Current Clinical and Medical Education

Received 12 Oct 2025 | Accepted 10 Nov 2025 | Published Online 9 Dec 2025

Published By: Vision Publisher

CCME 3 (12), 33-43

ISSN 2942-0792

Antimicrobial Properties of Ethanolic and Other Components Chromatographic Analysis of Artemisia annua Leaf Extract and Its Functional Groups

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Abstract:

Numerous studies have demonstrated that the essential oil and extracts of *Artemisia annua* has antibacterial characteristics, rendering them efficient against many bacteria and fungi. Some researchers think that the plant's ability to kill both Gram-positive and Gram-negative bacteria is due to the bioactive chemicals it contains, including as artemisinin and terpenes. Using chromatographic analysis of functional groups and ethanolic *Artemisia annua* leaf extract, this study intends to assess the antibacterial capabilities of the plant. The results of the GC-MS analysis proved the presence of the compounds, which are 9-Octadecenoic acid, (-)-delta-cadinene, Tetraneurin E, 1-tert-Butyl-4,4-diphenylpiperidine, Lucenin-2, Germacrene D-4-ol, 4-(2,3-Diphenylcyclopropyl)phenol, Farnesene, Tetracosahexaenoic acid, beta-Caryophyllene oxide, and Isochinol. According to (methanol, Ethyl acetate, and Ethanol fraction), Ampicillin (AMP) and Cefotaxime (CTX). Recorded (28.00±0.45, 18.00±0.28, 23.00± 0.40, 33.01±0.51 and 30.00±0.49) respectively in Staphylococcus aureus. While recorded (22.91±0.81, 14.09±0.23, 24.00±0.41, 29.00±0.48 and 26.98±0.45) for Escherichia coli. Record (24.19±0.38, 18.09±0.27, 21.26±0.29, 31.08±0.50 and 28.16±0.49) Streptococcus pyogenes. While (21.95±0.38, 25.96±0.42, 27.00±0.44, 27.95±0.48 and 23.00±0.45) for Staphylococcus epidermidis. The metabolites of *Artemisia annua* exhibited significant activity against Staphylococcus aureus (28.00±0.45).

Keywords: Chromatographic Analysis, Antimicrobial Properties, *Artemisia annua*, Functional Groups

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INTRODUCTION

Herbs and shrubs of the genus Artemisia are mostly found in semiarid (steppe climate) areas. According to what is known from published accounts, this genus contains about 500 species. As a result of the specimens' many geographic origins, it exhibits a high degree of morphological and phytochemical heterogeneity. Furthermore, the taxonomic and evolutionary relationships of the genus Artemisia are the subject of numerous investigations. Sesquiterpenes, terpenoids, and monoterpenes in essential oils are among the biologically active components of the Artemisia plant that give it scientific significance [1, 2]. A broad range of intriguing biological actions are associated with these types of phytochemicals. Emergence and spread of fungal and bacterial infectious illnesses have become a major cause of death for all forms of life on Earth. The primary weapon in the battle against infectious diseases is the antibiotic. It is becoming increasingly difficult to treat these infections due to the rise of multidrug-resistant microbes, which is having a major impact on healthcare costs. Oxidative stress, which occurs when the production of ROS outpaces their elimination, is another major threat to human health. Cell membrane oxidative damage, destruction of various biological substances, and numerous serious diseases are all results of this occurrence [3]. As a result, developing novel, potent compounds to combat free radicals and drug-resistant bacteria is of the utmost importance. To get around these issues, researchers have been focusing more on using natural components to create novel medications that are both effective and safe. A gift from Mother Nature, medicinal and aromatic plants (MAP) enable humans to live long, healthy lives free from illness. Natural medicine has a long and storied history of use in human and veterinary medicine, dating back to the dawn of recorded history. An increasing number of people are considering using natural medicines instead of synthetic ones because of the potential side effects and high cost of the former. Natural plants belonging to the Asteraceae family have a long history of widespread use in popular cuisine around the world due to their high nutritional content [4-7]. Moroccan tea is a popular herbal tea among diabetics. It is often used as an appetizer, condiment, spice, or in salads. They have chemical components that can be used to create medications that can compete with synthetic pharmaceuticals; in fact, some of these compounds have already been given the green light for clinical use [8-10]. The objective of this research is to analyze the antimicrobial characteristics of *Artemisia annua* leaf extract, its functional groups, and various ethanolic fractions.

MATERIALS AND METHODS

Methods for Extracting Ethanol from Herbs and Other Plant Materials The plant parts used in this study were the leaves and aerial portions of *Artemisia annua* L. plants, which are native to Hillah City in Iraq. The plant's aerial parts were cut into medium-sized pieces and then dried in a chamber at room temperature for two weeks at the University of Babylon. The final product was dried using anhydrous sodium sulfate (Na2SO4), then put in amber worm bottles and kept at 4 ◦C until it was time for testing. After drying the extract, it was preserved at a temperature of -18 degrees Celsius until needed.

Chemical Composition Analysis with GC-MS

Artemisia annua's chemical composition was determined using GC-MS analysis. In order to identify and quantify the components, 1 µL was passed through a gas chromatography–mass spectrometer (GC-MS–TQ8040 NX, brand Shimadzu, Tokyo, Japan) fitted with an apolar capillary column (RTxi-5 Sil MS-30m × 0.25 mm ID × 0.25 µm). Starting at 50 ◦C for two minutes, the oven temperature was gradually increased at a rate of 5 ◦C per minute until it reached 160 ◦C. After that, it was ramped up to 280 ◦C for two minutes. It is worth mentioning that the analysis was conducted for 50 minutes using 1 mL/min of high-purity helium as the carrier gas, with a split ratio of 1:20. A temperature of 280 ◦C was maintained for the detector, while an injection temperature of 250 ◦C was maintained. This experiment used a spectral range of m/z 40-650, an electron ionization energy of 70 eV, and an ion source temperature of 200 ◦C. The volume of the sample that was injected was 1 µL. We used the NIST-MS Search Version 2.0 application to compare the mass spectra of the chemical compounds in the sample to those in the MS library. This

allowed us to identify the chemicals in the sample. In addition, the Adams database served as a reference for comparing their Kovats index..

Antimicrobial Activity Determining the Minimum Inhibitory Concentration (MIC)

Traditional harmful bacterial strains like S. aureus, E. coli, Streptococcus pyogenes, and S. epidermidis have already undergone experimental screening. For twenty-four hours at 37 degrees Celsius, they were actually cultured in a petri dish with 10 milliliters of sterile nutritional broth. Only one day was all the incubation time for the plates at 37°C. In the experiment, an optical microscope was utilized to confirm the form of the bacterium. Meanwhile, sterile saline was used to disperse the number of colonies actually belonging to each strain. This was then adjusted in the lab so that it matched the turbidity of the 0.5 McFarland standard, which was made on Mueller-Hinton agar and contained 1.5 x 108 colony-forming units per milliliter.

Data Analysis by Statistic

The data is shown as the mean plus or minus the standard deviation. A program called GraphPad Prism 9 was used to conduct the analysis. We employed analysis of variance (ANOVA) and Tukey's extremely significant difference test to compare the results. When the p-value was lower than 0.05, it was assumed that there was a statistically significant difference.

RESULTS AND DISCUSSION

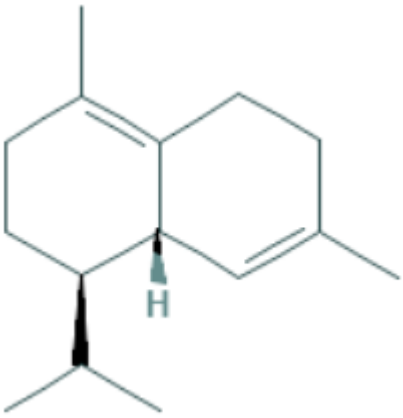
According to the World Health Organization, traditional medicine is currently practiced by 80% of the population in developed countries. An important genus of herbs, Artemisia is officially recognized as Asteraceae annua L. Species identified in this group are mostly associated with northern temperate climates, where the average yearly rainfall is between zero and fifty centimeters [11, 12]. Sweet wormwood, or *Artemisia annua* L., is a member of the Asteraceae family of plants that grows wild mostly in Asia, more specifically in Korea, Japan, and China. It has also spread to other nations, where it is now grown. The results of the GC-MS analysis proved the presence of the compounds, which are v9-Octadecenoic acid, (-)-delta-cadinene, Tetraneurin E, 1-tert-Butyl-4,4-diphenylpiperidine, Lucenin-2, Germacrene D-4-ol, 4-(2,3-Diphenylcyclopropyl)phenol, Farnesene, Tetracosahexaenoic acid, beta-Caryophyllene oxide, and Isochinol.

9-Octadecenoic acid

MF: $C_{18}H_{34}O_2$

MW: 282.5 g/mol

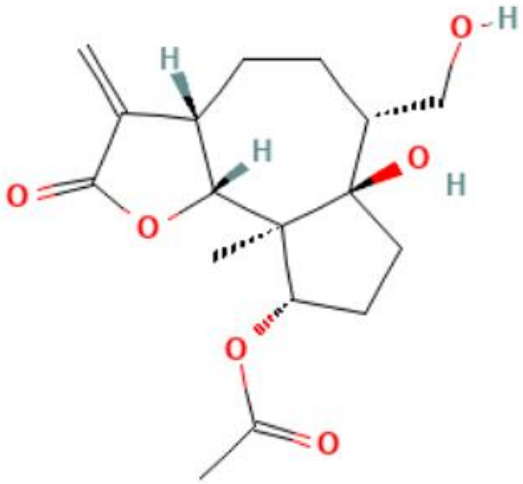
(-)-delta-cadinene



MF: $C_{15}H_{24}$

MW: 204.35 g/mol

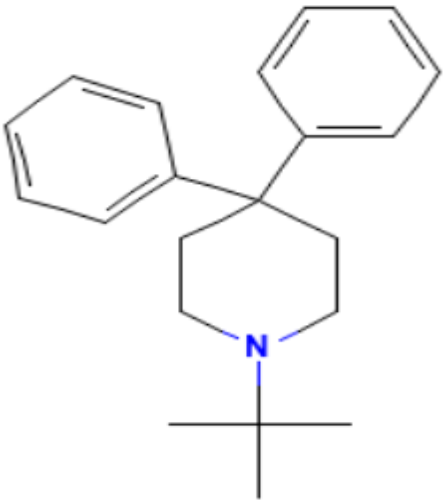
Tetraneurin E



MF: $C_{17}H_{24}O_6$

MW: 324.4 g/mol

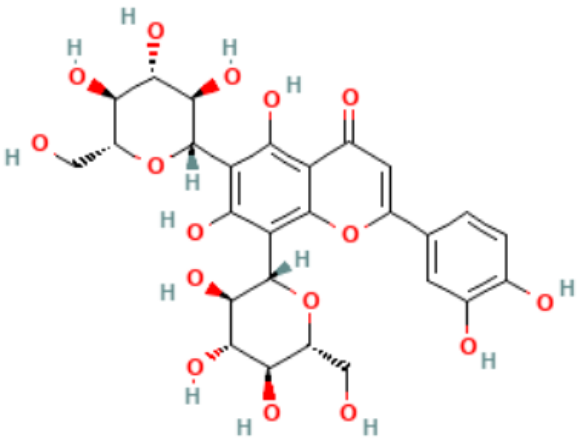
1-tert-Butyl-4,4-diphenylpiperidine



MF: $C_{21}H_{27}N$

MW: 293.4 g/mol

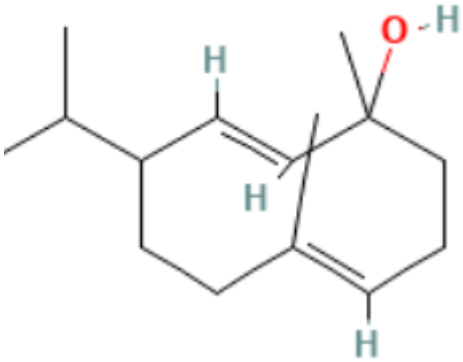
Lucenin-2



MF: $C_{27}H_{30}O_{16}$

MW: 610.5 g/mol

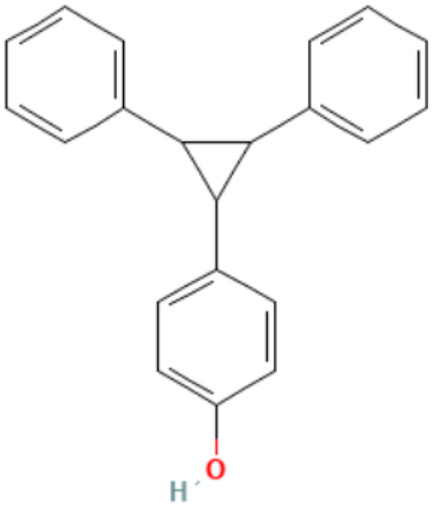
Germacrene D-4-ol



MF: $C_{15}H_{26}O$

MW: 222.37 g/mol

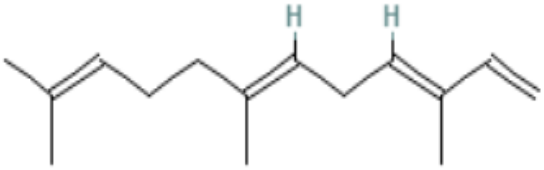
4-(2,3-Diphenylcyclopropyl)phenol



MF: $C_{21}H_{18}O$

MW: 286.4 g/mol

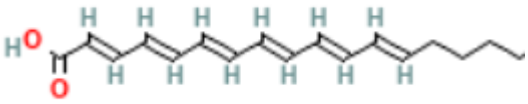
Farnesene



MF: $C_{15}H_{24}$

MW: 204.35 g/mol

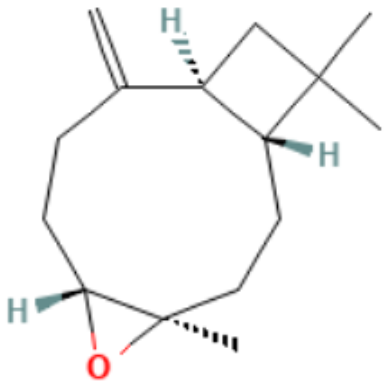
Tetracosahexaenoic acid



MF: $C_{24}H_{36}O_2$

MW: 356.5 g/mol

beta-Caryophyllene oxide



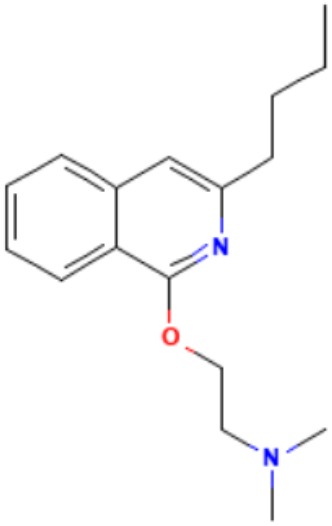
MF: $C_{15}H_{24}O$

MW: 220.35 g/mol

Isochinol

MF: $C_{17}H_{24}N_2O$

MW: 272.4 g/mol



In vitro tests were conducted on four distinct microbial species to determine the antibacterial activity of leaf extracts of *Artemisia annua*. Cefotaxime (CTX) and Ampicillin (AMP) are based on the (methanol, ethyl acetate, and ethanol portion). Staphylococcus aureus tested positive for (28.00±0.45, 18.00±0.28, 23.00±0.40, 33.01±0.51 and 30.00±0.49) respectively. Although the results for Escherichia coli were recorded as (22.91±0.81, 14.09±0.23, 24.00±0.41, 29.00±0.48, and 26.98±0.45). The Streptococcus pyogenes record includes values of (24.19±0.38, 18.09±0.27, 21.26±0.29, 31.08±0.50, and 28.16±0.49). For Staphylococcus epidermidis, the values are (21.95±0.38, 25.96±0.42, 27.00±0.44, 2795±0.48, and 23.00±0.45). Staphylococcus aureus was significantly inhibited by the metabolites of *Artemisia annua* (28.00±0.45).

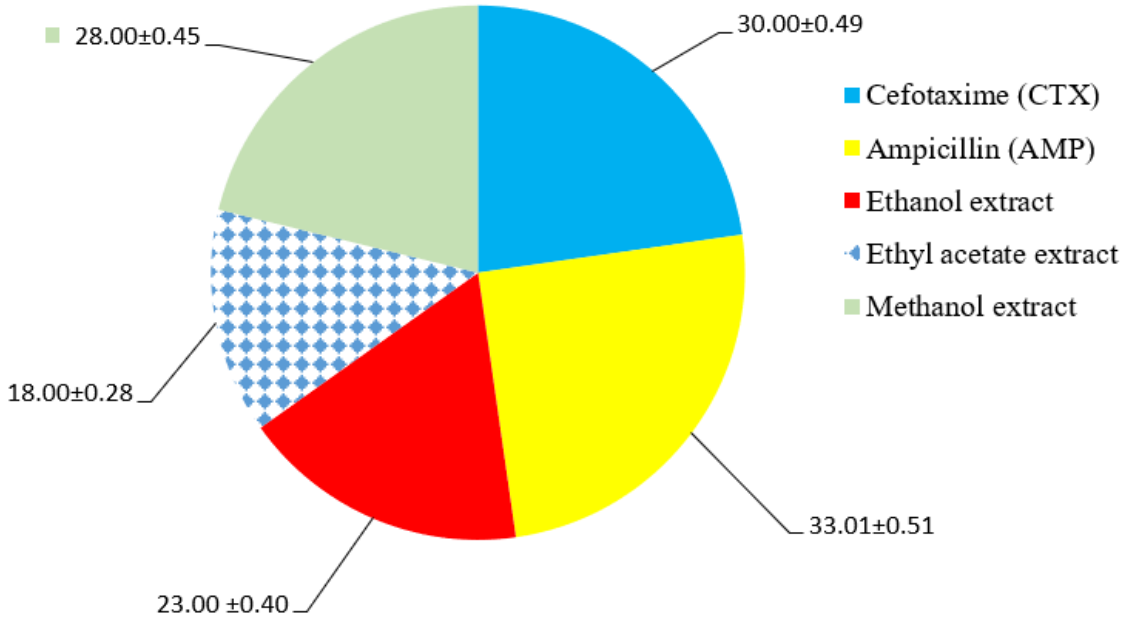


Figure 1. Antibacterial activity of bioactive compounds of *Artemisia annua* and standard antibiotics against *Staphylococcus aureus*

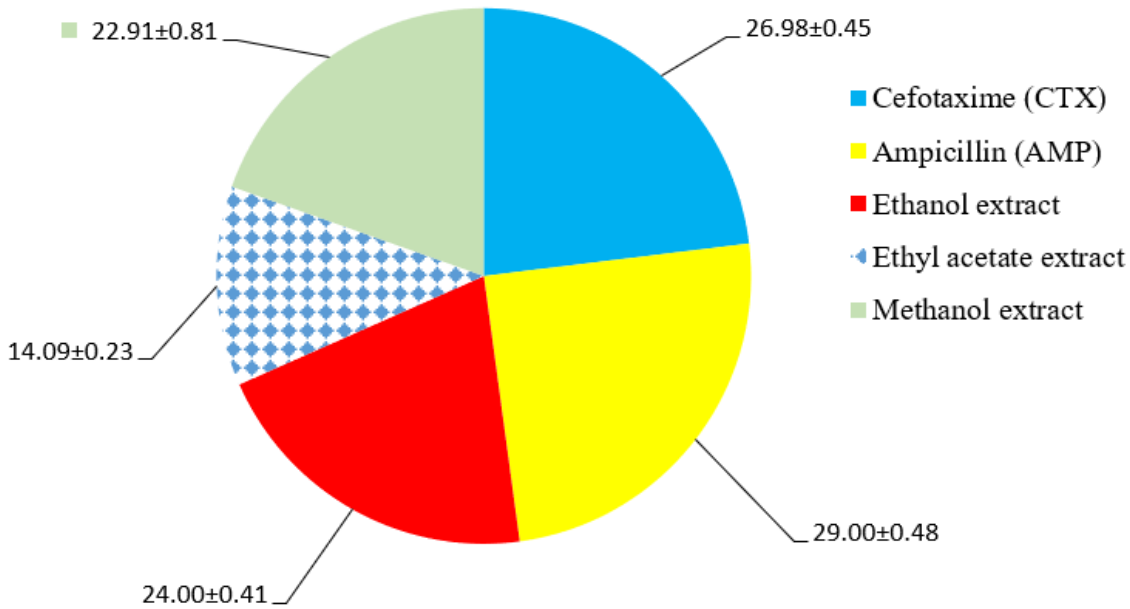


Figure 2. Antibacterial activity of bioactive compounds of *Artemisia annua* and standard antibiotics against *Escherichia coli*

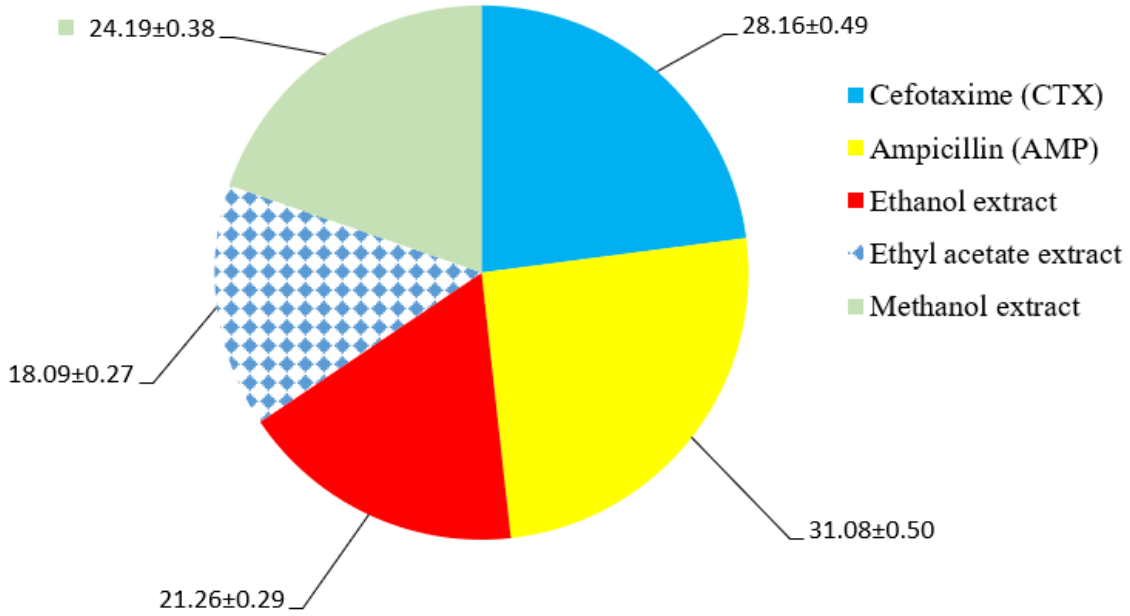


Figure 3. Antibacterial activity of bioactive compounds of *Artemisia annua* and standard antibiotics against *Streptococcus pyogenes*

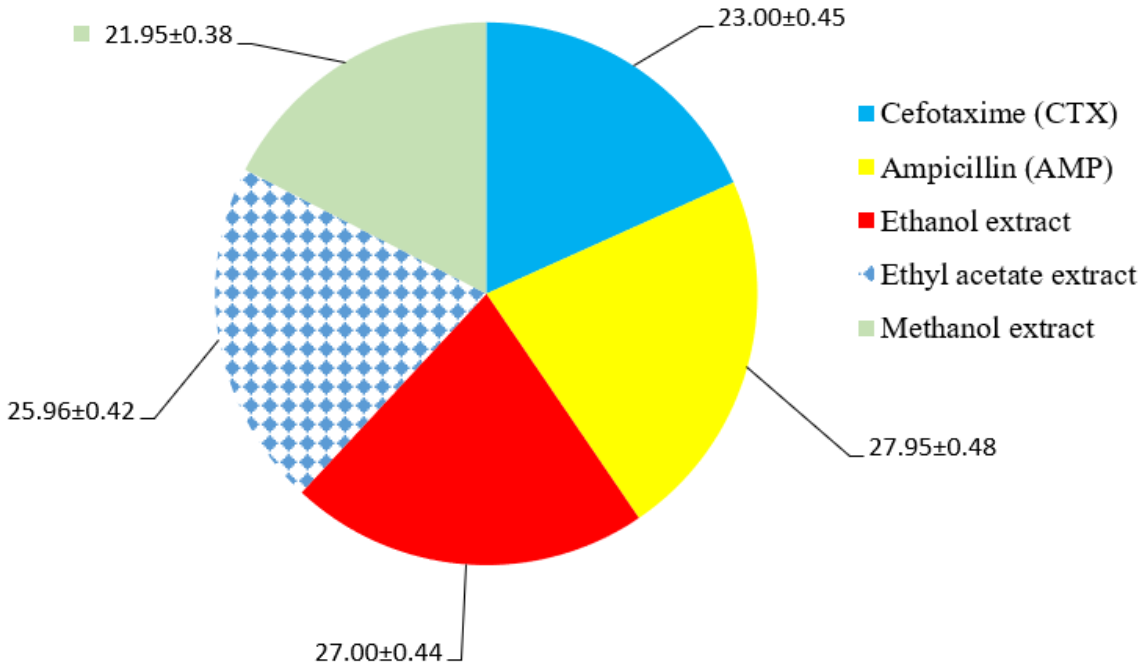


Figure 4. Antibacterial activity of bioactive compounds of *Artemisia annua* and standard antibiotics against *Staphylococcus epidermidis*

Metabolites with antibacterial characteristics are produced by multiple Artemisia species. The ethanolic extract also had a high concentration of chlorogenic acid, which was discovered in a tall variety of Asteraceae. Recent studies have shown that chlorogenic acid can induce cell death by binding to the outer membrane [13-15], disrupting it, decreasing the intracellular potential, and releasing macromolecules from the cytoplasm. Disappointing patient outcomes and increased healthcare costs are the results of antibiotic resistance, a worldwide problem that is only getting worse. The increasing resistance to commonly prescribed antibiotics like tetracycline and sulfamethoxazole has made it increasingly challenging to combat severe bacterial infections caused by Enterobacteriaceae, such as Escherichia coli, and other Gram-negative bacteria. This is especially true given the limited therapeutic options available for multidrug-resistant strains and the lack of new drug development. Additionally, resistance to ampicillin, chloramphenicol, gentamicin, ciprofloxacin, trimethoprim/sulfamethoxazole, and tobramycin has significantly increased during the past few years [16-19]. The use of traditional medicinal plants to treat a wide range of illnesses is becoming increasingly popular around the world. It is worth mentioning that Artemisia is one of the biggest genera in the Asteraceae family and is found all over the northern hemisphere. To enhance treatment procedures and perhaps supplant conventional medicines, medicinal plants can be combined with active pharmacological and nutritional components [20, 21].

Essential oils extracted from plants have grown in popularity as a result of their low toxicity and widespread use as treatments for serious illnesses. To that end, we tested the antioxidant and antibacterial capabilities of essential oils extracted from the Mediterranean-dwelling *Artemisia annua* L. Thanks to our EO extraction process, we were able to achieve a yield of about 0.51%. We found that the yield varies depending on when it is harvested, where the seeds are grown, how the leaves or flowers are used, and the location of the culture. Importantly, chemotype, plant part, genotype, drying circumstances, harvest season, geographic location, fertilizer, soil pH, and extraction process are some of the factors that can affect chemical compound variance [22-25]. There were a lot of oxygenated monoterpenes (55.35 percent) and hydrocarbon sesquiterpenes (13.43%) in the EOAA analysis. While noting the lack of oxygenated monoterpenes, a smaller quantity of oxygenated monoterpenes (1.62%) was found. Essential oils (EOs) derived from the *Artemisia* genus, including species found in the Mediterranean (A. arborescens, A. caerulescens subsp., and A. annua), are rich in the majority of these components. Other regions that produce EOs

include North America, Turkey, India, and France [26, 27]. Also, EOAA and the chemicals found in essential oils of the Moroccan-endemic A. mesatlantica are somewhat similar. It follows that EOAA's high concentration of artemisia ketone may account for its antioxidant power. According to the literature and the DPPH test, artemisia ketone is more active than camphor and 1,8-cineole. Research has shown that the active ingredients in essential oils are often responsible for their antioxidant properties [28-30]. The antioxidant powder of essential oils is determined by the synergistic action of their minor components. When comparing OEs with and without hydrocarbon terpenes, those rich in oxygenated monoterpenes tend to have stronger antiradical effects. The antioxidant results align with earlier research that indicated Artemisia to have promising antioxidant activity across all bioassays, including DPPH, β-carotene bleaching, and total antioxidant capacity. This is an important finding. At the moment, scientists are trying to figure out how artemisinin, the active ingredient in *A. annua* L., works to prevent malaria. Additionally, *A. annua* L. has been the subject of research into its possible medicinal benefits for a wide range of illnesses in the last several decades. These include inflammatory and malignant disorders [31], infections caused by bacteria, viruses, and parasites, and more. According to our research, the antibacterial activity of the ethanolic *A. annua* extract was greater.

CONCLUSION:

Antibacterial activity against Staphylococcus aureus, Escherichia coli, Streptococcus pyogenes, and Staphylococcus epidermidis was demonstrated by means of the polyphenolic compounds that are prevalent in the leaves of the Artemisia annua L. species. The level of efficiency against Staphylococcus aureus (28.00±0.45) was much higher when Artemisia annua was used. Based on our findings, it is worth further investigation to explore the possibilities of using Artemisia extracts in new therapy regimens for infectious disorders that are resistant to current treatments. Artemisia annua extracts showed significant antibacterial action against multidrug-resistant Escherichia coli, according to the study, which provides hope for a natural substitute for traditional antibiotics and food preservatives. Natural extracts from Artemisia may play a pivotal role in creating new treatments and preventative measures against multidrug-resistant bacteria, providing a sustainable and environmentally friendly answer to the problem of antibiotic resistance. These results provide strong evidence in favor of additional research into the therapeutic use of Artemisia extracts against resistant infections.

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