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Evaluation of Antimicrobial Activities of Solenostemma arga and Acacia nilotica fruit Against some Bacterial in Elobeid – Sudan

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ABSTRACT

This study aimed to evaluate the antibacterial activity of extracts from Peganum harmala and the fruit of Acacia nilotica against some bacterial strains in the city of El Obeid, given the rising bacterial resistance to conventional antibiotics and the need for natural therapeutic alternatives. The study focused on three bacterial strains: Escherichia coli, Staphylococcus aureus and Pseudomonas aeruginosa. Plant extracts were prepared using water and ethyl alcohol, and their efficacy was assessed by measuring the diameters of inhibition zones and comparing the results with standard antibiotics. The results showed that Pseudomonas aeruginosa was the most inhibited (34.7%), while the inhibition rate for both Escherichia coli and Staphylococcus aureus was 32.7%. The Acacia nilotica (Qard) plant recorded the highest usage rate among the studied plants. The plant extracts demonstrated varying degrees of antibacterial activity, with inhibition zone diameters ranging from 0 to 56 mm; this indicates that some extracts possess high efficacy in inhibiting bacterial growth, alongside a clear variation in the sensitivity of the bacterial species to the extracts used. Statistical analyses using the chi-square test revealed no significant differences between the bacterial species and the variables studied ($P > 0.05$), a result attributed to the small sample size and the distribution of data across a large number of categories. Nevertheless, the findings confirmed that Harjal and Qard extracts exhibit promising natural antibacterial activity, supporting their potential use in developing therapeutic alternatives or supplements to conventional antibiotics. The study recommended further research to isolate active compounds, investigate their toxicity, and evaluate their efficacy against antibiotic-resistant bacterial strains using advanced analytical techniques.

Keywords: Peganum harmala, Acacia nilotica, antibiotics, plant, antibacterial activity

المستخلص :

هدفت هذه الدراسة إلى تقييم النشاط المضاد للبكتيريا لمستخلصات نبات الحرجل (Peganum harmala) وثمرة نبات القرض (Acacia nilotica) ضد بعض الأنواع البكتيرية المعزولة في مدينة الأبيض، وذلك في ظل تزايد مقاومة البكتيريا للمضادات الحيوية التقليدية والحاجة إلى بدائل علاجية طبيعية. شملت الدراسة ثلاث سلالات بكتيرية هي Escherichia coli، وStaphylococcus aureus، وPseudomonas aeruginosa. وتم تحضير المستخلصات النباتية باستخدام الماء والكحول الإيثيلي، ثم تقييم فعاليتها بطريقة قياس أقطار مناطق التثبيط ومقارنتها ببعض المضادات الحيوية القياسية.

أظهرت النتائج أن الزائفة الزنجارية كانت الأكثر تثبيط بنسبة (34.7%)، بينما بلغت نسبة كل من

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الإشريكية القولونية والمكورات العنقودية الذهبية (32.7%). كما سجل نبات القرض أعلى نسبة استخدام بين النباتات المدروسة، وأظهرت المستخلصات النباتية نشاطًا مضادًا للبكتيريا بدرجات متفاوتة، حيث تراوحت أقطار مناطق التثبيط بين 0 و56 مم، مما يدل على امتلاك بعض المستخلصات فعالية عالية في تثبيط نمو البكتيريا، مع اختلاف واضح في حساسية الأنواع البكتيرية للمستخلصات المستخدمة. وأوضحت التحليلات الإحصائية باستخدام اختبار مربع كاي عدم وجود فروق معنوية بين الأنواع البكتيرية والمتغيرات المدروسة (P>0.05)، وهو ما يُعزى إلى صغر حجم العينة وتوزيع البيانات على عدد كبير من الفئات. ومع ذلك، أكدت النتائج أن مستخلصي الحرجل والقرض يمتلكان نشاطًا واعدًا كمضادات طبيعية للبكتيريا، مما يدعم إمكانية الاستفادة منهما في تطوير بدائل أو مكملات علاجية للمضادات الحيوية التقليدية. وأوصت الدراسة بإجراء مزيد من الأبحاث لعزل المركبات الفعالة، ودراسة سميتها، وتقييم فعاليتها ضد السلالات البكتيرية المقاومة للمضادات الحيوية باستخدام تقنيات تحليل متقدمة.

الكلمات المفتاحية: البكتيريا، المضادات الحيوية، المستخلصات، التثبيط.

1. INTRODUCTION

Bacterial diseases represent one of the major health problems affecting both humans and animals, causing numerous infections and illnesses that lead to significant health and eco-nomic losses. Since their discovery, antibiotics have contributed to limiting and controlling the spread of these diseases. However, the excessive and unregulated use of these drugs has led to the emergence of bacterial strains resistant to many antibiotics, posing a global threat to public health. Therefore, researchers have increasingly focused on finding new treatment alternatives, particularly from natural sources, which are characterized by their availability and fewer side effects compared to synthetic compounds.

Medicinal plants have occupied an important place in traditional medicine and herbal remedies worldwide. Eighty percent of the world's population still relies on them due to their availability, accessibility, low cost, promising effectiveness, and to avoid the negative effects of chemical drugs. Most medicinal plants are non-toxic, although some are highly toxic to both humans and animals (Okoye et al.). (2014) Medicinal plants are among the most important natural sources of biologically active compounds, containing numerous secondary chemicals such as alkaloids, phenols, flavonoids, terpenes, and tannins, which possess antibacterial, antifungal, and antiviral properties. Several studies have demon-strated that plant extracts can be a promising source for developing novel antimicrobials capable of addressing the growing problem of bacterial resistance (Cowan, 1999). The plant Peganum harmala L is widespread in arid and semi-arid environments and is used

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in traditional medicine to treat various ailments. It contains a range of active alkaloids, such as harmine and harmalin, which have exhibited diverse biological activity, including antimicrobial effects. The fruit of the Acacia nilotica plant is also of significant medicinal importance, containing high concentrations of tannins and phenolic compounds known for their ability to inhibit the growth of many pathogenic microorganisms. (El-Kamali & Mahjoub, 1997)

North Kordofan State, particularly the city of El Obeid, boasts a rich plant diversity, including many local medicinal plants whose therapeutic properties still require further scientific study (El-Kamali & Mahjoub, 1997). Despite the widespread traditional use of the plants Peganum harmala and Acacia nilotica in folk practices, scientific information regarding their effectiveness against locally isolated bacteria remains limited. This necessitates rigorous laboratory studies to determine the impact of their extracts on different bacterial species (Harborne, 1998). The study also seeks to enhance scientific knowledge of local medicinal plants and highlight their potential in supporting efforts to find effective therapeutic alternatives to address the growing challenges of bacterial resistance (Sofowora, 2008)

2.MATERIALS AND METHODS

2.1 Collection of plants:

Peganum harmala seeds and Acacia nilotica fruits were collected from various locations in North Kordofan State, then cleaned, dried at room temperature, and ground into a fine powder.

2.2 Preparation of extracts:

The extracts were prepared using 70% ethanol and distilled water via the cold maceration method; 250 g of plant powder was soaked in 500 mL of solvent for 72 hours with continuous agitation. The extract was then filtered and concentrated using a rotary evaporator to obtain the crude extract.

Harjal plant material was obtained from a herbalist in the El Obeid city market and ground using an electric grinder. A 200-gram sample of the ground material was soaked in 500

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ml of 70% ethyl alcohol for 24 hours. The mixture was then filtered using filter paper and a glass funnel, and the filtrate was poured into Petri dishes and left to dry completely.

One gram of the powder was dissolved in 5 mL of distilled water to serve as a standard solution for determining other concentrations; the same steps were followed to prepare the aqueous extract of *Solenostemma argel* (Hargal).

2.3 Preparation of the bacterial growth medium:

Forty grams of blood agar medium were weighed and dissolved in 1,000 mL of distilled water in a volumetric flask. The mixture was placed in a water bath at 45°C until the solution was homogeneous, then sterilized using a steam sterilizer (autoclave) at 121°C and 15 psi of pressure for 15 minutes. The solution was allowed to cool to 47°C before being poured into clean, sterile Petri dishes.

2.4 Bacterial bioassay

Three parts of plant (leaves, root and bark) extraction solution methanol concentration (25mg/ml, 50mg/ml and 100mg/ml) were tested against isolated bacterial. For bioassay a bacterial suspension of approximately 1.0×10^6 cell/ml in sterile normal saline was prepared as described by (Forbes et al., 1990). 1 ml of bacterial suspension was uniformly seeded on the Mueller-Hinton agar medium 15 ml, 4 cm thickness in Petri dishes. Wells of 8 mm in diameter and about 4 cm apart were punctured in the culture media using cork borers (8mm).

2.4.1 Treatments

One ml of each the plant extract concentrations were dispensed in to the corresponding well on the inoculated agar plate .The inoculated plate was incubated aerobically at 37c° over night. Then examine for inhibition zone around the well conking the all concentration of extracts.

2.4.2 Determination of inhibition zone

The plates were then incubated at 37°C for 48 hrs. After incubation, bioactivity was determined by measuring the diameter of inhibition zone (DIZ) in millimeter. All samples were tested in triplicate. Susceptible organisms showed inhibition zone where as resistant organisms showed growth tightly at the edge of the disc. Zone of inhibition gave relative activity of the extract against each test organism. Susceptibility test were carried out ac-

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cording to standard methods (Monica, 2000). At the end of incubation period, inhibition zone were recorded in millimeter as the diameter of growth free zones around the wells using a transparent meter ruler.

2. Results

Table 1 shows the types of bacteria and the analysis results for the samples studied.

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	Pseudomonas	17	34.7	34.7	34.7
	Staphylococcus aureus	16	32.7	32.7	67.3
	Escherichia	16	32.7	32.7	100.0
	Total	49	100.0	100.0	

Table 2 shows the distribution of plant species and the antibiotics used.

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	Acacia plant	24	49.0	49.0	49.0
	Solenostemma argel	6	12.2	12.2	61.2
	Ampocillin	3	6.1	6.1	67.3
	Ciprofloxacin	3	6.1	6.1	73.5
	Arthrothromycin	3	6.1	6.1	79.6
	FC. Fusidic Acid	2	4.1	4.1	83.7
	Gentamicin	2	4.1	4.1	87.8
	Chlormafenicol	3	6.1	6.1	93.9
	Vancomycin	3	6.1	6.1	100.0
	Total	49	100.0	100.0	

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Table 3 shows the distribution of the types of extracts used in the study.

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	Water	15	30.6	30.6	30.6
	Cohil	15	30.6	30.6	61.2
	Antibiotic	19	38.8	38.8	100.0
	Total	49	100.0	100.0	

Table 4 shows the distribution of concentration values used in the study.

Focus Value					
		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	1	6	12.2	12.2	12.2
	5	9	18.4	18.4	30.6
	10	13	26.5	26.5	57.1
	20	12	24.5	24.5	81.6
	30	9	18.4	18.4	100.0
	Total	49	100.0	100.0	

Table 5 shows the diameter of the zone of inhibition.

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		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	0	9	18.4	18.4	18.4
	5	1	2.0	2.0	20.4
	6	2	4.1	4.1	24.5
	9	1	2.0	2.0	26.5
	10	2	4.1	4.1	30.6
	12	1	2.0	2.0	32.7
	13	2	4.1	4.1	36.7
	15	2	4.1	4.1	40.8
	16	1	2.0	2.0	42.9
	18	1	2.0	2.0	44.9
	19	4	8.2	8.2	53.1
	20	1	2.0	2.0	55.1
	21	3	6.1	6.1	61.2
	22	1	2.0	2.0	63.3
	23	1	2.0	2.0	65.3
	24	1	2.0	2.0	67.3
	25	1	2.0	2.0	69.4
	29	1	2.0	2.0	71.4
	30	2	4.1	4.1	75.5
	32	1	2.0	2.0	77.6
	40	2	4.1	4.1	81.6
	42	1	2.0	2.0	83.7
	44	2	4.1	4.1	87.8
	46	3	6.1	6.1	93.9
	52	1	2.0	2.0	95.9
	54	1	2.0	2.0	98.0
	56	1	2.0	2.0	100.0
	Total	49	100.0	100.0	

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4. DISCUSSION

The study results demonstrated that extracts from the Peganum harmala and Acacia nilotica plants possess varying degrees of antibacterial activity, a finding consistent with numerous previous studies indicating that these plants contain active compounds such as tannins, phenols, and alkaloids.

The results also revealed variations in the diameters of the zones of inhibition across the different treatments, indicating differences in the sensitivity of the bacterial species to the plant extracts and antibiotics.

On the other hand, Chi-square tests showed no significant differences between the bacterial species and the variables under study; this may be attributed to the small sample size and the distribution of frequencies across a large number of categories, factors that reduce the test's statistical power to detect actual differences.

The results of the current study demonstrated that extracts from Peganum harmala and Acacia nilotica possess antibacterial activity against the tested bacterial strains, with varying degrees of susceptibility among the different species. These findings align with those of Koç et al. (2024), who showed that Peganum harmala extract exhibits significant efficacy against several medically important bacteria, including Escherichia coli, Staphylococcus aureus, Klebsiella pneumoniae and Pseudomonas aeruginosa. This efficacy may be attributed to the presence of various bioactive compounds in Peganum harmala, such as alkaloids, flavonoids, and phenolic compounds. which have the ability to disrupt the bacterial cell wall and plasma membrane, leading to the inhibition of bacterial growth or the elimination of microbial cells. The efficacy of the Qarad fruit demonstrated in the current study may be attributed to its high content of phenolic compounds, tannins, and antioxidants—substances known for their ability to bind to bacterial cell proteins and disrupt the enzymatic systems essential for bacterial growth. The observed variation in the diameters of inhibition zones across different bacterial species may be linked to structural differences between Gram-positive and Gram-negative bacteria; Gram-negative bacteria possess an additional outer membrane that limits the permeability of antimicrobial compounds, thereby rendering them more resistant than Gram-positive bacteria. Consequently, the alignment of the current study's findings with those of Koç et al. (2024) reinforces the importance of medicinal plants as a promising source of antibacterial compounds. It also supports the potential use of Peganum harmala and Acacia nilotica extracts to develop natural alterna-

tives or therapeutic supplements to conventional antibiotics, particularly given the ongoing rise of antibiotic resistance.The findings of the current study align with those reported by Zihao Zhu et al. (2022), who indicated that Peganum harmala exhibits broad-spectrum antimicrobial activity against various pathogenic bacteria and fungi. Their review demonstrated that the plant's major alkaloids—such as harmine and harmaline—play a pivotal role in this antimicrobial effect by disrupting cell membranes, increasing intracellular oxidative stress, and interfering with DNA synthesis and biological processes essential for growth and reproduction. Furthermore, the study showed that these compounds are capable of inhibiting a wide range of microorganisms, thereby explaining the notable efficacy of Peganum harmala extracts against the bacteria tested in the present study. These results also indicate the need for further studies to isolate the active compounds, precisely determine their mechanisms of action, and evaluate their efficacy for future medical applications.

5.Conclusion

1.The Peganum harmala extract exhibits antibacterial activity against the tested bacteria.

2.Acacia nilotica fruits possess an inhibitory effect on bacterial growth.

3.The efficacy of the extracts varies depending on the bacterial species and the extract concentration.

4.There are significant differences between the effects of the two extracts on certain bacterial species.

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