Unique Mastic Resin from Chios

Chadzopulu A.,[1*] Koukouliata A.,[2] Theodosopoulou E.,[3] Adraniotis J.[1]

1 General Hospital of Kavala, Greece
2 Labour Inspectorate of Kavala, Greece
3 University of Athens, Faculty of Nursing, Greece

ABSTRACT

Authors presented analysis of the literature on medical use *Pistacia lentiscus var. Chia.* Mastic tree thrives and gives mastic only in the south part of the island of Chios (Greece) and nowhere else in the Word. Mastic oil has been found to be effective in both preventing and treating various cancers. It heals also pectic ulcers by killing *Hellicobacter pylori.* Natural mastic gum has also been proven to absorb cholesterol thus diminishing chances of heart attacks and high blood pressure, and helps reduce triglyceride and total lipid levels of the organism.

Key words: *Pistacia lentiscus var. Chia,* medicine, cancer, *Hellicobacter pylori*

***Corresponding author:**
General Hospital of Kavala
Agios Silas 65-500 Kavala
Greece
E-mail: antygonah@interia.pl (Chadzopulu Antigoni)

Received: 17.06.2011
Accepted: 24.06.2011
Progress in Health Sciences
Vol. 1(1) · 2011 · pp 131- 136.

The Mastic tree (*Pistacia lentiscus* var. *Chia*) is a dioecious evergreen shrub or small tree belonging to the Anacardiaceae family [1]. It is cultivated for its aromatic resin on the Greek island of Chios, in the Aegean Sea. Mastic tree thrives and gives mastic only in the south part of the island of Chios and nowhere else in the Word. Fossils of mastic tree leaves found on the island reveal its existence on the island six thousand years ago suggesting that its origin comes from this island [1].

Herodotos, on the 5th century BC, mentions that the resin product from the phloem of the mastic tree was used for chewing [1]. Hippocrates suggests the mastic resin as a cure and Diomides mentions its therapeutic usage in his work. The Greek physician and botanist Dioscorides wrote about the medicinal properties of mastic in his classic treatise *De Materia Medica* [1].

Mastic resin has been used as a chewing gum and as a medicine for gastrointestinal ailments for several thousand years [1]. Mastic is known to have been popular in Roman times when children chewed it. In Medieval times it was highly prized for the Sultan's harem both as a breath refreshener and for cosmetics The spice's use was widened when Chios became part of the Ottoman Empire [1].

Nowadays, about 2.000.000 mastic trees are cultivated in the south part of the island [1]. Mastic is collected in raw form and it is concentrated, standardized, packed and traded by the Chios' Gum Mastic Growers Association having 4,850 members.

The Association was created in 1938 to help commercialize the product and ensure the income of the growers. The Association ensures the quality of the product by utilizing modern technology in process and packing, and by constantly increasing the production volumes encouraging new cultivations [1].

Pistacia lentiscus var. *Chia* can grow as irregular shrub or a single to multistemmed tree, up to 3-4 m tall [1]. The flowers grow in dense panicles or spikes and the anthers are dark red for male flowers and grenish for female. The leaves are dark green, with 6-18 leaflets, leathery pinnate and distinctive for the lack of an end leaflet Blooming time is in mid May to July.

The chemical analysis of mastic resin: essential oil (mastic oil) 1 - 3%; a- and b-masticinic acid 4%; masticholic acid 0.5%; - a-mastichonic acid 20%; b mastichonic acid 18%; a-mastic resin 30%; b-mastic resin 20%. The mastic oil contains dozens of identified chemical compounds [1].

The aim of this paper was analysis of literature on medical use *Pistacia lentiscus.*

Arabino-Galactan Proteins (AGPs) were isolated from Chios mastic gum (CMG) by using a buffer containing 0.1 M NaCl, 20 mM Tris-HCl, pH 7.5 [2]. Protein analytical methods, combined with specific procedures for carbohydrate characterization, indicated the presence of highly glycosylated protein backbone. In particular, staining by Yariv reagent of the electrophoretically separated molecules revealed the existence of arabinose and galactose and such a modification is characteristic for AGPs. After experiments involving extensive dialysis of the isolated extracts against water and atomic absorption, there was evidence of the existence of zinc ions that are probably covalently bound to the AGPs [2]. By using anion-exchange chromatography, capillary electrophoresis, colorimetric methods and GC-MS, it was found that the extracts were separated into three major populations (A, B, and C), which were consistent with their respective negative charge content namely, uronic acid. The characterization of neutral sugars that was investigated with GC-MS showed the existence of arabinose and galactose in different amounts for each group. Experiments concerning the inhibition of growth of Helicobacter pylori in the presence of AGPs, as is shown for other CMG constituents, showed that the extracts of at least 1.4 g CMG affected the viability of the bacterium. There is no evidence as to whether the AGPs provoke abnormal morphologies of *Heliocobacter pylori*, as is reported for the total CMG, or for O-glycans that possess terminal alpha1, 4-linked N-acetylglucosamine and are expressed in the human gastric mucosa; this has to be further investigated [2].

The group ingesting Chios mastic powder (high-dose group) exhibited a decrease in serum total cholesterol, LDL, total cholesterol/HDL ratio, lipoprotein (a), apolipo-protein A-1, apolipoprotein B (apoB/apoA-1 ratio did not change), SGOT, SGPT and gamma-GT levels; in the second (low-dose) group, glucose levels decreased in males [3].

Kim and Neophytou [4] investigated the anti-inflammatory properties of mastic (*Pistacia lentiscus var. Chia*) to help reduce intestinal inflammation in inflammatory bowel disease patients. Mastic and mastic resin were obtained from the Chios Mastiha Growers Association (www. mastihashop. com). The resin was ground into a fine powder using a pestle and mortar and formulated in factorial design manner. Evaluation of the efficacy of specific anti-inflammatory/ antioxidant compounds in mitigating the clinical colitis parameters in a mouse model of colitis were performed with mastic itself and combination of tocopherol compounds. Colonic drug delivery system was developed consisting of two compartment model and its release profile was also investigated [4].

For a long time, mastic has been esteemed for its aphrodisiac properties [5]. To test this hypothesis, the trace element zinc was determined while the quantity released after a certain time of chewing was studied. For comparison, three commercial chewing-gums were analyzed as well. A portion of natural mastic or commercial gum was uniformly chewed for 1, 2, 3, and 4 h and the zinc content measured. The zinc content of mastic from *Pistacia lentiscus var. Chia* was compared to that of other natural resins from the same genus (*Pistacia terebinthus L.*) or conifer [*Pinus halepensis Mill. (Pinaceae*)], having a different secretion mechanism and also used as an additive in human nutrition [5]. Secreted resin and plant tissues from the above trees were sampled and the zinc content was determined. Zinc concentrations in the resin were lower than in the plant tissues. The Chios mastic showed a slightly greater zinc content compared to the other analyzed specimens. Among all gums studied, only the Chios mastic released a small amount of about 0.7 mg kg(-1) zinc in the mouth and gastrointestinal system after 4 h chewing time. With commercial gums, the zinc content increased to a large degree (up to 2 mg kg(-1)) after the same treatment, a fact which was attributed to the zinc uptake from salivary secretions, indicating zinc deprivation for the human organism [5].

Gum of Chios mastic (*Pistacia lentiscus var. Chia*) is a natural antimicrobial agent that has found extensive use in pharmaceutical products and as a nutritional supplement [6]. The molecular mechanisms of its anti-inflammatory activity, however, are not clear. In this work, the potential role of antioxidant activity of Chios mastic gum has been evaluated. Spin trapping study did not show significant scavenging of superoxide by mastic gum itself. However, mastic gum inhibited cellular production of superoxide and $_{H2O2}$ in dose dependent manner in TNF-alpha treated rat aortic smooth muscle cells but did not affect unstimulated cells. TNF-alpha significantly increased the cellular superoxide production by NADPH oxidase, while mastic gum completely abolished this stimulation. Mastic gum inhibited the activity of purified PKC, decreased PKC activity in cell homogenate, and attenuated superoxide production in cells stimulated with PKC activator PMA and PKC-dependent angiotensin II in endothelial cells [6].

In a search [7] for more effective and safe anti-diabetic compounds, developed a pharma-cophore model based on partial agonists of PPARγ The model was used for the virtual screening of the Chinese Natural Product Database, a library of plant-derived natural products primarily used in folk medicine. From the resulting hits, we selected methyl oleanonate, a compound found, among others, in *Pistacia lentiscus var. Chia* oleoresin (Chios mastic gum). The acid of methyl oleanonate, oleanonic acid, was identified as a PPARγ agonist through bioassay-guided chromatographic fractionations of Chios mastic gum fractions, whereas some other sub-fractions exhibited also biological activity towards PPARγ. The results from the present work are two-fold: on the one hand we demonstrate that the pharmacophore model we developed is able to select novel ligand scaffolds that act as PPARγ agonists; while at the same time it manifests that natural products are highly relevant for use in virtual screening-based drug discovery [7].

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 [8]. In this work, the chemical composition of mastic oil and gum was studied by GC-MS, and the majority of their components was identified. alpha-Pinene, beta-myrcene, beta-pinene, limonene, and beta-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 [8]. Several trace components that appear to contribute significantly to the antibacterial activity of mastic oil have been identified: verbenone, alpha-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 [8].

The chemical composition of the three essential oils obtained by steam distillation of the mastic gum, leaves and twigs of *Pistacia lentiscus var. Chia*, was studied by GC-MS [9]. Sixty nine constituents were identified from the oils. alpha-Pinene, myrcene, trans-caryophyllene and germacrene D were found to be the major components. The in vitro antimicrobial activity of the three essential oils and of the resin (total, acid and neutral fraction) against six bacteria and three fungi is reported [9].

The antibacterial, antifungal, and antiviral properties of 15 lipohylic extracts obtained from different parts (leaf, branch, stem,

kernel, shell skins, seeds) of *Pistacia vera* were screened against both standard and the isolated strains of *Escherichia coli*, *Pseudomonas aeruginosa*, *Enterococcus faecalis*, *Staphylococcus aureus*, *Candida albicans* and *C. parapsilosis* by microdilution method [10]. Both *Herpes simplex* (DNA) and *Parainfluenza* viruses (RNA) were used for the determination of antiviral activity of the *P. vera* extracts by using Vero cell line. Ampicilline, ofloxocine, ketoconazole, fluconazole, acyclovir and oseltamivir were used as the control agents. The extracts showed little antibacterial activity between the range of 128–256 μg/ml concentrations whereas they had noticeable antifungal activity at the same concentrations [10].

Entire Helicobacter Pylori Neutrophil Activated Protein (HPNAP) and its truncated forms NH(2)-terminal region HPNAP(1-57) and C-terminal region HPNAP(58-144) after cloning into pET29c vector, purification and removal of LPS traces were subjected to human neutrophil activation [11]. They found that the C-terminal region of HPNAP is indispensable for human neutrophil stimulation and their further adhesion to endothelial cells - a step necessary to *Helicobacter pylori* inflammation - in a ratio equal to that exhibited by the entire protein. In addition, experiments concerning the implication of Arabino-Galactan-Proteins (AGPs) derived from Chios Mastic Gum, the natural resin of the plant *Pistacia lentiscus var. Chia* revealed the inhibition of neutrophil activation and therefore their adhesion to endothelial cells, in vitro. Both, the involvement of HPNAP C-terminal region in stimulation-adhesion of neutrophils to endothelial cells as well as the inhibition of this process by AGPs have to be further investigated and may be exploited in a future anti-inflammatory therapy for *Helicobacter pylori* patients [11].

Helicobacter pylori infection is among the most common human infections and the major risk factor for peptic ulcer disease and gastric cancer [12]. They presented the implication of C-terminal region of *Helicobacter pylori* neutrophil activating protein in the stimulation of neutrophil activation as well as the evidence that the C-terminal region of *Helicobacter pylori* activating protein is indispensable for neutrophil adhesion to endothelial cells, a step necessary to H. pylori inflammation.

Herbal remedies are increasingly popular for the treatment of functional dyspepsia [13]. Chios mastic gum is a resinous exudate from the stem of *Pistacia lentiscus var. Chia.* It is a traditional natural remedy used throughout the eastern Mediterranean. Dabos et al. [13] assessed the efficacy of Chios mastic gum in patients with functional dyspepsia. The symptom score after treatment was significantly lower in the Chios mastic gum than in the placebo group (14.78±1.78) vs (19.96±1.83)) ($p<0.05$). There was a marked improvement of symptoms in 40% of patients receiving placebo and in 77% of patients receiving Chios mastic gum ($p<0.02$). Individual symptoms that showed significant improvement with Chios mastic gum were: stomach pain in general, stomach pain when anxious, dull ache in the upper abdomen and heartburn (<0.05 for all four symptoms). Chios mastic gum significantly improves symptoms in patients with functional dyspepsia compared to placebo [13].

Doi et al. [14] investigated the modifying effects of Chios Mastic Gum on rat liver carcinogenesis, 6-week-old male F344 rats were subjected to the established rat liver medium-term carcinogenesis bioassay (Ito-test). At the commencement, rats (groups 1-4) were intraperitoneally injected with 200 mg/kg body weight of diethylnitrosamine (DEN). After two weeks, mastic was added to CRF (Charles River Formula)-1 powdered basal diet at doses of 0, 0.01, 0.1 and 1% in groups 1-4, respectively. At week 3, all rats were underwent two-thirds partial hepatectomy. The experiment was terminated at week 8. As results show, liver weights were significantly increased in a mastic dose-dependent manner among groups 1-4 [14]. The numbers (/cm(2)) and the areas (mm(2)/cm(2)) of glutathione S-transferase placental form (GST-P)-positive cell foci (> or=0.2 mm in diameter) were significantly increased in the DEN-1% group compared to the DEN-alone group, along with the average areas per foci and larger-sized foci (> or =0.4 mm). 5-Bromo-2'-deoxyuridine (BrdU)+GST-P double-immunohistochemistry showed the highest BrdU-labeling indices within GST-P foci in the DEN-1% group. 8-hydroxydeoxyguanosine (8-OHdG) levels in liver DNA did not vary, while real-time quantitative polymerase chain reaction (PCR) analysis of livers revealed many up- or down-regulated genes in the DEN-1% group. In conclusion, this is the first report to display a promotion potential of Chios Mastic Gum on the formation of preneoplastic lesions in the established rat liver medium-term carcinogenesis bioassay [14].

Mastic oil from *Pistacia lentiscus var. Chia* has been extensively studied for its antimicrobial activity attributed to the combination of its bioactive components [15]. One of them, perillyl alcohol displays tumor chemopreventive, chemotherapeutic, and antiangiogenic properties. It was investigated whether mastic oil would also suppress tumor cell growth and angiogenesis. It was observed that mastic oil concentration and time dependently exerted an antiproliferative and proapoptotic effect on K562 human leukemia cells

and inhibited the release of vascular endothelial growth factor (VEGF) from K562 and B16 mouse melanoma cells. Moreover, mastic oil caused a concentration-dependent inhibition of endothelial cell (EC) proliferation without affecting cell survival and a significant decrease of microvessel formation both in vitro and in vivo. Investigation of underlying mechanism(s) demonstrated that mastic oil reduced 1) in K562 cells the activation of extracellular signal-regulated kinases 1/2 (Erk1/2) known to control leukemia cell proliferation, survival, and VEGF secretion and 2) in EC the activation of RhoA, an essential regulator of neovessel organization. The authors concluded mastic oil may be a useful natural dietary supplement for cancer prevention [15].

It was demonstrated that a 50% ethanol extract of the plant-derived product, Chios mastic gum (CMG), contains compounds which inhibit proliferation and induce death of HCT116 human colon cancer cells in vitro [16]. CMG-treatment induces cell arrest at G(1), detachment of the cells from the substrate, activation of pro-caspases-8, -9 and -3, and causes several morphological changes typical of apoptosis in cell organelles. These events, furthermore, are time- and dose-dependent, but p53- and p21-independent. Apoptosis induction by CMG is not inhibited in HCT116 cell clones expressing high levels of the anti-apoptotic protein, Bcl-2, or dominant-negative FADD, thereby indicating that CMG induces cell death via a yet-to-be identified pathway, unrelated to the death receptor and mitochondrion-dependent pathways. The findings presented here suggest that CMG (a) induces an anoikis form of cell death in HCT116 colon cancer cells that includes events associated with caspase-dependent pathways; and (b) might be developed into a chemotherapeutic agent for the treatment of human colon and other cancers [16].

CONCLUSIONS

1. Mastic resin is a highly commercialized product due to its: medicinal, cosmetic, pharmaceutical and industrial applications.
2. Mastic oil contains perillyl alcohol, that has been found to be effective in both preventing and treating various cancers.
3. Mastic resin heals also pectic ulcers by killing *Hellicobacter pylori* which causes peptic ulcers, gastritis and duodenitis.
4. Natural mastic gum has also been proven to absorb cholesterol thus diminishing chances of heart attacks and high blood pressure, and helps reduce triglyceride and total lipid levels of the organism.

REFERENCES

1. Hagidimitriou M. The resin of Chios mastic tree. An old product with modern [cited 2011 May 30] Available from: www.unipa.it/arbor/varie/Presentation_M.Hagidimitriou_2
2. Kottakis F, Mamari F, Matragkou Ch, Zachariadis G, Karamanos N, Choli-Papadopoulou T. Arabino-galactan pro-teins from Pistacia lentiscus var. chia: isolation, characterization and biological function. Amino Acids. 2008 Apr; 34(3): 13-20.
3. Triantafyllou A, Chaviaras N, Sergentanis TN, Protopapa E, Tsaknis J. Chios mastic gum modulates serum biochemical parameters in a human population. J Ethnopharmacol. 2007 Apr; 111(1): 43-9.
4. Kim HJ, Neophytou C. Natural anti-inflammatory compounds for the management and adjuvant therapy of inflammatory bowel disease and its drug delivery system. Arch Pharm Res. 2009 Jul; 32(7): 997-1004.
5. Sawids T, Yurukova L, Askitis T. Chios mastic, a natural supplement for zinc to enhance male sexuality and prostate function. Pharm. Biol. 2010 Jan; 48(1): 48-54.
6. Triantafyllou A, Bikineveva A, Dikalova A, Nazarewicz R, Lerakis S, Dikalov S. Anti-inflammatory activity of Chios mastic gum is associated with inhibition of TNF-alpha induced oxidative stress, Nutr J. 2011 Jun; 6; 10(1): 64 [Epub ahead of print].
7. Petersen RK, Christensen KB, Assimo-poulou AN, Fretté X, Papageorgiou VP, Kristiansen K, Kouskoumvekaki I. Pharmacophore-driven identification of PPARγ agonists from natural sources J. Comput Aided Mol Des. 2011 Feb; 25(2): 107-16.
8. Koutsoudaki C, Krsek M, Rodger A. Chemical composition and antibacterial activity of the essential oil and the gum of Pistacia lentiscus Var. chia. J Agric Food Chem. 2005 Oct; 53(20): 7681-85.
9. Magiatis P, Melliou E, Skaltsounis AL, Chinou IB, Mitaku S. Chemical composition and antimicrobial activity of the essential oils of Pistacia lentiscus var. chia. Planta Med. 1999 Dec; 65(8): 749-52.
10. Özcelik B, Aslan M, Orhan I, Karaoglu T. Antibacterial, antifungal, and antiviral activities of the lipophylic extracts of *Pistacia vera.* Microbiol Res. 2005 April; 160(2): 159-64.
11. Kottakis F, Befani C, Asiminas S, Kontou M, Koliakos G, Choli-Papadopoulou T. The C-terminal region of HPNAP activates neutrophils and promotes their adhesion to endothelial cells. Helicobacter. 2009 Jun; 14(3): 177-9.

12. Choli-Papadopoulou T, Kottakis F, Papadopoulos G, Pendas S. Helicobacter pylori neutrophil activating protein as target for new drugs against H. pylori inflammation. World. J. Gastroenterol. 2011 Jun; 7(17): 2585-91.
13. Dabos KJ, Sfika E, Vlatta LJ, Frantzi D, Amygdalos Gl, Giannikopoulos G. Is Chios mastic gum effective in the treatment of functional dyspepsia? A prospective randomised double-blind placebo controlled trial. J. Ethnopharmacol. 2010 Feb; 127(2): 205-9.
14. Doi K, Wei M, Kitano M, Uematsu N, Inoue M, Wanibuchi H. Enhancement of preneoplastic lesion yield by Chios Mastic Gum in a rat liver medium-term carcinogenesis bioassay. Toxicol Appl Pharmacol. 2009 Jan; 234(1): 135-42.
15. Loutrari H, Magkouta S, Pyriochou A, Koika V, Kolisis FN, Papapetropoulos A, Roussos C. Mastic oil from Pistacia lentiscus var. chia inhibits growth and survival of human K562 leukemia cells and attenuates angiogenesis. Nutr Cancer. 2006; 55(1): 86-93.
16. Balan KV, Prince J, Han Z, Dimas K, Cladaras M, Wyche JH, Sitaras NM, Pantazis P. Antiproliferative activity and induction of apoptosis in human colon cancer cells treated in vitro with constituents of a product derived from Pistacia lentiscus L. var. chia. Phytomedicine. 2007 Ap; 14(4): 263-72.