weighing after complete dryness.

2.3. Determination of constants and other constituents

Water content was estimated according to the method described by Rowell (1994), the total, soluble and insoluble carbohydrates were determined according to Chaplin and Kennedy (1994). The total nitrogen and protein contents were determined according to James (1995) and the total lipids were determined according to Christie (1982). The total ash content, acid and water insoluble ash and the crude fiber contents were determined according to Askar and Treptow (1993).

2.4. Determination of active constituents

Total flavonoids (Karawya and Aboutable, 1982), total alkaloids Balbaa (1986) and Woo *et al.* (1977), total phenolics (Pulido *et al.* 2000) and total tannins (Makkar and Googchild 1996). were determined.

2.5. Investigation of carbohydrates

Free and combined sugars were determined during winter season using paper chromatography (Chaplin and Kennedy, 1994).

2.6. Identification and fractionation of free and protein amino acids

The free and hydrolyzed protein-amino acids were determined during winter using amino acid analyzer according to the method described by Pellet and Young (1980).

2.7. Determination of crude lipids

The lipids were extracted from the powdered plant collected at winter with petroleum ether (B.P. 40-60ºC): ether (1:1 v/v) for 24 hours using soxhlet apparatus. The lipids were obtained by distilling off the solvent. The last traces of the solvents were removed by heating the liquid sample in vacuum oven a 50 ºC to constant weight.

2.7.1. The fundamental chemical properties

Acid value (A. V.), Iodine value (I. V.), ester value (E. V.) and saponification value (S. V.) were determined according to Mohamed and Amer (1965).

2.7.2. Investigation of fatty acids and unsaponifiable matter

The extracted lipids of *Reaumuria hirtella* were saponified and purified according to British Pharmacopoeia (1980). The unsaponifiable fraction was removed from the soapy solution by shaken in a separating funnel for several times with fresh portions of peroxide-free ether until complete extraction was obtained.

The soapy solution was converted into the corresponding free fatty acids by using of 2.5 % sulphuric acid. When the acid was completely liberated, they were collected by ether extraction. The ether extracted was washed several times with distilled water until free from acids, dried over anhydrous Na_2SO_4, then filtered and the ether was removed by distillation. The last traces of ether were removed under vacuum at 60° C, and then cooled in a desiccator.

2.7.3. GLC of fatty acids

The extracted fatty acids and the standards were converted to the corresponding methyl

esters using ethereal solution of diazomethane (Farag *et al.*, 1986). The methyl ester of the fatty acids was analyzed with a GCV. Pye-Unicam gas chromatography apparatus. The fraction of fatty acid methyl esters were conducted using a coiled glass column (**150m4mm**), packed with diatomite C (100-120 mesh) and coated with 10% polyethylene glycol.

The column oven temperature was programmed at 10 °C /minute for 100, 200°C, then isothermally at 200 °C for 15 minutes with nitrogen at 30 ml/ minutes. Peak identification was performed by comparing the relative retention time of each compound with those of standard material. The relative proportion of each individual compound was estimated as the ratio of the partial areas of the total area as mentioned by Fryer *et al.* (1960), Nelson *et al.* (1969), Farag *et al.*, (1986) and Khalil (1987).

2.7.4. GLC of unsaponifiable matter

The relative percentage of each unsaponifiable compound was determined using triangulation method according to Nelson *et al.* (1969). The results of Itoh *et al.* (1973) and Farag *et al.* (1986) were used as a guide to characterize some of the unknown compounds.

2.8. Anti-microbial activity

The effect of different extracts of *Reaumuria hirtella* on the growth of some micro-organisms (bacteria and fungi) was studied by a method described by Zahra (1990).

3. RESULTS AND DISCUSSION

3.1. Preliminary phytochemical screening

The preliminary phytochemical screening of *Reaumuria hirtella* extract showed that, it contains sterols, tannins, alkaloids, flavonoids, carbohydrates and / or glycosides, sulphates and chlorides. Neither volatile oil, saponins, coumarins nor anthraquinones were detected as represented in Table (1).

Table (1): Preliminary phytochemical screening of *R. hirtella*.

Test	Result
Volatile oil	-
Sterols	+
Tannins	+
Alkaloids	+
Saponins	-
Flavonoids	+
Carbohydrates and/or glycosides	+
Sulphates and chlorides	+
Coumarins	-
Anthraquinones	-

3.2. Extraction using organic solvents

3.2.1. Successive extraction

Data presented in Table (2) showed that ethyl alcohol 70% extract was the highest value (4.89%), followed by ethyl alcohol 96% extract. The minimum value of residues was that of chloroform extract (0.25%).

3.2.2. Selective extraction

Data presented in Table (2) showed that , ethyl alcohol 70% had the highest value (12.08%) while petroleum ether extract had the lowest value (1.02%).

3.3. Constants and constituents

Table (3) showed that, the moisture content of *Reaumuria hirtella* reached its maximum value (43.28%) during winter and its minimum value (30.26 %) during the summer. The decrease of water content in the summer may be due to the increase in the rate of transpiration of the plant and evaporation accompanied with increasing of wind velocity and temperature (Jain , 1997). The ash content of *Reaumuria hirtella* plant reached its maximum value (12.20 %) during summer, while its minimum value (10.14%) during winter season. This may be attributed to the increase in total ion accumulation because of increasing soil moisture stress during summer. This agrees with the findings of Larcher (1995), Al-Owaimer *et al.* (2008) and Ahmed and Emam. (2009). Water soluble ash reached its maximum value (7.82%) during autumn, while acid insoluble ash reached its maximum value (13.50%) during the summer season.

The percentages of total nitrogen and total protein reached their maximum values (3.50% and 21.86%, respectively) during winter and their minimum values (1.25% and 7.81%, respectively) during the summer season. This may be due to the increase in the metabolic rate of the plant as a result of high water resources of the soil during winter than that during the dry period. Aganga *et al.* (2003) reported that, *Atriplex nummularia* contains high concentrations of nitrogen in winter as compared to summer when it has high concentration of sodium.

The total lipid content of *Reaumuria hirtella* reached its maximum value (4.06%) during winter and its minimum value (1.87%) during summer. This may be due to the increase in metabolic rates of *Reaumuria hirtella* during winter which leads to an increase in carbohydrate content, which is converted to lipid by oxidation reaction.

Table (2): Percentage of residues of *Reaumuria hirtella* using successive and selective extraction techniques.

Extraction	Solvents						
	Petroleum ether	Ether	Chloroform	Acetone	Ethyl acetate	Ethyl alcohol	Ethyl alcohol 70%
Successive	1.02	1.18	0.25	0.86	1.98	2.28	4.89
Selective	1.02	4.12	2.81	7.28	9.92	9.22	12.08

There was a tendency to a gradual decline in total carbohydrates from winter to summer samples, where the percentage of the total carbohydrate content reached its maximum value (26.62%) during winter, while its minimum value (22.78%) during the summer season. Abo-Kassem *et al.* (2002) reported that, high salt concentration can result in osmotic adjustment by regulating the accumulation of solutes especially sugars and proteins. Crude fiber content of *Reaumuria hirtella* reached its maximum value of (38.00%) in winter, while its minimum value (20.98%) was recorded in autumn season as shown in Table (3).

The results show that the presence of total phenolics reached its maximum value (1.52%) during summer, while its minimum value (0.81%) during winter. This may be due to increase activity of esterase and peroxidase enzymes which are responsible for the oxidation of phenolic compounds in plant tissues.

Also, the percentages of total alkaloids reached their maximum value (0.38%) during summer and their minimum value (0.18%) during autumn. This agrees with the findings of El-Lamey (2005), who reported that there was a tendency of some medicinal plants to accumulate alkaloids by stress, where the accumulation of alkaloids was significantly increased in dry seasons.

Table (3): Mean values of constants and constituents of the studied plant in different seasons.

Constants and constituents	Winter	Spring	Summer	Autumn
Moisture content %	43.28	41.32	30.26	36.82
Ash content %	10.14	10.30	12.20	11.18
Water-soluble ash %	6.25	5.36	4.28	7.82
Acid-insoluble ash%	12.06	11.86	13.50	12.60
Total nitrogen content mg/g dry wt.	3.50	2.72	1.25	1.89
Total protein content mg/g dry wt.	21.86	16.94	7.81	11.77
Total lipid content %	4.06	3.40	1.87	2.58
Soluble carbohydrates content mg/g dry wt.	11.82	13.69	18.80	17.60
insoluble carbohydrates content mg/g dry wt.	14.80	11.31	3.98	5.10
Total carbohydrates mg/g dry wt.	26.62	25.30	22.78	23.70

3.4. Active constituents

Total flavonoids, total phenolics, total alkaloids and total tannins contents are showen in Table (4). The percentage of flavonoids reached its maximum value (2.08%) during winter and its minimum value (1.58%) during summer season. Flavonoids and tannins are phenolic compounds and plant phenolics are a major group of compounds that act as primary antioxidants or free radical scavengers (Polterait, 1997).

On the other hand, the highest content of tannins (8.63%) was detected during winter season and the lowest percentage (7.13%) during autumn season. The high percentage of tannins in *Reaumuria hirtella* may encourage their probable use in diarrhea, piles, as well as tanning (Ghazanfer, 1994).

Table (4): Percentages of the active constituents of *R. hirtella* in different seasons during the period of study (2013).

Item %	Winter	Spring	Summer	Autumn
Total flavonoids	2.08	2.02	1.58	1.62
Total phenolics	0.81	0.88	1.52	1.48
Total alkaloids	0.32	0.30	0.38	0.18
Total tannins	8.63	8.16	7.52	7.13

3.5. Identification of free and combined sugars

Data shown in Table (5) illustrate the result of investigation of free and combined sugars of *Reaumuria hirtella* which revealed the presence of glucose, maltose, ribose, arabinose and sucrose as free sugars but glucose, ribose, arabinose and rhamnose were present as combined sugars.

Table (5): Free and combined sugars of *Reaumuria hirtela*.

Sugar	Free sugar	Combined sugar
Glucose	+	+
Maltose	+	-
Ribose	+	+
Arabinose	+	+
Sucrose	+	-
Rhamnose	-	+
Raffinose	-	-

3.6. Free and protein amino acid contents of *R.hirtella*

The investigation of the free and hydrolyzed protein amino acids was achieved using amino acid analyzer, where each component was calculated and presented in Table (6), twelve free amino acids were presented in *Reaumuria hirtella*. Proline had the highest concentration (0.12 mg/100gm protein) while tyrosine had the lowest value (0.0026 mg/100gm protein). The obtained results also revealed that, the detected protein amino acids present in *Reaumuria hirtella* were 15 amino acids, where aspartic acid, glutamic acid and serine represent the highest concentrations of the separated protein amino acids.

3.7. Lipid content

3.7.1. Fundamental chemical constants of *R.hirtella*

The fundamental chemical constant of the extracted lipids of *Reaumuria hirtella* are presented in Table (7). The acid value reached the value of 76.24, the ester value was 826.54, the saponification value was 902.68 and the iodine value was 23.08.

Table (7): Acid, iodine, esterification and saponification values of lipids of *Reaumuria hirtella*.

constant	Value
Acid value	76.24
Esterification value	826.54
Saponification value	902.68
Iodine value	23.08

3.7.2. Investigation of Unsaponifiable matter fraction

The unsaponifiable matter content (hydrocarbons and sterols) of *Reaumuria hirtella* was determined using GLC technique, where the relative of each component was calculated and tabulated in Table (8). The obtained results indicated that, *Reaumuria hirtella* contained n-octadecane, n-eicosane, n-docosane, n-tricosane, n-tetracosane, n-pentacosane, n-hexacosane, n-octacosane, n-tricontane, n-dotriacontane and β-sitosterol, while n-octacosane had the highest concentration followed by n-hexacosane.

Table (6): Free and protein amino acids of *Reaumuria hirtella* **using amino acid analyzer.**

Amino acid	R.t.	Free amino acid (mg/100 gm protein)	Protein amino acid (mg/100 gm protein)
Aspartic acid	11.34	0.0082	0.2068
Threonine	14.51	-	0.0387
Serine	15.77	0.0152	0.0972
Glutamic acid	17.62	0.0044	0.1295
Proline	20.43	0.1200	0.0546
Glycine	24.46	0.0122	0.0732
Alanine	27.48	0.0118	0.0968
Valine	32.75	0.0058	0.0335
Isoleucine	44.38	0.0042	0.0286
Leucine	45.65	0.0028	0.0169
Tyrosine	49.20	0.0026	0.0787
Phenyl alanine	51.69	0.0082	0.0862
Histidine	58.14	0.0158	0.0329
Lysine	62.50	-	0.0278
Argenine	67.60	-	0.0486

Rt : Retention time

Table (8): Gas liquid chromatography of hydrocarbons and sterols of *Reaumuria hirtella*.

compounds	Retention time	Number of carbon atom	Relative conc.
Unknown	**12.920**	**-**	**0.129**
n-Octadecane	**14.117**	**18**	**0.152**
n-Eicosane	**16.480**	**20**	**0.157**
Unknown	**17.483**	**-**	**0.102**
n-Docosane	**18.650**	**22**	**2.806**
n-Tricosane	**19.230**	**23**	**2.008**
n-Tetracosane	**20.400**	**24**	**5.652**
n-Pentacosane	**21.418**	**25**	**1.920**
n-Hexacosane	**22.283**	**26**	**8.723**
n-Octacosane	**24.133**	**28**	**12.308**
Unknown	**25.332**	**-**	**3.812**
n-Tricontane	**26.800**	**30**	**5.312**
n-Dotriacontane	**28.410**	**32**	**7.123**
Unknown	**30.900**	**-**	**1.128**
Unknown	**33.816**	**-**	**0.672**
***β*-Sitosterol**	**43.800**	**27**	**8.325**

3.7.3. Investigation of saponifiable matter fraction

Relative percentage of each component was calculated and tabulated in Table (9). The obtained result revealed that, the saturated fatty acid (stearic) represented the highest percentage of fatty acids (17.502%) while the lowest one was the saturated fatty acid (pelargonic) which had the value of (0.086%).

Table (9): Gas liquid chromatography of fatty acids of *Reaumuria hirtella*.

Name of fatty acids	Retention time	No. of carbon atom	Relative conc.
Unknown	**5.384**	**-**	**0.120**
Caprylic acid	**5.820**	**8.0**	**0.262**
Pelargonic acid	**6.717**	**9.0**	**0.086**
Capric acid	**9.717**	**10.0**	**0.482**
Unknown	**10.216**	**-**	**0.208**
Lauric acid	**11.083**	**12.0**	**0.826**
Myristic acid	**13.683**	**14.0**	**2.012**
Pentadecyclic acid	**15.100**	**15.0**	**0.828**
Palmitic acid	**16.200**	**16.0**	**11.270**
Unknown	**18.600**	**-**	**5.216**
Stearic acid	**20.583**	**18.0**	**17.502**
Linoleic acid	**21.560**	**18.2**	**8.813**
Unknown	**24.800**	**-**	**9.118**

3.8. Anti-microbial activity

The obtained data of the effect of different successive extracts, petroleum ether, chloroform, ethyl acetate, acetone, ethyl alcohol 70% and water on the inhibition of growth of some microorganisms such as bacteria (*Proteus vulgaris*, *Proteus mirabilis* and *Staphylococcus aureus*) and fungi (*Candida albicans* and *Fusarium oxysporum*) are shown in Table (10). The diameters of growth zone of fungi were measured whereas the total number of colonies of bacteria were counted. It can be concluded that, all the used extracts did not possess any antimicrobial activity except for the high concentration of ethyl acetate (2000 ppm) which had some effect on the three types of bacteria beside *Fusarium oxysporum*.

The present study provides a scientific basis of the use of these plant extracts in traditional health care system. Detailed work by using different methods will be the aim of further investigation. Also, the present study will be helpful for further research in the field of pharmaceuticals.

Table (10): Effect of different successive extracts of *Reaumuria hirtella* on growth of some bacterial strains and fungi.

Plant extract	Extract conc. (ppm)	Bacteria			Fungi	
		Proteus vulgaris	*Proteus mirabilis*	*Staphylococcus aureus*	*Candida albicans*	*Fusarium oxysporum*
Petroleum ether	250	-ve	-ve	-ve	-ve	-ve
	500	-ve	-ve	-ve	-ve	-ve
	1000	-ve	-ve	-ve	-ve	-ve
	2000	-ve	-ve	-ve	-ve	-ve
Ether	250	-ve	-ve	-ve	-ve	-ve
	500	-ve	-ve	-ve	-ve	-ve
	1000	-ve	-ve	-ve	-ve	-ve
	2000	-ve	-ve	-ve	-ve	-ve
Chloroform	250	-ve	-ve	-ve	-ve	-ve
	500	-ve	-ve	-ve	-ve	-ve
	1000	-ve	-ve	-ve	-ve	-ve
	2000	-ve	-ve	-ve	-ve	-ve
Ethyl acetate	250	-ve	-ve	-ve	-ve	-ve
	500	-ve	-ve	-ve	-ve	-ve
	1000	-ve	-ve	-ve	-ve	-ve
	2000	+ve	+ve	+ve	-ve	+ve
Acetone	250	-ve	-ve	-ve	-ve	-ve
	500	-ve	-ve	-ve	-ve	-ve
	1000	-ve	-ve	-ve	-ve	-ve
	2000	-ve	-ve	-ve	-ve	-ve
Ethyl alcohol 70%	250	-ve	-ve	-ve	-ve	-ve
	500	-ve	-ve	-ve	-ve	-ve
	1000	-ve	-ve	-ve	-ve	-ve
	2000	-ve	-ve	-ve	-ve	-ve
Water	250	-ve	-ve	-ve	-ve	-ve
	500	-ve	-ve	-ve	-ve	-ve

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دراسات كيميائية وميكروبية على نبات مليح

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ملخص

يهدف هذا البحث إلى دراسة التركيب الكيميائى لنبات مليح النامى بمنطقة الساحل الشمالى الغربى (منطقة عجيبة) خلال فصول السنة المختلفة وذلك للتعرف على المركبات الفعالة الموجودة به ومدى أختلافها خلال فصول السنة الأربعة. وجد بعد الأطلاع على المراجع والدوريات العلمية أن هذا النبات لم يتعرض لدراسة مستفيضة ولذلك تم أختيارة وقد أشتملت الدراسة على:

- مسح كيميائى أولى للنبات اتضح أنه يحتوى على أستيرولات وتانينات وقلويدات وفلافونيدات وسكريات مختزلة و/ أو جليكوسيدات بالأضافة إلى الكبريتات والكلوريدات.
- تقدير نسب المستخلصات للنبات باستخدام مذيبات عضوية مختلفة وبطرق استخلاص مختلفة.
- تقدير بعض الثوابت والمكونات الكيميائية المختلفة للنبات والتي أظهرت بعض الاختلافات في القيم في المواسم المختلفة .

- تقدير الفلافونيدات والفينولات والقلويدات والتانينات خلال فصول السنة الأربعة.
- التعرف على السكريات الحرة والمرتبطة باستخدام كروماتوجرافيا الورق.
- التعرف على الأحماض الأمينية الحرة والمرتبطة الموجودة بالنبات باستخدام جهاز تحليل الأحماض الأمينية.
- دراسة محتوى النبات من الدهون ودراسة خواصها الطبيعية والكيميائية باستخدام طرق تحليل الكروماتوجراف الغازى.
- دراسة تأثير تركيزات مختلفة لعدد من مستخلصات النبات المتتالية القطبية على بعض أنواع البكتريا والفطريات حيث أوضحت الدراسة أن مستخلصات النبات ليس لها تأثير مضاد للميكروبات ماعدا فى حالة التركيز العالى لمستخلص خلات الايثايل حيث وجد بعض التأثير على أنواع البكتريا الثلاث ونوع واحد من الفطريات.

اتضح من هذه الدراسة أنه تحت الظروف القاسية يميل نبات مليح إلى تراكم مواد الأيض الثانوية والتى ربما تكون عاملا هاما للتأقلم مع الظروف البيئية غير المناسبة لنمو النبات.

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