The Therapeutic Potential of the Essential Oil of *Thymbra capitata* (L.) Cav., *Origanum dictamnus* L. and *Salvia fruticosa* Mill. And a Case of Plant-Based Pharmaceutical Development

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This review performs a comprehensive assessment of the therapeutic potential of three native herbs of Crete (*Thymbra capitata* (L.) Cav., *Salvia fruticosa* Mill. and *Origanum dictamnus* L.), their phytochemical constituents, health benefits and issues relevant to their safety, within a translational context. Issues discussed comprise: 1) Ethnopharmacological uses of the three herbs, reviewed through an extensive search of the literature; 2) Systematic analysis of the major phytochemical constituents of each plant, and their medicinal properties; 3) To what extent could the existing medicinal properties be combined and produce an additive or synergistic effect; 4) Possible safety issues. We conclude with a specific example of the use of a combination of the essential oils of these plants as an effective anti-viral product and the experience gained in a case of a plant-based pharmaceutical development, by presenting the major steps and the continuum of the translational chain.

Keywords: traditional medicine, Southeastern Europe, Mediterranean, Near East, synergy, regulatory affairs, clinical trials, antiviral

INTRODUCTION

Plants are traditionally used, in different forms, for the treatment of diseases, since the Neolithic Era (Hardy, 2019). Different populations used native flora for the production of preparations, efficient in curing different diseases and conditions (Fabricant and Farnsworth, 2001). The Cretan area (KK, Crete and Karpathos floristic region) is such an example, as its evolutionary history preserved a very diverse flora, with more than 2000 indigenous species, including a high number of endemics (Dimopoulos et al., 2013). Cretan people abundantly use plants and greens in different aspects of their life. Cretan diet, a specific entity of the Mediterranean diet, uses a diversity of plants for culinary purposes (Renaud, 1995 and references herein), while a variety of plants are used as decoctions and infusions for recreational or medical purposes. We systematically analyzed the habits of a rural population in Crete and reported that use of different plant infusions, alone or in combination, resulted in a significant protection from common cold and influenza infections (Lionis et al., 1998).
Based on this study, we have focused on three of the most efficient plants (Coridothymus capitatus, Salvia fruticosa and Origanum dictamnus) and developed a supplement, efficient at combating upper respiratory tract infections (Duikler et al., 2015; Anastasaki et al., 2017).

In this review, we analyze in depth the health benefits of these plants, their most prominent active compounds and their combination. In addition, we review our experience with the development of a nutraceutical product, discussing potential bottlenecks encountered during the process.

THE PLANTS

Taxonomy and Distribution

According to the Euro + Med plantbase, **Coridothymus capitatus** (L.) Reichenb. fil. is a dwarf-shrub. It is the only member of the monospecific genus *Coridothymus*. According to the Euro + Med plantbase (Accessed at June 2017), the name *Coridothymus capitatus* (L.) Reichenb. fil. is synonym of *Thymbra capitata* (L.) Cav., which is included in Kingdom–Plantae, Division–Tracheophyta, Subdivision–Spermatophytina, Class–Magnoliopsida, Superorder–Asterales, Order–Lamiales, Family–Lamiales, Genus–*Coridothymus* L.

**Accepted name: Thymbra capitata** (L.) Cav., Homotypic synonyms: *Coridothymus capitatus* (L.) Reichenb. fil., *Origanum capitatum* (L.) Kuntze, *Satureja capitata* L., *Thymbus capitatus* (L.) Hoffmanns. & Link.

**Salvia fruticosa Mill.** is also a member of the Labiatae family. The taxon is included in Kingdom–Plantae, Division–Tracheophyta, Subdivision–Spermatophytina, Class–Magnoliopsida, Superorder–Asterales, Order–Lamiales, Family–Lamiaceae Lindl., Genus–*Salvia* L.

**Accepted name: Salvia fruticosa** Mill, Heterotypic synonyms: *Salvia baccifera* Etl., *Salvia clausi* Jacq., *Salvia cypria* Unger & Kotschy, *Salvia fruticosa* subsp. *cypria* (Unger & Kotschy) Holmboe, *Salvia fruticosa* subsp. *thomasi* (Lacaita) Brullo and al., *Salvia incarnata* Etl., *Salvia libanotica* Boiss. & Gaill., *Salvia lobryana* Azn., *Salvia marrubioides* Vahl, *Salvia ovata* F. Dietr., *Salvia sipylea* Lam., *Salvia subtriloba* Schrank, *Salvia syphilea* Lam., *Salvia thomasi* Lacaita, *Salvia triloba* L. f., *Salvia triloba* subsp. *calpeana* (Dautetz & Debeaux) P. Silva, *Salvia triloba* var. *calpeana* Dautetz & Debeaux, *Salvia triloba* subsp. *libanotica* (Boiss. & Gaill.) Holmboe, *Sclarea triloba* (L.) Raf.

**Origanum dictamnus** L. is a local endemic of Crete, member of Labiatae family. The taxon is included in Kingdom–Plantae, Division–Tracheophyta, Subdivision–Spermatophytina, Class–Magnoliopsida, Superorder–Asterales, Order–Lamiales, Family–Lamiaceae Lindl., Genus–Origanum L.

**Accepted name: Origanum dictamnus** L., Heterotypic synonyms: *Amaracus dictamnus* (L.) Benth., *Majorana dictamnus* (L.) Kostel, Heterotypic synonyms: *Amaracus tomentosus* Moench, *Origanum dictamnifolium* St.-Lag., *Origanum saxatile* Salisb.

**Coridothymus capitatus** is found throughout the Mediterranean region distributed in all Mediterranean countries with the exception of France. *Salvia fruticosa* is distributed in Italy, Sicily, and the eastern Balkans, including Cyprus, while *Origanum dictamnus* is local endemic of Crete (Fielding and Turland, 2005).

METHODOLOGY

In the context of this review, PubMed, Scopus and Google Scholar were used for the retrieval of the relevant bibliography. The following inclusion criteria were used for the systematic review of the three plants: articles published in English, articles concerning countries of the Mediterranean basin and also including Jordan, Syria, Iran and Iraq, as well as studies with the following keywords in their text: *Coridothymus capitatus, Salvia fruticosa* and *Origanum dictamnus*. The PRISMA flow diagram, presenting the results of our search, is depicted in Figure 1. The primary search identified 1,162 articles, plus 10 articles from other sources. After the implementation of the eligibility criteria, 26 articles were finally incorporated. Any evaluation of studies concerning the plant quality was out of scope and has not been included in this review.

Concerning the synergistic interactions of the main constituents of the essential oil (see below) the review was carried out through a literature searching of the same databases using the following keywords in all fields: “synerg” AND (carvacrol OR eucalyptol OR 1,8-cineole OR beta-Caryophyllene).” The selection of manuscripts was based on the following criteria: articles published in English and articles where only documented synergy between pure substances was reported. The primary search identified 4,101 articles, with 1,538 from PubMed, 1,910 from Google Scholar and 653 from Scopus and after the implementation of the eligibility criteria, excluding the repetitions, 25 articles were finally included in the qualitative analysis.

ETHNOPHARMACOLOGICAL USES OF THE PLANTS

*Coridothymus capitatus, Origanum dictamnus* and *Salvia fruticosa* are renown since antiquity for their pharmaceutical properties (Karousou et al., 1998). The first two are included in the Dioscorides book “*De Materia Medica.*” *Salvia fruticosa* was introduced in the Iberian Peninsula for cultivation by Greeks and Phoenicians and elements of these cultivations can be discovered today in several parts of the Iberian coast (Rivera et al., 1994). *O. dictamnus* is also mentioned from the Minoan era, through the centuries from several physicians and philosophers including Asklepios, Euripides, Aristotle, Hippocrates, Theophrastus, Virgil and Galen (Vrachnakis, 2003).

*Coridothymus capitatus* is known in local cultures with several common names among which Spanish oregano, thyme, headed thyme, conehead thyme, agrio thymari (αγριό θυμαρί), thymari (θυμάρι), tomilho de Creta and tomilho de Dioscórides, *Salvia fruticosa* is known with the names Greek sage and faskomilo
Origanum dictamnus is known as diktamo, dittany of Crete and dictamo de Creta. 

The ethnobotanical and ethnopharmacological studies concerning the medical use of Coridothymus capitatus, Salvia fruticosa and Origanum dictamnus, as well as from studies on local herbal markets in Eastern Mediterranean region, are presented in Tables 1–3 respectively, together with the plant part and their mode of preparation/use. Based on this information, medicinal uses, in an ethnobotanical context, suggest that the three species have a highly antimicrobial and anti-inflammatory action. In addition, they are active against various targets and diseases, including diseases of the respiratory, digestive and urinary systems.

**PHYTOCHEMICAL CONSTITUENTS**

The essential oils of the three plants is produced from collected plant material, air dried in the dark, at room temperature (25°C) for 10 days. For analysis, after steam distillation, 1 ml of volatile oils were diluted with 2 ml of ether and filtered through anhydrous sodium sulfate to remove water traces and were stored at 4°C. Analysis was performed, as described previously (Duikler et al., 2015), by Gas Chromatography-Mass Spectroscopy (GC-MS, Shimadzu, QP 5050 A), with a MDN-5 column and a Quadrupole Mass Spectrometer as detector, after injection of 2 μL. The carrier gas was helium, the flow rate 0.9 ml/min. The sample was measured in a split mode procedure (1:35). For GS-MS detection an electron ionization system was used with ionization energy at 70 eV. The interested reader should refer to Duikler et al. (2015) for further analytical details.

The chemical constituents of the essential oils of the three plants, as well as their combination (1.5% v/v of pure essential oils in olive oil carrier), used in the context of upper respiratory tract infections are presented in details as Supplemental Material in Duikler et al. (2015). According to this description (see also Figure 2) carvacrol (52.7%) is the main constituent of the mixture, followed by eucalyptol (12.77%) and β-caryophyllene (3.41%). The compounds p-cymene, γ-terpinene, borneol and α-terpineol participate with concentrations 1.32, 1.17, 1.68 and 1.06% respectively, while the rest 15 compounds participate with less than 1% (Figure 2).

Carvacrol (5-isopropyl-2-methylphenol) is an aromatic monoterpen with molecular weight 150.2 g/mol. It is a constituent of many essential oils, especially those found in plants of the family Lamiaceae, where the Origanum vulgare subsp. hirtum (Link) (Kokkini and Vokou, 1989). Eucalyptol (1,8-cineole) is a cyclic ether monoterpen (molecular weight 154.3 g/mol), which is found as a constituent of plant essential oils (its name is derived from its presence in essential oils of Eucalyptus globulus). It is also considered as a major monoterpen emitted by vegetation into the atmosphere (Guenther et al., 1994).
| Area                                      | Local name | Plant part | Medicinal use                                                                 | Mode of preparation/use                                                                 | Literature                      |
|-------------------------------------------|------------|------------|------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------|---------------------------------|
| Israel (Golan heights and west bank region) | Foliage    | Foliage    | Heart diseases, paralysis, diabetes, track pain and inflammation and respiratory system | An infusion of one tsp foliage in a cup of water is made and taken 1/3 times/day until improvement occurs | Said et al. (2002)              |
| Turkey (west Anatolia)                    | Kekik      | Herbs      | For cough colds, stomachache                                                 | Internally, infusion, tea, 3 days on empty stomach                                        | Honda et al. (1996)             |
| Jordan (northern Badia region)            | Zater faresy | Leaves    | Heart and respiratory diseases, diabetes, inflammation and used as rubefacient | —                                                                                       | Al-Shayeh et al. (2000)         |
| Palestinian area                          | Thyme      | —          | Respiratory and urinary digestive systems and inflammation                    | —                                                                                       | Palevitch et al. (1986)         |
| Israel                                   | —          | —          | Cardiovascular system and digestive system                                    | —                                                                                       | Lev (2002)                      |
| The levant countries                      | Headed thyme | —        | Internal diseases, eye diseases, stomach intestine                             | —                                                                                       | Lev and Amar (2002)             |
| Jordan                                    | Conehead thyme | Branch  | Digestive system, respiratory system                                          | —                                                                                       | Lardos (2006)                   |
| Jordan                                    | Conehead thyme | Aerial parts | Respiratory tract diseases (cataarrh and common cold)                         | —                                                                                       |                                 |
| Jordan (Showbak)                         | Thyme      | Aerial parts | Antispasmodic                                                                 | Syrup                                                                                   | Al-Qura’n (2009)                |
| Israel (northern Israel)                  | —          | Leaves    | Pectoral, stomachic, and to treat urinary tract infections                    | Infusion                                                                                                 | Al-Khalil (1995)               |
| Spain (Barros area, Badajoz province)     | —          | Leaves    | Heart disorders. Swelling and drops. Indigestion. Local paralysis              | Tisane. Taken as needed decoction. Used as a drink for 40 days. Steam-bath. Treatment is given for a duration of a month | Dafni et al. (1984)             |
| Cyprus                                    | Throumpin, agrio thymari | Leaves | Digestive, tonic, carminative                                                 | —                                                                                       | Vázquez et al. (1997)           |
| Turkey                                    | Bali kelik, bal kekik, zahter, beyazkek | Flowering branches, essential oil, fixed oil | Diabetes, analgesic, Pharyngitis, cold, flu, pleasure and medicinal tea        | Drink onetea cup 2–3 times a day for 3–4 weeks/apply 2–3 times a day for 1–2 Weeks in, Eso.Lb, Lpw,Spc | Sargin (2015)                   |
| Egypt (Sinai peninsula)                   | Zaatar, Hasha | Flowering tops, leaves | Infusion as analgesic, sedative, digestive diseases, spasms, vomiting, flatulence, Liver diseases (jaundice) | —                                                                                       | Elissa et al. (2014)            |
### Table 2 | Medicinal uses of Salvia fruticosa derived from ethnobotanical and ethnopharmacological studies.

| Area (country)           | Local name          | Plant part       | Medicinal use                                                                 | Mode of preparation/use                                                                 | Literature                        |
|--------------------------|---------------------|------------------|-------------------------------------------------------------------------------|----------------------------------------------------------------------------------------|-----------------------------------|
| Israel (Golan heights and West Bank region) | Foliage             | Stomach ache, intestinal gas and inflammation, diabetes and sexual weakness | An infusion of 50 g in 1 L is prepared and taken orally, 150 cc, 1–3 times/day until improvement occurs | Said & Amar (2000)                |
| Turkey (West Anatolia)   | Almiya çalbasi = almiya yağ  | Herbs            | For flatulence and constipation for babies. For colds, cough, stomachache    | Internally, volatile oil is applied on nipples before nursing to alleviate the flatulence and constipation of a baby. Infusion, as herbal tea | Honda et al. (1996)              |
| Greece                   | Faskomilo, elelifakos | Erial parts      | Hypotension, diabetes, laryngitis, pharyngitis, tonsillitis, constipation, diarrhoea, spasmolytic, anemia, brain stimulant, calming, depression, common cold, arthritis, hair loss, hair tonic, stomatitis, dysmenorrhea, stimulant | Infusion, decoction, external application (footbath, massage, washings) | Hanlidou et al. (2004)           |
| Israel                   | Sage                | Leaf             | Hemorrhages, intestinal diseases and pains                                  | —                                                                                       | Lev & Amar (2000)                |
| Jordan                   | Sage                | Leaf             | Hemorrhages, intestinal diseases and pains                                  | —                                                                                       | Lev & Amar (2002)                |
| Palestinian area         | White sage          | —                | Digestive system, prostate disorders, skin disorders                        | —                                                                                       | Al-Shtayeh et al. (2000)         |
| Israel (northern Israel) | —                   | Leaves           | Indigestion. Coughs and colds                                               | —                                                                                       | Dafni et al. (1984)              |
| Jordan (northern Badia region) | Merameih           | Foliage          | Stomachache, flatulence, inflammation, diabetes and sexual weakness         | Decoction                                                                             | Alzweiri et al. (2011)           |
| Cyprus                   | Tree lobed sage     | Leaf, tip of shoot | Febrile conditions (not specified), gastro-intestinal tract disorders (tenesmus), malaria fever, absent or delayed menstruation, repellent against arachnids, insects, snakes, respiratory tract diseases (catarh and common cold, cough), supporting treatment in pleurisy and pneumonia, wounds | External (topical applications, baths), oral                                          | Lardos (2006)                    |
| Jordan (Showbak)         | Sage, clary         | Leaves stems     | Sedative, for wounds healing                                                | —                                                                                       | Al-Qura’n (2009)                 |
| Jordan (Mujib nature reserve) | Meriamiah         | —                | Astringent and antidianduff                                                  | —                                                                                       | Al-Khalil (1995)                 |
| Jordan (Palestinian area) | —                   | Leaves           | Spasm, common cold, intestinal gases                                         | Infusion (50 g in 1 L of water) is made and taken orally 3 times a day. Infusion is also prepared with tea by adding 3–5 g to a cup of tea | Hudaib et al. (2008)             |
| Cyprus                   | Spatzia, faskomilo  | —                | Hypotension, diabetes, estrogen action, diarrhea, dyspepsia, spasmolytic, anti-aging, anti-peripariment, brain stimulant, depression, and nervous tonic, aphthae, gingivitis, dysmenorrhea, antiseptic, diuretic, tonic | Decoction, infusion/oral (potion), external (Compress, washings)                        | Karousou & Derimontzoglou (2011) |
| Jordan (Ailoun heights region) | Meriamia, Mirameihe | Leaves          | Antispasmodic                                                                | —                                                                                       | Abruji et al. (2007)             |
| Palestinian area         | White sage          | Leaes            | Anti inflammatory gangle, antiseptic, antitussive, antihemorrhoids pain, antihemattemic, anti stomach, disturbances, astringent, carminative, hypotensive | —                                                                                       | Al-Shtayeh et al. (1998)         |
| Israel (northern Israel) | —                   | Leaves           | Indigestion goughs and colds                                                 | —                                                                                       | Dafni et al. (1984)              |
| Turkey (Marmaris, Muğla) | Alicaçay, almakayik, almageyk | Leaves          | Stomachache, flatulence, cold, tonsillitis, laxative, antipyretic             | Infusion, int                                                                         | Gürdal & Kültür (2013)           |
| Palestine/west bank      | —                   | Leaves           | Diarrhea                                                                      | —                                                                                       | Jaradat et al. (2016)            |
### TABLE 3 | Medicinal uses of Origanum dictamnus derived from ethnobotanical and ethnopharmacological studies.

| Area      | Local name      | Plant part | Medicinal use                                                                                                                                                                                                 | Mode of preparation/use                                      | References                                      |
|-----------|-----------------|------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------|------------------------------------------------|
| Greece    | Diktamos, erontas | Eerial parts | Diabetes, liver disorders, spasmyolytic, stomach ulcer, cholesterol, brain stimulant, headache, antiseptic, sanative, diuretic, dysmenorrhea, antibacterial activities, aphrodisiac, stimulant          | Infusion, external application (washings, compress)            | Hanlidou et al. (2004)                           |
| Iran      | Poneh kouhi      | —          | Dyspnea, bronchitis, allergy, depression, itch, dementia, abortifacient                                                                                                                                   | Decoction                                                   | Naghibi et al. (2005)                           |
| Cyprus    | Diktamo          | Eerial parts | Liver disorders, headache, neuralgia, cough, antiseptic, sanative, aphrodisiac, tonic                                                                                                                     | Infusion/oral (potion), external (compress)                  | Karousou and Deirmentzoglou (2011)              |
| Europe    | Dictamnus        | —          | Diabetes mellitus, obesity, disorders of lipoprotein metabolism and other lipidaemias, mood disorders, sexual dysfunction (not caused by organic disorder disease), epilepsy, acute nasopharyngitis (common cold), acute pharyngitis (unspecified), acute tonsillitis (unspecified), gingivitis and periodontal diseases, other specified disorders of teeth and supporting structures (toothache NOS), gastric ulcer, gastritis and duodenitis, diseases of liver, xerosis cutis, inflammatory polyarthropathies, pain in joint, acute nephritic syndrome, renal tubule-interstitial diseases, calculus of kidney and ureter, disorder of urinary system (unspecified), absent scanty and rare menstruation, pain and other conditions associated with female genital organs and menstrual cycle, dysmenorrhea unspecified, spontaneous abortion, long labor, cough, headache, convulsions (not elsewhere classified), multiple superficial injuries (unspecified), multiple open wounds (unspecified) | —                                                           | Martínez-Francés et al. (2015)                 |
**β-caryophyllene** is a natural bicyclic sesquiterpene with molecular weight 204.4 g/mol that is a constituent of many essential oils, such as clove or rosemary oil (Bernardes et al., 2010).

Concerning the other compounds, **p-cymene** is a naturally occurring organic compound that is characterized as a hydrocarbon related to a monoterpenes. Its molecular weight is 134.2 g/mol and it is a constituent of several essential oils, such as the oil of cumin (Allahghadri et al., 2010). **γ-terpinene** is a terpene with molecular weight 136.3 g/mol. It is a major component of essential oils made from *Citrus* fruits (Lücke et al., 2002). **Borneol** is a bicyclic monoterpenone with molecular weight 154.3 g/mol containing exactly two rings which are fused to each other. **α-Terpineol** is a naturally occurring monoterpenone alcohol, derived from several sources, including oil of pines. Terpineol, due to its pleasant odor (similar to lilac), is used as an ingredient in perfumes, cosmetics, and flavors (Kim and Chung, 2000).

### USES OF PHYTOCHEMICAL CONSTITUENTS

Apart from the ethnobotanical and ethnopharmacological uses of plants mentioned above, several uses of the aforementioned phytochemical constituents are also documented:

**Carvacrol**

Carvacrol is the basic constituent of many essential oils, mainly of oregano species (*Labiatae*) (Kokkini and Vokou, 1989; Kirimer et al., 1995; Baydar et al., 2004; Stefanaki et al., 2016); it is characterized by its strong antioxidant properties (similar to those of vitamin E and ascorbic acid) (Mastelic et al., 2008). It is also known for its pronounced antimicrobial and antibacterial action (Suntres et al., 2015). The antimicrobial activity of several essential oils has been related to their content of carvacrol (García-Beltrán and Esteban, 2016), while, in a number of studies, the antimicrobial and the antibacterial action of the pure compound has also been investigated (Ben Arfa et al., 2006).

The antibacterial action of carvacrol extends to a variety of Gram-positive and negative bacteria (Kim et al., 1995; Rattanachaikunsopon and Phumkhachorn, 2010a; Ravishankar et al., 2010; Rivas et al., 2010). The compound acts as a transmembrane monovalent cation (hydroxyl proton for a potassium cation) exchanger (Suntres et al., 32,015). Therefore, in addition to its hydrophobic characteristics, allowing its accumulation in the membrane, the presence of free hydroxyls is essential for its antibacterial and antimicrobial activity (Ben Arfa et al., 2006).

An anti-inflammatory action of carvacrol is also documented (Landa et al., 2009; Hotta et al., 2010) and relies to the decreased
production of inflammatory mediators, including cytokines, prostaglandins, enzymes, nitric oxide (NOS) and reactive oxygen species (ROS) (da Silva Lima et al., 2013). The authors suggest that carvacrol’s anti-inflammatory effects specifically induced IL-10 release, leading, subsequently, to the reduction of IL-1β and prostanoids production. In addition, it was reported that the inhibition of prostaglandin synthesis (Wagner et al., 1986) by carvacrol, is the basis of its anti-inflammatory activity. Additional mechanisms of anti-inflammatory actions of carvacrol include its agonistic activity for Transient Receptor Potential Vanilloid 3 (TRPV3), an ionic channel implicated in hyperalgasia and possibly skin sensitization (Xu et al., 2006). In addition, carvacrol activates and promptly desensitizes the specific sensor of environmental irritants TRPA1 ion channel/receptor (Xu et al., 2006). Klauke et al., 2014). Therefore, CB2 receptor-selective agonists, like β-caryophyllene is a widespread plant volatile compound, which is a selective ligand of the CB2 cannabinoid receptor, specifically related to analgesia and inflammation, and devoted of psychotrophic actions, mediated by CB1 (Gertsch et al., 2008; Klauke et al., 2014). Therefore, CB2 receptor-selective agonists, like β-caryophyllene, are potential analgesic drug candidates, in various types of pain (such as neuropathic pain) (Guindon and Johnston, 2000).

β-Caryophyllene
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β-Terpinene
The antimicrobial activity of γ-terpinene is debatable, with positive (Sato et al., 2007) and negative (Sivropoulou et al., 1996) reports, against strains of Gram-positive or Gram-negative bacteria.

Borneol
Borneol is a known medicinal substance in Chinese and Indian traditional medicine. Borneol and its derivatives, possess antimicrobial (Corrêa et al., 2012), anti-inflammatory (Ehrnhöfer-Ressler et al., 2013) and antiviral activity. It also possesses a highly stimulatory action at GABA\(\text{A}\) receptors (Granger et al., 2005; Manayi et al., 2016). GABA is the predominant inhibitory transmitter in the mammalian central nervous system and stimulation of GABA\(\text{A}\) receptors produces anxiolyis, sedation, anesthesia and myorelaxation (Chebib and Johnston, 2000).

β-Terpinene also expressed topical analgesic action (Wang et al., 2017), providing evidence for a topical analgesic efficacy in humans; TRPM8 was identified as its main molecular target. Moreover, borneol demonstrated anti-thrombotic effects, related to its anticoagulant properties (Li et al., 2008; Chen et al., 2015), possibly related to its potent modulation of the nitrite/nitrate reductase activity (Tang et al., 2009).

α-Terpinene
α-terpinene is characterized by a moderate antibacterial activity, with a broad antibacterial spectrum. It is worth mentioning that α-terpinene showed antibacterial activities against penicillin-resistant bacterial strains (Kotan et al., 2007).

α-terpinene exerts central and peripheral antinociceptive activity (Quintans-Júnior et al., 2011). It also inhibits NF-κB and subsequently down-regulates the expression of proinflammatory IL-1β and IL-6 cytokines (Held et al., 2007; Hassan et al., 2010), mainly explaining the compound’s antinociceptive and anti-inflammatory properties (Oliveira et al., 2012).

α-terpinene also exhibited a gastroprotective action in ethanol-induced gastric ulcers (Souza et al., 2011) and beneficial effects in the cardiovascular system (Ribeiro et al., 2010). The proposed mechanism of action included endothelial NO-related vasorelaxation and activation of the NO–cGMP pathway.

REGISTERED MEDICAL APPLICATIONS
A series of clinical studies (most of them double-blind placebo-controlled trials, Table 4) evaluated the efficacy of 1,8-cineole (eucalyptol), in several medical issues. 1,8-cineole (eucalyptol) is a licensed medicinal product in Germany, since many years, in intestine-soluble capsules, for the treatment of acute and chronic...
bronchitis, sinusitis, respiratory infections and rheumatoid-like joint diseases (Juergens et al., 2003; Guimarães et al., 2013).

Borneol and p-cymene have also been subjects of clinical studies for induced analgesia and treatment of the Fish Tapeworm disease respectively (Vartiainen, 1950; Wang et al., 2017).

SYNERGY OF PHYTOCHEMICAL CONSTITUENTS

According to a classical definition, synergy occurs when the combined effect of two or more substances is greater than the sum of the individual agents (Breitinger, 2012). When the registered effect is an add up of individual actions, the action is reported as additive. Recently, the definition of synergy has been clarified from two points of view, the pharmacodynamic (enhanced therapeutic actions on the same target) and pharmacokinetic (no direct interaction but a multi-target behavior) (Yang et al., 2014; Zhou et al., 2016). The main reason of employing combinations of active substances with synergistic interactions is to reduce the administered amount of each compound and to increase the biological activity of a preparation/mixture against a specific target. In addition, this strategy diminishes the chance of pharmaceutical resistance of the pathogenic organism (Araújo et al., 2016).

Synergistic interactions were known for specific substances long time ago. For example, Barbaste et al. (2002) proposed a “cascade” reaction from lipophilic to hydrophilic antioxidants, enhancing their biological effects, while Vardar-Ünlü et al. (2003) suggested that the crude essential oil of Thymus pectinatus var pectinatus was more effective as antioxidant (DPPH assay) than its main active components (thymol and carvacrol). Testing the antioxidant activity of thymol, carvacrol and p-cymene, Milos and Makota (2012) showed synergistic effects in any combination of any two of the above compounds. Moreover, p-cymene, the precursor of carvacrol was found to enhance the bactericidal activity of carvacrol when used in combination (Kiskó and Roller, 2005; Rattanachaikunsopon and Phumkhachorn, 2010b). Synergistic effects of p-cymene have also been reported in relation to thymol and γ-terpinene (Daqan and Abdullah, 2017).

Synergistic interactions of carvacrol, eucalyptol (1,8-cineole) and β-caryophyllene are presented in Table 5. Synergy was also reported between carvacrol and other minor constituents, such as, p-cymene, carvacrol and thymol. Eucalyptol also appeared to have synergistic effects with other constituent, including camphor, terpinene-4-ol and caryophyllene oxide. Additionally, synergy was also reported between minor constituents, such as between camphor and p-cymene.

SAFETY ISSUES

Carvacrol, eucalyptol and β-caryophyllene, p-cymene, γ-terpinene, borneol and α-terpineol have been approved by the Food and Drug Administration (FDA) for food use (Code
for Federal Regulation: 21CFR172.515). Moreover, the European Commission Implementing Regulation (EU No 872/2012) of October 1, 2012, based on the evaluations of EFSA, included carvacrol, eucalyptol and β-caryophyllene, in the Union’s List of Flavorings and Source Materials (Table 6). Their use is therefore permitted, in accordance with good agricultural and manufacturing practices and without given specific restrictions. This EU list takes also into consideration the reports of the Chemical Abstracts Service (CAS), the Joint FAO/WHO Expert Committee on Food Additives (JECFA) and the Council of Europe. The rest of the constituents included in the essential oil’s composition of the mixture of Coridothymus capitatus, Salvia fruticosa and Origanum dictamnus (Duijker et al., 2015), are also included in the same list, concerning Food supplements among others, as defined in Directive 2002/46/EC of the European Parliament and the Council, excluding food supplements for infants and young children.

However, in addition to these beneficiary compounds, the extract of the three aromatic plants includes also 0.74 and 0.52% of cis- and trans-thujone respectively (Duijker et al., 2015). Alpha and beta thujone are, according to the Regulation EC No 1334/2008 of the European Parliament and of the Council on December 15, 2008, among the substances which shall not be added as such, to food or food supplements. Maximum concentrations of thujone, naturally present in flavorings and food ingredients with flavoring properties, have been introduced. According to Regulation EC No 1334/2008, “The maximum levels shall not apply where a compound food contains no added flavourings and the only food ingredients with flavoring properties which have been added are fresh, dried or frozen herbs and spices. After consultation with the Member States and the Authority, based on data made available by the Member States and on the newest scientific information, and taking into account the use of herbs and spices and natural flavoring preparations, the Commission, if appropriate, proposes amendments to this derogation.” EMA/HMPC (2010) reported that exposures in the range between 3 and 7 mg/day do not pose special concerns. For higher concentrations, a case-by-case benefit/risk assessment is necessary (Lachenmeier and Uebelacker, 2010; Pelkonen et al., 2013; Sotiropoulou et al., 2016). Finally, Dettling et al. (2004) showed that a single dose of 0.28 mg/kg in men (20 mg/70 kg) and of 0.24 mg/kg (17 mg/70 kg) in women provided “borderline relevance” of adverse effects, mainly related to perturbations in driving, operating machinery, etc.

### A CASE STUDY OF THE USE OF PLANT EXTRACTS AS A PHARMACEUTICAL PRODUCT: LESSONS LEARNED

The idea of using herb extracts for the treatment of upper respiratory infections was raised several years ago, when...
epidemiological observations revealed that people who consumed certain herbs infusions had low rates of respiratory infections and rarely suffered from common colds or influenza infections. The efficacy of the selected herbs was attributed to their antioxidant properties (Lionis et al., 1998). After years of in vitro and in vivo research, an essential oil combination was developed, based on the extracts of the three plants discussed in the present review (Coridothymus capitatus, Salvia fruticosa and Origanum dictamnus) in extra-virgin olive oil (Duijker et al., 2015); we showed that this combination exerted a synergistic effect against viral upper respiratory tract infections, including influenza (Anastasaki et al., 2017). Moreover, an in vitro study revealed that this combination exhibited a remarkable direct antiviral activity against influenza A/H1N1 viral strains, influenza B and human rhinovirus 14 (HRV14), related to a defective trafficking of influenza A Nucleoprotein (NP) (Ts Eliou et al., 2019). In this respect, the “one drug, one target, one disease” approach (Zhou et al., 2016) was “violated.” Indeed, the combinatorial use of herbal preparations resulted in an synergistic effect, beyond the reported properties of each plant. Hence, here we address the case of the development and commercialization of a product, containing this essential oils’ combination.

The scheme of the translational chain, from the step of biodiversity to the development of a commercial product, is presented in Figure 3. The first edge of the chain, leading from a plant extract to a final product, relies on the choice of plants, as well as the choice of secondary metabolites, whose biological activity is expected to ensure the desired health benefit (drug design). For example, the preparation containing the essential oils of Thymbra capitata (L.) Cav., Salvia fruticosa Mill. and Origanum dictamnus L. avoided plant extracts rich in alkaloids, as preliminary (Lionis et al., 1988) and clinical (Duijker et al., 2015) or laboratory evidence (Ts Eliou et al., 2019) documented a potent health benefit of the preparation.

Another part of the continuum is related to the implementation of a clinical study, which is one of the main obstacles in the translational process. In the presented case, it was valuable not only for the demonstration of an antiviral action, but also for the evaluation of the amelioration of the symptoms of the disease, due to supplementary actions of the substances involved in the combination of essential oils.

The supply of raw material is an important part of the translational chain. It is well known that several restrictions rule the natural collections (harvests) and trade of herbs and spices, especially within the framework of the EU environmental policy, as well as within the framework of the United Nations Convention on Biological Diversity. For example, 232 medicinal and aromatic plant species are listed on Appendix II of CITES, which regulates the international trade in endangered species. Worldwide, the increased needs for medicinal plants, in combination with the strengthening of the regulatory framework for their collection and trade, have caused supply constraints of medicinal plants of high ethnopharmacological interest, which introduced a serious constrain in the development and commercialization of plant-derived products (Amirkia and Heinrich, 2014). In the presented case, the plants are cultivated under controlled and monitored conditions, while local farmers have an important income from this agricultural activity. This activity constitutes a viable developmental axis for the local communities.

Recently, a new tool based on Ecological Niche Modeling has been developed, in order to support local farmers in the decision-making process, concerning the suitability of the area where their land is located, for cultivation of medicinal taxa of high ethnopharmacological interest (Bariotakis et al., 2019). A web-based, easy-to-use application was created in the framework of precision agriculture, where the predicted suitability scores for each area of interest can be made accessible to anyone, by the use of its GPS coordinates. So, in our case, the raw material is
produced from organic farming, with Global GAP and precision agriculture.

Finally, it should be clear that the continuum in plant-based drug development, is not terminated at the step of release on the market. A follow-up of the final product, after its market release and additional supporting research is necessary. In the example case presented here, a follow up was made through a pragmatic prospective observational study (Anastasaki et al., 2017). In our opinion, this “supporting research” step is necessary as, in many cases, there are further research queries which need clarification even after the pharmaceutical evidence of an essential oil combination. In the example case, the effectiveness of the combination of essential oils in humans was documented (Duijker et al., 2015), but the mode of biological action was not understood. Limited in vitro data was available with ongoing research (Tsleiou et al., 2019), providing a possible mechanism, at a cellular level.

In summary, two main lessons emerged from the development of a new pharmaceutical product: The first is resumed in the words “mind the gap,” a well-known phrase from the London underground, as it reveals the necessity of the bridging between successional steps, or successional links of the translational chain. The second concerns the time lag in bridging some successional steps. Indeed, in practice, many subsequent successive steps are fulfilled before others, imposing several loops in the development of the final preparation, which should be treated and resolved accordingly.

However, in spite of the successful use of the three plants, further research is required, in order to decipher additional multi-target action(s), in view of supplementary beneficial effects, which need to be investigated/screened not only in vitro but also in preclinical and clinical studies in the context of Evidence Based Medicine (Stavrou et al., 2013). The results of this screening are expected to clarify whether the ethnopharmacological gap, between reported traditional uses in ethnobotanical studies and the tested properties, is due to a noise of data collection in ethnobotanical practice or reflects underline biological activities, which should be incorporated in the formal therapeutic practice. Moreover, there are no preclinical or clinical evidence about the exposure of sensitive groups (i.e., pregnant women, children, etc.).

A logical subsequent step might be the development a drug, instead of a dietary supplement. A number of items are required for this shift, especially: 1) the repetition of phase I/II trial, with a greater number of participants, together with a detailed pharmacokinetic study of the major active compounds; 2) the performance of a phased III trial. Phase III studies, undertaken in large numbers of patients, often in multiple centers, assess real outcomes in a variety of patients, approximating the global population of patients, who will receive the drug. Their aim is to compare new treatments with existing ones and to demonstrate long-term safety and tolerance (Hobbs and McCarthy, 2009).

Finally, taking into consideration reported variability of the plants’ chemical fingerprint, at least in two of the three native Cretan herbs, which have been used for the development of the active extract (see Karousou et al., 2000; Karousou et al., 2005), it becomes clear that the biological variability in nature does not conform to the requirements for stability in the composition required by market regulations. Thus, further study is suggested concerning the interaction of environmental, chemical, genetic and epigenetic factors, for the quality assurance process. Last but not least, further studies on the cultivation and storage of the

FIGURE 3 | The flow-chart of a translational chain development.
above species are required for the standardization and quality control measures, along the whole supply chain.

**AUTHOR CONTRIBUTIONS**

EC, SP, CL contributed conception and design of the study, SP and EC wrote the first draft of the manuscript, CL, GS, MK and MB wrote sections of the manuscript. All authors contributed to manuscript revision, read and approved the submitted version.

**FUNDING**

This work was partially supported by a grant from OLVOS Pharmaceuticals. During the peer review of this manuscript, an additional study of our group was published (Kalyvianaki K, Malamos P, Mastrodimou N, Manoura-Zonou I, Yamvoukaki R, Notas G, Malliaraki N,oustou E, Tzardi M, Pirintsos S, Lions C, Sourvinos G, Castanas E and Kampa M. Toxicity evaluation of an essential oil mixture from the Cretan herbs Thyme, greek sage and cretan dittany, npj Science of Food (2020) 4:20; doi:10.1038/s41538-020-00080-1, suggesting the absence of acute or sub-chronic toxicity of the three herb preparation.

**ACKNOWLEDGEMENTS**

We are grateful to Michael Heinrich for his valuable comments on an earlier version of this manuscript.

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