The North African Journal of Scientific Publishing (NAJSP)
مجلة شمال إفريقيا للنشر العلمي (NAJSP)
EISSN: 2959-4820
Volume 2, Issue 1, January - March 2024, Page No: 63-78
Website: https://najsp.com/index.php/home/index
SJIFactor 2023: 3.733 معامل التأثير العربي (AIF) 2023: 0.63 ISI 2023: 0.383

Evaluation of the Antimicrobial Activity of Selected Fabaceae Plants

Ahmed Abulqasim Alwagih [1*], Aml Ahmed Salih [2], Rabea Ali Ibrahim [3], Seham Esharif Salhin [4]
[1] Libyan Medical Research Centre, Zawiya, Libya
[2] Department of Botany, Faculty of Science, Bani Waleed, University of Bani Walid, Libya
[3] Department of Zoology, Faculty of Science, Bani Waleed, University of Bani Walid, Libya
[4] Department of Plant Protection, Faculty of Agriculture, Bani Waleed, University of Bani Walid, Libya

*Corresponding author: alwagiahahmed@gmail.com

Received: December 28, 2023	Accepted: February 09, 2024	Published: February 20, 2024

Abstract:
The objective of this study was to investigate the antimicrobial properties of several distinct species belonging to the Fabaceae family, namely Astragalus sigmoideus, Genista albida, Astragalus syringus, Ononis hirta, Astragalus lydius, Astragalus wiedemannianus, and Anthyllis tetraphylla. Extracts from the plant samples were prepared using a solvent mixture of 60% ethanol and 40% water. These extracts were then tested against 15 different microorganisms, including Bacillus subtilis DSMZ 1971, Enterobacter aerogenes ATCC 13048, Enterococcus faecalis ATCC 29212, Enterococcus faecium, Escherichia coli ATCC 25922, Klebsiella pneumoniae, Pseudomonas aeruginosa DSMZ 50071, Pseudomonas fluorescens P1, Salmonella enteritidis ATCC 13075, Salmonella infantis, Salmonella kentucky, Salmonella typhimurium SL 1344, Staphylococcus aureus ATCC 25923, Staphylococcus epidermidis DSMZ 20044, and Candida albicans DSMZ 1386.
The research utilized the disk diffusion (DD) and minimum inhibitory concentration (MIC) methods. The results revealed that different plant extracts exhibited activity against specific microorganisms. Notably, the extracts showed inhibitory effects against E. faecalis, K. pneumoniae, P. fluorescens, S. aureus, and S. typhimurium, with varying sizes of inhibition zones. The MIC values for all microorganisms that displayed antimicrobial activity in the DD assay consistently measured at 10 µg/mL, except for P. fluorescens, where it was 5.0 µg/mL when treated with the A. sigmoideus extract.

Keywords: Evaluation, Antimicrobial Activity, Fabaceae, Microorganisms, Bacteria, Yeast.

Cite this article as: A. A. Alwagih, A. A. Salih, R. A. Ibrahim, S. E. Salhin, "Induction of electric field in human bodies moving near MRI: An efficient BEM computational procedure," *The North African Journal of Scientific Publishing (NAJSP)*, vol. 2, no. 1, pp. 63–78, January-March 2024.

تقييم النشاط المضاد للميكروبات لنباتات مختارة من الفصيلة البقولية

أحمد أبو القاسم الوجيه [1*]، أمل أحمد صميدة [2]، ربيعة علي إبراهيم عبدالصمد [3]، سهام الشارف صالحين [4]
[1] المركز الليبي للأبحاث الطبية، الزاوية، ليبيا
[2] قسم النبات، كلية العلوم، جامعة بني وليد، بني وليد، ليبيا
[3] قسم علم الحيوان، كلية العلوم، جامعة بني وليد، بني وليد، ليبيا
[4] قسم وقاية النبات، كلية الزراعة، جامعة بني وليد، بني وليد، ليبيا

الملخص

هدفت الدراسة إلى فحص الخصائص المضادة للميكروبات لسبعة أنواع متميزة من عائلة البقوليات، وهي Astragalus sigmoideus، وGenista albida، وAstragalus syringus، وOnonis hirta، وAstragalus lydius، وAstragalus wiedemannianus، وAnthyllis tetraphylla. تم استخدام الإيثانول (60%) والماء (40%) كمذيبات لتحضير المستخلصات من العينات النباتية. تم اختبار هذه المستخلصات لاحقًا ضد 15 كائناً دقيقاً مختلفاً، بما في ذلك Bacillus subtilis DSMZ 1971، Enterobacter aerogenes ATCC 13048، Enterococcus faecalis ATCC 29212، Enterococcus faecium، Escherichia coli ATCC 25922، Klebsiella pneumoniae، Pseudomonas aeruginosa DSMZ 50071، P. سيدوموناس فلوريسسينس P1، السالمونيلا المعوية ATCC 13075، السالمونيلا الطفلية، السالمونيلا كنتاكي، السالمونيلا تيفيموريوم SL 1344، المكورات العنقودية الذهبية ATCC 25923، المكورات العنقودية البشروية DSMZ 20044، والمبيضات البيضاء DSMZ 1386.

استخدم البحث طريقة انتشار القرص (DD) والحد الأدنى للتركيز المثبط (MIC). أشارت النتائج إلى أن المستخلصات النباتية المختلفة أظهرت نشاطًا ضد كائنات دقيقة معينة. ومن الجدير بالذكر أن المستخلصات أظهرت تأثيرات مثبطة ضد E. faecalis، K. pneumoniae، P. fluorescens، S. aureus، وS. typhimurium، بأحجام مختلفة من مناطق التثبيط. وقد لوحظ باستمرار أن قيم MIC لجميع الكائنات الحية الدقيقة التي أظهرت نشاطاً مضاداً للميكروبات في اختبار DD تبلغ 10 ميكروغرام/مل، باستثناء P. fluorescens، حيث كانت 5.0 ميكروغرام/مل عند معالجتها بمستخلص A. sigmoideus.

الكلمات المفتاحية: تقييم، نشاط مضادات الميكروبات، الفصيلة البقولية، الكائنات الحية الدقيقة، البكتيريا، الخميرة.

Introduction

In a study conducted by Kanaan et al. (2017), the antimicrobial effects of an ethanol and water extract from Astragalus angulosus, an endemic Lebanese plant, were investigated. The researchers examined the impact of the extracts on three Gram-positive bacterial strains: Staphylococcus epidermidis (CIP 444), Staphylococcus aureus (ATCC 25923), and Enterococcus faecalis (ATCC 29212), as well as two Gram-negative strains: Escherichia coli (ATCC 35218) and Pseudomonas aeruginosa (ATCC 27853) using microdilution assays.

The findings revealed that all ethanolic extracts of the plant exhibited the most significant antimicrobial activity against S. epidermidis at a concentration of 12.78 mg/mL, while the water extract demonstrated the highest activity at 0.2 mg/mL. Staphylococcus aureus exhibited the highest level of inhibition, followed by species of Streptococcus and E. coli, whereas no zone of inhibition was observed for K. pneumoniae.

The study also determined the Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) values for five microorganisms: E. coli, K. pneumoniae, S. aureus, Streptococcus species, and Bacillus subtilis. The results indicated that Lathyrus species, specifically L. aphaca and L. ratan, had MIC values below 100 µg/mL, indicating good activity against the selected bacteria. However, B. subtilis exhibited the highest MIC value for both species, indicating it was the least susceptible. The lowest MIC value was observed for S. aureus, suggesting that the seed extracts were particularly effective against this bacterium.

In their study, Alrumman, Moustafa, and Alamri (2012) utilized the agar well diffusion method to examine the antimicrobial activity of different extracts derived from A. atropilosulus subsp. abyssinicus leaves. The extracts tested included acetone, ethanol, methanol, 1/1 ethanol/methanol, 1/1 ethanol/acetone, 1/1 acetone/methanol, as well as hot and cold-water extracts.

With the exception of the cold-water extract, all the extracts demonstrated broad-spectrum activity against the tested bacteria. The inhibition zones ranged from 9.33 mm to 35.0 mm. The minimal inhibitory concentration (MIC) values of the various plant extracts against the bacteria ranged between 12.50 mg/mL and 17.5 mg/mL, except for the cold water extract.

Furthermore, all the extracts exhibited antifungal activity against Candida sp., Drechslera halodes, Fusarium oxysporum, and Pythium ultimum. The inhibition zones for the antifungal activity ranged from 6.56 mm to 20.3 mm.

In the study conducted by Teyeb et al. (2012), the antimicrobial activity of various extracts from the aerial parts and roots of wild Astragalus gombiformis was investigated. Methanol, chloroform, and ethyl acetate extracts from both the aerial parts and roots were examined, along with water and methanol extracts from Astragalus gombiformis. The study focused on six types of bacteria: Listeria monocytogenes, Staphylococcus epidermidis, Pseudomonas aeruginosa, Bacillus subtilis, Escherichia

coli, and Salmonella typhimurium. The paper disk agar diffusion method was employed to evaluate antimicrobial activity, and minimal inhibition concentration was determined.
The results indicated that the roots of Astragalus gombiformis exhibited inhibition zone diameters of 12 mm or more at a concentration of 150 µg/disk. The minimal inhibition concentrations ranged from 233 µg/mL to 1250 µg/mL. Spectrophotometric and HPLC analyses revealed that the methanolic extract of the aerial parts contained higher levels of total polyphenols and flavonoids, as well as greater antioxidant activity, compared to the roots. However, these extracts had no effect against the tested bacteria.
In another study by Albaqawi and Selim (2015), the antimicrobial activity of distilled methanol (80%) extracts from Anziroat (Astragalus sp) was examined using the agar disk diffusion method. The extracts were tested against six bacterial strains and one yeast strain, including Aeromonas hydrophila, Proteus mirabilis, Pseudomonas aeruginosa, Staphylococcus aureus, Streptococcus species, and Escherichia coli.
The findings indicated that most of the extracts did not exhibit antimicrobial activity against Proteus mirabilis, Pseudomonas aeruginosa, or Streptococcus species. However, several extracts were found to be active against Staphylococcus aureus, with the highest inhibition zone measuring 20 mm at a concentration of 5 mg of extract. Additionally, some extracts demonstrated activity against Escherichia coli, with the highest inhibition zone measuring 15 mm at a concentration of 5 mg of extract.
In a study conducted by Arias, Gomez, Cudmani, Vattuone, and Isla (2004), the antimicrobial activity of ethanolic extracts from different parts of Acacia aroma (leaves, stems, and flowers) was investigated against both gram-positive and gram-negative bacteria. The gram-positive bacteria tested included Enterococcus faecalis, Staphylococcus aureus, coagulase-negative staphylococci, Streptococcus pyogenes, Streptococcus agalactiae, Staphylococcus aureus ATCC 29213, and Enterococcus faecalis ATCC 29212. The gram-negative bacteria tested were Escherichia coli, Klebsiella pneumoniae, and Proteus mirabilis.
The results showed that the leaf and flower extracts exhibited the highest activity against all gram-positive bacteria and demonstrated good activity against gram-negative bacteria. Both the ethanolic and aqueous extracts showed activity against Staphylococcus aureus, coagulase-negative staphylococci, Enterococcus faecalis, Enterococcus faecium, and all tested gram-negative bacteria. The Minimum Inhibitory Concentration (MIC) values ranged from 0.067 mg/mL to 0.308 mg/mL.
In another study conducted by Altuner et al. (2010), the antibacterial activity of a 75% aqueous ethanol extract from Ononis spinosa (Leguminosae) was tested using agar diffusion. The extract was evaluated against various microorganisms, including Escherichia coli ATCC 23556, Pseudomonas aeruginosa ATCC 10145, Bacillus subtilis ATCC 6633, Staphylococcus aureus ATCC 25923, Candida albicans ATCC 10231, Candida galabrata (isolate), and Candida krusei ATCC 6258. Both Staphylococcus aureus and Bacillus subtilis exhibited a 15-mm inhibition zone when exposed to the extracts. The extracts demonstrated significant activity against Candida albicans and Candida krusei, with inhibition zones measuring 16 mm and MIC values of 1.25 µg/mL. The ethanol extract effectively inhibited Candida glabrata, displaying a 10-mm inhibition zone and a MIC value of 5 µg/mL. Unfortunately, the provided text does not mention the primary contribution of the article. The remaining sections of the article are organized as follows: Section 2 provides a description of the materials and methods used in the study. Sections 3 and 4 conclude the article and include a list of references.

Material and methods

a. Materials

The Materials used in this study are shown in Table 1.

Table 1. Materials used in this study.

	Material	Description
1.	Petri Dishes	A total of 100 glass Petri dishes sized 15 cm in diameter were procured from Labor Technical. Each petri dish underwent thorough cleaning and sterilization before being utilized. These glass petri dishes were employed to culture bacterial and other microorganism samples, as well as for loading and drying empty sterile antibiotic discs.
2.	Filter Paper	125-millimeter diameter filter paper (Schleicher & Schuell) was used to filter the extracts

3.	Test Tubes	18 x 100mm borosilicate glass test tube from Isolab, Broth cultures and stock microorganisms. Before use, each was cleaned and sterilized
4.	Sterile Loops	Sterile loops were taken from Loop Plast (Italy) for the transfer and isolation of microorganisms
5.	Empty Sterile Antibiotic Disks	A disc with a diameter of 6 mm was collected from a bioassay-free sterile antibiotic (Turkey) and the extract was used to test the antimicrobial activity of the plant extract, used to load the extract and test the plant extract for its antimicrobial activity
6.	Sterile Cotton Swabs	We obtained sterile cotton swabs from Cultiplast, a supplier based in Italy. These swabs were used to ensure the even distribution of microorganisms on the surface of the media.
7.	Flasks	Evaporation bottles were ordered from S & H Labware (USA) and both solvents were used for evaporation and freeze drying
8.	Ethanol Absolute	plant compounds
9.	Nutrient Agar	We procured nutrient agar from OR-BAK, a supplier located in Ankara, Turkey. This nutrient agar was employed for the cultivation and growth of bacteria.
10	Mueller Hinton Agar	We acquired Mueller Hinton agar from OR-BAK, a supplier based in Ankara, Turkey. This specific agar was obtained for the purpose of conducting the paper disk diffusion test.
11	Saboraud Dextrose Agar	We obtained Saboraud Dextrose Agar from OR-BAK, a supplier located in Ankara, Turkey. This particular agar was utilized for the cultivation of fungi.

b. Equipment:

The equipment used in this study is shown in Table 2.

Table 2. Materials used in this study.

	Equipment	Description
1.	Blender	The laboratory-type blender (Waring, USA) was used to grind plant samples.
2.	Scale	For the test procedure, the Precision Scale from Precisa, a Swiss manufacturer, is employed to accurately measure all substances and materials used.
3.	Shaker	o blends the obtained solvent with milled plant samples, a laboratory shaker from Wise Shake, a Korean manufacturer, was utilized.
4.	Vortex	To generate a microorganism culture in accordance with McFarland standards, a vortex from Velp Scientific, a European manufacturer, was employed.
5.	Rotary Evaporator	To remove the alcohol from the extract, a rotary evaporator manufactured by Heidolph in Germany was utilized.
6.	Distilled Water	The distilled water utilized in the operation was generated using a distilled water apparatus manufactured by Human Corporation of Korea.
7.	Autoclave	The medium and other materials employed in the study were sterilized using an autoclave from Wise clave, a Korean manufacturer.
8.	Freeze Dryer	lyophilizer (German Christ) used to drying extract
9.	Mortar and Pestle	The mortar and pestle ordered from RTM (Germany) were crushed using plant samples prior to the extraction procedure.
10	Biosafety Cabinet.	The Biosafety Cabinet (China). For all the work that needs to be done in a sterile environment.
11	Incubator	Incubators (Selecta, Spain) are used to grow bacteria and fungi at a steady temperature.
12	Pipettes	Pipettes are ordered from Socorex (Switzerland) for the transfer of extracts and microorganisms.

c. Plant Samples:

The plants were gathered from various regions in Turkey, including both northern locations like Kastamonu and southern areas like Muğla.

Figure 1. Astragalus sigmoideus.

Figure 2. Ononis hirta.

Figure 3. Anthyllis tetraphylla.

Figure 4. Atragalus lydius.

Figure 5. Astragalus syringes.

Figure 6. Genista albida.

Figure 7. Astragalus wiedemannianus.

Results and discussion:

If available, an autoclave from Wise clave, a Korean manufacturer, was used to sterilize the medium and other materials used in the study.

1. Astragalus sigmoideus:

The results indicated that A. sigmoideus displayed significant antimicrobial activity against E. faecalis, K. pneumoniae, P. fluorescens, S. aureus, and S. typhimurium. However, no activity was observed against B. subtilis, C. albicans, E. aerogenes, E. coli, E. faecium, P. aeruginosa, S. enteritidis, S. epidermidis, S. infantis, and S. kentucky. Figure 8 provides detailed information regarding the antimicrobial activity of A. sigmoideus.

The results presented in figure 8 is clearly indicate the antimicrobial activity of A. sigmoideus against specific microorganisms. The inhibition zones for E. faecalis were measured to be 7.00 mm, 9.00 mm,

and 9.67 mm when treated with 10 µL, 50 µL, and 100 µL of extracts, respectively. Similarly, for K. pneumoniae, the inhibition zones were 7.00 mm and 10.33 mm when using 50 µL and 100 µL of extracts, respectively.
Concerning S. aureus, the extracts exhibited inhibition zones measuring 7.33 mm, 8.67 mm, and 10.00 mm for 10 µL, 50 µL, and 100 µL volumes, respectively. In the case of P. fluorescens, the inhibition zones were measured as 10.00 mm and 13.33 mm for 50 µL and 100 µL volumes of extracts, respectively. Lastly, for S. typhimurium, the extracts displayed inhibition zones of 7.00 mm, 9.67 mm, and 13.67 mm for 10 µL, 50 µL, and 100 µL volumes, respectively.

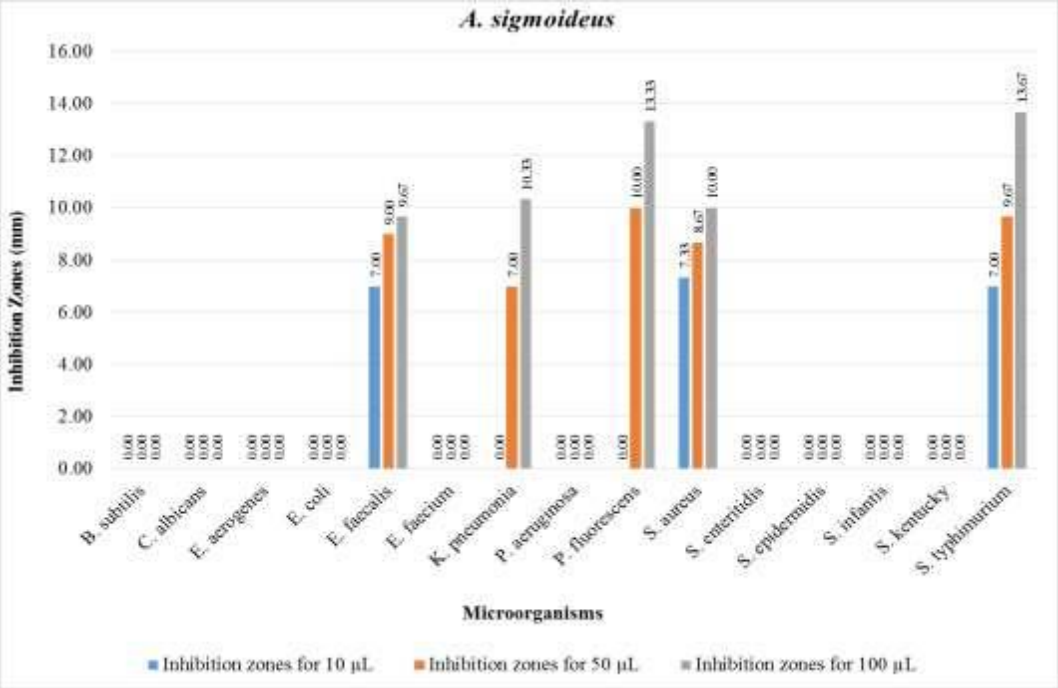

Figure 8. Disk diffusion results of A. sigmoideus.

Consistent with the earlier statement, no antimicrobial activity was detected against B. subtilis, C. albicans, E. aerogenes, E. coli, E. faecium, P. aeruginosa, S. enteritidis, S. epidermidis, S. infantis, and S. kentucky.

1. Genista albida:
The results demonstrated that G. albida exhibited antimicrobial activity solely against E. faecalis. No activity was observed against B. subtilis, C. albicans, E. aerogenes, E. coli, E. faecium, K. pneumoniae, P. aeruginosa, S. aureus, S. enteritidis, S. epidermidis, S. infantis, S. kentucky, and S. typhimurium. Detailed information concerning the antimicrobial activity of G. albida can be found in Figure 9. This figure 9 is clearly illustrates that the inhibition zones for G. albida against E. faecalis were measured as 7.00 mm, 9.00 mm, and 10.00 mm for 10 µL, 50 µL, and 100 µL volumes of extracts, respectively.
The study also found that there was no observed antimicrobial activity against B. subtilis, C. albicans, E. aerogenes, E. coli, E. faecium, K. pneumoniae, P. aeruginosa, S. aureus, S. enteritidis, S. epidermidis, S. infantis, S. kentucky, and S. typhimurium.

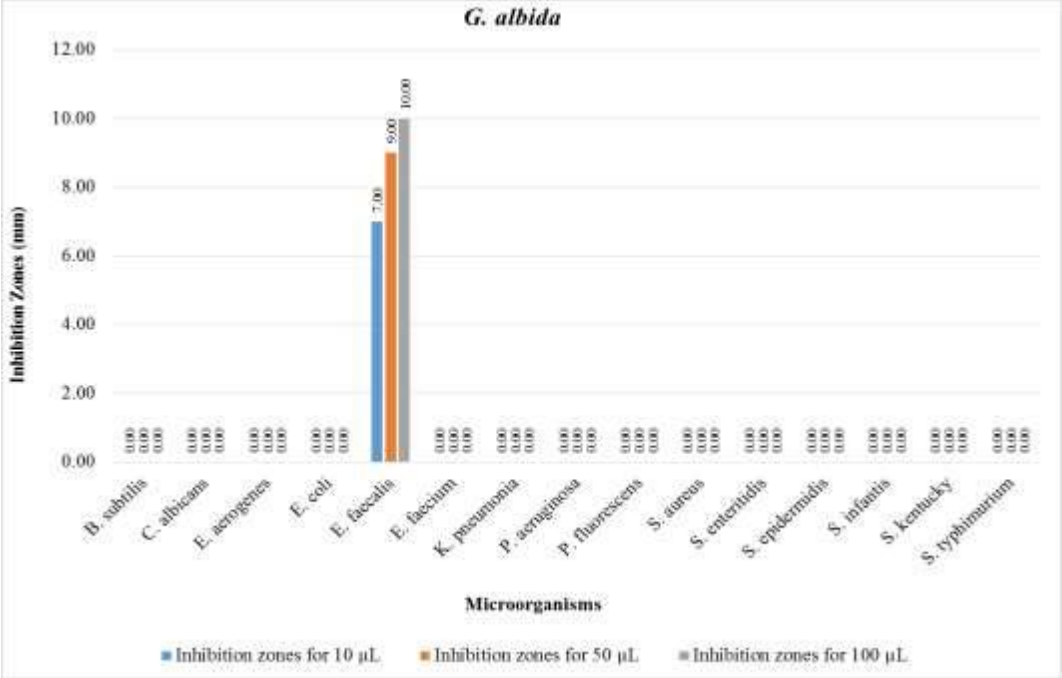

Figure 9. Disk diffusion results of G. albida.

2. Astragalus syringus:

The results demonstrated that A. syringus exhibited antimicrobial activity against E. faecalis and P. fluorescens. No activity was observed against B. subtilis, C. albicans, E. aerogenes, E. coli, E. faecium, K. pneumoniae, P. aeruginosa, S. aureus, S. enteritidis, S. epidermidis, S. infantis, S. kentucky, and S. typhimurium. Detailed information regarding the antimicrobial activity of A. syringus can be found in Figure 10. This figure is clearly illustrates that the inhibition zones for A. syringus against E. faecalis were measured as 7.33 mm, 8.67 mm, and 9.33 mm for 10 µL, 50 µL, and 100 µL volumes of extracts, respectively. Additionally, the activity against P. fluorescens resulted in inhibition zones of 10.00 mm for both 50 µL and 100 µL volumes of extracts.

As mentioned earlier, no activity was observed against B. subtilis, C. albicans, E. aerogenes, E. coli, E. faecium, K. pneumoniae, P. aeruginosa, S. aureus, S. enteritidis, S. epidermidis, S. infantis, S. kentucky, and S. typhimurium.

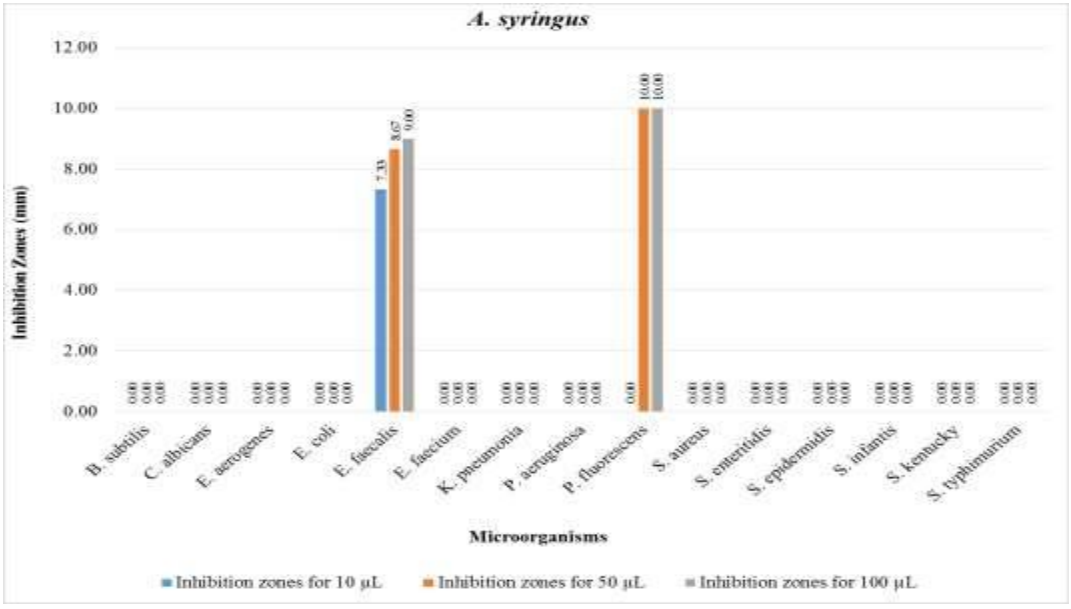

Figure 10. Disk diffusion results of A. syringus.

3. Ononis hirta:

The results indicated that O. hirta displayed antimicrobial activity against C. albicans, E. faecalis, P. aeruginosa, and S. infantis. No activity was observed against B. subtilis, E. aerogenes, E. coli, E. faecium, K. pneumoniae, S. aureus, S. enteritidis, S. epidermidis, S. kentucky, and S. typhimurium. Detailed information regarding the antimicrobial activity of O. hirta can be found in Figure 11. This figure is clearly shows that the inhibition zones for O. hirta against C. albicans were measured as 13.00 mm and 14.00 mm for 50 µL and 100 µL volumes of extracts, respectively. Additionally, the activity against E. faecalis resulted in inhibition zones of 15.33 mm, 18.00 mm, and 20.00 mm for 10 µL, 50 µL, and 100 µL volumes of extracts, respectively. The activity against P. aeruginosa was observed to be 8.00 mm and 11.00 mm for 50 µL and 100 µL volumes of extracts, respectively. Furthermore, the activity against S. infantis resulted in inhibition zones of 8.33 mm and 10.00 mm for 50 µL and 100 µL volumes of extracts, respectively.
As mentioned earlier, no activity was observed against B. subtilis, E. aerogenes, E. coli, E. faecium, K. pneumoniae, S. aureus, S. enteritidis, S. epidermidis, S. kentucky, and S. typhimurium.

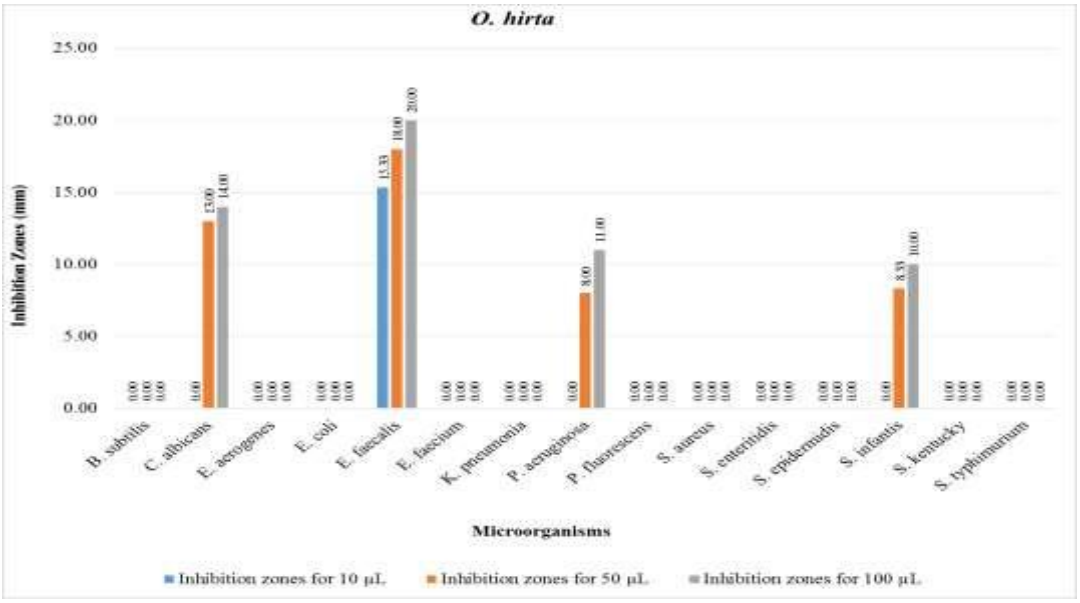

Figure 11. Disk diffusion results of O. hirta.

4. Astragalus lydius

The results indicated that A. lydius exhibited antimicrobial activity against E. faecalis, S. aureus, and S. infantis. No activity was observed against B. subtilis, C. albicans, E. aerogenes, E. coli, E. faecium, K. pneumoniae, P. aeruginosa, P. fluorescens, S. enteritidis, S. epidermidis, S. kentucky, and S. typhimurium. Detailed information regarding the antimicrobial activity of A. lydius can be found in Figure 12. This is clearly shows that the inhibition zones for A. lydius against E. faecalis were measured as 7.00 mm, 8.00 mm, and 7.33 mm for 10 µL, 50 µL, and 100 µL volumes of extracts, respectively. Additionally, the activity against S. aureus resulted in inhibition zones of 9.67 mm, 10.67 mm, and 13.33 mm for 10 µL, 50 µL, and 100 µL volumes of extracts, respectively. The activity against S. infantis was observed to be 7.00 mm, 7.33 mm, and 7.67 mm for 10 µL, 50 µL, and 100 µL volumes of extracts, respectively.

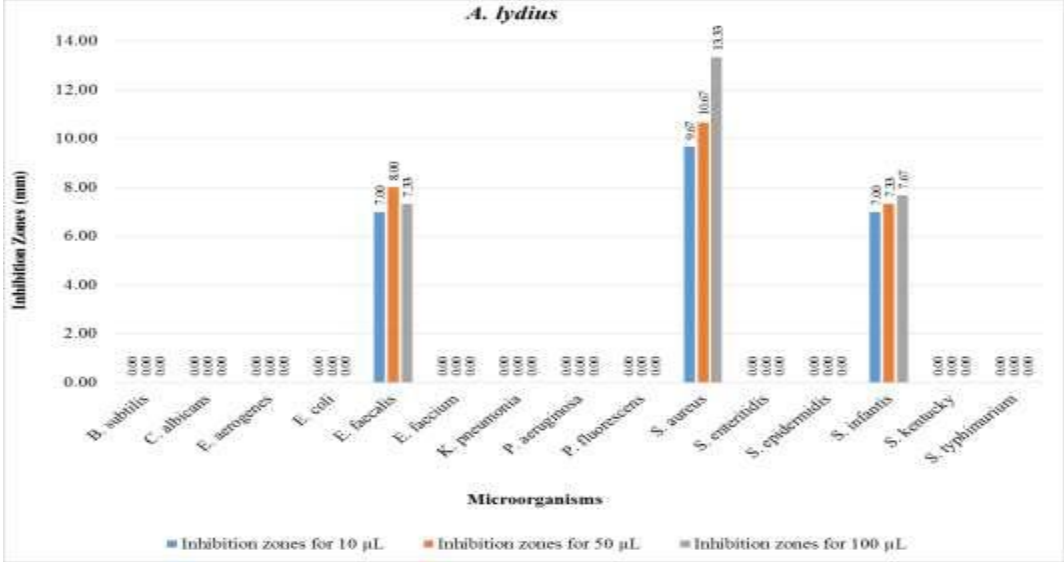

Figure 12. Disk diffusion results of A. lydius.

There was no observed activity against B. subtilis, C. albicans, E. aerogenes, E. coli, E. faecium, K. pneumoniae, P. aeruginosa, P. fluorescens, S. enteritidis, S. epidermidis, S. kentucky, and S. typhimurium.

5. Astragalus wiedemannianus:

The results showed that A. wiedemannianus had antimicrobial activity against B. subtilis, E. faecalis, P. aeruginosa, and P. fluorescens. No activity was found against C. albicans, E. aerogenes, E. coli, E. faecium, K. pneumoniae, S. aureus, S. enteritidis, S. epidermidis, S. infantis, S. kentucky, and S. typhimurium. Figure 13 provides detailed information on A. wiedemannianus' antimicrobial activity. The figure clearly shows that the inhibition zones for A. wiedemannianus against B. subtilis were measured as 7.00 mm, 10.33 mm, and 12.00 mm for 10 µL, 50 µL, and 100 µL volumes of extract, respectively. The inhibition zones against E. faecalis were 7.00 mm, 7.67 mm, and 8.33 mm at 10 µL, 50 µL, and 100 µL concentrations, respectively.

No activity was observed against C. albicans, E. aerogenes, E. coli, E. faecium, K. pneumoniae, S. aureus, S. enteritidis, S. epidermidis, S. infantis, S. kentucky and S. typhimurium as mentioned before.

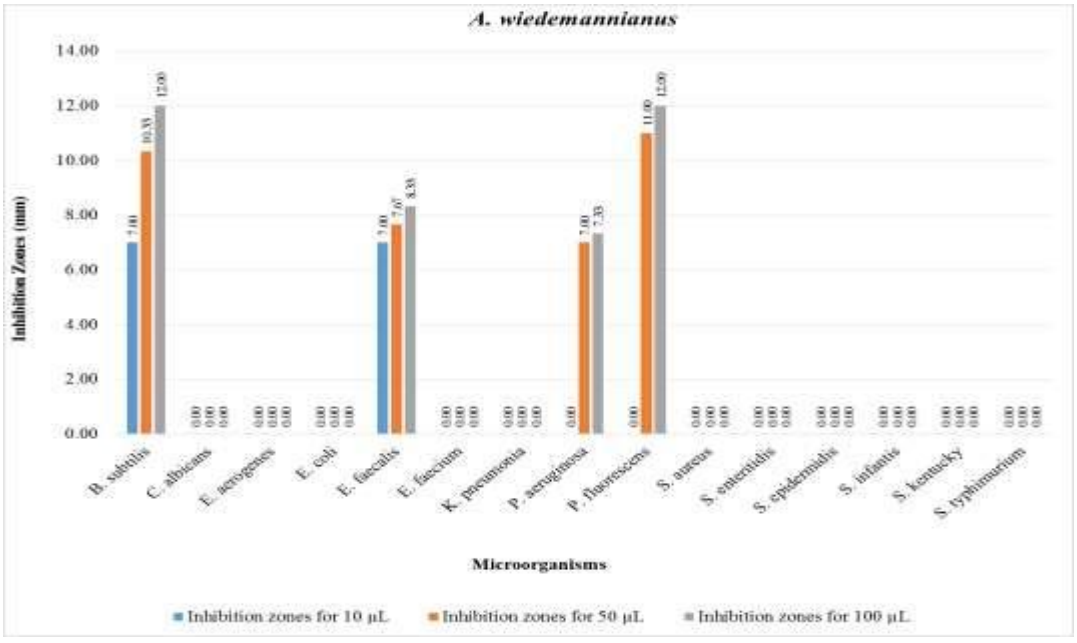

Figure 13. Disk diffusion results of A. wiedemannianus.

6. Anthyllis tetraphylla:

The results showed that Anthyllis tetraphylla had antimicrobial activity against E. faecalis and P. aeruginosa. No activity was found against B. subtilis, C. albicans, E. aerogenes, E. coli, E. faecium, K. pneumoniae, P. fluorescens, S. aureus, S. enteritidis, S. epidermidis, S. infantis, S. kentucky, and S. typhimurium. Figure 14 provides detailed information on Anthyllis tetraphylla's antimicrobial activity. The inhibition zones for Anthyllis tetraphylla against E. faecalis were measured as 7.00 mm, 8.33 mm, and 9.33 mm for extract volumes of 10 µL, 50 µL, and 100 µL, respectively. Furthermore, the activity against P. aeruginosa resulted in inhibition zones of 8.00 mm, 8.67 mm, and 9.67 mm for 10 µL, 50 µL, and 100 µL volumes, respectively.

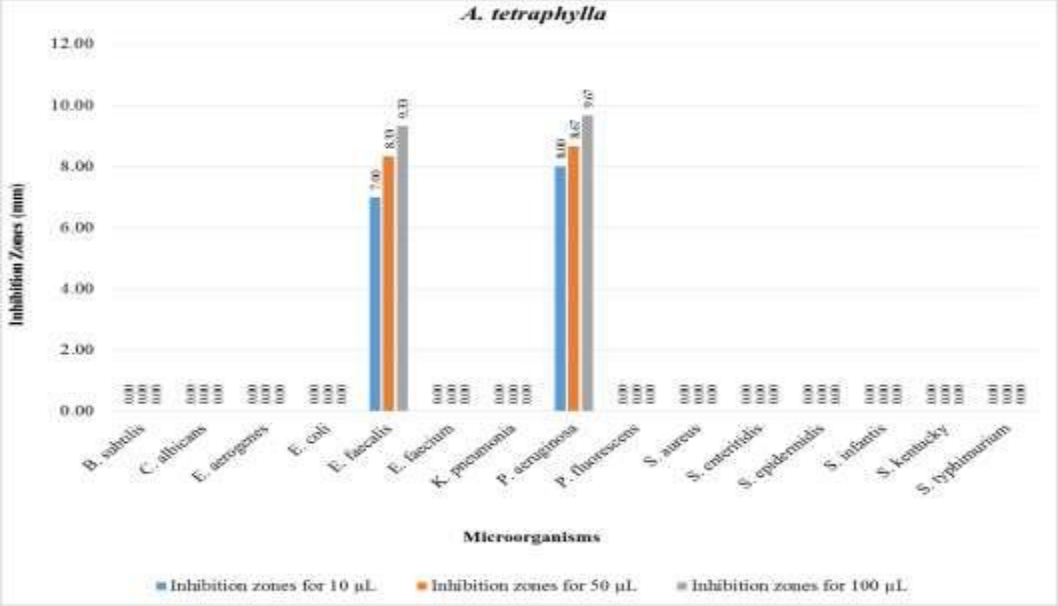

Figure 14. Disk diffusion results of A. tetraphylla.

No activity was observed against B. subtilis, C. albicans, E. aerogenes, E. coli, E. faecium, K. pneumoniae, P. fluorescens, S. aureus, S. enteritidis, S. epidermidis, S. infantis, S. kentucky, and S. typhimurium, as previously stated.

7. Results of MIC Tests:

Table 3 and Table 4 provide the results of the Minimum Inhibitory Concentration (MIC) tests.

Table 3. MIC values of plant extracts against microorganisms.

	MIC Values (µg/mL)		
	A. sigmoideus	**G. albida**	**A. syringus**
▪ B. subtilis	-	-	-
▪ C. albicans	-	-	-
▪ E. aerogenes	-	-	-
▪ E. coli	-	-	-
▪ E. faecalis	10	5	10
▪ E. faecium	-	-	-
▪ K. pneumoniae	10	-	-
▪ P. aeruginosa	-	-	-
▪ P. fluorescens	5	-	-
▪ S. aureus	10	-	-

▪ S. enteritidis	-	-	-
▪ S. epidermidis	-	-	-
▪ S. infantis	-	-	-
▪ S. kentucky	-	-	-
▪ S. typhimurium	10	-	-

Table 4. MIC values of plant extracts against microorganisms

	MIC Values (µg/mL)			
	O. hirta	**A. lydius**	**A. wiedemannianus**	**A. tetraphylla**
▪ B.subtilis	-	-	5	-
▪ C.albicans	2,5	-	-	-
▪ E.aerogenes	-	-	-	-
▪ E.coli	-	-	-	-
▪ E.faecalis	5	2,5	1,25	1,25
▪ E.faecium	-	-	-	-
▪ K.pneumoniae	-	-	-	-
▪ P.aeruginosa	1,25	-	2,5	1,25
▪ P.fluorescens	-	-	5	-
▪ S.aureus	-	1,25	-	-
▪ S.enteritidis	-	-	-	-
▪ S.epidermidis	-	-	-	-
▪ S.infantis	5	1,25	-	-
▪ S.kentucky	-	-	-	-
▪ S.typhimurium	-	-	-	-

Table 3 shows the results of the Minimum Inhibitory Concentration (MIC) tests for Astragalus sigmoideus. The MIC values for E. faecalis, K. pneumoniae, S. aureus, and S. typhimurium were found to be 10 µg/mL. The MIC value for P. fluorescens was 5 µg/mL. Astragalus sigmoideus did not receive a MIC test against B. subtilis, C. albicans, E. aerogenes, E. coli, E. faecium, P. aeruginosa, S. enteritidis, S. epidermidis, S. infantis, or S. kentucky because no activity was observed in the disk diffusion test. Table 4 shows Genista albida's MIC values against E. faecalis at 5 µg/mL. There was no MIC test for Genista albida against B. subtilis, C. albicans, E. aerogenes, E. coli. No MIC test was conducted for Astragalus syringus against B. subtilis, C. albicans, E. aerogenes, E. coli, E. faecium, K. pneumoniae, P. aeruginosa, S. aureus, S. enteritidis, S. epidermidis, S. infantis S. kentucky and S. typhimurium because no activity was observed at these combinations in disk diffusion test.

Results given in Table 9 showed the MIC values for Ononis hirta against C. albicans was 2,5 µg/mL. Against E. faecalis, and S. infantis were observed to be the same, which was 5 µg/mL. Against P. aeruginosa was 1,25 µg/mL. No MIC test was conducted for Ononis hirta against B. subtilis, E. aerogenes, E. coli, E. faecium, K. pneumoniae, S. aureus, S. enteritidis, S. epidermidis, S. kentucky and S. typhimurium because no activity was observed at these combinations in disk diffusion test. Table 9 presents the MIC values for Astragalus lydius. The MIC value against E. faecalis was 2.5 µg/mL, while the MIC values for S. aureus and S. infantis were both 1.25 µg/mL. No MIC test was conducted for Astragalus lydius against B. subtilis, C. albicans, E. aerogenes, E. coli, E. faecium, K. pneumoniae, P. aeruginosa, P. fluorescens, S. enteritidis, S. epidermidis, S. kentucky, and S. typhimurium due to the absence of activity observed for these combinations in the disk diffusion test. In the same table, the MIC values for Astragalus wiedemannianus were determined. The MIC value against E. faecalis was 1.25 µg/mL, while the MIC values for B. subtilis and P. fluorescens were both 5 µg/mL. The MIC value against P. aeruginosa was 2.5 µg/mL. No MIC test was conducted for Astragalus wiedemannianus against C. albicans, E. aerogenes, E. coli, E. faecium, K. pneumoniae, S. aureus, S. enteritidis, S. epidermidis, S. infantis, S. kentucky, and S. typhimurium due to the absence of activity observed for these combinations in the disk diffusion test. Furthermore, Table 9 presents the MIC values for Anthyllis tetraphylla. The MIC values against P. aeruginosa and E. faecalis were both observed to be 1.25 µg/mL. No MIC test was conducted for Anthyllis tetraphylla against B. subtilis, C. albicans, E. aerogenes, E.

coli, E. faecium, K. pneumoniae, P. fluorescens, S. aureus, S. enteritidis, S. epidermidis, S. infantis, S. kentucky, and S. typhimurium due to the absence of activity observed for these combinations in the disk diffusion test.

Results of Statistical Analysis

The parallel study accepted the H0 hypothesis: the results of the three parallel studies were statistically similar. When the statistical analysis results were compared, it was found that the parallel p-value for each concentration was between 0.9688 and 1 for all plant extracts. The H0 hypothesis was accepted because of a P-value > 0.05, indicating no difference between the results. The analysis section of the appendix is given in detail.

Upon comparing the results of the statistical analysis, it was observed that all plants, concentrations, and microorganisms (including Bacillus subtilis, Candida albicans, Enterobacter aerogenes, Escherichia coli, Enterococcus faecium, Enterococcus faecalis, Klebsiella pneumoniae, Pseudomonas aeruginosa, Pseudomonas fluorescens, Staphylococcus aureus, Salmonella enteritidis, Staphylococcus epidermidis, Salmonella infantis, Salmonella typhimurium, and Salmonella typhimurium) exhibited p-values ranging from 0.9587 to 1.

Furthermore, upon comparing the effects of p-values, it was observed that all tested microorganisms exhibited the following results: Bacillus subtilis = 0.9374, Candida albicans = 0.6139, Enterobacter aerogenes = 1, Escherichia coli = 1, Enterococcus faecium = 1, Enterococcus faecalis = 0.4995, Klebsiella pneumoniae = 0.3366, Pseudomonas aeruginosa = 0.7725, Pseudomonas fluorescens = 0.1385, Staphylococcus aureus = 0.9423, Salmonella enteritidis = 1, Staphylococcus epidermidis = 1 (for all concentrations: 10, 50, and 100 µL), Salmonella infantis = 0.7144, Salmonella kentucky = 1, and Salmonella typhimurium = 0.9030.

The statistical analysis revealed that Salmonella exhibited an impact on all five different concentrations of microorganisms, and the p-values for all plant extracts were 0.9929, 0.9586, and 0.9661 for 10, 90, and 100 µL, respectively. The null hypothesis (H0) was accepted since the p-value was greater than 0.05.

Furthermore, when comparing the statistical analysis results, it was found that all plant extracts affected only one microorganism, specifically Candida albicans, across different concentrations. The p-values ranged from 0.9878 to 1 for 100 µL. Again, the null hypothesis (H0) was accepted because the p-value was greater than 0.05.

Statistical analysis showed that the alpha-necked neck animals had two different microorganisms with different concentrations and parallel to all plant extracts, with p-values of 0.9938 for 10, 90, and 100 µL, respectively. 0,9980 and 0.9981. The H0 hypothesis was accepted because the p-value was > 0.05.

Statistical analysis showed that, compared with the results, all plant extracts O. hirta affected p-values of 0, 986, 10, 50, and 100 µL, respectively, for four microorganisms of different concentrations and parallel to microorganisms. 0,9688 and 0,9707. The H0 hypothesis was accepted because the p-value was > 0.05.

The statistical analysis revealed that A. lydius exhibited an impact on three different microorganisms across various concentrations. The p-values for the plant extracts were 0.9980, 0.9950, and 0.9948 for 10, 50, and 100 µL, respectively. Based on these results, the null hypothesis (H0) was accepted since the p-value was greater than 0.05.

Similarly, the statistical analysis demonstrated that A. wiedemannianus affected four different concentrations of microorganisms. The p-values for the plant extracts were 1, 0.9963, 0.9960, and 0.99982 for 1, 10, 50, and 100 µL, respectively. Once again, the null hypothesis (H0) was accepted as the p-value exceeded 0.05.

Furthermore, the statistical analysis indicated that A. tetraphylla had an impact on two microorganisms across different concentrations. The p-values for the plant extracts were 0.9960, 0.9928, and 0.9941 for 10, 90, and 100 µL, respectively. The null hypothesis (H0) was accepted since the p-value was greater than 0.05.

According to statistical analysis, the effect of each plant extract at different concentrations on microorganisms increases in parallel.

1. Results of Standard Antibiotic:

The results of disk diffusion test of standard antibiotics against working microorganisms are given in Table 5.

Table 5. Antimicrobial effect of standard antibiotics against studied strains by disk diffusion method.

Antibiyotik →	K	S	MEM	VA	AM	CN	OFX	L	CAZ	TE
Mikroorganizma	**30µg**	**10µg**	**10µg**	**30µg**	**10µg**	**10µg**	**5µg**	**5µg**	**30µg**	**30µg**
▪ C. albicans	-	-	-	-	-	-	-	-	-	-
▪ E. faecalis	11	-	15	-	15	-	14	-	-	-
▪ E. aerogenes	18	21	25	-	-	21	23	-	20	17
▪ E. coli	15	20	30	12	7	20	-	-	14	-
▪ E. faecium	-	-	-	-	-	-	-	-	-	-
▪ K. pneumonia	15	11	22	-	-	10	20	-	-	18
▪ L.monocytogenes	19	-	-	26	-	15	19	-	-	30
▪ P. aeruginosa	-	18	30	-	-	20	18	-	11	-
▪ P. fluorescens	-	-	-	-	-	-	-	-	-	-
▪ S. aureus	20	15	30	17	22	22	24	20	-	22
▪ S. enteritidis	21	15	35	14	13	24	24	20	-	10
▪ S. epidermidis	18	10	30	7	15	14	25	-	18	17
▪ S. infantis	-	13	22	8	15	18	15	-	18	-
▪ S. kentucky	15	10	25	7	14	10	20	-	18	17
▪ S. typhimurium	20	-	30	8	13	21	23	-	15	15

(-): No effect, K: Kanamisin, S: Streptomisin, MEM: Meropenem, VA: Vankomisin, AM: Ampisilin, CN: Gentamisin, OFX: Ofloksazin, L: Linkomisin, CAZ: Seftazidim, TE: Tetrasiklin.

2. Disk Diffusion Test:

In the present study, E. faecalis is the most sensitive microorganism because it is affected by all plant extracts in different amounts tested. In addition, the most resistant microorganisms to all plant extracts were observed to be E. coli, E. aerogenes, E faecium, S. enteritidis, S. epidermidis and S. kentucky, which weren't affected by any plant extract at any volumes tested.

The results indicate that microorganisms exhibit varying sensitivities to different plant extracts. For instance, certain microorganisms were found to be sensitive to specific plant extracts. For example, B. subtilis showed sensitivity to Astragalus wiedemannianus across all volumes (10, 50, and 100 µL), C. albicans was affected by Ononis hirta in 50 and 100 µL, K. pneumoniae was affected by Astragalus sigmoideus in 50 and 100 µL, P. fluorescens was affected by Astragalus wiedemannianus in 50 and 100 µL, and S. typhimurium was affected by Astragalus sigmoideus across all volumes (10, 50, and 100 µL).

Furthermore, some microorganisms exhibited sensitivity to two different types of plant extracts. For example, P. aeruginosa was affected by A. wiedemannianus in 50 and 100 µL, as well as by A. tetraphylla across all volumes (10, 50, and 100 µL). S. aureus showed sensitivity to both A. sigmoideus

and A. lydius across all volumes (10, 50, and 100 µL), while S. infantis was affected by O. hirta in 50 and 100 µL, as well as by A. lydius across all volumes (10, 50, and 100 µL).
Additionally, the results indicate that A. sigmoideus had an impact on all five tested microorganisms, while the strongest plant extract, Genista albida, only affected one of the microorganisms tested.
In conclusion, it can be inferred that all plants, regardless of their strength, demonstrated some level of activity against certain microorganisms.
The results presented in this thesis represent the initial findings of the tested plant samples. Currently, there are no existing reports in the literature regarding the antimicrobial activity of these specific plants, making it impossible to compare the results with those of the same plants.
In a study conducted by Teyeb et al. (2012), they examined the antimicrobial activity of methanol, chloroform, and ethyl acetate extracts from the aerial parts and roots of wild Astragalus gombiformis. They also tested water and methanol extracts from Astragalus gombiformis on six different types of bacteria, namely Listeria monocytogenes, S. epidermidis, P. aeruginosa, B. subtilis, E. coli, and Salmonella typhimurium. The researchers employed the paper disk agar diffusion method and determined the minimal inhibition concentration. Their findings revealed that the tested extracts exhibited antimicrobial activity against all six bacteria. The three extracts from the aerial parts (methanol, chloroform, and ethyl acetate) produced inhibition zones ranging from 10 mm to 15 mm, while the two extracts from the roots (water and methanol) yielded inhibition zones between 10 mm and 14 mm. In the present thesis study, it was discovered that the methanol extract of Astragalus sigmoideus demonstrated activity against E. faecalis, K. pneumoniae, P. fluorescens, S. aureus, and S. typhimurium, with the highest inhibition zones recorded as 9.67 mm, 10.33 mm, 13.33 mm, 10.00 mm, and 13.67 mm, respectively. The primary reason for these differences is that although the plant samples used in both studies belong to the same genus, they are distinct species.
In another study by Albaqawi and Selim (2015), they investigated the antimicrobial activity of 100 mL of distilled methanol (80%) extracts from Anziroat (Astragalus sp) against various pathogenic microorganisms. They employed the agar disk diffusion method on six bacterial strains and one yeast strain, including Aeromonas hydrophila, Proteus mirabilis, P. aeruginosa, S. aureus, Streptococcus sp, and E. coli. Their findings indicated that the extract exhibited antimicrobial activity against S. aureus and E. coli, resulting in inhibition zones of 20 mm and 15 mm, respectively. In the present thesis study, it was found that the methanol extract of Astragalus syringus did not demonstrate activity against S. aureus and E. coli. The main reason for this discrepancy is that, although the plant samples used in both studies belong to the same genus, they represent different species.
Çitoğlu and Altanlar (2003) conducted a study on the antimicrobial activity of 75% aqueous ethanol extracted from Ononis spinosa (Leguminosae) using the agar diffusion method. They discovered that the extract exhibited antimicrobial activity against E. coli, P. aeruginosa, S. aureus, and B. subtilis, resulting in inhibition zones of 11 mm. They also observed activity against C. albicans and C. krusei, with inhibition zones of 16 mm. In the present thesis study, it was found that the methanol extract of Ononis hirta demonstrated antimicrobial activity against C. albicans, E. faecalis, P. aeruginosa, and S. infantis, with the highest inhibition zones recorded as 14.00 mm, 20.00 mm, 11.00 mm, and 10.00 mm, respectively. The primary reason for these differences is that, although the plant samples used in both studies belong to the same genus, they represent different species.
Balachandar, Jagadeeswari, Dhanabalan, and Meenachi (2012) investigated the antimicrobial activity of methanolic and ethanolic extracts of Astragalus membranaceus using the disk diffusion method. They found that the methanolic extract exhibited antimicrobial activity against E. coli and S. enteritidis, resulting in inhibition zones of 13 mm and 12 mm at 5 mg/disk, respectively. They also observed that the ethanolic extract had antimicrobial activity against E. coli and S. enteritidis, with inhibition zones of 12 mm and 13 mm at 5 mg/disk, respectively. In the present thesis study, it was found that the methanol extract of Astragalus lydius demonstrated antimicrobial activity against E. faecalis, S. aureus, and S. infantis, with the highest inhibition zones recorded as 8.00 mm, 13.33 mm, and 7.67 mm, respectively. However, in this thesis study, the methanol extract did not affect E. coli and S. enteritidis. The main reason for these differences is that, although the plant samples used in both studies belong to the same genus, they represent different species.
Küçükboyaci, Özkan, and Tosun (2012) examined the antibacterial and antifungal activities of alkaloid extracts from Genista sandrasica. The extract demonstrated antimicrobial activity against E. coli, P. aeruginosa, B. subtilis, S. aureus, and C. albicans, with minimum inhibitory concentration (MIC) values of 125 µg/mL, 125 µg/mL, 31.25 µg/mL, 62.5 µg/mL, and 125 µg/mL, respectively. In the present thesis study, it was observed that the methanol extract of Genista albida exhibited antimicrobial activity against only E. faecalis, with a MIC value of 5 µg/mL. The main reason for these differences is that, although

the plant samples used in both studies belong to the same genus, they represent different species and utilize different methods.

Alrumman, Moustafa, and Alamri (2012) conducted a study on the antimicrobial activity of various extracts, including acetone, ethanol, methanol, 1/1 ethanol/methanol, 1/1 ethanol/acetone, 1/1 acetone/methanol, and water (hot and cold) extracts, from the leaves of A. atropilosulus subsp. abyssinicus. They employed the agar well diffusion method to test against pathogenic bacteria and fungi. They observed that all the extracts exhibited antimicrobial activity against P. aeruginosa, S. aureus, E. coli, Proteus sp, K. pneumoniae, Micrococcus sp, and S. epidermidis, with inhibition zones ranging from 9.33 mm to 35.0 mm. In the present thesis study, it was observed that the methanol extract of Astragalus wiedemannianus displayed antimicrobial activity against B. subtilis, E. faecalis, P. aeruginosa, and P. fluorescens, with the highest inhibition zones recorded as 12.00 mm, 8.33 mm, 7.33 mm, and 12.00 mm, respectively. The main reason for these differences is that, although the plant samples used in both studies belong to the same genus, they represent different species.
In a study conducted by Kiruthiga, Rakkimuthu, and Aravinthan (2014), the antibacterial activity of the methanolic leaf extract of Crotalaria pallida Aiton was investigated against five strains of human pathogenic bacteria, including Gram-negative strains (P. aeruginosa, E. coli, and K. pneumoniae) and Gram-positive strains (S. aureus and Bacillus sp.). The agar well diffusion method was employed for this purpose. The researchers observed that the extract exhibited antimicrobial activity against E. coli, K. pneumoniae, P. aeruginosa, Bacillus sp., and S. aureus, resulting in inhibition zones of 19 mm, 18 mm, 11 mm, 13 mm, and 15 mm, respectively, at a concentration of 25 mg/mL. In the present thesis study, it was observed that the methanol extract of Anthyllis tetraphylla demonstrated antimicrobial activity against E. faecalis and P. aeruginosa, with the highest inhibition zones recorded as 9.33 mm and 9.67 mm, respectively. The primary reason for these differences is that, although the plant samples used in both studies belong to different genera, they represent different species.
MIC Test: In the current study, the MIC (minimum inhibitory concentration) values were determined. The results indicated that E. faecalis was the most susceptible microorganism, as it was affected by all plant extracts, namely Astragalus sigmoideus, Genista albida, Astragalus syringus, Ononis hirta, Astragalus lydius, Astragalus wiedemannianus, and Anthyllis tetraphylla, with MIC values of 10 µg/mL, 5 µg/mL, 10 µg/mL, 5 µg/mL, 2.5 µg/mL, 1.25 µg/mL, and 1.25 µg/mL, respectively.
On the other hand, it was observed that the most resistant microorganisms against all plant extracts were E. coli, E. aerogenes, E. faecium, Salmonella enteritidis, S. epidermidis, and S. Kentucky.
Based on the results, Astragalus sigmoideus, Ononis hirta, and Astragalus wiedemannianus exhibited the highest potency in terms of MIC values, ranging from 1.25 µg/mL to 10 µg/mL. In contrast, Genista albida showed the lowest potency among the tested plants, as it only affected one microorganism, namely E. faecalis, with a MIC value of 5 µg/mL.

Conclusion:
In recent years, the pharmaceutical industry has increasingly recognized the potential of plant-based alternatives for treating infectious diseases. These plants offer a valuable source of novel anti-infective compounds. The investigation of plant antimicrobial activity is an ongoing endeavor aimed at discovering new and alternative approaches to disease treatment.
The findings of the current study are expected to contribute to the existing knowledge in drug research. However, further in-depth investigations are necessary to establish the plants used in this study as potential natural medicines.
Interestingly, all plant extracts tested in this study exhibited some level of activity against the microorganisms. Among them, Genista albida demonstrated the weakest activity, showing resistance to E. faecalis. Conversely, Astragalus sigmoideus displayed the strongest activity against a range of microorganisms, including E. faecalis, K. pneumoniae, P. fluorescens, S. aureus, and S. typhimurium.

References:

[1] Kanaan, H., El-Mestrah, M., Sweidan, A., As-Sadi, F., Al Bazzal, A., & Chokr, A. (2017). Screening for antibacterial and antibiofilm activities in Astragalus angulosus. Journal of intercultural ethnopharmacology, 6(1), 50.

[2] Khan, N. A., Quereshi, S., Pandey, A., & Srivastava, A. (2009). Antibacterial Activity of Seed Extracts of Commercial and Wild Lathyrus Species. Turkish Journal of Biology, 33(2), 165-169.

[3] Alrumman, S. A., Moustafa, M. F., and Alamri, S. A. (2012). Anti-bacterial and antifungal investigation of Astragalus atropilosulus subsp. abyssinicus. African Journal of Microbiology Research, 6(34), 6365-6369.

[4] Teyeb, H., Houta, O., Najjaa, H., Lamari, A., Neffati, M., Douki, W., & Najjar, M. F. (2012). Biological and chemical study of Astragalus gombiformis. Zeitschrift für Naturforschung C, 67(7-8), 367-374

[5] Arias, M. E., Gomez, J. D., Cudmani, N. M., Vattuone, M. A., & Isla, M. I. (2004). Antibacterial activity of ethanolic and aqueous extracts of Acacia aroma Gill. ex Hook et Arn. Life sciences, 75(2), 191-202.

[6] Altuner, E. M., Ceter, T., and İşlek, C. (2010). Investigation of antifungal activity of Ononis spinosa L. ash used for the therapy of skin infections as folk remedies. Mikrobiyoloji Bülteni, 44(4), 633-639.