The progress of essential oils as potential therapeutic agents: a review

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Abstract

Essential oils are products obtained from a natural raw material of plant origin, by steam distillation, mechanical processes of citrus fruit epicarp, or dry distillation after separation of the aqueous phase by physical processes. They are usually composed by secondary metabolites of aromatic plants with oxygenated structures such as alcohols, ketones, aldehydes, and esters, presenting therapeutic properties such as antibacterial, antifungal and antioxidant activities. Essential oils represent a small fraction of plant composition, but confer the characteristics for which aromatic plants are used in the pharmaceutical, food, and fragrance industries.

The increasing use of plants by the pharmaceutical industry makes the study of essential oils crucial for the development of new bioactive delivery systems. This paper presents a literature review that summarizes the best advanced data regarding the use of essential oils and their volatile constituents for biomedical applications and focuses on innovative pharmaceutical formulations covering essential oils. Nonetheless it seems clear that more clinical evaluations are required until essential oils can be considered as possible applications in pharmacy or as adjuvants to current medications.

Keywords: Essential oils**,** biomedical potential, pharmaceutical formulations

1. Introduction

Aromatic plants have long been used in traditional medicine due to their preservative and therapeutic properties (as digestives, diuretics, expectorants, and sedatives), in foodstuffs to impart aroma and flavor and also as antioxidant and antimicrobial agents (1). They can be found in the market as infusions, tablets and/or extracts. Several pharmaceutical properties attributed to aromatic plants are somewhat found in essential oils (EOs) and are related to their composition (2).

Essential oils are produced in more than 17.500 aromatic species and are stored in various plant organs, e.g., flowers (*Citrus bergamia,* bergamot orange), leaves (*Cymbopogon citratus,* lemon grass), wood (*Santalum acuminatum*, sandalwood), roots (*Chrysopogon zizanioides*, vetiver), rhizomes (*Zingiber officinale,* ginger; *Curcuma longa,* turmeric), fruits (*Pimpinella anisum,* anise), and seeds (*Myristica fragrans,* nutmeg) (3).

Essential oils are defined as secondary metabolites from plants, characterized by a strong odor, being natural multi-component systems composed mainly of volatile terpenes and hydrocarbons. The chemical profile of the EOs products differs not only in the number of molecules, but also in their stereo-chemical types extracted (4). The extraction product can vary in quality, quantity and composition according to climate, soil composition, plant organ, age and vegetative cycle stage (5, 6). So, in order to obtain the same composition of EOs, they have to be extracted under the same conditions, i.e. from the same organ of the plant, which must been growing on the same soil, under the same climate and picked in the same season (4). Most of the commercialized EOs are chemo-typed by gas chromatography and mass spectrometry analysis. Analytical monographs have been published by several world organizations such as European Pharmacopoeia, International Organization for Standardization, World Health Organization (WHO), and Europe Council to ensure good quality of EOs.

Several techniques encompassing conventional or modern methodologies can be used to obtain EOs and/or extracts from different parts of the aromatic plant, including water or steam distillation, solvent extraction, expression under pressure, supercritical fluid and subcritical water extractions (7). Recent trends have largely focused on green extraction technologies that minimize the use of solvents while also enabling process intensification and a cost-effective production of high quality extracts (8). For instance, the interest for supercritical fluid extraction has increased in last

years as an alternative to the traditional solvent extraction methods. This technique provides high speed and efficiency extraction, while eliminates the concentrating steps and avoid the use of organic solvents, which are potentially harmful in terms of environmental impact (9). http://www.sciencedirect.com/science/article/pii/S0896844616300808 - bib0050

Essential oils and their individual volatile constituents are crucial compounds for biomedical or pharmaceutic purposes due to their antiseptic (i.e. bactericidal, virucidal, and fungicidal) and medicinal (i.e. can be used in embalmment, analgesic, sedative, anti-inflammatory, spasmolytic, and locally anesthetic remedies) properties (2).

Nowadays, approximately 3000 EOs are known, 300 of which are commercially important, especially for the pharmaceutical, agronomic, food, sanitary, cosmetic and perfume industries (4). Some EOs appear to exhibit particular medicinal properties that have been claimed to prevent or even cure some organ dysfunctions or systemic disorders (10, 11).

The study comprises data for the last ten years, considering all EOs as biological products in different drug delivery systems and their main applications. This review article intends to underline the great therapeutic potential of EOs and their volatile compounds in new pharmaceutical dosage forms. The isolation and extraction methods, the chemical composition, and the synergism between the components are highlighted regarding its understandable clinical importance. The improvement of biopharmaceutics by delivery systems was also focus of this work, as well as the safety performance of EOs. In order to help interested readers to understand the regulatory issues and limitations of new pharmaceutical drug delivery systems formulated with EOs and their potential biomedical benefits, this subject is also comprehensively reviewed.

2. Essential oils

Plants may contain EOs in their composition and their diversity may vary with the part of the plant employed as raw material. Essential oils may represent 5% of the vegetal dry matter and could be obtained from roots, rhizomes, leaves, bark, branches, flowers, fruits, and seeds (12). Nevertheless, cultivation, soil and climatic conditions and harvesting time can also determine the composition and quality of the EOs (13). The chemical composition, as well as the biocompounds synergic effect may compromise the good pharmaceutical formulation development and its

biomedical application. So, it is absolutely crucial to understand the EOs composition in order to predict its specific mode of action, and therefore the possible therapeutic outcome.

2.1. Chemical composition

Essential oil composition is highly diverse across different plant species. For instance, 1,8-cineole is the major constituent of *Eucalyptus globulus* (eucalyptus), whereas linalool is abundant in *Coriandrum sativum* (coriander). Within the same plant species, chemo-types are very common. For example *Thymus vulgaris* (thyme) has numerous chemo-types named according to the major compound, e.g., thymol, carvacrol, terpineol, and linalool (3).

Essential oils are complex natural mixtures that can contain different components (in terms or nature and number), being extremely difficult to precisely know and describe its content. Nonetheless, in a generally way, data available reported about 20-60 components at different concentrations varying from 20 to 70% (14).

The components include two groups of distinct biosynthetically origin, being the major constituents generally responsible for the biological properties of the EOs (15). The main group is composed of terpenes, that are made from combinations of several 5-carbon-base (C5) units called isoprene (4) and terpenoids, which are terpenes containing oxygen (4) (Figure 1). Main hydrocarbon terpenes may constitute more than 80% of the plant EOs like monoterpenes (C_{10}) and sesquiterpenes (C_{15}), presenting acyclic structures as mono-, bi- or tricyclic. Furthermore, terpenoids present in EOs comprise a wide variety of chemical organic functions, such as alcohols, aldehydes, ketones, acids, phenols, ethers, and esters (12).

Terpenes and phenylpropanoids may have different biosynthetic pathways concerning their derivatives and generally are separated in plants, but may coexist in some, with one major pathway taking over (4). These groups are also chemically instable (due to the C-C bonds) and thus molecules present different chemical reorganizations (isomerization). Some substances comprising EOs have similar boiling points and are, for this reason, difficult to isolate. On the other hand, extraction and fractionation of these substances should be carried out at moderate temperatures, in order to prevent thermal degradation. In general, terpenes are relatively insoluble in water, and present a poor flavor and aroma impact to the oil, while they are easily decomposed by light and heat, being quickly oxidized (12).

Aromatic compounds are less common in EOs than terpene and are derived mainly from the shikimate pathway, for example, the phenylpropanoid dillapiole. Nevertheless, a few phenols, such as carvacrol and cuminaldehyde, are a rare group derived from terpene biosynthesis by desaturation. Products of nonvolatile compounds, e.g., compounds derived from fatty acids (jasmonic acid) or glycosides of volatiles (e.g., linalool glucoside), are also identified in EOs (3).

Figure 1. Chemical structures of selected components of essential oils adapted from Bakkali et al. 2008 (4).

2.2. Synergism between the components

Essential oils are eco-friendly, being biodegradable in nature. The specific advantage of these compounds appears to be in synergistic effects of their components as evidenced in greater activity when applied as natural EOs, compared with the effects sum of the individual substances. It is well known that the activity of the main components could be potentiated by other minor molecules present in EOs composition (4).

A number of reports established a good correlation between strong antibacterial activity and the presence of monoterpenes, eugenol, cinnamaldehyde, carvacrol, and thymol in EOs (16-18). Other example is the higher repellent activity of the EOs against insects and arthropod, when compared with the effect of the components isolated. The repellent activity of EOs has been linked to the presence of mixtures of hydrocarbons (i.e. monoterpenes and sesquiterpenes) with a diversity of functional groups. Synthetic chemical compounds have been used to protect human from insect bites, but they may cause environmental and human health problems. Therefore, there has been a growing interest for natural and eco-friendly repellents. Some EOs have demonstrated to be comparable to, or even better than synthetic chemicals (19).

Synthetic blends formulated with major components of EOs demonstrated much lesser activity than those of the corresponding essential oil. The minor components that are present in low amounts in EOs may display an crucial role as synergists, thus improving the effectiveness of the major components (19). Apart from this, the main components of EOs such as sesquiterpenoid bisabolol, nerolidol, farnesol and apritone compounds have been associated with a variety of

148 important biological functions as food-grade antimicrobials. These compounds were even
149 investigated for their ability to enhance the bacterial permeability and susceptibility of several
150 strains (i.e. *Escherichia coli* and *Staphylococcus aureus*) to exogenous antimicrobial agents as
151 traditional antibiotics by affecting the membrane permeability of these bacteria (20). The major
152 compounds, generally, were used because reflect quite well the biophysical and biological
153 features of the EOs from which they were isolated, and for that reason, in most cases, only such
154 compounds were analyzed. However, the activity of major compounds can be modulated by other
155 minor molecules, and the numerous compounds of the EOs, sometimes present in trace amounts,
156 are important to the fragrance, density, texture, color, cell penetration, lipophilic or hydrophilic
157 attraction and fixation on cell walls or membranes, as well as to cellular distribution. These
158 characteristics are relevant since the distribution of the oil in the cells define the radical reactions
159 to be produced, which are dependent of their compartment into the cell (4).
160 Therefore, the study of entire oil instead of its major components is crucial to assess all potential
161 biological impacts due to the relevant synergism effect previously reported.

162 ***2.3. Properties and clinical importance***

163 It is essential to underline that regarding their biological properties, EOs are complex mixtures of
164 numerous molecules, and, consequently, their biological effects may be due to a synergism of all
165 molecules or reflect the main molecules present at the highest levels.
166 Essential oils have the capacity to interfere with mitochondrial functions, and in certain cases may
167 add pro-oxidant effects, becoming for this reason an effective anti-cancer agent (7). The anti-
168 mutagenic capacity, which could well be linked to an anti-carcinogenic activity of these natural
169 oils, is well documented (11). Some studies have demonstrated that the pro-oxidant activity is
170 also related to this anti-cancer biological activity, which is mainly related to the activity of some
171 polyphenols present in EOs(21-25). Essential oils are very efficient in reducing local tumor volume
172 or tumor cell proliferation by apoptotic and/or necrotic effects (26-28).
173 Zedoary turmeric oil is an EOs extracted from the dry rhizome of *Curcuma zedoaria*; it is
174 composed by a mixture of structurally diverse compounds, which are volatile and unstable under
175 ambient condition (29). Pharmacological and clinical studies indicated that Z. turmeric EOs
176 exhibits a wide array of therapeutic activities, such as hepatoprotection, tumor suppression, and

177 antioxidant and antibacterial action (30-32). *Myrica gale* EOs has an anticancerogenic activity on
178 the lung and colon cancer cell lines (33). *Nigella sativa* EOs has showed an anti-proliferative
179 activity and inhibition of 1,2-dimethylhydrazine-induced cancer in *in vivo* studies conducted in rats
180 (26).
181 Geraniol inhibits also colon cancer cell proliferation by inducing membrane depolarisation and
182 interfering with ionic canals and signaling pathways (24). *Satureja khuzestanica* EOs also
183 demonstrated a significant effect decreasing the fast blood glucose level in diabetic rats (34).
184 Also, oral administration of a combination of EOs including *Cinnamomum verum* (cinnamon),
185 *Cuminum cyminum* (cumin), *Foeniculum vulgare* (fennel), *Origanum vulgare* (oregano), *Myrtus*
186 *communis* (myrtle) besides others, showed to enhance insulin sensitivity in type 2 diabetes, in
187 addition to lowering circulating glucose in the tolerance testing in rats (35).
188 In the case of EOs, radical production could be very well controlled and targeted without
189 presenting by itself any toxic or mutagenic side-effects to healthy tissues. This beneficial pro-
190 oxidant activity and their associated cytotoxic capacity can make them also excellent antiseptic
191 and antimicrobial agents for personal use, i.e. for purifying air, personal hygiene, or even internal
192 use via oral consumption (4).
193 Other potent biological benefit of EOs is the potential to improve transdermal drug delivery by the
194 interaction of EOs with liquid crystals of skin lipids. They are known as penetration enhancers,
195 sorption promoters or accelerants. These oils are able to penetrate into the skin and to decrease
196 the barrier resistance (36).
197 Thus, EOs could exert their benefits from the traditional to the modern medical domain. Beyond
198 all these examples cited above, other plat EOs and their main biological properties are provided
199 in Table 1.
200
201 **Table 1.** Some plant essential oils and their major biological activity.
202

203 ***2.4 Isolation and extraction methods***

204 Essential oils are defined as the products obtained from hydrodistillation, steam distillation, dry
205 distillation, or mechanical cold pressing of epicarp of citrus fruit (3). The extraction procedure

applied to recover EOs from plant matrix is fundamental and must maintain the natural proportion of its original components. New effective technological approaches to extract and isolate these substances from raw materials are gaining much attention in the research and development field. Traditional approaches to recover essential oil and extracts from plant matrix include steam- and hydrodistillation, liquid–solvent extraction and distillation. However, the hydrodistillation methods may undergo chemical alterations and the heat-sensitive compounds can easily be destroyed at the boiling temperature of water. Therefore, the quality of the essential oil extracts is extremely impaired (57).

The essential oil extract components using solvents at high pressure, or supercritical fluids, has received much attention in the last years, especially in food, pharmaceutical and cosmetic industries, because it presents an alternative to conventional processes such as organic solvent extraction and steam distillation (58, 59). Modern methods of extraction include microwave-assisted process and supercritical fluid extraction, which may have great advantages such as lower oxidation of compounds, a feature especially useful for flower fragrances (3). Supercritical fluid extraction is definitely the most widely studied application. This methodology is performed generally using carbon dioxide (CO_2) for several practical reasons: CO_2 has moderately low critical pressure (74 bar) and temperature (32 ºC), is non-toxic, non-flammable, available in high purity at relatively low cost, and is easily removed from the extract (60). Supercritical plant extracts are being intensively investigated as potential sources of natural functional ingredients due to their favorable effects on diverse human diseases, with the consequent application in the production of novel functional foods, nutraceuticals and pharmacy products. The general agreement is that supercritical extracts proved to be of superior quality, i.e. better functional activity, in comparison with extracts produced by hydro-distillation or using liquid solvents (12, 60, 61).

3. Essential oils in pharmaceutical dosage forms

Essential oils are good penetrants due to the their mode of action, which promotes the disruption of lipid bilayers in cells, thus increasing their own bioavailability and that of co-administered products, making them good synergists A considerable literature on penetrants is derived from

235 the pharmacognosy literature (62). Nevertheless, they can be easily degraded when subjected to
236 undesired environmental conditions such as high oxygen concentration, light and moderate
237 temperatures (62). Moreover, they are usually insoluble in water, which difficult its use, and for
238 some applications is required a controlled release to guarantee its efficiency. These aspects may
239 limit their application, so they should be considered for the production of an adequate formulation
240 of the essential oil in order to maximize their commercial applications. In an essential oil
241 formulations development several criteria should be strictly followed i.e. they must be protected
242 from degradation or from losses by evaporating during processing, should be controlled release
243 in specific sites of action and easy handled.
244 Formulations of EOs comprise liquid (emulsions, micelles, liquid solutions), semi-solid (gels,
245 liposomes), solid (microcapsules or microcomposites) and aerosol forms (Table 2) (63).
246
247 **Table 2.** Examples of pharmaceutical formulations for essential oils application.
248

249 ***3.1. Liquid systems***

250 Essential oils demonstrated antimicrobial activity against cariogenic and periodontopathic
251 bacteria, therefore they have been incorporate into rinses or mouthwashes (66). The major
252 advantage of the use of this type of products is their facility to deliver antimicrobial benefits to all
253 accessible surface in the mouth, whereas remaining active for an extended period of time (66).
254 The antibacterial properties of EOs and their components are also exploited in such diverse
255 commercial products as dental root canal sealers (67).
256 Mouthwashes containing EOs with chlorhexidine gluconate are used as pre-procedural
257 preparations to decrease postoperative infection, oral bacterial load and aerosolization of bacteria
258 (7). These products are also used as a part of plaque-control routine because they penetrate the
259 plaque biofilm and inhibit pathogenic microorganisms by disrupting their cell walls or inhibiting
260 their enzymatic activity. Furthermore, EOs in mouthwashes prevent bacterial aggregation, slow
261 the multiplication and extract bacterial endotoxins (7). For example, Listerine ™ is a mouthwash
262 containing a combination of 4 EOs (0.064% thymol, 0.092% eucalyptol, 0.060% methyl salicylate
263 and 0.042% menthol) and has been report to prevent and reduce supragingival plaque and

gingivitis in orthodontic patients (68). This product holds a stronger bactericidal effect against *Streptococcus mutans* biofilm (68).

Essential oils and their individual volatile substances have also been used in aromatherapy. The term ‘aromatherapy’ is used to describe a wide range of practices involving odorous substances. The word ‘aromatherapy’ combines two words: aroma (a fragrance or sweet smell) and therapy (a treatment). Aroma and massage therapy are the practice of using EOs and their volatile constituents for psychological and physical well-being via inhalation or massage. The clinical use of EOs and their volatile constituents via inhalation or massage has expanded worldwide (7).

3.2. Semi-solid systems

Several drugs have been applied to the skin for systemic treatment, like creams and ointments. As EOs are fat soluble, they have the capability to penetrate the membranes of the skin, before being captured by the micro-circulation, and then drained into systemic circulation, which attain all organs (69). Tea tree oil has been used to prevent methicillin-resistant *Staphylococcus aureus* colonization or infection (70), as well as for the treatment of recurrent herpes labialis because have activity against herpes simplex virus. This essential oil is currently used as aqueous gel (71) or ointment (72), being topically applied.

Essential oils have been also used for the treatment of wounds, an ointment formulation containing *Hypericum perforatum* (EOs, sage and oregano Eos) was developed to supply more efficient wound healing activity. This formulation demonstrated a highest activity when compared with a standard ointment used as reference (73). The ointment formulated did not reduce elastase activity *in vitro*, but inhibited the collagenase activity, and also demonstrated bactericidal and candicidal activities (73).

The EOs of *Lippia sidoides* has been used as a topical antiseptic agent in skin for treatment of wounds and superficial infections. To investigate this effect, ointments at 6% and 12% were applied daily on cutaneous wounds of rats. The applications of the ointment with this essential oil increased the inflammatory response without delay the lesions closure (74).

In another study, a tea tree oil ointment was formulated to treat the ocular itching associated with ocular demodicosis. In this sense, twenty-four patients with ocular itching and ocular Demodex

were administered with chlortetracycline hydrochloride eye ointment lid massage for 4 weeks and then treatment was changed to 5% of tea tree oil ointment for more 4 weeks. It was observed a symptomatic resolution and reduction of Demodex by massage with the tea tree oil ointment formulated (75).

Semi-solid preparations are also widely used for vaginal drug delivery, because in addition to their consistency, these preparations have the ability of adhesion to surfaces for a reasonable period of time before being removed by washing or by natural factors (76). There are some studies on the use semi-solid preparations containing herbal antifungal EOs as an alternative to conventional drugs because of side effects associated to their use, namely toxicity issues and resistance acquired by pathogens. Das Neves et al. (2009) showed that a polycarbophil-based gel with thyme EOs (1%, w/w) exhibited antimicrobial activity against *Candida* spp. This result suggests that such natural product (polycarbophil-based gel with thyme essential oil) may play an important role in the management of vulvovaginal candidiasis (77). Moreover, The commercial available vaginal gel known as “Saugella gel” including in their formulation the EOs of *T. vulgaris* and *Eugenia cariophylla*, containing as active molecules thymol and eugenol, have useful antibacterial and antimycotic activities (78).

3.3. Solid systems

The transformation of liquid compounds into crystalline form can make such materials suitable for the manufacture of powders, granules and tablets (79).

After drying or precipitation, the EOs can be used in different solid forms, such as soft pill, tablets, gums, and most recently, “chewy squares” (80). Lavender EOs from *Lavandula angustifolia* is a popular essential oil that has been used to alleviate anxiety. Bradley et al. (2009) formulated gelatin capsules containing sunflower oil with 100 or 200 µL of organic lavender oil, which were posteriorly administered to 97 patients in a randomized between-subjects double blind test. In this research the lavender capsules showed anxiolytic effects to conditions of low anxiety, although these effects were not observed under conditions of high anxiety (81).

In other study, capsules with *Mentha balsamea* Willd EOs (peppermint) were used to test their effectiveness in patients with irritable bowel syndrome. Peppermint oil was prepared in enteric-

coated, gastro-protected capsules that resists during the passage through the stomach and only dissolve in intestinal which the pH is 7.0 or higher. The capsules included the essential oil and a particular starch that absorbs oils in solid powder (Natrasorb). In this double blind study, 57 patients were treated with two enteric-coated capsules twice per day for 4 weeks. At the end of administration was concluded that peppermint oil improves abdominal symptoms in patients with this pathology (82). In another study were used enteric-coated peppermint oil capsules to test their effect in 42 children with irritable bowel syndrome. After 2 weeks of administration, 75% of patients had reduced severity of pain associated with this disease (83).

More recently, films containing EOs (e.g. oregano essential oil) have been developed demonstrating antimicrobial and antioxidant properties (84). The combined effects of EOs from lemon, thyme, cinnamon on the physical and structural properties of chitosan-based films were also investigated and it was concluded that matrix blended with EOs provides a better formulation option to develop an antimicrobial film (85).

4. Essential oils for aerosols

Essential oils and their volatile constituents can be inhaled through the respiratory tract, which can distribute them into the bloodstream. Inhalation of EOs and the volatile compounds has an important role in controlling the central nervous system. Essential oils have been used in the treatment of epilepsy, as tranquilizer and analgesic (7).

The essential oil from SuHeXiang Wan is used in traditional Chinese medicine for treating epilepsy. Fragrance inhalation inhibited brain lipid peroxidation, to which the anticonvulsive action is attributed. This oil showed an inhibitory effect on the central nervous system via the gamma-aminobutyric acid (GABA)-ergic system (86). The inhalation of fragrances in oolong tea (cis-jasmone and methyl jasmonate) increased the sleeping time of mice induced by pentobarbital, suggesting that these compounds have a tranquillizing effect on the brain and thereby potentiated the GABA receptor response (87). In a study conducted by Barocelli et al. (2004), rodents inhaled lavender EOs (100 mg/kg) during 60 min and analgesic effects on rats were observed. The inhalator treatment with analgesic doses of EOs did not affect mice spontaneous locomotor activity, and doses administered were devoid of sedative side effects (88).

Lavandula angustifolia EOs may decrease the use of required analgesics following tonsillectomy in pediatric patients. In a randomized controlled prospective clinical trial, post-tonsillectomy patients received acetaminophen to relieve pain and, simultaneously, they inhaled lavender EOs. It was observed that the inhalation of EOs reduced the use of acetaminophen (89).

Aromatherapy also represents an effective treatment option for postoperative nausea. To analyze the effect of aromatherapy, 121 patients with postoperative nausea were subjected to a treatment with an aromatic inhaler containing a blend of lavender, peppermint, ginger, and spearmint Eos. It was observed that aromatherapy was efficient in the treatment group comparing with placebo group (90).

5. Improvement of biopharmaceutics by delivery systems

All findings confirm that most EOs are rapidly absorbed after dermal, oral, or pulmonary administration and cross the blood-brain barrier, interacting with receptors in the central nervous system, and thus promoting relevant biological functions (Abdelouaheb et al., 2012). Despite this, it is also well known that most of EOs components are fast metabolized and the half-life of the active compounds, in some cases results in poor bioavailability (Kesarwani et al., 2013). The bioavailability reflects the fraction of the drug that reaches the systemic circulation. If a compound is extensively metabolized it will be poorly absorbed. This means that only a limited fraction of the dose administered will reach the systemic circulation (Abdelouaheb et al., 2012). Nowadays, with the advancement and improvement in the drug delivery technologies, the poor bioavailability obtained for most active compounds could be overcome by the use of novel drug delivery systems. Recently, chemical modifications, coupling agents, liposomes, microparticles, nanoparticles, transferosomes, ethosomes, lipid-based systems, and gel-based systems, as well as cyclodextrins and prodrugs have been explored for successful modified delivery of various herbal drugs (Kesarwani et al., 2013). Encapsulation consists in the entrapment of a substance (i.e. active compound) within a carrier material. The delivery of an active compound can be improved by means of its encapsulation, for instance by enhancing their solubility (i.e. allows solubilizing a hydrophilic compound in hydrophobic matrices or vice versa) or protecting from undesired environmental conditions (i.e. oxygen, light or heat) during processing and storage by

enabling to slow down the degradation processes; protecting its functionality; or permitting targeted release in sites where absorption is desired (91, 92). Moreover, nanoencapsulation can provide some important advantages. The stability of labile bioactive substances (i.e. vitamins) can be enhanced by means of increasing their solubility through nanoencapsulation, which implies solubilizing a hydrophilic compound in hydrophobic matrices or vice versa. Nanoencapsulation can also act as a barrier between bioactive molecules with limited stability and the environment, thus protecting against undesired permeation of gases like CO_2, O_2, H_2O, reducing the evaporation and degradation of volatile substances (i.e. aromas), as well as prevent unpleasant sensorial properties (i.e. bitter taste, astringency or smell) when consuming the food products. Furthermore, this technology can be used for improved delivery, controlled release and bioavailability of bioactive compounds to cells and tissues in the GI tract (93). The encapsulation allows an improvement of the molecular stability (such as the retardation of the crystal growth) and chemical stability by the deceleration/suppression of the chemical reactivity (such as volatility, photodegradation, dehydration, hydrolysis, sublimation, oxidation, thermal decomposition, stereochemical transformations and isomerization), thus protecting the functionality of labile active substances and permitting the targeted release in sites where absorption is desired (79). This enables the production and use of numerous active substances that are currently not in use, due to stability, compatibility and absorption problems (79). Essential oils are in liquid form at room temperature. For that reason, the encapsulation by emulsifying or dispersing the components in an aqueous solution is the simplest form. The major problem of this formulation is the handle difficulty, but this can be solved by producing a dry formulation by microencapsulation, entrapping the oil drops in a carrier material. The techniques of encapsulation can be divided in three main classes: chemical processes (molecular inclusion or interfacial polymerization), physicochemical techniques (coacervation and liposome encapsulation) and physical processes (spray drying, spray chilling/cooling, co-crystallization, extrusion or fluidized bed coating) (63). Some studies have been performed to maintain or even increase the activity and stability of EOs using different encapsulation techniques (Table 3). There are also several data about the potential offered by polymers to achieve the encapsulation goal to improve oils bioavailability. Nonetheless, only a limited number of polymers can be used as constituent of nanoparticles designed to deliver drugs *in vivo* (94, 95). This may be due to the several requirements that the polymer needs to

fulfil for a medical application. It is understandable that the biodegradable character of the polymer is fundamental to guarantee its total elimination from the body in a short period of time. This will allows the repeat administration without any risk of uncontrolled accumulation. Moreover, the polymer and its degradation products must be also non-toxic and non-immunogenic. At least, the polymers should be capable to form nanoparticles, in order to achieve the drug delivery goal for which the nanoparticles are designed. For these clauses only a few of polymers are accepted by health authorities for parenteral administration. Others received agreements to be used in oral or topical formulations or in the food industry. Recently, a large number of copolymers including one part of poly(ethylene glycol) or polysaccharides have been developed. This comes out from the need of nanoparticles with tunable surface properties to modulate their interactions with blood proteins and with mucosa, hence controlling their *in vivo* fate (96). Other different approach to deliver EOs is by complexing with other molecules such as cyclodextrins to improve their solubility and stability. Cyclodextrins complex with various compounds changing their chemical, physical and biological properties. In this way, the use of complexation technique in therapeutically formulations can protect the EOs against decomposition, eliminate undesirable tastes, prevent gastric-intestinal irritation, avoid drug-drug interactions or transform oils into powders (97). Prodrugs are alternative delivery system to increase the bioavailability of EOs. One example of this is the use of linalyl acetate as prodrug of linalool, that potentiate the anti-inflammatory effect on male albino Wistar rats (98). Novel drug delivery systems may improve the efficacy of therapy, generally by improving the bioavailability of EOs. Implication of novel delivery systems for EOs is ruled by physicochemical characteristics, biopharmaceutics and pharmacokinetic parameters of the oils to be formulated (99). All these delivery systems have been beneficial to the pharmaceutical industries as it is a strategic tool for expanding drug market and patent life (100).

Table 3. Examples of essential oils, encapsulation methodologies employed, main results obtained and potential applications.

6. Safety performance of essential oils

The therapeutic potential of biopharmaceuticals are based on their efficacy and safety performance, and for this reason, it must be guarantee their quality and stability until the time of usage or until their expiration date (108). The quality should be kept under the various conditions that pharmaceuticals run into, during production, transportation and storage in warehouses, hospitals, community pharmacies, as well as in consumer's home. Therefore, it is crucial to understand the factors that alter the stability of oils and identify the ways to guarantee their stability. All this should be considered before formulating any dosage form (109). If the application is topical, for example using creams, ointments and mouthwashes, the use is generally safe, but sometimes that appears skin reactions and can cause irritation. If EOs are administrated using aerosols, it is important limit the time of exposure of strong oils because the vapors can cause eye irritation, and some EOs and their volatile constituents are not even suggested to use for direct inhalation (110). In the formulation of tablets, EOs did not showed any degradation in studies conducted for the peppermint and caraway oils for a month time period (108). The drug stability study showed that the both oils were sufficiently stable and suitable for solid dosage forms. Almost all pharmaceutical dosage forms include excipients. Excipients interact with the active pharmaceutical ingredients in the final formulated dosage, which may affect the critical quality (111). This excipients interaction also influences the safety of drugs depending on the route of administration. For this reason, qualitative and quantitative understanding of their composition is a critical factor to understand the dosage form bioavailability and bio-equivalence (109).

Besides the dosage forms, using ways and excipients interaction the blending effect of chemical components in the EOs natural matrix, must also be evaluate. The biological effects of individual chemical components of EOs are known, although the toxic kinetics of their blends is much more difficult to assess. Nevertheless, one of the most attractive features of EOs is that they are, in general, low-risk products. It is documented that their mammalian toxicity is low and they are relatively well-studied experimentally and clinically because of their use as medicinal products (3). In the last few years several studies on the biological properties and therapeutic applications of EOs have been published such as one covering the scientific literature from 2000 onwards, up to the first half of 2007 (62). The toxicity of EOs components has been divided into three structural

466 classes based on toxicological potential (3). Class I compounds with little functionality, such as
467 the aliphatic compound limonene, have a low order of oral toxicity; Class II compounds with some
468 functionality are intermediate toxicity; and Class III compounds have a high potential toxicity owing
469 to reactive functionality. For instances, elemicin, which is an allyl-substituted benzene derivative
470 with a reactive benzylic/allylic position, is assigned to Class III (3). Even so, more studies are
471 necessary to analyze the safety performance of these natural products, for their safe medical
472 application. It's absolutely crucial to understand their mechanism of action and possible collateral
473 damages. For this concern, the study conducted by Monzote et al. (2007) (112) was developed
474 to examine the activity of the EOs from *Chenopodium ambrosioidesin* in mice infected with
475 *Leishmania amazonensis*. The infected animals received two cycle of treatment by different
476 routes (intraperitoneal, oral or intralesional route). The data clearly demonstrated that this natural
477 product could be an alternative for the development of a new drug against cutaneous
478 leishmaniasis based in the ethnomedical information (112). Other study developed a sensitive,
479 accurate and reproducible analytical method to investigate the kinetics of diffusion of 8 selected
480 terpenes (i.e. camphor, carvone, 1,8-cineole, linalool, menthol, α-thujone, menthone, *t*-anethole),
481 chosen as analytical markers of a mixture of plant EOs contained in a cosmetic formulation
482 through Reconstructed Human Epidermis *in vitro*. The method represents a convenient model for
483 safety/quality assessment of cosmetic formulations (113). Data reported that lemon myrtle EOs
484 from *Backhousia citriodora* and its major component citral possess significant antimicrobial
485 activity against a range of Gram-positive and Gram-negative bacteria, yeast, and mold given a
486 MIC range of 0.03-2.0% (v/v). Nevertheless, preliminary toxicity studies using an *in vitro*
487 cytotoxicity assay suggest that neat lemon myrtle oil and citral are toxic to human liver derived
488 cells (HepG2), skin cells (F1-73) and skin fibroblasts. Moreover, product containing 1% lemon
489 myrtle oil was significantly less toxic to human skin cells and skin fibroblasts. This study
490 demonstrated that more studies are needed to assess the toxicity of lemon myrtle oil such as the
491 mechanisms by which the oil may penetrate the human skin barrier and the potential effects it
492 may have at the cellular level (114). The EOs l and solvent extracts of *Salvia africana-caerulea*,
493 *Salvia africana-lutea* and *Salvia lanceolata*, collected at the same locality throughout the 2005
494 growing season, were compared in terms of EOs composition, yields and biological activities. All
495 the solvent extracts prepared from the winter collection exhibited the lowest toxicity, while the

three EOs obtained from autumn collection were more toxic to human kidney epithelial cell (115). Researchers have investigated the acute, sub chronic and genotoxicity of turmeric EOs from *Curcuma longa* L. No effects of mutagenicity to *Salmonella typhimurium* TA-98, TA-100, TA-102 and TA-1535 were observed, as well as turmeric EOs did not produce any chromosome aberration or micronuclei in rat bone marrow cells, and did not produce any DNA damage as seen by comet assay, thus confirming its non-toxicity (116). The chemical composition of *Ligusticum chuanxiong* rhizomes EOs was analyzed, as well as its ability to induce acute toxicity and skin sensitivity. The experimental results indicate that short term application of essential oil is probably safe within the range of its clinical doses, but the doses should be controlled for external use due to its slight skin irritation effect (117).

7. Market and regulatory issues

The greatest use of EOs and their volatile compounds in the European Union (EU) is in pharmaceuticals (due to their functional properties), food (as flavorings and antioxidants) and perfumes (fragrances and aftershaves) (67). Nevertheless and considering the EOs therapeutic potential it is important for a new formulation development to know the clinical definition of a drug by the regulatory entities as Food and Drug Administration (FDA) (118): drugs, are "articles intended for use in the diagnosis, cure, mitigation, treatment, or prevention of disease" and "articles (other than food) intended to affect the structure or any function of the body of man or other animals" (FDA - Regulatory Information, section 201, chapter II). Considering the close relation of EOs between the pharmaceutical and cosmetics domains, it is important to distinguish when it is considered for one or other applications. For that reason (118) cited that the principle holds to: for instance, a fragrance marketed for promoting attractiveness is a cosmetic. But a fragrance marketed with certain "aromatherapy" claims, such as assertions that the scent will help the consumer sleep or quit smoking, meets the definition of a drug because of its health propose. The same happens with a massage oil that is simply intended to lubricate the skin and impart fragrance is a cosmetic, but if the product is intended for a therapeutic use, such as relieving muscle pain, it's a drug. So (118) makes decisions concerning the regulation of EOs on a case-by-case basis. Despite all this, there are some issues that should be considered in the use of

525 EOs. Essential oils come from suppliers or companies all over the world that usually obtain these
526 oils from farmers or wholesalers whose practices and integrity they have come to trust over time.
527 It is for that reason understandable that the quality of EOs can vary widely. The end consumer is
528 not aware of this relationship and practice and even if they knew, it is very difficult to assess the
529 quality. To regulate and assure this feature parameter various agencies abroad have been
530 proactive in setting standards for essential oil quality. Examples of these agencies include: French
531 national organization (119). They provide directives and standards for members of the European
532 Union (EU) states to facilitate intra-community trade. All companies that wish to exchange goods
533 within Europe are therefore obliged to comply with their directives. In the case of EOs, they
534 provide guidelines and information on various topics including determination of water content,
535 chromatographic profiles, determination of acid value, content of phenols, among others.
536 International Organization for Standardization (120), promotes the development of
537 standardization in the areas of intellectual, scientific, technological, and economic activity. For
538 EOs, they provide guidelines for packaging, conditioning, storage, labeling, sampling, and testing.
539 ISO also provides, for a fee, quality standards for individual EOs. This regulatory issue seems to
540 be crucial since the quality of EOs can be compromised from the growing conditions of the plants
541 to their harvesting, distillation, manufacture, distribution, and storage (Figure 2).
542
543 **Figure 2.** Parameters affecting the quality control of essential oils, from raw materials to storage
544 conditions.
545

546 **8. Conclusions**

547 This review attempts to highlight the potential of EOs as new bioactive compounds and their
548 therapeutic potentials, based on modern scientific knowledge of their mode of action, constitution,
549 extraction pathway and their safety issues. The data revised in this work is aimed to underline the
550 need to inquire for new biodrugs for pharmaceutical application. Thus, EOs and their volatile
551 constituents can hopefully be considered in the near future for more clinical evaluations and
552 possible applications, and as adjuvants to current medications. Essential oils could make their
553 way from the traditional into the modern medical domain. Nonetheless, it is important to know that

every compound can be toxic at high concentrations, and EOs are not an exception. The possible toxicity of EOs can also be entirely different to that of the herb, not only due to the common high concentration, but also because of their ability to pass across membranes very efficiently – due to their lipophilicity. Besides this, EOs with no toxicity described may have a toxic effect on some people, and this may be influenced by previous sensitization to a given essential oil. Regarding all this, and considering the huge biomedical potential of EOs, as in all drugs a deep knowledge of the oil should be taken into account before its use in the pharmaceutical field. New legislations are now being a reality, as well as, the entities responsible are aware of the possible toxicity issues or safety performance of every natural product.

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Conflict of Interest statement: The authors declare that there are no conflicts of interest

Table 1.

Plants essential oils	Biological activity	Reference
Achillea ligústica	Anti-cariogenic activity, showed antimicrobial activity against the cariogenic Gram-positive *Streptococcus mutans*	(Maggi, Bramucci et al. 2009)
Aquilaria crassna	Anti-pancreatic cancer properties	(Dahham, Tabana et al. 2016)
Citrus aurantium L.	Anxiolytic effect	(FC, MF et al. 2016)
Citrus limon	Antidepressant effect	(Hao, Lai et al. 2013)
Cymbopogon citratos	Reduce the blood cholesterol level	(Costa, Bidinotto et al. 2011)
Cymbopogon citratos	Acetylcholinesterase and butyrylcholinesterase inhibitory activities	(Chaiyana, Saeio et al. 2010)
Cymbopogon flexuosus	Anticancer activity and causes loss in tumor cell viability by activating the apoptotic process	(Sharma, Mondhe et al. 2009)
Eremanthus erythropappus	Antinociceptive and anti-inflammatory effects	(Sousa, Silvério et al. 2008)
Eucalyptus globulus	Antimicrobial activity against *Escherichia coli* and *Staphylococcus aureus*	(Bachir and Benali 2012)
Hyptis spicigera	Gastroprotective and ulcer healing effects	(Takayama, de-Faria et al. 2011)
Lippia alba	Anti-proliferative effect on human leukemia cells (K562)	(García, Leal et al. 2017)
Lippia multiflora	Anti-inflammatory activity	(Soro, Munier et al. 2016)
Myristica fragrans	Antiangiogenic activity	(Piaru, Mahmud et al. 2012)
Myristica fragrans	Anti-Parasitic Activity against *Toxoplasma gondii*	(Pillai, Mahmud et al. 2012)

Ocimum gratissimum	Anesthetic effects	(Silva, Parodi et al. 2012)
Ocimum gratissimum	Enhance normal hair growth and promote follicular proliferation in cyclophosphamide-induced hair loss	(Orafidiya, Agbani et al. 2004)
Salvia leriifolia benth	Inhibition of LPS-induced NO production, antioxidant activity and cholinesterase inhibitory activity	(Loizzo, Menichini et al. 2009)
Schinus areira	Decrease inflammatory responses and decrease systolic pressure and cardiac contractility	(Bigliani, Rossetti et al. 2012)
Thymus algeriensis	Inhibitory activity towards angiotensin I converting enzyme, antifungal activity, these essential oil can also act as radical scavengers and displayed a lipid peroxidation inhibitory activity	(Zouari, Fakhfakh et al. 2011)
Trachyspermum ammi	Spermicidal potential	(Paul and Kang 2011)

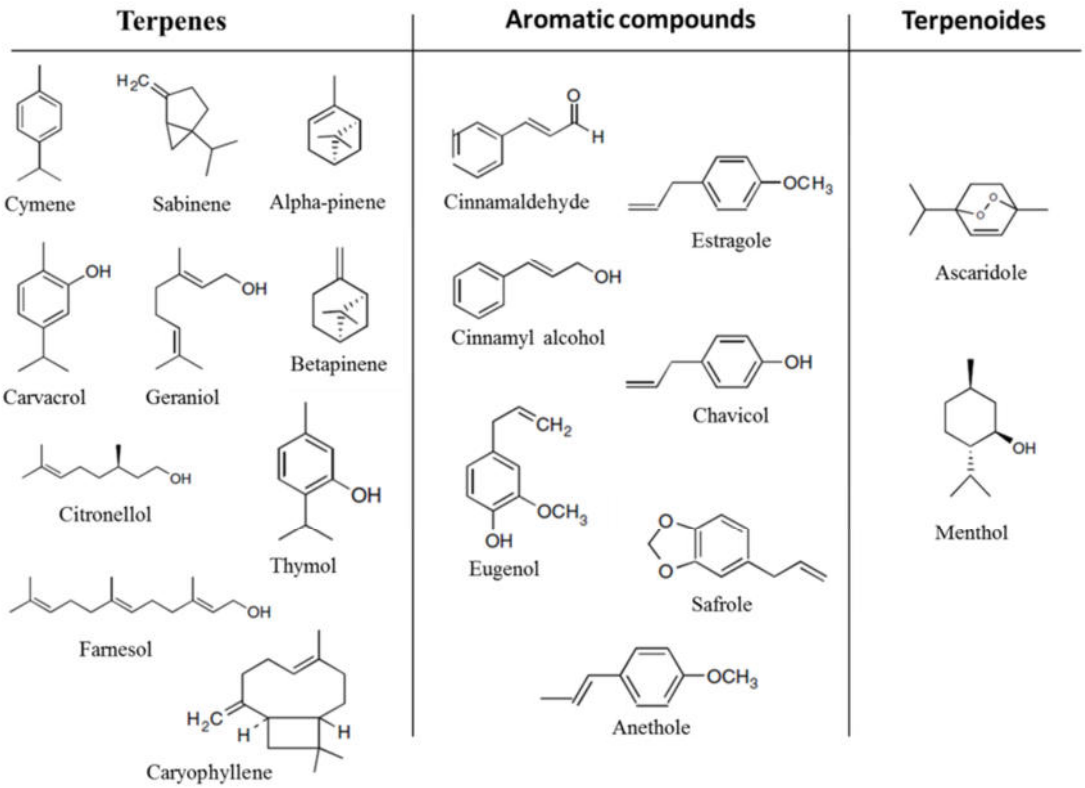
Table 2

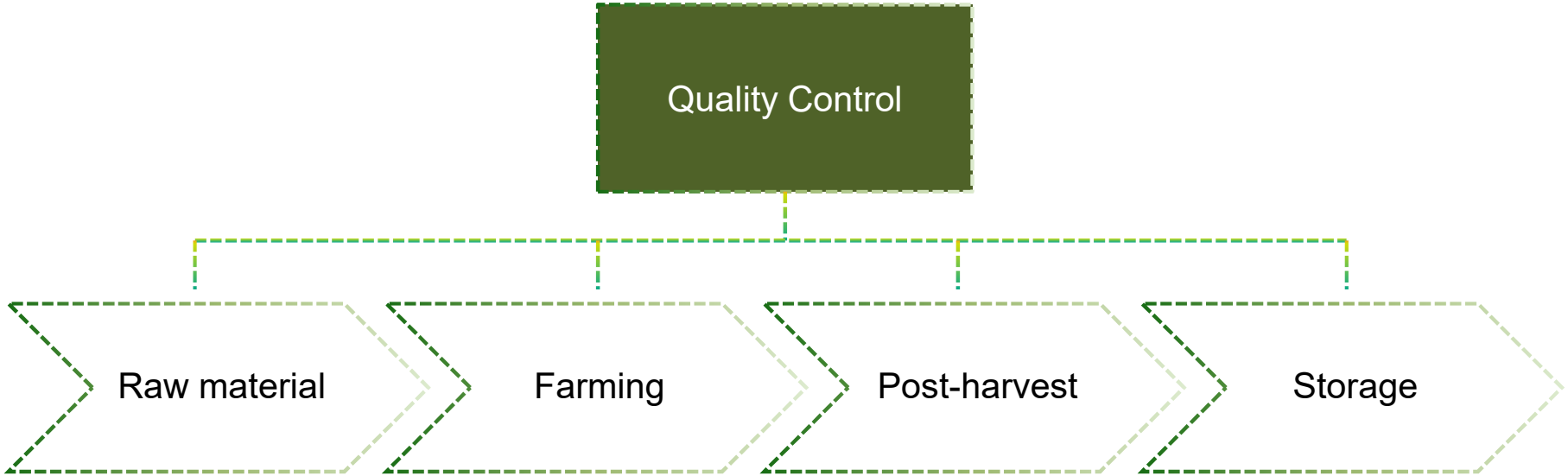
Pharmaceutical dosage forms	Application	Essential oils	Reference
Liquid systems	Rinses or mouth washes	Thymol, Eucalyptol, methyl salicylate, menthol	(Chen et al. 2011)
	Aromatherapy (Inhalation and massage)	Several essential oils such as tea tree oil, lavender oil, geranium essential oils, among others	(Edris 2007)
		Satureja brevicalyx and *Satureja boliviana*	(Yahyaoui, Gordobil et al. 2016)
Semi-solid systems	Aqueous gel or ointment for herpes labialis	Tea tree oil	(Carson et al. 2001) (Carson et al. 2008)
	Ointment for wounds	Hypericum perforatum oil, sage and oregano essential	(Süntar et al. 2011)
		Essential oil of *Lippia sidoides*	(Oliveira et al. 2014)
		Lavender	(Ben Djemaa, Bellassoued et al. 2016)
	Polycarbophil-based gel for vaginal delivery	Thyme essential oil	(das Neves et al. 2009)
		Thymol and eugenol	(Braga et al. 2009)
Solid systems	Capsules	Lavender	(Bradley et al. 2009)
		Peppermint oil	(Cappello et al. 2007) (Kline et al. 2001)
	Films	Oregano essential oil	(Jouki et al. 2014)
		Lemon, thyme and cinnamon	(Peng et al. 2014)
		Essential oils from Rosemary, Myrtle and Thyme	(Yahyaoui, Gordobil et al. 2016)
Aerosols	Inhalation	Several essential oils such as essential oil from SuHeXiang Wan, lavender, peppermint, ginger, spearmint oils.	(Koo et al. 2004) (Barocelli et al. 2004) (Soltani et al. 2013) (Hodge et al. 2014)

Table 3

Essential oil	Method	Results	Application	Reference
Carvacrol, limonene and cinnamaldehyde	Compounds were encapsulated in the sunflower oil droplets of nanoemulsions prepared by high pressure homogenization and stabilized by different emulsifiers: lecithin; pea proteins; sugar ester and a combination of Tween 20 and glycerol monooleate.	The measure antimicrobial activity was significantly affected by the formulation of the nanoemulsion. The effect of the delivery systems on the antimicrobial activity was correlated to the concentration of the essential oil components in the aqueous phase in equilibrium with the nanoemulsion droplets.	Antimicrobial application	(Donsì, Annunziata et al. 2012)
Essential oil from *Atractylodes macrocephala* Koidz	Preparation of lipossomes by a modified technique of rapid expansion of supercritical solutions.	The liposomes appeared as double-layered spheres with a uniform and narrow size distribution. The entrapment efficiency, drug loading, and average particle size of liposomes were found to be 82.18%, 5.18% and 173nm, respectively.	The freeze-dried liposomes can be used as a pharmaceutical ingredient for the injection and other oral formulations	(Wen, Liu et al. 2010)
Lippia sidoides oil	Encapsulation with chitosan and cashew gum by a coacervation method	*In vitro* release profiles showed that nanoparticles presented slower and sustained release. The larvicide effect was improved	Larvicide effect	(Abreu, Oliveira et al. 2012)
Oregano essential oil	Oregano was encapsulated in rice starch porous spheres, inulin and gelatine/sucrose capsules by spray and/or freeze drying.	The three tested materials are able to encapsulate oregano. Gelatine/sucrose microparticles exhibit high antioxidant and antimicrobial activity while inulin and rice starch microencapsulates ensure higher stability.	Food preservative (antimicrobial and antioxidant)	(Beirão da Costa, Duarte et al. 2012)
Santolina	For the preparation of liposomes,	The presence of the glycols improved vesicle stability	Enhancing the delivery	(Castangia, Manca et

insularis	propylene glycol (PG), ethylene glycol (EG) -PEVs, P90H, cholesterol and *S. insularis* essential oil were dispersed in PBS or PG/PBS or EG/PBS and then, the dispersions were sonicated.	and pig skin treated with *S. insularis* lipossomes revealed a penetration capability. All formulations exhibited *in vitro* biocompatibility in human keratinocytes.	of essential oils to the skin	al. 2015)
Thymol and carvacrol	Essential oils were encapsulated in the nanoparticles of zein using the liquid-liquid dispersion method.	Nanoparticles enhance the solubility of essential oils up to 14 fold without hindering their ability to scavenge free radicals or to control *Escherichia coli* growth.	Potential for use in food preservation and control human pathogenic bacteria	(Wu, Luo et al. 2012)
Zedoary turmeric oil	Self-nanoemulsification (isotropic mixture composed of oil, surfactant, co-surfactant and drug).	Nanoemulsion improved aqueous dispersibility, stability and oral bioavailability.	Oral delivery	(Zhao, Wang et al. 2010)
Yuxingcao essential oil	Yuxingcao essential oil loaded solid lipid nanoparticles were prepared by a high-shear homogenization method.	*In vitro* assays displayed that the nanoparticles led to sustained drug release up to 48 h. Nanoparticles prolonged pulmonary retention up to 24 h and improve local bioavailability, after intratracheal administration to rats.	Pulmonary delivery	(Zhao, Chang et al. 2017)


Terpenes
Aromatic compounds
Terpenoides
Cymene
Sabinene
Alpha-pinene
Cinnamaldehyde
Estragole
Ascaridole
Carvacrol
Geraniol
Betapinene
Cinnamyl alcohol
Chavicol
Citronellol
Thymol
Eugenol
Safrole
Menthol
Farnesol
Anethole
Caryophyllene



- ✓ Species variety
- ✓ Cultivar chemotypes
- ✓ Special characters

- ✓ Growing site
- ✓ Crop rotation
- ✓ Production technique and maintenance
- ✓ Fertilization
- ✓ Plant protection

- ✓ Transport
- ✓ Washing
- ✓ Drying

- ✓ Storehouse conditions
- ✓ Packaging