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Synergistic Antibacterial Activity of *Argyranthemum Foeniculaceum* Extract with Antibiotics on Bacteria Causing Periodontal Diseases

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Abstract

Background: *Argyranthemum foeniculaceum* is a traditional herb used in Yemeni folk medicine to treat mouth infections. Phytochemical acetylenes and sesquiterpene lactones were isolated from this plant. *A. foeniculaceum* possesses antibacterial activity, but there is no current data on its activity alone or combined with antibiotics against bacteria causing periodontal diseases. **Objective:** This study aimed to evaluate the antibacterial activity of ethanol extract of *A. foeniculaceum* and antibiotics (ampicillin, gentamicin and levofloxacin) as well as their combination against *S. aureus*, *S. mutans*, *S. pyogenes*, and *A. actinomycetemcomitans.* **Materials and Methods**: The antibacterial activities of ethanol extract and antibiotics were evaluated using the agar-well diffusion method. The minimal inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of plant extract against selected bacteria were assessed using the microdilution method. The synergistic effect of plant extract in combination with antibiotics was evaluated by measuring the diameter of the zone of inhibition. **Results**: In this study, ethanol extract of *A. foeniculaceum* showed good antibacterial activity against four bacteria that cause periodontal diseases. The Maximum antibacterial effect was exhibited by *A. foeniculaceum* extract against *A. actinomycetemcomitans* (MIC = 6.25 µg/ml, and MBC = 12.5 µg/ml), whereas the minimum activity was displayed by *A. foeniculaceum* extract against *S. aureus* (MIC = 50 µg/ml and MBC = 50 µg/ml). The interactions between *A. foeniculaceum* crude extract and the antibiotics used varied, with synergistic, additive, and antagonistic effects observed depending on the bacteria strain. The best synergism was displayed by the plant extract with gentamicin against *A. actinomycetemcomitans* (the inhibition zone was 33 mm). Combinations of Levofloxacin and ethanol extract showed an additive action against *A. actinomycetemcomitans* **Conclusion:** *A. foeniculaceum* extract can be used alone or in combination with antibiotics in the fight against bacterial infections that cause periodontal disease.

Keywords: *Argyranthemum foeniculaceum*; Antibacterial activity; Periodontopathic bacteria; Synergism

1. Introduction

Oral health, a pivotal component of overall health, holds significant importance as a primary health concern for humans. In developing and underdeveloped nations, periodontal disease, characterized by alveolar bone loss, is a major reason for tooth loss [1-3]. Periodontal diseases are infectious diseases caused by more than 300 bacterial species in the oral cavity [4]. The microbial film on tooth surfaces, known as dental plaque, significantly contributes to caries and periodontal diseases. The major pathogens bacteria responsible for periodontal disease are anaerobic bacteria such as *Prevotella intermedia*, *Porphyromonas gingivalis*, *Tannerella forsythia*, *Aggregatibacter actinomycetemcomitans*, and *Treponema denticola* [5]; other facultative anaerobic bacteria, such as Streptococcus sp, *Staphylococcus aureus* and *Escherichia coli* have also been identified as colonizer associated with periodontitis [6, 7]. Effective treatment for infectious diseases involves removing bacterial buildup through dental cleaning or prophylaxis. Widely used antibacterial agents such as fluorides, phenol derivatives, vancomycin, erythromycin, penicillin, ampicillin, and tetracycline are commonly implemented in dentistry for inhibiting bacterial growth [8-10]. Overuse of these chemicals can lead to disruptions of the oral and intestinal bacteria, resulting in side effects including microorganism resistance, vomiting, diarrhea, and tooth staining [10, 11]. There's a need to discover more natural antibacterial agents targeted at oral pathogens that are safe for human use. However, due to the increasing incidence of oral diseases, there has been a global need for safe and effective alternative prevention and treatment methods. It has been reported that several plants are used to treat periodontal diseases. These plants have significant anti-inflammatory, antioxidant, and antibacterial effects against various microorganisms and have fewer side effects compared to standard treatments [12, 13]. *Argyranthemum*

foeniculaceum is daisy-like bushy plant that is grown as an evergreen herbaceous perennial, with creamy-white ray florets and yellow disc flowers, belonging to the family Asteraceae (Figure **1**). Investigations on *A. foeniculaceum* have revealed the presence of various compounds including frutescin, capillol acetate, capillon, foeniculacin, 6-(2-thienyl)-2,4-hexadienoic acid N-isobutylamide and α-tetrahydrosentonin [14, 15]. Recently, an in vitro study has reported that *A. foeniculaceum* possesses antimicrobial and cytotoxic activities against HeLa and Hep-2 cell lines [15], but there is no current data on its activity against bacteria that cause periodontal disease. The ability to function synergistically with plant extracts and antibiotics could be a new approach to solving the problem of bacterial resistance and less resistant bacteria [16]. This work was carried out to demonstrate the in *vitro* antibacterial activity of an ethanol extract of *A. foeniculaceum* against four bacteria that cause periodontal disease and study the synergistic effect of the combination of the plant extract with standard antibiotics, including gentamicin, levofloxacin, and ampicillin.

Figure 1: leaves and flowers of *A. foeniculaceum.*

2. Material and Methods

2.1 Plant Material

A. foeniculaceum (aerial part) was collected in December 2023 in the garden of a house in Dhamar city, Yemen. The plant identity was confirmed by Dr. Abdullah Al-Najjar from the agricultural research center in Dhamar. The plant material was cleaned, cut into small pieces, and dried in the shade. It was then ground into powder. Figure **2** shows a flow chart that presents the complete study methodology.

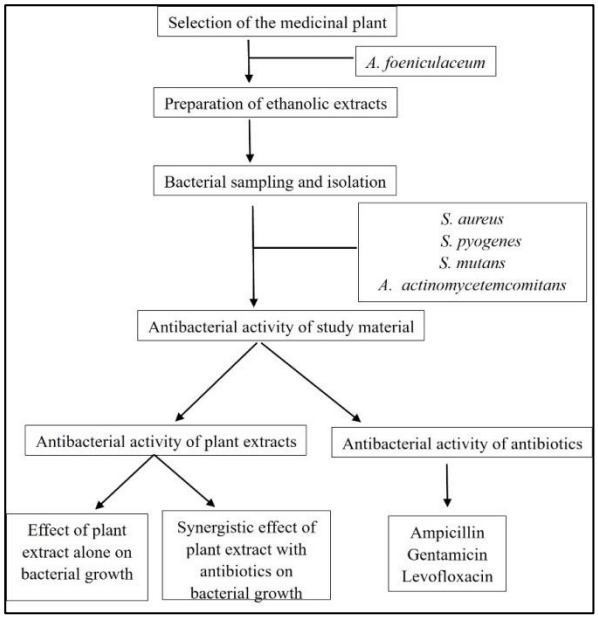

Figure 2: Flow chart of the study design.

2.2 Preparation of Plant Extract

One hundred grams of the plant material's dry powder was immersed in ethanol alcohol for 48 hours at room temperature. This process was repeated three times. The resulting crude extracts were obtained by evaporating the solvent at 45°C under reduced pressure using a vacuum rotary evaporator [17].

2.3 Antimicrobial Activity Assay:

2.3.1 Antibacterial Activity of Plants Extract

The Antibacterial effects of the extract were tested at different concentrations ranging from 100 to 6.25 µg/ml. The ethanol extract of *A. foeniculaceum* was weighed and dissolved in DMSO to prepare a 100 µg/ml storage solution. This stock solution was then diluted to obtain the desired concentrations of 50, 25, 12.5 and 6.25 µg/ml, using the equation $C_1V_1 = C_2V_2$ [17].

2.3.2 Microorganisms

The microorganisms used in this study included *Staphylococcus aureus* (*S. aureus*), *Streptococcus mutans* (*S. mutans*), *Streptococcus pyogenes* (*S. pyogens*) and *Aggregatibacter actinomycetemcomitans* (*A. actinomycetemcomitans*). These organisms were isolated from periodontitis patient. The isolates were grown on nutrient agar medium and selectively cultured at 37 °C for 24 hours. The bacterial strains were identified using standard biochemical tests [18] and stored in microbiological collection at the Laboratory of Microbiology, Faculty of Applied Sciences, Thamar University.

2.3.3 Antibacterial Activity Screening

The bacterial suspension of each target species for the study was prepared [19] by taking a needle package from each newly growing bacterial culture incubated for 24 hours. It was then inoculated into a sterile test tube containing 5 ml of normal saline. A bacterial suspension was prepared on the MacFarland scale. A swab smear was taken from each bacterial suspension and spread on the petri dishes containing Muller-Hinton agar. In the medium fed and inoculated with bacteria, holes were made with a cork borer, with four holes in each plate. The pits were filled with the plant alcoholic extract. The dishes were incubated at 37 °C for 24 hours. The appearance of an inhibition zone around the pits containing the tested extract was considered evidence of the extract's effect on the tested bacteria, and the absence of such a zone was scored negatively [19]. The effect of the extracts on bacteria was determined by measuring the diameter of the inhibition zone on the underside of the plate using a transparent ruler [20].

2.3.4 Determination of Minimum Inhibitory Concentration (MIC)

The minimum inhibitory concentration (MIC) of the plant extract against bacterial strains was determined by micro-broth dilution assays using Muller Hinton broth. Different concentrations of plant extracts were prepared. Consequently, 100 µl of culture of all bacterial strains were transferred into the wells of 96-well plates. Ten µl of the plant extract dilution were loaded to the wells for each strain. The starting inoculum for each strain was 1.5×105 CFU/ml. After 24 hours of anaerobic incubation at 37°C, after incubation, the MIC values were determined as the lowest concentration of plant extracts that inhibited visible bacterial growth [21].

2.3.5 Determination of Minimum Bactericidal Concentration (MBC)

To determine minimum bactericidal concentration (MBC), 10 µl from each concentration of plant extracts in the MIC method was poured and spread onto Muller-Hinton agar plates and incubated at 37ºC for 18-24 hours. The minimum inhibitory concentration was represented by the lowest concentration of plant extracts at which no viable bacteria cells were observed on the agar plates [22].

2.3.6 Synergistic Antibacterial Assays

The synergistic antimicrobial activity of the plant extract was determined in combination with different antibiotics by the agar-well diffusion method. Sterilized Muller Hinton agar was poured into Petri dishes, and then suspension from grown bacteria was prepared, adjusted to a 0.5 MacFarland solution, and spread on the MHA. 8 mm wells were prepared using sterile puncture. Then, 200 µl of the plant extracts (at the MIC value) and antibiotic solution (32 µg/ml) mixture were loaded into the wells, and the plates were incubated at 37 ºC for 24 hours. The diameter of the inhibition zone was measured, and results were recorded for each bacterium. The interaction was defined as synergistic if the zone

of combination treatment was greater than the zone of the plant extract plus the zone of the corresponding antibiotic; antagonistic if the zone of combination treatment was less than the zone of the plant extract plus the zone of the corresponding antibiotic; and additive if the zone of combination treatment was equal to the zone of the plant extract plus the zone of the corresponding antibiotic [3, 23].

3. Results and Discussion

The extracts of many plants have beneficial health effects, as they have been used for years in daily life to treat diseases worldwide. The present study investigated the antimicrobial properties of the ethanol extract of *A. foeniculaceum* against periodontal pathogens, including *A. actinomycetemcomitans, S. aureus*, *S. pyogenes*, and *S. mutans*. In addition, the study also evaluated the synergistic effect of *A. foeniculaceum* extracts in combination with antibiotics against these periodontal pathogens.

3.1 Antibacterial Activity of the *A. foeniculaceum* Extract on Bacteria that Cause Periodontal Disease Using Well Diffusion Method.

In the present study, the ethanol extracts of *A. foeniculaceum* showed a variable degree of antimicrobial activity against different microorganisms that cause periodontal disease. These results are presented in Table 1. The results showed that the antibacterial activity of plant extracts increased with an increase in the concentration of crude extracts. Although all four concentrations (100, 50, 25, and 12.5 µg/ml) of *A. foeniculaceum* extracts revealed antimicrobial activity, they differed in their relative activities against the tested microorganisms (Figure 3). At a concentration of 100 µg/ml, the effect ranged from 24 to 34 mm. The highest zone of inhibition was recorded by *A. foeniculaceum* extract against *S. aureus* (34 mm), followed by 33 mm inhibition zone against *S. pyogenes*. On the other hand, at a concentration of 12.5 µg/ml, the greatest effect was observed on *S. aureus* with a zone of inhibition of 18 mm, while the lowest effect was recorded on *S. mutans* with 14 mm zone of inhibition. Gonzalez et al. [15] reported the activity of acetone extract of *A. foeniculaceum* against *S. aureus* with a diameter of the inhibition zone of 23 mm. Many compounds have been isolated from *A. foeniculaceum*, such as frutescin, capitol acetate, foeniculacin, and α-tetrahydrosentonin [14], but their antimicrobial activities have not been evaluated. Therefore, the present study provides additional information on the potential of this plant against bacterial diseases causing periodontal diseases.

The MIC analysis of plant extracts showed the optimum bacteriostatic and bactericidal concentrations for the ethanol crude extract of *A. foeniculaceum*. The MIC values indicated that the strongest antibacterial activity was seen against *A. actinomycetemcomitans* with MIC value of 6.25 µg/ml, followed by *S. pyogenes* and *S. mutans* with MIC of 25 µg/ml. The results of MIC and MBC for all pathogens are presented in Table 1.

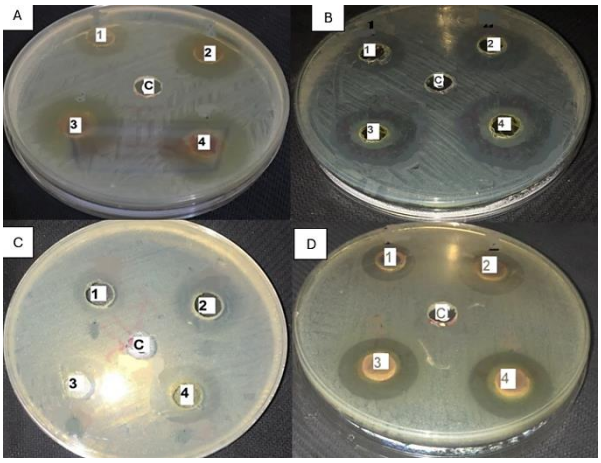

Figure 3: Inhibition zones of *A. foeniculaceum* extract on (A) *S. aureus*, (B) *S. pyogenes*, and (C) S. mutans, (D*) A. actinomycetemcomitans*. 1 = 12.5 µg/ml; 2 = 25 µg/ml; 3 = 50 µg/ml; 4 = 100 µg/ml; C = negative control (DMSO).

3.2 The Effect of Antibiotics on Bacteria Causing Periodontal Diseases.

In this study, the antibiotics showed varying diameters of inhibition zones as shown in Table 2, the results indicated that the highest effect was on *S. pyogenes* with an inhibition zone of 20 mm obtained from levofloxacin, whereas the lowest effect was on *A. actinomycetemcomitans* with an inhibition zone of 7 mm obtained from gentamicin. However, all the isolated bacteria were resistant to ampicillin.

Table 1: Zone of inhibition (mm), different concentrate of *A. foeniculaceum* extract against bacteria cause periodontal disease with MIC and MBC values.

Microorganisms	Concentration				MIC (µg/ml)	MBC (µg/ml)
	100 µg/ml	50 µg/ml	25 µg/ml	12.5 µg/ml		
	Zone of inhibition (mm)					
S. aureus	34	29	24	18	50	50
S. pyogenes	33	28	22	17	25	50
S. mutans	24	20	16	14	25	50
A. actinomycetemcomitans	30	24	19	15	6.25	12.5

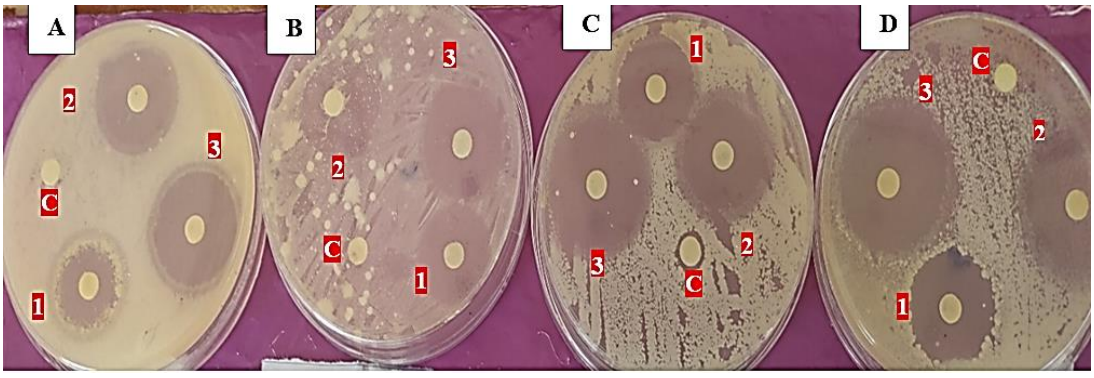

Figure 4: Inhibition zones of ethanol extract of *A. foeniculaceum* with antibiotics on (A) *S. aureus*, (B) *S. pyogenes*, and (C) *S. mutans*, and (D*) A. actinomycetemcomitans.* 1= Ampicillin, 2 = Levofloxacin, 3 = Gentamicin with *A. foeniculaceum* extract; C = negative control (DMSO).

Table 2: Synergistic effects of the ethanol extract of *A. foeniculaceum* with antibiotics.

Bacteria	Antibiotics	Zone of inhibition with Antibiotics (mm)	Zone of inhibition with *A. foeniculaceum* (mm)	Zone of inhibition with Antibiotics and *A. foeniculaceum* (mm)	Outcome
S. aureus	Gentamicin	15	29	30	Antagonistic
	Levofloxacin	11		30	Antagonistic
	Ampicillin	0		20	Antagonistic
S. mutans	Gentamicin	13	16	28	Antagonistic Synergistic
	Levofloxacin	8		25	Synergistic
	Ampicillin	0		17	
S. pyogenes	Gentamicin	9	22	31	Additive
	Levofloxacin	20		28	Antagonistic
	Ampicillin	0		20	Antagonistic
A. actinomycetemcomitans	Gentamicin	7	15	33	Synergistic Additive
	Levofloxacin	15		30	Synergistic
	Ampicillin	0		20	

3.3 The Synergy of *A. foeniculaceum* with Antibiotics

The concept of drug synergy between established antibiotics and bioactive plant extracts is innovative and has the potential for both advantageous outcomes, such as synergistic or additive interactions, as well as detrimental effects, such as antagonistic or toxic outcomes [24, 25]. The synergistic effect of ethanol extract of *A. foeniculaceum* with levofloxacin, ampicillin, and gentamicin in oral bacteria is presented in Table 2 and Figure 4.

In addition, the combination of ampicillin with *A. foeniculaceum* extract was more effective against *S. mutans* and *A. actinomycetemcomitans* than ampicillin alone. Teethaisong et. al. [26] reported that the plant extract has a high potential to reverse bacterial resistance to ampicillin drug susceptibility. The interaction between *A. foeniculaceum* extracts and antibiotics holds clinical potential for treating *A. actinomycetemcomitans* and *S. mutans* infections. Acetylenes and sesquiterpene lactones are the main phytoconstituents of this plant. Therefore, the synergistic activity of *A. foeniculaceum* extracts may be credited to acetylenes' capacity to disrupt cell walls, depolarize membranes, and enhance the penetration of antibiotics into bacteria. [14, 27]. However, the interaction between plant extract compounds and antibiotics remains unexplained.

In our study, we also found that the combination of *A. foeniculaceum* extract and antibiotics (ampicillin, gentamicin, and levofloxacin) exhibited antagonistic activity against *S. aureus*, while Darwish et al. [28] reported that several plant extracts from Jordan had enhanced gentamicin activity against *S. aureus*. On the other hand, the result showed an additive effect when *A. foeniculaceum* extract was combined with gentamicin and levofloxacin against *S. pyogenes* and *A. actinomycetemcomitans* respectively. In a previous study [29], the combined effects of the MeOH extract of *Ficus carica* leaves with gentamicin were shown to be additive against the oral bacterium *S. pyogenes*.

4. Conclusions

The study indicates that plant extracts serve as effective sources of antimicrobial agents. When combined with various antimicrobials, plant extracts can be valuable in fighting relatively resistant endodontic microorganisms. The current in *vitro* study discovered that ethanolic extracts from the *A. foeniculaceum* plant exhibit antibacterial activity against all the experimented periodontal pathobionts. The results of the synergistic tests showed a significant antimicrobial effect when plant extracts are combined with antibiotics. The strongest antibacterial activity against *A. actinomycetemcomitans* and *S. mutans* was observed when *A. foeniculaceum* extract was combined with gentamicin and ampicillin, respectively. However, further studies are required to identify and isolate the active compounds in this plant extract, assess their biocompatibility, efficacy against biofilms, and determine mechanisms responsible for synergy.

Data Availability

The datasets used and analyzed during the current study are available from the corresponding author upon reasonable request.

Conflict of Interest

The authors declare no conflict of interest.

Acknowledgments

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References

[1] Sheiham, A. (2005) Oral health, general health and quality of life, SciELO Public Health, pp. 644-645.

[2] De Pablo, P., Chapple, I.L., Buckley, C.D., Dietrich, T. (2009) Periodontitis in systemic rheumatic diseases, *Nature Reviews Rheumatology* **5**: 218-224.

[3] Saquib, S.A., AlQahtani, N.A., Ahmad, I., Arora, S., Asif, S.M., Javali, M.A., Nisar, N. (2021) Synergistic antibacterial activity of herbal extracts with antibiotics on bacteria responsible for periodontitis, *The Journal of Infection in Developing Countries* **15**: 1685-1693.

[4] Mayrand, D., Grenier, D. (1998) Bacterial interactions in periodontal diseases, *Bulletin de l'Institut Pasteur* **96**: 125-133.

[5] Han, J., Wang, P., Ge, S. (2017) The microbial community shifts of subgingival plaque in patients with generalized aggressive periodontitis following non-surgical periodontal therapy: a pilot study, *Oncotarget* **8**: 10609.

[6] Uribe-García, A., Paniagua-Contreras, G.L., Monroy-Pérez, E., Bustos-Martínez, J., Hamdan-Partida, A., Garzón, J., Alanís, J., Quezada, R., Vaca-Paniagua, F., Vaca, S. (2021) Frequency and expression of genes involved in adhesion and biofilm formation in Staphylococcus aureus strains isolated from periodontal lesions, *Journal of Microbiology, Immunology and Infection* **54**: 267-275.

[7] Estemalik, J., Demko, C., Bissada, N.F., Joshi, N., Bodner, D., Shankar, E., Gupta, S. (2017) Simultaneous detection of oral pathogens in subgingival plaque and prostatic fluid of men with periodontal and prostatic diseases, *Journal of periodontology* **88**: 823-829.

[8] Wara-aswapati, N., Krongnawakul, D., Jiraviboon, D., Adulyanon, S., Karimbux, N., Pitiphat, W. (2005) The effect of a new toothpaste

containing potassium nitrate and triclosan on gingival health, plaque formation and dentine hypersensitivity, *Journal of clinical periodontology* **32**: 53-58.

[9] Bidault, P., Chandad, F., Grenier, D. (2007) Risk of bacterial resistance associated with systemic antibiotic therapy in periodontology, *Journal of the Canadian Dental Association* **73**: 721-725.

[10] Allaker, R.P., Douglas, C.I. (2009) Novel anti-microbial therapies for dental plaque-related diseases, *International journal of antimicrobial agents* **33**: 8-13.

[11] Van Strydonck, D., Timmerman, M., Van Der Velden, U., Van Der Weijden, G. (2005) Plaque inhibition of two commercially available chlorhexidine mouthrinses, *Journal of clinical periodontology* **32**: 305-309.

[12] Halawany, H.S. (2012) A review on miswak (*Salvadora persica*) and its effect on various aspects of oral health, *The Saudi dental journal* **24**: 63-69.

[13] Dhaliwal, J.S., Gambhir, R.S., Sodhi, S.K., Shaheed, G., Binti, D., HajiPG, K.M. (2017) Herbs and their use in oral care: A Review, *Brunei Darussalam Journal of Health* **7**: 5-17.

[14] Gonzalez, A., Barrera, J.B., Dìaz, J.G., Garcia, T.Z., De Paz, P.P. (1988) Distribution of acetylenes and sesquiterpene lactones in Argyranthemum from Tenerife, *Biochemical systematics and ecology* **16**: 17-21.

[15] Gonzalez, A.G., Estevez-Reyes, R., Estevez-Braun, A., Ravelo, A.G., Jimenez, I.A., Bazzocchi, I.L., Aguilar, M.A., Moujir, L. (1997) Biological activities of some Argyranthemum species, *Phytochemistry* **45**: 963-967.

[16] Stefanovic, O., Comic, L. (2012) Synergistic antibacterial interaction between *Melissa officinalis* extracts and antibiotics, *Journal of applied pharmaceutical science* **2**: 01-05.

[17] Al-Mekhlafi, N.A., Al-Badaii, F., Al-Ezzi, M.S., Al-Yamani, A., Almakse, E., Alfaqeeh, R., Al-Hatar, G., Al-Twity, M., Al-Masadi, M., Abdullah, M. (2023) Phytochemical Analysis and Antibacterial Studies of Some Yemeni Medicinal Plants against Selected Common Human Pathogenic Bacteria, *Thamar University Journal of Natural & Applied Sciences* **8**: 14–18.

[18] De la Maza, L.M., Pezzlo, M.T., Bittencourt, C.E., Peterson, E.M. (2020) Color atlas of medical bacteriology, 3rd Edition ed., John Wiley & Sons, New York, USA.

[19] Khan, M.M., Harunsani, M.H., Tan, A.L., Hojamberdiev, M., Poi, Y.A., Ahmad, N. (2020) Antibacterial studies of ZnO and Cu-doped ZnO nanoparticles synthesized using aqueous leaf extract of Stachytarpheta jamaicensis, *BioNanoScience* **10**: 1037-1048.

[20] Saif, M.M.S., Alodeni, R.M., Alghamdi, A.A., Al-Odayni, A.-B. (2022) Synthesis, spectroscopic characterization, thermal analysis and in vitro bioactivity studies of the N-(cinnamylidene) tryptophan Schiff base, *Journal of King Saud University-Science* **34**: 101988.

[21] Bose, D., Chatterjee, S. (2016) Biogenic synthesis of silver nanoparticles using guava (*Psidium guajava*) leaf extract and its antibacterial activity against Pseudomonas aeruginosa, *Applied Nanoscience* **6**: 895-901.

[22] Tippayawat, P., Phromviyo, N., Boueroy, P., Chompoosor, A. (2016) Green synthesis of silver nanoparticles in *Aloe vera* plant extract prepared by a hydrothermal method and their synergistic antibacterial activity, *Peer Journal* **4**: e2589.

[23] Saquib, S.A., AlQahtani, N.A., Ahmad, I., Kader, M.A., Al Shahrani, S.S., Asiri, E.A. (2019) Evaluation and comparison of antibacterial efficacy of herbal extracts in combination with antibiotics on periodontal pathobionts: an in vitro microbiological study, *Antibiotics* **8**: 89.

[24] Adwan, G. (2008) Synergistic effects of plant wxtracts and antibiotics on Staphylococcus aureus strains isolated from clinical specimens, *Middle-East Journal of Scientific Research* **3**: 134-139.

[25] Hemaiswarya, S., Kruthiventi, A.K., Doble, M. (2008) Synergism between natural products and antibiotics against infectious diseases, *Phytomedicine* **15**: 639-652.

[26] Teethaisong, Y., Autarkool, N., Sirichaiwetchakoon, K., Krubphachaya, P., Kupittayanant, S., Eumkeb, G. (2014) Synergistic activity and mechanism of action of Stephania suberosa Forman extract and ampicillin combination against ampicillin-resistant Staphylococcus aureus, *Journal of Biomedical Science* **21**: 1-11.

[27] Siddiq, A., Dembitsky, V. (2008) Acetylenic anticancer agents, *Anti-Cancer Agents in Medicinal Chemistry (Formerly Current Medicinal Chemistry-Anti-Cancer Agents)* **8**: 132-170.

[28] Darwish, R.M., Aburjai, T., Al-Khalil, S., Mahafzah, A. (2002) Screening of antibiotic resistant inhibitors from local plant materials against two different strains of Staphylococcus aureus, *Journal of Ethnopharmacology* **79**: 359-364.

[29] Jeong, M.-R., Kim, H.-Y., Cha, J.-D. (2009) Antimicrobial activity of methanol extract from *Ficus carica* leaves against oral bacteria, *Journal of Bacteriology and Virology* **39**: 97-102.