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The effect of *Euphorbia milii* and *Sansevieria trifasciata* plant extracts with antibiotics against Streptococcus pyogenes bacteria

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Abstract

The study aimed to evaluate the antibacterial activity of both *Euphorbia milii* and *Sansevieria trifasciata* plant extracts and their synergistic effect with antibiotics against *Streptococcus pyogenes.* A total of 100 samples were collected from patient with tonsillitis, where 10 bacterial isolates of the target species were identified using chemical tests and PCR technique. The plant extracts were prepared using 70% ethanol, and their effect on bacteria was tested at different concentrations (25%,50%,75%,100%). The results showed that *Euphorbia milii* extract was more effective, with an average diameter of the inhibition zone of 18.8 mm at 100% concentration, compared to 14.6 mm for *Sansevieriatrifasciata* extract. When the extracts were combined with antibiotics Azithromycin(15mg), Tetracycline(10mg), Vancomycin (30), Clindamycin (10), a significant synergistic effect was observed, especially with azithromycin. The study showed that low concentrations of the extracts were safe for red blood cells, while 100% concentration of Euphorbia milii extract was toxic. The results suggest the potential use of these plants as supplements to enhance the effectiveness of antibiotics, with the importance of controlling the concentration to avoid toxic effects.

Introduction

Streptococcus pyogenes is one of the most important human pathogens, causing a wide range of diseases ranging from simple infections such as sore throat and tonsillitis to life-threatening diseases such as rheumatic fever and toxic shock syndrome. This bacterium is characterized by its high ability to spread and produce many pathogenic factors such as toxins and enzymes that attack host tissues [1]. Antibiotic resistance is a major challenge in the treatment of bacterial and fungal infections [2]. Many antibiotics that were considered safe havens have become ineffective due to their misuse and the development of bacteria, which has increased the resistance of microbes to them, and this has required the search for new antimicrobial therapeutic alternatives [3]. One of these alternatives is medicinal plants, which are an important source of biologically active compounds used to protect from microbes, insects and herbivores [4].

Euphorbia is an important medicinal plant, this plant belongs to the Euphorbiaceae family, as it constitutes the third largest genus of flowering plants, and includes approximately two thousand species [5] Euphorbia milii is an important species, this plant has had medical importance since ancient times, it has been used as a traditional medicinal herb in China and India and is found in all countries of the world except cold regions. It is used to treat tumors, cough, warts, asthma, and is an antiviral. Its chemical compounds help in its biological activity as an antibacterial [6]. The genus *E.milii* contains a large number of biologically active compounds, the most important of which are terpenes, flavonoids, tannins, and others [7]. The antibacterial activities of the plant were studied on some Gram-positive and Gram-negative species [6,7]. *Sanseviaratrifasiata* plant belongs to the Asparagaceae family, this plant is known for its importance in absorbing pollutants from the air and has been used for a long time as a traditional medicine throughout Asian and African countries, where it has been used to treat coughs, influenza, snake bites, and to bandage wounds [8]. It is a perennial ornamental plant with broad, dark green, usually pale. The plant is characterized by its possession of phytochemical compounds that are naturally formed within it and give the plant its color, flavor and smell. They are part of the plant's defense system and are useful in protecting humans and fighting diseases [9]. The biologically active compounds in the plant include alkaloids, flavonoids, saponins, glycosides, and others. The plant can be used as an antibacterial, antioxidant, and anticancer agent [8] Studied indicate that the plant extract can be used to prevent the growth of bacteria *E. coli* and *Pseudomonas* [10]. The plant extracts under study were prepared using alcoholic extraction, with a study of its effect on bacteria and its synergy with antibiotics in an attempt to improve the action of antibiotics. The objectives of this study were to evaluate the antibacterial effect of plant extracts on bacteria, test the interaction between plant extracts at different concentrations with antibiotics, and evaluate the toxicity of the concentrations used for plant extracts

Materials and Methods

Sample Collection

The 100swabs were collected from patients with acute and non-acute tonsillitis from Ramadi Teaching Hospital and Women's and Children's Hospital in Ramadi city. The samples included both sexes and their ages ranged from 5-45 years, during the period from August 2024 to January 2025. Swabs were taken from the tonsil area using cotton swabs containing a carrier medium, and were transferred to the laboratory within one hour.

Dignosis and molecular diagnosis the samples were diagnosed using biochemical tests, these tests included microscopic examination, evaluation of hemolytic activity on blood agar, a catalase test, and Bacitracin sensitivity test. The results are determined as stated in the source[11]. in addition to PCR technology by detecting the diagnostic gene *16SrRNA*, with size 407bp, its sequences are shown in Table. 1. Antibiotic sensitivity and resistance assessments were conducted using the disk diffusion method on Mueller-Hinton agar enriched with 5% sheep blood. A total of ten antibiotics were evaluated: Ampicilin (AMP,25mg), Azithromycin (AZ,15mg), Tetracycline (TE,10mg), Amoxicillin/clavulanicacid (AMC,30mg), Ciproflaxcin (CIP,10mg), Vancomycin (VA,30mg), Erythromycin (E,10mg), Gentamycin (CN,10mg), Clindamycin (DA,10mg), Levoflaxacin (L,5mg). The results were analyzed in accordance with the Clinical and Laboratory Standards Institute (CLSI) guidelines for determining bacterial resistance. The sensitivity and resistance of the isolates to antibiotics were also determined and compared with clsi2024[12]. Five isolates that were multi drug to antibiotics were selected to test the effect of plant extracts.

TABLE 1. Shows the primers used to identify the *16srRNA*gene in *S.pyogenes* bacteria, manufactured by the Korea company Macrogen, with a size of 407 base pairs and a fusion temperature of 50 °C.

	Gene sequences
SPY1	AAAGACCGCCTTAACCACCT
SPY2	TGCCAAGGTAAACTTCTAAAGCA

Plant Preparation

Euphorbia milii and *Sansevieria trifasciata* plants were purchased from a nursery in Ramadi city (Figure.1) and (Figure.2). The leaves of the two plants were collected, cleaned of dust, and dried in a ventilated room away from sunlight*; Euphorbia milii* was dried for two weeks and *Sansevieria trifasciata* for one month. The leaves were turned to ensure complete dryness, then ground using an electric grinder and stored in tightly sealed glass jars until used.

Fig.1. Euphorbia milii

Fig.2. Sanseviearia trifasciata

Leaves extraction of *Euphorbia milii* and *Sansevieria trifasciata*

The plant extracts were prepared using a mixture of 70% ethanol and 30% distilled water according to the method in [13]. 50 g of powder of each plant was weighed and added to 250 ml of solvent mixture in a 1000 ml beaker (Figure.3). The mixture was placed in sterile flasks and closed with medical gauze, then placed in a shaking water bath at 50-60 °C for 24 h. Then, the solution was filtered through medical gauze and filter paper, and the resulting solution was left on a magnetic device to evaporate the solvent and concentrate to become a gelatinous substance. The material was poured into glass dishes and left to dry in an incubator at 40 °C for three days. The stock solution was prepared by dissolving 500 mg of each plant extract in 5 ml of DMSO solvent with a concentration of less than 1%, then left on the roller mixer for 24 hours to dissolve the extracts well. After ensuring that the extracts were dissolved, they were filtered using filters with a diameter of 0.45 micrometers, where the extract was drawn using a sterile syringe and placed in its designated place in the filter and the extract was pushed into the filter and then into a sterile glass bottle. After that, three concentrations (25, 50, and 75) % of each extract were prepared from the stock solution, which was considered the basic concentration and its concentration was 100%.

Fig.3. Preparation of plant extracts using water bath

Determination of the plant extracts antibacterial activity using disk diffusion

A bacterial suspension was prepared and spread evenly on Mullar Hinton Agar medium with 5% sheep blood added. Holes were made using a cork piercer(8mm) and 100 microliters of each concentration and both extracts were added. The plates were then incubated at 37°C for 24 hours, and the diameter of inhibition was measured in millimeters.

Synergy test between plant extracts and antibiotics

To examine the synergistic effect of ***Euphorbia milii*** **and** ***Sansevieria trifasciata*** with antibiotics, the antibiotics utilized in our study were selected to encompass almost all relevant antibiotic classes suitable for the bacteria *S.pyogenes*. The Kirby-Bauer method[14]. On Mueller Hinton agar with 5% sheep blood added was used to assess susceptibility to four antibiotics, as follows: Azithromycin (15µg), Tetracycline (10µg), Vancomycin (30µg), Clindamycin (10µg), conforming to clinical laboratory standards institute. The results were recorded, and the resistance and susceptible isolates were determined by measuring the diameter of the inhibition zone according to CLSI (2024). Synergism was performed by adding fixed volume of 25 µL from each concentration of the plant extract directly into the antibiotic. The resulting inhibition diameter was compared with the inhibition diameter of the antagonist alone.

Toxicity test of plant extracts

The cytotoxicity of plants *Euphorbia milii* and *Sansevieriatrifasciata* extract was determined by the hemolysis method, which works to inactivate human red blood cells (RBC) in the laboratory by plant extracts. The blood of a healthy, non-smoking human was used in this test, as stated in [14, 15] .

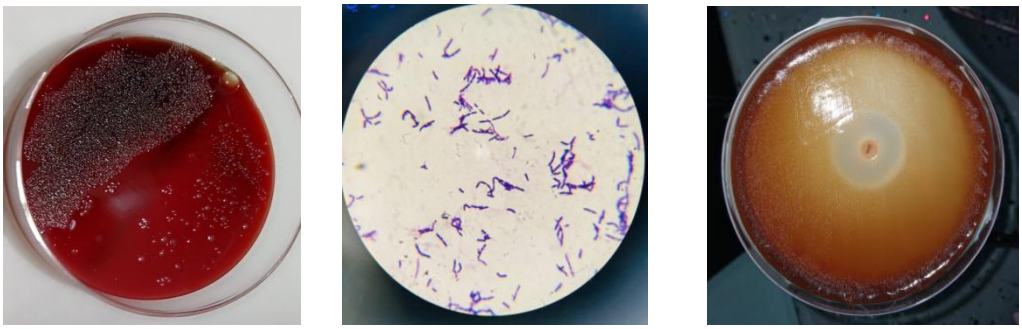
Data Analysis

The Statistical Packages of Social Sciences-SPSS (2019) program was used to detect the effect of difference groups in study parameters. Least significant difference-LSD was used to significant compare between means (ANOVA) in this study [16].

Results and Discussion

Isolation and diagnosis

The 100 samples were collected from of patients with tonsillitis who have consulted who visited Ramadi Teaching Hospital and Women's and Children's Hospital in Ramadi city. The results showed the diagnosis of 10 bacterial *Streptococcus pyogenes* isolates. Bacteria were diagnosed using phenotypic diagnosis (Figure.4 A). The isolates appeared to be completely hemolytic and were positive for Gram stain. Microscopic examination showed that they were cocci arranged in chains (Figure.4 B). The results showed that the bacteria were catalase negative and sensitive to bacitracin (Figure.4 C). They were diagnosed using the polymerase chain reaction PCR (Figure.5).



(A**)** **(**B**)** **(**C**)**

***Fig.4*.A**-*Streptococcus pyogenes* on blood agar, **B**-*Streptococcus pyogenes* under the microscope, , **C**. Bacterial sensitivity to bacitracin

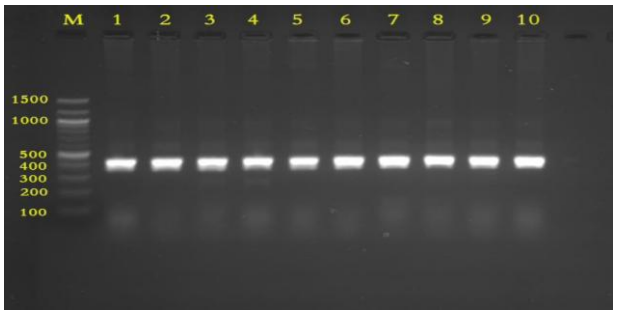


***Fig.5*.**Electrophoresis of DNA (*16SrRNA*)for *S.pyogenes*

Evaluation of Antibacterial Activity of **Selected Plant Extracts against *S.pyogenes***

Table.2 illustrates a clear concentration-dependent increase in the inhibition zones for both plant extracts *E. milii* and *S.trifasciata*, *E. milii* exhibited a stronger antibacterial activity, with the inhibition zone increasing from 8 mm at 25**%** concentration to 10,15 ,18.8 mm at 50%,75% and 100**%** respectively.In contrast, *S. trifasciata* demonstrated a weaker inhibitory effect, with inhibition zones ranging from 4.0 mm at 25**%** to 10.8,14,14.6 mm at 50**%,**75**%** and 100**%**respectively.

The LSD values indicate significant differences between the effects of different concentrations of extracts at a significance level of (P≤0.05). *E. milii* contains alkaloids, triterpenes, and phenolic compounds known for their antibacterial properties, Compounds such as Flavonoidshave an inhibitory effect on bacterial growth by interacting with bacterial cell wall proteins, It also prevents the formation of biofilms, which reduces cell resistance [7]. *S.trifasciata* contains saponins, terpenoids, and flavonoids, which are known for their ability to inhibit bacterial growth, the compounds damage the cell membrane of bacteria, resulting in the loss of vital substances. Inhibit bacterial enzymes necessary for the production of energy and proteins [8].Researchers believe that the chemical composition of this plant may fight various diseases, including its potential as an antibacterial agent[17].The ethanol extract of *S.trifasciata* can inhibit the reproduction of *Pseudomonas aeruginosa* and Neophyladiene compound identified by GC-MS is suspected to play an important role in this activity[18].

Table2: Inhibition diameters of *Euphorbia milii* and *Sanseviaria trifasciata* at their four concentrations on bacterial isolates

Treatment	Damping diameter rate				LSD value
	Concentration 25%	Concentration 50%	Concentration 75%	Concentration 100%	
E. milii	8	10	15	18.8	3.58 *
S. trifascia	4	10.8	14	14.6	4.02 *

*(P≤0.05).

***** LSD Least Significant Difference

(*) Significant at P ≤ 0.05 according to the LSD test

Synergism test between plant extracts of *S.trifasciata* and antibiotics:

In Table.3 the isolates showed high resistance to the antibiotic Azithromycin, as the average diameter of inhibition for the antibiotic alone was 4 mm, and with the addition of the extract, the average diameter of inhibition increased, The mean diameter of inhibition of azithromycin increased significantly with increasing concentration of *E. milii* extract, increasing from 14.6 mm at 25% concentration to 15.6,16.4 and 23.8 mm at 50%,75% and 100% concentration respectively. Diameter of inhibition for tetracycline was 15.4mm, With the addition of concentrations 25%,50%,75% and 100%from *E. milii* extract, the rate of inhibition diameter increased to 17.2,20.4,19.6 and 22.6mm respectively. Diameter of inhibition rate of the antidotic vancomycin was 0 and when we add *E. milii* extract 25%,50%,75% and 100%, The rate of inhibition diameter increased to 3.6,6.4,6.4,14.2mm respectively, as shown in the Figure.6. As for the antibiotic clindamycin, the isolates were 100% resistant to it. Then, a concentration of 25% was added, and the rates of inhibition diameters increased to 13 mm, reaching 16.6 at a concentration of 100%. The results show that the isolates were resistant to antibiotics, but the addition of the extract improved the antibiotic effect. Figure.7 shows the synergistic effect between *E. milii* extract and the antibiotics.

Table 3. Inhibition diameters in mm for the synergy of *Euphorbia milii* with antibiotics

Antibiotic	**Diameter rate**					**LSD value**
	Antibiotic (mm))(	**Synergism *Euphorbia milii* 25% with the antibiotic**	**Synergism *Euphorbia milii* 50% with the antibiotic**	**Synergism *Euphorbia milii* 75% with the antibiotic**	**Synergism *Euphorbia milii* 100% with the antibiotic**	
AZ	4	14.6	15.6	16.4	23.8	5.52 *
TE	15.4	17.2	20.4	19.6	22.6	4.78 *
VA	0	3.6	6.4	6.4	14.2	4.41 *
DA	0	13	12	14.4	16.6	5.68 *
LSD value	4.39 *	5.18 *	4.63 *	4.72 *	4.29 *	---

*P≤0.05

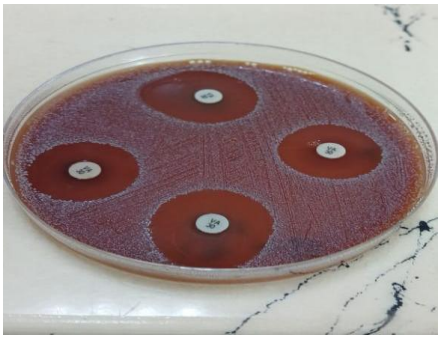


Fig.6.synergistic effect between *E. milii* extract and antibiotics on *S.pyogenes*

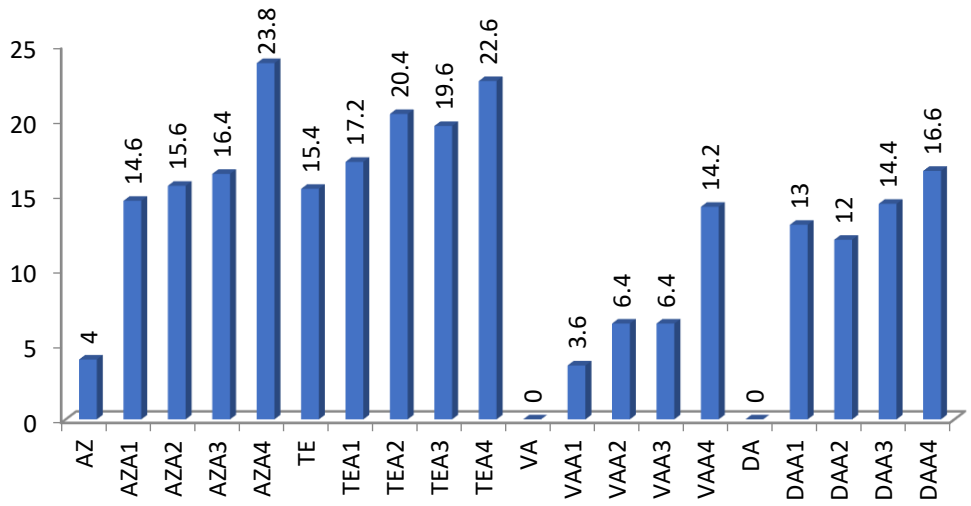


Fig.7. It shows the rates of inhibition diameters of the four antibiotics and their synergy with *E.milii* extract indicated by the symbol A (A1: indicates the concentration of *E.milii* extract 25%, A2: indicates the concentration of *E.milii* extract 50%, A3: indicates the concentration of*E.milii* extract 75%, A4: indicates the concentration of *E.milii* extract 100%.) , antibiotics (Azithromycin-AZ, Tetracyclin-TE, Vancomycin-VA, Clindamycin-DA) against *Streptococcus pyogenes*

Synergism test between plant extracts of *S. trifasciata* and antibiotics:

The isolates showed high resistance to Azithromycin with an average diameter of 4 mm. With the addition of *S. trifasciata* extract at a concentration of 25%, the average diameter of inhibition increased to 14 mm and increased with increasing concentrations of 50%, 75% and 100%, to be a result of the average diameter of inhibition 15.2, 16.2, 23.4mm respectively. Tetracycline was effective alone 15.4mm, but adding the plant extract increased the rate of inhibition diameters, which was 16.8 at a concentration of 25%, and increased with increasing concentration to reach 19.4,19.6 and 22.8 mm at a concentration of 50%,75% and 100%, as shown in table 4.

Figure.8 show the synergy between *S. trifasciata* and antibiotics on mueller hinton agar. The isolates showed 100% resistance to vancomycin,when add the extract at a concentration of 25%, the average diameter of inhibition was 3.6 mm and increased with increasing extract concentration to reach 12.6 mm at a concentration of 100%. As for the antibiotic clindamycin, the isolates were 100% resistant to it, and the rate of inhibition diameter increased at

concentrations of 25%, 50%, 75% and 100% to 9.8, 11.8, 13.4 and 15 mm, respectively, as shown in the Figure.9.

The results showed that the best concentration for inhibition is 100% and 75%, and the rate of inhibition diameters decreases at low concentrations. The study showed that the synergy of the extract with azithromycin was the best, which may be due to the compatibility of the mechanism of action of azithromycin with plant compounds in killing bacteria.Mechanisms by which plant compounds can synergize with antibiotics by targeting the cell wall and facilitating the task of transporting the antibiotic [19].

Table 4. Inhibition diameters in mm for the synergy of *S. trifasciata* with antibiotics

Antibiotic	Diameter rate					LSD value
	Antibiotic (mm))(	Synergism *S.trifascita* 25%with the antibiotic	Synergism *S.trifasciata* 50% with the antibiotic	Synergism *S.trifasciata* 75% with the antibiotic	Synergism *S.trifasciata* 100% with the antibiotic	
AZ	4	14	15.2	16.2	23.4	5.49 *
TE	15.4	16.8	19.4	19.6	22.8	5.16 *
VA	0	3.6	6.6	6.6	12.6	3.94 *
DA	0	9.8	11.8	13.4	15.0	4.16 *
LSD value	4.39 *	5.02 *	4.22 *	4.71 *	4.07 *	---

*(P≤0.05)

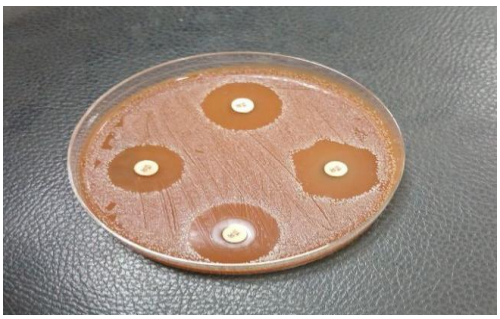


Fig.8. Demonstrates the synergistic effect between the *S. trifasciata* and antibiotic on *S.pyogenes*

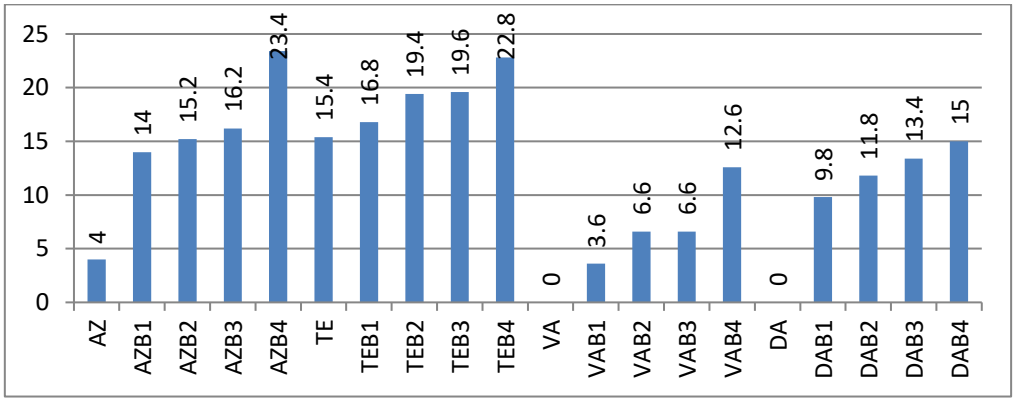


Figure.9-shows the rates of inhibition diameters of the four antibiotics and their synergy with *S.trifasciata* extract indicated by the symbol B (B1: indicates the concentration of *S.trifasciata* extract 25%, B2: indicates the concentration of *S.trifasciata* extract 50%, B3: indicates the concentration of *S.trifasciata* extract 75%, B4: indicates the concentration of *S.trifasciata* extract 100%.) , antibiotics (Azithromycin-AZ, Tetracyclin-TE, Vancomycin-VA, Clindamycin-DA) against *Streptococcus pyogenes* isolates

Toxicity test of plant extracts

The results of the hemolysis toxicity test showed that the *S. trifascita* extract prepared in this study at all concentrations was safe for human cells, as the percentages were less than 10%, which is considered safe according to studies[20]. As for the *E. milii* extract, the results showed that the 100% concentration is a toxic concentration, as its result was more than 25%, and this toxicity is due to a group of compounds, the most important of which is latex([21]. As for the remaining concentrations of the *E. milii* extract, the test results showed that they are safe for red blood cells due to their slight effect on red blood cells.

Conclusions

The results of the present study demonstrated that both *E. milii* and *S.trifasciata* plant extracts possess antibacterial activity against *S.pyogenes*, with a concentration-dependent increase in inhibitory effect. *E.milii* exhibited a stronger antibacterial potential compared to *S.trifasciata*, as indicated by larger inhibition zones across all tested concentrations.

This study provides indispensable evidence of a type of antibacterial interaction that exists between the extracts E.*milii* and *S.trifasciata* with antibiotics against *S.pyogenes*. This synergy may be of therapeutic importance for infections caused by*S.pyogenes*, with the importance of choosing concentrations that are safe for human cells.

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تأثير مستخلصات نبات *Euphorbia milii* و *Sansevieria trifasciata* مع المضادات الحيوية ضد بكتيريا *Streptococcus pyogenes*

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الخلاصة:
هدفت الدراسة إلى تقييم النشاط المضاد للبكتيريا لمستخلصي نبات *Euphorbia milii* و *Sansevieriatrifasciata* وتأثيرهما التآزري مع المضادات الحيوية ضد البكتيريا *Streptococcus pyogenes*. تم جمع 100 عينة من مريض مصاب بالتهاب اللوزتين، حيث تم تحديد 10 عزلات بكتيرية من الأنواع المستهدفة باستخدام الاختبارات الكيميائية وتقنية PCR، وتم تحضير المستخلصات النباتية باستخدام 70% إيثانول، واختبار تأثيرها على البكتيريا بتراكيز مختلفة (25%-100%). أظهرت النتائج أن مستخلص نبات *Euphorbia milii* كان أكثر فعالية، حيث بلغ متوسط قطر منطقة التثبيط 18.8 ملم عند التركيز 100%، مقارنة بـ 14.6 ملم لمستخلص *Sansevieriatrifasciata*. عند دمج المستخلصات مع المضادات الحيوية (أزيثروميسين، تتراسيكلين، فانكومايسين، كليندامايسين)، لوحظ تأثير تآزري كبير، خاصة مع أزيثروميسين. وأظهرت الدراسة أن التراكيز المنخفضة من المستخلصات آمنة لخلايا الدم الحمراء، في حين أن التركيز 100% من مستخلص *Euphorbia milii* سام. وتشير النتائج إلى إمكانية استخدام هذه النباتات كمكملات غذائية لتعزيز فعالية المضادات الحيوية، مع أهمية التحكم في تركيزها لتجنب التأثيرات السامة.