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THE ANTIBACTERIAL ACTIVITIES OF *SYZYGIUM AROMATICUM* (L.) MERR. & PERRY AGAINST ORAL BACTERIA AND ITS ANTIOXIDANT AND ANTIMUTAGENIC ACTIVITIES

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Keywords:

Syzygium aromaticum, Antibacterial activity, Antioxidant activity, Antimutagenic activity

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ABSTRACT: Plants are an important source of substances which are claimed to induce biological activities. Although there are a few studies on antimicrobial and antioxidant activities of this plant, antimutagenic activity has not been studied and there is no study in Turkey. Antibacterial activities of *Syzygium aromaticum* against oral pathogens have not been reported until today. The scope of this work was to investigate the biological activities of *S. aromaticum* different extracts. The various extracts were screened for antibacterial activity. The bacteria were isolated from oral flora by traditional methods. The plant extracts were tested by Kirby-Bauer method. Other antibacterial activities tests are MIC (minimum inhibitory concentration) and MBC (minimum bactericidal concentration). In addition to, the antioxidant activities of plant extracts were screened by the stable DPPH (2, 2-diphenyl-1-picrylhydrazyl hydrate) free-radical. The antimutagenicity of the plant extracts were determined by Ames test using *Salmonella typimurium* strains. The highest antibacterial activity was determined as 20 mm inhibition zone from methanol extracts. The highest DPPH scavenging activity was found as 82% from aqueous extract. *S. aromaticum* extracts have antibacterial, antioxidant and antimutagenic potentials. Our results support the use of this plant in traditional medicine and show that some of the plant extracts possess compounds with good biological activities.

INTRODUCTION: Dental caries are one of the public health concerns for several reasons. Teeth affected with dental caries are sources of infection, which can cause an inflammation of dental pulp, periodonteum and gums. If left untreated, this disease gradually leads to teeth loss, which causes chewing difficulties and aesthetic problems [1]. It remains one of the most widespread diseases of the mankind. In developing countries, dental caries is often at epidemic proportions, especially among the poor.

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Nowadays microorganisms have become resistance to many antibiotics due to increased use of drugs, which is decreasing efficiency of conventional medicines. So, it has become necessary to find out new antimicrobial agents. Prevention of pathogenic microorganisms in dental caries are usually achieved by using chemical preservatives but they are responsible for many carcinogenic and teratogenic attributes as well as residual toxicity and with growing concern of microbial resistance towards conventional preservatives, consumers tend to be suspicious of chemical additives and thus the exploration of naturally occurring antimicrobial for mouth preservatives receives increasing attention [2]. Presently, the major problem is that we can not use chemical preservatives safely now a day due to carcino-genic nature of these chemicals [3].

Higher aromatics plants have traditionally been used in folk medicine; antimicrobial properties of these plants are well documented against bacteria, fungi and yeasts [4]. Most of the medicinal properties of these plants are directly correlated with the essential oils produced by these plants. Essential oils and extracts of these plants are able to control microorganisms related to skin diseases, dental caries and food spoilage [5].

Syzygium aromaticum (clove) is one of the most valuable spices that have been used from centuries as food preservative and for many medicinal purposes. Nowadays cloves are cultivated in several parts of the World [6]. *Syzygium* species (Fam. Myrtaceae) have been reported to possess biological activities [7]. Clove's Botanical name is *Caryophyllus aromaticus* which is derived from the Latin "clavus", which means nail due to its resemblance with the shape. The clove tree is an evergreen tropical plant, which flowers twice every year. Cloves are the unopened buds and harvested when the outer green leaves have changed from green to a yellow pink [8]. The cloves are highly antiseptic [9], antimutagenic, anti-inflammatory, antioxidant, anti-ulcerogenic, antithrombotic, anti-parasitic [10], antibacterial [11], antifungal [12], and antiviral [13]. Bud oil of clove has natural behavior and the main properties include antioxidant, insecticidal, antifungal and antibacterial properties [3]. Flower bud have many medicinal properties like antimicrobial, general stimulating, carminative and anesthetic [14, 15].

The active ingredients of plants against microorganisms are mostly some of the secondary metabolites [8]. The most important constituent of clove is the eugenol due to which it has strong characteristic aroma [16]. Several compounds from *S. aromaticum* have been found to possess growth inhibitory activity against oral pathogens, namely 5, 7-dihydroxy-2-methylchromone-8-*C*-*β*- D-gluco-pyranoside, biflorin, kaempferol, rhamnocitrin, myricetin, gallic acid, ellagic acid and oleanolic acid [17]. Recently, flavonoide triglycosides have been isolated [18]. The present study was scoped to evaluate the antibacterial, antioxidant and antimutagenic potency of *Syzygium aromaticum* plant from Turkey, therefore justifying the use of this plant in ethno-medicine for treatment of various ailments.

MATERIALS AND METHODS:

Organisms: In this study, 8 bacteria were used in experiments. Bacteria were isolated aseptically from mouth flora of different people. The identifications of bacteria were studied by traditional methods by Assoc. Prof. Dr. Gulten Okmen [19, 20, 21]. The bacterial growth were provided at Mueller- Hinton Broth (MHB; Merck).. Incubation was at 37 ºC for 24 h.

Plant Material: *Syzygium aromaticum* dried flower table was obtained from akhtars in Mugla on October 2017. The identity of plant was confirmed by Prof. Dr. Guven Gork. The specimens were stored at the Herbarium of Department of Biology, Mugla Sitki Kocman University (Voucher no: OC 1250). The identification of the plant was carried out with the Flora of Turkey [22].

Plant Extraction: The dried flower tables were washed with flowing water and once with sterile distilled water. This process was done 2-3 times. Then this material was powdered in a blender. All samples were stored at ambient temperature until initial sample preparation, after which they were stored at 4 ºC until required for analysis. The samples (30 g) were extracted with ethanol, methanol and aqueous (350 mg/mL) using by Soxhlet. These experiments were continued for 4 h. All of the extracts were evaporated and then the extracts were dissolved in their solvent and then kept in small sterile opac bottles under refrigerated conditions until used. All of the extracts concentrations were set to 350 mg/mL.

Cultivation of Microorganisms: The extracts were tested against oral pathogens. The oral pathogens were grown at 37 ºC at Mueller- Hinton Broth (Merck). Duration of incubation was 24 h.

***In-vitro* Antibacterial Activity:** Antibacterial activity studies were done with Kirby-Bauer method [23]. The extracts of plant were tested by disc diffusion assay. The concentration and quantity of extracts were taken as 350 mg/ml and 30 µL. In this study, ethanol, methanol and aqueous were used as organic solvents. The active cultures of bacteria were inoculated on Mueller-Hinton agar plates (MHA, Merck). The concentrations of cultures were adjusted to 0.5 McFarland. The experiments were performed in triplicate. The

incubation of bacteria were done at 37 ºC in 24 h. Then, the inhibition zone values were measured. Ethanol, methanol and water are negative controls. In this study, a lot of antibiotics used for positive control.

Determination of Minimum İnhibitory Concentration (MIC): The other antibacterial activity study is MIC. The broth dilution method was done as described in the CLSI standards [24, 25]. In this test, final concentrations of each extract were performed as 26000, 13000, 6500, 3250 and 1625 µg/mL.

Determination of Minimum Bactericidal Concentration (MBC): MBC was determined by using the broth dilution technique [26] by assaying the test tubes resulting from MIC determinations. A loopful of the content of each test tube was independently inoculated by streaking on a solidified nutrient agar plate incubated at 37 ºC for 24 h and then observed for bacterial growth. The lowest concentration of the subculture with no growth was considered the minimum bactericidal concentration.

Determination of Non-Enzymatic Antioxidant Activity: The stable 2,2-diphenyl-1-picrylhydrazyl hydrate radical (DPPH) was used for determination of free radical scavenging activities of the extracts. Extract (0.1 mL) was added to 3.9 mL of a 0.1 mM methanol DPPH solution. After incubation for 30 min, absorbance of extract was measured at 515 nm by spectrophotometer. Methanol is blank. The methanol with DPPH solution was used as control [27]. Trolox is reference antioxidant. The DPPH scavenging capacity expressed in percentage (%) was calculated using the formula.

Determination of Antimutagenic Activity: Antimutagenic activity tests were evaluated by the *Salmonella* - microsome method. *Salmonella typimurium* tester strains TA98 and TA100 were used in this study. The bacteria kindly provided by Dr. B. N. Ames (Berkeley, CA, USA), without (−S9) metabolization by the pre-incubation method [28]. In this study, these strains included *Salmonella typimurium* TA98 and TA100. The *Salmonella* histidine point mutation assay of Maron and Ames [28] was used to determine the antimutagenic activities of *Syzygium aromaticum* extracts without S9 mix. The percentage of inhibition was calculated according to the formula given by Ong *et al.,* [29] Sodium azide was used as positive control. Methanol is negative control.

Concurrently, a positive control (where mutagen but no extract was added) and a negative control (where no mutagen was added) were also set. The test sample was dissolved in methanol. But mutagen was dissolved in distilled water. In our study, non-toxic concentrations of the test sample used for investigating were 50000, 25000, 12500, 6250, 3125 and 1562 µg/plate.

RESULTS: The antibacterial activities of extracts of *Syzygium aromaticum* were tested against 8 microorganisms, which are known to cause diseases in teeths. MBKK1 and MBKK2 were Gram negative bacteria. The other bacteria were *Staphylococcus* and Gram positive. These bacteria are including *Serratia* sp. MBKK1 and MBKK2, *Staphylococcus* sp. MBKK3, *S. aureus* MBKK4 and MBKK5, *Staphylococcus epidermidis* MBKK6, MBKK7 and MBKK8. The table of identification not shown. Results of antibacterial activities of used plant extracts against the test pathogens are shown in **Table 1**. Besides, the inhibition zone diameters of the reference antibiotics to the test organisms are shown in **Table 2**.

TABLE 1: ANTIBACTERIAL ACTIVITIES OF *SYZYGIUM AROMATICUM* EXTRACTS AGAINST ORAL PATHOGENS (350 mg/mL)

Bacteria	Inhibition zone diameters (mm)			Solvents		
	EE	ME	AE	E	M	A
Serratia sp. MBKK1	(-)	(-)	10	-	-	-
Serratia sp. MBKK2	8	(-)	(-)	-	-	-
Staphylococcus sp. MBKK3	19	20	16	-	-	-
S. aureus MBKK4	9	11	10	-	-	-
S. aureus MBKK5	10	10	11	-	-	-
Staphylococcus epidermidis MBKK6	13	16	14	-	-	-
S. epidermidis MBKK7	12	12	10	-	-	-
S. epidermidis MBKK8	11	13	15	-	-	-

EE: Ethanol extract ME: Methanol extract AE: Aqueous extract (-): zone did not occur E: Ethanol M: Methanol A: Aqueous

The results of zones of inhibition were recorded as in mm for all the materials used as follows. Results show that *Syzygium aromaticum* extracts inhibit the growths of bacteria and the inhibition zones were between 8 to 20 mm. The highest inhibition zone was found against *Staphylococcus sp.* MBKK3.

The inhibition zone was 20 mm. Additionally, all of the extracts were determined antibacterial effects against used test bacteria **Table 1**. Reference antibiotics used as positive control. A lot of antibiotics very strongly inhibited the bacterial growths **Table 2** and **3**.

TABLE 2: REFERENCE ANTIBIOTICS PROFILES OF ORAL PATHOGENS

Bacteria	Inhibition zone diameter (mm)						
	Gentamicin (10µg)	Aztreonam (30µg)	Amikacin (30µg)	Nalidixic acid (30µg)	Penicillin (10µg)	Methicillin (5µg)	Novobiocin (30µg)
Serratia sp. MBKK2	16	20	20	22	nt	nt	nt
S. aureus MBKK4	nt	nt	nt	nt	15	14	28
S. aureus MBKK5	nt	nt	nt	nt	16	14	18
S. epidermidis MBKK6	nt	nt	nt	nt	26	13	40
S. epidermidis MBKK7	nt	nt	nt	nt	26	11	38
S. epidermidis MBKK8	nt	nt	nt	nt	47	13	36

nt: not tested

TABLE 3: REFERENCE ANTIBIOTICS PROFILES OF OTHER PATHOGENS

Antibiotics	*Serratia sp.* MBKK 1	*Staphylococcus sp.* MBKK 3
Gentamicin (10 µg)	19	nt
Aztreonam (30 µg)	-	nt
Amikacin (30 µg)	21	nt
Nalidixic acid (30 µg)	-	nt
Chloramphenicol (30 µg)	-	-
Streptomycin (10 µg)	14	-
Bacitracin (73 U/mg)	-	-
Ampicillin (10 µg)	-	-
Penicillin (10 µg)	nt	-
Methicillin (5 µg)	nt	-
Novobiocin (30 µg)	nt	-
Tetracycline (30 µg)	nt	8
Streptomycin (10 µg)	nt	-
Vancomycin (30 µg)	nt	9
Oxacillin (5 µg)	nt	9

(-): No inhibition nt: Not tested

Table 4 shows MIC values of *Syzygium aromaticum* extracts. The lowest MIC value was 1625 µg/mL for two bacteria.

TABLE 4: MINIMUM INHIBITORY CONCENTRATIONS OF *SYZYGIUM AROMATICUM* EXTRACTS (µg/mL)

Bacteria	EE	ME	AE
Serratia sp. MBKK1	(nt)	(nt)	-
Serratia sp. MBKK2	1625	(nt)	(nt)
Staphylococcus sp. MBKK3	1625	3250	-
S. aureus MBKK4	3250	3250	-
S. aureus MBKK5	3250	3250	-
S. epidermidis MBKK6	3250	3250	-
S. epidermidis MBKK7	6500	3250	-
S. epidermidis MBKK8	3250	3250	-

nt: Not tested (-): No inhibition EE: Ethanol extract ME: Methanol extract AE: Aqueous extract

Table 5 shows MBCs of *Syzygium aromaticum* extracts obtained by the broth dilution method. The lowest MBC value was 3250 µg/mL for two bacteria.

TABLE 5: MINIMUM BACTERICIDAL CONCENTRATIONS OF *SYZYGIUM AROMATICUM* EXTRACTS (µg/mL)

Bacteria	EE	ME	AE
Serratia sp. MBKK1	(nt)	(nt)	-
Serratia sp. MBKK2	3250	(nt)	(nt)
Staphylococcus sp. MBKK3	3250	6500	-
S. aureus MBKK4	6500	6500	-
S. aureus MBKK5	6500	6500	-
S. epidermidis MBKK6	6500	6500	-
S. epidermidis MBKK7	13000	6500	-
S. epidermidis MBKK8	6500	6500	-

nt: Not tested (-): No inhibition EE: Ethanol extract ME: Methanol extract AE: Aqueous extract

Table 6 shows the percent of DPPH radical scavenging capacity with trolox as reference. The aqueous extract showed 82.7% inhibition at 350 mg/mL concentration. Trolox equivalent value was 2.2 mM/g Table 6.

TABLE 6: ANTİOXİDANT ACTİVİTİES OF *SYZYGİUM AROMATİCUM* (350 mg/mL)

Plant extracts	DPPH inhibition (%)	TE
EE	59	1.7
ME	68.2	1.9
AE	82.7	2.2

TE: Trolox equivalent (mM/g DW); DW: Dry weight EE: Ethanol extract ME: Methanol extract AE: Aqueous extract

In this study, these concentrations were categorized as non-toxic because they showed a well-developed

lawn, almost similar size of colonies and no statistical difference in the number of spontaneous revertants in test and control plates. The antimutagenic activities of the extracts were evaluated by the against NaN_3 (sodium azide) by Ames test in absence of rat microsomal liver enzyme (-S9). **Table 7** and **8** shows the percent of inhibition. The methanol extracts of *Syzygium aromaticum* (6250 µg/plate) was found to have its lowest antimutagenic activity for *Salmonella typhimurium* TA98. This inhibition value is 15% **Table 7**. *S. aromaticum* extracts (6250 µg/plate) detected a moderate positive effect (27 %) for *S. typhimurium* TA100 **Table 8**.

TABLE 7: ANTIMUTAGENIC ACTIVITY OF *SYZYGIUM AROMATICUM* EXTRACTS (6250 µg/plak)

***Salmonella typhimurium* TA98**						
Test substances	**Ethanol extract**		**Methanol extract**		**Aqueous extract**	
	Revertant	**% Inhibition**	**Revertant**	**% Inhibition**	**Revertant**	**% Inhibition**
Control	56		56		56	
Negative control	53		49		55	
Positive control	60		60		60	
Sa	50	17	51	15	73	mutagenic

Sa: *Syzygium aromaticum*

TABLE 8: ANTIMUTAGENIC ACTIVITY OF *SYZYGIUM AROMATICUM* EXTRACTS (6250 µg/plak)

***Salmonella typhimurium* TA100**						
Test substances	**Ethanol extract**		**Methanol extract**		**Aqueous extract**	
	Revertant	**% Inhibition**	**Revertant**	**% Inhibition**	**Revertant**	**% Inhibition**
Control	119		119		119	
Negative control	104		112		107	
Positive control	135		135		135	
Sa	98	27	106	21	134	1

Sa: *Syzygium aromaticum*

DISCUSSION: Herbal medicines have been shown to have genuine utility and about 80% of rural population depends on its primary health care. Bioactive compounds are playing an important role for the treatment of different diseases. As a results of findings, the *Syzygium aromaticum* flower tables contain bioactive compounds that explain the importance of *S. aromaticum* as medicinal plant. Results show that the *S. aromaticum* extracts inhibit bacterial growths. The highest inhibition zone was found against MBKK and the inhibition zone was 20 mm **Table 1**. Soni and Dahiya [30] reported that antimicrobial activities of *Syzygium caryophyllatum* essential oil was found between 7 to 22 mm inhibition zone. High levels of eugenol present in *S. caryophyllatum* essential oil is responsible for strong antimicrobial activity. This phenolic compound can denature proteins and reacts with cell membrane phospholipids changing their permeability [31, 32, 33].

In this work, antibacterial activities of *S. aromaticum* extracts were found against Gram positive bacteria **Table 1**. All of these bacteria are *Staphylococcus.* Abdelkader and Halawani [34] reported that *Staphylococcus aureus* ATCC 25923 were affected strongly from *S. aromaticum* extracts. A previous study in Turkey [35] showed that the chemical composition of *S. aromaticum* oil had about 87% eugenol, 8% eugenyl acetate and 3.6% *β*-caryophyllene. The modes of action by which microorganisms are inhibited by essential oil and their chemical compounds seem to involve different mechanisms. It has been hypothesized that the inhibition involves phenolic compounds, because these compounds sensitize the microbial cytoplasmic membrane causing increased permeability, unavailability of vital intracellular ingredients [36] and / or impairment of bacterial enzymes systems [37]. Previous studies also showed that clove had strong antibacterial activity against Gram positive bacteria [38, 39, 40, 41]. Our results are in agreement with those reported by these studies.

In our study, extracts were affected two Gram negative bacteria **Table 1**. The antimicrobial activity of *Syzygium caryophyllatum* oil showed strong antibacterial activity against all bacterial isolates tested with maximum activity against *Pseudomonas aeruginosa. Klebsiella pneumoniae, Serratia marcescens, Salmonella typhi, Shigella dysentriae* and *Vibrio cholerae* were found resistant

[13]. The antibacterial activity of *S. caryophyllatum* is attributed to eugenol. High tannin content (10 - 19%) in *S. caryophyllatum* also provides additional antimicrobial activity [42]. The antibacterial activity of flavonoids can be explained by the toxicity of this compound towards non- specific interactions in showed susceptibility, such as the establishment of hydrogen bonds with the cell walls proteins or enzymes, the chelation of metal ions, inhibition of bacterial metabolism, sequestration of substances necessary for the growth of bacteria.

Also, the *β*-ring of flavonoids is important in the intercalation with nucleic acids, thus inhibits DNA and RNA synthesis. It can also inhibit the DNA gyrase of *Escherichia coli* [43, 44]. Previous studies also found that clove had strong antibacterial activity against Gram positive bacteria [13, 38, 39, 40, 41, 45, 46]. Our results are in agreement with those reported by these studies.

In this study, two bacteria were showed the lowest sensitivity to extracts of *Syzygium aromaticum* (1625 µg/mL). Therefore MBC value was 3250 µg/mL **Table 4**. Abdelkader and Halawani [34] reported that ethanolic extracts of *S. aromaticum* exhibited maximum activities against *S. aureus* ATCC 25923 with MIC = 62.5 µg/mL while MBC value was 125 µg/mL. Barakat [38] reported that MIC value for *S. aureus* was 1500 µg/mL. Dua *et al*., [41] reported that MIC values for *S. aureus* and *E. coli* were 0.98 and 3.90 mg/mL, respectively. Whereas Karunamoonthy *et al*., [47] were studied by *S. benthamianum*, and MIC values were found 250 and 500 µg/mL for *E. coli* and *S. aureus*, respectively. Results of our study are similar with this results.

In these study, the extracts of *S. aromaticum* have different free radical inhibition. The aqueous extract of flower tables showed 82.7% inhibition at 350 mg/mL concentration **Table 6**. Previous studies also showed that clove had strong antioxidant activity, and a high level of phenolics [48, 49]. Our results are in agreement with those reported by these studies. These DPPH scavenging activity differences might be caused by geographic origins, climatic and seasonal conditions, the time of collection, the stage of development, the method of extraction and even might be correlated to the existence of new chemotypes [13].

In our study, the extracts of *S. aromaticum* (6250 µg/plate) were found to have its low antimutagenic activity for *Salmonella typhimurium* TA98. This inhibition value is 17% **Table 6**. Whereas, ethanol extract of plant were shown moderate effect for *S. typhimurium* TA100 **Table 7**. In determining the antimutagenic potential of a sample, a value smaller than 20% inhibition of the mutagen activity indicates a weak or non-antimutagenic effect, a moderate effect when the value is between 20 and 40% and strong antimutagenicity when the value is greater than 40% [50]. Karunamoonthy *et al*., [47] reported anticancer activity for *S. benthamianum.* This also supports our results.

CONCLUSION: In the present study, extracts of *Syzygium aromaticum* inhibited the bacterial growths, but their effectiveness varied. Ethanolic, methanolic and water extracts of *S. aromaticum* showed considerable antibacterial properties against the tested organisms. The results obtained in this report clearly demonstrate that greater part of tested extracts exhibited strong antioxidant activities, particularly, to scavenge free radicals generated from DPPH reagent, especially *S. aromaticum* aqueous extract. The aqueous extract of *S. aromaticum*, should be useful as an antioxidant protection system. Furthermore the extracts of *S. aromaticum* have weak anti-mutagenic activity for *Salmonella typhimurium* TA98. Whereas, the extracts of *S. aromaticum* have moderate antimutagenic activity for *S. typhimurium* TA100.

It may be suggested from the present findings that *S. aromaticum* extracts can be used as a potential source of natural antimicrobial compound possessing strong antioxidant potential. However, further research is needed for the identification of biologically active compounds present and *in vivo* studies using animal model. In subsequent researches, fractionation and characterization of the active components should be do further works to investigate.

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CONFLICT OF INTEREST: The authors declare no conflict of interest.

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