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Article

Evaluation of Antimicrobial Activity and Molecular Docking of Artemisia herba-alba Growing in the Al-Muthanna Desert, Iraq

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Abstract: Medicinal plants have gained an important source in traditional medicine, and drug production in various countries of the world. A percentage 80% of the world's population depends on them due to their availability, ease of access, and low cost. Hence, the importance of our study emerged, which dealt with the effect of the extract of the white *Artemisia herba-alba* plant, which is widespread in the desert of Al-Muthanna Governorate, on the microorganisms isolated from patient with gingivitis. A moleculer docking simulation was also carried out for the flavonoids that are abundant in the *Artemisia herba-alba* extract. In the laboratory, agar diffusion experiments were conducted to assess the inhibitory effects of *Artemisia herba-alba* extract and some selected antibiotics on bacterial growth. while the in-silico approach involved consensus sequence and structural analyses of topoisomerase and DNA gyrase through docking and molecular-dynamics simulation. The results revealed that *Artemisia herba-alba* extracts, particularly flavonoids, displayed significant antimicrobial activity against all types of bacteria ($P \leq 0.05$). *Artemisia herba-alba* extract exhibited the most potent effects, while ciprofloxacin showed comparatively lower activity. Docking analysis demonstrated strong interactions between the inhibitors and topoisomerase and DNA gyrase, with the inhibitors binding within the domain regions. These findings underscore the promising antimicrobial properties of *Artemisia herba-alba* extracts, with flavonoids being particularly effective against antibiotic-resistant bacteria. Continued research in this domain holds the potential for developing novel and effective antimicrobial agents based on *Artemisia herba-alba*, thereby potentially reducing reliance on conventional antibiotics.

Keywords: Asteraceae, Antibiotic Resistance, Extraction Methods, Medicinal Plant, Molecular Docking

Introduction

Artemisia herba-alba is an aromatic plant that grows naturally in the mountainous regions of North Africa (Algeria, Morocco, Tunisia) and some country in Asia such as Iraq[1] . The aerial parts of this plant are widely used in traditional medicine to treat bronchitis, diarrhea, high blood pressure, and diabetes. A recent study demonstrated that white wormwood (*Artemisia herba-alba*) possesses promising biological properties in combating colorectal cancer, one of the most common and deadly cancers worldwide. An extract of this plant was tested on eight different colon cancer cell lines, and the results showed an inhibitory effect on cancer cell growth with a clear induction of apoptosis[2], [3], [4].

The white wormwood extract was shown to directly affect the cell cycle, inhibiting the expression of important proteins such as Cyclin B1 and CDK1, which play a pivotal role in regulating cancer cell division. The extract also demonstrated its ability to disrupt the PI3K/AKT/mTOR molecular pathway, a pathway known to play a role in cell growth, proliferation, and resistance to death, thus supporting the hypothesis that the active compounds in this plant influence the internal mechanisms of tumor growth. Furthermore, the extract's toxic effect was observed to be selective, targeting cancer cells without causing significant damage to healthy cells. This suggests its potential for future development as a supportive natural therapeutic agent with acceptable efficacy and limited side effects compared to conventional chemotherapy. The findings of this study reinforce the growing interest in natural products as a potential source of anticancer drugs and pave the way for future clinical trials to evaluate the efficacy and safety of Artemisia herba-alba extract in living models and in patients[5], [6], [7], [8].

The use of antibiotics such as Ampicillin, Clindomcin, Tetracycline, Kanamycin, Trimethoprim, and Erythromycin has played a significant role in treating bacterial infections, including gingivitis, for many years. However, several problems have arisen with their use, such as the emergence of resistant strains. It is worth noting that bacteria possess two types of resistance: natural resistance and acquired resistance. Gram-negative bacteria have natural resistance to many highly effective antibiotics that are active against Gram-positive bacteria. This resistance is due to the presence of a lipopolysaccharide layer in their cell wall, which prevents the penetration of inhibitory concentrations of antibiotics into the bacterial cell (Brooks et al., 1998). The problem lies in acquired resistance, which has been increasing rapidly in recent years (Merck, 2002). Therefore, recent studies have focused on the use of Plant extracts that are used in treatment due to their effectiveness, ease of access, low cost, and environmental friendliness[9], [10], [11], [12].

The use of combination antibiotics has several benefits, including reducing the chances of resistant strains emerging, increasing the effectiveness of antibiotics, and reducing toxicity in treating infections caused by multiple microorganisms [12].Gum disease is one of the most common oral diseases, but most sufferers do not notice it initially because they experience little to no pain. Gum disease sometimes appears during puberty and in women during pregnancy due to hormonal reasons. However, gum disease is most prevalent after the age of forty in both sexes[13]. Al-Bustani (2003) stated that the epidemiology of gingivitis is influenced by a multifactorial environment, and that heredity, diet, and personal hygiene all play a role in susceptibility to inflammation[14], [15].

Plants currently occupy an important place in agricultural and industrial production. They are the primary source of medicinal drugs and active ingredients used in the preparation of medicines or as raw materials to produce numerous chemical compounds used in the manufacture of several important medications [16], [17]. It is now known that plants contain essential substances such as carbohydrates and proteins that play a role in medicine. It has also been noted that the active ingredients in plants are affected by several factors, including soil and climate conditions, and the methods of harvesting and preservation[3], [18]. The gall is a medicinally important plant. Its fruits and oil have been used worldwide for generations to relieve toothaches. It possesses anti-inflammatory properties due to its eugenol content, which acts as an analgesic and antibiotic [19]. Numerous studies have been conducted on the effects of microorganisms on teeth and gums. These studies have shown that Streptococcus sp. bacteria are responsible for inflammation and decay of the teeth. Some studies have also found that consuming cranberries and sugars promotes the adhesion of Streptococcus bacteria to the teeth and gum wall [20], [21]. A study by Al-Dhahab (1998) confirmed the antagonistic activity of extracts from Myrtus communis (sweet wormwood) and Peganum harmala (wild rue) against several types of bacteria, including Escherichia coli, Staphylococcus aureus, and Proteus mirabilis, comparing them to a group of synthetic antibiotics. The study showed that Gram-positive bacteria were more sensitive to the extract than Gram-negative bacteria. Another study of garlic extract revealed that it contains active compounds such as allyl mercaptan and allyl alcohol, which are effective against the parasite Giardia lamblia [22]. Furthermore, Al-Jumaili (2005) demonstrated the inhibitory effect of plant extracts from the stems and leaves of Urtica urines and Quercus infection on E. coli and Pseudomonas aeruginosa. The results showed that the alcoholic and acetone extracts of nettle were effective. The study showed an inhibitory effect on all types of bacteria studied, while the alcoholic and acetone extracts of tannins showed a weaker inhibitory effect on all types of bacteria studied[23]. The study also showed that the mixture of tea and *Artemisia herba-alba* influences bacteria causing gingivitis. Abbas's study (2010) confirmed that the volatile oils of clove and eucalyptus plants have an inhibitory effect on fungi isolated from cabbage roots. The results showed that a concentration of (100) micrograms/ml of the volatile oil of the clove plant inhibited all types of isolated fungi by 100%, while the volatile oil extracted from the eucalyptus plant gave a result of 90% against fungi in this study.

Materials and Methods

Plant Samples Collection

The leaves of *A.herba-alba* were collected from a desert in Al-Muthanna Governorate (Figure 1) and brought to the Pharmacognosy and Medicinal Plants Laboratory at the College of Pharmacy, Al-Muthanna University. There, they were identified, classified, and a sample was deposited in the College of Pharmacy's herbarium. The sample was then washed with distilled water to remove dirt and impurities, placed in paper bags, and oven-dried at 55°C for 24 hours. Finally, it was ground using a blender to obtain a dry powder ready for extraction.

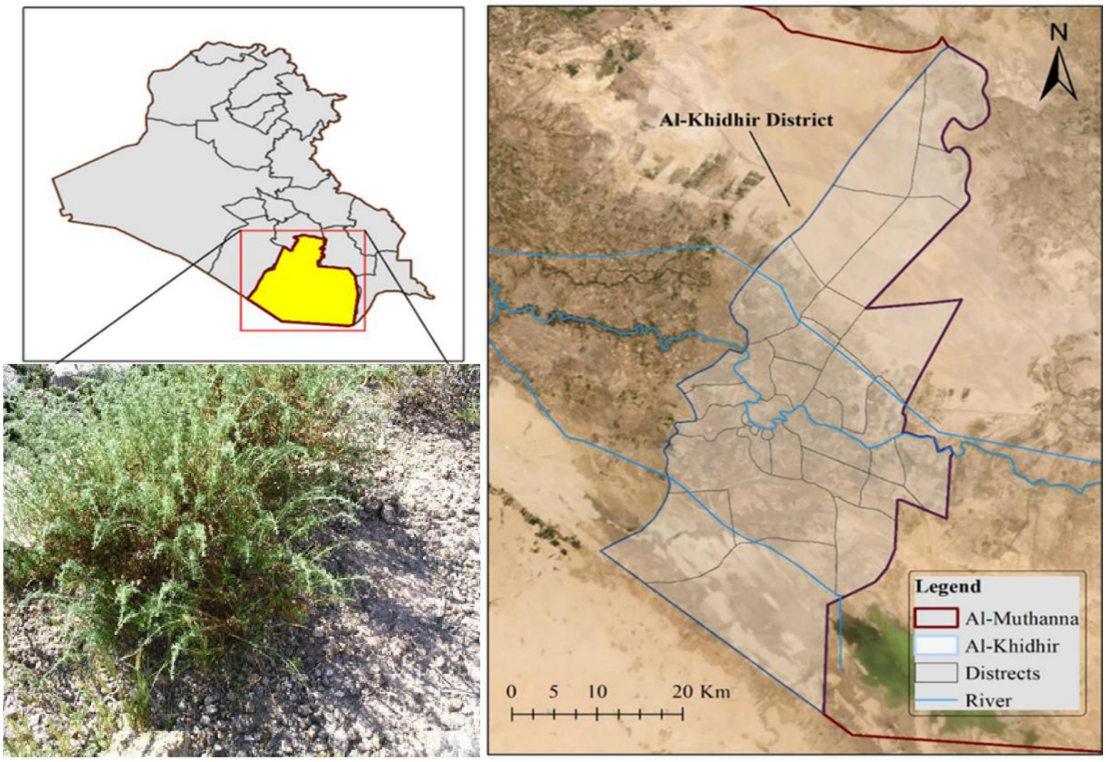


Figure 1. The study area in the desert of Al-Muthanna, Iraq

Preparation of the plant Extract and Phytochemical Screening

The aqueous extract was prepared according to the method of [24]. In this process, one-part dry plant powder was diluted with five parts distilled water (1:5 ratio) in a flask and heated to boiling for 15 minutes. The mixture was then filtered using filter paper No. 1. The resulting extract was sterilized by autoclaving at 121°C for 15 minutes and then stored in sterile test tubes at 4°C until use. While the alcoholic extract was prepared using a Soxhelet apparatus. 50 grams of the previously prepared dry powder were placed in a thimble, then in the Soxhelet, and 80% ethanol was added to initiate the extraction process. After completing the process and obtaining the extract, we remove any traces of alcohol. The extract is then placed in an oven at 50°C for one hour. Following this, it is transferred to test tubes and stored at 4°C until use. After preparing both the alcoholic and aqueous extracts, we performed phytochemical screening tests to detect the presence of certain chemical compounds.

Bacterial Isolation and antibiotic resistance test

Bacterial isolates were obtained from the dental clinics of the College of Dentistry, Al-Muthanna University. They were activated, cultured, and their bioactivity was tested after preparing the dilutions of the plant extract. A series of half-dilutions were performed for both the alcoholic and aqueous extracts. The sensitivity test method (Leven et al., 1997) was then applied. The bacteria were cultured on Mueller-Hinton sensitivity test medium[24]. Paper discs prepared from Watman (NO1) filter paper, saturated with different concentrations of the aqueous and alcoholic extracts to be tested, were fixed to the surface of the medium and incubated at 37°C for 24 hours. After this period, the areas of inhibition were measured. The sensitivity test method followed the method described by Leven et al. (1997). The bacteria were cultured on Mueller-Hinton sensitivity test medium. Paper discs prepared from Watman (NO1) filter paper, impregnated with different concentrations of the aqueous and alcoholic extract to be tested, were then fixed to the surface of the medium and incubated at 37°C for 24 hours. Afterward, the areas of inhibition were measured.

The Molecular Docking process

The SDF files of target chemical structures were downloaded from the PubChem database (https://pubchem.ncbi.nlm.nih.gov) and then converted to PDB format using Convert V2. The protein crystal structures for antimicrobials were downloaded from the protein databank (https://www.rcsb.org/) using a previously published molecular docking protocol (Mohamed et al., 2020).

Results

The preliminary chemical investigation of the active compounds of the aqueous and alcoholic extract of the jujube plant. In light of the results of our study on the biological activity towards some fungal and bacterial isolates of the aqueous and alcoholic extracts of the jujube plant, an investigation was conducted into their content of active compounds, and Table (1) shows that the two extracts contain all the active compounds that were detected, except for the resinous compounds.

Table 1. shown the Phytochemical Constituents for both Aqueous Extract Alcoholic Extract in *Artemisia herba-alba*

No.	Phytochemical Constituents	Aqueous Extract	Alcoholic Extract
1	Alkaloids	+	+
2	Glycosides	+	+
3	Saponins	+	+
4	Carbohydrates	+	+
5	Flavonoids	+	+
6	Flavones and Flavanols	+	+
7	Tannins	+	+
8	Amino Acids and Primary & Secondary Amines	+	+
9	Phenols	+	+
10	Resins	−	−
11	pH Value	5	4
12	Coumarins	−	+

The reason for the higher efficacy of the alcoholic extract may be the absence of fucoxanthin in the aqueous extract and their presence in the alcoholic extract. fucoxanthin are a type of phenolic compound capable of degrading bacterial biofilms (Al-Dhahab, 1998). Alternatively, the alcoholic extract may be more effective than the aqueous extract due to its lower pH compared to the aqueous extract (Table 1). Acidity plays a role in increasing efficacy, and these results align with Al-Sarraf's (1992) study, which demonstrated that high acidity alters the nature of living matter, particularly proteins in cell membranes, through denaturation. This causes these proteins to lose their function, leading to cell membrane rupture and the death of fungal and bacterial cells. The presence of plant acids also contributes to lowering the pH of the extract. Al-Sarraf and his colleagues (2006) indicated that the leaves of the jujube plant contain several organic acids, such as malic, citric, tannic, and acetic acids, which are responsible for the lower pH. In Table 2 showed that alcoholic extracts are more effective than aqueous extracts. Since the extraction conditions and method are the same, the difference is attributed to the difference in the polarity of the two solvents, which is due to the difference in the dielectric constant of both types. The dielectric constant of water is (78.54), while for ethyl alcohol it is (24.25) (Table 4).

Table 2. showed the MIC (mg/mL)MBC (mg/mL) for both alcoholic extracts and aqueous extracts.

Organism	Aqueous Extract		Alcoholic Extract	
	MIC (mg/mL)	MBC (mg/mL)	MIC (mg/mL)	MBC (mg/mL)
Staphylococcus aureus	0.220	1.00	0.625	1.25
Staphylococcus	1.00	2.00	1.25	2.5
Bacillus subtilis	0.320	1.00	0.625	1.25
Escherichia coli	1.5	3.0	2.5	5.0
Klebsiella pneumoniae	1.5	3.0	2.5	5.0
Pseudomonas aeruginosa	4.0	5.0	5.0	10.0
Candida albicans	1.25	2.5	1.25	2.5

Table 3. explained the *Artemisia herba-alba* alcoholic extract antibacterial activity compared with some standard selection antibiotics.

Microorganism	Artemisia herba-alba EO (mm)	Ciprofloxacin (5 µg)	Gentamicin (10 µg)	Amoxicillin (25 µg)	Cefotaxime (30 µg)	Imipenem (10 µg)
Staphylococcus aureus	24.3 ± 0.8	30.2 ± 0.5	27.8 ± 0.6	18.4 ± 0.7	25.1 ± 0.5	31.4 ± 0.4
Staphylococcus	21.7 ± 0.6	24.5 ± 0.7	23.8 ± 0.8	10.2 ± 0.4	18.9 ± 0.6	29.6 ± 0.5
Bacillus subtilis	26.1 ± 0.7	31.7 ± 0.4	29.2 ± 0.5	21.8 ± 0.8	26.4 ± 0.7	32.3 ± 0.3
Escherichia coli	18.9 ± 0.5	29.6 ± 0.5	25.4 ± 0.6	15.1 ± 0.7	24.7 ± 0.4	30.5 ± 0.4
Klebsiella pneumoniae	17.4 ± 0.6	28.8 ± 0.6	23.9 ± 0.5	13.4 ± 0.5	22.6 ± 0.6	29.7 ± 0.3
Pseudomonas aeruginosa	14.2 ± 0.4	27.1 ± 0.4	21.7 ± 0.7	R	20.4 ± 0.5	28.9 ± 0.4
Candida albicans	20.8 ± 0.5	ND	ND	ND	ND	ND

Values are expressed as **mean ± SD, R** = Resistant. **ND** = Not determined.

Table 4. The activity of Artemisia herba-alba extracted in combination with some selected antibiotics.

Antibiotic	Target strain	Antibiotic Alone (mm)	Combination with EO (mm)	Increase (%)
Ciprofloxacin	Staphylococcus	24.5	31.4	28.2
Gentamicin	Staphylococcus	23.8	30.6	28.6
Amoxicillin	S. aureus	18.4	27.3	48.4
Cefotaxime	E. coli	24.7	30.1	21.9
Imipenem	P. aeruginosa	28.9	32.7	13.1

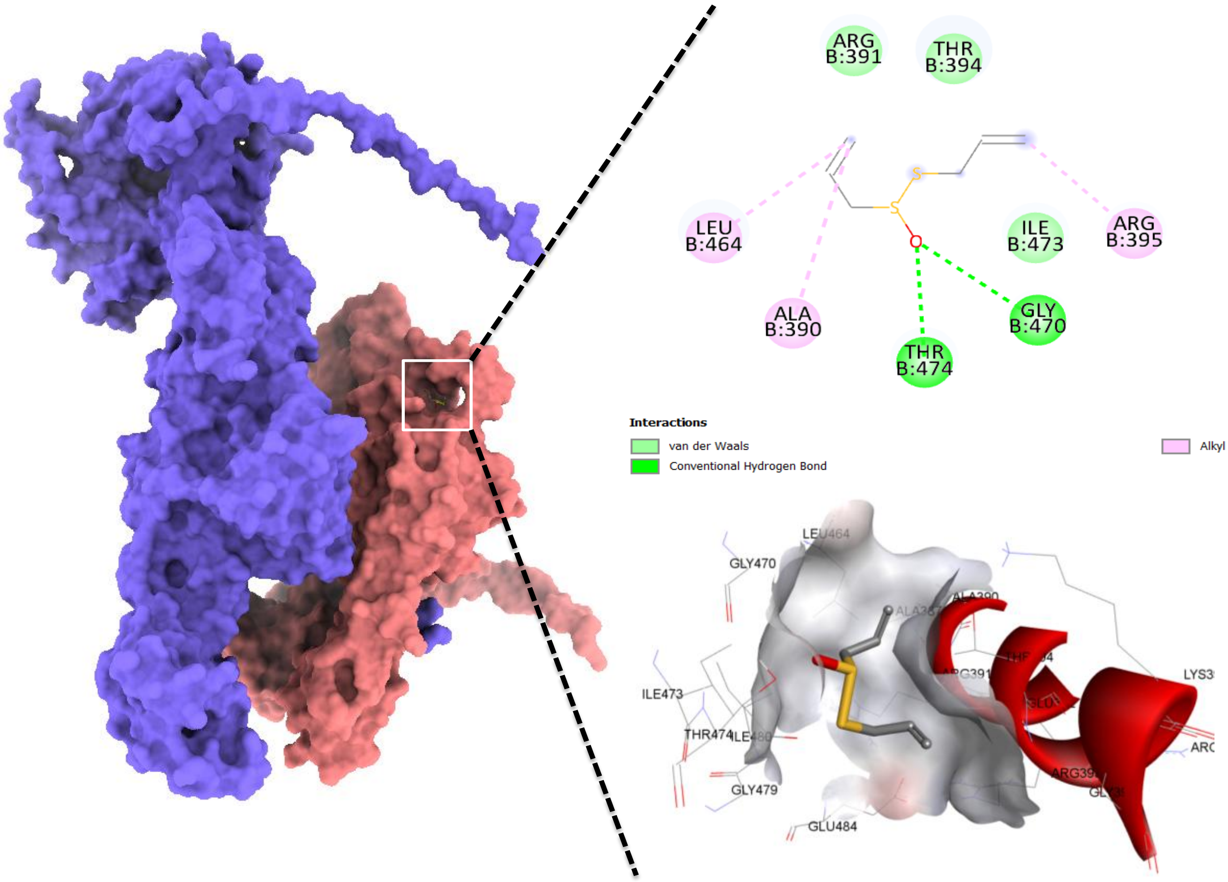
Binding Interaction Analysis of Proteins Against Inhibitors

The Autodock 4.0 is utilized for DNA gyrase and topoisomerase docking against compounds Artemisia herba-alba and Ciprofloxacin. The docking analysis discovered a substantial interaction between proteins and inhibitors. The binding score for DNA gyrase-A. herba-alba is -8.9 kcal/mol. In contrast, DNA gyrase-Ciprofloxacin has -7.5 kcal/mol. It was observed that Artemisia herba-alba formed conventional hydrogen bonds, and ciprofloxacin formed hydrogen bonds with, ARG482 residues of DNA gyrase receptor. In the case of DNA Topoisomerase, Artemisia herba-alba forms 2 hydrogen bonds and ciprofloxacin forms 3 hydrogen bonds with with binding energies -8.6 kcal/mol and -7.4 kcal/mol, respectively(Table 5). Moreover, multiple sequence alignment of DNA gyrase and

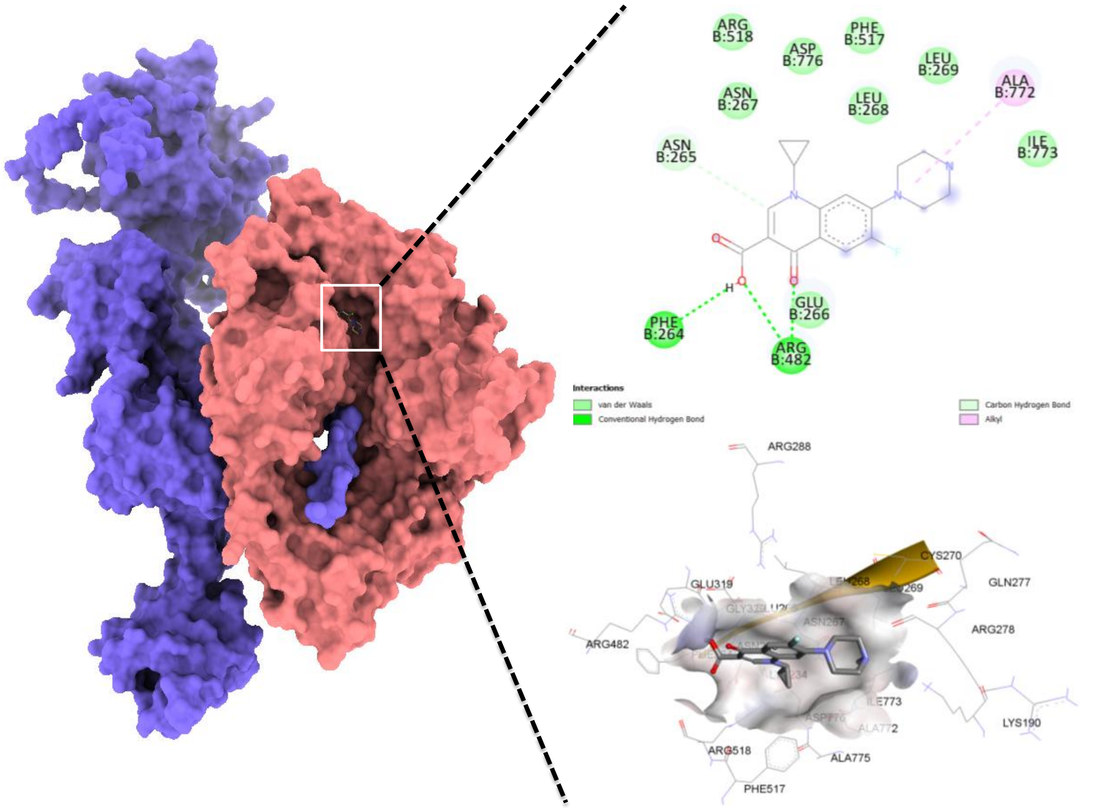
Topoisomerase proteins from 5 different bacterial strains showed binding of these two inhibitors is also conserved in all other species.

Table 5. Shown the molecular docking results for Artemisia herba-alba extracted as target molecule.

Compound	Relative Abundance (%)	DNA Gyrase	PBP2a	β-Lactamase
Camphor	22.5	-5.4	-5.2	-5.0
Eucalyptol	14.6	-5.1	-4.9	-4.8
α-Thujone	12.4	-6.0	-6.1	-5.9
β-Thujone	9.8	-6.2	-6.3	-6.1
Borneol	6.7	-6.3	-6.0	-6.0
Chrysanthone	4.3	-7.1	-7.0	-6.8
Davanone	3.9	-7.4	-7.6	-7.3

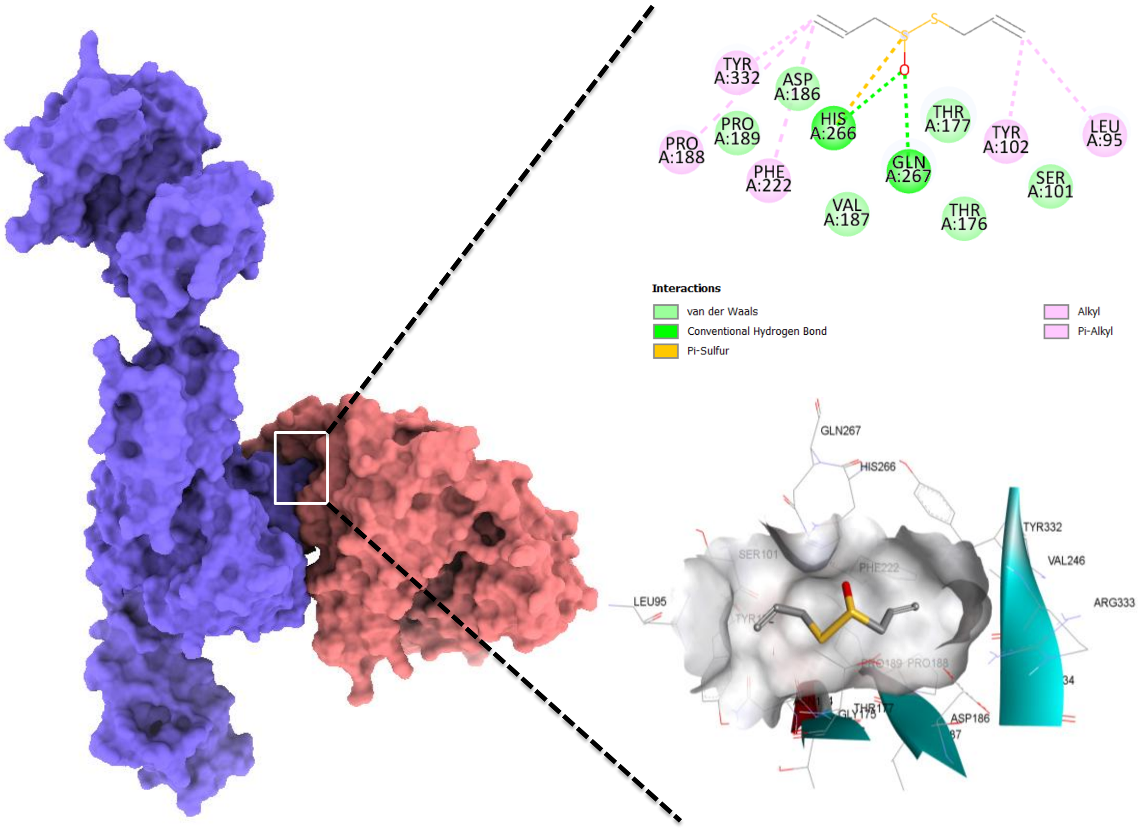


Gyrase+ Flavonoid

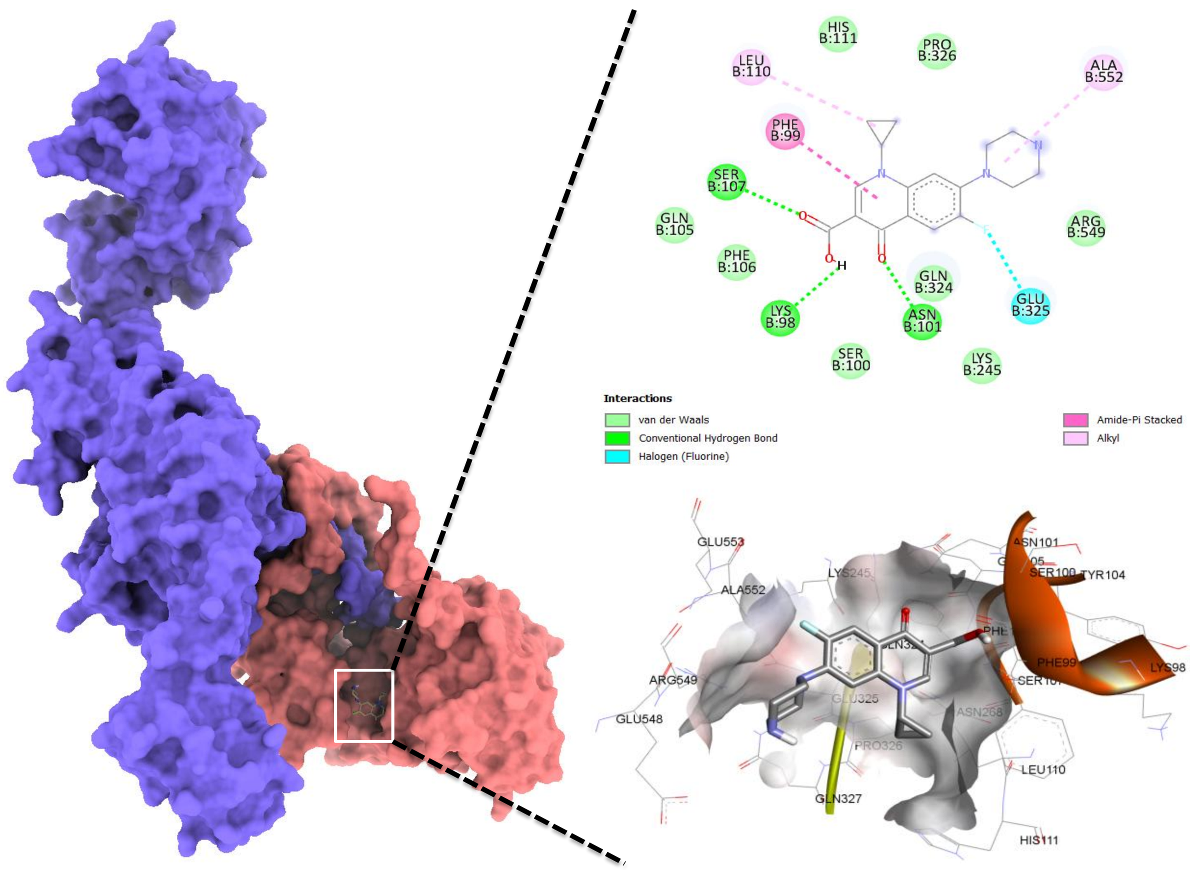


Gyrase+Ciprofloxacin

A



Topoisomerase+ Flavonoid



Topoisomerase+Ciprofloxacin
B

Discussion

The results showed that Streptococcus species, which cause gingivitis, are the most common. They play a significant role in tooth decay and are called caries-causing bacteria. Furthermore, they predispose to opportunistic infections caused by another group of Gram-positive bacteria, namely Staphylococci, due to their natural presence on the skin, nose, and digestive tract [25]. Their prevalence in the mouth is attributed to their rapid resistance mechanisms and ease of propagation via plasmids through conjugation and transformation processes, or to their possession of surface antigens and lytic enzymes that facilitate their penetration into body tissues, as seen in Staphylococcus bacteria [26], [27]. As for Gram-negative bacteria, they constituted a smaller percentage compared to Gram-positive bacteria. Several studies, including Waltimo et al. (1997), have confirmed that most Gram-negative bacteria originate from respiratory or gastrointestinal infections and appear in the mouth. This aligns with the findings of Al-Maysari (2002), Al-Rubaie (1998), Al-Mousawi (2006), and Al-Absi (2009).
Gram-negative bacteria constituted a smaller percentage compared to Gram-positive bacteria. The results also showed that Gram-positive isolates exhibited significant variation in their antibiotic sensitivity. This may be due to the production of beta-lactamase enzymes, the genes for which may be chromosomally located on plasmids [1], [16]. Alternatively, bacterial resistance may be attributed to one of three mechanisms: modification of the antibiotic target site, reduced permeability, or production of inhibitory enzymes (Sanders, 1992). Another possibility is that the increased and indiscriminate use of antibiotics has led to the development of resistance in bacteria due to the use of subtherapeutic doses, resulting in the emergence of mutant isolates.

The results also showed that the test bacteria were sensitive to the aqueous extract, and their sensitivity may be greater or equal to that of antibiotics. This can be explained either by the bacteria's unfamiliarity with these extracts and therefore their inability to resist them, or by the extracted substances having a chemical affinity for reacting with cell components, or by the presence of specific receptors on the bacterial cell wall and suitable transporters that carry their molecules into the cell to inhibit the action of coenzymes and other bioactive molecules[28], [29]. This is consistent with the findings of Abbas and Al-Abadi. Furthermore, gallnut contains high levels of eugenol, ranging from 70-90% of its composition, which acts as an analgesic for toothaches [22]. The combination of the antibiotic Amikacin with the aqueous extract also demonstrated a synergistic inhibitory effect against the test bacteria.

Conclusion

In this study, the antimicrobial properties and chemical contents of *A. herba-alba* were identified by using traditional methods. Also, computational tools and a molecular docking protocol were applied to evaluate the pharmacognosy action of *A. herba-alba*. The results explained that the A. herba alba extraction solution had a significant inhibitory effect using different tests. The phytochemical evaluation of *A. herba-alba* proved it contains secondary metabolite material such as glycosides, alkaloids, tannins, flavonoids, resins, saponins, terpenes, and phenols. However, work on toxicological assessments is required to verify the safety of these extractions. Furthermore, pharmacodynamics and pharmacokinetic investigations of *A. herba alba* extract are then necessary to confirm the beneficial effects in human healthcare and industrial applications as potential sources of natural antioxidants and preservative agents.

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