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Review article

Synergistic antimicrobial potential of essential oils in combination with nanoparticles: Emerging trends and future perspectives

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ABSTRACT

The development of resistance to different antimicrobial agents by bacteria, fungi, viruses, parasites, etc. is a great challenge to the medical field for the treatment of infections caused by them, and therefore, there is a pressing need to search for new and novel antimicrobials. The antimicrobial activity of essential oils and biogenic nanoparticles is well known. Recent studies have demonstrated that nanoparticles functionalized with essential oils have significant antimicrobial potential against multidrug- resistant pathogens. The aim of the present review is to discuss various studies on the broad-spectrum antimicrobial activity of essential oils used singly and in combination with nanoparticles. The brief explanation of their mechanism has also been discussed.

Contents

1. Introduction

There has been global emergence of multidrug- resistant microorganisms due to over- or underuse of antibiotics. Many microbes including bacteria, fungi, viruses, parasites, protozoans, etc. have developed resistance to antibiotics, and therefore, this problem is now recognized as an emergent global challenge (Roca et al., 2015). Ubiquitous occurrence of multidrug-resistant bacteria reduce the efficacy of the current antibiotic therapy, which results in thousands of deaths. Therefore, new and novel alternative antimicrobials are urgently required to address the problem of multidrug resistance (Allahverdiyev et al., 2011). In this context, the use of essential oils (EOs) in combination with nanoparticles may exert synergistic antimicrobial activity, leading to the development of novel approach for treatments.

EOs are volatile, natural, fragrant liquids that can be extracted from different parts of the plants especially leaves and flowers. They are synthesized through complex metabolic pathways with an aim of protecting plants from diverse pathogenic microbes. Due to their remarkable bioactivities, several efforts have been made to utilize them for the treatment of a wide range of microbial infections (Alwan et al., 2016; Belay et al., 2011; El-Baz et al., 2015; Hennebelle et al., 2008; Rai et al., 1999; Shaaban et al., 2012; Wagh et al., 2007; Zeedan et al., 2014).

The production of EOs is limited mainly to Myrtaceae, Myristicaceae, Piperaceae, Rutaceae, Asteraceae and Lamiaceae,

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Table 1
Antimicrobial activity of EOs against various bacterial, fungal and viral pathogens.

Sr. no.	EOs of plants	Part used	Major constituents of EO	Microbes inhibited	References
1	*Satureja thymbra* *Thymbra spicata*	dried inflorescences and the upper leaves	thymol, γ-terpinene, p-cymene carvacrol, γ-terpine	*Escherichia coli, Pseudomonas aeruginosa, Salmonella typhimurium, Listeria monocytogenes, Enterococcus faecalis, Bacillus cereus, Micrococcus flavus, Staphylococcus aureus,* *Aspergillus fumigatus, A. niger, A. versicolor, A. ochraceus, Penicillium funiculosum, Penicillium ochrochloron, Trichoderma viride*	Marković et al. (2011)
2	*Syzygium aromaticum, Myroxylon balsamum, Thymus vulgaris, Melaleuca alternifolia*			Biofilms formed by *P. aeruginosa, P. putida, S. aureus*	Kavanaugh and Ribbeck (2012)
3	*Curcuma longa*	rhizomes	turmerone	*S. aureus, P. aeruginosa, Candida albicans, A. niger*	Singh et al. (2012)
4	*Cinnamomum verum, Cymbopogon citratus, Thymus vulgaris, Origanum compactum, Satureja montana*			*Streptococcus pyogenes*	Sfeir et al. (2013)
5	*Lavandula x intermedia, Laurus nobilis*	-	1,8-cineole, camphor, linalool	*E. coli, Mycobacterium smegmatis*	Flores et al., 2014
6	*Struchium sparganophora*	leaves, stem	Germacrene A, a-humulene, Germacrene D	*Salmonella typhi, B. cereus, Proteus mirabilis, P. aeruginosa, Bacillus subtilis*	Kasim et al. (2014)
7	*Achillea fragrantissima*	leaves, flowers	trans2,7Dimethyl4, 6octadien2ol, A-Thujone, 2,5,5trimethyl3,6he ptadien2ol, eucalyptol	*S. aureus, Staphylococcus epidermidis* (MRSA), *E. coli* Orf virus	Zeedan et al. (2014)
8	*Pelargonium graveolens, Cinnamomum zeylanicum, Cymbopogon flexuosus, Cinnamomum zeylanicum, Citrus bergamia, Cymbopogon flexuosus, Thymus vulgaris*	-	-	Influenza virus A1/Denver/1/57 (H1N1)	Vimalanathan and Hudson (2014)
9	*Salvia sclarea*	-	linalyl acetate, linalool, germacrene D	*E. coli, S. aureus, Bacillus pumilus, Klebsiella pneumonia, B. subtilis, S. typhimurium, P. aeruginosa*	Cui et al. (2015)
10	*Origanum vulgare, Salvia officinalis, Thymus vulgaris*	leaves, inflorescences	-	MDR strains of *Klebsiella oxytoca, K. pneumoniae, E. coli*	Fournomiti et al. (2015)
11	*Origanum vulgare, Thymus vulgaris*	leaves	carvacrol	32 erythromycin-resistant Group A streptococci	Magi et al. (2015)
12	*Eucalyptus smithii*	leaves	1,8-cineole	*Microsporum canis, Microsporum gypseum, Trichophyton mentagrophytes, Trichophyton rubrum*	Baptista et al. (2015)
13	*Plectranthus neochilus*	leaves	α-pinene, β-pinene, *trans*-caryophyllene, caryophyllene oxide	*Streptococcus salivarius, S. sanguinis, S. mitis, S. mutans, S. sobrinus, E. faecalis, Lactobacillus casei*	Crevelin et al. (2015)
14	*Eucalyptus camaldulensis*	leaves	-	*S.t faecalis, B. subtilis, L. monocytogenes, E. coli, S. typhi, P. aeruginosa,* *Saccharomyces cerevisiae, C. albicans, A. niger* Rotavirus, adenovirus type 7, Coxsackie virus B4, Herpes Simplex Virus Type 1	El-Baz et al. (2015)
15	*Zataria multiflora, Artemisia kermanensis, Eucalyptus caesia, Satureja hotensis, Rosmarinus officinalis*	aerial parts	-	Herpes simplex virus-1 (HSV-1)	Govanji et al. (2015)
16	*Hyssopus officinalis*	-	cis-pinocamphone, b-pinene, *trans*-pinocamphone, b-phellandrene	*C. albicans, C. glabrata, C. tropicalis, C. parapsilosis, C. krusei*	Hristova et al. (2015)
17	*Zataria multiflora*	-	carvacrol, thymol	Newcastle disease virus (NDV)	Mohammadi et al. (2015)
18	*Foeniculum vulgare*	seeds	trans-anethole, pinene, fenchone	*Trichophyton tonsurans, T. rubrum, T. mentagrophytes, Microsporum gypseum*	Zeng et al. (2015)
19	*Micromeria barbata*	aerial part	Pulegone, limonene, Neomenthol, menthol β-Pinene	*E. coli* (β-lactamases producer), *E. coli* (carbapenemases producer), *S. aureus* (MRSA), *K. pneumonia* (cephalosporinase producer), *Salmonella spp., Listeria innocua, E. faecalis, C. albicans*	Alwan et al. (2016)
20	*Citrus aurantium*	leaf, ripe and unripe peel	Linalool, limonene	*S. aureus, B. cereus, Streptococcus faecium, E. coli, S. typhi, Shigella dysentery, S. cerevisiae*	Azhdarzadeh and Hojjati (2016)
21	*Rosmarinus eriocalyx*	aerial parts	camphor, 1,8-cineole, camphene, α-pinene	*P. aeruginosa, Salmonella montevideo, S. enteritidis, E. faecalis, S. aureus, B. subtilis, B. cereus, L. monocytogenes C. albicans*	Benbelaid et al. (2016)
22	*Cymbocarpum erythraeum*	-	(*E*)-2-decenal, (*E*)-2-decen-1-ol, (*E*)-2-dodecenal, decanal	*Acinetobacter lwoffi, Alcaligenes faecalis, Bacillıs cereus, B. subtilis, Enterobacter cloacea, E. coli, Flavobacterium indologenes, K. pneumoniae, L. monocytogenes, P. mirabilis, Proteus vulgaris, Providencia alkalifaciens, P. aeruginosa, P. pseudoalkaligenes, P. putida, S. typhimurium, S. aureus, Staphlococcus hominis, S. pyogenes, Yersinia enterocolitica, A. niger, Geotrichum candidum, Penicillium jensenii, C. albicans, Sacharomyces boulardii, S. cerevisiae*	Cetin et al. (2016)

Table 1 (*Continued*)

Sr. no.	EOs of plants	Part used	Major constituents of EO	Microbes inhibited	References
23	*Echinophora tenuifolia*		methyl eugenol, *p*-cymene, α-phellandrene	*A. lwoffi, E. cloacea, E. coli, F. indologenes, K. pneumoniae, P. mirabilis, P. aeruginosa, P. fluorescens, P. putida, S. typhimurium, S. aureus, S. pyogenes, C. albicans*	Cetin et al. (2016)
24	*Citrus karna*	fruit peels	D-limonene, β-pinene	*B. subtilis, E. coli, P. aeruginosa, S. aureus*	Dar et al. (2016)
25	*Rhaphiodon echinus*	leaves	monoterpenes, sesquiterpenes	*E. coli, S. aureus, P. aeruginosa C. albicans, C. krusei, C. tropicalis*	Duarte et al. (2016)
26	*Origanum vulgare*	leaves	terpinen-4-ol, γ − Terpinen, o-cymene, *cis*-β-Terpineol, α-Terpinen, β-Phellandrene, α-Terpieol, carvacrol	*E. coli, P. aeruginosa, S. aureus, B. cereus, C. albicans.*	Jnaid et al. (2016)
27	*Syzygium aromaticum, Myristica fragrans, Zingiber officinale*	flower buds, fruits, rhizome	-	Clinical isolates of *Clostridium difficile.*	Justin and Antony (2016)
28	*Artemisia herba-alba*	leaves, flowers	camphor, chrysanthenone, 1,8-cineole, α-thujone, borneol, bornyl acetate	*P. aeruginosa, S. aureus, K. pneumoniae, B. cereus, E. coli*	Lakehal et al. (2016)
29	*Thymus schimperi, Eucalyptus globulus, Rosmarinus officinalis*	leaves	-	*S. typhi, S. paratyphi, S. typhimurium, Shigella sp., P. aeruginosa, S. aureus, E. coli Trichophyton spp., Aspergillus* spp.	Mekonnen et al. (2016)
30	*Ligularia persica*	flowers	cis-ocimene, β-myrcene, β-ocimene, γ-terpinene	*S. aureus, S. sobrinus, E. coli, P. aeruginosa*	Mohadjerani et al. (2016)
		stem	β-phellandrene, β-cymene, valencene		
		roots	fukinanolid, α-phellandrene, β −selinene		
		leaves	cis-ocimene, β-ocimene, linolenic acid methyl ester		
31	*Myrtus communis*	leaves	1, 8-Cineole, α-Pinene, linalool, geranylacetate	*E. faecalis, S. aureus, C. albicans*	Nabavizadeh et al. (2014)
32	*Ocimum tenuiflorum*	leaves, inflorescence	camphor, eucalyptol, eugenol	*S. aureus* (including MRSA), *E. coli, P. aeruginosa*	Yamani et al. (2016)
33	Plants from families Labiatae and Verbenaceae	whole plant	-	Human Herpes virus 1, Human Herpes virus 2	Brand et al. (2016)

etc. The broad-spectrum biological activities of EO could be attributed to the complexity and variability of their chemical composition that are influenced by many factors such as local climate, seasonal and experimental conditions (Rasooli et al., 2005). The studies carried out demonstrated that there was synergetic effect when two or more EOs are mixed together (Doran et al., 2009; Edwards-Jones et al., 2004; Padalia et al., 2015). Moreover, there are also reports of synergistic activity of EOs when used in combination with known antibiotics (Duarte et al., 2016; Karpanen et al., 2008, 2010; Hemaiswarya et al., 2008; Magi et al., 2015; Rosato et al., 2007). When blended with other antimicrobial agents, the constituents of EOs can unlock the cell membrane channels, thus opening the passage of antimicrobial agents to reach their target sites. Now-a-days, vapours of EOs are also being studied extensively as their antimicrobial effect may have potential use for decontaminating environment, preserving food and in wound dressings (Fisher and Phillips, 2006; Laird and Phillips, 2012). In addition, EOs from different plants can be exploited as natural additives for food.

Nanotechnology is one of the most important and emerging technologies, which has generated revolution. Nanoparticles are very important tools for the treatment of different diseases in general and microbial diseases in particular due to their unique antimicrobial properties and high surface area/volume ratio. Among these, metal nanoparticles play an important role in various biomedical applications (Potara et al., 2015; Składanowski et al., 2016). In this context, silver nanoparticles have already demonstrated their potential and hence hoped to be a new generation of antimicrobials (Rai et al., 2009, 2016). Apart from silver, nanoparticles of other noble metals such as gold and platinum have also demonstrated different biological activities (Rai et al., 2016).

The aim of the present review is to discuss broad-spectrum antimicrobial activity of EOs alone and in combination with nanoparticles against different pathogens including multidrug-resistant organisms. In addition, possible explanation for their mechanism of action and the development of future strategies in this area have also been discussed.

2. EOs as novel antimicrobial agent

The ability of various EOs to safeguard human beings and food from pathogenic and food spoiling microbes has been studied by many researchers (Akthar et al., 2014; Alwan et al., 2016; Cui et al., 2015; Friedman, 2006). Recent studies showing antimicrobial activities of EOs have been summarized in Table 1. The utilization of various EOs to combat pathogenic as well as multidrug resistant microbes is a promising approach in antimicrobial research (Knezevic et al., 2016; Mulyaningsih et al., 2010; Yap et al., 2014).

EOs contain different chemical components, whose main structures are shown in Fig. 1. The chemical profile of EOs differ in number and stereo-chemical properties of the components that depends on the type of extraction (Akthar et al., 2014). The EOs may vary in quantity, quality and composition depending on plant organs, age and its vegetative cycle stage, climate and soil composition (Angioni et al., 2006; Masotti et al., 2003). Recently,

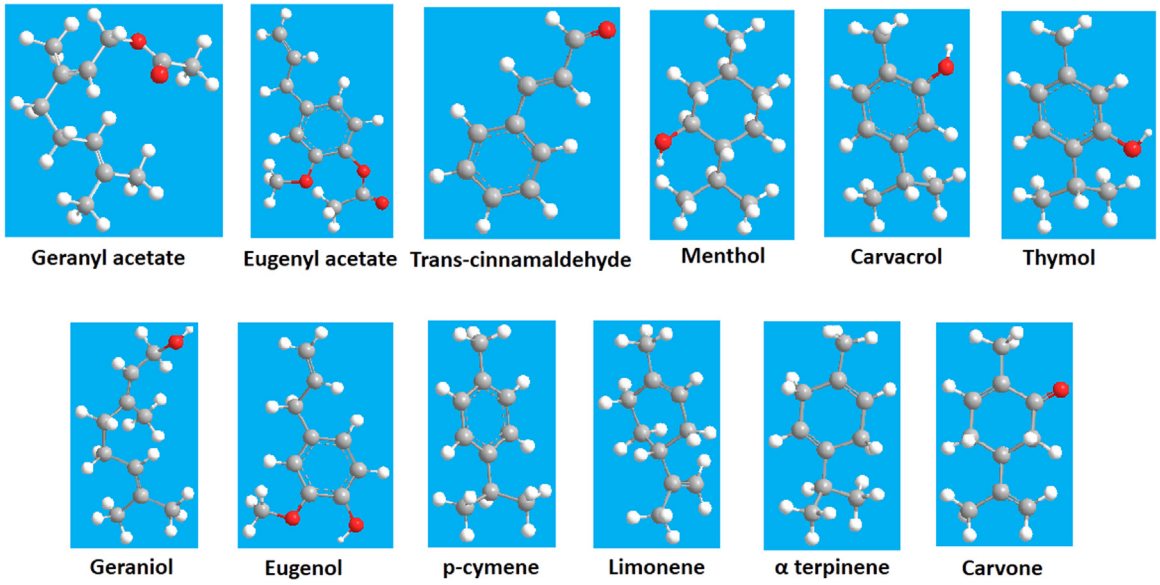

Fig. 1. Structure of some important chemical components of EOs.

Eze (2016) reported various important functional groups of essential oils including aldehydes (citral, citronellal, cinnamaldehyde, benzaldehyde, vanillin), alcohols (geraniol, citronellol, menthol, linalool, terpineol, borneol), esters (benzoates, acetates, salicylates, cinnamates), ketones (camphor, carvone, menthone, pulegone, thujone), oxides (cineol), phenol ethers (anethol, safrol), phenols (eugenol, thymol, carvacrol), hydrocarbons (cymene, myrcene, sabinene, storene), terpenes (limonene, phellandrene, pinene, camphene, cedrene) and acids (benzoic, cinnamic, myristic, isovaleric). All essential oils consist of one or more of these components as key element and play vital role as antimicrobials.

Carvacrol is the main constituent of Oregano (60% to 74%) and Thyme EOs (45%) (Arrebola et al., 1994). EOs rich in carvacrol, have been widely reported to possess remarkable antimicrobial activity (Aligiannis et al., 2001; Baydar et al., 2004; Botelho et al., 2007; Cetin et al., 2011; Magi et al., 2015). Jianu et al. (2013) isolated caryophyllene (24.1%), beta-phellandrene (16%) and eucalyptol (15.6%) from EO of *L. angustifolia,* while camphor (32.7%) and eucalyptol (26.9%) from EO of lavandin (*Lavandula x intermedia*) as major constituents which showed significant antibacterial activity.

The antibacterial activity of various EOs have been extensively studied against various pathogenic bacteria including multidrug-resistant bacteria, foodborne pathogens, oral pathogens and their biofilms. Fournomiti et al. (2015) demonstrated antibacterial activity of EOs of oregano, sage and thyme against 32 multidrug-resistant bacterial strains of *Escherichia coli*, *Klebsiella oxytoca* and *K. pneumoniae*. Thyme, cinnamon, rose, and lavender EOs exhibited excellent bactericidal activity against *Propionibacterium acnes* (which causes acne on skin) at a concentration of 0.25% (v/v), where *P. acnes* was completely killed after 5 min (Zu et al., 2010).

EOs can serve as potential antimicrobial agents against a broad range of oral bacteria responsible dental caries. Benbelaid et al. (2014) reported that oregano and thyme EOs can be used to treat intractable oral infections, caused by multidrug-resistant *Enterococcus faecalis*. Freires et al. (2015) systematically reviewed activity of various EOs and their isolated components against cariogenic bacteria including streptococci and lactobacilli considering menthol and eugenol as outstanding compounds.

Several pathogenic fungi including yeasts are susceptible to various EOs (Marković et al., 2011; Mekonnen et al., 2016; Singh et al., 2012). The fungi *Aspergillus niger, Geotrichum candidum, Penicillium jensenii, Candida albicans, Saccharomyces boulardii* and *S. cerevisiae* were remarkably inhibited by oil of *Cymbocarpum erythraeum,* whereas the same fungi were resistant to *Echinophora tenuifolia* oil (Cetin et al., 2016). EO extracted from *Hyssopus officinalis* (Hyssop) demonstrated significant antifungal activity against 52 clinically isolated strains of *Candida albicans, C. glabrata, C. tropicalis, C. parapsilosis* and *C. krusei* including both fluconazole sensitive and resistant strains (Hristova et al., 2015).

Numerous EOs have demonstrated high efficacy against dermatophytes. Among 22 samples of EOs from 11 species of *Cinnamomum*, the oil of *C. suvabenium* was found to be most effective against *Trichophyton mentagrophytes, T. rubrum, Microsporum canis*, *Candida albicans* and *C. glabrata*, showing MIC values in the range of 470–2500 μg/ml (Mastura et al., 1999). Tansy (*Tanacetum parthenium*) oil showed fungistatic (MIC 31.2 and 15.6 μg/ml) and fungicidal effect (62.5 and 31.2 μg/ml) against *M. gypseum* and *T. mentagrophytes* respectively (Kalodera et al., 1997). EO obtained from fennel seeds demonstrated antifungal activity against *T. rubrum, T. tonsurans, T. mentagrophytes* and *M. gypseum*, which was greater than commonly used antifungal agents including fluconazole and amphotericin B (Zeng et al., 2015).

In addition, several EOs have been found active against different viruses. EOs of lemongrass (*Cymbopogon flexuosus*) and Roman chamomile (*Chamaemelum nobile*) demonstrated a high degree of inhibition against two types of bacteriophages (Chao et al., 2000). Vimalanathan and Hudson (2014) evaluated antiviral activity of various EOs and their isolated compounds in vapor phase as well as liquid phase. In vapor phase, citronellol and eugenol obtained from *Citrus bergamia* and *Eucalyptus globulus* exhibited remarkable activity against influenza virus (H1N1) with only 10 min exposure while oils of *Cinnamomum zeylanicum, Pelargonium graveolens* and *Cymbopogon flexuosus* were effective at 30 min exposure. In liquid phase, EOs of *C. zeylanicum, Citrus bergamia, C. flexuosus* and *Thymus vulgaris* displayed 100% inhibition at 3.1 μL/ml concentration. Roy et al. (2015) reported 80% and 40% inhibition of Japanese encephalitis virus (JEV) when treated with 0.5 mg/ml of

Trachyspermum ammi (ajwain) oil in pre-exposure and post-exposure treatments respectively. Further, antiviral effect of EOs have been investigated on Orf virus (Zeedan et al., 2014), Rotavirus, Adenovirus type 7, Coxsackie virus B4, Herpes Simplex virus type 1 (El-Baz et al., 2015), Newcastle disease virus (Mohammadi et al., 2015), Human Herpes virus 1, Human Herpes virus 2 (Brand et al., 2016).

Thus, EOs from selected plants can be used as antimicrobial agents for food and human applications; however, their mode of action depends on the type and composition. EOs are novel and safe antimicrobials and have been reported to show a little or no toxicity. In this context, important *in vivo* studies performed on different EOs have been briefly discussed here. Recently, Dahham et al. (2016) demonstrated *in vivo* toxicity of EOs of agarwood (*Aquilaria crassna*) in female and male Swiss mice. They focused mainly on acute and sub-chronic toxicity studies. The acute toxicity test allowed the estimation of median lethal dose (LD50), which represented the dose that killed 50% of the tested population. There was no treatment related mortality observed in both male and female mice even at the dose concentration of 2000 mg/kg during the treatment of 14 days. In addition, no other symptoms such as apathy, hyperactivity, dizziness, vomiting, diarrhea, excessive salivation, loss of fur, anxiety, convulsions, lethargy and morbidity were reported among the tested animals. Thus, the study confirmed that EOs are safe at the dose level of 2000 mg/kg, and hence, the LD50 value for oral toxicity has been considered to be more than 2000 mg/kg. Similarly, Sudhakaran and Radha (2016) studied acute and dermal toxicity of EO of *Etlingera fenzlii* in Wistar albino rats. The results did not show any toxicity, mortality or significant changes in the body weight and wellness parameters of tested animals at various dose concentrations i.e. 175, 550 and 2000 mg/kg body weight. Further, the dermal absorption study with essential oil also did not show any treatment-related changes in body weight, food consumption and water intake in any of the animals tested. In another similar study, *in vivo* dermal absorption and sub-acute toxicity studies of EO extracted from *Blumea eriantha* was reported (Pednekar et al., 2013). The observations reported for dermal absorption study did not show presence of any essential oil component in the rabbit plasma samples. Similarly, no significant differences in the body weight, food consumption, hematological and biochemical parameters were observed in both female and male groups when subjected to sub-acute treatment. However, in the first week of study skin irritation was observed only in animals treated with high dose (15%). Apart from these, there are a few reports, which highlights the carcinogenic nature of EOs (Dweck, 2009). Thus, EOs can be used safely considering percentage use, type of product, its application, toxicological data and the target consumer.

3. Synergistic antimicrobial efficacy of nanoparticles and EOs

Different nanoparticles (NPs) such as silver, gold, zinc, chitosan, platinum, iron, copper, carbon nanotubes and many others have been used in combination with EOs for evaluation of their antimicrobial activity (Gaspar et al., 2017; Jogee et al., 2017; Van Long et al., 2016; Rai et al., 2009; Sadat-Shandiz et al., 2016; Schmitt et al., 2016; Wang et al., 2016). Schmitt et al. (2016) prepared super-lattice of EO droplet in emulsion with gold NPs, whereas, Duncan et al. (2015) synthesized capsules with peppermint EOs and cinnamaldehyde in the core part, which were stabilized by NPs encapsulation. Similar study using different techniques was reported by Paula et al. (2016) who prepared chitosan-gum NPs loaded with thymol containing EO of *Lippia sidoides*. This nano-encapsulation was developed by spray-drying

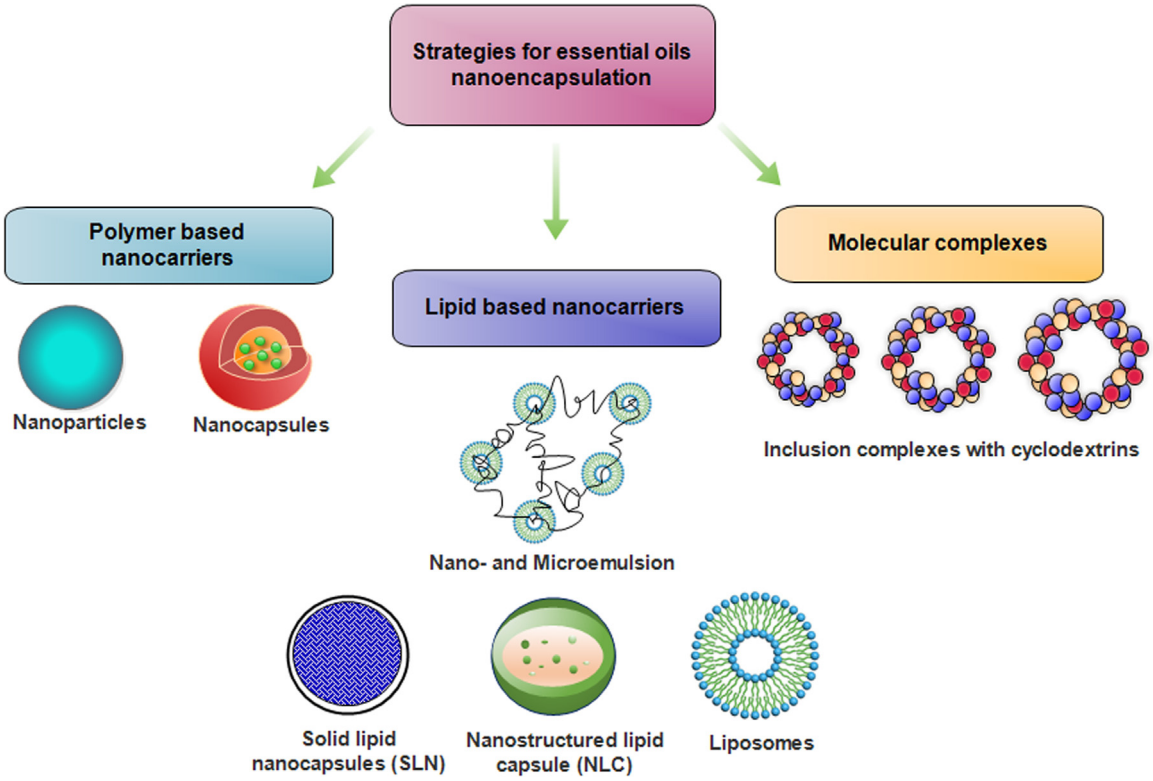

Fig. 2. Schematic illustration of different approaches for the encapsulation of EOs using different nanomaterials.

Table 2
Different combinations of EOs and nanoparticles used for antimicrobial purpose.

Sr. No.	Source of essential oils/EOs constituents	Type of NP	Combination type	Antimicrobial activity	References
1	*Santolina insularis*	Phosphatidylcholine	Liposomal encapsulation	Herpes Simplex Virus 1 (HSV-1)	Valenti et al. (2001)
2	*Artemisia arborecens*	Hydrogenated soy phosphatidylcholine	Encapsulation	Herpes Simplex Virus 1 (HSV-1)	Sinico et al., (2005)
3	*Artemisia arborescens*	Solid lipid	Encapsulation	Herpes Simplex Virus 1 (HSV-1)	Lai et al. (2006)
4	*Thymus boissieri, T. longicaulis, T. ocheus, T. leucospermus*	Phosphatidylcholine and cholesterol	Encapsulation	Gram-positive and Gram-negative bacteria, human pathogenic fungi and food pathogenic bacteria	Gortzi et al. (2006)
5	*Artemisia arborescens*	Solid lipid	Encapsulation	Herpes Simplex Virus 1 (HSV-1)	Lai et al. (2007)
6	*Origanum dictamnus*	Lipid	Liposome encapsulation	Gram-positive and gram-negative bacteria, and fungus (*Listeria monocytogenes*)	Gortzi et al. (2007)
7	*Vegetable oil*	Metal NPs	Dispersion	*Staphylococcus aureus, E. coli*	Kumar et al. (2008)
8	Eugenol, Carvacrol	Chitosan	Encapsulation	*S. aureus, E. coli*	Chen et al., 2009
9	*Zanthoxylum tingoassuiba*	Phosphatidylcholine	Encapsulation	*S. aureus*, Dermatophytes	Detoni et al. (2009)
10	*Origanum dictamnus*	phosphatidyl choline-based liposomes	Encapsulation	Gram-positive and Gram-negative bacteria and human pathogenic fungi	Liolios et al. (2009)
11	*Glycine max*	Lipid	Encapsulation	Malerial infections	Aditya et al., (2010)
12	Terpenes	Food grade ingredients	Nanoemulsion	*Lactobacillus delbrueckii, Saccharomyces cerevisiae, E. coli*	Donsi et al. (2011)
13	Cinnamaldehyde, Eugenol	Poly (DL-lactide-co-glycolide) (PLGA)	Nanoencapsulation	*Salmonella* spp., *Listeria* spp.	Gomes et al. (2011)
14	Carvacrol	Poly (DL-lactide-co-glycolide) (PLGA)	Encapsulation	*S. epidermidis* biofilms	Iannitelli et al. (2011)
15	Carvacrol	Chitosan	Encapsulation	*E. coli, S. aureus, B. cereus*	Keawchaoon and Yoksan (2011)
16	*Rosmarinus officinalis*	Magnetic	Nanobiosystem	*Candida albicans, C. tropicalis*	Chifiriuc et al. (2012)
17	*Eugenia carryophyllata*	Magnetite/oleic acid-core/shell NP	Biofilm	Fungi	Grumezescu et al. (2012)
18	*Syzygium aromaticum*	Eugenol NP	Microemulsion	*E. coli, S. aureus, Salmonella typhi, Pseudomonas aeruginosa, Bacillus cereus, Listeria monocytogenes*	Hamed et al. (2012)
19	Thymol	Zein−Sodium Caseinate (SC)	Antimicrobial film	*E. coli, Salmonella* sp.	Li et al. (2012)
20	Thymol	Methyl and Ethylcellulose NP	Nanosphere	*S. aureus, E. coli, P. aeruginosa*	Wattanasatcha et al. (2012)
21	Thymol, Carvacrol	Zein	Encapsulation	*E. coli*	Wu et al. (2012)
22	*Oreganum* spp.	Chitosan	Encapsulation	Antimicrobial	Hosseini et al. (2013)
23	*Artemisia argyi*	Hydroxyapatite	Microcapsule	*S. aureus, E. coli*	Hu et al. (2013)
24	*Oreganum* spp.	Silver	Edible film	*S. aureus, Listeria monocytogenes*	Khalaf et al. (2013)
25	*Anethum graveolens, Salvia officinalis*	Magnetic	Nanobiocoated wound dressings	*C. albicans*	Anghel et al. (2013)
26	*Prunus dulcis*	Solid lipid	Encapsulation	*C. albicans*	Cerreto et al. (2013)
27	*Melaleuca alternifolia*	Polymeric	Nanocapsule	*Trychophyton rubrum*	Flores et al. (2013)
28	*Lippia sidoides*	Alginate/cashew gum	Encapsulation	Fungicide and bactericide	De Oliveira et al. (2014)
29	*Cocos nucifera*	Polymeric	Nanoencapsulation	*C. albicans, C. glabrata*	Santos et al. (2014)
30	Thymol	Silver	Combination	Microbial infections in tissue culture	Taghizadeh and Solgi (2014)
31	Thymol	Zein NP	Encapsulation	Gram-positive bacteria	Zhang et al. (2014)
32	*Oreganum* spp.	Zinc oxide, silver	Film	*S. aureus, Salmonella typhimurium, Listeria monocytogenes, E. coli*	Morsy et al. (2014)
33	*Cananga odorata, Pogostemon cablin, Vanilla planifolia*	Magnetic	Nanostructure	*S. aureus, Klebsiella pneumoniae*	Bilcu et al. (2014)
34	*Ricinus communis*	Solid lipid	Suspension	*S. aureus*	Chen et al. (2014)
35	*Cinnamomum Cassia*, lemon, basil, thyme, geranium, clove	Silver	Nanocomposite	*Candida krusei, C. albican, C. glabrata*	Szweda et al. (2015)
36	Peppermint oil and cinnamaldehyde hybrid	Silica	Nanocomposite	*E. coli, P. aeruginosa, Enterobacter cloacae* complex, MDR *S. aureus* biofilms	Duncan et al. (2015)
37	Terpenoides	Non-polar functionalized NP	Nanocomposite	Microbial biofilm	Mogosanu et al. (2015)
38	*Cymbopogon citratus, C. martini, Eucalyptus globules, Azadirachta indica, Ocimum sanctum*	Silver	Nano functionalized antimicrobial oil	Bacterial and fungal pathogens on animals skin	Bansod et al. (2015)
39	*Copaifera* spp.	Solid lipid	Nanoencapsulation	*Candida krusei, C. parapsilosis, Trichophyton rubrum, Microsporum canis*	Svetlichny et al. (2015)
40	*Lippia sidoides*	Chitosan	Nanoencapsulation	Food microbes	Paula et al. (2016)

Table 2 (*Continued*)

Sr. No.	Source of essential oils/EOs constituents	Type of NP	Combination type	Antimicrobial activity	References
41	*Origanum vulgare*	Silver	–	Gram-positive and Gram-negative bacteria, including MDR	Scandorieiro et al. (2016)
42	Lemongrass	Cellulose acetate	Nanoencapsulation	*S. aureus*	Liakos et al. (2016)
43	Cinnamon, garlic, clove	zinc oxide, silver-copper alloy NP	Polylactide (PLA) based films	*L. monocytogenes*, *S. typhimurium*	Ahmed et al., 2016
44	Thyme	Chitosan	Nanoencapsulation	*Enterobacter* sp., S. *aureus*	Ghaderi-Ghahfarokhi et al. (2016)
45	Cinnamon, clove	Mesoporous silica NP	Nanoencapsulation	*S. aureus*, methicillin resistant *S. aureus, E. coli, P. aeruginosa, C. albicans*	Lillie (2016)

of EOs emulsion and gum solution in various concentrations. These types of nano-encapsulations are used in chemical, pharmaceutical and food industries, etc.

Mashwani et al. (2016) reported the involvement of plant secondary metabolites and terpenoides in the synthesis of NPs. Gaspar et al. (2017) synthesized magnetic nano-fluid by incorporation of EOs in iron oxide NPs. *Carum copticum* EO was encapsulated in chitosan NPs by an emulsion ionic gelatinization method in the study of Esmaeili and Asgari (2015) for evaluation of its biological activity and release profile. Similar study was performed by Hosseini et al. (2013) who developed the combination of chitosan NPs and oregano EO for the study of its antimicrobial potential and also to determine the release pattern of EO particles adsorbed onto NPs. This report was also supported by the study that nano-encapsulation of thymol by zein-sodium caseinate NPs have enhanced the antimicrobial potential of thymol (Li et al., 2012).

There are different types of NPs used for the preparation of nano-complexes which enhance the bactericidal and fungicidal activity of EOs. Gomes et al. (2011), prepared poly DL-lactide-co-glycolide (PLGA) NPs complex by the incorporation of eugenol and cinnamaldehyde which proved to be potential bactericidal agent against *Salmonella* and *Listeria.* The combination of EOs and NPs was used by many scientists; for example, combination of lipid based liposome NPs and *Origanum dictamnus* EO was used by Gortzi et al. (2007) against Gram-positive and Gram-negative bacteria.

Valenti et al. (2001) used different constituents of *Santolina insularis* EOs viz. carvacrol, thymol, γ-terpinine, p-cymene and EO in combination with phosphatidylcholine liposome NPs. In addition, novel nano-complexes have been used for efficient inhibition of microbial growth. For such type of formulations mostly three kinds of nano-carries are employed, which are categorized as (i) polymer-based nanoparticles, (ii) lipid-based nanoparticles and (iii) molecular complexes (Fig. 2). Pedro et al. (2013) presented detailed account of different formulations used for EOs and nanoparticles combination.in order to use them in biomedical field (Table 2**).**

4. Mechanism of EOs activity used singly and in combination

The activity of EO depends on composition, functional groups present on active components and their synergistic interactions (Dorman and Deans, 2000). Many EOs commonly contain phenolic compounds such as carvacrol, thymol, eugenol and their antibacterial activity is attributed to the phenolic hydroxyl present on their structures (Veldhuizen et al., 2006). The removal of the aromatic ring substituent of carvacrol reduced the antimicrobial activity to some extent. The bioactive molecule 2-Amino-*p*-cymene has similar structure to cavacrol, except hydroxyl group. The lower activity by 3-fold of 2-amino-*p*-cymene, as compared to carvacrol, indicated the essential role of the hydroxyl group in the antimicrobial activity of carvacrol (Veldhuizen et al., 2006). The relative position of hydroxyl group is crucial for the bioactivity of these components, which explains the superior antimicrobial action of carvacrol as compared to other plant phenolics (Veldhuizen et al., 2006).

Hydrophobicity is the significant characteristic of EOs and the lipophilic nature of their components allow them to easily interact with fatty acids of the dense microbial cell membrane (Blaszyk and Holley, 1998; Helander et al., 1998). The antimicrobial mechanism of action varies with the type of EO or the strain of the microorganism used. It is well known that Gram-positive bacteria are more susceptible to EOs as compared to Gram-negative bacteria (Azhdarzadeh and Hojjati, 2016; Huang et al., 2014; Okoh et al., 2010; Trombetta et al., 2005). This may be due to the fact that Gram-negative bacteria have more complex and rigid outer membrane rich in lipopolysaccharide (LPS), which limits the diffusion of hydrophobic compounds through it, while Gram-positive bacteria lack this extra complex membrane, instead are surrounded by a thick peptidoglycan wall, which is not dense enough to resist small antimicrobial molecules, facilitating the access to the cellular membrane (Holley and Patel, 2005; Hyldgaard et al., 2012; Zinoviadou et al., 2009). Moreover, the lipophilic ends of lipoteichoic acids in cell membrane of Gram-positive bacteria may ease the infiltration of hydrophobic compounds of EOs (Cox et al., 2000).

There are several reports, which supports that the bioactive components present in EOs might attach to the cell surface, and thereafter, penetrate to the phospholipid bilayer of the cell membrane. Their accumulation disturbs the structural integrity of cell membrane, which can detrimentally influence the cell metabolism and lead to cell death (Bajpai et al., 2013; Lv et al., 2011). Zhang et al. (2016) have elucidated antibacterial activity mechanism of cinnamon EO against *E. coli* and *S. aureus.* It was found that EO at MIC level, damaged the bacterial cell membrane and at MBC level, cells were destroyed, which was evidenced by scanning electron microscopy. Cinnamon EO caused leakage of electrolytes and rapid increase in the electric conductivity of cell suspension samples in a few hours. Also, the concentration of nucleic acids and proteins in cell suspension was increased with cinnamon EO. Membrane potential study reflected 3–5 fold decrease in bacterial metabolic activity (Zhang et al., 2016). Similar mode of action was noted by other researchers. EOs in general, act on cell membrane integrity by changing the membrane permeability which leads to leakage of electrolytes and loss of vital intracellular contents like proteins, reducing sugars, ATP and DNA while inhibiting the energy (ATP) generation and related enzymes leading to the destruction of cell (Cui et al., 2015; Huang et al., 2014; Li and Yu, 2015; Lakehal et al., 2016; Skocibušic et al., 2006).

Therefore, antimicrobial activity of EOs is attributed to a cascade of reactions that involves the entire bacterial cell (Macwan et al., 2016).

Ultee and Smid (2001) and De Souza et al. (2010) reported that the exposure of *B. cereus* to carvacrol and *S. aureus* to oregano EO inhibited the enterotoxin production. The modifications in the bacterial membrane due to attachment of active components of EO that disturbs the phospholipid bilayer and limiting the transmembrane transport process can inhibit the secretion of toxins to the environment. Besides this, the EOs may have more specific targets to interact with microbes, which may vary between the microorganism and the individual components of EO (Boire et al., 2013).

Microarray data analysis revealed 24-fold increase in expression of gene *cwr A* in methicillin-resistant *Staphylococcus aureus* (MRSA) when exposed to citrus oil, which was similar to the effect of antibiotics; penicillin G, imipenem, phosphomycin, oxacillin and vancomycin, which inhibits cell wall synthesis (Balibar et al., 2010; McAleese et al., 2006; Muthaiyan et al., 2012; Sobral et al., 2007). Citrus oil also increased the expression of penicillin binding protein-4 (PBP 4) which is involved in peptidoglycan synthesis and genes in operon dltABCD. This operon is responsible for alanylation of teichoic acids in the cell wall that may bring out autolysin activity of *S. aureus* (Muthaiyan et al., 2012). Autolysin activity was also noted by Carson et al. (2002) by tea tree oil that caused release of membrane-bound, cell wall autolytic enzymes which lead to cell lysis and death.

EOs alone are good antimicrobial agents while their effect seems to be enhanced when studied in synergy that can be resulted from the mixture of two or more different EOs or their combination with other antimicrobial agents such as organic acids, commercial antibiotics (Moon et al., 2011; Van Vuuren et al., 2009) and nanoparticles that are new generation of antimicrobials (Rai et al., 2009). Delaquis et al. (2002) observed synergistic effect of combination of cilantro and eucalyptus EO fractions against *Y. enterocolitica* which resulted in 75 times reduction in MIC value. This may be the result of interactions of different active components of both EOs.

Yap et al. (2014) have listed combinations of EOs and antibiotics with synergistic activities against different microorganisms. Usually the mechanism for synergism involve inhibition of protective enzymes, a common biochemical pathway and use of cell wall active agents that enhance the uptake of other antimicrobials agents (Santiesteban-Lopez et al., 2007). Pei et al. (2009) have hypothesized that synergism shown by eugenol/thymol and eugenol/carvacrol might be due to activity of carvacrol and thymol that disintegrate the outer cell membrane of *E. coli* and facilitates the entry of eugenol into the cytoplasm which finally interacts with proteins and enzymes. Zhou et al. (2007) explained similar mechanism for synergistic effects of cinnamaldehyde/carvacrol or cinnamaldehyde/thymol against *S. typhimurium*. Thymol or carvacrol may increase the cell membrane permeability by increasing the size, number of pores and possibly enable cinnamaldehyde to transport easily into the cell. EOs thus can act as membrane permeabilizers, disorganize the anionic lipopolysaccharides and thereby sensitizing the bacterial cell to antibiotics (Helander et al., 1998; Vaara and Vaara, 1983; Veras et al., 2012).

Zeng et al. (2015) established the mechanism for antifungal activity of EO of fennel seeds. The results of flow cytometry and transmission electron microscopy (TEM) suggested that the EO of fennel damaged the plasma membrane and intracellular organelles of dermatophytes, *T. rubrum, T. tonsurans, M. gypseum* and *T. mentagrophytes* and further the EO also inhibited mitochondrial enzyme activities like succinate dehydrogenase, malate dehydrogenase and ATPase. Vimalanathan and Hudson (2014) endeavored to elucidate the antiviral mechanism of EOs against influenza virus. The vapours of EO were evaluated for direct effects on the major external proteins of influenza virus which include HA (hemagglutinin) and NA (Neuraminidase). The vapour of most EO inhibits only HA activity. Therefore, it was suggested that interaction of EO with HA seems to be is a possible mechanism.

Besides EOs, there are numerous reports available on antimicrobial efficacy of nanoparticles. Prabhu and Poulose (2012) reviewed various mechanisms of antimicrobial action of NPs especially silver NPs, which are potential antimicrobial agents (Rai et al., 2015). Silver NPs anchor and penetrate cell wall, forming pits

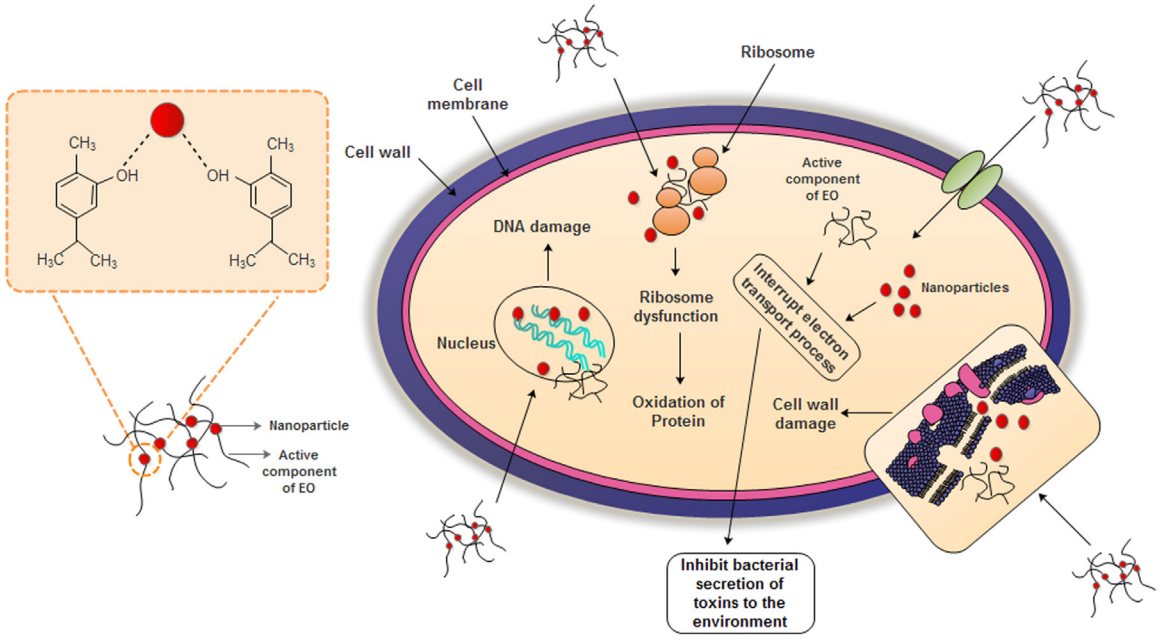

Fig. 3. Schematic representation of possible mechanism of actions nano-encapsulated EOs (this figure is modified version of figure from Bilia et al., 2014).

and release of free radicals followed by structural change in the cell membrane, due to which the cell permeability increases leading to more influx of antibacterial agent through cytoplasmic membrane which results into cell death. They can also inhibit signal transduction and cell wall formation, attack on DNA bases, interact with respiratory enzymes liberating reactive oxygen species followed by cell death.

Since the last decade, several research studies have been focusing on synergy between EOs and various types of NPs for their superior antimicrobial efficacy (Table 2). Ghosh et al. (2013) reported strong synergistic activity of cinnamaldehyde, a representative of EO and silver NPs against spore forming *Bacillus cereus* and *Clostridium perfringens.* This combination of antimicrobial agents exerted rapid bactericidal action revealed by bacterial kill curve analysis while electron and atomic force microscopy evidenced extensive damage to cell envelop. Fig. 3 is the schematic representation of possible mechanism of synergistic action of EOs and NPs.

The antimicrobial potential of EOs can be enhanced by encapsulating with various nanomaterials; for example; solid lipid NPs, liposomes, polymeric NPs and nano-emulsions, where the nanomaterial forms the outer nano-capsule while inside core consists of EO. This represents a promising approach in order to modulate drug release i.e. burst release and/or controlled release (Bilia et al., 2014). Hosseini et al. (2013) encapsulated oregano EO with chitosan NPs and the *in vitro* release study revealed an initial burst effect followed by slow release of drug (EO). Abreu et al. (2012) observed a slow and sustained release of EO by chitosan/cashew gum nano-encapsulation, which demonstrated efficient biocidal activity against *Stegomyia aegypti* larvae. Thymol encapsulated in zein NPs suppressed Gram-positive bacterium more efficiently as compared to encapsulated thymol for a longer period of time (Zhang et al., 2014). In another study, the antimicrobial activity of carvacrol loaded in polylactic glycolic acid nanocapsules was enhanced as the NPs significantly transformed rheological characteristic of bacterial biofilm that potentially facilitated the activity of carvacrol (Iannitelli et al., 2011).

Nano-encapsulation increases the physical stability of EOs, decrease volatility and protect it from environmental interactions (e.g. light, oxygen, moisture, pH), enhance bioactivity, reduce toxicity and also improve the patient's compliance and convenience (Ravi Kumar, 2000). The nanocarriers protect EOs from enzymatic degradation, transform them into powder and help to achieve the desired therapeutic levels to the target tissues for required time duration with lower number of doses and may also ensure an optimal pharmacokinetic profile (Bilia et al., 2014). Thus, various EOs with their inherent antimicrobial activity when used in combination with other potent antimicrobial agents like NPs, may greatly enhance their antimicrobial activity by complementing each other against different type of pathogens involving various mechanisms. Such combinations of different antimicrobials appear to be the best strategy to control multidrug resistant microbes.

5. Conclusions and future perspectives

The problem of multidrug- resistance in pathogens is increasing with alarming rate, which is evidenced by the high rate of morbidity and mortality. It is one of the great challenges faced by researchers and clinicians. The inefficacy of existing medical treatments has necessitated to search for novel and efficient drugs to tackle the problem. EOs possess important volatile compounds with diverse bioactivities including antimicrobial potential. Due to this property, EOs have been used in food, drug and cosmetics. However, there are certain limitations, such as, low water solubility, strong organoleptic flavor and low stability.

The promising antimicrobial activity of EOs have attracted the researchers to use them in combination with nanomaterials as potential antimicrobial agents. The encapsulation of NPs by EO increases the chemical stability and solubility. Moreover, the rapid evaporation and degradation of their active components are minimized. The application of encapsulated NPS with EO also supports their controlled and sustained release, which enhance the bioavailability and efficacy against multidrug resistant pathogens. Thus, the nano-encapsulation of EOs is a promising strategy to facilitate its application as antimicrobial agents.

Conflicts of interest

The authors report that they have no conflicts of interest.

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