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Natural Solutions for Bacterial Infections: Evaluating the Efficacy of Sedum Album sp Plant Extract against Gram-Positive and Gram-Negative Bacteria

Rezgallah Younis Mahmoud Rezgallah [1*], Garnasah Ahmed Asmeedah [2], Aml Ahmed Asmeedah [3], Seham Alsharif Salheen [4], Afya Atoumi Baroud [5]
[1] Department of Botany, Faculty of Science, University of Benghazi, Libya
[2,5] Department of Chemistry, Faculty of Science, Bani Waleed University, Libya
[3] Department of Botany, Faculty of Science, Bani Waleed University, Libya
[4] Department of Plant Protection, Faculty of Agriculture, Bani Waleed University, Libya

*Corresponding author: rezgalah.rezgalah@uob.edu.ly

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Abstract:
With the rise of antibiotic resistance, there is an urgent need for alternative and effective treatments for bacterial infections. Natural compounds derived from plants have gained considerable attention for their potential antimicrobial properties. This study aims to explore the efficacy of Sedum album sp plant extract against both Gram-positive and Gram-negative bacteria, shedding light on its potential as a natural solution for bacterial infections.
The study concluded the antimicrobial activity observed in the extract of Sedum album sp indicates the presence of bioactive compounds that hold promise for potential therapeutic applications it also indicated that the application of Sedum album sp plant extract has demonstrated significant potential in combating both Gram-positive and Gram-negative bacteria. The observed antimicrobial activity suggests the presence of bioactive compounds with therapeutic applications. However, further studies are needed to identify and isolate the specific constituents responsible for these effects, as well as to elucidate their mechanisms of action.

Keywords: Albums, Sedum, Crassulaceae, antimicrobial activity

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الحلول الطبيعية للعدوى البكتيرية: تقييم فعالية مستخلص نبات ألبوم السيدوم ضد البكتيريا إيجابية الجرام وسالبة الجرام

رزق الله يونس محمود رزق الله [1*]، قرناصة أحمد اصميده [2]، أمل أحمد اصميده [3]، سهام الشارف صالحين [4]، عافية التومي بارود [5]
[1] قسم النبات، كلية العلوم، جامعة بنغازي، بنغازي، ليبيا
[5،2] قسم الكيمياء، كلية العلوم، جامعة بني وليد، بني وليد، ليبيا

[3] قسم النبات، كلية العلوم، جامعة بني وليد، بني وليد، ليبيا
[4] قسم وقاية النبات، كلية الزراعة، جامعة بني وليد، بني وليد، ليبيا

الملخص

مع ارتفاع مقاومة المضادات الحيوية، هناك حاجة ملحة لعلاجات بديلة وفعالة للعدوى البكتيرية؛ فقد. اكتسبت المركبات الطبيعية المشتقة من النباتات اهتماماً كبيراً لخصائصها المضادة للميكروبات. هدفت هذه الدراسة إلى استكشاف فعالية مستخلص نبات ألبوم السيدوم ضد كل من البكتيريا إيجابية الجرام وسالبة الجرام، وتسليط الضوء على إمكاناته كحل طبيعي للعدوى البكتيرية.

خلصت الدراسة إلى أن النشاط المضاد للميكروبات الذي لوحظ في مستخلص نبات ألبوم السيدوم يشير إلى وجود مركبات نشطة بيولوجياً تبشر بالتطبيقات العلاجية المحتملة. كما أشارت الدراسة إلى أن تطبيق مستخلص نبات ألبوم السيدوم قد أظهر إمكانات كبيرة في مكافحة كل من بكتيريا إيجابية الجرام، والبكتيريا سالبة الجرام. كما أشارت النتائج إلى أن النشاط المضاد للميكروبات الملحوظة وجود مركبات نشطة بيولوجياً لها تطبيقات علاجية. ومع ذلك، هناك حاجة إلى مزيد من الدراسات لتحديد وعزل المكونات المحددة المسؤولة عن هذه التأثيرات، وكذلك لتوضيح آليات عملها.

الكلمات المفتاحية: ألبومات، نبات السيدوم، عائلة الدهنيات، نشاط مضادات الميكروبات.

Introduction

The Crassulaceae family comprises a diverse range of flowering plants, including succulents, herbs, and shrubs. These plants are renowned for their adaptability to various environmental conditions, particularly in arid and semi-arid regions. Many species within this family have a long history of traditional use in folk medicine, owing to their therapeutic properties [1]. One notable member of the Crassulaceae family is Sedum album sp, commonly known as White Stonecrop. This perennial plant features small, fleshy leaves and white flowers. It has a wide distribution and can be found in different regions across the globe. In traditional medicine, Sedum album sp has been utilized for its medicinal benefits, such as aiding in wound healing and exhibiting anti-inflammatory properties [2].

Antimicrobial activity refers to the capacity of a substance to inhibit the growth or eliminate microorganisms, including bacteria, fungi, and viruses. This attribute is crucial in the search for potential therapeutic agents in the field of medicine. Exploring the antimicrobial properties of natural sources, such as plants, offers a promising avenue for the discovery of novel antimicrobial compounds [3].

Previous Studies:

Saleem et al. (2015) used a disc diffusion test to investigate the antimicrobial activity of a petroleum ether extract from the Kalanchoe pinnata genus against four bacteria types (Bacillus subtilis, Pasteurella multocida, Staphylococcus aureus, and Escherichia coli) and two fungus types. They discovered that the highest activity was against G. lucidum and E. Coli, with inhibition zones measuring 23.5 mm and 22.5 mm, respectively. They discovered that the lowest activity was against B. Subtilis, with an inhibition zone measuring 11.2 mm. The plant's petroleum ether extract showed no antimicrobial activity against P. multocida or S. aureus. [4].

In addition, the researchers examined the antimicrobial properties of the chloroform extract derived from the same plant. Their findings revealed that the extract exhibited the most significant effect against Pasteurella multocida, resulting in an inhibition zone diameter of 30.2 mm. Conversely, the chloroform extract displayed the lowest activity against S. aureus, with an inhibition zone diameter of 10.7 mm.

Furthermore, the ethyl acetate extract demonstrated the highest antimicrobial activity against both P. multocida and S. aureus, with an inhibition zone diameter of 30 mm. Similarly, the n-butanol extract of the plant exhibited notable antimicrobial activity, particularly against P. multocida, with an inhibition zone diameter of 26 mm. Although the anhydrous (absolute) methanol extract of the plant did not exhibit any activity against G. lucidum, it displayed a significant antimicrobial effect against A. alternata, resulting in an inhibition zone of 22.7 mm. Conversely, its activity against B. subtilis was the lowest, with an inhibition zone of approximately 6 mm.

On the other hand, the methanol extract (95%) showed inhibition activity solely against G. lucidum, with an inhibition zone of 22.5 mm, while it did not demonstrate notable antimicrobial activity against other microorganisms.

In their research, Pattewar, Patil, and Dahikar (2013) conducted a study to examine the antimicrobial properties of ethanol and methanol extracts derived from the plant genus Kalanchoe using the disk diffusion test. Their findings revealed that the ethanol extract exhibited antimicrobial activity against S. aureus, Pseudomonas aeruginosa, E. coli, and Candida albicans, resulting in inhibition zones with diameters of 15 mm, 18 mm, 18 mm, and 15 mm, respectively.

Similarly, the methanol extract of the Kalanchoe genus demonstrated antimicrobial activity against S. aureus (with an inhibition zone diameter of 21 mm), P. aeruginosa (approximately 21 mm), E. coli (around 25 mm), and C. albicans (18 mm) according to the disk diffusion test

In a separate study conducted by Biswas, Chowdhury, Raihan, Akbar, and Mowla (2012), they examined the antimicrobial efficacy of the chloroform extract from the K. pinnata plant against eight

bacterial strains using the disk diffusion method. The results showed that the extract exhibited the highest antimicrobial activity against E. coli. On the other hand, it displayed the lowest effect against B. subtilis, S. aureus, P. aeruginosa, Salmonella typhi, and Shigella dysenteriae. Notably, no antimicrobial activity against Vibrio cholera was observed in their study [5].

In a study conducted by Tosun, Bahadir, and Altanlar (2006), the antimicrobial activity of an 80% ethanol extract derived from Sedum acre was examined against six bacteria using the disc diffusion method. The extract displayed antimicrobial activity against C. albicans, resulting in an inhibition zone of 13 mm, and against Candida krusei, with an inhibition zone of 12 mm. However, no antimicrobial activity was observed against S. aureus, B. subtilis, E. coli, and Candida glabrata [6].

In another study by Ramesh, Manikandan, and Shanmugam (2016), the antimicrobial effect of an ethanol extract from K. pinnata was investigated using the agar well diffusion method against four types of pathogenic bacteria, namely Streptococcus sp, Klebsiella planticola, Klebsiella pneumoniae, and S. aureus. The results showed that the extract exhibited 6 mm inhibition zones against all tested microorganisms

In a study conducted by Rovčanin, Ćebović, Stešević, Kekić, and Ristić (2015), the antimicrobial activity of an ethanol extract derived from Sempervivum tectorum was investigated using the well diffusion method against E. coli. The extract exhibited antimicrobial activity, resulting in a 28 mm inhibition zone when tested against E. coli [7].

In another study by Nwadinigwe (2011), the antimicrobial efficacy of ethanol and water extracts from Bryophyllum pinnatum was examined using the agar well diffusion method. The study also determined the Minimum Inhibition Concentration (MIC) values for six microorganisms, namely S. typhi, P. aeruginosa, S. aureus, B. subtilis, C. albicans, and Aspergillus niger. The results showed that the ethanol extract displayed significant antimicrobial activity against B. subtilis (20-25 mm) and S. aureus (17-22.5 mm) at concentrations of 100, 50, and 25 mg/mL in the agar diffusion test ($p < 0.01$). The water-containing extract also exhibited significant antimicrobial activity against S. typhi (9.5-18 mm) and B. subtilis (15.5-24 mm) at the same concentrations ($p < 0.01$). However, both extracts did not show any antimicrobial activity against P. aeruginosa, C. albicans, and A. niger. The lowest MIC value was observed against S. aureus, with a value of 6.29 mg/mL for the ethanol extract, while S. typhi displayed the highest MIC value of 9.98 mg/mL for the water-containing extract ($p < 0.01$ significant). Overall, the results indicated that B. pinnatum exhibits clear antimicrobial activity [8]. In a study conducted by Wafa and Sofiane (2016), the antimicrobial activity of tannins extracted from the North Africa endemic species Sedum pubescens was investigated using the disk diffusion test against three bacterial strains and three fungal strains. The extracts exhibited varying degrees of antimicrobial activity against the microorganisms tested. Specifically, the extracts showed a 7 mm inhibition zone against E. coli and a 9 mm inhibition zone against S. aureus. However, no activity was observed against Salmonella typhimurium. Furthermore, the tannins obtained from S. pubescens displayed antimicrobial activity against Aspergillus flavus (with a 13 mm inhibition zone), Aspergillus niger (with a 10 mm inhibition zone), and Candida albicans (with a 9 mm inhibition zone) [9]. In another study by Muiruri and Mwangi (2015), the antimicrobial efficacy of ethanol and water extracts from Crassula ovata was examined using the disk diffusion test against five bacterial strains. The ethanol extract of C. ovata showed antimicrobial activity against E. coli, resulting in a 7 mm inhibition zone, while the water extract exhibited an average inhibition zone of 6 mm against E. coli [10].

Literature Review

1. Crassulaceae Family

The Crassulaceae family encompasses approximately 1,400 to 1,500 plant species that belong to 33 different genera worldwide [11]. In Turkey, this family is represented by 8 species, 79 types, and 93 taxons at the type and sub-type levels. Among the taxons grown in Turkey, 28 are endemic, accounting for an endemism rate of 30.1% [12]. The distribution of family members in Turkey, based on phytogeographical regions, is as follows: Mediterranean region (47%), Europe-Siberia (35%), and Iran-Turan (18%) [13]. The plants in this family are dicotyledonous, characterized by fleshy leaves. While most species are herbaceous, the family also includes shrubs, tree-like plants, and even species suitable for aquariums. They are predominantly found in the Northern Hemisphere, but their distribution extends worldwide (see Map 1.1) [14]. Although no member of this family is considered a significant cultivar, many of them are popular in horticulture. Crassulaceae members often exhibit striking visual characteristics and are generally robust plants that require minimal care. The family comprises succulent herbs and small bushes, with many species commonly grown as house plants. Physiologically, members of the Crassulaceae family exhibit Crassulacean Acid Metabolism (CAM). The plants in this family have fleshy, herbaceous leaves arranged in a basic, complete, fleshy, and alternating sequence, or similar patterns. In many species, leaves can be used to propagate new

individuals. The flowers of Crassulaceae species typically display radial symmetry and have an equal number of sepals and petals, usually five of each, although variations can occur [14].
The sepals in the Crassulaceae family can either be distinct or fused together, and the same applies to the petals, which sometimes form a tubular corolla. The stamens are arranged in one or two whorls, with the number matching that of the petals. The ovary is positioned above the other floral parts (superior), and the 4-5 carpels are mostly free, although they may be partially united at the base in certain species. Each carpel is accompanied by a nectary and contains numerous ovules on axile placentae. As the carpels mature, they develop into follicles. The Crassulaceae family is found in a range of habitats, from tropical to boreal regions, often thriving in arid environments. Many boreal species of this family grow amidst rocks, which quickly absorb and radiate heat from the sun onto the plants [15]. Figure (1) illustrates the distribution of the Crassulaceae family.

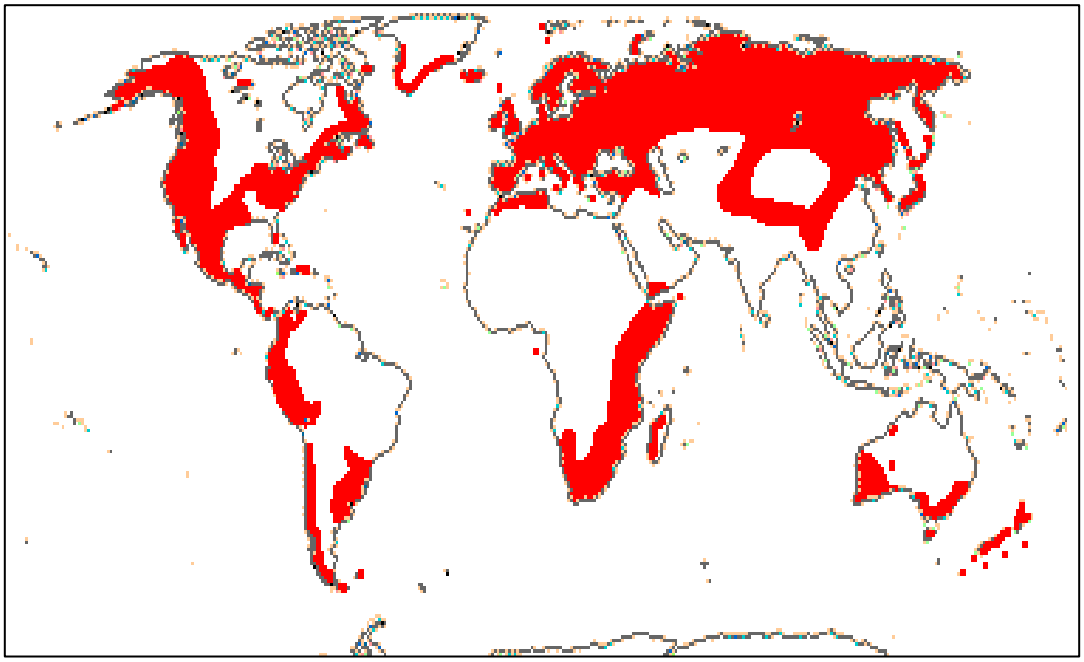

Figure 1. Distribution of Crassulaceae family [15].

2. Genus Sedum:
Turkey stands as a highly significant region for plants within the temperate zone, boasting an impressive diversity of approximately 10,765 vascular plant taxa. Among them, 34.4% are exclusive to the area. The country not only harbors a rich flora but also encompasses a wide array of habitats, including coastal dunes, peatlands, wetlands, heathlands, grasslands, and ancient forests [16].
Regrettably, the distinctive flora and habitats of Turkey face grave threats and have experienced a rapid decline over the past four decades [17]. The Sedum genus, commonly known as Orpine, belongs to the Crassulaceae family and is found across Europe, Asia, and the Americas. With a staggering 428 species, Sedum stands as the largest genus within the family [18]. This genus thrives not only in arid regions but also in subarid, sub-tropical, and both cool and warm areas [4] [19] [20]. In Europe alone, there are fifty-three Sedum species belonging to the subgenus Sedum [18] [21] [22].
Within Turkey, the Sedum genus (Crassulaceae) is represented by 33 species and 36 taxons at the genus and subgenus levels [12]. Several species, such as Sedum acre L., Sedum telephium L., and Sedum pallidum, are utilized by certain communities in Anatolia for treating wounds, hemorrhoids, and constipation. Additionally, they are employed as emollients and diuretic medicines [23].

3. Antimicrobial Activity of Plants
In developing countries with lower incomes, individuals residing in remote areas far from towns and local populations often rely on ethnopharmacological remedies to address various ailments [17]. These remedies primarily involve the use of plant-based ethnomedicines, which are consumed by directly drinking plant juices, ingesting plant parts, or preparing infusions to alleviate respiratory issues, headaches, and stomach problems. Additionally, plants are utilized to create creams or dressings for covering burned or damaged skin that carries a risk of infection. Consequently, the limited access to

modern medicine and medical facilities, coupled with the trust placed in local healers who prepare medicines from natural sources, leads people to turn to plant-based remedies. Moreover, these local healers' prescriptions are believed to be not only devoid of side effects but also as effective, if not more so, than modern medicines, while being much more affordable. Local healers often assert that plant-based treatments exhibit greater efficacy than chemically synthesized antimicrobial medications. However, it is crucial to scientifically evaluate this claim in order to explore the potential of ethnomedicines in combating diseases caused by microorganisms [19].
It is widely recognized that pharmaceutical plants serve as an alternative treatment option for non-serious cases of infectious diseases. They not only offer a promising source for discovering potent antimicrobial agents effective against non-resistant strains of pathogens but also hold the potential to contain active compounds with activity against resistant pathogens [21]. In this thesis study, five species belonging to the Crassulaceae family were selected to evaluate their antimicrobial activities against various microorganisms.

Methods and Procedures:

1. Experimental Techniques:

The analysis of the antimicrobial activity of Sedum album sp involved several experimental techniques. The plant material was collected and dried to facilitate extraction. Different solvent systems, such as ethanol or methanol, were employed to extract the bioactive compounds from the plant material.

2. Study Sample:

This study aimed to assess the antimicrobial activity of plant extracts by testing them against a total of 15 microorganisms. Among the gram-positive bacteria used in the study were Bacillus subtilis DSMZ 1971, Enterococcus faecalis ATCC 29212, Enterococcus faecium, Staphylococcus aureus ATCC 25923, and Staphylococcus epidermidis DSMZ 20044. The gram-negative bacteria included Enterobacter aerogenes ATCC 13048, Escherichia coli ATCC 25922, Klebsiella pneumonia, Salmonella infantis, Salmonella kentucky, Salmonella enteritidis ATCC 13075, Salmonella typhimurium SL 1344, Pseudomonas aeruginosa DSMZ 50071, and Pseudomonas fluorescens P1. Additionally, the fungus Candida albicans DSMZ 1386 was also included in the study. Prior to testing, the collected plants were thoroughly cleaned using distilled water.

2.1 Extraction Process:

150 g of fresh- plant samples were weighed into a flask and 300 mL of 60% ethanol (reference) was transferred into the flask and placed on a shaker (WiseShake, Korea) and shaken at 100 rpm for three days at room temperature (figure 2).

Figure 2. Extraction Process (Shaker)

Following a three-day period, the mixture was filtered (see figure 3) into evaporating flasks. These flasks were then connected to a rotary evaporator (Heidolph, Germany), and the alcohol present in the extract was eliminated by rotating the samples at temperatures ranging from 35 to 45 ºC (see figure 3). Once all the alcohol was removed from the extract, the filtrate was fully frozen prior to being connected to a freeze dryer (Christ, Germany).

Figure 3. Filtration process.

The frozen extracts attached to the freeze dryer, which was set to - 82 ºC and 0.12 atm vacuum and left for one to three days until the extract completely dried Figure (4)**.**

Figure 4. Rotary evaporator.

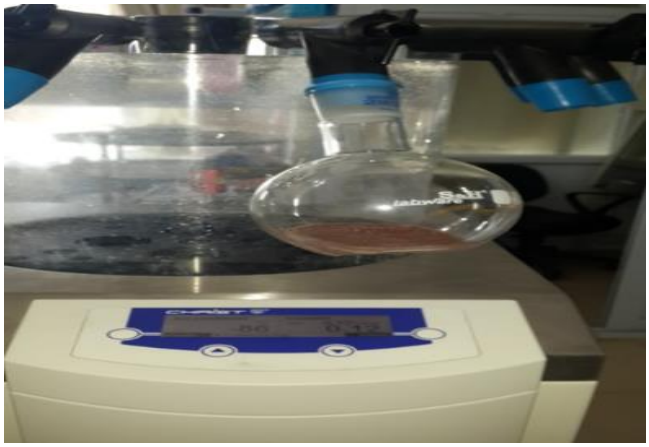

Figure 5. Freeze drying process.

2.2 Preparation of Inoculate

For each microorganism, which will be used in the study an inoculum was prepared. To prepare the inoculum morphologically similar colonies of the microorganism were transferred in 0,9% sterile NaCl solution and the turbidity was adjusted to 0,5 McFarland standard.

2.3 Loading Extracts to Empty Disks

Different volumes (10 µL, 50 µL and 100 µL) of extract stocks figure (5), which were prepared previously loaded on empty sterile antibiotic disks in aseptic conditions. And the ethanol was removed by leaving disks for 24 h at 40 ºC, to prevent any interaction with mic.

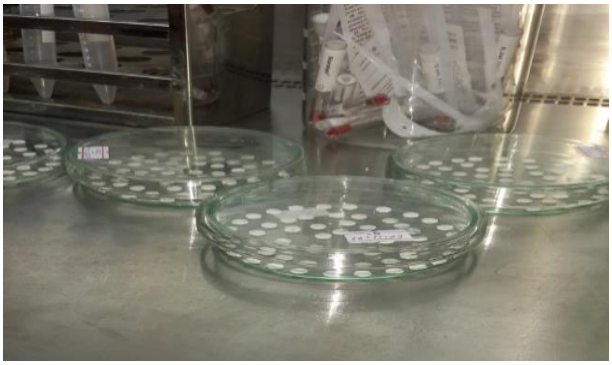

Figure **6.** Disks loaded.

Figure 7. Incubation.

2.4 Disk Diffusion Test:

The disk diffusion test involved the use of inocula from fifteen microorganisms and disks loaded with extracts. The surface of Mueller Hinton Agar (MHA) was inoculated with the inoculum using a sterile cotton swab. Subsequently, four disks were applied to the MHA surface, including one empty disk, one disk containing 10 µL of extract, one disk containing 50 µL of extract, and one disk containing 100 µL of extract. The plates were then incubated at 37 ± 1 ºC for 24 hours for bacteria and at 27 ± 1 ºC for 48 hours for fungi. Following incubation, the diameters of the inhibition zones were measured using a ruler, and the results were recorded in millimetres (see Figures 7 & 8).

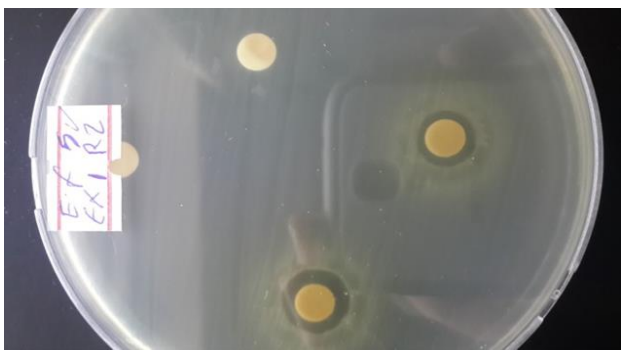

Figure 7. A sample inhibition zone**.**

2.5 Minimum Inhibitory Concentration (MIC) Test

The determination of the minimum inhibitory concentration (MIC) of an antimicrobial agent involves identifying the lowest concentration that visually inhibits the growth of microorganisms. The MIC values were assessed by incubating a known quantity of bacteria with serial dilutions of the extracts. Careful steps were followed to ensure valid and reliable results. The extracts were added to a prepared Mueller Hinton Broth, which was made from a dehydrated base. The pH of the broth needed to be maintained between 7.2 and 7.4 at room temperature. Each well of a microtiter plate (96 wells) contained a different concentration of the extracts.

Within 15 minutes of adjusting the inoculum to the 0.5 McFarland turbidity standard, the suspension was mixed and diluted to achieve an approximate final concentration of 5x10^8 cfu/mL. Initially, 100 µL of Mueller Hinton Broth (MHB) was transferred to all wells of the microtiter plate, numbered from 1 to 12. Next, 100 µL of the extract stock solution was added to the first well and mixed thoroughly and cautiously. Then, 100 µL of the content from well number 1 was transferred to well number 2, and the contents were mixed carefully. This serial dilution process was repeated until well number 10, and the content of well number 10 was discarded.

After completing the serial dilution, 10 µL of the inoculum was transferred to all wells except well number 12. Wells number 1 to 10 were used to test the activity of the plant extract, while well number 11 served as the positive control for the microorganism, and well number 12 acted as the negative control for the culture media (MHB). Precautions were taken during the inoculation of the MIC panel to prevent splashing between wells. The 96-well plates were then incubated at 37 ± 1°C for 24 hours for bacteria and at 27 ± 1°C for 48 hours for fungi. The MIC value was determined as the lowest concentration of the extract that completely inhibited the visible growth of the microorganism (see Figure 8).

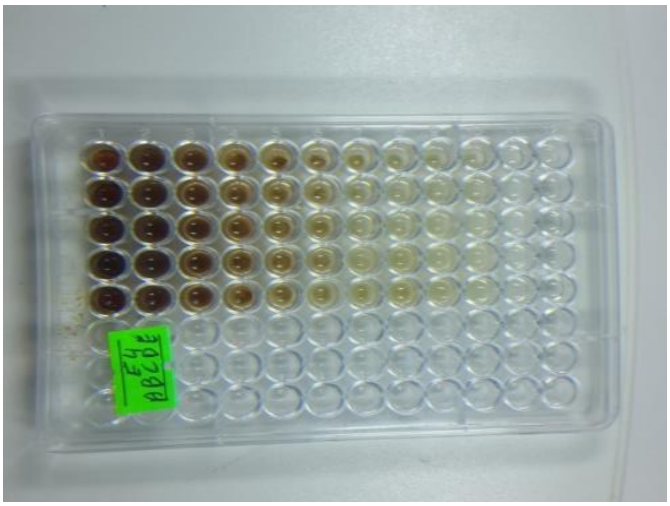

Figure 8. MIC Test.

Statistical Analysis:

In this study One way ANOVA was used to compare the parallel studies and the differences between different concentrations and p-value was accepted as p>0.05. One way ANOVA Calculator in the following link was used to conduct statistical analysis.

Null hypothesis for parallel studies was set as H_0: The results of three parallels are statistically similar.

When the results of the statistical analysis compared, it is found that for all plants extracts, for all concentrations the *p*-values for the parallels was found as 0,9281 to 1. Since p-value > 0.05, we accept the null hypothesis H_0, which means there is no difference between the results. Detailed analysis were given in the appendix section.

When the results of the statistical analysis compared, it is found that for all plants, all concentrations, all microorganisms (*B. subtilis, C. albicans, E. aerogenes, E. coli, E. faecium, E. faecalis, K. pneumonia, P. aeruginosa, P. fluorescens, S. aureus, S. enteritidis, S. epidermidis, S. infantis, S. kentucky and S. typhimurium*) the p value for the parallel was also between 0,9281 to 1.

Moreover, the results of comparison of the effect of the p values for all plant extract against every microorganisms were tested for all concentration (10, 50 and 100 µL), with B. subtilis =0.6161, C. albicans =0.2968, E. aerogenes =0.1840, E. coli=0.3022, E. faecalis=0.7876, E. faecalis=0.0155, K. pneumonia=0.5041, P. aeruginosa=0.0250, P. fluorescens=0.3803, S. aureus=0.3358, S.

enteritidis=0.0162, S. epidermidis=0.2680, S. infantis=0.0082, S. kentucky=0.2727 and S. typhimurium=0.5583.
When the results of the statistical analysis compared, for all plant extracts against microorganisms with different concentrations and parallels, was found that for S. album affected three microorganisms, the p-values were 0.9971 and 0.9984 for 50 and 100 µL respectively. Since p-values > 0.05, we accept the null hypothesis H0. For 10 µL. When the results of the statistical analysis compared, for all plant extracts against microorganisms with different concentrations and parallels, was found that for S.pallidum var bitynicum affected eight microorganisms were tested, the p-values were 0.9873 and 0.999 for 50 and 100 µL respectively. Since p-values > 0.05, we accept the null hypothesis H0. For 10 µL
When the results of the statistical analysis compared, for all plant extracts against microorganisms with different concentrations and parallels, it was found that for S. pallidum var pallidum affected twelve microorganisms, the p-values were 1, 0.9948 and 0.9504 for 10, 50 and 100 µL respectively. Since *p*-values > 0.05, we accept the null hypothesis H_0. When the result of the statistical analysis compared, for all plant extracts against microorganisms with different concentrations and parallels, was found that for *S.* sediforme affected eight microorganisms, the p-values were 0.9976, 0.9894 and 0.99982 for 10, 50 and 100 µL respectively. Since p-values > 0.05, we accept the null hypothesis H0. When the results of the statistical analysis compared, for all plant extracts against microorganisms with different concentrations and parallels, was found that for *S. armenum* affected eight microorganisms were tested, the p-values were 0.9971 and 0.9281 for 50 and 100 µL respectively. Since *p*-values > 0.05, we accept the null hypothesis H_0. According to the statistical analysis compared, for every plants extracts against microorganisms with different concentrations and parallels, since increasing the concentration it increases the effect.

Results:

1. Results of Sedum Album:

The findings revealed that S. album exhibited antimicrobial activity against C. albicans, E. faecalis, and E. faecium. However, no activity was observed against B. subtilis, C. albicans, E. aerogenes, E. coli, K. pneumonia, P. aeruginosa, P. fluorescens, S. aureus, S. enteritidis, S. epidermidis, S. infantis, S. kentucky, and S. typhimurium. The antimicrobial activity results for S. album are presented in Figure 9.

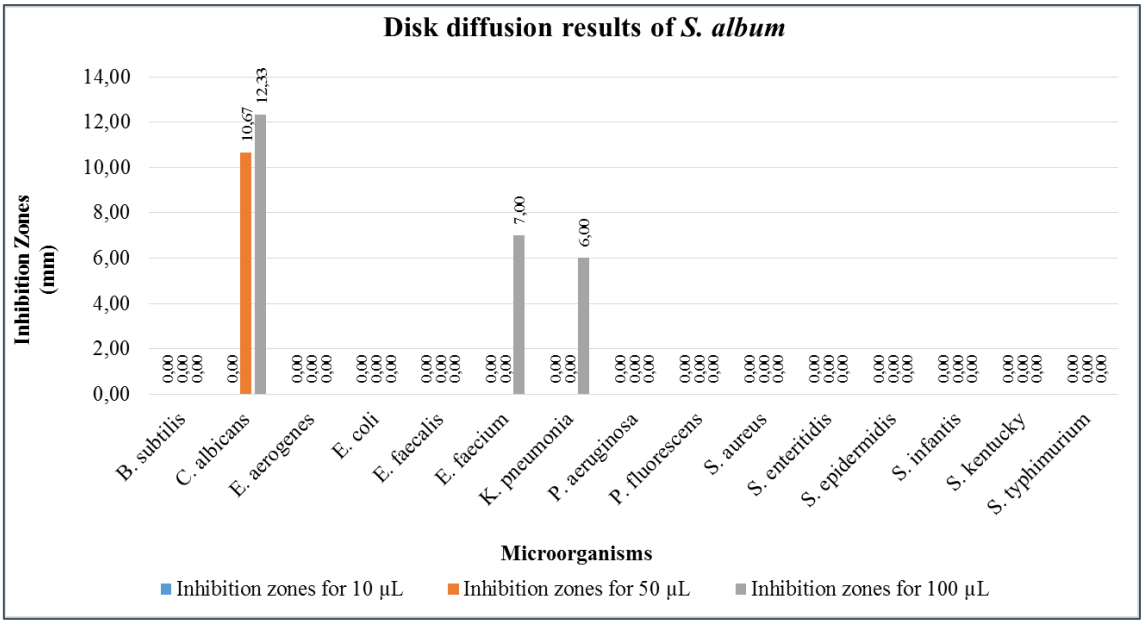

Figure 9. Disk diffusion results of S. album.

As mentioned previously, there was no observed activity against the following microorganisms: B. subtilis, E. aerogenes, E. coli, K. pneumonia, P. aeruginosa, P. fluorescens, S. aureus, S. enteritidis, S. epidermidis, S. infantis, S. kentucky, and S. typhimurium.

2. Results of Sedum Pallidum var Bitynicum

The results indicated that S. pallidum var bitynicum exhibited antimicrobial activity against E. aerogenes, E. faecalis, P. aeruginosa, P. fluorescens, S. aureus, S. enteritidis, S. infanti, and S. typhimurium. However, no activity was observed against B. subtilis, C. albicans, E. coli, E. faecium, K. pneumonia, S. epidermidis, and S.

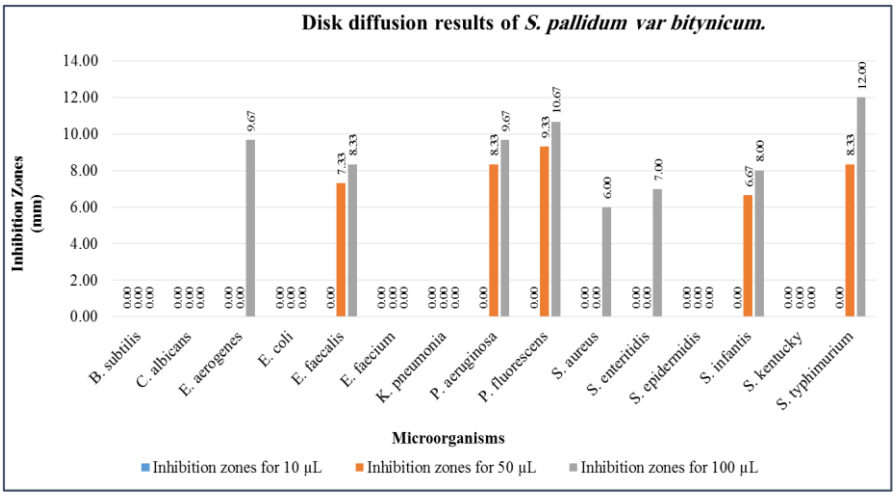

Figure 10. Disk diffusion results of S. album.

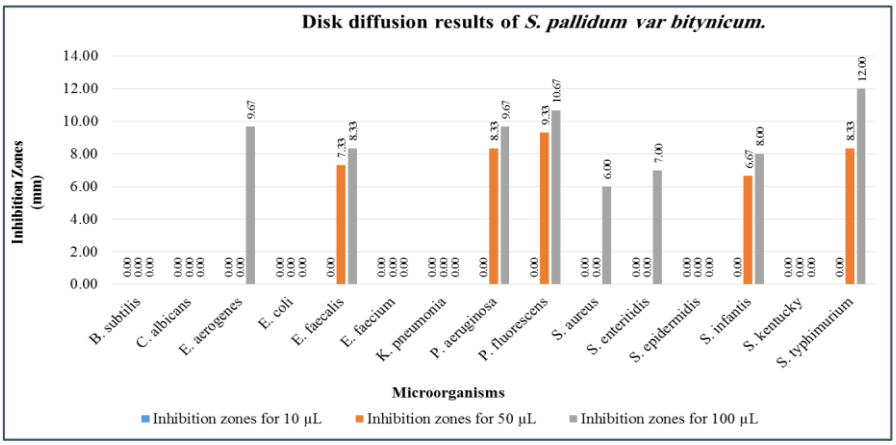

Figure 11. Disk diffusion results of S. pallidum var bitynicum.

The antimicrobial activity of S. pallidum var bitynicum against E. aerogenes was clearly observed in Figure 11, with an inhibition zone of 9.67 mm observed for only 100 µL of extract. The activity against E. faecalis showed inhibition zones of 7.33 mm and 8.33 mm for 50 µL and 100 µL of extracts, respectively. Furthermore, the activity against P. aeruginosa exhibited inhibition zones of 8.33 mm and 9.67 mm, while for P. fluorescens, inhibition zones of 9.33 mm and 10.67 mm were observed for 50 µL and 100 µL of extracts, respectively.

The antimicrobial activity of the extract against S. aureus resulted in an inhibition zone of 6.00 mm, observed only for 100 µL of extract. Similarly, against S. enteritidis, an inhibition zone of 7.00 mm was observed, again only for 100 µL of extract.

For S. infantis, the activity showed inhibition zones of 6.67 mm and 8.00 mm for 50 µL and 100 µL of extracts, respectively. Moreover, against S. typhimurium, inhibition zones of 8.33 mm and 12.00 mm were observed for 50 µL and 100 µL of extracts, respectively.

As mentioned earlier, no activity was observed against B. subtilis, C. albicans, E. coli, E. faecium, K. pneumonia, S. epidermidis, and S. kentucky.

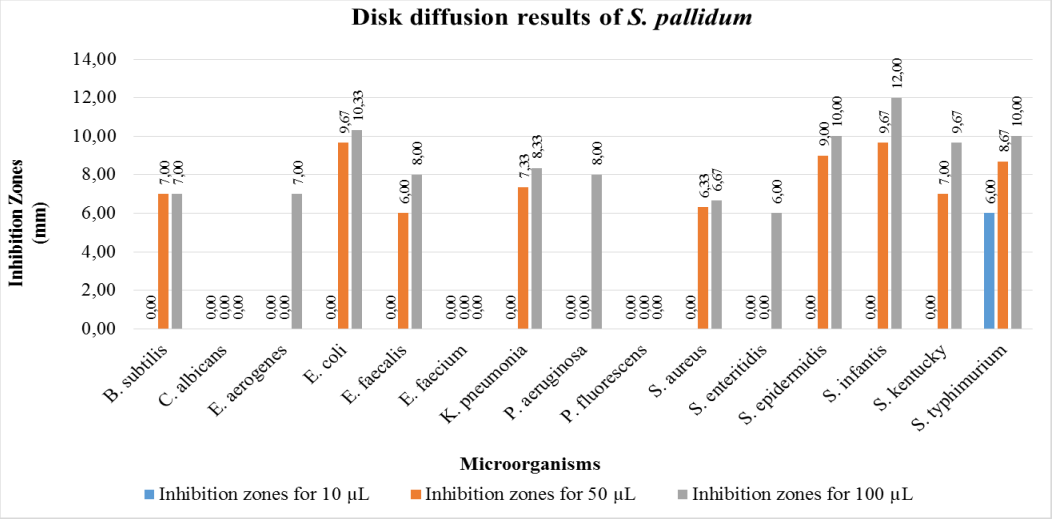

Figure 12. Disk diffusion results of S. pallidum.

Figure 12 above illustrates the antimicrobial activity of S. pallidum against various microorganisms. The inhibition zone against E. aerogenes was observed to be 7.00 mm, exclusively for 100 µL of extract. Additionally, the activity against P. aeruginosa and S. enteritidis resulted in inhibition zones of 8.00 mm and 6.00 mm, respectively, for 100 µL of extracts. Furthermore, a consistent 7 mm inhibition zone was observed against B. subtilis for both 50 µL and 100 µL of extract. The activity against E. coli exhibited inhibition zones of 9.67 mm and 10.33 mm for 50 µL and 100 µL of extracts, respectively. Similarly, the activity against E. faecalis showed inhibition zones of 6.00 mm and 8.00 mm; K. pneumonia resulted in inhibition zones of 7.33 mm and 8.33 mm; S. aureus exhibited inhibition zones of 6.33 mm and 6.67 mm; S. epidermidis showed inhibition zones of 9.00 mm and 10.00 mm; S. infantis displayed inhibition zones of 9.67 mm and 12 mm; and S. kentucky demonstrated inhibition zones of 7.00 mm and 9.67 mm for 50 µL and 100 µL of extracts, respectively. In the case of S. typhimurium, inhibition zones of 6.00 mm, 8.67 mm, and 10.00 mm were observed for 10 µL, 50 µL, and 100 µL of extracts, respectively. As mentioned earlier, no activity was observed against C. albicans, E. faecium, and P. fluorescens.

3. Sedum Sediforme:

The findings revealed that S. sediforme exhibited antimicrobial activity against C. albicans, E. coli, E. faecalis, E. faecium, P. aeruginosa, P. fluorescens, S. enteritidis, and S. infantis. However, no activity was observed against B. subtilis, E. aerogenes, K. pneumonia, S. aureus, S. epidermidis, S. kentucky, and S. typhimurium. The antimicrobial activity results for S. sediforme are presented in Figure 13.

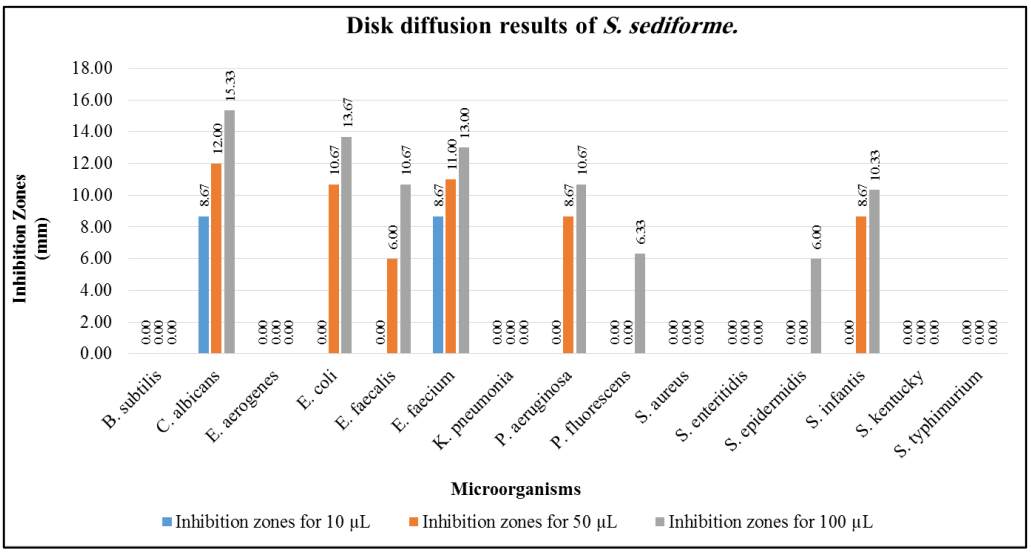

Figure 13. Disk diffusion results of S. sediforme.

Figure 13, depicted above, presents the antimicrobial activity of S. sediforme against various microorganisms. The inhibition zones against C. albicans were observed to be 8.67 mm, 12.0 mm, and 15.33 mm for 10 µL, 50 µL, and 100 µL of extracts, respectively. Additionally, the activity against E. coli exhibited inhibition zones of 10.67 mm and 13.67 mm for 50 µL and 100 µL of extracts, respectively.
For E. faecalis, inhibition zones of 6.0 mm and 10.67 mm were observed for 50 µL and 100 µL of extracts, respectively. The activity against E. faecium resulted in inhibition zones of 8.67 mm, 11.0 mm, and 13.0 mm for 10 µL, 50 µL, and 100 µL of extracts, respectively.
Moreover, the activity against P. aeruginosa exhibited inhibition zones of 8.67 mm and 10.67 mm for 50 µL and 100 µL of extracts, respectively. Furthermore, a 6.33 mm inhibition zone was observed against P. fluorescens for 100 µL of extract. The activity against S. enteritidis showed an inhibition zone of 6.00 mm, exclusively for 100 µL of extract.
For S. infantis, inhibition zones of 8.67 mm and 10.33 mm were observed for 50 µL and 100 µL of extracts, respectively. As mentioned earlier, no activity was observed against B. subtilis, E. aerogenes, K. pneumonia, S. aureus, S. epidermidis, S. kentucky, and S. typhimurium.

4. Sempervivum Armenum:
Results showed that S. armenum presented antimicrobial activity against C. albicans, E. aerogenes, P. aeruginosa, S. epidermidis, S. infantis and S. kentucky, no activity was observed against B. subtilis, E. coli, E. faecalis, E. faecium, K. pneumonia, P. fluorescens, S. aureus, S. enteritidis and S. typhimurium. The antimicrobial activity results for S. armenum are given in figure 14.

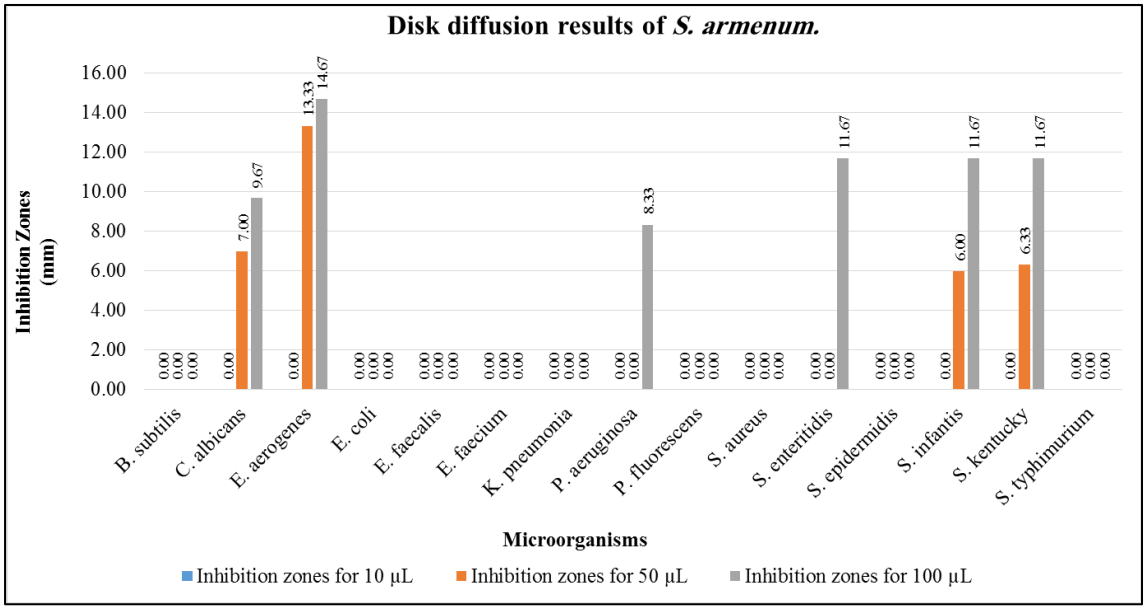

Figure 14. Disk diffusion results of S. armenum.

Figure 15 provides a clear representation of the antimicrobial activity of S. armenum against different microorganisms. The inhibition zones against C. albicans were measured to be 7.00 mm and 9.67 mm for 50 µL and 100 µL of extracts, respectively. Additionally, the activity against E. aerogenes resulted in inhibition zones of 13.33 mm and 14.67 mm for 50 µL and 100 µL of extracts, respectively. Furthermore, an 8.33 mm inhibition zone was observed against P. aeruginosa for 100 µL of extract. The activity against S. epidermidis displayed an inhibition zone of 11.67 mm for 100 µL of extract. For S. infantis, inhibition zones of 6.00 mm and 11.67 mm were observed for 50 µL and 100 µL of extracts, respectively. Similarly, the activity against S. kentucky exhibited inhibition zones of 6.33 mm and 11.67 mm for 50 µL and 100 µL of extracts, respectively. As mentioned earlier, no activity was observed against B. subtilis, E. coli, E. faecalis, E. faecium, K. pneumonia, P. fluorescens, S. aureus, S. enteritidis, and S. typhimurium.

5. Results of MIC Tests
The MIC (Minimum Inhibitory Concentration) test results are presented in Table 1. According to the results in table 1, the MIC values for S. pallidum var pallidum against B. subtilis, E. aerogenes, E. coli, E. faecalis, K. pneumonia, P. aeruginosa, S. aureus, S. enteritidis, S. epidermidis, and S. kentucky were

all found to be 10 µg/mL. However, for S. infantis and S. typhimurium, the MIC values were 5.0 µg/m. No MIC test was conducted for S. pallidum var pallidum against C. albicans, E. faecium, and P. fluorescens because no activity was observed for these combinations in the disk diffusion test.

Table 1. MIC values of plant extracts against microorganisms.

	MIC Values (µg/mL)				
	S. pallidum	*S. pallidum* var *bitynicum*	*S. sediforme*	*S. armenum*	*S. album*
B. subtilis	10	-	-	-	-
C. albicans	-	-	2,5	10	10
E. aerogenes	10	10	-	10	-
E. coli	10	-	10	-	-
E. faecalis	10	10	10	-	10
E. faecium	-	-	10	-	10
K. pneumonia	10	-	-	-	-
P. aeruginosa	10	10	10	10	-
P. fluorescens	-	10	10	-	-
S. aureus	10	10	-	-	-
S. enteritidis	10	10	10	-	-
S. epidermidis	10	-	-	10	-
S. infantis	5,0	-	10	10	-
S. kentucky	10	-	-	10	-
S. typhimurium	5,0	10	-	-	-

The MIC (Minimum Inhibitory Concentration) values obtained from Table 1 are as follows:

- For S. pallidum var bitynicum, the MIC values were 10 µg/mL against E. aerogenes, E. faecalis, P. aeruginosa, P. fluorescens, S. aureus, S. enteritidis, and S. typhimurium. No MIC test was conducted against B. subtilis, C. albicans, E. coli, E. faecium, K. pneumonia, S. epidermidis, S. infantis, and S. kentucky due to the absence of activity in the disk diffusion test.
- For S. sediforme, the MIC value against C. albicans was 2.5 µg/mL. The MIC values for E. coli, E. faecalis, E. faecium, P. aeruginosa, P. fluorescens, S. enteritidis, and S. infantis were all 10 µg/mL. No MIC test was conducted against B. subtilis, E. aerogenes, K. pneumonia, S. aureus, S. epidermidis, S. kentucky, and S. typhimurium due to the absence of activity in the disk diffusion test.
- For S. armenum, the MIC values were 10 µg/mL against C. albicans, E. aerogenes, P. aeruginosa, S. epidermidis, S. infantis, and S. kentucky. No MIC test was conducted against B. subtilis, E. coli, E. faecalis, E. faecium, K. pneumonia, P. fluorescens, S. aureus, S. enteritidis, and S. typhimurium due to the absence of activity in the disk diffusion test.
- For S. album, the MIC values were 10 µg/mL against C. albicans, E. faecalis, and E. faecium. No MIC test was conducted against B. subtilis, E. aerogenes, E. coli, K. pneumonia, P. aeruginosa, P. fluorescens, S. aureus, S. enteritidis, S. epidermidis, S. infantis, S. kentucky, and S. typhimurium due to the absence of activity in the disk diffusion test.

6. Results of Statistical Analysis

Null hypothesis for parallel studies was set as H_0: The results of three parallels are statistically similar. When the results of the statistical analysis compared, it is found that for all plants extracts, for all concentrations the *p*-values for the parallels was found as 0,9281 to 1. Since p-value > 0.05, we accept the null hypothesis H_0, which means there is no difference between the results. Detailed analysis were given in the appendix section. When the results of the statistical analysis compared, it is found that for all plants, all concentrations, all microorganisms (B. subtilis, C. albicans, E. aerogenes, E. coli, E. faecium, E. faecalis, K. pneumonia, P. aeruginosa, P. fluorescens, S. aureus, S. enteritidis, S. epidermidis, S. infantis, S. kentucky and S. typhimurium) the p value for the parallel was also between 0,9281 to 1.

Moreover, the results of comparison of the effect of the p values for all plant extract against every microorganisms were tested for all concentration (10, 50 and 100 µL), with *B. subtilis* =0.6161, *C. albicans* =0.2968, *E. aerogenes* =0.1840, *E. coli*=0.3022, *E. faecalis*=0.7876, *E. faecalis*=0.0155, *K. pneumonia*=0.5041, *P. aeruginosa*=0.0250, *P. fluorescens*=0.3803, *S. aureus*=0.3358, *S.*

enteritidis=0.0162*, S. epidermidis*=0.2680*, S. infantis*=0.0082*, S. kentucky*=0.2727 and *S. typhimurium*=0.5583*.*
When the results of the statistical analysis compared, for all plant extracts against microorganisms with different concentrations and parallels, was found that for *S. album* affected three microorganisms, the p-values were 0.9971 and 0.9984 for 50 and 100 µL respectively*.* Since *p*-values > 0.05, we accept the null hypothesis H_0. For 10 µL. There are no results to compare.
When the results of the statistical analysis compared, for all plant extracts against microorganisms with different concentrations and parallels, was found that for *S.pallidum* var *bitynicum* affected eight microorganisms were tested, the p-values were 0.9873 and 0.999 for 50 and 100 µL respectively*.* Since *p*-values > 0.05, we accept the null hypothesis H_0. For 10 µL there are no results to compare.
When the results of the statistical analysis compared, for all plant extracts against microorganisms with different concentrations and parallels, it was found that for *S. pallidum* var *pallidum* affected twelve microorganisms, the p-values were 1, 0.9948 and 0.9504 for 10, 50 and 100 µL respectively*.* Since *p*-values > 0.05, we accept the null hypothesis H_0.
When the result of the statistical analysis compared, for all plant extracts against microorganisms with different concentrations and parallels, was found that for *S. sediforme* affected eight microorganisms, the p-values were 0.9976, 0.9894 and 0.99982 for 10, 50 and 100 µL respectively*.* Since *p*-values > 0.05, we accept the null hypothesis H_0.

7. Results of Statistical Analysis:
The null hypothesis for the parallel studies, denoted as H0, states that the results of the three parallels are statistically similar. Upon comparing the results of the statistical analysis, it was determined that the p-values for the parallels ranged from 0.9281 to 1 for all plant extracts and concentrations. As the p-value is greater than 0.05, we accept the null hypothesis H0, which suggests that there is no significant difference between the results. A detailed analysis of the results can be found in the appendix section. Furthermore, when examining the results of the statistical analysis for all plants, concentrations, and microorganisms (B. subtilis, C. albicans, E. aerogenes, E. coli, E. faecium, E. faecalis, K. pneumonia, P. aeruginosa, P. fluorescens, S. aureus, S. enteritidis, S. epidermidis, S. infantis, S. kentucky, and S. typhimurium), it was observed that the p-values for the parallels also fell between 0.9281 and 1.
Additionally, the effect of the p-values for all plant extracts on each microorganism was examined for all concentrations (10, 50, and 100 µL). The resulting p-values were as follows: B. subtilis = 0.6161, C. albicans = 0.2968, E. aerogenes = 0.1840, E. coli = 0.3022, E. faecalis = 0.7876, E. faecalis = 0.0155, K. pneumonia = 0.5041, P. aeruginosa = 0.0250, P. fluorescens = 0.3803, S. aureus = 0.3358, S. enteritidis = 0.0162, S. epidermidis = 0.2680, S. infantis = 0.0082, S. kentucky = 0.2727, and S. typhimurium = 0.5583. Since all of these p-values are greater than 0.05, we accept the null hypothesis H0.
Further analysis comparing all plant extracts against microorganisms with varying concentrations and parallels revealed the following findings:
For S. album, which had an impact on three microorganisms, the p-values were 0.9971 and 0.9984 for 50 and 100 µL, respectively. Since the p-values are greater than 0.05, we accept the null hypothesis H0. Unfortunately, there are no results available to compare for 10 µL.
For S. pallidum var bitynicum, which affected eight microorganisms, the p-values were 0.9873 and 0.999 for 50 and 100 µL, respectively. Since the p-values are greater than 0.05, we accept the null hypothesis H0. Again, no results are available for 10 µL.
For S. pallidum var pallidum, which affected twelve microorganisms, the p-values were 1, 0.9948, and 0.9504 for 10, 50, and 100 µL, respectively. Since the p-values are greater than 0.05, we accept the null hypothesis H0. Lastly, for S. sediforme, which affected eight microorganisms, the p-values were 0.9976, 0.9894, and 0.99982 for 10, 50, and 100 µL, respectively. Since the p-values are greater than 0.05, we accept the null hypothesis H0.
Upon comparing the results of the statistical analysis for all plant extracts against microorganisms with different concentrations and parallels, it was observed that S. armenum affected eight microorganisms that were tested. The corresponding p-values were 0.9971 and 0.9281 for 50 and 100 µL respectively. Since both p-values are greater than 0.05, we accept the null hypothesis H0. However, no results are available for comparison. Furthermore, based on the statistical analysis comparing all plant extracts against microorganisms with different concentrations and parallels, it can be concluded that increasing the concentration leads to an increase in the observed effect.

Conclusions & Recommendations
Numerous studies have investigated the antimicrobial properties of Sedum album sp plant extract against different strains of bacteria. Gram-positive bacteria, such as Staphylococcus aureus and

Streptococcus pneumoniae, have been found to be susceptible to the extract's antimicrobial effects. Additionally, Gram-negative bacteria, including Escherichia coli and Pseudomonas aeruginosa, have shown varying degrees of sensitivity to the extract.

The study concluded that:

1. The antimicrobial activity observed in the extract of Sedum album sp indicates the presence of bioactive compounds that hold promise for potential therapeutic applications.
2. The application of Sedum album sp plant extract has demonstrated significant potential in combating both Gram-positive and Gram-negative bacteria.
3. The observed antimicrobial activity suggests the presence of bioactive compounds with therapeutic applications. However, further studies are needed to identify and isolate the specific constituents responsible for these effects, as well as to elucidate their mechanisms of action.

The recommended the following:

1. It is necessary to conduct further investigations to identify and isolate the specific constituents responsible for the observed antimicrobial effects. By elucidating the structures and characterizing these compounds, we can gain valuable insights into their mechanisms of action.
2. This knowledge can further facilitate the development of novel antimicrobial agents. The results obtained from the S. album sp extract indicate varying levels of antimicrobial.
3. Identification of Bioactive Compounds: Isolate and identify the specific bioactive compounds present in the plant extract responsible for its antimicrobial activity. This can be achieved through various techniques, such as chromatography and spectroscopy.
4. Mechanistic Studies: Conduct in-depth studies to elucidate the precise mechanisms of action of the bioactive compounds against different bacterial strains. This knowledge will help optimize the extract's application and potentially lead to the development of targeted antimicrobial therapies.
5. Synergistic Effects: Investigate the potential synergistic effects of Sedum album sp plant extract when used in combination with existing antibiotics. This could enhance the overall antimicrobial efficacy and potentially reduce the development of antibiotic resistance.
6. Formulation Development: Explore the development of formulations or delivery systems that can enhance the stability, bioavailability, and targeted delivery of the bioactive compounds from Sedum album sp plant extract.

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