Assessment report on *Pistacia lentiscus* L., resin (mastix)
Draft

Based on Article 16d(1), Article 16f and Article 16h of Directive 2001/83/EC as amended (traditional use)

Herbal substance(s) (binomial scientific name of the plant, including plant part)	*Pistacia lentiscus* L., resin (mastix)
Herbal preparation(s)	Powdered herbal substance
Pharmaceutical form(s)	Powdered herbal substance in solid dosage form for oral use Powdered herbal substance in semi-solid dosage form for cutaneous use
Rapporteur(s)	Ioanna Chinou
Peer-reviewer	Marisa Delbò

Note: This draft assessment report is published to support the release for public consultation of the draft European Union herbal monograph on *Pistacia lentiscus* L. resin, (mastix). It is a working document, not yet edited, and which shall be further developed after the release for consultation of the monograph. Interested parties are welcome to submit comments to the HMPC secretariat, which will be taken into consideration but no 'overview of comments received during the public consultation' will be prepared on comments that will be received on this assessment report. The publication of this <u>draft</u> assessment report has been agreed to facilitate the understanding by Interested Parties of the assessment that has been carried out so far and led to the preparation of the draft monograph.

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1. Introduction

1.1. Description of the herbal substance(s), herbal preparation(s) or combinations thereof

- Herbal substance(s)

According to the specific Eur. Ph. Monograph (01/2008:1876), mastic is the dried resinous exudate obtained from stems and branches of *Pistacia lentiscus* (var latifolius Coss. under revision) with a content of minimum 10 ml/kg of essential oil (anhydrous drug). .

Mastic is more correctly an oleoresin obtained from a cultivated variety of cultivated clone of the mastic tree (*Pistacia lentiscus* L.).

The mastic gum has also a monograph in The Ayurvedic Pharmacopoeia of India (1999) which referrs as well to the resin of *Pistacia lentiscus* L. called as "Rumimastangi"

Beng.: Rumi-Mastungi

Guj. : Rumi Mastagee

Hindi: Rumi Mastagee; Rumi Mastiki; Mastagee

Mar.: Rumaa Mastakee

Urdu.: Rumee Mastagee

The mastic tree (*Pistacia lentiscus* L.), from the Anacardiaceae family, is naturally distributed in areas that enclose the coastal regions of the Mediterranean, Portugal and tropical Africa (PDR 2007). Flower and fruit is compact and spike-like. The flowers are yellowish or purplish. The drupe approximately 4 mm, globose, apiculate and is red, but later turns black.

The plant is an evergreen dio-oecious tree or shrub 1-8 m high. The leaves are bipinnate. The 8 to 12 leaflets measure 1 to 5 cm by 0.5 to 1.5 cm. They are lanceolate to ovate-lanceolate, mucronate and coriaceaous. The rachis is broadly winged. The petioles are glabrous (Evans 1989)

The plant variety (*Pistacia lentiscus* L. var l*atifolius* Coss.) described in Eur. Ph Monograph Mastic (01/2008:1876) has been referred in the Botanical Book of the Botanist K.H. Rechinger (1943) as an endemic variety of the Greek islands of Crete (Chania, Ierapetra, Sitia, Toplou, Mirambelou) and the island of Karpathos. To the knowledge of Greek botanists this variety has never been collected on the aforementioned islands and has also never been phytochemically studied since this report. In the same book of Flora Aegea (1943), KH Rechinger has reported that, on Chios Island, a woody form of pistachio - *Pistacia chia* Desf. is cultivated which supplies a special resinous gum, the pharmaceutically and commercially used resin, so-called mastic or mastix as it is well known all over Europe and the world (Gennadios 1914) . Mastix or mastic is a unique natural product from the Greek island of Chios (with a Protected Designation of Origin 317707/14-01-1994 (gaz. 17/14-01-94) and plays a substantial role in the island's as well as Greece's economy.

The botanical name *Pistacia lentiscus* var. *Chia* (Desf. Ex Poiret) DC has been also used in the great majority of the existing publications for this plant. In a more recent and well documented botanical study of Prof Kazimierz Browicz (*Plant Systematics and evolution*, 1987), it is proposed that, instead of the widely used botanical name of *Pistacia lentiscus* var. *Chia*, the name *Pistacia lentiscus* **cv**. C*hia*

should be used, as cv means cultivated clone. In the proposal for revision from Greece, it is documented that the best scientific way, could be the acceptance in the Eur Pharmacopoeia's monograph the species *Pistacia lentiscus* L. without any further specified variety or cultivar.

The3 cultivar *Pistacia lentiscus* L. cv Chia (synonym *Pistacia lentiscus* var. *Chia*) from the Greek island Chios, commercially is the major source of Chios Mastiha (Chios Gum Mastic; CGM) all over the world. The *P. lentiscus* cv. *Chia*, is cultivated and grown only in Southern Chios Area of Mastihohoria, (Savvides, 2000). The resin of that plant is obtained as an exudate after "hurting" the trunk and branches (Paraschos *et al*., 2007). A fully grown tree of the *Pistacia lentiscus* (*Pistacia lentiscus* L. cv Chia) species produces 1 kg of resin yearly. As it drips, this sap appears as a sticky and translucent liquid which, 15-20 days later, is solidified into irregular shapes influenced by the area's weather conditions in summertime that is intense drought and sunlight. After being solidified, it has a crystal form, while it is rather bitter taste quickly subsides to leave a distinctive aroma.

After the resin is harvested, it is washed with water to remove impurities and then mastic gum is sorted, classified and graded according to the colour and size of the granule (Dabos *et al*., 2010).

Chios island exports approx 250 000 kg annually (Perrikos 1986; Belles 2005) mainly in France, USA, Emirates Saudi Arabia, UK, Australia etc. Since 1997, mastic from the island of Chios has been characterized a s a Product of Protected Designation of Origin (PDO), on the basis of Regulation No. 123/1997 (L0224/24-1-97) of the European Union and it has been registered on the relevant European Union List of PDO Products.

The rapporteur of the AR does not have any further knwolende about commercial production of resin of Pistacuia lentiscus from other coubntries, which may exists and used for medicinal purposes.

Mastic occurs in yellow or greenish yellow rounded or pear-shaped tears of about 3 mm in diameters. The shape of the tears is sufficient to distinguish them from those of sandarac (resin of *Tetraclinia articulata* which is used for its adulteration). The tears of mastic are brittle but become plastic when chewed. Odour slightly balsamic; taste, mildly terebinthinate (Evans 1989) while the ones of sandarac when chewed remain gritty, showing no tendency to form a plastic mass, with a faint terebinthinate odour and somewhat bitter taste (distinction from mastic)

Chemical constituents

- Natural polymer (van den Berg *et al*. 1998)

- Triterpenes (tetracyclic euphane- and dammarane skeleton type and of the pentacyclic oleanane and lupane skeleton type such as mastic acid, isomastic acid, oleanolic acid, tirucallol etc (PDR 2007)

- Monoterpene hydrocarbons, 20% oxygenated monoterpenes and sesquiterpenes

- Polyphenols, phytosterols

The first research on the chemical composition of mastic is reported back to 1930, however and despite the extended range of its compounds' identification, until today is not entirely determined yet. The resin appears to consist of a variety of organic ingredients including a natural polymer, volatile and aromatic ingredients that constitute the essential oil (Mastic oil), terpenic acids, phytosterols, polyphenolic molecules and a large number of other potentially active secondary metabolites, some of which have been isolated and determined in nature for the first time. This combination of more than 80 ingredients justifies the multiple uses of mastic not only in the food but also in the health & personal care area.

cis-1,4-poly-β-myrcene
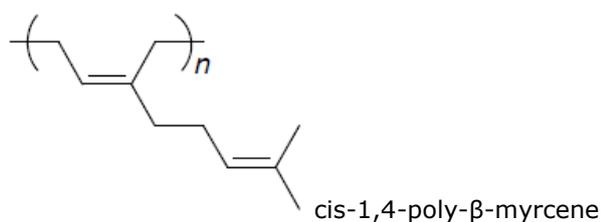
The polymer of mastic was identified as cis-1,4-poly-β-myrcene (van den Berg *et al*. 1998), while mastic also contains a small fraction (approximately 2%) of essential oil, which was analyzed by several scientists mainly by Papageorgiou *et al*. (1981, 1997, Magiatis *et al*. 1999.

The triterpenoids present in mastic resin are of the tetracyclic euphane- and dammarane skeleton type and of the pentacyclic oleanane and lupane skeleton type

oleanane:
R_1=H; R_2=CH_3
ursane:
R_1=CH_3; R_2=H

dammarane

hopane

euphane

lupane

bicyclic triterpenoid

tricyclic triterpenoid
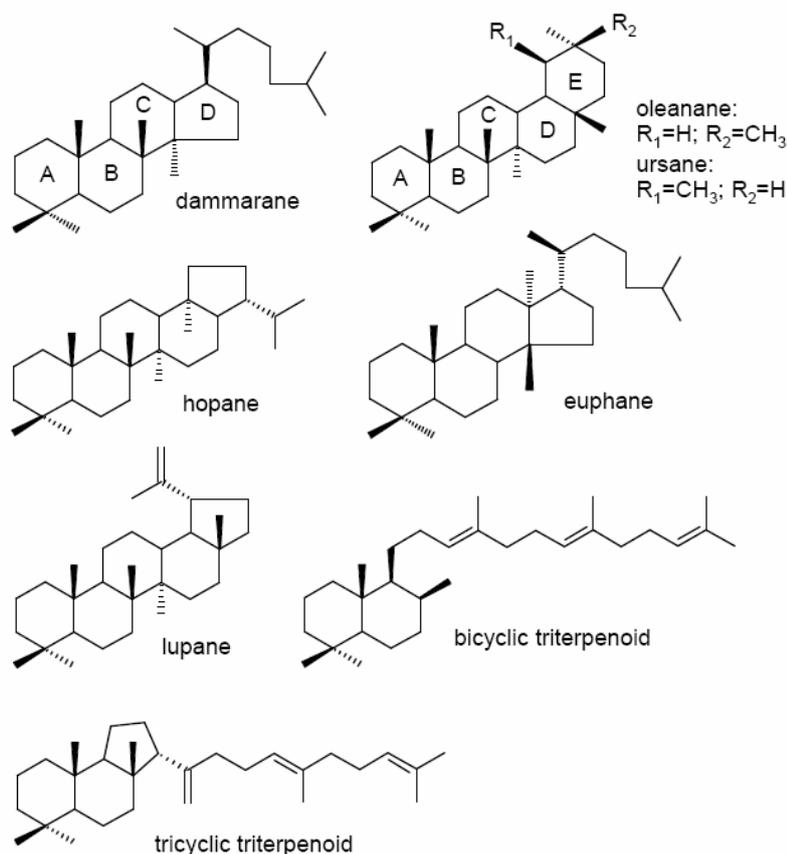
Triterpenoids present in mastic resin

Overall, the main non-volatile natural products reported in the literature that have been isolated from the gum are: a) masticadienonic acid, b) tirucallol, c) oleanolic acid, d) isomasticadienonic acid, e) 3-o-28-norolean-12-en, f) 20(S)-3β-acetoxy-20-hydroxydammar-24-en, g) 3-oxo-dammara-20(21),24-diene, h) 3β-hydroxymalabarica-14(26),17E,21-triene, i) 3-oxo-malabarica-14(26),17E,21-triene, j) 3-β-hydroxy-28-norolean-12-en, k) 3-oxo-28-norlup-20(29)-en, l) (8R)-3-Oxo-8-hydroxy-polypoda-13E,17E,21-triene, m) 1,4-poly-β-myrcene (The Review of Natural Products 2005; Assimopoulou & Papageorgiou 2005i and 2005ii; Paraschos *et al*. 2007).
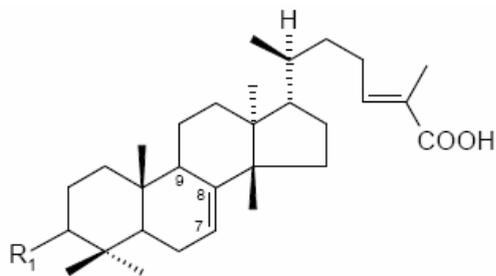
R1: O (masticadienonic acid)
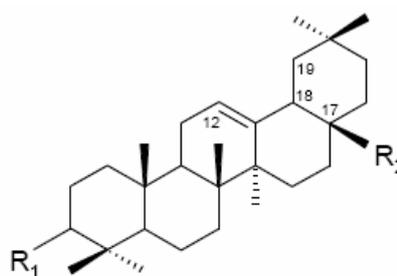
R1: O R2: COOH (oleanolic acid)
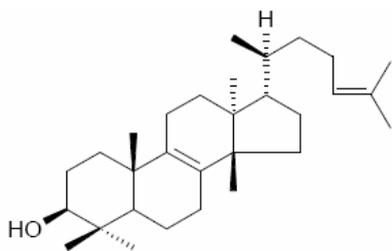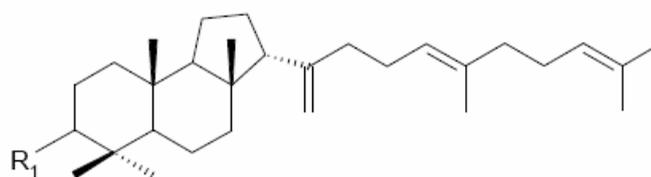
O 3-oxo-malabarica-14(26),17E,21-triene

Tirucallol

Polyphenols

There are also reported traces of the compounds of tyrosol, p-hydroxy-benzoic acid, p-hydroxy-phenyl acetic acid, vanillic acid and gallic acid (PDR 2007)

Volatiles secondary metabolites from *Pistacia lentiscus* L.

During the decades of the 90's and 2000's, the volatile components of mastic were the subject of several studies in the context of the analysis of the composition and activity of the essential oil of mastic (Papageorgiou et al. 1981; 1997) . In 1991, Boelens & Jimenez comparatively studied the chemical composition of essential oils of resin, leaves and unripe and ripe fruits of mastic, identifying a total of 90 components (50% monoterpene hydrocarbons, 20% oxygenated monoterpenes and sesquiterpenes 25%) with major components of the essential oil of resin the α-pinene (79%) and the myrcene (3%).

A very considerable study of the three essential oils of mastic gum, leaves and branches of mastic was published in 1999 by Magiatis *et al*. In this study the major components identified were α-pinene (66.48%), myrcene (8.34%) and β- pinene (3.29%), the essential oil of leaves myrcene (20,58%), germacrene D (13.30%), L-caryophyllene (8.33%), α-cadinol (7.33%) and δ-cadinene (7.00%), while the essential oil of the branches were myrcene (47.92%), germacrene D (15.46%) and E-caryophyllene (4.75%). In 2005 (Koutsoudaki *et al*.), indicated the α-pinene, β-myrcene, β-pinene, limonene and trans-caryophyllene as the major components. The above results have been verified by a recent work (Kokolakis *et al*., 2010), in which the authors identified α-pinene, myrcene and β-pinene as the main ingredients an indicator of origin of mastic one or another collection, but also as an indicator of storage time.

- Herbal preparation(s)

Comminuted herbal substance

- Combinations of herbal substance(s) and/or herbal preparation(s) including a description of vitamin(s) and/or mineral(s) as ingredients of traditional combination herbal medicinal products assessed, where applicable.

The request for information exchange concerning preparations from *Pistacia lentiscus* L. resin, mastic revealed that the powder is widely distributed in the food sector all over the world, also in self care products (tooth paste etc) in Balkan area as well as in cosmetics area. There are also products where can be found in combination. However, such combinations are not subject of this assessment report.

1.2. Search and assessment methodology

The assessment is based on the sources mentioned in the list of references. Publications in other languages than English (at least abstract in English or other language available) were precluded from assessment.

Search engines used: Google; key words: mastix, mastic, *Pistacia lentiscus* cv Chia, *Pistacia lentiscus*, mastic gum.

Scientific databases: Scifinder, Scopus; search date 29.10.2014; key words: "mastix", "mastic", "*Pistacia lentiscus* cv Chia", "*Pistacia lentiscus*", "mastic gum", mastic oil"

Medical databases: Pubmed, Cochrane library; key words: "mastix", "mastic", "*pistacia lentiscus* cv Chia", "*Pistacia lentiscus*", "mastic gum", mastic oil"

Toxicological databases: Toxnet; key words: "mastix", "mastic", "*Pistacia lentiscus* cv Chia", "*Pistacia lentiscus*", "mastic gum", mastic oil"

Pharmacovigilance resources: Not applicable.

Data from EU and non-EU regulatory authorities:

Other resources: Library of the University of Athens (Pharmacy and Pharmacognosy library)

2. Data on medicinal use

2.1. Information about products on the market

2.1.1. Information about products on the market in the EU/EEA Member States

Information on medicinal products marketed in the EU/EEA

In Greece and other Balkan countries the herbal drug "mastic resin" or "mastic gum" as synonym are widely distributed for use according to folk medicine and sold via pharmacies especially after '80s and international publications of the healing activities of mastic on gastrointestinal disorders (functional dyspepsia, as an adjuvant in Helicobacter pylori therapy), skin healing activities, products for mouth hygiene.

The request for information exchange concerning preparations from *Pistacia lentiscus* L. resin, mastic, revealed that there are no medicinal products authorized or registered in the EU/EEA containing preparations from *Pistacia lentiscus* L. resin, mastic as a single active substance.

Information on relevant combination medicinal products marketed in the EU/EEA

In Greece there is one combination medicinal (?) product (healing cream) containing Alkanna tinctoria and powder of *Pistacia lentiscus* L. resin, mastic which was registered first at 1976, with-drown some years later and then re-registered in 90's under a different brand name and slight changes in its content.

Moreover, there are certain galenic products used through pharmacies containing mastic powder and mastic essential oil widely used for cutaneous and oromucosal uses (gargling) marketed since '50s and '90s respectively.

Information on other products marketed in the EU/EEA (where relevant)

The request for information exchange concerning preparations from *Pistacia lentiscus* L. resin, mastic revealed that the powder is widely distributed in the food sector as well in cosmetics sector all over Europe and in Arabian countries, also in combination products.

2.1.2. Information on products on the market outside the EU/EEA

No data

2.2. Information on documented medicinal use and historical data from literature

Powdered herbal substance for oral use

Ancient Greek physicians (Hippocrates, Dioscorides, Galenos) reported for first time the properties of Chios Mastiha and recommended its use for its distinctive flavour and its therapeutic properties. Documents show that it was the first natural chewing gum of the ancient world, used to clean the teeth and to freshen the breath. It was even used in cosmetology for cleansing the face and body. Then the resin was used as an active ingredient in a series of pharmaceutical formulas and nostrums, many of which have been recorded from time to time in international pharmacopeias (Gennadios 1914; Perikos 1986; Savvidis 2000).

Dioscorides (1st century AD.) in *Materia Medica* referred the therapeutic properties of mastic from the island of Chios (Chios Mastiha), mentioning that it helps in the cases of indigestion, in blood problems, in chronic coughing, while at the same time it acted as tranquilizer. He proposed the healing properties of chewing mastic resin to support oral hygiene as well as to clean and fresh breath. He mentioned the use of Mastiha oil, the essential oil of mastic, to be applied in multiple ways for affections of the uterus, as well as for its styptic activities (Genandios 1914).

From the 1st until the 7th century AD mastic was used by medical practitioners and botanists mainly for the treatment of stomach disorders like gastralgia, dyspepsia and peptic ulcer for more than 2500 years. Ancient Greek physicians, such as Hippocrates, Dioscorides, Theophrastos and Galenos mentioned its properties and recommended its use (Paraschos *et al*., 2007). In the view of the people at that time, the use of mastic contributed to the smooth operation of the gastric and intestinal system. More specifically and from the various sources results that the Mastiha was used for soothing the pain of the stomach as well as indigestion and stomach disorders (Oribasius, Aetius, Galen, Pilen). The effect of mastic in atonic and inflammation of the stomach, intestine and liver, and the emollient properties, is reported by Galen in «Simpliciun medicamentorum temperamentis ac faculatibus libri XI». (Perikos 1986; Belles 2006).

The Jerusalem Balsam - In the pharmacy of the Franciscan Monastery of Saint Savior in Jerusalem, where the monk Antonio Menzani di Cuna worked, after twenty-four years of experimentation he succeeded in creating an effective balsam named "The Jerusalem Balsam". It was presented in Milan, in 1712, as an unguent to heal wounds abdominal pain, dermatitis, intestinal worms, toothache, haemorrhoids etc. Menzani's formula contained four ingredients: aloe, frankincense, myrrh and mastic dissolved in ethanol. It was referred by Gilbertus Anglicus, (13th century in England) in his Compendium Medicinae nostrum for the spleen called "diacerasus", which contains cherry juice, cinnamon and mastic resin. Later, Giovanni de Vigo Franciscan monk-physician to Pope Julius II, prescribed a Balsam for Itching, containing egg white, linseed, poplar buds, and mastic powder, in olive oil (Perikos 1986).

Paracelsus in his Der grossen Wundartzney (Great Surgery Book) proposes mastic resin to heal wounds." In 18th and 19th centuries mastic resin was used for filling of dental cavities, dissolving 4 parts mastic and 1 part ether in a flask: the solution forming a yellowish colour and oily consistency would be used to moisten a cotton bud and applied to the cavity to fill and seal it. These uses of mastic, were rescued in traditional healing people of Eastern Mediterranean and Middle East, where the use of mastic is extremely widespreadMoreover, Al-Razi, Abu Yusuf Ya'qub ibn Ishaq al-Kindi and Abu Marwan'Abd al-Malik have prescribed mixtures in medical formulas to fill decayed teeth, to fortify stomach, aid the liver (Perikos 1986, Belles 2006)

Many medical practitioners, pharmacist and botanists, referred to the therapeutic properties of mastic resin, that they used for preparing therapeutic formulas and widely used preparations. The use of mastic continued to spread successfully during Byzantine times. In many European Pharmacopeias of 16th – 18th century P.C.

Since last century, mastic is currently used as a seasoning in Mediterranean cuisine, in the production of chewing gum, in perfumery, in dentistry and by the local population of Chios island for the relief of epigastric pain and protection against peptic ulcer disease

In our days, the potential therapeutic activities of mastic gum have been scientifically studied and showed that especially mastic from the island Chios (as the majority of the studies referred to that one) displays positive action against digestive disorders, contributes to oral hygiene, displays antimicrobial and anti-inflammatory action, is a natural antioxidant, and also potentially aids in trauma healing.

Today, a series of reports in international medical journals corroborate the historically recorded properties of mastic, which are based on the results of laboratory studies as well as on small clinical trials carried out by independent researchers in Greece and abroad, and have revealed that mastic resin possesses interesting bioactive properties.

In particular, mastic gum (in accordance of the existing studies, mainly from Chios island CGM) and its essential oil, according current research suggests that they possess mainly antimicrobial and antioxidant properties. The antibacterial activity (Magiatis *et al*., 1999; Lauk *et al*., 1996; Koutsoudaki *et al*., 2005), as well as and its *in-vivo* antiplaque action in the oral cavity has been attributed to its inhibitory action against overall bacterial growth (Takahashi *et al*., 2003), especially against *Streptococcus mutans* and *Helicobacter pylori* (Aksoy *et al*., 2006). Due to these findings, it can be assumed that the powdered herbal substance of *Pistacia lentiscus* L. resin, mastic, was used for oral and cutaneous use at least since 1980 in considerable amounts in dyspeptic and gastrointestinal disorders, and for cutaneous use for its healing properties, within the European Union. Thus the requirements for the period of medicinal use according to Directive 2001/83/EC as amended with respect to "traditional use" are regarded as fulfilled.

The mastic gum has also a monograph in The Ayurvedic Pharmacopoeia of India (1999) which referrs as well to the resin of *Pistacia lentiscus* L. called as "Rumimastangi" and its medicinal uses have been propsoed for doses 1-2 g daily

Oral use

The recommended posology is, according to Perikos (1986) and by Al-Habbal *et al*. (1984), 0,35-05 g of powdered mastic divided in doses of 0.5 g up to 6 times daily. Daily dose up to 2 g which is in accordance with recent clinical studies.

Table 1: Overview of historical data

Herbal preparation	Documented Use / Traditional Use	Pharmaceutical form Strength (where relevant) Posology Duration of use	Reference
Mastic powder	Traditional use, folk medicine for gastrointestinal disorders/mild dyspeptic/gastrointestinal disorders	oral use: a) 0.5 g twice per day for a period of 2-3 weeks b) Single dose 0.5-1 g before breakfast and 1 g before sleeping at night duration of use: 2-4 weeks c) 0.35g x 3 (1,05 g) For 2 weeks	a) Al-Habbal *et al*. 1984 ; Perikos 1986; PDR 2007 b)Huwez, Al-Habbal, 1986 c) Dabos *et al*,. 2010i and 2010ii
Mastic powder	Traditional use, folk medicine for the symptomatic treatment of minor inflammations of the skin and as an aid in healing of minor wounds.	cutaneous use: Semisolid cream containing 9-11% mastic powder 1 week on the affected skin area Up to three times daily	(commercial product Histoplastin Red®, HELIXDERM®) 1978 Perikos 1986 Mikhail *et al*. 1986, 1989; Lesegne 1992 Yavuzer *et al*. 2005
Mastic powder	Traditional use, folk medicine for the symptomatic treatment of minor inflammations in the mouth	in gargling solutions/toothpastes	Perikos 1986 Topitsoglou-Themeli *et al*. 1984
Mastic essential oil (?)	Traditional use, folk medicine for the symptomatic treatment of minor inflammations in the mouth	Not adequately specified	Perikos 1986 Topitsoglou-Themeli *et al*. 1986

Herbal preparation	Documented Use / Traditional Use	Pharmaceutical form Strength (where relevant) Posology Duration of use	Reference
Mastic water	Traditional use, folk medicine for the symptomatic treatment of minor inflammations in the mouth	Mastic water in gargling solutions/toothpastes	Perikos 1986 Topitsoglou-Themeli *et al.* 1984; 1985

2.3. Overall conclusions on medicinal use

As discussed in 2.2 since the end of the 1970s/the beginning of the 1980s the medicinal use of the powder of the resin of *Pistacia lentiscus* L. mastic, has become very popular in folk medicine in Greece and other Balkan and Asian countries as well as in the USA for its healing properties. Thus the requirements for the period of medicinal use according to Directive 2001/83/EC as amended with respect to "traditional use" regarded fulfilled for the indications

- Traditional herbal medicinal product used in mild dyspeptic disorders

- Traditional herbal medicinal product used for the symptomatic treatment of minor inflammations of the skin and as an aid in healing of minor wounds

Table 2: Overview of evidence on period of medicinal use

Herbal preparation Pharmaceutical form	Indication	Strength Posology	Period of medicinal use
Mastic powder	gastrointestinal disorders – mild dyspeptic/gastrointestinal disorders	oral use: a) 0.5 g twice per day for a period of 2-3 weeks b) Single dose 0.5-1 g before breakfast and 1 g before sleeping at night duration of use: 2-4 weeks c) 0.35g x 3 (1,05 g) For 2 weeks	a)Al-Habbal *et al.* 1984; Perikos 1986; PDR 2007 b) Huwez, Al-Habbal, 1986 c) Dabos *et al*, 2010i and 2010ii*et alet al*
Mastic powder	for treatment of minor inflammations of the skin and as an <u>aid in healing of</u> minor wounds	cutaneous use: Semisolid cream containing 9-11% mastic powder 1 week on the affected skin area Up to three times daily	commercial product 1978Perikos 1986 Mikhail *et al.* 1986, 1989; Lesegne 1992 Yavuzer *et al.* 2005

Based on available literature references as well as recent clinical trials the following posologies are proposed:

Oral use

Powder

Single dose: 0,35 - 0.5 g 3-4 times daily

Daily dose:1.05 -2 g

Duration of use 2 weeks

Cutaneous use

Semisolid cream containing 9-11% mastic powder

Up to three times daily

Duration of use: 1 week, to be applied as a thin layer on the affected skin area

3. Non-Clinical Data

3.1. Overview of available pharmacological data regarding the herbal substance(s), herbal preparation(s) and relevant constituents thereof

3.1.1. Primary pharmacodynamics

Antimicrobial activity

Several studies have been conducted to investigate mastic's antimicrobial properties. Mastic gum has been proven to have a wide range of antimicrobial activity against Gram-positive (+) and Gram-negative (-) bacteria, as well as in other pathogenic microorganisms.

Daifas _et al_. (2004) investigated the effect of mastic gum and its essential oil, alone and in conjunction with ethanol, on the growth of proteolytic strains of _Clostridium botulinum_. They proved that mastic and its mastic oil can effectively be used as factors against the appearance of _botulinum_ neurotoxin in nutrition goods. More specifically, the results of the laboratory tests showed that the addition of only 0.3% mastic oil was required for the inhibition of proteolytic strains of _Clostridium botulinum_. The study concluded that mastic gum and mastic oil could potentially be used as natural preservative in bakery products.

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. (Tassou & Nychas 1995; Magiatis _et al_. 1999; Koutsoudaki _et al_., 2005).

Bactericidal effect of mastic against Helicobacter pylori

On 1983 Marshall and Warren isolated and cultured successfully a bacterium, initially called _Campylobacter pylori_ and later _Helicobacter pylori,_ that was demonstrated to be able colonizing the stomach and thereby cause gastric inflammation. _H. pylori_ is a Gram-negative bacterium and it is responsible for 75% of digestive ulcer cases, while the respective amount in the case of duodenal ulcer amounts to 90%.

A number of studies have shown that mastic gum and mastic oil exhibit actions on gastrointestinal lesions. After discovery of *Helicobacter pylori* and correlation with gastrointestinal disease, the interest for the determination of mechanism of action of mastic and mastic oil for these disorders focused on the exploration and eventual finding of anti-*H. pylori* properties.

The first study that proves mastic's anti- H. pylori activity is published in the New England Journal of Medicine in 1998 (Huwez et al., 1998). Mastic proved to kill the H. pylori NCTC 11637 strain and the six clinical isolates. In this study which made at the University Hospital of Nottingham in England, it was concluded that mastic (from Chios) had a proven action against Helicobacter pylori. The study showed the findings that suggest that even 1 g of mastic per day, for a time period of two weeks could cure digestive ulcer. This beneficial action, was due to the fact that mastic exterminated Helicobacter pylori which is liable for the majority of the digestive ulcer cases. In the specific study fresh samples were used with the presence of Helicobacter pylori, which were isolated from patients and the minimum bactericidal concentration (MBC) of mastic was searched, which means the minimum concentration required in order to exterminate 99.9% of the bacterium within 24 hours. Mastic exterminated the bacterium in all the examined samples, regardless of the size of the population. The minimum bacterial concentration (MBC) of mastic was 60 μg/ml, but even in smaller concentrations, the antibacterial action was especially important. The anti- H. pylori activity shown in this study, may explain a very rapid positive therapeutic effect in patients with peptic ulcers, even with low doses of mastic gum (1 g per day for two weeks).

Marone *et al*. (2001) and Bona *et al*. (2001) assessed the antibacterial effect of mastic on the isolated clinical isolates of *Helicobacter pylori* at concentrations of 2000 to 1.9 μg/ml. The minimum bactericidal concentrations, calculated by microdilution method showed that mastic exhibited remarkable bactericidal effect on 12 strains isolated from patients of *H. pylori*, killing 50% of the executives in concentration of 125 μg/ml and 90% at concentration of 500 μg/ml. Furthermore, the microscopic observation of the morphology of the bacteria by electron emission led to the conclusion that the resin induces the release of air bubbles, the challenge morphological anomalies and segmentation of cells of *H. pylori*. It was also attempted to place the bactericidal property of the mastic on *H. pylori* in-arabino-galactan proteins (AGP's) isolated from the resin (Kottakis *et al*., 2009). Specifically, the inhibition of growth of *H. pylori* in the presence of aqueous mastic extracts containing AGPs was studied. The results showed that the extracts of at least 1,4 g gum affect the viability of bacterium, preventing cell growth. There were no indications if AGPs cause abnormal morphology in *H. pylori*, as mentioned for total mastic (Bona *et al*, 2001)

Paraschos *et al*. (2007) utilized an established *H. pylori* infection model to evaluate the potential therapeutic effect of continuous total mastic extract without polymer (TMEWP) administration on *H. pylori* colonization and development of associated gastritis. Initially, TMEWP was obtained from crude mastic gum in a 70% proportion and it was further divided into two fractions, an acidic and a neutral one. The acidic fraction of TMEWP after chromatographic separations afforded the major triterpenic acids oleanonic acid (515 mg), moronic acid (338 mg), 24Z-masticadienonic acid (1.1 g), 24Z-isomasticadienonic acid (1.0 g), 24Z-masticadienolic acid (95 mg), and 24Z-isomasticadienolic acid (102 mg). The neutral fraction, after similar treatment, afforded five neutral triterpenic compounds: tirucallol (110 mg), dammaradienone (128 mg), 28-norolean- 12-en-3-one (206 mg), oleanonic aldehyde (152 mg), and oleanolic aldehyde (98 mg). Then, the antimicrobial activities of all these fractions, as well as that of mastic total extract, against a panel of 10 clinical isolates of *H. pylori* and the CCUG 38771 reference strain were tested over a period of three months. Mastic extracts exhibited concentration- and strain-dependent bactericidal activities. More specifically, in all strains tested, the acidic fraction exhibited the highest activity, with a mean MBC of 0.136 mg/ml, followed by the TMEWP (MBC, 0.256 mg/ml). Reduced activity was observed for the neutral fraction of the TMEWP (0.638

mg/ml). Up to twofold differences were observed in the MBC between individual strains tested, and only in the case of LAVHP-7 strain was a higher susceptibility against the TMEWP and its acidic fraction observed. Having obtained the highest activity with the acidic fraction of the TMEWP, they proceeded to test the isolated pure acidic compounds for anti-*Helicobacter* activity. Highest overall activity was obtained consistently and for all 11 of the *H. pylori* strains tested with isomasticadienolic acid, with a mean MBC of 0.202 mg/ml (0.443 mM), followed by masticadienolic (0.220 mg/ml [0.482 mM]), oleanonic (0.292 mg/ml [0.643 mM]), and moronic acid (0.310 mg/ml [0.683 mM]). Interestingly, the 3-oxo derivatives, isomasticadienonic and masticadienonic acids showed reduced activity compared to the corresponding 3-hydroxyl derivatives. It was verified that the chemical consistency of TMEWP was virtually identical to that of crude mastic gum, except for the absence of the polymer, and it also presented better solubility properties and increased concentration of active constituents. The experiments showed that the mastic total extract could moderately reduce *H. pylori* colonization in the antrum and corpus of the stomach. The reduction in colonization levels calculated was approximately 30-fold. These results were in concurrence with the visible reduction in *H. pylori* colonization observed in the histopathology evaluations. Finally, the results also suggest that habitual long term mastic consumption may be effective in moderating *H. pylori* colonization.

Following the above study, (Kottakis *et al*., 2009) investigated the effect the AGPs mastic (arabino-galactan proteins) derived from CGM, both *in vitro* and *in vivo*, in the presence of neutrophil-activating protein *H. pylori* (HP-NAP), on intrinsic activators of cellular immunity (activators neutrophils), comparing patients carriers of *H. pylori* to healthy volunteers, receiving 1 g gum daily for two months. The *H. pylori* virulence factors are three conserved antigens, namely the vacillating cytotoxin A (VacA), the cytotoxin-associated antigen (GagA), and HPNAP. The VacA interacts with the membrane of epithelial cells and enters therein, wherein forms a low conductivity ions channel. HPNAP is known to attract and activate neutrophils, monocytes, and mast cells, resulting in the release of pro-inflammatory mediators. Pull-down experiments in this study showed, for the first time, a specific binding of AGPs to two membrane proteins of neutrophils, possibly resulting in inhibition of neutrophil activation. Although these two neutrophil proteins were not characterized in this study, the authors state that further studies are needed to elucidate their characteristics and involvement in neutrophil activities. Neutrophil activation was reduced when incubated *in vitro* with HP-NAP (P=0.0027) and AGP plus HP-NAP (P=0.0004) in *H. pylori*-positive patients who consumed AGP for two months. Similar results were also obtained when neutrophils were incubated with AGP plus HP-NAP (P=0.0038) but not with HP-NAP (P>0.05) in controls.

Sharifi *et al*. (2009) investigated the anti-*H. pylori* activity of mastic in another study that indicated that most of the active fractions of mastic is a polymer, followed by the acid and the same resin, while the neutral fragment was inactive. Notably, the increase in reactivity was observed by both the oxidation of the polymer (doubling) and by mastication for 4 hours the resin (50% increase).

Choli-Papadopoulou *et al*. (2011) evidenced that the broad C-terminal region of HPNAP stimulates neutrophil adhesion and that the AGPs from CGM disrupt the process of neutrophil-endothelial cell attachment caused by HPNAP, an effect that should be further investigated and may be exploited in a future anti-inflammatory therapy for *H. pylori* patients. The HPNAP is one of a number of virulence factors produced by the bacterium *H. pylori*. Free radicals produced by neutrophils are a key component of the innate immune system and an effective antimicrobial agent against *H. pylori* as well as a factor that perpetuates mucosal damage and gastritis. A possible blocking of reactive species production may lead to improvement of *H. pylori* induced chronic gastritis and reduction of signs of inflammation.

A very recent study (Miyamoto *et al*., 2014) examined which component of mastic gum is responsible for anti-*H. pylori* activity. GC–MS analysis of the essential oil of mastic gum led to the identification of 20 components among which α-pinene (82.26 %) was the most abundant. Then, the authors examined which component inhibits the growth of *H. pylori*. Ten commercially available compounds were tested for antibacterial activities against *H. pylori* strains that were established from patients with gastritis, gastric ulcer and gastric cancer. Some of them showed antibacterial activity against clarithromycin (CAM)- and/or metronidazole (MNZ)-resistant strains. α-terpineol and (E)-methyl isoeugenol showed anti *H-pylori* activity not only against drug sensitive strains (#09-292 from gastric cancer) but also against drug resistant strains (#09-87 derived from atrophic gastritis, #09-224 from gastric ulcer, #09-243 from atrophic gastritis). These 10 compounds also showed antibacterial activity against three different strains (*E. coli, S. aureus, B. subtilis*).The authors concluded that these components could be useful to overcome the drug-resistance *H. pylori* growth in stomach

Oral care

Mastic gum was a traditional remedy since antiquity for oral malodour, and oral hygiene and this knowledge has been assessed in more recent studies. Mastic gum showed selective antibacterial action against oral bacteria *Porphyromonas gingivalis* (Sakagami *et al.*, 2009, Sterer 2006) and *Prevotella melaninogenica* (Sakagami *et al.*, 2009).

In vivo activities

Anti-inflammatory & Anti-oxidant properties

Heo *et al*., 2006, studied *in vivo* the effect of mastic in reducing the damage induced by diclofenac bowel and bacterial translocation in rats, a phenomenon caused by non-steroidal anti-inflammatory drugs (NSAIDs) in general.

For this purpose rats were divided into four groups; a control group, diclofenac group, diclofenac with 0.3 ml/kg mastic group and diclofenac with 1.0 ml/kg mastic group. Mastic oils (not further information has been given) were administered 3 hours before diclofenac administration (100 mg/kg orally for 2 days). The parameters they were measured were intestinal permeability, enteric aerobic bacterial counts in the distal ileum and cecum, intestinal adhesion, lipid peroxidation of distal ileum, and bacterial translocation to mesenteric lymph nodes, liver, spleen, kidney and heart, respectively. All parameters which were increased by administration of diclofenac, was found that they were decreased after administration of mastic at a dose of 1 ml/kg weight.

The anti-inflammatory properties of mastic to help reduce intestinal inflammation in inflammatory bowel disease patients were investigated by Kim and Neophytou in 2009. The dextran-sulfate sodium (DSS) model of colitis was used to assay the anti-inflammatory properties of mastic *in vivo*. Two experiments were performed. In the first trial, the animals were fed on diets containing a combination of mastic resin (0.2%) and mastic oil (0.02%) for 14 days; treated with 3% DSS for 5 days then with normal drinking water. After 14 days on the special diet experimental colitis was induced in the mice by treatment with 3% DSS in drinking water for 7-10 days while still receiving mastic or other treatment. In the second trial animals were fed on different diets as follows: Group 1 (control) mice received a normal diet, Group 2 mice received a diet containing 0.02% mastic oil, Group 3 mice received a diet containing 0.30% γ -tocopherol, Group 4 mice received a diet containing 0.02% mastic oil and 0.30% γ -tocopherol.

The animal data indicated that supplementation with **mastic oil** delayed the onset and progression of the disease and it helped prevent weight loss caused by the disease. It was concluded that mastic oil

provides some protection against acute colitis. Using mastic oil in combination with γ-tocopherol gave similar results to using mastic or γ-tocopherol alone.

Anti ulcer activity

In 1986 Al-Said *et al*. conducted an *in vivo* study in guinea-pigs in order to evaluate the effectiveness of mastic against gastric ulcer and duodenal ulcer. For this purpose, with the use of the appropriate chemicals ulcer (aspirin, cysteamin hydrochloride, etc) was formed in the stomach. Afterwards, through their food, mastic was administered to them in the proportion of 500 mg per kg. The results of the study have shown that the administration of Chios mastic has produced an important decrease in the expansion and intensity of the formed ulcer in the gastric membrane of the guinea-pigs, suggesting that it can be used as a treatment of the locally formed ulcer.

Table 3: Overview of the main non-clinical data/conclusions of mastic

Herbal preparation tested	Strength Dosage Route of administration	Experimental model In vivo/In vitro	Reference Year of publication	Main non-clinical conclusions
mastic gum and its essential oil	Several % dises of mastic oil Mastic gum	In vitro, Clostridium botulinum	Daifas et al.,(2004)	0.3% mastic oil required for the inhibition of proteolytic strains of Clostridium botulinum. Mastic gum and mastic oil could potentially be used as natural preservative
mastic essential oil	Not specified	In vitro, Gram-positive (+) and Gram-negative (-) bacteria	Magiatis et al. 1999; Koutsoudaki et al., 2005	antibacterial activity of mastic oil can be attributed to the combination of several components rather than to one particular compound
Mastic gum powder	Not specified	in vitro, H. pylori NCTC 11637 strain and the six clinical isolates strains from patients	Huwez et al., 1998	MBC of mastic required in order to exterminate H. pylori was 60 µg/ml
Mastic gum	2000 to 1.9 µg/ml	In vitro 12 isolated clinical strains of Helicobacter pylori	Marone et al. (2001) and et al	remarkable bactericidal, killing 50% of the executives in concentration of 125 µg/ml and 90% at concentration of 500 µg/ml
Aqueous mastic extracts	Aqueous mastic extracts containing arabino-galactan proteins (no further specification)	In vitro 12 isolated clinical strains of Helicobacter pylori	Bona et al. (2001),	the extracts of at least 1,4 g gum affect the viability of bacterium, preventing cell growth
Total mastic extract without polymer (TMEWP)	Total mastic extract without polymer (TMEWP) (no further specification) and its acidic and neutral fraction	In vitro 10 clinical isolates of H. pylori and the CCUG 38771 reference strain tested over a period of three months	Paraschos et al. 2007	Mastic extracts exhibited concentration- and strain-dependent bactericidal activities. The acidic fraction exhibited the highest activity, with a mean MBC of 0.136 mg/ml, followed by the TMEWP (MBC, 0.256 mg/ml) and the neutral fraction of the TMEWP (0.638 mg/ml)

Herbal preparation tested	Strength Dosage Route of administration	Experimental model In vivo/In vitro	Reference Year of publication	Main non-clinical conclusions
Mastic gum	oral 1 g daily for 1 month	*In vitro* *and in vivo* Incubation in the presence of neutrophil-activating protein *H. pylori* (HP-NAP). Patients carriers s of *H. pylori* compared to healthy volunteers	Kottakis *et al* *et al.*, 2009	Specific binding of arabino-galactan proteins (AGPs) to two membrane proteins of neutrophils, possibly resulting in inhibition of neutrophil activation. Neutrophil activation reduced when incubated *in-vitro* with HP-NAP (P=0.0027) and AGP plus HP-NAP (P=0.0004) in *H. pylori*-positive patients who consumed AGP for two months. Similar results were also obtained when neutrophils were incubated with AGP plus HP-NAP (P=0.0038) but not with HP-NAP (P>0.05) in controls.
Mastic	Not specified	*In vitro* *H. pylori*	*et al.* (2009	most active fractions of mastic was polymer, followed by the acid and the whole resin, while the neutral fragment was inactive.
Mastic gum	Not specified	*In vitro*	Triantafyllou *et al.* 2011	Mastic gum inhibited the activity of purified PKC (Inhibition of protein kinase C), decreased PKC activity in cell homogenate, and attenuated superoxide production in cells stimulated with PKC activator PMA (phorbol 12-myristate 13-acetate) and PKC-dependent angiotensin II in endothelial cells
10 commercially available compounds of mastic oil	Not specified	*In vitro* Inhibition of growth of *H. pylori* strains from patients with gastritis, gastric ulcer and gastric cancer.	Miyamoto *et al*, 2014	Some of them showed antibacterial activity against clarithromycin (CAM)- and/or metronidazole (MNZ)-resistant strains. α-terpineol and (E)-methyl isoeugenol showed anti *H-pylori* activity also against drug resistant strains derived from atrophic gastritis, from gastric ulcer, from atrophic gastritis. These 10 compounds showed antibacterial activity against three different

Herbal preparation tested	Strength Dosage Route of administration	Experimental model In vivo/In vitro	Reference Year of publication	Main non-clinical conclusions
Mastic gum	Not specified	*In vitro* antibacterial action against oral bacteria *Porphyromonas gingivalis* and *Prevotella melaninogenica*	Sakagami *et al.*, 2009, Sterer 2006	strains (*E. coli, S. aureus, B. subtilis*). potential non-toxic local agent in treating oral malodour and gum disease
Mastic oil (no further information)	oral administration of mastic oil 3 hours before diclofenac administration (100 mg/kg orally for 2 days) a) 0.3 ml/kg b) 1.0 ml/kg c) only diclofenac d) control group	*in vivo* in rats	Heo *et al.*, 2006	Reduced intestinal damage induced by diclofenac bowel and bacterial translocation, caused by non-steroidal anti-inflammatory drugs. After administration of mastic at a dose of 1 ml/kg weight, decrease of all parameters which were increased by administration of diclofenac.
Mastic gum and mastic oil	Trial 1) on rats: diets containing mastic (0.2%) and mastic oil (0.02%) for 14 days; treated with 3% DSS for 5 days. After 14 days experimental colitis was induced in the mice with 3% DSS in for 7-10 days while still receiving mastic or other treatment. Trial 2) on mice : Group 1 (control) normal diet, Group 2 : diet containing	*in vivo* dextran-sulfate sodium (DSS) model of colitis Trial 1) in rats Trial 2) in mice	Kim and Neophytou 2009	Supplementation with mastic oil delayed the onset and progression of the disease and it helped prevent weight loss caused by the disease. It was concluded that mastic oil provided some protection against acute colitis. Using mastic oil in combination with γ-tocopherol gave similar results to using mastic or γ-tocopherol alone.

Herbal preparation tested	Strength Dosage Route of administration	Experimental model In vivo/In vitro	Reference Year of publication	Main non-clinical conclusions
	0.02% mastic oil, Group 3 : diet containing 0.30% γ -tocopherol, Group 4 : diet containing 0.02% mastic oil and 0.30% γ -tocopherol			
Mastic	oral administration of 500 mg/ kg	*In vivo* in guinea-pigs with the use of the appropriate chemicals ulcer in the stomach	1986 Al-Said *et al. in vivo* in guinea-pigs (mice),	The results have shown that the administration of Chios Mastic has produced an important decrease in the expansion and intensity of the formed ulcer in the gastric membrane of the guinea-pigs

3.1.2. Secondary pharmacodynamics

Cytotoxic activity

The cytotoxic activities of mastic gum and its major compounds has been reviewed by Giaginis & Theoharis (2011) in *in vitro* studies that have proved that CMG (Chios mastic gum) inhibited cell proliferation of cancer cells derived from several types of human neoplasia including mainly prostate, colon, lung, pancreatic carcinoma and haematological malignancies.

Plasma Lipid and blood sugar reduction

Georgiadis *et al*, 2014 have investigated *in vivo* the effect of crude CMG on metabolic parameters in diabetic mice. Streptozotocin-induced diabetic 12- week-old male C57bl/6 mice were assigned to three groups: NC (n=9) control; LdM (n=9) animals receiving low dose mastic for 8 weeks (20 mg/kg body weight [bw]); and HdM (n=9) animals receiving high dose mastic (500 mg/kg bw) for the same period. Serum lipid and glucose levels were determined at baseline, at 4 and 8 weeks. Serum total protein, adiponectin, and resistin levels were also measured at the end of the experiment. Histopathological examination for liver, kidney, aorta and heart lesions was performed. After 4 weeks, CMG administration resulted in decreased serum glucose and triglyceride levels in both LdM and HdM, whereas bw levels were reduced in LdM group compared with controls. At the end of the experiment, LdM presented significantly lower serum glucose, cholesterol, low-density lipoprotein cholesterol and triglyceride levels and improved high-density lipoprotein cholesterol levels compared with control group. HdM group had ameliorated serum triglyceride levels. Hepatic steatosis observed in control group was partially reversed in LdM and HdM groups. CMG administered in low dosages improves glucose and lipid disturbances in diabetic mice while alleviating hepatic damage.

The *in vivo* hypolipidemic properties of the essential oil of CGM were also evaluated (Vallianou *et al* 2011). The hypolipidemic effect of CGM was investigated in naive as well as in rats susceptible to detergent-induced hyperlipidemia. CGM administration into naive rats resulted in a dose-dependent reduction in the constitutive synthesis of serum cholesterol and triglycerides. In hyperlipidemic rats, CGM treatment had also a strong hypolipidemic effect. By testing various components of mastic essential oil, it was shown for the first time that the hypolipidemic action is associated with camphene. Administration of camphene at a dose of 30 ƒÊg/g of body weight in hyperlipidemic rats resulted in a 54.5% reduction of total cholesterol (p<0.001), 54% of Low Density Lipoprotein (LDL)-cholesterol (p<0.001) and 34.5% of triglycerides (p<0.001). Treatment of HepG2 cells with camphene led to a decrease in cellular cholesterol content to the same extend as mevinolin, a known HMG-CoA reductase inhibitor. The hypolipidemic action of camphene is independent of HMG-CoA reductase activity, suggesting that its hypocholesterolemic and hypotriglyceridemic effects are associated with a mechanism of action different than that of statins. Given the critical role that the control of hyperlipidemia plays in cardiovascular disease, the results of that study provided insights into the use of mastic essential as an alternative lipid lowering agent and merits further evaluation.

Protection against atherosclerosis

The potential of antiatherogenic effect of mastic gum has been also investigated. The biological action of the saliva coming from the chewing of natural CMG, but also the chewing of commercial gums (with synthetic perfumes and artificial antioxidant BHT) was examined in the suspension of oxidation procedure of low-density lipoprotein (oxLDL). The biological activity of the saliva from five different chewing gums, on the inhibition of low density lipoprotein (LDL) oxidation, produced in vitro by copper ions, was demonstrated and quantitatively expressed as % protection (% Pr) (Andrikopoulos et al., 2002). Crude CMG was found to be the most effective (74.6% Pr) followed by commercial CMG (64.3%

Pr). The biologically active substances present in CMG(3 g) extracts and in the respective saliva (1 h chewing) were characterized as (poly)phenolic compounds in quantities of 0.3 and 0.2 mg, respectively. Its protective action was slightly higher even than the respective action of vitamin E that was used as a basis for comparative reasons.

Andrikopoulos *et al*., (2003) also showed that triterpenes present in mastic gum exhibited remarkable antioxidant effect on low-density lipoprotein (LDL). The results of the tests have led to the conclusion that CMG *(Pistacia lentiscus* var. *Chia)* was the most effective natural product of all those that have been examined (*P. terebinthus* resin, dammar resin, acacia gum, tragacanth gum, storax gum) in the protection against the oxidation of human LDL. The minimum and maximum doses for the saturation phenomena of inhibition of LDL oxidation were 2.5 mg and 50 mg CMG (75.3% and 99.9%, respectively).

Dedoussis *et al*. 2004 examined the effect of the total polar extract in the survival of peripheral blood mononuclear cells (PBMC), under oxidant stress conditions, which is created by the oxidized low-density lipoprotein (oxLDL). During the experimental study, the exposition of cells in the oxidized form of LDL, has led to the fast apoptosis and necrosis of the aforementioned cells. It was also investigated the molecular mechanisms through which total polar extract of the resin inhibits oxLDL cytotoxic effect on PBMC. The exposure of cells in the oxidized form of LDL, has led to the fast apoptosis and necrosis of the aforementioned cells. When culturing cells with oxLDL and the polar extract concurrently, inhibition of both the phenomena was observed.

Antioxidant activity

Mastic has been used as a preservative for fats and oils by various people. Such a use by Egyptian villagers, trigger the first study on mastic's antioxidant activity in 70's where Abdel Rahman & Youssef Soad 1975; Abdel-Rahman *et al.*1975 showed that mastic possessed antioxidant activity similar to that of butylated hydroxyanisol. Moreover, the molecular mechanisms of the anti-inflammatory activity and the potential role of antioxidant activity of CMG has been evaluated (Triantafyllou *et al*., 2011) where it was found that GMG inhibited the activity of purified PKC (Inhibition of protein kinase C), decreased PKC activity in cell homogenate, and attenuated superoxide production in cells stimulated with PKC activator PMA (phorbol 12-myristate 13-acetate) and PKC-dependent angiotensin II in endothelial cells.

3.1.3. Safety pharmacology

3.1.4. Pharmacodynamic interactions

Induction of CYP enzymes

Katsanou *et al*. (2014) investigated whether mastic gum modulates expression of Cyp1a1 and Cyp1a2 mRNA (measured by reverse transcription real-time polymerase chain reaction) and Cyp1a-linked ethoxyresorufin O-deethylase (EROD) activity in rat liver following oral administration of CMG extract 1428 and 2000 mg/kg bw. For the evaluation of potential modulation of CYP1A1/ 2 on rat liver for human risk assessment, a well-known bioactive natural compound, caffeine, was studied for detection of potential comparative effects on liver enzymes. Administration of CMG extract at the doses used does not cause significant transcriptional modulation of Cyp1a1/2 and subsequent CYP1A enzyme activity whereas administration of caffeine as a high dose of 100 mg/kg bw induced both the mRNA expression and the enzyme activity. The authors anticipate that administration of CMG extract at doses exceeding the recommended pharmaceutical doses does not modulate the CYP1A-catalyzed metabolic activation of several pro-carcinogens, thus considered to be of no biological or toxicological significance as compared to the respective effects observed after the treatment by caffeine.

3.1.5. Conclusions

3.2. Overview of available pharmacokinetic data regarding the herbal substance(s), herbal preparation(s) and relevant constituents thereof

As the gum of *Pistacia lentiscus* L. (from the island of Chios) is used extensively as a constituent of herbal drugs or in food's area, the oral absorption of its major constituents still remained unclear. In the context of identifying the features of CMG that could be attributed for either therapeutic effects or effects of nutritional value, a methodology based on high-performance liquid chromatography (HPLC) coupled to tandem mass spectrometry (MS/MS) was developed and applied for the quantification of mastic gum triterpenic acids, 24Z-isomasticadienonic acid (IMNA), and 24Z-isomasticadienolic acid (IMLA) in mouse plasma (Lemonakis *et al*. 2011). The specific compounds were selected based on their biological activity and potential against *H. pylori*. Concentrations were determined simultaneously in mouse plasma after oral administration of mastic gum or total mastic extract without polymer (TMEWP) in order to evaluate the role of the natural polymer, poly-β-myrcene, in the absorption process. Following TMEWP administration in mice, circulating IMNA and IMLA plasma levels were significantly higher (approximately 10-fold) in comparison to IMNA and IMLA plasma levels following total CMG administration, suggesting that the polymer plays a critical role in the absorption process. More specifically following TMEWP administration, C_{max} plasma values were 3300 ± 859 ng/ml for IMNA and 163 ± 58 ng/ml for IMLA. In comparison, following CMG administration, C_{max} plasma values were 329 ± 57 ng/ml for IMNA and 28 ± 8 ng/ml for IMLA.

3.3. Overview of available toxicological data regarding the herbal substance(s)/herbal preparation(s) and constituents thereof

3.3.1. Single dose toxicity

No data available.

3.3.2. Repeat dose toxicity

Dietary toxicity of mastic gum was studied in male and female F344 rats (Kang *et al*. 2007) fed 0%, 0.22%, 0.67% and 2% levels mixed into powdered basal diet for 13 weeks. No mortality or obvious clinical signs were observed in any of the animals throughout the experimental period. Body weights were reduced in the high dose-treated group from week 2 to the end in males, and at weeks 8 and 13 in females. There were increased absolute and relative liver weights in a dose-related manner or limited to the high dose group males or females, along with changes in haematological parameters, including increased WBC and platelet in high dose males. Altered serum biochemistry parameters included increases of total proteins, albumin, and total cholesterol in both sexes, and γ-GTP in females only. However, macroscopic examination at necropsy revealed no gross lesions, and microscopic examination also revealed no treatment-related findings in any organs examined. As dietary treatment of mastic gum for 13 weeks in this study caused decreased body weights at the high dose, especially in males, and increased liver weights in a dose-related manner in both genders without any morphological findings, it is concluded that the administration of it has a no observed adverse effect level (NOAEL) of 0.67% in the diet.

3.3.3. Genotoxicity

No data available for mastic resin.

An aqueous extract, called Chios mastic water (CMW) widely used in oral hygiene marketed products, was studied for its potential genotoxic activity, as well as its antigenotoxic properties against the mutagenic agent mitomycin-C (MMC). Genotoxicity was evaluated by employing the *in vitro* Cytokinesis Block MicroNucleus (CBMN) assay and the *in vivo* Somatic Mutation And Recombination Test (SMART). In the former assay, lymphocytes were treated with 1, 2 and 5% (v/v) of CMW with or without MMC at concentrations 0.05 and 0.50 µg/ml.

No significant micronucleus induction was observed by CMW, while co-treatment with MMC led to a decrease of the MMC-induced micronuclei, which ranged between 22.8% and 44.7%. (Vlastos *et al*. 2013)

3.3.4. Carcinogenicity

No data available.

3.3.5. Reproductive and developmental toxicity

No data available.

3.3.6. Local tolerance

No data available.

3.3.7. Other special studies

No data available.

3.3.8. Conclusions

In the 3-month repeat dose study in rats the only significant finding was the decreased body weight in the high-dose group. Consequently the NOAEL value was 0.67% of mastic gum in diet. Otherwise data on toxicity are not available. As only *in vitro* micronucleus and SMART tests with MGW were performed for evaluating genotoxicity, a List Entry is not proposed.

3.4. Overall conclusions on non-clinical data

The *in vitro* studies show the antimicrobial activity of mastic against a panel of Gram-positive and Gram-negative bacteria as well as its particular strong activity against *H, pylori*, with a minimum bacterial concentration (MBC) , i.e. the minimum concentration of mastic required in order to exterminate 99.9% of the *H. pylori* within 24 hours, at 60 µg/ml. These findings together with the results of the *in vitro* antioxidative and anti inflammatory activities give a positive signal to the proposed therapeutic indication of mastic against mild dyspeptic disorders. Moreover, the results of the *in vivo* experimental models give adequate plausibility to the longstanding traditional medicinal use of mastic in the proposed therapeutic indication.

One single study investigated on the pharmacokinetic of two of the most abundant constituents of the mastic gum, the triterpenic acids, 24Z-isomasticadienonic acid (IMNA), and 24Z-isomasticadienolic

acid (IMLA), while other data on pharmacokinetics and interactions are not available. Mastic gum is not an inducer of CYP1A1/2 in rat liver.

Non-clinical information on the safety of mastic gum is not available.

The 13-week repeat dose toxicity study in rats showed no observed adverse effect level (NOAEL) of 0.67% in the diet.

No signals of clinical safety concern during the long-standing use of mastic are raised against medicinal use of mastic oil in the proposed condition of use.

As there is no information on reproductive and developmental toxicity, the use during pregnancy and lactation cannot be recommended.

Adequate studies to evaluate genotoxicity are not available. Tests on reproductive toxicity and carcinogenicity have not been performed.

4. Clinical Data

4.1. Clinical pharmacology

4.1.1. Overview of pharmacodynamic data regarding the herbal substance(s)/preparation(s) including data on relevant constituents

No data available.

4.1.2. Overview of pharmacokinetic data regarding the herbal substance(s)/preparation(s) including data on relevant constituents

No data available.

4.2. Clinical efficacy

No data available.

4.2.1. Dose response studies

No data available.

4.2.2. Clinical studies (case studies and clinical trials)

Prevention of ulcers due to action against *Helicobacter pylori*

Thirty-eight volunteers with symptoms and endoscopic confirmation of duodenal ulcer participated in the first clinical study (Al- Habbal *et al*. 1984]) in the University clinic of Mosul University in Iraq.. For the comparison of the effectiveness of Chios mastic, the volunteers were divided in two groups: group 1 consumed Chios mastic for two weeks (1 g per day) and group 2 consumed the same dosage of placebo powder (lactose) for the same time period. After the lapse of two weeks all the volunteers were endoscopically examined, in order to see the progress of the ulcer. The results showed that in the group that consumed mastic there was an alleviation of the symptoms in 80% of the cases, while the endoscopic examination has confirmed that duodenal ulcer was cured in 70% of the cases. The conclusions of the clinical study recommend mastic as drastic element for the alleviation and the

treatment of ulcer symptoms. Another important conclusion of the research was that the use of Chios mastic produced no unwanted side-effect.

The same research team has published (Huwez *et al*. 1986) the findings of a new clinical study in patients who suffered of gastric ulcers, of benign nature. For this purpose, Chios Mastic was administered in the dosage of 2 g per day for four weeks (1 g before breakfast and 1 g before sleeping at night) to 6 patients with gastric ulcer diagnosed by means of gastroscopy. No patient was administered another type of pharmaceutical treatment, for a time period of at least two months before the initiation of the clinical study. For the evaluation of the action of mastic gastroscopies were conducted as well as routine laboratory controls in the blood, urine and other biochemical parameters, before the initiation of the treatment, two weeks after, four weeks after and two months after the initiation of mastic administration. The results of the study have shown that the administration of mastic has relieved all six patients that participated in the search from the symptoms, while the treatment was even endoscopically confirmed in five of them. During the study, but also two months after its completion no type of unwanted effect was found or any unusual result in the laboratory analysis.

In 2002 Roe *et al*. published a clinical study concerning the action of mastic against gastritis caused by *H. pylori* in the *South Korea Society of Gastroenterology* magazine. Forty-eight volunteers found to be infected by *H. pylori* using UBT test (UREA BREATH TEST technique for detecting *H. pylori),* participated to the study that was conducted by the Medical School of Dan-kook University in South Korea. The participants were divided in two groups: the first group used Chian chewing gum for 90 days, while the second one used placebo gum. The UBT test was applied on patients before the initiation of the study as well as in intervals of 30 and 90 days following the completion. The results of the tests showed that the use of mastic is especially effective in limiting the concentration of *H. pylori* as well as the gastritis due also to *H. pylori*, reaching the conclusion that it can be used as an additional means for containing the bacterium and its effects.

In 2009 Kottakis *et al*. investigated the effects of mastic gum (from the island of Chios) on innate cellular immune effectors. The *in-vivo* effect of AGPs/CMG under the presence of *HP*-NAP in neutrophil activation was investigated in five (5) *H. pylori*-infected patients and (3) three healthy volunteers who received 1 g daily consumption of CMG for 2 months. All participants did not receive any immunosuppressive medication before or during the trial; patients with infectious diseases that could modify their immunologic status were excluded. *In-vitro* studies with pull-down experiments to assess the effect of AGPs/CMG under the presence of *HP*-NAP on the neutrophil activation were also carried out. Neutrophil activation was estimated by nicotinamide adenine dinucleotide phosphate-oxidase assays and optical microscopy methods by measurement of cytochrome C reduction. The studies suggested that the AGPs/CMG inhibit neutrophil activation in the presence of *HP*-NAP, playing a crucial role in *H. pylori*-associated pathologies in gastric mucosa.

Recent studies (Dabos *et al*. 2010i;) have shown that CMG provided relief from heartburn to people who suffer from gastroesophageal reflux disease (GERD), providing symptomatic relief of pain in the stomach area but also in patients with epigastric pain. In the same study 54 patients obtain 1 g of CMG over three weeks. The results showed that: 75% of patients showed recession of symptoms. Symptoms such as stomach pain, heartburn and belching improved significantly.

Interesting are the results of Dabos *et al*. (2010ii), which was designed to evaluate the effect of mastic gum on *H. pylori in vivo*. (52) Fifty-two patients participated in the clinical trial. They were randomized to receive either 350 mg three times a day (tid) of pure mastic gum for 14 days (Group A), or 1.05 g of pure mastic gum (Group B) for 14 days, or pantoprazole 20 mg twice a day (bd) plus pure mastic gum 350 mg tid for 14 days (Group C) or pantoprazole 20 mg bid plus amoxicillin 1 g bid plus

clarithromycin 500 mg bid for 10 days (Group D). They were also asked to keep a log of adverse events. *H. pylori* eradication was tested 5 weeks after completion of the eradication regime. The results showed that mastic gum has some effect on *H. pylori in vivo*. Nine patients in the monotherapy groups achieved eradication while in ten more patients the urea breath test (UBT) value decreased compared to the pre-treatment reading. Eradication of H. *pylori w*as confirmed in 4/13 patients in Group A and in 5/13 in Group B. No patient in Group C achieved eradication whereas 10/13 patients in Group D had a negative result. There were no statistically significant differences between Groups A, B, C although there was a trend in Group A (p= 0.08) and in Group B (p =0.064). The difference was significant in Group D (p= 0.01). The combination of mastic gum and pantoprazole was ineffective in eradicating *H. pylori* or did it have effect on bacterial load either. The surprising failure of combination gum and pantoprazole probably is explained by the fact that the pump inhibitors protons increase the pH of the stomach, thereby decreasing the possible activity of acidic components of mastic, which is probably due to the anti-*H. pylori* action. It was concluded that although even the high dose monotherapy did not achieve acceptable eradication rates it could be used as an alternative regime in patients unwilling to undergo eradication with the triple therapy regime. All patients tolerated mastic gum well and no serious adverse events were reported.

Recently Kaliora *et al*. 2007 and 2007ii clinical trials made by the Harokopio University of Athens in collaboration with the Gastroenterology Department of the General State Hospital Nicea proved that a dose of 2.2 g/day of mastic gum from Chios island (CMG), equal to ≈ 40mg/Kg body weight, improves the clinical status of patients with Crohn's disease of mild to moderate severity as well as better laboratory indexes. Results from a recent study by the same research group, prove the anti-inflammatory and antioxidant properties of mastic in Idiopathic Inflammatory Bowel Disease IBD (Gioxari *et al*. 2011; Papalois *et al*. 2012). In the same study CMG showed to be active as a whole molecule rather than its individual fractions in order to reduce inflammation via NF-κB regulation.

Relief of Gastro oesophageal reflux disease and functional dyspepsia

In a clinical study of Dabos *et al*, 2010i the symptoms of gastro-esophageal reflux disease were evaluated after treatment with mastic gum. Patients received 350 mg mastic gum three times daily or placebo. After 3 weeks of treatment the severity of the symptoms was assessed using the Hong Kong index of dyspepsia. Patient's global assessment of efficacy was also evaluated. At the placebo group 32 patients had either acid reflux or heartburn or both and 32 patients in the treatment arm had either acid reflux or heartburn or both. Only 6 patients in the placebo group showed some improvement of their symptoms at the end of the study period, whereas 25 patients in the treatment group reported benefit from the treatment (p<0.01).

In 2010ii Dabos *et al*., performed the first double-blind placebo controlled trial to assess the effects of CMG in functional dyspepsia. The study was conducted in Chios District General Hospital Skylitsion, Chios, Greece. One hundred and forty eight patients (**148**) fulfilling Rome II criteria for functional dyspepsia were randomly assigned to receive either CMG 350 mg three times daily or placebo. After 3 weeks of treatment the change from baseline in the severity of symptoms of functional dyspepsia was assessed using the Hong Kong index of dyspepsia (HKID). The following twelve dyspepsia symptoms on a five point scale each (0 for absent, 1 for mild, 2 for moderate, 3 for severe and 4 for very severe): stomach pain in general, bloating of the upper abdomen, dull ache of the upper abdomen, stomach pain before meals, stomach pain when anxious, vomiting, nausea, belching, acid regurgitation, heartburn, acidity in the stomach, loss of appetite. Patients' global assessment of efficacy was also evaluated at the end of the 3 weeks' trial period. The symptom score after treatment was significantly lower in the CMG than in the placebo group ((14.78+/-1.78) vs (19.96+/-1.83)) (p<0.05). There was significant improvement in the actively treated group ((23.68±1.64) vs

(14.78±1.78)) (p < 0.03). There was no significant improvement in the placebo group ((23.27±1.73) vs 19.96±1.69)) (p = 0.23). With regards to patients' own global assessment of efficacy, 40% (30/74) of patients showed improvement on the placebo arm, while 77% (57/74) of patients in the active treatment group showed improvement of symptoms (p < 0.02). Individual symptoms that showed significant improvement with CMG were: stomach pain in general, stomach pain when anxious, dull ache in the upper abdomen and heartburn (<0.05 for all four symptoms). It was proved that CMG significantly improved the perception of symptoms in patients with functional dyspepsia over 3 weeks of treatment compared to placebo. The table that follows shows the results for the twelve individual symptom scores that form part of the HKID.

There were no significant differences in symptoms' improvement in eight of the twelve symptoms. Differences in stomach pain in general (1.05±0.05 vs 0.43±0.03), stomach pain when anxious (0.91±0.06 vs 0.33±0.04), heartburn (0.77±0.03 vs 0.21±0.01) and dull ache in the upper abdomen (0.87±0.05 vs 0.23±0.03) were significantly in favour of the treatment group (p < 0.05 for all four symptoms). CMG was well tolerated by the patients.

Crohn's Disease

A study (Kaliora et al., 2007i and 2007ii) was performed in order to assess the effects of mastic on patients with Crohn's disease. The study was conducted in patients with established mildly to moderately active Crohn's disease and in healthy controls. Ten patients and 8 controls, recruited to a 4 week treatment with mastic caps (6 caps/d, 0.37 g/cap). Interleukin-6 (IL-6), tumour necrosis factor-alpha (TNF-alpha), monocyte chemotactic protein-1 (MCP-1), macrophage migration inhibitory factor (MIF) and intracellular antioxidant glutathione (GSH) were evaluated in peripheral blood mononuclear cells (PBMC) before and after treatment. Treating CD patients with mastic resulted in the reduction of TNF-alpha secretion (2.1 +/- 0.9 ng/ml vs 0.5 +/- 0.4 ng/ml, P = 0.028). MIF release was significantly increased (1.2 +/- 0.4 ng/ml vs 2.5 +/- 0.7 ng/ml, P = 0.026) meaning that random migration and chemotaxis of monocytes/macrophages was inhibited. No significant changes were observed in IL-6, MCP-1 and GSH concentrations. The study showed that mastic acts as an immunomodulator on PBMC, acting as a TNF-alpha inhibitor and a MIF stimulator. This finding provides strong evidence that mastic might be an important regulator of immunity in Crohn's disease.

They also assessed the effects of mastic administration on cytokine production of circulating mononuclear cells of patients with active Crohn's disease (Kaliora et al., 2007B). Interleukin-6 (IL-6), tumor necrosis factor-alpha (TNF-alpha), monocyte chemotactic protein-1 (MCP-1), macrophage migration inhibitory factor (MIF) and intracellular antioxidant glutathione (GSH) were evaluated in peripheral blood mononuclear cells (PBMC) before and after treatment. Treating CD patients with mastic resulted in the reduction of TNF-alpha secretion (2.1 +/- 0.9 ng/ml vs 0.5 +/- 0.4 ng/ml, P = 0.028). MIF release was significantly increased (1.2 +/- 0.4 ng/ml vs 2.5 +/- 0.7 ng/ml, P = 0.026) meaning that random migration and chemotaxis of monocytes/macrophages was inhibited. No significant changes were observed in IL-6, MCP-1 and GSH concentrations.

It was proven that mastic acts as an immunomodulator on PBMC, acting as a TNF-alpha inhibitor and a MIF stimulator. Although further double-blind, placebo-controlled studies in a large number of patients is required to clarify the role of this natural product, this finding provides strong evidence that mastic might be an important regulator of immunity in Crohn's disease.

Wound healing activity

The adhesive properties of mastic, as well as its beneficial presence in healing wounds and post-operative incisions have been identified and studied by researchers for at least twenty years now. The natural resin of mastic is used very often as ingredient in bandages, plasters, compresses and other

healing means, applied in the protection and healing of wounds or postoperative incisions. The results of relevant publications show, that CMG (mastic gum of Chios presents exceptional adhesive properties, when used in covering means and wound and incisions healing means, while at the same time, it contributes to the effective regeneration of the skin and to the wound healing, while it does not have undesirable side-effects on the skin (irritation, itching, dermatitis, skin depigmentation, etc), as the conventional ingredients used in healing means.

Since 1986 and 1989 in relevant studies (Mikhail *et al*. 1986; 1989) published a comparison made of the adhesive properties of three categories of bandages. In the first category the adhesive bandages did not contain any additional ingredient, in the second category the bandages contained additionally the widely used ingredient for such applications: benzoin, USP and in the third category Chios mastic solution (no further specification?) was used as reinforcing means. The specific study has shown that despite the use of benzoin, USP in the bandages has resulted in improving their adhesive properties; the use of mastic has brought an even more impressive improvement, confirming that Chios Mastic can be used with great success in the specific application.

In a relevant study (Lesesne 1992) a comparison was made between the adhesive properties, as well as of the undesirable effects of mastic as an ingredient of the adhesive bandages, and benzoin, USP. The study has been applied to 300 volunteers (100 men and 200 women), who were submitted to plastic surgeries. The volunteers were divided in two groups: in the first group adhesive bandages with benzoin, USP were applied, while in the second group bandages with Chios Mastic ingredient were used. The volunteers were examined postoperatively after a period of 6 days, 1, 3, 6 and 12 months. The evaluation was based on elements such as: the attentive study of the condition of the wound, the appearance of infections, the effluence of the wound, the depigmentation and irritation of the skin, as well as the premature loss of the adhesive properties of the bandage. The findings of the specific study have reached the conclusion that CMG (mastic gum of Chios offers not only exceptional adhesive properties to the healing means, as compared to benzoin, USP, but also it has an advantage over this latter one, in that it has exceptionally lower possibility of problems to arise due to dermatitis, and irritation or depigmentation of the skin. The results of the specific study were confirmed in a relevant announcement (Yavuzer *et al*. 2005) by another research team, which reaches the conclusion that mastic substantially increases the adhesive action of the self-adhesive bandages, when these are used as the only means for covering wounds and incisions.

Oral hygiene

In another study chewing mastic gum (MG) was proven to significantly reduce plaque index and gingival index. These results suggested that CMG is a useful antiplaque agent and that chewing CMG reduces both bacterial growth in saliva and plaque formation on teeth (Takahashi *et al*., 2006).

In other studies mastic gum exhibited antibacterial activity against *Streptococcus mutans* (Aksoy *et al*., 2006 and *mutans streptococci* both *in vitro* and *in vivo*, lactobacilli, and total cultivated bacteria (Aksoy *et al*., 2006). The results showed that chewing mastic gum decreased the total number of viable bacteria, *S. mutans* and *lactobacilli* in saliva. Therefore, chewing CMG might be useful in preventing caries.

A relevant clinical study (Topitsoglou-Themeli *et al*. 1984; 1985), which was conducted in the Dental faculty of the Univ of Thessaloniki, Greece in 1985, demonstrated that if mastic from Chios is used systematically, it may result in important decrease in the amount of formatting or already formatted dental plaque. In this study ten (**10**) volunteer students participated, with low caries rate and were divided in two groups: the first of which chewed CMG for ten days, while the second chewed a placebo chewing gum. The results of the study have confirmed that in the group that used CMG the amount of

microbial plaque was largely diminished and hence it can be used effectively in the prevention of caries, peridental disorders and buccal cavity diseases in general.

A similar clinical study (Fukazawa *et al*. 2001; Takahashi, *et al*. 2003), that was published in 2003 in *Journal of Periodontology* magazine, conducted by Dental Faculty of the University of Meikai in Japan, examined the action of chewing gum with natural CMG against the bacteria of saliva and the buccal cavity in general. For this purpose 20 orally healthy volunteers participated, who were divided in two groups. The first group used CMG, while the second one used a placebo gum. In the saliva that was concentrated, before and after the chewing, the total number of bacterial colonies were identified and compared. At the same time, before and after the systematic chewing for 7 days, in the two groups the level of gingivitis – dental plaque as well as gum irritation degree – were studied. The results led to the conclusion that CMG leads to suspension of bacterial development in the buccal cavity, responsible for causing peridental diseases as well as the formation of dental plaque. At the same time, CMG led to a significantly lower degree of gum irritation, in comparison with placebo gum, confirming that it constitutes a drastic and safe means for improving oral hygiene.

Recently, Aksoy *et al*. 2006 from three universities of Turkey published in *Archive of Oral Biology* magazine a study that investigates the *in vitro*, as well as *in vivo* action of mastic against pathogenic bacteria of *streptococcus mutans* family, which constitute one of the most basic reasons for the appearance of the caries and diseases of the buccal cavity in general. For the laboratory study of the antimicrobial action of mastic (*in vitro*) model *streptococcus mutans* samples wereused. Respectively, the clinical study was applied in 25 peridentally healthy volunteers, who were divided in two groups: the first group consisted of those who used mastic and the second of those who used placebo gum for comparative reasons. The appraisal of the effectiveness of mastic in limiting *streptococcus mutans* development, has been conducted by comparing samples of saliva that were taken from the two groups of volunteers before and after 15, 45, 75, 105 and 135 minutes from the moment they started chewing mastic and the placebo gum. In each of the aforementioned five intervals it was discovered that in the saliva samples of the volunteers of mastic group there was an important, gradual decrease (15 minutes: 37%, 45 minutes:48.5%, 75 minutes:56.7%, 105 minutes:62.7%) of the total population of bacteria that reached 62.1% after 135 minutes of chewing. On the contrary, in the case of placebo gum group, there was no type of containment of the bacteria population. In the conclusions of the study it is established that mastic presents an exceptionally interesting antibacterial action, which can be compared to the action of antibiotics (vancomycin) in the case of S*treptococcus mutans*. This action of mastic appears as especially important, as it concerns the limitation of the frequent and dangerous bacteria of the mouth, *streptococcus mutans*, which are responsible for the decalcification of the enamel of the teeth, also responsible for a number of surface diseases of the denture. The results of the study reach the conclusion that the frequent use of mastic constitutes an important factor (natural chewing gum) in improving oral hygiene, always in combination with the frequent teeth brushing.

In another recent study (Watanabe *et al*. 2010) it was also proved the useful effect of the mastic essential oil for chronic periodontitis.

Plasma Lipid and blood sugar reduction

Triantafyllou *et al.* (2011) investigated the effect of mastic powder in reducing plasma lipids and glucose levels. Subjects (n=133, aged over 50) were randomly assigned to two groups, the first (high-dose group) ingesting daily 5 g of mastic powder and the second receiving daily a Chios mastic solution (low-dose group). Serum biochemical parameters were determined on a monthly basis for an 18-month (high-dose group) and a 12-month (low-dose group) follow-up period. Generalized least squares random-effects linear regression was performed. The group ingesting Chios mastic powder

(high-dose group) exhibited a decrease in serum total cholesterol, LDL, total cholesterol/HDL ratio, lipoprotein (a), apolipoprotein A-1, apolipoprotein B (apoB/apoA-1 ratio did not change), SGOT, SGPT and gamma-GT levels. A decrease in glucose levels in males in the second (low-dose) group was observed.

Type (aim) and objective(s) of Study Reference	Study Design and Type of Control Study duration (if available)	Test Product(s): herbal preparation, pharmaceutical form; Dosage Regimen; Route of Administration Duration of treatment	Number of Subjects (including age, sex, drop out)	Healthy Subjects or Diagnosis of Patients (inclusion criteria)	Outcomes (primary and secondary endpoints)	Statistical analysis (e.g. ITT yes/no, CI 95%) Quality score e.g. Jadad score	Comments on clinical relevance of results
Al- Habbal et al. 1984 in the University clinic of Mosul University in Iraq	Uncontrolled	Mastic for **two weeks** (1 g per day)	**38** volunteers were divided in two groups: in those that consumed Chios and those that consumed the same dosage of placebo powder (lactose) for the same time period		After the lapse of **two weeks** all the volunteers were endoscopically examined, in order to see the progress of the ulcer.		The results showed that in group consumed mastic there was an alleviation of the symptoms in 80% of the cases, while the endoscopic examination confirmed that duodenal ulcer was cured in 70% of the cases. The use of mastic produced no unwanted side-effect
Huwez et al. 1986	Uncontrolled	**where mastic** was administered in the dosage of **2 g per day for four weeks** (1 g before breakfast and 1 g before sleeping at night) for **two months.**	**6**	patients to whom gastric ulcer was diagnosed by means of gastroscopy, Chios	For the evaluation of mastic, gastroscopies were conducted as well as routine laboratory controls in the blood, urine, and other biochemical parameters, before the initiation of the		The results have shown that the administration of mastic has relieved all six patients from the symptoms, while the treatment was even endoscopically confirmed in five of them. During the study, but also two months after no type of

Type (aim) and objective(s) of Study Reference	Study Design and Type of Control Study duration (if available)	Test Product(s): herbal preparation, pharmaceutical form; Dosage Regimen; Route of Administration Duration of treatment	Number of Subjects (including age, sex, drop out)	Healthy Subjects or Diagnosis of Patients (inclusion criteria)	Outcomes (primary and secondary endpoints)	Statistical analysis (e.g. ITT yes/no, CI 95%) Quality score e.g. Jadad score	Comments on clinical relevance of results
Roh et al. 2002		The participants were divided in two groups the first of which used Chian chewing gum for **90** days, while the second one used placebo gum	**48** volunteers participated, and it was discovered that they were infected by the *helicobacter,*	The UBT test was applied on patients before the initiation of the study as well as in intervals of **30** and **90** days following the completion	treatment, two weeks after, four weeks after and two months after the initiation of mastic administration.		The results have shown that the use of mastic was effective in limiting the concentration of *helicobacter pylori* as well as the gastritis due also to helicobacter
2009 Kottakis et al.		The participants who received **1 g** daily consumption of CMG for **2** months.	in five (**5**) *H. pylori*-infected patients and (**3**) three healthy volunteers			unwanted effect was found, nor any unusual result in the lab analysis.	The studies suggested that the AGPs/CMG inhibit neutrophil activation in the presence of *HP-NAP*, playing a crucial role in *H. pylori*-associated

Type (aim) and objective(s) of Study Reference	Study Design and Type of Control Study duration (if available)	Test Product(s): herbal preparation, pharmaceutical form; Dosage Regimen; Route of Administration Duration of treatment	Number of Subjects (including age, sex, drop out)	Healthy Subjects or Diagnosis of Patients (inclusion criteria)	Outcomes (primary and secondary endpoints)	Statistical analysis (e.g. ITT yes/no, CI 95%) Quality score e.g. Jadad score	Comments on clinical relevance of results
Kaliora et al. 2007		**2.2g/day** of Chios Mastiha, equal to ≈ 40mg/Kg body weight	**54** patients obtain	people who suffer from *Gastroesophageal reflux disease (GERD)*, providing symptomatic relief of pain in the stomach area but also in patients with epigastric pain			Symptoms such as *stomach pain, heartburn and belching improved significantly.*
Dabos et al. 2010i; 2010ii		**1** g of Chios Mastiha over **three weeks**					pathologies in gastric mucosa. 75% of patients showed recession of symptoms.;
Dabos et al. (2010ii),	double-blind placebo	350 mg three times a day (tid) of pure mastic gum for 14 days (Group A), or 1.05 g of pure mastic gum	One hundred and forty eight patients (148) fulfilling Rome II criteria were randomly assigned to receive either Chios mastic gum 350 mg three times daily or placebo	fulfilling Rome II criteria for functional dyspepsia	After 3 weeks of treatment the change from baseline in the twelve individual symptom scores that form part of the HKID. No	The table that follows shows the results for the actively treated group ((23.68±1.64) vs (14.78±1.78)) (p < 0.03). There was no significant improvement in the	There was significant improvement in the

Type (aim) and objective(s) of Study Reference	Study Design and Type of Control / Study duration (if available)	Test Product(s): herbal preparation, pharmaceutical form; Dosage Regimen; Route of Administration Duration of treatment	Number of Subjects (including age, sex, drop out)	Healthy Subjects or Diagnosis of Patients (inclusion criteria)	Outcomes (primary and secondary endpoints)	Statistical analysis (e.g. ITT yes/no, CI 95%) Quality score e.g. Jadad score	Comments on clinical relevance of results
		(Group B) for 14 days, or pantoprazole 20 mg twice a day (bd) + mastic 350 mg tid for 14 days (Group C) or pantoprazole 20 mg bid + amoxicillin 1 g bid + clarithromycin 500 mg bid for 10 days (Group D).			was assessed using the Hong Kong index of dyspepsia (HKID). The following twelve dyspepsia symptoms on a five point scale each (0 for absent, 1 for mild, 2 for moderate, 3 for severe and 4 for very severe):	significant differences in symptoms' improvement in 8 of the 12 symptoms. Differences in stomach pain in general (1.05±0.05 vs 0.43±0.03), stomach pain when anxious (0.91±0.06 vs 0.33±0.04), heartburn (0.77±0.03 vs 0.21±0.01) and dull ache in the upper abdomen (0.87±0.05 vs 0.23±0.03) were	placebo group ((23.27±1.73) vs 19.96±1.69) (p = 0.23). With regards to patients' own global assessment of efficacy, 40% (30/74) of patients showed improvement on the placebo arm, while 77% (57/74) of patients in the active treatment group showed improvement of symptoms (p < 0.02). Individual symptoms that showed significant improvement with Chios mastic were: stomach pain in general, stomach pain when anxious, dull ache in the upper abdomen and heartburn (<0.05 for all four symptoms). It was

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					significantly in favour of the treatment group of symptoms in patients with functional dyspepsia (p < 0.05 for all four symptoms). Mastic well tolerated by the patients.	proved that mastic improved the perception over 3 weeks of treatment compared to placebo.	
Kaliora et al., 2007i	Controlled study Crohn's Disease	The study was conducted in patients with established mildly to moderately active Crohn's disease and in healthy controls 6 caps/d, 0.37 g/cap	**10** patients and **8** controls, recruited to a **4** week treatment with mastic caps (6 caps/d, 0.37 g/cap				Treating CD patients with mastic resulted in the reduction of TNF-alpha secretion (2.1 +/- 0.9 ng/ml vs 0.5 +/- 0.4 ng/ml, P = 0.028). MIF release was significantly increased (1.2 +/- 0.4 ng/ml vs 2.5 +/- 0.7 ng/ml, P = 0.026) meaning that random migration and chemotaxis of monocytes/macrophages

Type (aim) and objective(s) of Study Reference	Study Design and Type of Control Study duration (if available)	Test Product(s): herbal preparation, pharmaceutical form; Dosage Regimen; Route of Administration Duration of treatment	Number of Subjects (including age, sex, drop out)	Healthy Subjects or Diagnosis of Patients (inclusion criteria)	Outcomes (primary and secondary endpoints)	Statistical analysis (e.g. ITT yes/no, CI 95%) Quality score e.g. Jadad score	Comments on clinical relevance of results
							was inhibited. No significant changes were observed in IL-6, MCP-1 and GSH concentrations.
Mikhail et al. 1986; 1989; Yavuzer et al. 2005	Wound healing activity, adhesive properties	The volunteers were divided in two groups: In the first group adhesive bandages with benzoin, USP were applied, while in the second group bandages with Chios mastic ingredient were used, volunteers were examined postoperatively after a period of 6 days, 1, 3, 6 and 12 months	300 volunteers (100 men and 200 women),	Volunteers who were submitted to plastic surgeries, examined postoperatively after a period of 6 days, 1, 3, 6 and 12 months of mastic	The evaluation was based on elements such as: the condition of the wound, the appearance of infections, the effluence of the wound, the depigmentation and irritation of the skin, as well as the premature loss of the adhesive properties of the bandage.		Mastic contributes to the effective regeneration of the skin and to the wound healing, with no undesirable side-effects on the skin (irritation, itching, dermatitis, skin depigmentation, etc), as the conventional ingredients used in healing means.
Topitsoglou-Themeli *et al.*	dental plaque	Ten (10) volunteer, with	In this study ten (10) volunteer				The results confirmed that in the group that used

Type (aim) and objective(s) of Study Reference	Study Design and Type of Control Study duration (if available)	Test Product(s): herbal preparation, pharmaceutical form; Dosage Regimen; Route of Administration Duration of treatment	Number of Subjects (including age, sex, drop out)	Healthy Subjects or Diagnosis of Patients (inclusion criteria)	Outcomes (primary and secondary endpoints)	Statistical analysis (e.g. ITT yes/no, CI 95%) Quality score e.g. Jadad score	Comments on clinical relevance of results
1984; 1985		low caries rate in two groups: the first of which chewed mastic for 10d, while the second chewed a placebo.					Mastic the amount of microbial plaque was decreased
Triantafyllou et al. 2007	Plasma Lipid and blood sugar reduction	The subjects were randomly assigned to 2 groups, the first (high-dose group) ingesting daily 5g of mastic powder and the second receiving daily a Chios mastic solution (low-dose group	n=**133**, aged over 50 years of age	Serum biochemical parameters were determined on a monthly basis for an 18-month (high-dose group) and a 12-month (low-dose group) follow-up period	Generalized least squares random-effects linear regression was performed		The mastic group i (high-dose group) exhibited a decrease in serum total cholesterol, LDL, total cholesterol/HDL ratio, lipoprotein (a), apolipoprotein A-1, apolipoprotein B (apoB/apoA-1 ratio did not change), SGOT, SGPT and gamma-GT levels. A decrease in glucose levels in males in the second (low-dose) group was observed

4.3. Clinical studies in special populations (e.g. elderly and children)

No data available.

4.4. Overall conclusions on clinical pharmacology and efficacy

The results have shown that the use of mastic was effective in limiting the concentration of *H. pylori* as well as the gastritis due also to *Helicobacter* as well as for the relief of symptoms such as *stomach pain, heartburn and belching.* In the only double blind placebo control clinical study (Dabos *et al*. (2010ii), to one hundred and forty eight patients (148) where 350 mg three times a day (tid) of pure mastic gum for 14 days (Group A), there was significant improvement in the actively treated group ((23.68±1.64) vs (14.78±1.78)) (p < 0.03). There was no significant improvement in the placebo group ((23.27±1.73) vs 19.96±1.69)) (p = 0.23). With regards to patients' own global assessment of efficacy, 40% (30/74) of patients showed improvement on the placebo arm, while 77% (57/74) of patients in the active treatment group showed improvement of symptoms (p < 0.02). Individual symptoms that showed significant improvement with mastic from the island of Chios were: stomach pain in general, stomach pain when anxious, dull ache in the upper abdomen and heartburn (<0.05 for all four symptoms). It was proved that mastic improved the perception of symptoms in patients with functional dyspepsia over 3 weeks of treatment compared to placebo.

Mastic was also clinically tried cutaneously (in 300 patients) for a period of time up to one year with generally positive results for its healing skin activities and no adverse reaction reported. Analytically 100 men and 200 women, volunteers who were submitted to plastic surgeries, being divided in two groups: in the first group adhesive bandages with benzoin, USP were applied, while in the second group bandages with Chios mastic ingredient were used, volunteers were examined postoperatively after a period of 6 days, 1, 3, 6 and 12 months. The evaluation was based on elements such as the condition of the wound, the appearance of infections, the effluence of the wound, the depigmentation and irritation of the skin, as well as the premature loss of the adhesive properties of the bandage. Mastic appeared to contribute to the effective regeneration of the skin and to the wound healing, with no undesirable side-effects on the skin (irritation, itching, dermatitis, skin depigmentation, etc), as the conventional ingredients mainly used in healing means.

In conclusion the existing results of the clinical trials support adequately both the proposed therapeutic indications in mild dyspeptic disorders as well as for the symptomatic treatment of minor inflammations of the skin and as an aid in healing of minor wounds

5. Clinical Safety/Pharmacovigilance

5.1. Overview of toxicological/safety data from clinical trials in humans

No data available.

5.2. Patient exposure

Aside from the known use in folk medicine and data from studies, there are no concrete data concerning patient exposure.

According to the above referred clinical trials patients (>350) have been exposed orally for a period of time from two weeks up to two months (8 weeks) taking a daily dose 6 x 370 mg up to 2.2 g with no signs of adverse effects.

Mastic was also clinically tried cutaneously (in 300 patients) for a period of time up to one year with generally positive results for its healing skin activities and no adverse reaction reported

5.3. Adverse events, serious adverse events and deaths

Adverse events, serious adverse events and deaths have not been reported so far.

Two case reports described allergic contact dermatitis (ACD) from medical adhesive bandages containing a commercial liquid adhesive combination product with mastic gum (Mastisol) (Worsnop *et al*. 2007; Meikle *et al*. 2012)

5.4. Laboratory findings

No data available.

5.5. Safety in special populations and situations

No data available.

5.5.1. Use in children and adolescents

No data available.

5.5.2. Contraindications

Hypersensitivity to the active substance.

5.5.3. Special Warnings and precautions for use

To ensure a safe use the following statement should be labelled:

If adverse reactions occur, a doctor or a qualified health care practitioner should be consulted.

5.5.4. Drug interactions and other forms of interaction

Drug interactions from clinical trials or case studies have not been reported so far.

5.5.5. Fertility, pregnancy and lactation

No fertility data available.

Safety during pregnancy and lactation has not been established. In the absence of sufficient data, the use during pregnancy and lactation is not recommended

5.5.6. Overdose

No data available.

5.5.7. Effects on ability to drive or operate machinery or impairment of mental ability

No data available.

5.5.8. Safety in other special situations

Not applicable.

5.6. Overall conclusions on clinical safety

No adverse events, serious adverse events or deaths as well as no drug interactions from clinical trials or case studies have been reported so far.

Two case reports described allergic contact dermatitis (ACD) from medical adhesive bandages containing a commercial liquid adhesive combination product with mastic gum

6. Overall conclusions (benefit-risk assessment)

There are insufficient data to support the "well-established medicinal use" of *Pistacia lentiscus* L. resin that has never been authorized in Europe as a medicinal product" and therefore the requirement of 10 years of marketing authorization is not fulfilled.

However the powder of *Pistacia lentiscus* L. resin, mainly Chios mastic has been used in folk-medicine in several countries all over the world (Iraq, Turkey, Japan, S, Korea, USA, etc) as well as in the European Union (Greece) for more than 30 years, Based on the available data from various literature sources the legal requirements for traditional use are met for the traditional medicinal use of mastic resin powdered.

Results of non-clinical *in vitro* and *in vivo* studies, which show the antimicrobial activity of mastic against a panel of Gram-positive and Gram-negative bacteria, its particular strong activity against *H, pylori* (MBC 60 µg/ml) and the *in vitro* antioxidative and anti inflammatory activities, complemented by the results of clinical studies, provide adequate plausibility to the longstanding traditional medicinal use of mastic resin powderedin the following indications, even though the well established use cannot be considered:

Indication 1)

Traditional herbal medicinal product used in mild dyspeptic disorders.

In the absence of data in children and adolescents under the age of 18 years, mastic should not be used in this target population and should be limited to adults and elderly

Indication 2)

Traditional herbal medicinal product used for the symptomatic treatment of minor inflammations of the skin and as an aid in healing of minor wounds.

The use in children under 12 years of age has not been established due to lack of adequate data.

No fertility data are available.

Safety during pregnancy and lactation has not been established. In the absence of sufficient data, the use during pregnancy and lactation is not recommended

According to the results of different clinical studies, no adverse reactions have been reported and the herbal preparation was well tolerated.

So far adverse events, serious adverse events and deaths as well as drug interactions from clinical trials or case studies have not been reported.

Toxicity studies are scarce, but no concerns in the proposed condition of use arise from repeat dose study in rats (NOAEL value was 0.67% of mastic in diet) and from the widespread long-standing medicinal use in folk medicine for more than 30 years.

No constituent with known therapeutic activity or active marker can be recognised by the HMPC.

A European Union list entry is not supported due to lack of adequate data on genotoxicity.

Annex

List of references