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Committee on Herbal Medicinal Products (HMPC)

## Assessment report on *Pistacia lentiscus* L., resina (mastic)

Final

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

Herbal substance(s) (binomial scientific name of the plant, including plant part)	<i>Pistacia lentiscus</i> L., resina (mastic)
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
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## ABBREVIATIONS

AGPs	Arabino-galactan proteins
bid	twice a day
CBMN	Cytokinesis Block Micro-Nucleus
CMG	Chios Mastic Gum
CMW	Chios mastic water
DSS	Dextran-sulfate sodium
EROD	Ethoxyresorufin O-deethylase
GagA	Cytotoxin-associated antigen
GSH	Glutathione
HKID	Hong Kong index of dyspepsia
HP-NAP	Neutrophil-activating protein <i>H. pylori</i>
IL	Interleukin
IMLA	24Z-isomasticadienolic acid
IMNA	24Z-isomasticadienonic acid
MBC	Minimum bactericidal concentration
MCP-1	Monocyte chemotactic protein-1
MIC	Minimum inhibitory concentration
MIF	Macrophage migration inhibitory factor
MMC	Mitomycin-C
NOAEL	No Observed Adverse Effect Level
NSAIDs	Non-Steroidal Anti-inflammatory Drugs
NF-κB	Nuclear factor-κB
oxLDL	oxidized low-density lipoprotein.
PBMC	Peripheral blood mononuclear cells
PDO	Product of Protected Designation of Origin
PKC	Protein kinase C
PMA	Phorbol 12-myristate 13-acetate
SMART	Somatic Mutation and Recombination Test
tid	three times a day
TMEWP	Total mastic extract without polymer
TNF-alpha	Tumour necrosis factor-alpha,

UBT	Urea Breath Test
VacA	Vacillating cytotoxin A
WBC	White blood cells

# 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 (mastix) is the dried resinous exudate obtained from stems and branches of *Pistacia lentiscus* L. with a content of minimum 10 ml/kg of essential oil (anhydrous drug).

Mastic is an oleoresin obtained from mastic tree (*P. lentiscus* L.). The terms mastic, mastix and mastic gum are used as synonyms in the Balkan area. It is also often referred to in the literature as Chios Mastic Gum (CMG) from its main origin, the Greek island of Chios.

The Ayurvedic Pharmacopoeia of India (1999) also contains a monograph referring to the resin of *P. lentiscus* L. called as "Rumimastangi". Its name in the different Indian languages is as follows:

Beng.: Rumi-Mastungi

Guj.: Rumi Mastagee

Hindi: Rumi Mastagee; Rumi Mastiki; Mastagee

Mar.: Ruma Mastakee

Urdu.: Rume Mastagee

The mastic tree (*P. lentiscus* L.), from the Anacardiaceae family, is naturally distributed in areas that enclose the coastal regions of the Mediterranean, Portugal and tropical Africa (Gruenwald *et al.* 2007). Flower and fruit is compact and spike-like. The flowers are yellowish or purplish. The drupe, approximately 4 mm, globose, apiculate, is red, but later turns black. The plant is an evergreen dioecious tree or shrub 1-8 m high. The leaves are bipinnate. The 8 to 12 leaflets measure 1 to 5cm by 0.5 to 1.5cm. They are lanceolate to ovate-lanceolate, mucronate and coriaceous. The rachis is broadly winged. The petioles are glabrous (Evans 1989).

Synonym with *P. lentiscus* L. (Gennadios 1914; Rechinger 1943) is *P. lentiscus* var. *Chia* (Desf. Ex Poiret) DC, which has been used in the great majority of the existing scientific publications for this plant. In a more recent and well documented botanical study of Prof Kazimierz Browicz, it is proposed that, instead of the widely used botanical name of *P. lentiscus* var. *Chia*, the name *P. lentiscus* cv. *Chia* should be used as cv, means cultivated clone. Very recently the species *P. lentiscus* L. without any further specified variety or cultivar was accepted in the European Pharmacopoeia's monograph (Browicz 1987).

The *P. lentiscus* L. from the Greek island of Chios commercially is among the major sources of mastic (Chios Mastic Gum (CMG)) worldwide with a specific geographic origin of Southern Chios Area of Mastihohoria, (Savvidis 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 *P. lentiscus* species produces approximately 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 its 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 is sorted, classified and graded according to the colour and size of the granule (Dabos *et al.* 2010i).

Only from Chios island, approximately 250,000 kg of mastic is annually exported (Perikos 1986; Belles 2006); mainly to France, USA, Emirates, Saudi Arabia, UK, Australia. Since 1997, mastic from the island of Chios has been characterized as 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.

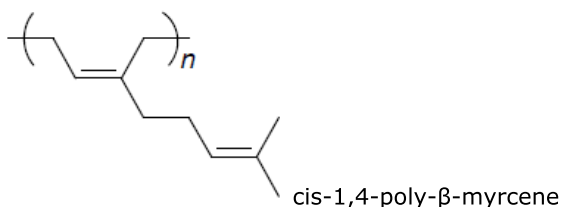
Not any further knowledge about commercial production of resin of *P. lentiscus* from other countries was available.

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 has been found to be used for its adulteration). The tears of mastic are brittle but become plastic when chewed, their odour is slightly balsamic and the taste mildly terebinthinate (Evans 1989) while the tears 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

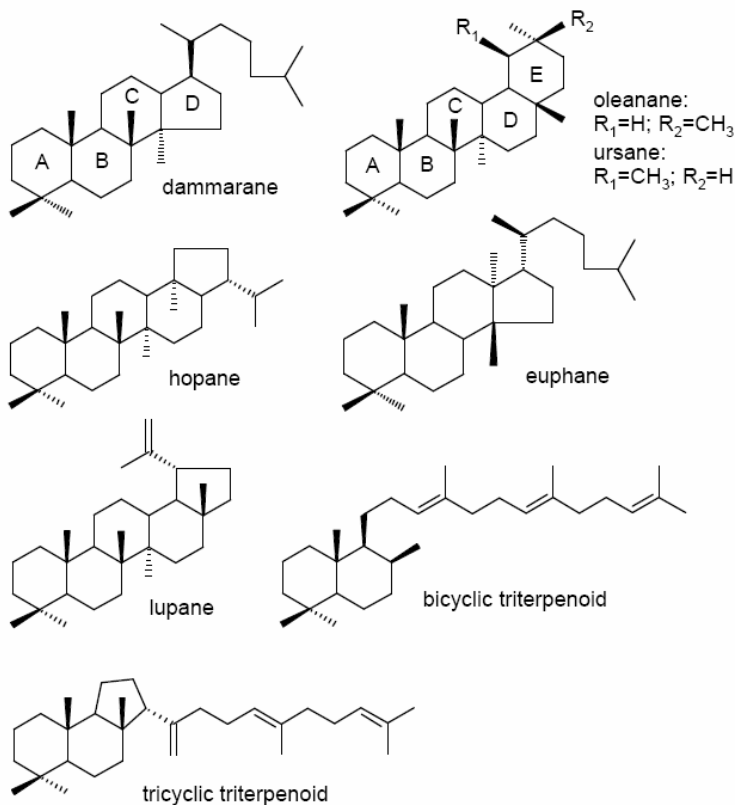
- Triterpenes (tetracyclic euphane and dammarane skeleton type and of the pentacyclic oleanane and lupane skeleton type such as mastic acid, isomastic acid, oleanolic acid, tirucalol, etc. (Gruenwald *et al.* 2007).
- Monoterpene hydrocarbons, 20% oxygenated monoterpenes and sesquiterpenes
- Polyphenols, phytosterols
- Natural polymer (cis-1,4-poly- $\beta$ -myrcene) (van den Berg *et al.* 1998)

The first research on the chemical composition of mastic is reported back in 1930. However, and despite the extended compounds' identification, until today the full composition 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.



The polymer of mastic was identified as cis-1,4-poly- $\beta$ -myrcene (van den Berg *et al.*, 1998).

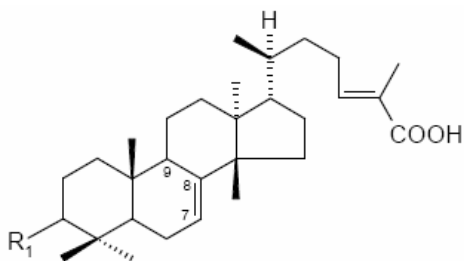
The triterpenoids present in mastic are of the tetracyclic euphane and dammarane skeleton type and of the pentacyclic oleanane and lupane skeleton type.



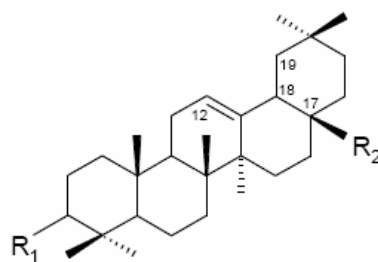
Triterpenoids present in mastic

### Non-volatile secondary metabolites from *Pistacia lentiscus* L., resina (mastic)

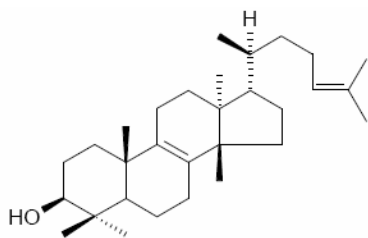
Overall, the main **non-volatile** natural products reported in the literature that have been isolated from the mastic are: a) masticadienonic acid, b) tirucallol, c) oleanolic acid, d) isomasticadienonic acid, e) 3-*o*-28-norolean-12-en, f) 20(*S*)-3 $\beta$ -acetoxy-20-hydroxydammar-24-en, g) 3-oxo-dammara-20(21),24-diene,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



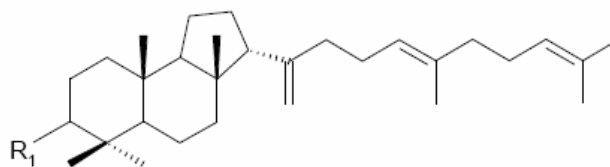
R<sub>1</sub>: O (masticadienonic acid)



R<sub>1</sub>: O R<sub>2</sub>: COOH (oleanolic acid)



Tirucalol



O 3-oxo-malabarica-14(26),17E,21-triene

Polyphenols: Traces of the compounds of tyrosol, p-hydroxy-benzoic acid, p-hydroxy-phenyl acetic acid, vanillic acid and gallic acid are also reported (Kaliora *et al.* 2004).

### Volatile secondary metabolites from *Pistacia lentiscus* L. (mastic and other plant parts)

During the decades of the 1990's and 2000's, the volatile components of the *P. lentiscus* L. tree were the subject of several studies in the context of the analysis of the composition and activity of the essential oil of the resin (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 25% sesquiterpenes) with  $\alpha$ -pinene (79%) and the myrcene (3%) as major components of the resin essential oil.

A considerable study of the three essential oils of mastic, leaves and branches of *P. lentiscus* L was published in 1999 by Magiatis *et al.* In this study the major components identified from the resin were  $\alpha$ -pinene (66.48%), myrcene (8.34%) and  $\beta$ -pinene (3.29%), while in the essential oil of leaves myrcene (20.58%), germacrene D (13.30%), L-caryophyllene (8.33%),  $\alpha$ -cadinol (7.33%) and  $\delta$ -cadinene (7.00%), while in 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  $\alpha$ -pinene, myrcene,  $\beta$ -pinene, limonene and trans-caryophyllene as the major components of mastic. The above results have been verified by a recent work (Kokolakis *et al.*, 2010), in which the authors identified the same main ingredients ( $\alpha$ -pinene, myrcene and  $\beta$ -pinene) and proposed them also as indicators of storage time.

- Herbal preparation(s)

Powdered herbal substance

Data presented in this AR are either from studies using the powdered herbal substance (as only preparation included in the monograph) or sometimes other preparations of mastic such as mastic oil, mastic water or mastic extracts. Often there is insufficient information available on the exact preparation/strength and posology.

**Mastic water** has been also referred to in the literature (Perikos 1986; Topitsoglou-Themeli *et al.*, 1984; 1985; Vlastos *et al.*, 2013). Mastic water is a flavouring water obtained in large quantities together with mastic oil during the steam distillation of mastic. It is a 100% natural aqueous extract that contains all the water-soluble components of mastic as well as a small amount (0.5–1% V/V) of mastic oil.

- 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 information from market overview concerning preparations from 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 the Balkan area as well as in cosmetics area. There are also products where it 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, e.g. several in Greek) were also included in the assessment.

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

Scientific databases: Scifinder, Scopus; search date August 2018; key words: "mastix", "mastic", "*Pistacia lentiscus* cv Chia", "*Pistacia lentiscus*", "mastic gum", mastic oil".

For the key word "mastix" no references were found.

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: Scopus research, others not available to Rapporteur.

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 substance "mastic" or "mastic gum" as synonym is widely distributed for use according to folk medicine and sold *via* pharmacies especially since at least '60s following international publications of the healing activities of mastic on gastrointestinal disorders (functional dyspepsia, as an adjuvant in *Helicobacter pylori* therapy), skin healing activities, and products beneficial for mouth hygiene.

The information exchange among the EU Regulatory Authorities concerning preparations from *P. lentiscus* L. resin (mastic) revealed that there are no medicinal products authorised or registered in the EU/EEA for oral or cutaneous use, containing preparations from mastic as a single active substance.

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

There are some products available for oromucosal uses (gargling) and mouth washes marketed since '50s and '90s respectively (Perikos 1986; Topitsoglou *et al.* 1984; 1985). No specific information regarding strengths and posology is available for these preparations

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

The powder is widely distributed in the food sector as well in cosmetics sector all over Europe for at least the last two centuries. This is confirmed by the exported quantities of mastic at least of Greek Chios island which are available by the Association of Mastic tree growers (Serpico 2000 and Doukas 2003 cited in Vlastos *et al.* 2013).

In Greece and Mediterranean area, there is a known use of mastic powder locally applied for wounds and burns, due to its healing properties (Perikos 1986; The review of natural products 2005, Paraschos *et al.* 2012), while semi-solid galenic preparation containing around 10% of the herbal substance have been used widely through Pharmacies and also prescribed by medical doctors.

Since many years there are creams containing low percentage of mastic powder (1-3%) for cosmetic products.

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

The information concerning preparations from *P. lentiscus* L. resin (mastic) as well as in combination products revealed that the powder is widely distributed in the food sector as well in cosmetics sector also in Turkey and in Arabian countries, mainly for women's use (Al-Habbal *et al.* 1984). This is confirmed by the export quantities of mastic of Greek Chios island mentioned by Perikos (1986) and Belles (2006 only abstract available) with reference to the Association of Mastic tree growers.

In the Mediterranean area (Tunisia, North Africa), Turkey (Turkish Codex as Mastix solute since 1940-1948), as well as in Iran and Iraq there is a known use of mastic preparations locally applied for wounds and burns.

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

### **Historical data**

The ancient Greek physician Hippocrates (4<sup>th</sup> century BC) and later Dioscorides and Galen (1<sup>st</sup> and 2<sup>nd</sup> century AD) reported for first time the properties of Chios Mastic and recommended its use for its distinctive flavour and its therapeutic properties (Paraschos *et al.*, 2007, 2012). 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 pharmacopeia (Gennadios 1914; Al-Habbal *et al.* 1984; Perikos 1986; Savvidis 2000).

Dioscorides (1st century AD.) in *Materia Medica* reported 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 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 (Gennadios 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 (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 mastic 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 "Simplicium medicamentorum temperamentis ac facultibus 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" (Perikos 1986). 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 (13<sup>th</sup> century in England) in his Compendium Medicinae nostrum for the spleen called "diacerasus", which contains cherry juice, cinnamon and mastic. 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). Some variations of the formula are included in some pharmacopoeias (B.P., 1998 (not included in List of references). Martindale: The Extra Pharmacopeia, 33<sup>rd</sup> ed. cited in Moussaieff *et al.* 2005)

For a period of over 250 years, the formula found in a manuscript of a monastery, containing 4 plants: olibanum (*Boswellia* spp.), myrrh (*Commiphora* spp.), aloe (*Aloe* sp.) and mastic (*P. lentiscus* L. gum) (16:3:9:12), appears to be widely used, and even today, for its anti-inflammatory, healing, as well as anti-oxidative, and anti-septic properties [e.g. commercial website <http://www.jerusalemalsam.com>].

Paracelsus in his Der grossen Wundartzney (Great Surgery Book) proposes mastic resin to 'heal wounds'. In the 18<sup>th</sup> and 19<sup>th</sup> 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 widespread. Moreover, 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; Al-Habbal *et al.* 1984).

In the early 20th century "Mastisol", a preparation of mastic used for wound healing, was reported in several papers with somewhat varying composition, that differs from the today Mastisol used as liquid adhesive (and containing mastic, stirax, methyl salicylate and alcohol). According to the references, Mastisol and its substitutes (analogous) are resinous solutions like a mucilage (McDill 1918) that are painted on the affected area and allowed to evaporate until the application has become sticky. Then the bandage may (or not) be applied and left in place until healing has resulted, unless suppuration occurs and this case the medication is renewed (The Merck's Annual Report 1913, Shaw 1914, McDill 1918). Several advantages were reported with this method in comparison with other antiseptic wound dressing, that made it popular also in the field hospitals during the wars: avoidance of all cleansing processing which are injurious to the wounds and cause pain; independence of water thus avoiding the moisture which is so favourable to bacterial growth; great simplicity of the process which can easily be learned by laymen; great saving of time; it can be used in those part of the body to which is difficult to apply bandages; dressing cannot be displaced; cheapness; less irritation of the skin compared to other disinfectants like iodine; security against infection as the asepsis effected with mastic dressing lasts for very long time. Moreover, it can be easily prepared in the hospitals (The Merck's Annual Report 1913, Shaw 1914, McDill 1918).

The Merck's Annual Report 1913 refers to Mastisol as a resinous preparation containing 5% of mastic (terebinth 15 g, mastic 12 g, resin (any) 25 g, resin alb. 8.0 g, alcohol 90% 180 g) recommended as a wound dressing by Dr von Oettingen of Berlin, as the director of a German field hospital in the Balkan war, who modified a previous formula introduced in 1900 through secret proprietary preparation by an orthopedic surgeon. The American Review of reviews (Shaw 1914) reports that the same German surgeon von Oettingen in the Russo-Japanese war used for the antiseptic wound treatment a solution of 20 g mastic in 50 g chloroform and 20 drops linseed oil, while a solution of mastic in benzol was used in Balkan hospitals for the same purpose.

Krebser used the formula containing about 28% mastic (20 g of mastic diluted in 50 g of chloroform, with addition of 1 g linseed oil) for the treatment of operation wounds (The Merck's Annual Report 1913).

Suter used successfully mastisol for the treatment of operation wounds, Wagner in the treatment of wounds and Naugebauer in burns and corrosions (The Merck's Annual Report 1913).

Furthermore, in Medical War manual No 5 "Lessons from the Enemy How Germany cares for her war disabled", McDill reported the same formula containing approx. 28% of mastic (mastic 20 g, 50 g and linseed oil 20 drops) as a mucilage used as surgical dressing (McDill 1918).

In the Booklet "The chemist and Druggist" No 1818, Vol LXXXV Nov 1914 the wound dressing Mastisol is referred together with its historical uses. It is reported as in use in Germany since 1913 (Pharmaceutisch Weekblad" 1913 p 104), as healing agent in a formula (as imitation of mastisol) containing about 4% mastic (Mastic 2g, Benzol 50 g, an unknown ether 20 drops). Other formulas for mastisol are given by the "Pharm. Ztg.", where again a solution of 20 g mastic in 50 g chloroform with addition of 20 drops linseed oil is mentioned (about 28% mastic), and by Nordmann who suggests 20 g mastic, 50 g benzol, 20 drops linseed oil, 10 g colophony and 7 g Venice turpentine (about 23% mastic) (The chemist and Druggist 1914).

These formulas for mastisol continued to be in use also later. In the Turkish Codex (1940-1948) Mastisol is referred as the fantasy name of the preparation Mastix solutus (Sakiz mahlolu) containing 28% of mastic resin (28 g mastic, 71 g benzol, 1 g linseed oil). This corresponds to the preparation used in the Balkan hospitals referred by Shaw (1914) in the American Review of reviews. The same solution containing 28% of mastic in benzol plus linseed oil is also reported in Medicamenta, an Italian theoretical-practical guide for healthcare professional (medical doctors and pharmacists (Medicamenta 1965)

Sollman (1957) reiterates that several formulas containing mastic have been used as surgical varnish to fix skin bacteria on the hands and operative area. Among them it reports the Borchat's one containing 40% mastic in organic solvent (40 g mastic, 60 g benzol, 20 drops of castor oil).

The book of Perikos 1986 refers to the healing properties of mastic cream (patented since 1958 from inventor Mr S Paradeisopoulos) containing 10-20% of pistacia's resin/powder against any kind of skin damages (such as burning, war's wounds, furuncles, psoriasis dermatological diseases etc.) There is correspondence between the Inventor and the Association of Mastic Growers and publications in Press of and Greek newspapers.

Mastic has been used traditionally by oriental women as a masticatory (British Pharmaceutical Codex 1949 cited by Al-Habbal *et al.* 1984) and as breath sweetener, as well as an ingredient of sweets and drinks (Tanker & Tanker cited in Al-Habbal *et al.* 1984). Mastic itself or alcoholic solutions are used in dentistry as a filling of carious teeth (Wren 1971, British Pharmaceutical Codex 1949; Martindale Extra Pharmacopoeia 1978 cited in Al-Habbal *et al.* 1984). Moreover it has been used (*Pigmentum Mastiche Compositum*) as a surgical varnish for protective covering of wounds (Martindale Extra Pharmacopoeia

1978 cited in Al-Habbal *et al.* 1984). So far, no side effects from the wide used of mastic have been mentioned in the literature.

The Merck Index, 10<sup>th</sup> Ed. 1983, reports the use of mastic as a dental aid in tooth cements, plasters, lacquers, chewing gum and incense (The Merck Index 1983).

Medicamenta 8<sup>th</sup> Ed. 1965 reports mastic for internal use as an infusion (2-8 g daily) as a tonic and astringent (in children diarrhoea), for chewing and components of tooth cements providing various formulas where mastic is dissolved in organic solvents (because of its insolubility in water) and the solution may be absorbed on clay or other support (Medicamenta 1965).

Many medical practitioners, pharmacists and botanists referred to the therapeutic properties of mastic resin, which they used for preparing therapeutic formulas and widely used preparations. The use of mastic continued to spread successfully during Byzantine times. Since last century, mastic is 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 (The Review of Natural Products 2005; Al-Habbal 1984). Several authors considered in the 1980s that today, the potential therapeutic activities of mastic have been scientifically studied and showed that especially mastic from the Greek 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 (Topitsoglou-Themeli *et al.* 1984; 1985; Al-Habbal *et al.* 1984; Huwez & Al-Habbal 1986).

It was reported that the mechanism of action of mastic in relieving symptoms and healing properties is not known. However, as mastic is not soluble in water, it was hypothesised that therefore it is possible that it may form complexes with proteins and produce a cytoprotective layer which further protects the gastric mucosa as well as skin wounds. This hypothesis may explain the various uses of mastic (*pigmentum mastiche compositum*) as a surgical varnish for protective covering of wounds (Huwez & Al-Habbal 1986).

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. (Al Habbal *et al.* 1984; Al Said *et al.* 1986; Huwez *et al.* 1986; 1998; Perrikos 1986; Dabos *et al.* 2010i; 2010ii; Kaliora *et al.* 2007i; 2007ii).

In particular, current research suggests that mastic (mainly from Chios Island, CMG, according with the existing studies) and its essential oil possess antimicrobial and antioxidant properties. The antibacterial activity (Magiatis *et al.*, 1999; Iauk *et al.*, 1996; Koutsoudaki *et al.*, 2005) and its *in vivo* antiplaque action in the oral cavity have 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).

For **oral use**, against gastrointestinal problems, the recommended posology based on tradition of mastic powder, according to Perikos (1986) is 0.5-1 g of powdered mastic as a single dose, up to 2 times daily, duration of 2 times a day (Daily dose 1-2 g).

Mastic was also used in small clinical trials by Al-Habbal *et al.*, (1984) and Huwez & Al-Habbal (1986). The daily dose is up to 2 g in accordance with one study (Huwez & Al-Habbal, 1986). These clinical trials were conducted in non-EU countries for another indication (duodenal or gastric ulcer) and used other posology. Therefore the results can be considered as supportive only from a safety perspective.

The Ayurvedic Pharmacopoeia of India (1999) contains also a monograph which refers to the resin of *P. lentiscus* L. called as "Rumimastangi" and its medicinal uses have been proposed at 1-2 g daily dose, comparable to that used in Europe.

For **cutaneous use**, mastic powder has been referred for centuries as a healing agent on the skin or on plasters and dressings (Perikos 1986, The Review of natural products 2005; Paraschos *et al.* 2012). Since 1912 there are references on the use of mastic diluted in organic solvents for skin injuries, serious skin wounds etc. (The Merck's Annual Report 1913; The Chemist and Druggist 1914, Shaw 1914, Sollmann 1957).

There are also galenic formulas (semisolid preparations) prepared in pharmacies and widely used (prescribed also by medical doctors) known for their healing purposes

**Table 1:** Overview of historical data

<b>Herbal preparation</b>	<b>Documented use / Traditional use</b>	<b>Pharmaceutical form, Strength Posology Duration of use</b>	<b>Reference</b>
Mastic powder	For wound dressing, treatment of operation wounds, wounds, burns and corrosion of the second and third degree.	Resinous solution ≈28% mastic in organic solvent (20 g mastic, 50 g chloroform and 1 g linseed oil) or 5% of mastic in other resin and ethanol (Terebinth 15 g, mastic 12 g, resin (any) 25 g, 8 g, alcohol 90% 180 g) Cutaneous use: Method of use: Paint the affected area (once until healing, unless suppuration occurs; in this case the medication should be renewed)	The Merck's Annual Report 1913
Mastic powder	Wound dressing	Resinous solution Various formulas: e.g. ≈28% mastic in organic solvent (20 g mastic, 50 g chloroform and 20	The Chemist and Druggist 1914

Herbal preparation	Documented use / Traditional use	Pharmaceutical form, Strength Posology Duration of use	Reference
		<p>drops linseed oil)</p> <p>≈23% mastic in organic solvent (20 g mastic, 50 g benzol, 20 drops linseed oil, 10 g colophony and 7 g Venice turpentine)</p> <p>20% mastic in organic solvent (mastic 20 parts, colophony 20 parts, castor oil 3 parts, methyl salicilate 1 parts and benzol 56 parts)</p> <p>Cutaneous use</p>	
Mastic powder	antiseptic wound treatment	<p>Resinous solution</p> <p>≈28% mastic in organic solvent (20 g mastic, 50 g chloroform, 20 drops linseed oil)</p> <p>(or mastic in benzol)</p> <p>Cutaneous use:</p> <p>To be painted to the affected area</p>	Shaw 1914 The American review of reviews
Mastic powder	Surgical dressing	<p>Solution like a mucilage (called mastisol)</p> <p>≈28% mastic in organic solvent (mastic 20 g, benzol 50 g, linseed oil 20 drops)</p> <p>Cutaneous use:</p> <p>To be painted to the affected area</p>	McDill 1918
Mastic	Not specified	28% mastic in organic solvent (mastic 28 g, benzol 71 g, linseed oil	Turkish Codex as (since 1940-1948),

<b>Herbal preparation</b>	<b>Documented use / Traditional use</b>	<b>Pharmaceutical form, Strength Posology Duration of use</b>	<b>Reference</b>
		1 g) Cutaneous use	Medicamenta 1965
Mastic powder	In surgery to fix the skin bacteria on the hands and operative area	≈40% in organic solvents (mastic 40 g, benzol 60 g, castor oil 20 drops)  Cutaneous use: as a varnish to be brushed on and allowed to dry	Sollmann 1957
Mastic powder	Traditional use, folk medicine for gastrointestinal disorders/mild dyspeptic/gastro-intestinal disorders	Oral use: 0.5-1 g twice per day Duration of use: 2-4 weeks	Perikos 1986
Mastic powder	Gastric ulcer	Oral use Daily dose: 1 g	The Review of Natural Products 2005
Mastic powder	Traditional use for inflammation, intestine disorders, loss of appetite	Oral use Daily dose: 1-2 g	The Ayurvedic Pharmacopoeia of India 1999
Mastic	<ul style="list-style-type: none"> <li>• as a tonic and astringent (in children diarrhoea)</li> <li>• for chewing</li> </ul>	Oral use: 2-8 g daily as an infusion	Medicamenta 1965
Mastic powder	Traditional use, folk medicine for the symptomatic treatment of minor inflammations in the mouth	Oromucosal use In gargling solutions/toothpastes Not adequately specified daily dose	Perikos 1986 Topitsoglou-Themeli <i>et al.</i> , 1984
Mastic essential oil	Traditional use, folk medicine for the symptomatic treatment of minor inflammations in the mouth	Oromucosal use Not adequately specified daily dose	Perikos 1986 Topitsoglou-Themeli <i>et al.</i> , 1985
Mastic water	Traditional use, folk	Oromucosal use	Perikos 1986

<b>Herbal preparation</b>	<b>Documented use / Traditional use</b>	<b>Pharmaceutical form, Strength Posology Duration of use</b>	<b>Reference</b>
	medicine for the symptomatic treatment of minor inflammations in the mouth	In gargling solutions/toothpastes  Not adequately specified daily dose	Topitsoglou-Themeli <i>et al.</i> , 1984; 1985
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	Galenic semisolid preparations containing powder of <i>P. lentiscus</i> L. resin (mastic 9-11%, mostly 10%)  Several times daily, on the affected skin area	Galenic preparations (copies from Pharmacies notebooks and Books of recipes and medical doctors prescriptions; not in List of references)
Mastic powder	Healing properties of against several kind of skin damages (such as burning, war's wounds, furuncles, dermatological diseases etc.)	Patented cream for dermatological disorders containing 10-20% mastic cutaneous use	Perikos 1986  (patent since 1958)
Mastic	Component of tooth cements	Various formulas containing mastic in organic solvents (and clay or other support for the cement)	Medicamenta 1965

### **2.3. Overall conclusions on medicinal use**

Since the end of the 1970s' the beginning of the 1980s the medicinal use of the powder of the resin of *P. lentiscus* L., has become popular in traditional medicine in Greece, Mediterranean and Asian (Middle East) countries as well as in the USA for its healing properties. Several supportive references with details on strength, posology and indications are available, for both oral and cutaneous uses.

**Table 2a:** Overview of evidence on period of medicinal use (oral use)

<b>Herbal preparation/ Pharmaceutical form</b>	<b>Indication</b>	<b>Posology, Strength</b>	<b>Period of medicinal use</b>
Mastic powder	Traditional use, folk medicine for gastrointestinal disorders/mild dyspeptic/ gastrointestinal	Oral use  0.5-1.g, twice per day  Duration of use: 2-4 weeks	Perikos 1986

Herbal preparation/ Pharmaceutical form	Indication	Posology, Strength	Period of medicinal use
	disorders		

The requirements for the period of medicinal use according to Directive 2001/83/EC with respect to "traditional use" by oral administration is regarded as fulfilled for the following indications:

- Traditional herbal medicinal product used in mild dyspeptic disorders

Based on available literature references the following posology is proposed for the Oral use of the powder:

- Single dose: 0.5-1 g
- Daily dose: 1.0-2 g
- Duration of use: 2 weeks considering that the product is for self-medication and if such kind of symptoms persist longer during the use of the medicinal product, a doctor or a qualified health care practitioner should be consulted (in consistency with other herbal medicines with same indications).

**Table 2b:** Overview of evidence on period of medicinal use (cutaneous use)

Herbal preparation Pharmaceutical form	Indication	Posology, Strength	Period of medicinal use
Mastic powder	For wound dressing, treatment of operation wounds, wounds, burns and corrosion of the second and third degree	Resinous solution ≈28% mastic in organic solvent (20 g mastic, 50 g chloroform and 1 g linseed oil)  Cutaneous use  Method of use:  Paint the affected area (once until healing, unless suppuration occurs; in this case the medication should be renewed)	The Merck's Annual Report 1913
Mastic powder	Antiseptic wound treatment	Resinous solution ≈28% mastic in organic solvent (20 g mastic, 50 g chloroform, 20 drops linseed oil)  Cutaneous use:	Shaw 1914 The American review of reviews

<b>Herbal preparation Pharmaceutical form</b>	<b>Indication</b>	<b>Posology, Strength</b>	<b>Period of medicinal use</b>
		To be painted to the affected area	
Mastic powder	Wound dressing	Resinous solution Cutaneous use: ≈28% mastic in organic solvent (20 g mastic, 50 g chloroform and 20 drops linseed oil)	The Chemist and Druggist 1914
Mastic powder	Surgical dressing	Solution like a mucilage (called mastisol) ≈28% mastic in organic solvent (mastic 20 g, benzol 50 g, linseed oil 20 drops) Cutaneous use: To be painted to the affected area	McDill 1918
Mastic powder	For dermatological disorders (healing purposes) (Personal communication with Prof F Demirci)	Resinous solution (called mastix solute or mastisol) External use Mastic 28% in organic solvents (mastic 28 g, benzol 71 g, linseed oil 1 g)	Turkish Codex (since 1940-1948) Medicamenta 1965
Mastic powder	In surgery to fix the skin bacteria on the hands and operative area	≈40% in organic solvents (mastic 40 g, benzol 60 g, castor oil 20 drops) Cutaneous use: as a varnish to be brushed on and allowed to dry	Sollmann 1957

Herbal preparation Pharmaceutical form	Indication	Posology, Strength	Period of medicinal use
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	Galenic Semisolid preparation containing powder of <i>P. lentiscus</i> L. resin (mastic 9-11%, mostly 10%).  Several times daily, on the affected skin area	Galenic preparations (copies from Pharmacies notebooks and Books of recipes and medical doctors prescriptions; not in List of references)
Mastic powder	Healing properties of against several kind of skin damages (such as burning, war's wounds, furuncles, dermatological diseases, etc.	Patented creams containing 10-20% mastic for dermatological disorders	Perikos 1986  (patent by S Paradeisopoulos since 1958)

#### Cutaneous use

The requirements for the period of medicinal use according to Directive 2001/83/EC with respect to "traditional use" is regarded as fulfilled for the cutaneous use for the following indications:

- 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 cutaneous medicinal use of mastic is substantiated by its general and continuous use as antiseptic medication in wounds healing and for surgical dressing in several preparations in Europe (since 1912) as well as outside EU (since 1918 in USA, 1940 in Turkey). The medicinal use refers to preparations consisting in various concentrations (the most mentioned one is  $\approx 28\%$ ) of mastic dissolved in organic solvents (due to its insolubility in water) which are nowadays not permitted in the medicinal use due to their toxicity. The use of organic solvents had also the purpose to allow them to evaporate and the residual sticky layer of mastic could also serve as adhesive for bandage, but it could simply be applied on small wounds on hands, fingers, and face remaining in the position without binding the medication (The Merck's Annual Report 1913, Shaw 1914, McDill 1918).

The galenic (magistral) preparation currently in use refers to a lower concentration (mostly around 10%) that is achievable with other solvents or mostly oils (e.g. in creams). This traditional use is proved from medical prescription as well as through Pharmacists Books or recipes nowadays (no reference included in List of references). The use of mastic in healing ointment for centuries is also reported by Paraschos *et al.* 2012, referring to ancient formulas and old pharmacopoeias. It is up to the applicant for registration to choose the most suitable excipient for the product to be placed on the market and to perform local tolerance studies for the cutaneous preparation if needed.

As Article 16c(c) of Directive 2001/83/EC requires documented medicinal use throughout a period of at least 30 years, including at least 15 years within the EU, which is satisfied even where the marketing of the product has not been based on a specific authorisation, the data relating to these products can be used to substantiate the traditional use for the cutaneous preparations of mastic.

The following posology is proposed for the cutaneous use of mastic:

Semisolid preparation containing 9-11%<sup>1</sup> mastic powder.

Up to 3 times daily.

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

The above-mentioned duration of use is proposed considering that the product is for self-medication and if such kind of symptoms persist or worsen during the use of the medicinal product, a doctor or a qualified health care practitioner should be consulted.

### 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**

###### ***In vitro* studies**

###### **Antimicrobial activity of mastic, mastic oil and other preparations**

Several studies have been conducted to investigate mastic antimicrobial properties.

Different *P. lentiscus* L. **leaves preparations** (10% decoction, petroleum ether extract, ethanol extract, infusion and maceration) were tested against selected Gram-positive (+) and Gram-negative (-) bacteria, but only the decoction showed some activity (MIC 312 mg/ml) while all four other preparations were practically inactive against *S. lutea*, *S. aurea* and *E. coli* (Iauk 1996).

Daifas *et al.* (2004) investigated the effect of mastic resin and its essential oil, alone and in conjunction with ethanol, on the growth of proteolytic strains of *Clostridium botulinum* in media, and on neurotoxin production in challenge studies with English-style crumpets. Preliminary studies, using a spot-on-the-lawn method, indicated that high levels of mastic resin in ethanol (~8% w/w) were required for complete inhibition of all strains of *C. botulinum* tested, but mastic resin in ethanol had a greater anti-botulin effect than ethanol alone. However, only low levels of mastic oil (~0.3% v/v) were required for inhibition of proteolytic strains of *C. botulinum*. Both studies showed a strain specific inhibition, with *C. botulinum* type A strains being more sensitive to mastic resin and its essential oil than type B strains. However, mastic resin in ethanol proved to be more effective when used as a vapor phase inhibitor applied to cotton pads and placed inside inoculated plates than when added directly to media. While both mastic resin and its essential oil inhibited the growth of proteolytic strains of *C. botulinum* in vitro, they failed to inhibit neurotoxin production in challenge studies with *C. botulinum* in English-style crumpets.

The antibacterial activity of **mastic essential oil** has been tested with heterogenous results. Tassou & Nychas (1995) found some activity in Gram-positive strains but none in Gram-negative. Magiatis *et al.*, (1999) reported some activity for the essential oil from the resin but no activity from the leaves or twigs and attributed it mainly to  $\alpha$ -pinene. Koutsoudaki *et al.* (2005) attributed it to the combination of several components and suggested that these work synergistically given that different bacteria are susceptible or not to different compounds of the essential oil.

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<sup>1</sup> Note: While references may point to a wider possible strength, this should be reconsidered with the next systematic revision of the monograph.

## **Bactericidal effect of mastic, mastic oil, or mastic fractions/constituents against *Helicobacter pylori***

A number of studies have shown that mastic and mastic oil exhibit actions on gastrointestinal lesions. After discovery of *Helicobacter pylori* and correlation with gastrointestinal disease in 1983, 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 report on mastic anti- *H. pylori* activity is published in the New England Journal of Medicine in a correspondence to the editor (Huwez *et al.*, 1998). Mastic proved to kill the *H. pylori* NCTC 11637 strain and the six clinical isolates. In the specific study fresh samples were used with the presence of *H. 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 MBC of mastic was 60 µg/ml, but even in smaller concentrations, the antibacterial action was observed.

Marone *et al.* (2001) and Bona *et al.* (2001) assessed the antibacterial effect of mastic on the clinical isolates of *H. pylori* at concentrations of 2000 to 1.9 µg/ml. The MBCs, 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 mastic on *H. pylori* in arabino-galactan proteins (AGPs) 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 resin 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) utilised an established *H. pylori* infection model to evaluate the potential therapeutic effect of continuous total mastic extract without polymer (TMEWP) administration on *H. pylori* colonisation and development of associated gastritis. Initially, TMEWP was obtained from crude mastic 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 contained 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, contained 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 a higher susceptibility against the TMEWP and its acidic fraction was observed. Having obtained the highest activity with the acidic fraction of the TMEWP, the authors 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, 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 histopathologic evaluations. According to the authors, the results also suggest that habitual long-term mastic consumption may be effective in moderating *H. pylori* colonisation.

Following the above study, Kottakis *et al.* (2009) investigated the effect the AGPs (arabino-galactan proteins) derived from mastic from Chios (CMG), 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 resin 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 HP-NAP. The VacA interacts with the membrane of epithelial cells and enters therein, wherein forms a low conductivity ions channel. HP-NAP 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 characterised 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 AGPs plus HP-NAP (P=0.0004) in *H. pylori*-positive patients who consumed AGPs for two months. Similar results were also obtained when neutrophils were incubated with AGPs plus HP-NAP (P=0.0038) but not with HP-NAP (P>0.05) in controls.

Sharifi & Hazell (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 of the resin for 4 hours (50% increases).

Choli-Papadopoulou *et al.* (2011) evidenced that the broad C-terminal region of HP-NAP stimulates neutrophil adhesion and that the AGPs from CMG disrupt the process of neutrophil-endothelial cell attachment caused by HP-NAP, an effect that should be further investigated and may be exploited in a future anti-inflammatory therapy for *H. pylori* patients. The HP-NAP 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 recent study (Miyamoto *et al.*, 2014) examined which component of mastic is responsible for anti-*H. pylori* activity. GC-MS analysis of the essential oil of mastic led to the identification of 20 components among which  $\alpha$ -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- and/or metronidazole-resistant strains.  $\alpha$ -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 (*Escherichia coli*, *Staphylococcus aureus*, *Bacillus subtilis*). The authors concluded that these components could be useful to overcome the drug-resistance *H. pylori* growth in stomach.

### **Oral care**

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

### **In vivo studies**

#### **Anti-inflammatory properties**

The anti-inflammatory properties of mastic to help reduce intestinal inflammation in inflammatory bowel disease patients were investigated by Kim & Neophytou (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 resin's essential 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%  $\gamma$ -tocopherol, Group 4 mice received a diet containing 0.02% mastic oil and 0.30%  $\gamma$ -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  $\gamma$ -tocopherol gave similar results to using mastic or  $\gamma$ -tocopherol alone.

Results from a recent study using colitic Wistar rats showed anti-inflammatory and antioxidant properties of mastic. The authors concluded that mastic could possibly have a therapeutic role in Crohn's disease, regulating oxidant/antioxidant balance and modulating inflammation (Gioxari *et al.*, 2011).

In a study by Papalois *et al.* (2012), mastic powder (100 mg/kg of body weight) or mastic components (i.e., inulin, acidic fractions AF, neutral necrosis factor NF, or the major triterpenic acid oleanolic acid OA) were individually administered in trinitrobenzene sulfonic acid-treated rats. Colonic damage was assessed microscopically, and levels of tumour necrosis factor- $\alpha$  (TNF- $\alpha$ ), interleukin (IL)-6, IL-8, and intercellular adhesion molecule-1 were measured. A model of inflammation in co-cultured human colon epithelial HT29 cells and monocytes/macrophages was established. Lactate dehydrogenase release and levels of TNF- $\alpha$ , IL-8, and nuclear factor- $\kappa$ B (NF- $\kappa$ B) p65 were measured. *In vivo*, histological amelioration of colitis and significant regulation in inflammation occurred with mastic powder, even at the mRNA level. Although no histological improvement was observed, AF and NF reduced levels of inflammatory markers. Inulin was ineffective. *In vitro*, mastic powder treatment down-regulated IL-8 and NF- $\kappa$ B p65. Neither fractions nor OA was the bioactive component solely. The authors concluded that most probably, the entire mastic powder rather than its individual fractions reduces inflammation via NF- $\kappa$ B regulation.

### **Anti-ulcer and antioxidant 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, cysteamine 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 showed that the administration of CMG 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.

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 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. It was found that all parameters increased by administration of diclofenac were decreased after administration of mastic at a dose of 1 ml/kg weight.

**Table 3:** Overview of the main non-clinical data/conclusions on mastic and mastic preparations

<b>Herbal preparation tested</b>	<b>Strength Dosage Route of administration</b>	<b>Experimental model</b>	<b>Reference</b>	<b>Main non-clinical conclusions</b>
Mastic and its essential oil	Several % doses of mastic oil Mastic	<i>In vitro</i> Antibacterial activity against <i>Clostridium botulinum</i>	Daifas <i>et al.</i> , 2004	0.3% mastic oil is required for the inhibition of proteolytic strains of <i>Clostridium botulinum</i>
Mastic (total, acid and neutral fraction) and 3 essential oils from mastic, leaves and twigs from <i>P. lentiscus</i> var. Chia	Not specified	<i>In vitro</i> Activity against <i>Staphylococcus aureus</i> , <i>Staphylococcus epidermidis</i> , <i>Pseudomonas aeruginosa</i> , <i>Enterobacter cloacae</i> , <i>Klebsiella pneumoniae</i> , <i>Escherichia coli</i> , <i>Candida albicans</i> , <i>Candida tropicalis</i> , <i>Torulopsis glabrata</i>	Magiatis <i>et al.</i> , 1999	<i>In vitro</i> antibacterial/fungistatic activity of the 3 essential oils and of mastic against 6 bacteria and 3 fungi, shows that mastic oil from resin is the most active against bacteria and fungi, while mastic exerts some activity only against bacteria
Mastic, mastic oil and its components	20 µl on 6-mm diameter paper disk on 20 ml agar Petri disk	<i>In vitro</i> Antibacterial activity against <i>S. aureus</i> , <i>S. epidermidis</i> , <i>B. subtilis</i> and <i>E. Coli</i>	Koutsoudaki <i>et al.</i> , 2005	Mastic oil from resin is the most active against the 3 strains tested. The antibacterial activity can be attributed to the combination of several components rather than to one particular compound.  Mastic components extractable by ethanol shows no effect against <i>S. aureus</i> , <i>B. subtilis</i> and <i>E. Coli</i>

Herbal preparation tested	Strength Dosage Route of administration	Experimental model	Reference	Main non-clinical conclusions
Mastic powder	Not specified	<i>In vitro</i> Activity against <i>H. pylori</i> NCTC 11637 strain and the six clinical isolates strains from patients	Huwez <i>et al.</i> , 1998	MBC of mastic required in order to exterminate <i>H. pylori</i> was 60 µg/ml
Mastic	2000 to 1.9 µg/ml	<i>In vitro</i> Activity against 12 isolated clinical strains of <i>H. pylori</i>	Marone <i>et al.</i> , 2001	Bactericidal activity; EC50, 50% of the strains were killed at 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)	<i>In vitro</i> Activity against 12 isolated clinical strains of <i>H. pylori</i>	Bona <i>et al.</i> , 2001	The extracts of at least 1.4 g mastic 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	<i>In vitro</i> Activity against 10 clinical isolates of <i>H. pylori</i> and the CCUG 38771 reference strain tested over a period of three months	Paraschos <i>et al.</i> , 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)
Mastic	Oral, 1 g daily for 1 month  2-part study	<i>In vitro</i> and <i>in vivo</i> Part 1: <i>in vitro</i> effect of AGPs on neutrophil activation by pull-down	Kottakis <i>et al.</i> , 2009	Specific binding of arabino-galactan proteins (AGPs) to two membrane proteins of neutrophils, possibly resulting in inhibition of neutrophil

Herbal preparation tested	Strength Dosage Route of administration	Experimental model	Reference	Main non-clinical conclusions
		<p>experiments and incubation of AGPs with HP-NAP and neutrophils in five <i>H. pylori</i> positive patients (3 women, age 21–74) and three <i>H. pylori</i> negative healthy men volunteers (age 23–72).</p> <p>Part 2: <i>in vivo</i> effect of mastic gum consumption on neutrophil activation in all participants is reported in Table 4 (Clinical studies)</p>		<p>activation</p> <p>Neutrophil activation reduced when incubated <i>in vitro</i> with HP-NAP (P=0.0027) and AGPs plus HP-NAP (P=0.0004) in <i>H. pylori</i>-positive patients who consumed AGPs for two months. Similar results were also obtained when neutrophils were incubated with AGPs plus HP-NAP (P=0.0038) but not with HP-NAP (P&gt;0.05) in controls</p>
Mastic	Not specified	<p><i>In vitro</i></p> <p>Activity against <i>H. pylori</i></p>	Sharifi & Hazell 2009	<p>The most active fraction of mastic was polymer, followed by the acid and the whole resin, while the neutral fragment was inactive</p>
Mastic	Not specified	<i>In vitro</i>	Triantafyllou <i>et al.</i> , 2011	<p>Mastic inhibited the activity of purified PKC (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</p>
Mastic	Not specified	<i>In vitro</i>	Sakagami <i>et al.</i> ,	Mastic showed selective antibacterial action against oral bacteria

Herbal preparation tested	Strength Dosage Route of administration	Experimental model	Reference	Main non-clinical conclusions
Mastic oils (no further information)	oral administration of mastic oils 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	Activity against <i>Porphyromonas gingivalis</i> and <i>Prevotella melaninogenica</i>  <i>In vivo</i> In rats	2009, Sterer 2006  Heo <i>et al.</i> , 2006	<i>Porphyromonas gingivalis</i> and <i>Prevotella melaninogenica</i>  Mastic oil at a dose of 1 ml/kg weight prevented intestinal damage and bacterial translocation induced by diclofenac
Mastic and mastic resin essential 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 0.02%	<i>In vivo</i> Dextran-sulfate sodium (DSS) model of colitis Trial 1) in rats Trial 2) in mice	Kim & 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 $\gamma$ -tocopherol gave similar results to using mastic or $\gamma$ -tocopherol alone

Herbal preparation tested	Strength Dosage Route of administration	Experimental model	Reference	Main non-clinical conclusions
Mastic	mastic oil Group 3: diet containing 0.30% γ-tocopherol Group 4: diet containing 0.02% mastic oil and 0.30% γ-tocopherol	<i>In vivo</i> In guinea-pigs with the use of the appropriate chemicals ulcer in the stomach	Al-Said <i>et al.</i> , (1986)	CMG 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 and its major compounds has been reviewed by Giaginis & Theoharis (2011) in *in vitro* studies that have proved that CMG (mastic from Chios) inhibited cell proliferation of cancer cells derived from several types of human neoplasia including mainly prostate, colon, lung, pancreatic carcinoma and haematological malignancies.

#### Protection against atherosclerosis

The potential of antiatherogenic effects of mastic 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. 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 hour 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 exhibited remarkable antioxidant effect on low-density lipoprotein (LDL). The results of the tests have led to the conclusion that CMG (resin from *P. 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 polar extract of the resin (methanol/water 60:40 V/V) in the survival of peripheral blood mononuclear cells (PBMC), under oxidant stress conditions, which is created by the oxidised low-density lipoprotein (oxLDL). During the experimental study, the exposition of cells in the oxidised 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. 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 antioxidant activity in 70's where Abdel Rahman & Youssef Soad (1975) showed that mastic possessed antioxidant activity similar to that of butylated hydroxyanisole. 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 CMG 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

No data available.

### 3.1.4. Pharmacodynamic interactions

#### Induction of CYP enzymes

Katsanou *et al.* (2014) investigated whether mastic 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-catalysed 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

Several *in vitro* and *in vivo* studies have been published over the last 30 years for mastic powder but also derived preparations, fractions and constituents (Huwez *et al.*, 1998; Magiatis *et al.*, 1999; Daifas *et al.*, 2004; Koutsoudaki *et al.*, 2005; Bona *et al.*, 2001; Marone *et al.*, 2001; Paraschos *et al.*, 2007). They support the plausibility of the use of mastic, which is the only herbal preparation proposed in the monograph. Some data showed antimicrobial (mainly against *H. pylori*) together with antioxidant and anti-inflammatory properties.

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

Even though the resin of *P. lentiscus* L. (from the island of Chios) is used 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 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 or total mastic extract without polymer (TMEWP) in order to evaluate the role of the natural polymer, poly- $\beta$ -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  $3300 \pm 859$  ng/ml for IMNA and  $163 \pm 58$  ng/ml for IMLA. In comparison, following CMG administration,  $C_{max}$  plasma values were  $329 \pm 57$  ng/ml for IMNA and  $28 \pm 8$  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 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 (white blood cells) and platelet in high dose males. Altered serum biochemistry parameters included increases of total proteins, albumin, and total cholesterol in both sexes, and  $\gamma$ -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 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 anti-genotoxic properties against the mutagenic agent mitomycin-C (MMC). Genotoxicity was evaluated by employing the *in vitro* Cytokinesis Block Micro-Nucleus (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  $\mu\text{g/ml}$ . 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). Mastic water is water obtained in large quantities together with mastic oil during the steam distillation of mastic resin, containing all the water soluble components of mastic as well as a small amount (0.5–1% V/V) of mastic oil.

#### **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 in diet. Otherwise data on toxicity are not available. As for evaluating genotoxicity *in vitro* micronucleus and SMART tests were performed only with mastic water but not with mastic. A List Entry for mastic is not proposed.

### **3.4. Overall conclusions on non-clinical data**

The *in vitro* studies showed some antimicrobial activity of mastic-derived preparations (mainly essential oil) against Gram-positive and partially Gram-negative bacteria as well as particularly an activity against *H. pylori*, with the most effective minimum bacterial concentration (MBC) reported 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 mastic, the triterpenic acids, 24Z-isomasticadienonic acid (IMNA), and 24Z-isomasticadienolic acid (IMLA), while other data on pharmacokinetics and interactions are not available. Mastic is not an inducer of CYP1A1/2 in rat liver.

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

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. Due to the lack of adequate tests on genotoxicity, an EU list entry is not recommended.

## **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**

### **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***

Sixty volunteers (60) with symptoms and endoscopic confirmation of duodenal ulcer participated in a first clinical study (Al-Habbal *et al.*, 1984). For the comparison of the effectiveness of CMG (mastic from Chios), the volunteers were divided in two groups: group 1 consumed CMG 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 thirty-eight (38) volunteers were endoscopically examined, in order to see the progress of the ulcer (10 in CMG group and 12 in the placebo group did not attend for the follow-up endoscopy and therefore were dropped from the trial). The results showed that in the group that consumed CMG 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, while the results of placebo group were 50% (alleviation of the symptoms) and 22% (cure of duodenal ulcer) respectively. The authors concluded that mastic was more active than placebo for the alleviation and the treatment of ulcer symptoms, while the use of CMG did not produced unwanted side-effect.

*Assessor comment:*

*The high rate of drop-out (almost 50%), duration of use too short and the lack post-treatment investigation preclude any reliable conclusion on efficacy and, as also stated by the Authors further studies are needed to establish the role of mastic in the treatment of ulcer.*

The same research team published (Huwez & Al-Habbal 1986) the findings of another clinical study in patients who suffered of gastric ulcers, of benign nature. For this purpose, CMG 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 other type of pharmaceutical treatment was administered 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 CMG administration. The results of the study have shown that the administration of CMG caused symptom relief in all six patients. The positive outcome was 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 2003, Roe *et al.*, published a clinical study concerning the action of mastic against gastritis caused by *H. pylori* in the *South Korea*. 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 chewing gum mastic (1 mg/piece) from the island of Chios 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 authors concluded that mastic has a beneficial effect on *H. pylori*-infected gastritis. In 2009, Kottakis *et al.* investigated the effects of CMG on innate cellular immune effectors. The *in vivo* effect of arabinogalactan proteins isolated from mastic (AGPs) under the presence of HP-NAP (*Helicobacter pylori* neutrophil activating

protein) in neutrophil activation was investigated in five *H. pylori*-infected patients and 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 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. It was found that neutrophil activation was reduced when neutrophils were incubated *in vitro* with HP-NAP ( $P=0.0027$ ) and AGPs plus HP-NAP ( $P=0.0004$ ), in *H. pylori*-positive patients who consumed AGPs for 2 months. Similar results were also obtained when neutrophils were incubated with AGPs plus HP-NAP ( $P=0.0038$ ) but not with HP-NAP ( $P>0.05$ ) in controls. The studies suggested that the AGPs inhibit neutrophil activation in the presence of HP-NAP, playing a crucial role in *H. pylori*-associated pathologies in gastric mucosa.

Another clinical trial evaluated the effect of mastic on *H. pylori in vivo* (Dabos *et al.*, 2010ii). Fifty-two patients participated in the clinical trial. They were randomised to receive either 350 mg three times a day (tid) of pure mastic for 14 days (Group A), or 1.05 g of pure mastic (Group B) for 14 days, or pantoprazole 20 mg twice a day (bid) plus pure mastic 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 performing UBT a mean of 8 days (4-14 days) before starting treatment and repeated a mean of 39 days (33-61 days) after completion of the study medication). The results showed that mastic has some effect on *H. pylori in vivo*. Nine patients in the monotherapy groups achieved eradication while in ten more patients the UBT value decreased compared to the pre-treatment reading. Eradication of *H. pylori* was 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 and pantoprazole was ineffective both in eradicating *H. pylori* and on bacterial load. The surprising failure of combination mastic 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 well and no serious adverse events were reported.

### **Relief of functional dyspepsia**

Dabos *et al.* (2010i) performed the first double-blind placebo-controlled trial to assess the effects of CMG in functional dyspepsia. 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 (p<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. 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**

Two studies (Kaliora *et al.*, 2007i and 2007ii) were 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 per day, 0.37 g per 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. 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.*, 2007ii). Authors concluded 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 provided a positive outcome for the role of mastic as a potential regulator of immunity in Crohn's disease.

### **Adhesive properties**

The adhesive properties of mastic have been identified and studied by researchers since last thirty years (Mikhail *et al.*, 1986, 1989; Lesesne, 1992; Yavuzer *et al.*, 2005). The natural resin of mastic is used very often 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 from Chios) presents adhesive properties, when used in covering means and wound and incisions healing means, 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.

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: tincture of benzoin USP (aloe, tolu balsam and styrax liquid in alcohol) and in the third category a mastic compound MC (mastic 45%, styrax liquid <5%, methyl salicylate <5% and alcohol) was used as reinforcing means. The specific study has shown that despite the use of benzoin, CMG in the bandages has resulted in improving their adhesive properties; the use of mastic has brought an even more improvement, confirming that CMG could be used with success in the specific application.

In another study (Lesesne 1992) a comparison was made between the adhesive properties, as well as of the undesirable effects of a patented preparation of mastic (mastic 45%, styrax liquid <5% and methyl salicylate <5%) and tincture of benzoin USP as ingredients of the adhesive bandages. 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 tincture of benzoin USP were applied, while in the second group bandages with the preparation of CMG 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 offers not only exceptional adhesive properties to the healing means, as compared to tincture of 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 by another research team (Yavuzer *et al.*, 2005), which reaches the conclusion that mastic substantially increased the adhesive action of the self-adhesive bandages, when these are used as the only means for covering wounds and incisions.

### **Oral hygiene**

Topitsoglou-Themeli *et al.* (1984; 1985) demonstrated that when CMG was used systematically, it may result in important decrease in the amount of forming or already formed dental plaque. Ten (10) volunteer students with low caries rate participated in this study. They 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 confirmed that in the group that used CMG the amount of microbial plaque was largely diminished. The authors concluded it can be used effectively in the prevention of caries, periodontal disorders and buccal cavity diseases in general.

A similar clinical study (Takahashi, *et al.*, 2003), that was published in 2003 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 periodontal 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.

Aksoy *et al.*, (2006) investigated the *in vitro*, as well as the *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 *S. mutans* samples were used. Respectively, the clinical study was applied in 25 periodontally 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 *S. 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. mutans*. This action of mastic appears as especially important, as it concerns the limitation of the frequent and dangerous bacteria of the mouth, *S. 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.

### **Plasma Lipid and blood sugar reduction**

Triantafyllou *et al.* (2007) investigated the effect of mastic powder in reducing plasma lipids and glucose levels. Subjects (n=133-93 women, 43 men-, aged over 50) were randomly assigned to two groups, the first (high-dose group) ingesting daily 5 g of CMG powder and the second (low-dose group) receiving daily a CMG solution containing one-seventh of the daily dose taken by the high-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. Generalised least squares random-effects linear regression was performed. The group ingesting CMG 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), serum glutamic oxaloacetic transaminase (SGOT), serum glutamate-pyruvate transaminase (SGPT) and gamma-glutamyltransferase (gamma-GT) levels. A decrease in glucose levels in males in the second (low-dose) group was observed.

**Table 4:** Clinical studies

Type of study	Study Design	Test Product(s)	Number of subjects	Type of subjects	Outcomes	Statistical analysis	Clinical relevance of results
Gastric or duodenal ulcer and effect of <i>H. pylori</i> infected patients							
Efficacy in duodenal ulcer Al-Habbal <i>et al.</i> 1984	Double blind placebo control Randomised 2 weeks	Group 1: 1 g mastic per os per day for two weeks Group 2: same dosage and duration of placebo (lactose)	60 volunteers aged 62-22 years randomly divided in two groups (treatment and placebo) 38 subjects at end, high drop-out (10 CMG and 12 placebo)	Patients with symptomatic and endoscopically proven benign duodenal ulcer, with no antiulcer medication in the previous month. No patients with pylori stenosis, pregnant or lactating women	In mastic group 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 results of placebo group were 50% (alleviation of the symptoms) and 22% (cure of duodenal ulcer) respectively, while the use of CMG produced no unwanted side-effect	z-statistics	The high rate of drop-out (almost 50%), duration of use too short and the lack post-treatment investigation preclude any reliable conclusion on efficacy
Efficacy in benign gastric	Uncontrolled study	mastic administered per os in the	6 patients: 5 men, 1 woman all >20 years of age	Patients with gastric ulcer diagnosed by	The results have shown that the administration of	Not performed (not statistical relevance)	Very small number of patients

Type of study	Study Design	Test Product(s)	Number of subjects	Type of subjects	Outcomes	Statistical analysis	Clinical relevance of results
ulcer Huwez & Al Habbal 1986	4 weeks; Follow up: 2 months	dosage of 2 g per day for four weeks (1 g before breakfast and 1 g before sleeping at night) for 4 weeks	(woman aged >70 years non responding to several cimetidine cycles)	means of gastroscopy, not receiving treatment in the last 2 months with H2 blockers, bismuth, carbenoxolone or sucralfate	mastic has relieved all six patients from the symptoms after 7 days of treatment (mean). The healing was even endoscopically confirmed in five of them after 4 weeks (including the elderly female patient and one patient with double gastric ulcer). During the study, but also two months after no type of unwanted effect was found, nor any unusual result in the lab analysis		
<i>In-vivo</i> efficacy study on neutro- phil	Control study 2 months	AGPs from 1 g CMG daily for 2 months	five (5) <i>H. pylori</i> - infected patients (3 women and 2 men; age range 21-74 years) and (three <i>H.</i>	<i>H. pylori</i> -infected patients and three <i>H. pylori</i> negative healthy men volunteers not	Neutrophil activation reduced when neutrophils incubated <i>in vitro</i> with HP-NAP	Mann- Whitney U test to compare the neutrophil	Small number of participants, further relative large-scale studies are needed to substantiate the potential

Type of study	Study Design	Test Product(s)	Number of subjects	Type of subjects	Outcomes	Statistical analysis	Clinical relevance of results
active-tion in <i>H. pylori</i> infected patients Kottakis <i>et al.</i> 2009			<i>pylori</i> negative healthy men volunteers	receiving any immunosuppressive medications before or during the trial, and not suffering from any other infectious diseases that could modify their immunological status	(P=0.0027) and AGPs plus HP-NAP (P=0.0004, in <i>H. pylori</i> -positive patients who consumed AGPs for 2 months. Similar results were also obtained when neutrophils were incubated with AGPs plus HP-NAP (p=0.0038) but not with HP-NAP (p>0.05) in controls.	activation as indicated by measurement of cytochrome C reduction between baseline and 2 months post-treatment period. Significance set at p<0.05.	benefit of mastic consumption in upper and lower gastrointestinal <i>H. pylori</i> -associated pathologies
Efficacy against <i>H. pylori</i> <i>in vivo</i> Dabos <i>et al.</i> (2010ii)	Prospective randomised controlled trial 14 days (UBT performed a mean of 8 days (4-14 days) before starting	Treatment group A: 350 mg three times a day (tid) of CMG for 14 days Treatment group B: 1.05 g of pure CMG for 14 days, Treatment group C:	52 patients randomly assigned to the for groups A, B, C and D Drop out: 1 patient from Group A completed the study but did not return for his follow up UBT 5 weeks later. 2 patients from Group C completed the study	Patients confirmed harbouring <i>H. pylori</i> by a 13C urea breath test (UBT). before entering the study	Eradication of <i>H. pylori</i> 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 UBT. There were	Intention to treat analysis Results shown as mean and SEM. Paired t-test for comparisons between UBT values before and after the	Only pilot study (small size)

Type of study	Study Design	Test Product(s)	Number of subjects	Type of subjects	Outcomes	Statistical analysis	Clinical relevance of results
	treatment and repeated a mean of 39 days (33-61 days) after completion of the study (medication)	pantoprazole 20 mg twice a day (bd)+CMG 350 mg tid for 14 days Control group D: or pantoprazole 20 mg bid+amoxicillin 1 g bid+clarithromycin 500 mg bid for 10 days	but did not return for their UBT 5 weeks later. 1 patient in Group D stopped because of side effects (diarrhoea and abdominal cramps).		no statistically significant differences in mean UBT values in 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).	interventional. Significance set at p<0.05.	
<b>Functional dyspepsia</b>							
Efficacy in functional dyspepsia Dabos <i>et al.</i> (2010)	Prospective randomised double-blind placebo controlled clinical trial 3 weeks; Follow up: 1 month	Treatment group: capsule containing 350 mg CMG 3 times daily per os before meals for 3 weeks Placebo group: capsule containing lactose 3 times daily per os before meals for	One hundred and forty-eight patients (148), males and females 18-75 years of age, randomly assigned to the treatment (74) or to the placebo (74) group. Drop out: 4 patients (1 in the active treatment and 3 in the placebo group)	Patients fulfilling Rome II criteria for functional dyspepsia with symptoms present for at least 12 weeks in the previous 9 months	Primary outcome: change in the summary HKID score relative to the baseline. Secondary outcome: patients' global assessment of efficacy at the end of the study. The symptom score after	Intention to treat analysis Wilcoxon signed ranked tests for comparisons between scores of HKID before and after the intervention. Fisher's	Good clinical relevance with statistically significant results despite its relatively small sample size Main limitations of the study: the HKID has not been validated yet in the study population.

Type of study	Study Design	Test Product(s)	Number of subjects	Type of subjects	Outcomes	Statistical analysis	Clinical relevance of results
		3 weeks	because of lack of efficacy. One patient in the active treatment was lost to follow up.		treatment was significantly lower in the CMG than in the placebo group ((14.78±1.78) vs (19.96±1.83)). There was an improvement of symptoms in 40% of patients receiving placebo and in 77% of patients receiving mastic(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).	exact test for the patients' global assessment to response. Results shown as mean± standard error of the mean	

Type of study	Study Design	Test Product(s)	Number of subjects	Type of subjects	Outcomes	Statistical analysis	Clinical relevance of results
<b>Crohn's Disease</b>							
Effects of mastic on Cytokine production of circulating mononuclear cells of patients with active Crohn's disease Kaliora et al., 2007i Kaliora et al. 2007ii	Controlled study Crohn's Disease 4 weeks	2 times 3 mastic caps per day (0.37 g/cap; d, 2.2 g in total) for 4 weeks	10 patients and 8 healthy controls No drop out	Patients with established mildly to moderately active Crohn's disease and healthy controls	Treating CD patients with mastic resulted in the reduction of TNF-alpha secretion ( $2.1 \pm 0.9$ ng/ml vs $0.5 \pm 0.4$ ng/ml, $p=0.028$ ). MIF release was significantly increased ( $1.2 \pm 0.4$ ng/ml vs $2.5 \pm 0.7$ 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.	Results expressed as mean $\pm$ SE. Mann-Whitney Test for comparing differences between patients and controls prior the intervention. Significance set at $p < 0.05$	Preliminary study. Further double-blind, placebo-controlled studies in a larger number of patients are required to clarify the role of this natural product in the treatment of patients with CD
<b>Dental plaque</b>							
Efficacy of	Placebo	Treatment	10 volunteers divided	Subjects with low	Decrease of dental	Not known	Small study, where

Type of study	Study Design	Test Product(s)	Number of subjects	Type of subjects	Outcomes	Statistical analysis	Clinical relevance of results
mastic chewing in reducing dental plaque Topi-tsoglou-Themeli et al. 1984; 1985	controlled study 10 days	group: mastic chewed for 10 days Placebo group: placebo chewed for 10 days	into a treatment group and a placebo group	caries rate	plaque in treatment group		statistics and outcome are not described in details
Plasma lipid and blood sugar reduction							
Efficacy in plasma lipid and blood sugar reduction Triantafyllou et al. 2007	Randomised control study Follow up period: High-dose group: 18-month Low-dose group: 12-month	High-dose group: 5 g of mastic powder daily per os. Low-dose group: CMG solution containing one-seventh of the daily dose taken by the high-dose group per os daily.	n=133 (93 women, 43 men), aged over 50 years randomly assigned to a high-dose group (45) and a low-dose group (83)	Healthy volunteers	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 High-dose group: decrease in serum total cholesterol, LDL, total cholesterol/HDL ratio, lipoprotein (a), apolipoprotein	Generalised least squares random-effects linear regression	Supportive to the safety of oral use of mastic

Type of study		Study Design		Test Product(s)		Number of subjects		Type of subjects		Outcomes	A-1, apolipoprotein B (apoB/apoA-1 ratio did not change), SGOT, SGPT and gamma-GT levels. Low-dose group: decrease in glucose levels in males	Statistical analysis		Clinical relevance of results	

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

No data available.

### **4.4. Overall conclusions on clinical pharmacology and efficacy**

#### **Oral use**

The existing trials (to approximately 300 persons) evaluated the oral use of mastic in alleviation of the symptoms associated with gastric or duodenal ulcer or to relief of the symptoms of functional dyspepsia.

Only one double blind placebo controlled clinical study was identified (Al-Habbal *et al.* 1984). The majority of trials are small with methodological weakness, such as: inclusion criteria not well defined, high drop-out rates (up to 50%), primary endpoint not always defined and no follow-up phase. Taking into account all these weaknesses the results have a limited value and are not considered sufficient to support a well-established medicinal use according to Article 10a of Directive 2001/83/EC.

#### **Cutaneous use**

A combination wound adhesive containing mastic with other compounds (styrax, methyl salicylate and ethanol) was also clinically tested cutaneously in comparison with wound adhesive containing tincture of benzoin USP (aloe, tolu balsam and styrax liquid in alcohol). The study was conducted in 300 patients (200 woman and 100 men), divided into two groups, undergone plastic surgical procedures and followed up for at least 16 months post-operatively. The authors reported better results in adhesive strength with no adverse reaction in the group using wound adhesive containing mastic. The positive outcome of these trials cannot support any therapeutic indication.

In conclusion, the existing results of the clinical trials are insufficient to support a well-established use indication, but are potentially supportive for the proposed oral use in mild dyspeptic disorders.

## **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 clinical trials, there are no concrete data concerning patient exposure.

Mastic, within a combination product, was also clinically tested cutaneously with no adverse reactions reported. This could support the safe cutaneous use.

### **5.3. Adverse events, serious adverse events and deaths**

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

Only two case reports described allergic contact dermatitis from medical adhesive bandages containing a commercial liquid adhesive combination product with mastic (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.

There is only limited information related to the safety, since only two case reports described allergic contact dermatitis from medical adhesive bandages containing a commercial liquid adhesive combination product with mastic.

## 6. Overall conclusions (benefit-risk assessment)

There are several clinical studies that investigated oral use of mastic (alone) or the cutaneous use of mastic (in combination). The majority are small with methodological weakness, therefore cannot support any well-established use indication of *P. lentiscus* L. resin (mastic). However, these trials can be supportive for the traditional use as they did not raise any safety concerns.

The powder of *P. lentiscus* L., resin has been used in traditional and folk-medicine in several countries (Iraq, Iran, Turkey, 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 requirements for traditional use according to Directive 2001/83/EC are considered fulfilled for the medicinal use of powdered mastic.

The mastic powder as herbal substance has been used for cutaneous use (4-40%) while according to information by the Rapporteur during the last years (80's) all galenic formulas through pharmacies and medical prescriptions are containing 9-11% for use in skin disorders such as minor inflammations.

Results of non-clinical *in vitro* studies show some antimicrobial activity. Mastic was reported to exhibit particular strong activity against *H. pylori*.

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 long-standing medicinal use in traditional medicine for more than 30 years. The following traditional use indications are included in the monograph:

### Indication 1)

Traditional herbal medicinal product used in mild dyspeptic disorders.

The use in children and adolescents under 18 years of age has not been established due to lack of adequate data.

### 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. Only two case reports described allergic contact dermatitis from medical adhesive bandages containing a commercial liquid adhesive combination product with mastic.

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