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The Effect of Aqueous and Alcoholic Extract of Asparagus (*Asparagus officinalis* L.) Roots on the inhibition of some Bacteria and Fungi

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Abstract: This study aimed to investigate the inhibitory leverage of the aqueous and alcoholic extracts from Asparagus (Asparagus officinalis L.) root as in inhibiting inhibit the growth of the pathogenic bacterium, and fungus in vitro study. Concentrations of 50, 100, and 150 mg.ml⁻¹ were used as aqueous extract and alcohol extract of Asparagus root against selected organisms, which tested using a drilling method, adding a 0.2 ml aqueous extract and alcohol extract of Asparagus root in every per well. Also 0.2 ml of distilled water and methanol alcoholic add as control and tetracycline as an antibiotic. GC/MS test results showed that the Asparagus root extract (ARE) contains various major phytochemical compounds such as flavonoids, phenols, alkaloids, glycosides, steroid, resin, saponins, and tannins. Also, it was observed that the alcoholic ARE had a higher inhibitory effect than aqueous ARE of Escherichia coli and Staphylococcus aureus. The highest diameter of inhibition at alcoholic extract concentrations of 50, 100 and 150 (mg.ml⁻¹) were 28, 32, 35, and 26, 31, 35 mm respectively for aqueous ARE, compared to other concentrations. Whereas the effect of the aqueous extract was higher for the Pseudomonas 17, 25 and 34 mm compared to other concentrations, except for the antibiotic, which obtained the highest value (41mm). Aqueous extract concentrations 50, 100 and 150 mg.ml⁻ ¹ showed higher efficiency than alcohol extract, antibiotic and control; when used against the fungus Aspergillus niger, and pathogenic yeast Candida albicans, the retarding diameters were 14, 17 and 23, and 11, 13 and 15 mm, respectively.

Keywords: Antimicrobial activity, Asparagus officinalis, Bioactive components, Quantitative analysis.

Introduction

The plant kingdom harbors an inexhaustible source of active ingredients invaluable in managing and treating many intractable diseases (Shariff, 2001). Even though, these complementary ingredients give the plant whole safety and efficiency much better than that of isolated and pure active components, ability to the agents which inhibit the microbial pathogens and (Husein *et al.*, 2019). There are many studies deals with the antimicrobial activity varied herbal extracts in various regions of the world. Because side effects and resistance pathogenic microorganisms build against antibiotics, lately, attention has been given herbal extracts and biologically active compounds isolated from plant species used in herbal medicine (Singh *et al.*, 2017; Al-Janae'e *et al.*, 2017 ; Aljazy *et al.*, 2019). *Asparagus officinalis* L. of orders Asparagales belonging to family Asparagaceae. The plant age reaches 20 years within the farmers' production fields (Kobus-Cisowska et al., 2019). A feature of this plant its tuberous roots keeps its vitality at the end of the germination season and remains until the next germination season during the winter season (Drost, 2018). Asparagus officinalis L. is a vegetable crop rich in bioactive compounds such as antioxidants and the most important characteristic of Asparagus is the multiple health benefits due to its high content of active compounds such as flavonoids, alkaloids, phenols, saponins, tannins. aromatic oils and others, which acts as antioxidant properties (Minh et al., 2019). It is used to treat wounds, cramps, allergies, malaria, leprosy, abortion, antipyretic and analgesic for pain, and contribute to the consolidation of the immune response (Seema et al., 2019). Besides, its role in treating constipation, diarrhea, osteoporosis, obesity, cardiovascular disease, rheumatism and diabetes (Iqbal et al., 2017). And for employing the extracts of this plant and its active compounds in the medical field and the possibility of using it in reducing diseases caused by pathogenic germs. This study was carried out to conduct a preliminary phytochemical screening and to know the inhibitory effect of the aqueous and alcoholic extracts of Asparagus root (Asparagus officinalis L.) against selected bacteria and fungi.

Materials & Methods

Collection of plant material

The Asparagus (*Asparagus officinalis* L.) plant roots were collected from the Eastern Island of Teeb, East of Maysan Governorate. The roots were cleaned from the dust, washed with tap water, cut into small pieces and dried in the shade. The roots were ground to a fine powder in an electric mixer, and then powders were stored in black plastic bags at room temperature (25°C), until the extraction process was performed. The plant is identified by Dr. Fatima Ali Hussein College of Agriculture, Dept. Horticulture and Garden Engineering.

Preparing the aqueous extracts

The aqueous extract was prepared according to the method described by Mbiantcha et al. (2011). First, the dried roots were cut and milled into a fine powder. Then 5g of fine dry powder of A. officialis was extracted with distilled water (500 ml), and then we do solution mix well to obtain a homogeneous mixture then leave for 4 hours in a horizontal shaker at medium speed, after leave which the sample is stabilize for an hour and then filter the mixture using filter paper (Whatman No.1). After that, the mixture was evaporated by using the oven at a temperature of 37 °C for seven days, then the concentrated extract obtained in a thick viscous form and the weight of the resulting extract was 3.6 g.

Preparation of alcoholic extract

A Harborne (1984) method is use to prepare the alcoholic extract of Asparagus officialis roots, after some modifications were made, by dissolving 50 g of the A. officialis roots powder with 250 ml of 70% methanol alcohol and leaving the mixture for 24 hours in a 37 °C water bath. Then the mixture was shaken for one hour, filtered with filter paper (Whatman No. 1). The filtrate solution was concentrated with a drying oven (37°C) until a very thick extract was obtained by evaporating the most significant possible amount of solvent. The extract yield from the weight of 50 g of Asparagus root is appreciate to be 5 g. Then dissolved in 50 ml of distilled water and kept in sealed glass bottles in the refrigerator until it will be used in vitro experiment for evaluating the inhibitory effect of the Asparagus aqueous

and alcoholic extract against selected bacteria and fungi in an *in vitro*.

Detection of active compounds by High Performance Liquid Chromatography (HPLC)

Detection of the most important active compounds of the Asparagus root by High Performance Liquid Chromatography (HPLC). In the laboratories of the Ministry of Science and Technology, Baghdad-Iraq, after making the aqueous and alcoholic extract of the *Asparagus* root. The relative percentage of each component according method of Reid & Smith (2000), were also measured quantity by method Al-Aloosi (2014), according to the

$$[Au \times \left(\frac{Vs}{Ws}\right)]$$
 Active ingredient = $Ac \times As$

Where Ac - area under the curve of the sample, As – Concentration of the standard compound, Au -Area under the curve of the standard compound,Vs-Volume of the sample (ml), Ws-Weight of the sample.

Analysis of the active compounds of the methylated extract of *Asparagus officinalis* root using GC-MS

Conducting the analysis of active compounds by GC-MS in the laboratories of the Ministry of Science and Technology, Iraq. Analysis of extract separation of the active these compounds of alcoholic and aqueous Asparagus root extract using GC-MS Gas Spectrometry Chromatography-Mass was carried out by following the method of Abdelrahman et al. (2020). The dimensions of the poetic column was (30 m' 0.25 mm ID' 1 m df, composed of 100% polydimethylsiloxane. Helium is use as the carrier gas at a flow rate of 1 m.min⁻¹, and the injector temperature 250°C; Ion-source temperature 280°C. The oven temperature was programmed from 110°C (isothermal for 2 min), with an increase of 10°C.min⁻¹, to 200°C, then 5°C.min⁻¹ to

280°C, ending with a 9 mins. Isothermal at 280°C. Mass spectra were taken at 70 eV, a scanning interval of 0.5 seconds and fragments from 45 to 450 Da. Total GC running time was 36 min.

Microbial testing

Microbial isolates used to test the effectiveness of inhibition

Three bacterial isolates taken were (Staphylococcus aureus, E. coli, Pseudomonas aeruginosa), the fungus Aspergillus niger, and pathogenic yeast Candida albicans. According to Gupta et al. (1996), the drilling method was followed in testing the sensitivity of bacteria to the plant extract. First, the pathogenic bacteria and fungi were activated on their culture media according to the recommendations of the supplier company and sterilized using a steam autoclave at 121 ° C for 15 min and poured into Petri dishes, 0.1 ml of the bacterial suspension of each type of microorganism was spread to these media and then placed in the incubator at 37 °C for 24 hours for bacteria and 27 °C for 48 hours for fungi. Then process of fixing the number of bacteria and fungi was carried out according by McFarland method using the liquid medium Nutrient Broth (Table 1), which is taken by the swab from the solid culture and added to sterile glass tubes containing 9 ml of liquid Nutrient Broth and then placed in the incubator at 37 ° C for 24 hours for bacteria and 27 ° C for a period 48 hours for fungi. The glass tubes are taken and placed in the centrifuge for 15 min, where the filter was neglected, and the precipitate is add to it, and 9 ml of sterile liquid peptone is added to it and shaken well. Then the measuring the absorbency by taking 2 ml of the contents of each tube. If it is close to the number of McFarland, then it is the required culture, and if it is more, dilute it with sterile peptone. Then we perform the inhibition process by planting

the bacterial and fungal (15×10^8) on the medium of Muller-Hinton at a rate of 0.1 ml and spread by L shape and then drilling using the cork drill. The extract is placed with the drill a level (1.5 ml) and incubated and then placed in the incubator at 37 ° C for 24 hours for bacteria and 27 ° C for 48 hours for fungi. Then the damping diameter is measured with a ruler.

Data analysis

All data be subject to analysis of variance (ANOVA) by completely randomized design (CRD) using SPSS software (2015). Treatment means were also assessed using the Least Significant Difference (LSD) test at $p \le 0.05$ (SPSS, 2015).

Results & Discussion

Preliminary phytochemical screening

After the preliminary phytochemical detection, it has been shown that aqueous and methanol extract of Asparagus (Asparagus officinalis L.) contains different types root of phytoconstituents, which contain such as phenols, saponins, glycosides, tannins, resin, flavonoids, steroid, and alkaloids as shown in the table (2), which shows the active substances in both extracts. The active compounds of Asparagus root extract revealed that the alcoholic extract containing the highest values of all the phytoconstituents except for saponins, resin, steroid and alkaloids compared to the aqueous extract (Table 2). Many recent studies have demonstrated the phytochemical component of A. officinalis L. root extracts. In this regard, Minh et al. (2019) reported active compounds content in Asparagus extract such flavonoids, alkaloids, phenols, saponins, as across, the bitter taste in some and tannins, types of Asparagus was attributed to an increased concentration of steroidal saponins a bitter taste (Al-Snafi, 2017). Also, the author

refers to Asparagus officinalis L. extract contents, rich in steroids, flavonoids, saponins, amino acids such as fructans, ferulic acid, minerals, and some vitamins and flavonoids compounds. According to Shaha & Anurag (2017) the active chemical compounds of A. officinalis were a group of important compounds with multiple effects such as alkaloids, glycosides, flavonoids, saponins, phenolic compounds, tannins, phytosterols, carbohydrates, proteins, amino acids, and vitamin B6. Phytochemical screening of the aqueous and alcoholic extracts of the Asparagus root revealed for the presence of glycosides, phenolic, alkaloids, c compounds, steroids, tannins, saponins, and flavonoids. The present study was planned to identify the number the active chemical compounds in the extract aqueous and alcoholic of the A. root by quantitative GC-MS officinalis analysis. Our results concluded that the dried aqueous and alcoholic extracts root have more or less similar phytoconstituents. In total 1, 5 compounds were identified in GC-MS analysis of dried aqueous extracts of Asparagus root and 7 compounds of alcoholic extracts. Exegesis mass spectrum GC-MS (Figs. 1, 2) these results were supported by Zhang et al. (2019) quantification of six major bioactive compounds, namely, 2-Propanone, 1.3dihydroxy 1), 2-Fruancarboxy aldehyde, 5-(hydroxymethyl) 2), Hexadecanoic acid 3), n-Hexadecanoic acid 4), Ethanol,2(Octyloxy)-5),1,9-Nonanediol 6) from Asparagus roots ethanol extract.

Composite

Planned

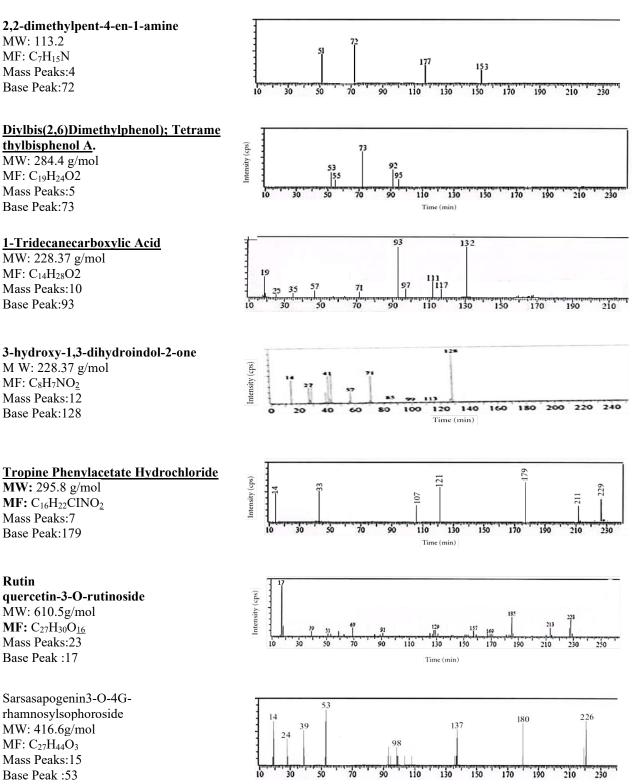


Fig. (1): The alcoholic extract content of the *A. officinalis* root powder from effective chemical compound using GC-MS analysis.

Composite

Planned

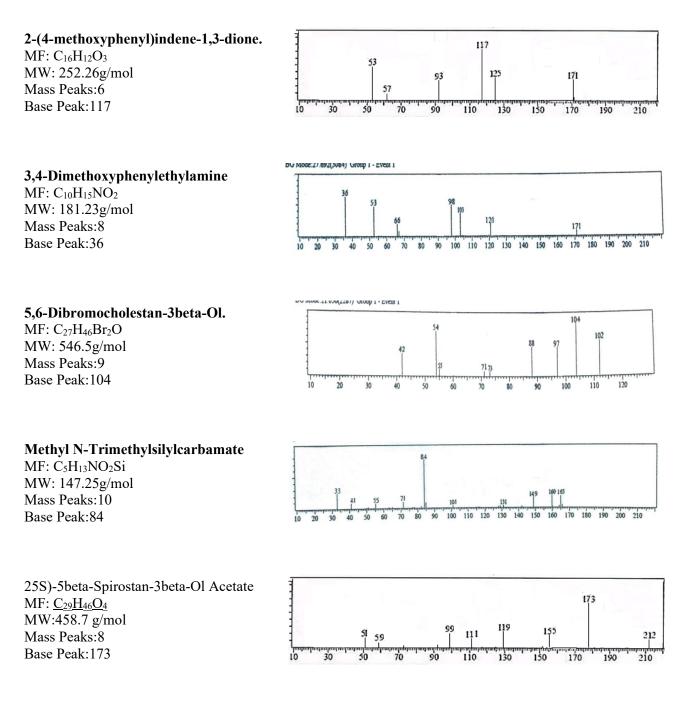


Fig. (2): The aqueous extract content of the *A. officinalis* L. root powder from effective compound using GC-MS analysis.

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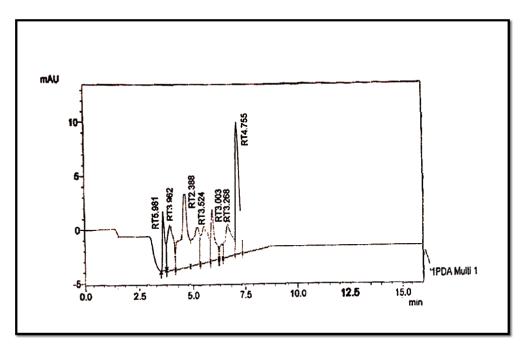


Fig. (3): Separation and characterization of the active compounds in the alcoholic extract using a technique HPLC.

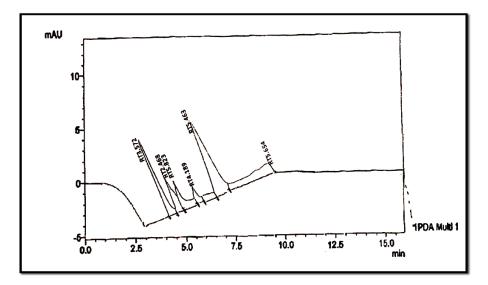


Fig. (4): Separation and characterization of the active compounds in aqueous extract using a technique HPLC.

Tube	1ml Aqueous barium chloride1%	1ml Sulfuric acid1%	Number of bacteria		
number	[BaCl2.2H2O]	[H2SO4]	per 10 ⁸ ml		
1	0.5	15			

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Active ingredient	Phenols %	Saponin %	Tannins %	Flavonoids %	Steroid %	Alkaloids %
Alcohol extracts	5.77	4.86	9.52	10.36	5.69	8.32
Aqueous extracts	4.61	7.85	8.26	6.11	6.31	9.57

Table (2): Estimation the active compounds in the Asparagus root extracts by GC/MS.

Table (3): Antibacterial activity of A. officinalis root extracts against selected pathogenic bacteria and fungi.

				Average of inhibition diameter (mm)*						
Test	Control		Tetracycline TE30	Aqueous extract (mg.ml ⁻¹)			Alcohol extract (mg.ml ⁻¹)			
organisms										significance
	Distilled water	Alcohol		50	100	150	50	100	150	level
P. aeruginosa	-	$29^b \pm 1.155$	41 ^a ±1.155	17 ° ±0.577	25 ° ±1.155	$34^{a}\pm0.577$	14 ° ±0.577	$24^{d}\pm 0.577$	$25^{bc}\pm 0.577$	0.05
E. coli	-	29 °±0.577	26°±0.577	$16^{\rm f} \pm 0.577$	29°±0.577	32 ^b ±0.577	28 ^d ±0.577	32 ^b ±0.577	35 ª±0.577	0.05
S. aureus	-	$34^{a}\pm 0.577$	11 ^f ±1.155	$11^{ef} \pm 0.577$	13°±0.577	23 ^d ±0.577	26 ° ±1.155	31 ^b ±0.577	35ª±0.577	0.05
A. niger	-	5 ^g ±0.577	9 ^f ±1.155	14 ^d ±0.577	17°±1.155	23ª±1.155	8e±1.732	17°±1.155	21 ^b ±1.155	0.05
C. albicans	-	9 ^d ±1.155	10°±0.577	11°±0.577	13 ^b ±1.155	15ª±1.732	7e±1.155	10°±0.577	13 ^{ab} ±0.577	0.05

*The diameters of the inhibition zones represent the reading rate of three replicates.

^{a-f} Means within each product in the same row with different subscripts have significantly differed at ($P \le 0.05$).

(-): Lack of inhibition efficacy.

These results were supported by Churanya *et al.* (2017) method was developed for simultaneous limitation of five saponin glycosides, asparacoside, shatavarin IX, shatavarin IV, asparanin A and shatavarin V in A. The growth inhibitory effects of the *A. officinalis* root extracts were presented in table (3).

The result indicated A. officinalis root extracts were shown antibacterial activities against all pathogenic microorganism note the clear variation of the concentration factor in influencing the growth of bacterial and fungal genera, it was observed that the alcoholic extract of the Asparagus root had a higher inhibitory effect than the aqueous extract against E. coli and Staphylococcus aureus, the highest diameter of inhibition was in within alcoholic extract concentrations of 50, 100 and 150 mg. ml⁻¹ were 28, 32 and 35 and 26, 31and 35 mm for both, compared to all other concentrations. It was observed that increasing affected the concentration increasing the inhibitory effect on bacterial growth.

The lowest effect was a 50 mg. ml⁻¹ aqueous and alcoholic extract concentration compared to the higher concentrations. While of the effect the aqueous extract concentrations was higher for the Pseudomonas (17, 25 and 34) mm compared to the rest of the other concentrations, except for the antibiotic, which obtained the highest value (41)mm. It is reported the concentration 150 of methanol extract prevented growth of types of bacteria and fungi used in test. It was the maximum significant inhibition zone against S. aureus bacteria positive for the Gram stain (35±0.577) mm and E. coli (35±0.577) mm and the lowest inhibition area for Gramnegative (25±0.577) mm. against bacteria P. aeruginosa. Aqueous extract concentrations 50, 100 and 150 mg. ml⁻¹ showed higher efficiency than the concentrations of alcohol extract and the remaining concentrations used. In the experiment against the fungi A. niger and pathogenic yeast C. albicans. The highest inhibition was (15 ± 1.732) for C. albicans treated with concentration 150 mg. ml⁻¹ of aqueous extract, while the sensitivity of yeast was less towards concentration of 150 mg. ml⁻¹ than the alcohol extract (13 \pm (0.577) and the rest of the other concentrations and the antibiotic. Also, the inhibitory effect of aqueous extract (23 ± 1.155) was higher at level 150 (mg. ml⁻¹) compared to level 150 (mg. ml⁻¹) of alcoholic extract (21 ± 1.155) and the rest of the other extracts for A. niger. The standard antibiotic (Tetracycline) tablets used in this study inhibited the growth of bacterial and fungal organisms used in the test. Where it was found that the area of inhibition produced by the tetracycline tablet was higher than the concentrations of the aqueous extracts and the alcohol extract used, for *P. aeruginosa*, the inhibition diameter was (41 ± 1.155) . While the rest of the isolates were less or close to the concentrations of extracts used in the experiment. From the above, we can say that the methanol extract was more efficient than the aqueous extract in extracting the active compounds. Probably, this is due to the polarity of the solvent, which plays a large role in extracting some active compounds without others, which results in greater precipitation of the active compounds. Our results are different from the researcher noticed, that showed about Zone of inhibition were obtained against E. coli bacteria, was less active inhibitory concentration at(mg.ml⁻ ¹), and was the aqueous extract of *Asparagus* racemosus root is effective against Grampositive and Gram-negative pathogens of Streptococcus spp., and S. aureus spp., E. coli, while alcoholic methanol extract showed a broad spectrum against Grampositive and negative bacteria, E. coli, P. aeruginosa and S. aureus, and showed ineffectiveness against Streptococcus isolates compared to ethanol extract and methanol acetate. These results collaborate well with the findings of Ismaeli *et al.* (2016) found that use different concentrations of the root extract of A. racemosus plant inhibited the growth of E. coli and S. aureus, while ethanol and acetone root extract Asparagus showed an inhibition spectrum on S. aureus and E. coli. Also, Tinrat & Sila-Asna (2017) indicated that efficacy of pathogenic strains, with minimum inhibitory concentration (MIC), values varying from 1,600 to more than 3,200 (mg.ml⁻¹). The MIC analysis of plant extracts showed the optimum bacteriostatic concentration for aqueous (distilled water and naturally distilled water) extracts of A. racemosus root; these results are consistent with that of the present study. The same results by Pravala & Venkafesham, (2017) who studied the inhibitory effect of the aqueous and alcoholic extract A. racemosus root agree against the effectiveness of some pathogenic bacterial isolates and resistance to some drugs and various antibiotics, as the ethanolic extract showed antibacterial activity against E. coli. Moreover, P. aeruginosa had no activity against Streptococcus species, and S. aureus, while the aqueous extract showed activity against Streptococcus spp., S. aureus spp., E. coli, and P. aeruginosa. Likewise attributed this to the presence of biologically active substances in high concentrations in these extracts, such as steroids, tannins, saponins, alkalis, phenolic compounds, glycosides, and the extract showed that it is a

safe anti. It has antimicrobial potential and without any known harmful effect, and it may be an alternative to antibiotics in some diseases. Moreover, many studies have demonstrated the ability of A. officinalis extracts to prevent or reduce the growth fungus pathogenic. In this regard, Chouhan & Pragati (2014) indicated possibility of the effect of the synergistic activity of the Asparagus plant with a group of medicinal plants such as Solanum torvum, Adhatoda vasica, Terminalia chebula and Asparagus as antifungal activity using methanol extract and ethanol in a ratio (1:1) against the fungal Aspergillus fungus pathogens and А. parasiticus, where plant extracts showed a certain degree of antimicrobial activity against A. fungus and highest area was obtained. Additionally, Shah et al. (2014) found that the aqueous extract of Asparagus officinalis, showed antifungal activity at a concentration of 5%, and attributed that to its saponins content.

Conclusion

The current study's findings indicate that an methanol and aqueous extract of Asparagus A. officinalis root contains phytochemical compounds such as flavonoids, phenols, saponins and tannins, which may be helpful in treating diseases as well as for preventing the growth of many pathogens. Also, it was observed that the alcoholic extract of the Asparagus root had a higher inhibitory effect than the aqueous extract, and the highest inhibition diameter of all bacterial tested and pathogenic fungi was within the alcohol extract at a concentration of 150 (mg.ml⁻¹).

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Conflict of interest

The authors declare that they have no conflict of interest.

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Contributions of Authors

R.J.A.: Research project proposal, Read and approved the manuscript. Contribute to the publication of the articles.

R.A.A.: Conducting the practical part of the research, Statistical analysis, Write the manuscript

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تأثير المستخلص المائي والكحولي لجذر نبات الهليون (.Asparagus Officinalis L) في تثبيط بعض المستخلص المائي والكحولي لجذر نبات الهليون

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المستخلص: هدفت الدراسة لمعرفة التأثير المثبط للمستخلص المائي والكحولي لجذر نبات الهليون (Asparagus officinalis) (ملغم مل⁻¹) لكلا (...) في تثبيط بعض البكتيريا والفطريات المختارة في المختبر. استخدمت التراكيز 50 (150,00 (ملغم مل⁻¹) لكلا المستخلصين ضد عزلات البكتريا والفطريات الممرضة, وتم الاختبار باستخدام طريقة الحفر وذلك إضافة 0.2 مل من المستخلص المائي والمستخلص الكحولي لكل حفرة واستخدم 2.0 مل من الماء المقطر وكحول الميثانول كمعاملة سيطرة, واضيفت اقراص الـ Tetracycline كمضاد حيوي. أظهرت نتائج فحص جهاز الـ GC/MS احتواء مستخلصي جذر نبات الهليون على العديد من المركبات الكيميائية النباتية الفعالة مثل (الفلافونويد والفينولات والقلويدات والجليكوسيدات والستيرويد والراتنج والصابونين والتانين). كما ظهر للمستخلص الكحولي لجذر نبات الهليون تأثير مثبط أعلى من تأثير المستخلص المائي ضد بكتريا والصابونين والتانين). كما ظهر للمستخلص الكحولي لجزر نبات الهليون تأثير مثبط أعلى من تأثير المستخلص المائي ضد بكتريا والصابونين والتانين). كما ظهر للمستخلص الكحولي لجزر نبات الهليون تأثير مثبط أعلى من تأثير المستخلص المائي ضد بكتريا والمابونين والتانين). كما ظهر للمستخلص الكحولي لجزر نبات الهليون تأثير مثبط أعلى من تأثير المستخلص المائي ضد بكتريا والمابونين والتانين). كما ظهر للمستخلص الكحولي لجزر نبات الهليون تأثير مثبط أعلى من تأثير المستخلص المائي ضد بكتريا والمابونين والتانين التثبيط (28، 32 و 25) و (60، 31 و 25) ملم على التوالي مقارنة بالتراكيز الأخرى. بينما كان تأثير مام مقارنة ببقية التراكيز أعلى بالنسبة لبكتريا *Pseudomonas aeruginosa* المستخلص المائي للذرى. بينما كان تأثير تراكيز المستخلص المائي لنفس التراكيز أعلى بالنسبة لبكتريا لعزلية البكتيرية اكثر حساسية تجاهه بقطر التثبيط (18 و 25 و 31) تراكيز المستخلص المائي ولفر المضاد الحيوي اذكانت العزلية البكتيرية اكثر حساسية تجاهه بقطر تثبيط 14 ملم. 20 و 10 تراكيز المستخلص المائي ماعد المضاد الحيوي المعرضة (*Candida albicans*) فكانت اقطار التثبيط (14 ، 16 و 25) و والمازنة ضد فطر عور) ملم على التوالي. والمقارنة ضد فطر عور) ملم على التوالي.

الكلمات المفتاحية: الفعالية المضادة للميكروبات, Asparagus officinalis, المكونات النشطة بيولوجيا، التحليل الكمي