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Chemical Composition and Antibacterial Activity of the Essential Oil and the Gum of *Pistacia lentiscus* Var. *chia*

CHRISTINA KOUTSOUDAKI,† MARTIN KRSEK,‡ AND ALISON RODGER*,†

Department of Chemistry and Department of Biological Sciences, University of Warwick, Warwick, Coventry CV4 7AL, United Kingdom

The essential oil and gum of *Pistacia lentiscus* var. *chia*, commonly known as the mastic tree, are natural antimicrobial agents that have found extensive uses in medicine in recent years. In this work, the chemical composition of mastic oil and gum was studied by GC−MS, and the majority of their components was identified. α-Pinene, β-myrcene, β-pinene, limonene, and β-caryophyllene were found to be the major components. The antibacterial activity of 12 components of mastic oil and the oil itself was evaluated using the disk diffusion method. Furthermore, attempts were made to separate the essential oil into different fractions in order to have a better picture of the components responsible for its antibacterial activity. Several trace components that appear to contribute significantly to the antibacterial activity of mastic oil have been identified: verbenone, α-terpineol, and linalool. The sensitivity to these compounds was different for different bacteria tested (*Escherichia coli*, *Staphylococcus aureus*, and *Bacillus subtilis*), which suggests that the antibacterial efficacy of mastic oil is due to a number of its components working synergistically. The establishment of a correlation between the antibacterial activity of mastic oil and its components was the main purpose of this research. Mastic gum was also examined, but it proved to be more difficult to handle compared to the essential oil.

KEYWORDS: Mastic oil; antibacterial activity; GC−MS; disk diffusion

INTRODUCTION

Mastic is a white, semitransparent, natural resin that is obtained as a trunk exudate from mastic trees. The mastic tree is an evergreen bush that thrives in the Eastern Mediterranean area, but only in the southern part of the island of Chios, a Greek island, does the plant produce resin that congeals. Its scientific name is *Pistacia lentiscus*, of the Anacardiaceae family. Mastic gum has numerous qualities and uses and is now exported to many countries. In medicine, a lot of research has been undertaken on the properties of mastic gum. For example, mastic gum has been used in clinical trials on patients with peptic ulcers (*1*). The administration of mastic (1 g daily) relieved the pain and healed the stomach and duodenal ulceration in the majority of the patients within 2 weeks. The same group of researchers (*2*) confirmed that mastic gum kills *Helicobacter pylori*, at concentrations as low as 0.06 mg/mL. In an earlier study (*3*), the effect of mastic has been studied on experimentally induced gastric and duodenal ulcers in rats. Mastic at an oral dose of 500 mg/kg produced a significant reduction of gastric secretions, protected cells, and reduced the intensity of gastric mucosal damage. The in vitro antimicrobial activity of *P. lentiscus* extracts has also been tested on bacteria and fungi (*4*). Of the different plant extractions (decoctions, infusions, macerations, and extracts from petroleum ether and from ethanol), decoctions showed the best antibacterial activity. It has also been found that chewing mastic gum prevents plaque formation or reduces it when it has already been formed on those teeth surfaces that can be reached by the mass of mastiche during its methodical chewing (*5*).

In surgery, byproducts of mastic gum are used for the production of special stitches that are eventually absorbed by the human body. In dentistry, mastic acts as an oral antiseptic and tightens the gums (*5*), and for that reason it is used in toothpastes and chewing gums. The essential oil of mastic gum is also used in perfumery and in the cosmetic industry (creams and other facial products) (*6*). Moreover, there are culinary uses of mastic, for example, in biscuits, ice cream, and mastic "sweets of the spoon".

The chemical composition of the mastic oil and mastic gum has recently been studied (*7*, *8*), but as yet no correlation between the antibacterial activity and the composition of mastic has been reported for the variety *chia*. The purpose of this study was to examine the chemical composition of this oil and gum and to examine the antibacterial activity of 12 individual components of mastic oil against three test organisms.

MATERIALS AND METHODS

Mastic oil (100% pure) and mastic gum, both of the harvest of 2002, were kindly provided by Chios Gum Mastic Growers Association

* Corrrsponding author. Telephone: +44(0)-24-76574696/76523234. Fax: +44(0)-24-76575795. E-mail: a.rodger@warwick.ac.uk.
† Department of Chemistry.
‡ Department of Biological Sciences.

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(Chios, Greece) and VIORYL S. A. (Athens, Greece). Five oil samples were used in the analysis: mastic oil as received, three successive fractions from distillation (see below), and the fraction of the oil that remained in the flask after the distillation. The oil was diluted in ethanol (50% v/v) in order to reduce the concentration of the pure oil and identify even its trace components. The distillate fractions (see below) were analyzed as collected. Mastic gum was ground with the help of a pestle and mortar and was then partially dissolved in ethanol (30 mg/mL), and the undissolved part of the gum was removed by filtration and disgarded. The ethanol soluble part of the gum was analyzed by GC−MS and GC−FID and was also tested for its antibacterial activity. Standards were also analyzed and tested for their antibacterial activity: α-pinene, β-myrcene, *p*-cymene, β-caryophyllene, verbenone, α-terpineol, methyl isoeugenol, limonene, β-pinene, linalool, γ-terpinene, and *trans*-anethole. All of the above components were of the highest purity available (above 97%) and were obtained from Sigma-Aldrich Chemical Co. (Dorset, UK), with the exception of α-terpineol and *trans*-anethole, which were obtained from Fisher Scientific (Leicestershire, UK). A 1% v/v solution in ethanol was prepared for each standard in order to be analyzed by GC−MS and GC−FID.

Distillation of Mastic Oil. A microdistillation setup was used to separate the mastic oil (2 mL) into fractions of differing volatilities. The sample was heated by an oil bath and the pressure in the distillation system was reduced to 20 mmHg. The first fraction was collected without any heating (maximum temperature 20 °C). When the distillation stopped, the mastic oil that remained in the flask was gradually heated to 21 °C and the distillate collected (fraction 2) until distillation ceased. No further distillate was collected, despite increasing the temperature of the oil bath to 140 °C. When the apparatus was removed from the oil bath, it was observed that the remaining essential oil had become very viscous and its color had changed from very pale yellow to very intense yellow. The flask was rinsed twice with ethanol. The first rinse became fraction 3 and the second fraction 4.

Chemical Composition of Mastic Oil and Gum. The GC−MS analysis of the samples was undertaken using a Shimadzu GC-17A, QP-5000 GC−MS system, operating in electron ionization (EI) mode with an ionization energy of 70 eV. The instrument was equipped with a Supelco SPB TM-1 capillary column (30 m, 0.25 mm i.d., 0.25 μm film thickness) with helium as carrier gas at 0.7 mL/min flow rate. Column temperature was initially kept for 1 min at 60 °C, gradually increased to 180 °C at a rate of 3.5 °C/min, and finally increased to 280 °C at a rate of 20 °C/min and kept there for 2 min. The injector and interface were set at 220 and 250 °C, respectively. The gas chromatograph operated in the split mode with a split ratio of 93:1. The mass spectrum was monitored starting at *m*/*z* 60 and ending at *m*/*z* 350, with a scan interval of 0.5 and a threshold of 400, and the solvent cut was set at 4 min. The injection volume was 1 μL. The injected solutions were (i) solution of mastic oil in ethanol (50% v/v), (ii) ethanol solutions of each standard (1% v/v), (iii) the ethanol-soluble part of mastic gum (30 mg/mL original sample before filtration), and (iv) the collected fractions from the distillation (see Distillation of Mastic Oil).

The chemical composition of mastic oil and gum was also analyzed using GC−FID, since the flame ionization detector is known to have higher sensitivity and the signal magnitude is to a reasonable approximation proportional to the analyte concentration, independent of its identity. The samples were prepared as for the GC−MS analysis. The GC−FID analysis of the samples was undertaken with a Shimadzu GC-17A, system, equipped with a capillary column SGE-BPX5 (30 m, 0.32 mm i.d., 0.5 μm film thickness). The carrier gas used was helium, the makeup gas was nitrogen, while hydrogen and air were used as ignition gases for the detector. The data system used was Shimadzu Class VP Chromatography Software. The method used was similar to that of GC−MS. Column temperature was initially kept for 1 min at 60 °C, gradually increased to 180 °C at a rate of 3.5 °C/min, and finally increased to 280 °C at a rate of 20 °C/min and kept there for 2 min. The injector and detector were set at 280 and 340 °C, respectively. The flow rate of the carrier gas in the column was kept constant at 1.0 mL/min, and the gas chromatograph operated in the split mode with a split ratio of 100:1. One microliter of the samples was injected manually, as for the GC−MS analysis.

Antibacterial Activity of Mastic Oil, Its Components, and Its Fractions. Three representative bacteria were selected for this study: *Escherichia coli* (Gram-negative rod), *Staphylococcus aureus* (Gram-positive cocci), and *Bacillus subtilis* (Gram-positive rod). The 12 individual components of mastic oil tested were α-pinene, β-myrcene, *p*-cymene, β-caryophyllene, verbenone, α-terpineol, methyl isoeugenol, limonene, β-pinene, linalool, γ-terpinene, and *trans*-anethole. All of the above components were of the highest purity available (above 97%) and were obtained from Sigma-Aldrich Chemical Co. (Dorset, UK), with the exception of α-terpineol and *trans*-anethole, which were obtained from Fisher Scientific (Fisher Scientific, Leicestershire, UK).

The disk diffusion susceptibility method (*11*−*13*) was used in order to examine the sensitivity of the bacteria of interest toward mastic oil, its components, and its collected fractions. Essential oils and many of their components have limited solubility in aqueous media, and this property was expected to cause difficulty in susceptibility test methods. To overcome this difficulty, a modified disk diffusion method was also tried. In preliminary experiments to examine the effect of solubilizing agent on the diffusion of components through the agar, two sets of media were prepared: one without the presence of detergent and one with the presence of Tween 80 detergent. Mueller−Hinton (MH) Agar was used, prepared according to the instructions of the manufacturer (OXOID Ltd., Basingstoke, Hampshire, UK).

All agar plates were prepared in 90-mm Petri dishes with 20 mL of agar, giving a final depth of 4 mm. Overnight broth cultures were prepared in Bacto heart infusion broth which was prepared according to the instructions of the manufacturer (Becton, Dickinson and Co., Sparks, MD), appropriately adjusted in PBS (phosphate-buffered saline) or in Saline 0.9% in order to yield approximately 1.0×10^6 cfu/mL (colony forming units/mL). Whatman paper disks (Whatman International Ltd, Maidstone, UK) of 6-mm diameter were placed on the inoculated agar surfaces and were impregnated with 20 μL of each chemical to be tested.

Standard antibiotics were used in order to provide a control for the sensitivity of the test organisms in the experiments. For each bacterium, two antibiotics (*15*, *16*) were chosen as controls: for *E. coli*, gentamicin and tetracycline, and for both *S. aureus* and *B. subtilis*, gentamicin and vancomycin. Standard graphs for each antibiotic were prepared by testing paper disks containing varying amounts of the antibiotic against a standard organism. The concentrations of each antibiotic used for that purpose were 500, 200, 100, 50, and 10 μg/mL (in 18.2 MΩ water). Each test was performed in duplicate and plates with and without Tween 80 were used for *E. coli*. Standard graphs for each antibiotic and each bacterium were prepared by plotting the logarithm of the concentration of the antibiotic versus the mean zone of inhibition, and a very good linearity was observed. The same procedure was followed for mastic oil, mastic gum, the mastic oil distillation fractions, selected standards (see below), and ethanol. Each sample (20 μL of the liquids and 20 μL of a 30 mg/mL solution of the gum extracts) was applied to the paper disks, and they were tested on plates containing MH agar (without Tween 80).

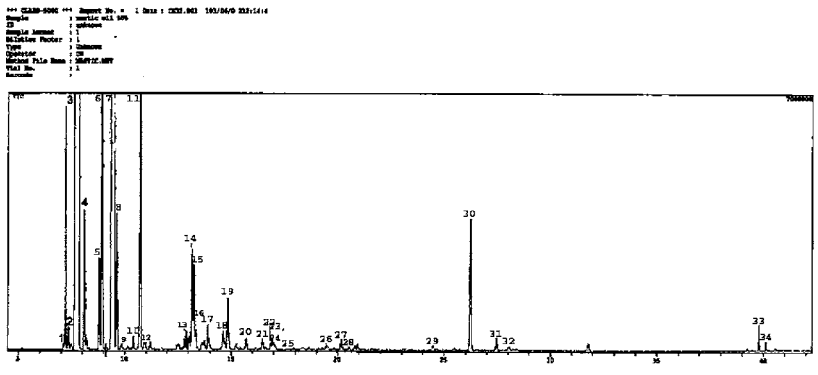
RESULTS AND DISCUSSION

Chemical Composition of Mastic Oil and Gum. The oil, gum, and distillation fractions were analyzed by GC−MS using the method described above. **Table 1** contains the identified peaks of fractions 1−4, along with their percentages, in comparison with those of mastic oil and of the ethanol-soluble components of mastic gum. GC−MS analysis of the oil and the gum led to the identification of the majority of the components, which are listed in Table 1 along with their semiquantitative data. A typical GC−MS chromatogram of mastic oil, obtained with the analytical method described above, is illustrated in **Figure 1**. The GC−FID analysis of mastic oil and gum showed no significant difference compared to that obtained by GC−MS. The identification of the components was based on comparison of their mass spectra with those of NIST12.LIB and NIST62.LIB libraries, as well as on comparison of their retention indices (*17*) and of the standard components analyzed (see Materials and Methods).

Table 1. Chemical Composition of Mastic Oil and Mastic Gum As Determined by GC–MS and GC–FID Analysis and Comparison of Their Chemical Composition with the Collected Fractions, Assuming That the Chromatogram Peaks Areas Are Proportional to the Concentration of Analytes

			percentage (%)[a]					
compound	RI[b]	identification methods[c]	mastic gum	mastic oil	fraction 1	fraction 2	fraction 3	fraction 4
octyl formate	923	MS	–[d]	tr[e]	tr	tr	tr	–
tricyclene	926	MS	–	0.1	–	–	–	–
α–pinene	939	GC–MS	40.9	63.3	66.3	52.6	2.4	3.1
camphene	953	MS	1.0	0.6	1.2	1.4	tr	–
sabinene	976	MS	0.3	0.4	0.8	1.5	0.1	0.2
β-pinene	980	GC–MS	1.7	3.3	5.4	8.8	0.6	1.2
β-myrcene	991	GC–MS	9.0	25.0	13.1	21.6	7.8	17.0
methyl-*o*-cresol	1009	MS	0.3	0.6	1.0	1.8	0.7	1.4
p-cymene	1026	GC–MS	–	0.1	0.1	0.2	0.1	0.3
limonene	1031	GC–MS	0.8	1.5	1.7	3.2	1.8	3.8
(*Z*)-*β*-ocimene	1040	MS	–	tr	tr	0.1	–	–
(*E*)-*β*-ocimene	1050	MS	–	tr	0.1	0.1	tr	0.2
α-terpinolene	1087	MS	–	tr	–	–	–	–
α-pinene epoxide	1095	MS	–	–	1.4	1.4	4.2	7.7
linalool	1098	GC–MS	0.8	0.5	0.4	0.3	2.7	3.2
perillene	1099	MS	0.8	0.5	0.8	0.8	2.6	4.3
cis-verbenol	1100	MS	–	0.1	0.4	0.4	3.5	5.2
α-campholene aldehyde	1126	MS	0.3	0.1	0.1	0.2	0.6	0.9
trans-pinocarveol	1139	MS	–	0.1	0.2	0.2	2.3	2.3
trans-verbenol	1143	MS	0.5	0.3	0.8	0.8	9.6	10.4
β-pinene epoxide	1156	MS	–	–	tr	tr	0.5	0.5
myrtenal	1193	MS	0.5	0.1	0.2	0.2	1.7	1.9
α-terpineol	1180	GC–MS	–	tr	–	–	–	–
myrtenol	1188	MS	–	tr	–	–	–	–
verbenone	1189	GC–MS	0.8	0.1	0.4	0.4	6.5	6.1
trans-carveol	1217	MS	–	tr	tr	tr	0.3	0.1
dihydrocarveol	1226	MS	–	–	–	–	0.2	0.2
neral	1240	MS	–	0.1	–	–	–	–
linalyl acetate	1257	MS	–	tr	tr	tr	0.5	0.2
(*E*)-anethole	1283	GC–MS	tr	0.1	tr	tr	0.5	0.4
α-fenchyl acetate	1290	MS	–	tr	–	–	0.2	0.1
neryl acetate	1365	MS	–	–	0.2	0.2	1.3	1.7
α-copaene	1381	MS	–	tr	–	–	0.2	0.1
β-caryophyllene	1419	GC–MS	5.3	0.9	0.1	0.1	2.5	2.1
α-humulene	1454	MS	0.1	0.1	–	tr	0.4	0.2
(*E*)-methylisoeugenol	1500	GC–MS	0.9	tr	–	–	0.4	0.1
(*Z*,*Z*)-farnesol	1713	MS	11.9	0.1	–	–	6.3	3.2
(*E*,*Z*)-farnesol	1742	MS	0.1	tr	–	–	1.8	1.6

[a] Percentages obtained by GC–FID. [b] Retention index relative to *n*-alkanes on SGE-BPX-5 capillary column (similar type to DB-5 capillary column). [c] Methods: GC, identification based on retention times of standard compounds on SGE-BPX-5 capillary column; MS, tentatively identified based on computer matching of the mass spectra of peaks with NIST12.LIB and NIST62.LIB libraries and published data. [d] An en-dash denotes that the percentage was below the limit of detection. [e] Less than 0.1%.



1. octyl formate
2. tricyclene
3. α – pinene
4. camphene
5. sabinene
6. β – pinene
7. β – myrcene
8. methyl o – cresol
9. (Z)-β-ocimene
10. p-cymene
11. limonene
12. (E)-β-ocimene
13. α-terpinolene
14. linalool
15. perillene
16. cis-verbenol
17. α – campholene aldehyde
18. trans – pinocarveol
19. trans-verbenol
20. neral
21. myrtenal
22. α-terpineol
23. myrtenol
24. verbenone
25. trans – carveol
26. linalyl acetate
27. (E)-anethole
28. α – fenchyl acetate
29. α – copaene
30. β – caryophyllene
31. α – humulene
32. (E)-methyl iso – eugenol
33. (Z,Z)-farnesol
34. (E,Z)-farnesol

Figure 1. GC–MS chromatogram of mastic oil obtained with the method described previously.

The major constituents of the essential oil of *P. lentiscus* var. *chia* were α-pinene (63%), *β*-pinene (3.3%), *β*-myrcene (25%), limonene (1.5%), and *β*-caryophyllene (1%), assuming that TIC (total ion current) as integrated over the peak in the GC−MS chromatogram is proportional to their concentration in the sample. Other constituents therefore account for 6.2% of the total concentration. For the gum, the major ethanol-soluble constituents were the same, but the relative percentages differed from those found in the oil: α-pinene (40%), *β*-pinene (1.5%), *β*-myrcene (9%), limonene (1.0%), and *β*-caryophyllene (5%). This difference is presumably due to the different ways the gum and the oil are produced.

As was intended, the distillation process separated the more volatile components from the less volatile ones. Fraction 1 has a similar chromatogram to that of mastic oil up to where verbenone (**Figure 1**) is eluted, although some peaks are more

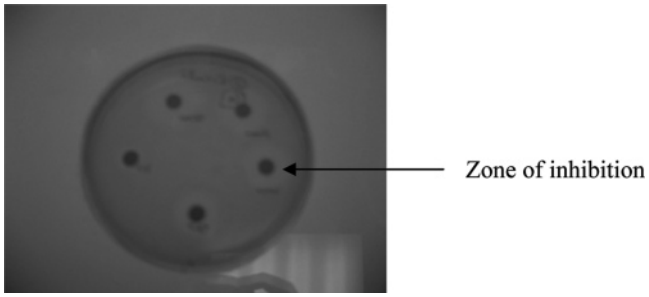


Figure 2. Zones of inhibition of *E. coli* against gentamicin on medium without Tween 80.

enhanced in the first fraction. The later eluting peaks of mastic oil either do not appear in the chromatogram of the fraction or they are very small. Fraction 2 is very similar to fraction 1, but some peaks are enhanced (e.g. β-myrcene, β-caryophyllene) and there is less of the major compound α-pinene. There is a clear enhancement of the later eluting peaks in fraction 3 (which was the first washing of the undistilled fraction) compared to mastic oil and fractions 1 and 2, which is consistent with the aims of distillation procedure. Fraction 4 is very similar to Fraction 3.

Antibacterial Activity of Mastic Oil, Its Components, and Its Fractions. *E. coli* and *S. aureus* were inoculated in agar plates with and without Tween 80, and several interesting observations were made: for *E. coli*, the growth of bacteria was quite similar on MH agar with and without the detergent, which indicated that the latter did not play a significant role. On the other hand, the addition of Tween 80 to the medium inhibited significantly the growth of *S. aureus* for reasons that were not clear. According to Carson et al. (*14*), the reduction in zone size observed when the detergent is added to the MH agar may be due to the Tween 80 allowing better distribution of components through the agar, resulting in a lower overall concentration. Alternatively, the Tween 80 may have enhanced the growth of the test organisms, as it is a source of oleic acid, or it could act as an antagonist to the oil components. Therefore, it was decided that plates containing the detergent would be used mainly in the disk diffusion tests with *E. coli*. As already mentioned, for that bacterium, there was essentially no difference between the detergent and nondetergent plates, suggesting that the solubility of the analytes was not the key issue in the different effectiveness of the analytes.

Figure 2 shows the Zones of Inhibition (ZoI) of growth of *E. coli* against gentamicin on medium without the detergent around the paper disks impregnated with different concentrations of the antibiotic.

The results of the antibacterial assays for *E. coli*, *S. aureus*, and *B. subtilis* are reported in **Table 2**, along with the corresponding amount of antibiotic (gentamicin, vancomycin, and tetracycline) that would give the same zone of inhibition as the chemical. The calculation of the corresponding amount of antibiotic is based on the respective equation of graph when plotting the logarithm of the concentration of the antibiotic versus the mean zone of inhibition.

As seen in **Table 2**, all three bacteria are resistant to α-pinene, which is the most abundant compound of mastic oil (65%), and this is in agreement with literature reference (*21*). A variation in the antibacterial activity of the other tested chemicals against the three bacteria is noticed; *E. coli* is resistant to β-myrcene, while *S. aureus* shows an intermediate response to that chemical and *B. subtilis* is sensitive to its presence. It should be noted that β-myrcene is the compound with the second highest percentage (25%) in the composition of mastic oil. *p*-Cymene, β-caryophyllene, methyl isoeugenol, limonene, γ-terpinene, and *trans*-anethole show only moderate antibacterial activity, and in some cases the bacteria are resistant to them. Furthermore, *E. coli* and *S. aureus* are resistant to β-pinene, while it inhibits only slightly the growth of *B. subtilis*. Verbenone (0.07%), α-terpineol (0.01%), and linalool (0.5%) are some of the trace components of mastic oil, but they show higher antibacterial activity than all other components, which is comparable to that of mastic oil itself.

A number of the fractions collected by microdistillation were also tested for antibacterial properties. Fractions 1 and 3 were chosen, since they were the ones that differed the most from a chemical point of view, and they were tested using the same procedure against the three bacteria. As observed in **Table 3**, fraction 3, which shows an enhancement of the later eluting peaks, has a stronger antibacterial activity than fraction 1, which, on the other hand, shows an enhancement of the earlier eluting peaks. It is, however, interesting that neither of them have the activity that mastic oil shows, although the antibacterial activity of fraction 3 is closer to that of the essential oil compared to the activity of fraction 1. This implies that the compounds that

Table 2. Comparison of Zones of Inhibition of the Three Bacteria against Each Compound and a Corresponding Amount of Antibiotic

	E. coli			*S. aure us*			*B. subtilis*		
compounds	ZoI (mm)	corresponding gentamicin (μg/mL)	corresponding tetracyclin (μg/mL)	ZoI (mm)	corresponding gentamicin (μg/mL)	corresponding vancomycin (μg/mL)	ZoI (mm)	corresponding gentamicin (μg/mL)	corresponding vancomycin (μg/mL)
α-pinene	0	–	–	3.5	–	–	0	–	–
β-myrcene	0	–	–	10	22	84	19.5	97	~4300[a]
p-cymene	0	–	–	3.5	–	–	9	6	34
β-caryophyllene	7	7	6	10.5	27	125	13.5	19	270
verbenone	12	40	42	21.5	~2000[a]	~820000[a]	17	49	~1360[a]
α-terpineol	17	218	292	13	72	9201	15.5	33	680
methylisoeugenol	8	10	9	7	7	8	10.25	8	60
limonene	7	7	6	0	–	–	9.5	7	42
β-pinene	0	–	–	0	–	–	6.75	–	12
linalool	12.5	47	51	14.5	130	~3050[a]	26	556	~87000[a]
γ-terpinene	7	7	6	8	10	17	12	13	135
trans-anethole	7	7	6	8	10	17	10	8	54
mastic oil	12	40	42	18.5	619	~75000[a]	17	49	~1360[a]
mastic gum in EtOH	0	–	–	0	–	–	0	–	–
ethanol	0	–	–	0	–	–	0	–	–

[a] The number is too high and it is off scale of the graph for the antibiotic calibration graph, therefore, is not reliable.

Table 3. Zones of Inhibition of the Three Bacteria against the Fractions of Mastic Oil and against the Essential Oil

	ZoI (mm) of bacteria		
fractions of mastic oil	*E. coli*	*S. aureus*	*B. subtilis*
mastic oil	12	18.5	17
fraction 1	0	7	7
fraction 3	6.5	10	11

contribute the most in the antibacterial activity of mastic oil are the ones that appear in the middle of its chromatogram (e.g. verbenone, linalool, α-terpineol) and are clearly enhanced in fraction 3. This conclusion is in agreement with the observations made from **Table 2** and **Figure 1**. However, we cannot overlook the fact that many of the components of mastic oil contribute to its antibacterial activity.

Reasons for the effectiveness of mastic oil against the bacterium *H. pylori* are of particular interest, given the difficulties encountered in treating such infections. As *H. pylori* and *E. coli* are both Gram-negative rods, the observations made for mastic oil and *E. coli* may be relevant for *H. pylori*.

In conclusion, the antibacterial activity of mastic oil can be attributed to the combination of several components rather than to one particular compound. It is also interesting to note that different bacteria are susceptible or not to different compounds of the essential oil. So it can be assumed that the antibacterial activity of mastic oil against the bacteria tested is due to its cocktail of components, including some of the trace elements, not all of which have been identified in this study.

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