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Study of Chios Mastic essential oil: Isolation, chemistry, and wound healing activity

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Abstract

Chios mastic gum (CMG) derived from *Pistacia lentiscus L. var. chia* family *Anacardiacea*e has shown beneficial effects on a wide range of human disorders beside other traditional uses. In this study the essential oil of CMG was isolated by hydrodistillation from *Pistacia lentiscus* L. var. Chia followed by the identification of its chemical composition revealing the dominancy of α-pinene (29.2%) and Caryophyllene (9.7 %). The oil acute wound healing activity was tested in vivo on excision wound model using albino rats and showed promising results since wounds were healed by almost 97% by the end of the 20 days compared to about 79% displayed by the untreated group.

Keywords: *Pistacia lentiscus L. var*. chia; *Anacardiaceae*; wound healing; GC-MS; essential oil

1. Introduction

Mastic gum (MG) is a white semitransparent oleogum obtained as a trunk exudate from the mastic tree *Pistacia lentiscus* L. (Anacardiaceae). A variety of mastic called Chios mastic gum (CMG) is obtained from *Pistacia lentiscus* L. var. chia trees cultivated extensively in a Greek island called Chios (Browicz 1987). Since ancient times CMG has been used in Middle Eastern countries as a flavoring additive in foods and drinks (Al-Habbal et al 1984). CMG is medically used to relieve many ailments including dyspepsia and for treatment of some GIT diseases including peptic ulcer (Al-Said et al. 1986).

Mastic essential oil contains unique therapeutic, aromatic, and flavoring ingredients. The oil is used as basic ingredient in some health care products and in food and beverages industry (Xynos et al. 2018). In 2017, Moudi, et al reported that mastic oleoresin (MO) was effective in reducing episiotomy pain and promoting wound healing. In 2015, The European Medicines Agency has recognized CMG as a herbal medicinal product for the symptomatic treatment of minor inflammations of the skin and as an aid in healing of minor wounds (Papada and Kaliora 2019). This directed us to separate and evaluate the mastic essential oil's activity compared to the oleoresin.

The dominance of a specific constituent over any other constituents in essential oils from the gum of *P. lentiscus* var. chia was reported by several authors based on literature survey from 1990 to 2020. It was observed that α-pinene (78.6%), β-pinene (3.3%), myrcene (3.2%) were the main components (samples collected from Spain), while, β-pinene (38.7%), α-pinene (21.7%), pinocarvone (5.3%), limonene (3.8%), n-nonanal (3.5%) were the main components of samples collected from Turkey, and α-pinene (67.5%), β-pinene (2.8%), verbenone (2.6%), trans-pinocarveol (2.5%), p-mentha-1,5-dien-8-ol (2.4%), myrcene (1.1%) were the main component of samples collected from Greece (Tabanca, et al. 2020)

This difference of the chemical composition of the same plant may be attributed to many factors that specifically affect the composition of their essential oils including geographical origin, soil type and climate zones such as humidity, temperature, and light. The composition of mastic gum oil is also influenced by the extraction methods and the storage duration (Paraschos et al. 2016).

Skin is one of the most readily accessible organs on human body representing the main route for topical drug delivery system. Gel is one of the topical formulations, which penetrates the skin, get absorbed by blood and transported to the site of action (Dhalkar et al. 2019).

The process of acute wound-healing consists of four phases: hemostasis, inflammation, proliferation, and tissue remodeling or resolution. For a wound to heal properly, the four phases must occur in the proper sequence and time limit (Guo and Dipietro 2010).

In 2021, it was demonstrated that topical treatment with mastic significantly ameliorate inflammatory and pruritic responses in a mouse model of allergic dermatitis. (Kishimoto et al. 2021)

2. Material and methods:

2.1. Experimental process:

Commercial mastic gum was supplied by the Chios Mastic Growers Association in Chios, Greece, which is the exclusive worldwide producer of the resin.

A quantity of mastic gum (40 g) was purchased from K. Monomachou, Chios, Greece in March 2020 submitted to hydrodistillation for 24 h, using a Clevenger-type apparatus. The essential oil was collected over anhydrous sodium sulfate then stored in the refrigerator. The produced essential oil constituted 2.5% of the dry resin weight. The oil was analyzed by Gas chromatography–mass spectrometry (GC-MS).

The chemical composition of mastic essential oil performed using Trace GC1310-ISQ mass spectrometer (Thermo Scientific, Austin, TX, USA) at the Regional Center for Mycology and Biotechnology (RCMB) at Al- Azhar University with a direct capillary column TG–5MS (30 m x 0.25 mm x 0.25 µm film thickness). The column oven temperature was initially held at 35 C and then increased by 3°C /min to 200°C hold for 3 min. Increased to the final temperature 280°C by 3°C /min and held for 10 min. The injector and MS transfer line temperatures were kept at 250, 260°C respectively; Helium was used as a carrier gas at a constant flow rate of 1 ml/min. The solvent delay was 3 min and diluted samples of 1 µl were injected automatically using Autosampler AS1300 coupled with GC in the split mode. EI mass spectra were collected at 70 eV ionization voltages over the range of m/z 40–1000 in full scan mode. The ion source temperature was set at 200 °C.

The components were identified by comparison of their retention times and mass spectra with those of WILEY 09 and NIST 11 mass spectral database.

2.2 Wound healing test:

Principle:

Acute wound healing activity is tested in vivo on excision wound model using albino rats. Healing potential is identified through measuring wound contraction and wound closure time (Yusufoglu, 2015).

Preparation of Topical Formulations:

Mastic essential oil was formulated as 1% gel according to a method by Khan et al 2013. Carbapol 934 (0.5g) was mixed with about 100 gm of distilled water and kept in an oven at 100°C for 20 min to obtain a homogenous viscous mixture, then cooled to room temperature with continuous stirring. Triethanolamine 10 ml was added dropwise with continuous stirring using mechanical stirrer. One gram of the oil was added and mixed using glass

rod. Other ingredients dimethyl sulfoxide (DMSO) 10ml, polyethylene glycol (PEG-200) 1.5g, polyethylene glycol (PEG-400) (1.5g) were added with constant stirring to prepare 100 g gel.

Procedure:

Two groups (5 animals each) of albino rats were used in the experiment. The dorsal skin was trimmed with an electric clipper. After 24 hours, all animals were anesthetized with diethyl ether and the shaved areas were sterilized with 70% alcoholic solution. A predetermined dorsal area (approximately 2.5 cm^2) was excised using toothed forceps, scalpel and pointed scissors. A fresh surgical cutting edge was used for the perpendicular cut in each animal and during the operation the tension of skin was kept constant.

Wound of the control group was treated with the base gel and the other group was treated with 1% gel preparation of the essential oil.

Wounds were fully covered topically with gels on the wound surface once a day for 20 days. The wound size was determined with the help of Vernier caliper immediately after the wound excision and at every 5 days until healing was accomplished. The reduction in the wound size was calculated according to the following formula:

$$\text{Wound contraction (\%)} = \frac{(\text{DWi} - \text{DWt})}{\text{DWi}} \times 100$$

Where:

- DWi = the wound area immediately after wound excision,
- DWt = the wound area on day t.

Small portions of healing area were cut and kept in formalin solution for photomicrograph study following (Yusufoglu, 2015).

3-Results and discussion:

3.1. Chemistry of Chios Mastic Gum Essential Oils:

GC-MS of the studied MCG oil resulted in the identification of 70 compounds. The identified compounds belong mainly to monoterpene hydrocarbons (14.3%), oxygenated monoterpenes (38.6%), sesquiterpene hydrocarbons (17.1%), oxygenated sesquiterpenes (14.3%) and others (15.7%). These compounds are dominated by α-pinene (29.2%), caryophyllene (9.7%), myrcene (6.2 %), m-camphorene (6.15%), and linalool (5.15%) representing (56.4%) of the identified compounds. The results of GC-MS analysis are shown in table 1.

These results are in accordance with those reported by Pachi et al. 2020 where a-pinene and myrcene were the major components but different percentages.

Table (1): Chemical composition of Chios mastic essential oil:

	compound name	Retention Time	Relative concentration.	Molecular Weights
1	5,5-Dimethyl-1-vinylbicyclo[2.1.1]hexane	10.35-10.91	0.45	136
2	α -Pinene	11.27-11.89/ 13.2	29.2	136
3	Camphene	12.06	0.83	136
4	Bicyclo[3.1.0]hex-2-ene, 4-methylene-1-(1-methylethyl)-	12.19	0.55	134
5	β-Pinene	12.95	0.5	136
6	α-Myrcene	13.8-14.07	6.2	136

7	Benzene, 1-methoxy-2-methyl-	14.22	1.83	122
8	2,6-Dimethyl-1,3,5,7-octatetraene, E,E-	14.38	0.18	134
9	2,4-Dimethyl-3-cyclohexene-1-carbaldehyde	14.8	0.12	138
10	p-Cymene	14.95-15.1	0.71	134
11	D-Limonene	15.39-15.58	2.07	136
12	Acetic acid, [4-(1-hydroxy-1-methylethyl) cyclohex-1-enyl]methyl ester	17.17	0.13	212
13	α-Campholenal	17.74/19.42	1.68	152
14	Benzene, 4-ethenyl-1,2-dimethyl	17.94	0.11	132
15	2-Carene	18.21	0.28	136
16	Linalool	18.63-18.88	5.15	154
17	Camphor	19.97	0.1	152
18	Pinocarveol	20.28	1.59	152
19	cis-Verbenol	20.67	1.79	152
20	Pinocarvone	20.88	0.28	150
22	p-Mentha-1,5-dien-8-ol	21.51	1.82	152
23	Terpinen-4-ol	22.07	0.14	154
24	p-Cymen-7-ol	22.24	0.46	150
25	Bicyclo[3.1.1]hept-2-ene-2-carboxaldehyde, 6,6-dimethyl-	22.33	0.99	150
26	L-α-Terpineol	22.73	0.52	154
27	(-)-Myrtenol	23	2.31	152
28	5,5-Dimethyl-4-[3-methyl-1,3-butadienyl]-1-O xaspiro[2.5]octane	23.12	0.11	206
29	cis-p-mentha-1(7),8-dien-2-ol	23.18	0.11	152
30	trans-Carveol	23.86	0.54	152
31	bicyclo[3.1.1]heptane,7,2,6,6-trimethyl-3-(2-propenyl)	24.05	0.17	178
32	Acorenone B	24.34	0.14	220
33	D-Carvone	24.47	0.1	150

34	2-Pyridinecarbonitrile, 3-ethyl-1,2,5,6-tetrahydro-1,2-dimethyl-	24.64	0.25	164
35	Benzene, 1,4-dimethoxy-2-methyl	24.95	0.65	152
36	Anethole	25.13\26.6	1.62	148
37	trans-Pinocarvyl acetate	25.32	0.14	194
38	3,5-Dimethoxytoluene	25.61	0.33	152
39	Linalyl acetate	25.87	0.33	196
40	Bornyl acetate	26.92	0.29	196
41	2-Undecanone	27.37	1.1	170
42	α-Terpinyl acetate	29.73	0.29	196
43	Widdrol	29.88	0.16	222
44	Copaene	30.29/31.36	0.41	204
45	Ylangene	31.17	0.34	204
46	Methyleugenol	31.56	0.55	178
47	(-)-α-Bourbonene	31.64	0.1	204
48	Caryophyllene	32.54-33.27	9.7	204
49	Methyl isoeugenol	33.65\35.36	3.63	178
50	Geranyl acetone	33.91	0.31	194
51	Humulene	34.42	1.74	204
52	Aromandendrene	34.65	0.16	204
53	α-muurolene	36.24	0.12	204
54	Cubebol	36.69	0.14	222
55	1-Isopropyl-4,7-dimethyl-1,2,3,5,6,8a-hexahydronaphthalene	37.1	0.48	204
56	Nerolidol	38.62	0.45	222
57	Caryophyllene oxide	39.1	2.18	220
58	2-Methyl-4-(2,6,6-trimethylcyc lohex-1-enyl)but-2-en-1-ol	39.38	0.1	208

59	Humulene epoxide	39.93	0.32	220
60	Cubenol	40.82	0.15	222
61	11,11-Dimethyl-4,8-dimethyle nebicyclo[7.2.0]undecan-3-ol	40.96	0.29	220
62	Isoaromadendrene epoxide	41.77	0.51	220
63	(1R,7S,E)-7-Isopropyl-4,10-di 8 methylenecyclodec-5-enol	42.27	0.6	220
64	Benzyl Benzoate	44.79	0.48	212
65	p-Camphorene	48.62-49.5\51.2\53.41	2.89	272
66	m-Camphorene	50.58/52.46/53.93	6.15	272
67	Hexadecanoic acid	52.73	0.18	256
68	Octadecanoic acid	59.14	0.17	284
69	Glycidyl palmitate	63.16\69,49	0.69	312
70	Glycidol oleate	68.75	0.13	338

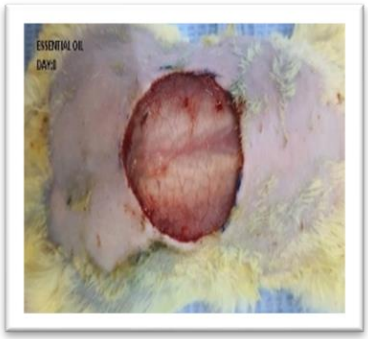
3.2. Wound healing test:

The observed percentage excised wound contraction with base control gel and essential oil gel were 40 ±1.4, and 71.79 ±1.2 on 5th day and 78.67 ±2.3, and 96.8 ±2.4 by the end of the 20 days as shown in Table 2. and Fig 1(while photomicrographs of skin sections were shown in (Fig 2 and 3)

Table 2: percent Reduction in wound size of excision wounds treated with base control and Chios Mastic essential oil formula

	Day			
fraction	5	10	15	20
Control	40 ±1.4	52 ±1.8	71.67 ±1.3	78.67 ±2.3
Essential oil	71.79 ±1.2	84.13 ±0.8	93.3 ±1.6	96.80 ±2.4

n = 5, mean ± SEM.



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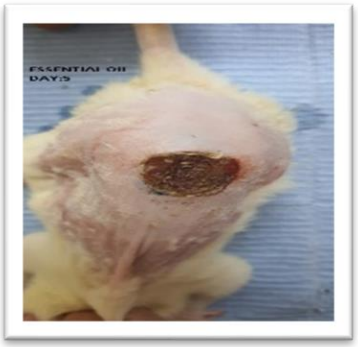

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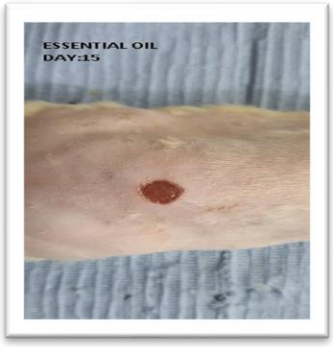

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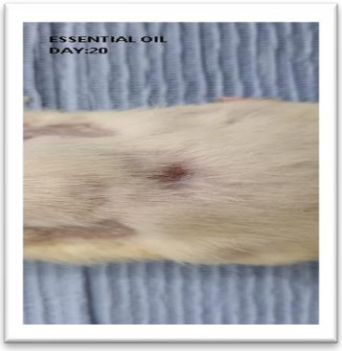

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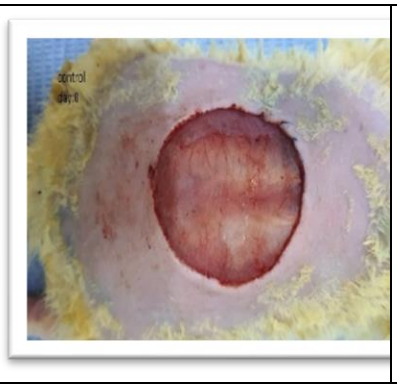

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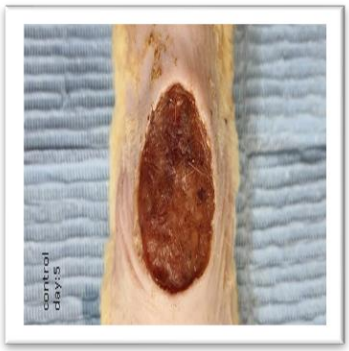

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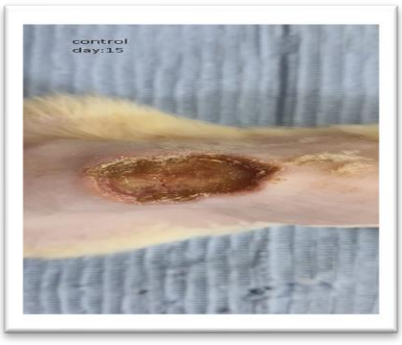

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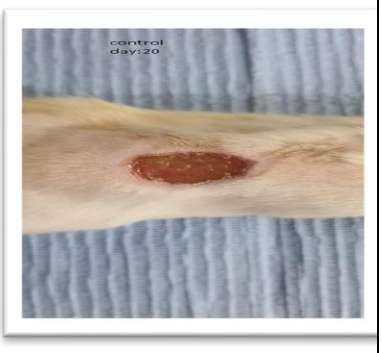

C20

(Fig. 1). Excision wound model of rats (0, 5, 10, 15 and 20 days) showing various phases of wound healing gel; E: Essential oil of Chios mastic gum gel C: control group based gel.

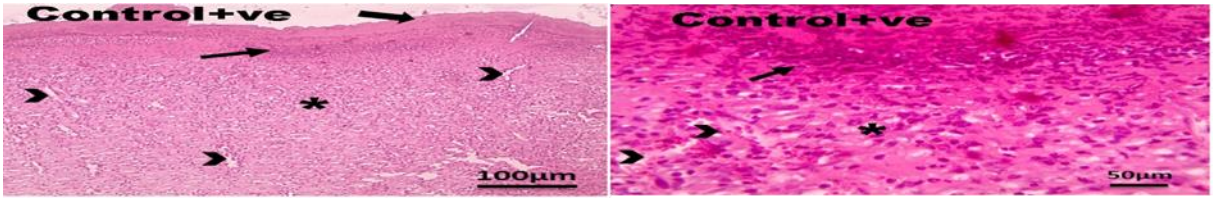

Fig 2: Photomicrograph of HE-stained skin sections of rats showing various histopathological changes on control +ve wounded group; showing wound area covered by dried necrotic scab (thick black arrows) underlined by inflamed hyalinized tissue (mainly neutrophils) (thin black arrows) followed by granulation tissue (*) containing many hyperemic capillaries (arrowheads) and many mononuclear cells infiltration. (Low magnification ×100 bar 100 and high magnification x400 bar 50µm)

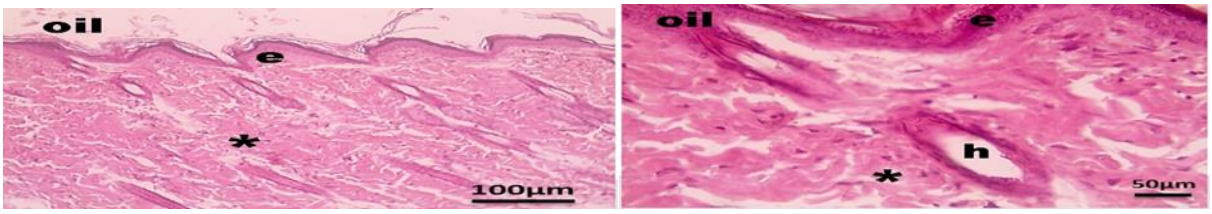

Fig 3: Photomicrograph of HE-stained skin sections of rats showing various histopathological changes on Skin sections from oil wounded and treated group showing normal epidermis (e) underlined by connective tissue (*) with regular deposition of fully mature collagen containing hair follicles (h). (Low magnification ×100 bar 100 and high magnification x400 bar 50 µm)

Microbes attack the wound when expose to the external environment. If a wound becomes infected, the acute phase of inflammation becomes pronounced leading to further production of tissues oxidants which damage cellular membranes, DNA, proteins, lipid, and extracellular matrix (Olugbuyiro et al.2010)

The process of wound healing involves the dynamic and complex restoration of the normal condition of cellular structures in wounded tissue. During the healing process, a mechanism known as wound contraction begin from the fibroblastic stage. The wound collapses during the last stage of wound healing, leaving a little tissue scar behind. The site of injury may be protected from microbial invasion by the free radicals produced there. (Shivhare et al.2010)

Treatment of the excision wounds with the mastic essential oil gel formulation gave good signs of the wound healing potency. It was observed that the wound contracting ability of the formulated gel were significantly greater than that of the control. The tested gel of Chios mastic essential oil showed a significant wound healing from the 5th days and onwards which was comparatively higher compared to the control drug.

Conclusion

Herbal medicine has been a fertile source for modern drug discovery. Following this approach, Chios Mastic Gum (CMG), was selected following a thorough literature review to evaluate its therapeutic effects. Previous studies reported its efficacy as wound healing agent. We decided to perform in-vivo acute wound healing model experiment on rats to evaluate the potential activity of the essential oil and to determine the active component of CMG. The results showed that essential oil is the active component of CMG for wound healing. Since it is much easier to incorporate the oil in many dosage forms and formulas compared to the resin itself or its total extract, we recommend using the oil rather than CMG for treating acute wounds.

Chemical profiling of the oil after the GC-MS experiment may help to pinpoint the active constituents responsible for the wound healing activity and/or the mechanism of action.

Further studies are required to investigate the correct mechanism.

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