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Effect of Menthe and Green Tea Volatile Oil Extract on Streptococcus mutans, a Bacterium Species Isolated from Dental Caries

Mohamed Abdulmunem Abdulafeet, Sura A. Jaber, Anes A. Alshamaa, Sohaib Mohameed Awad

College of Dentistry, University of Al Maarif, Anbar, Iraq

Abstract

Objectives: To identify phytochemical components, evaluate the yield or volatile oil extraction, and assess the impact of volatile oil extract on a strain of *Streptococcus mutans* that was isolated from a dental caries.

Materials and Methods: *Menthe spicata* (menthe) and *Camellia sinensis* (green tea) were harvested fresh during the flowering period of 2022, and their volatile oils were extracted for about 24 hours using a sox let apparatus. Green tea and menthe were prepared at four different concentrations (50, 75, 100, and 150 mg/ml). *Streptococcus mutans* growth isolates were inhibited, and the results were compared with extracts from both kinds of plants.

Results: Menthe produced the most volatile oil extract (3%), while green tea produced the least (2%). Alkaloid, phenol, glycoside, and flavonoid showed positive results in the phytochemical screening of *M. spicata* volatile oil extract, but saponin showed negative results. The results also showed that the volatile oil of green tea and menthol oils (100 and 150 mm/ml) were more inhibitory than those of 50 and 75 mm/ml. Overall, given these results, volatile oil of menthe has greater effect on dental caries compared to volatile oil of green tea.

Conclusions: The volatile oil extract on menthe inhibitor is more effective on the *S. mutans* bacteria compared to volatile oil of the green tea.

Keywords: Dental Caries; M. spicata; Streptococcus mutans; Volatile Oils; Green Tea.

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Email: mohamadjasem@yahoo.com

Introduction

One of the most prevalent oral conditions affecting people worldwide is dental caries. It is a chronic infectious disease caused by series microbial activities and chemical reactions linked to form a biofilm on dental plaque. Thus, minerals will be removed from the calcified tissues of teeth and cause decomposition of organic ingredients [1]. The presence of bacteria, consuming carbohydrates and producing acids by bacteria are the main causes of dental caries [2].

There are many different types of bacteria in the mouth, but only few types can cause dental caries [2,3]. One of the most well-known biofilm-forming bacteria, *Streptococcus mutans*, is a member of the oral micro flora and is thought to be the main cause of dental caries (or tooth decay) [3,4]. It is quite concerning that infectious diseases are on the rise and that harmful bacteria and fungi are rapidly developing medication resistance. The mortality and morbidity related to microbial infections remain high despite improving the knowledge about

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microbial pathophysiology and the use of different methods of treatments [5]. As a result, scientists in this sector had to come up with alternatives, including plant extracts or therapeutic herbs, as the primary sources of pharmaceuticals to treat a variety of bacterial illnesses, including gingivitis [6]. The *M. spicata* (menthe) plant is common in tropical and subtropical climates areas which consider as a source of terpene rich essential oils [7].

M. spicata are members of the Lamiaceae family and defined as the key sources of physiologically active chemicals [8]. The dried leaves are used commonly for herbal tea and may use as a medication, whereas the fresh leaves are used as a flavoring herb or raw vegetable [9]. Several biological effects of mint have been related to them, including anti-inflammatory, antioxidant, anti-cancer, and antibacterial properties [9]. Different esters are present in the volatile oil in different ratios; phenol reaches 5%, while menthol 5% and ketones 3% [9,10].

In this study, green tea (*Camellia sinensis*), which is made from a tree whose leaves grow in Southeast Asia and has been consumed by the Chinese for thousands of years, was chosen [11]. It contains phenolic compounds, also known as flavonoids, which are biologically effective compounds as antioxidants and anti-bacterial. These compounds bind free radicals, which are produced when food is burned for energy and whose presence works in cell membranes to stress the cell and make it vulnerable to DNA damage, causing the cell to become stressed [12].

Material and Methods

Plant collection

The plants *menthe* and green tea from Karbalaa city in Hindia, Iraq were collected during the flowering stage. The *M. spicata* were dried at oven degree 45 Cº and then were powdered and grinded by using mechanical grinder [13,14].

Plant Extraction

80 gram of plant was placed in thimble with 200 ml of ether 75% in flask round volume 1000 ml for 24 hours. Then, a rotary evaporation apparatus was used to evaporate the extract at 45 Cº [15,16]. The percentage is evaluated using the equation listed below [17-19].

$$\boldsymbol{Yield}\ (\boldsymbol{Wt.}\%) = \frac{\boldsymbol{Weight\ of\ oil\ produced}}{\boldsymbol{Weight\ of\ seed\ powder\ used}}\boldsymbol{x100}$$

Reagents were prepared according to previous work [20-24]. We examined the impact of various doses on the development of specific pathogenic microorganisms [22].

Statistical Analysis

The experiments were conducted and analyzed as factorial experiments with three replications using a Completely Randomized Design (CRD) with two or three factor tested by Least

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Significant difference (L.S.D) at probability of 1% ($P \leq 0.01$) [25].

Results

Percentage yield of volatile oils extracted

Results in Table 1 showed the percentage yield of volatile oils offered the height percentage of the mentha volatile oils extract (3%), and green tea has a variety of volatile oil components that are naturally produced and accumulate in plants (2%).

Table 1. Percentage yield of volatile oils extracted.

Oils extracted	Percentage yield of oils extracted
M. spicata	3/100g *100 = 3%
Green tea	2/100 g*100= 2%

Secondary metabolism screen study

Table 2 displays the findings of the secondary metabolism screen, which was conducted to identify the active chemicals extracted from *M. spicata*. The results showed positive results for alkaloids, phenols, glycosides, and flavonoids but negative results for saponin. While tests on green tea's alkaloid, phenol, glycoside, flavonoid, and saponin yielded a favorable result.

Table 2. Secondary metabolism screen of *M. spicata*.

Compounds	Menthe	Green tea
Alkaloid	+	+
Phenol	+	+
Glycoside	+	+
Flavonoid	+	+
Saponin	-	+

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Effect of menthe and green tea volatile oil extract on *S. mutans*

The goal of the study was to determine how the volatile menthol and green tea oils affected the development of the S. mutans bacteria by measuring the widths of the inhibition zones 48 hours after incubation, and then the findings were determined. The results showed that the effect of 100 and 150 mm/ml of green tea volatile oil on the dental caries were more inhibitory than those of 50 and 75 mm/ml as explained in Table 3.

Table 3. Inhibitory activity of the green tea volatile oil extract of the *S. mutans*.

Volatile oil concentration	Inhibitor zone
Control	0
50 %	18
75%	23
100%	28
150%	30
L.S.D (0.01)	1.52

The results in Table 4 listed the effect of menthe volatile oil on dental caries which showed that the inhibitory effect of treatment volatile oil (100 and 150 mm/ml) was greater than that of (50 and 75 mm/ml).

Table 4. Inhibitory activity of the menthe volatile oil extract of the *S. mutans*.

Volatile oil concentration	Inhibitor zone
Control	0
50 %	19
75%	25
100%	31
150%	32
L.S.D (0.01)	2.13

Discussion

The application of herbal treatments to treat dental caries is of potential interest [26]. The inhibition process in bacteria is directly proportional to the rise in concentration. The effect of the active extract on the bacterial species increases with the concentration of the extract [27]. The extract's potency is a result of the presence of active ingredients, which enhance its ability to stop the growth of microorganisms by blocking the function of the cell wall and its effects on biological processes [28]. For example, the extract's capacity to form hydrogen bonds with proteins results in the destruction of protein

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synthesis in bacterial cells, which stops the growth of bacteria. It also has an impact on the genetic makeup of these bacteria [28].

Previous work [29] showed that an inhibition diameter of 30 mm was caused by the green tea extract, compared to 28 mm for hot water extracts and 26 mm for cold water extracts. The inhibitory activity was due to the nature of the substances present in the plant, such as the presence of alkaloid compounds, anti-substances include phenols, tannins, flavonoids, saponins, and resins. For bacteria, tatins work to restrict the growth of bacteria by interfering with DNA and having a demonstrable impact on the bacterial cell. The cell membrane's carrier enzymes are present, and phenols are distinguished by their capacity to combine with other molecules complexes between extracellular proteins and cells [30].

The findings of current study were consistent with previous research [31], which demonstrated that green tea can be used as mouthwash to prevent cavities and is an inhibitor of *S. mutans* isolates. The outcomes concurred with those of a team of researchers [32] who discovered that green tea has the power to suppress the S. mutans bacteria that causes caries in teeth by stopping their attachment to teeth surfaces and thus reducing the production of essential membranes.

The results in this study were also agreed with a previous study [33] where they concluded that green tea extracts possess an inhibitory activity against *S. mutans*. The inhibitory activity is due to the nature of the substances contained in the plant, such as the presence of alkaloid compounds phenols, tannins, flavonoids, saponins, and resins which considered as anti-substances for bacteria. They have a clear effect in inhibiting the growth of bacteria to the bacterial cell and interfere with the DNA the carrier enzymes present in the cell membrane, while phenols are characterized by their ability to form a complex with Extracellular proteins complex with the cell wall and lead to disruption of the cell membrane of bacteria.

Conclusion

The volatile oil extract of menthe inhibitor is more effective on the *S. mutans* compared to volatile oil of the green tea. Overall, using 100 and 150 mm/ml of Volatile oil (wither menthe or green tea) are more effective in decreasing dental carries compared to lower concentrations.

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Ethical Approval

Because this research was conducted using samples unrelated to humans or animals, ethical approval was not necessary.

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Conflicts of interest

Authors have no conflicts of interest to declare.

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