

Studying the Effect of the Effectiveness of Antibiotics and the Alcoholic Extract of the Plant *Mentha spicata* in Eliminating Tooth Decay Bacteria *Streptococcus mutans* and its Effective Role as an Antioxidant

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ABSTRACT. *The study was conducted in Nasiriyah, Dhi Qar Governorate, Iraq, during 2025, from April to September. The study spanned approximately six months, encompassing the period from plant cultivation to drying prior to extraction.*

*The study demonstrated that alcoholic extracts of *Mentha spicata* possess excellent inhibitory activity against pathogenic bacteria and are safe for pharmaceutical use. Therefore, medicinal plants are used as antimicrobial agents.*

*An alcoholic extract of *Mentha spicata* was prepared. Twenty grams of the dried plant powder were weighed and 200 mL of ethanol was added to it at 45°C for 24 hours. The extract was then placed in a Soxhlet extractor and filtered using Whatman No. 1 filter paper (England). The sample was poured into glass Petri dishes, and the dry powder was stored in airtight glass containers and refrigerated at 4°C until use.*

*The efficacy of the ethanolic plant extracts against pathogenic *Streptococcus mutans* was tested at concentrations of 25, 50, and 100 mg/ml. The bacteria's susceptibility to the three commonly used antibiotics—amikacin (AK), gentamicin (CN), and ciprofloxacin (CIP)—was also tested. Because *Streptococcus mutans* is inherently less resistant to amikacin and gentamicin, other commonly used antibiotics, such as ciprofloxacin, were added to the treatment regimen for a broad spectrum of infections. Information on the reference system for interpreting antibiotic susceptibility should be provided, The DPPH assay is one of the most widely used techniques for evaluating the antioxidant properties of plant extracts. The alcoholic extract exhibited higher levels of inhibition than ascorbic acid, indicating the plant's high antioxidant activity.*

*The ethanolic extract showed concentration-dependent inhibitory activity against *Streptococcus mutans*, reaching a maximum concentration of 100 mg/ml. In antibiotic studies, ciprofloxacin was found to be the most effective antibiotic. Specifically, the plant extract at a concentration of 100 mg/ml demonstrated an inhibitory effect similar to that of ciprofloxacin. The ethanolic extract also showed good DPPH activity (87.84%) at 100 mg/ml, while ascorbic acid showed 82.04% DPPH activity at the same concentration.*

Keywords: *Mentha spicata*, *Streptococcus mutans*, antibacterial activity, antioxidant activity, ethanolic extract

1. INTRODUCTION

Plants of this genus are perennial herbaceous plants with a strong, pleasant aroma. They are found almost worldwide, in Europe, Africa, Asia, Australia, and North America, and are rarely annual. They are widespread in various regions. Their leaves are usually smooth with serrated edges, and their colors range from dark green and blue to gray-green, and sometimes light yellow. They are square or lanceolate in shape.[1] Their stems are square, branching, and erect [2].

As illustrated in Figure (1), *Mentha spicata* is a plant species belonging to the mint genus of the mint family (Lamiaceae) [3]. *Mentha spicata* leaves are extensively used in culinary applications, either fresh or dried, as well as in the production of chewing gum, toothpaste, and various pharmaceutical products [4]. Peppermint preparations, such as syrup, are traditionally used in the treatment of gallbladder disorders, intestinal colic, abdominal cramps, menstrual pain, and flatulence. In addition, mint has been reported to exhibit hepatoprotective, cardiogenic, nervine, and digestive benefits. It is also known to facilitate respiration, promote diuresis, and reduce gastric mucosal irritation. Moreover, it possesses anti-inflammatory, anti-bacterial, anti-fungal and anti-oxidative effects [5, 6].

The human oral cavity has a diverse range of microorganisms including more than 700 species of prokaryotes, as do the warm, moist, nutrient rich environments in which they live [7]. *Streptococcus mutans* bacteria are known to be one of the key factors for dental caries, these bacteria use fermentable carbohydrates originating from food as their energy source [8]. There have been recent studies which showed that extracts from medicinal plants, particularly *Mentha spicata*, are effective against *Streptococcus mutans*. [9, 10]. In recent studies, the therapeutic efficacy of *Mentha spicata* as an antibacterial and antioxidant agent has been greatly highlighted. complementing the traditional uses described in the main introduction. The phenolic acids, flavonoids, and essential oils found in *Mentha spicata* are responsible for its key role in inhibiting the proliferation of pathogenic oral bacteria like *Streptococcus mutans* and minimizing oxidative stress [11, 12]. *Mentha spicata* has the ability to disrupt biofilms of *Streptococcus mutans* bacteria, suggesting it could be a useful component in oral hygiene products.

Concurrently, *Mentha spicata* extracts exhibit high antioxidant activity, eliminate DPPH and have anti-oxidation effects on oral tissue [14, 15].

Besides, the combination of various phytochemicals promotes their antibacterial and antioxidant activity, sometimes to a concentration similar to those of conventional medicinal drugs [16,17].

In this extended context, the importance of the use of *Mentha spicata* extracts in preventive dentistry and oral health care products, as a natural alternative to conventional antibiotics and antioxidants, has been highlighted. The results confirm the scientific rationale for the current study, and suggest that the ethanolic extract of *Mentha spicata* could be investigated for its anti-*S. mutans* and antioxidant properties.

Table 1. Classification Of *Mentha Spicata*

Kingdom	Plantae
Phylum	Magnoliophyta

Class	Magnoliopsida
Order	Lamiales
Family	Lamiaceae
Genus	Mentha
Species	<i>Mentha spicata</i>



Figure 1. powder *Mentha spicata*

2. MATERIALS AND METHODS

2.1 Preparation of the plant powder

Spearmint leaves (*Mentha spicata* L.) were collected from local markets and the herbalists in Nasiriyah, Dhi Qar Governorate, Iraq. The plant material was washed and then finely ground using an electric grinder. The powder was kept in opaque glass bottles having tight fitting lids until use as shown in figure.1 [19].

2.2 Short-term storage

Streptococcus mutans isolates were maintained on slanted-sided brain and heart nutrient agar at 4°C after 24 hours of growth at 37°C. The isolates were kept for routine use for (1-3 months). To maintain bacterial viability, re-culture was performed every two weeks to reactivate the isolates throughout the study period [20].

2.3 Preparation of ethanolic extract

An ethanolic extract of *Mentha spicata* was prepared as follows: Twenty grams of dried plant powder were weighed and mixed with 200 mL of ethanol at 45 °C for 24 hours. The mixture was then subjected to Soxhlet extraction. Afterwards, the extract was filtered using Whatman-N0-1 (England) filter paper, and the sample was poured into glass Petri dishes. The dry sample

was stored in tightly sealed glass containers at a temperature of 4 degrees Celsius in the refrigerator until use. [21, 22]

2.4 Antibacterial activity assay

The antibacterial activity of the ethanolic extract of *Mentha spicata* was evaluated against the Gram-positive bacterium *Streptococcus mutans*. The bacterial cultures were first activated, and a standardized bacterial suspension was prepared. Mueller-Hinton agar was used for the antibacterial tests. To assess the antibacterial effect, the pitting diffusion method was used. Pits 6 mm in diameter were made in agar plates, and 50 μ L of the ethanolic extract at concentrations of 25, 50, and 100 mg/mL was added to each pit. The plate was then incubated, and the diameter of the inhibition zones around each pit was measured using a ruler. Each experiment was performed three times, and the average diameter of the inhibition zone was calculated [23, 24, 25].

2.5 Statistical Analysis

Using Two-way ANOVA, the data were statistically analyzed according to the design of completely randomized blocks and the least significant difference test was used to compare means under a probability level of $P \leq 0.05$ (SPSS ver.23).

The inclusion of triplicate measurements for each treatment ensured the reliability and reproducibility of the results. The statistical evaluation confirmed the significant antibacterial and antioxidant effects of the *Mentha spicata* compared to both standard antibiotics and ascorbic acid [26, 27].

3. RESULTS AND DISCUSSION

3.1 Effect of Ethanol Extract *Mentha spicata* Against *Streptococcus mutans*

The antibacterial activity of *Mentha spicata* ethanolic extract against *Streptococcus mutans* was investigated on agar medium. The inhibitory effect increased with increasing extract concentration. Table 1 shows the diameters of the inhibition zones for each concentration. The highest activity was recorded at a concentration of 100 mg/ml.

The strong effectiveness of the extract is attributed to an increase in the concentrations of active ingredients, including phenols and glycosides. [28]. Additionally, the presence of certain fatty acids, such as palmitic acid, oleic acid, may contribute to bacterial inhibition by disrupting cell membranes [29, 30]. These results are consistent with previous studies on the inhibitory efficacy of *Mentha spicata* against oral pathogens [31, 32].

Moreover, recent confirmed that the ethanolic extracts of *Mentha spicata* can inhibit biofilm formation, a key virulence factor of *S. mutans*, which enhances bacterial adherence to the tooth surface and contributes to dental caries development [33, 34]. It is important such inhibition persists to avoid plaque development and have a healthy mouth. Moreover, the findings indicate that *Mentha spicata* extract has a multi-target mechanism of action against bacteria, since some studies have reported that it also can inhibit the bacterial quorum sensing mechanism that interferes with bacterial communication pathways required for the formation of biofilms and virulence [35].

The antibacterial activity reported in this study also corroborates the trend of the interest in plant-derived natural products as alternative/complementary antimicrobial agents in the place

of synthetic antibiotics. Herbal extracts offer several benefits, such as minimal side effects, multi-target activity and possible antioxidant properties, giving them dual protection for oral health by regulating microbial growth as well as oxidative stress. [36].

Table 2. Effect of ethanolic extract (diameter of inhibition zone, mm) of *Mentha spicata* against *Streptococcus mutans*

Type Bacteria	Solvent	Concentration ml/ mg			L.S.D =1.96
		25	50	100	
<i>Streptococcus mutans</i>	Alcoholic	16.33± 2.51	19.33 ±2.08	23.00 ±2.64	

*The results indicate a dose-dependent antibacterial activity of the ethanol extract of spearmint against *Streptococcus mutans*, with the highest inhibition at a concentration of 100 mg/ml.

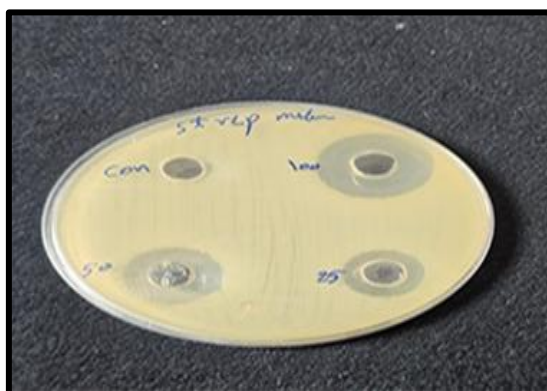


Figure 2. Shows the efficacy of ethanolic extracts of *Mentha spicata*.

3.2 Effect of Antibiotics on *Streptococcus mutans*

Then, the diameters of the inhibition zones were measured using the method of spreading the discs on the medium and the results showed that the antibiotic Ciprofloxacin had the highest antibacterial activity followed by Gentamicin and the lowest activity among the three antibiotics was that of Amikacin. These results are consistent to previously reported ones [37].

Table 3. Effect of antibiotics on *Streptococcus mutans*

Bacteria	Antibiotic			L.S.D =1.08
	AK	CN	CIP	
<i>Streptococcus mutans</i>	23.00±1.00	27.22±0.57	30.33 ± 1.15	

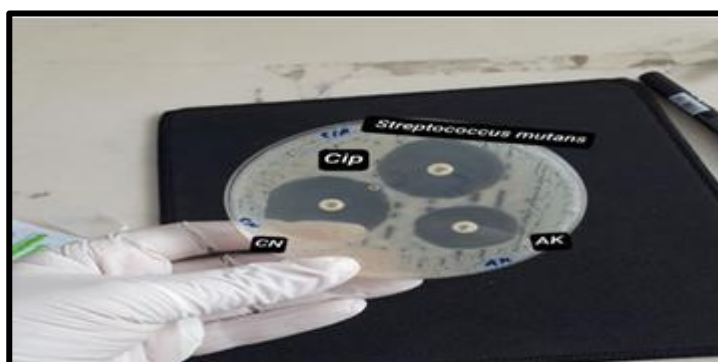


Figure 3. Shows the efficacy of antibiotics against *Streptococcus mutans*.

The antibacterial activity of Ciprofloxacin and ethanolic extract of *Mentha spicata* was compared and ciprofloxacin was slightly more active under the tested conditions. The plant extract, however, showed significant inhibitions, supporting the fact of its use as an anti-bacterial agent [38].

The ethanolic extract of *Mentha spicata* also exhibited significant inhibition and even demonstrated slightly improved antibacterial activity under the conditions tested, demonstrating the potential of *Mentha spicata* as a natural antibacterial agent [39]. This comparison is important because continued or chronic exposure to traditional antibiotics can result in the development of resistance in *S. mutans* and other oral pathogens [40]. However, plant-based extracts frequently contain several bio-active compounds with different modes of action, such as enzyme inhibition, anti-quorum sensing activity and membrane disruption, that would decrease the chances of resistance developing [41].

Gentamicin, an aminoglycoside, was moderately active, as expected. The lesser efficacy of Amikacin than Ciprofloxacin or Gentamicin may be attributed to intrinsic resistance mechanisms of *S. mutans* such as efflux pumps and diminished permeability of cell wall [42]. These data highlight the need for exploring natural alternatives, partially replacing or even substituting traditional antibiotics, in oral care. Peppermint extract, for example, has been shown to be a similar inhibitor *in vitro* and also possesses antioxidant and anti-inflammatory properties. effects, which are not present in conventional antibiotics [43, 44]. Finally, the use of plant extracts together with low concentrations of antibiotics might give synergistic effect, increasing the effect of antibiotics without increasing side effects or resistance [45]. Overall, the results indicate a potential role for *Mentha spicata* in the development of oral hygiene products such as herbal mouthwashes and toothpaste formulations which may be used to help prevent dental caries, especially in people who cannot access conventional antibiotic treatments.

3.3 Antioxidant Activity (DPPH)

One of the widely used tests used to measure the antioxidant capacity of plant extract is called DPPH test. The statistical analysis showed statistically significant differences in terms of the antioxidant effect of the extract compared to the standard antioxidant. The ethanolic extract had strong antioxidant activity with 87.84% inhibition at 100mg/mL while ascorbic acid had 82.04% inhibition at 100mg/mL. Based on these findings, it can be concluded that ethanolic extract of *Mentha spicata* has strong antioxidant activities which may be attributed to the presence of biologically active compounds like flavonoids, phenolic acids and terpenoids [46, 47].

Table 4. Effect of plant extracts in inhibiting free radicals

No	Concentration (mg/mL)	Ethanollic extract (%)	Ascorbic acid (%)
1	25	50.8	48.8
2	50	68.7	58.8
3	60	73.75	64.36
4	80	80.38	72.09
5	100	87.84	82.04

*The results confirm that *Mentha spicata* is a promising source of natural antioxidants, which can help reduce oxidative stress and may have therapeutic applications in oral health [48, 49].

CONCLUSION

The study concludes that the ethanolic extract recorded a high inhibition rate at concentrations of (25, 50, 100) mg/ml, and that increasing the effectiveness of the concentration leads to an increase in the inhibition of *Streptococcus mutans*. The crude extract was found to be more effective than isolated single compounds, likely due to a synergistic interaction among multiple bioactive constituents [50, 51].

At a concentration of 100 mg/ml, the ethanolic extract shows high inhibition, similar to the activity of ciprofloxacin, indicating its effectiveness in treating tooth decay.

Furthermore, the extract displayed potent antioxidant activity, surpassing that of ascorbic acid at the same concentration, indicating a high content of bioactive antioxidant compounds in the plant [52, 53, 54, and 55]. Overall, the findings highlight *Mentha spicata* as a promising natural alternative for oral healthcare applications, including the development of herbal mouthwash formulations and the management of oxidative stress.

Competing Interests

There are no competing interests.

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