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Nanoencapsulation of essential oils with enhanced antimicrobial activity: A new way of combating antimicrobial Resistance

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Abstract
Microorganisms have over the years evolved and developed resistance to available antimicrobials. This has cause a great impact to the world over health wise and socioeconomically. They are developing resistance at a very fast rate that current technologies and new drug developments in the pharmaceutical industries are unable to catch up. Therefore, alternative technologies and new drug innovations are necessary to find lasting solution to this problem. And one such technology could be nanoencapsulation of bioactive compounds with highlighted antimicrobial activities. Plants and their constituents, like essential oils, can be a great source of unique bioactive compounds that will be able to tackle this growing drug resistance of pathogenic microorganisms that threaten the very existence of humankind. Nanoencapsulation is one part of nanotechnology that has seen growing application in several industries including pharmaceuticals and medicine, with resultant great impact. This review focus mainly on application of encapsulated essential oils as a possible solution to drug resistance of some microbes by enhancement of their antimicrobial activities.

Keywords: Essential oils, nanoencapsulation, drug resistance, microbes.

Introduction
For centuries, essential oils have traditionally been used in different applications including treatment of various ailments/diseases such as cough, malaria, diarrhoea, severe headaches; seizures etc. Essential oils are commonly found in plants and usually have a wide and complex chemical composition. Studies have shown that their physicochemical characteristics enable them to have great and diverse biological activities. Researchers have also highlighted that the high content of bioactive compound such as phenols, flavonoids, terpenes and their derivatives which are responsible for their inhibition or killing of microorganisms amongst other properties seen in essential oils (garlic oil) and other plants part (Huzaifa *et al.*, 2014) [46]. Activities against multi-resistant bacteria has also recently been elucidated, which has drawn attention to the possibility of them being a source of novel compounds to be used in combating microbial drug resistance. The fast growing drug resistance of infectious organisms has caused great concerns due to its huge impact globally. No society is safe from this health threat, and pharmaceutical companies are unable to deal with it using conventional technologies and also due to decrease in new antibiotic development. This has given rise to the need for new technologies, drug designs and drug alternatives that can bring lasting solutions to this problem.

Nanoencapsulation has been proposed as one such technology that has great potential to solving this problem. It is a branch of nanotechnology that has received a lot of attention by researchers recently especially in the pharmaceutical and biotechnology field. There are already approved products from such studies for clinical use in diagnostics, drug delivery, medical devices and imaging (Sumita *et al.*, 2003) [42]. Nanotechnology has seen remarkable interest in its applications in dealing with virtually all facet of microbial infection such as microbial diagnosis, antibiotic delivery, therapeutics, vaccination and medical devices. Nanoencapsulation involves the use of delivery vessels, also referred to as nano carriers to encapsulate substances or bioactive molecules. They protect these molecules from environmental factors such as pH, oxygen, light etc., serving as barrier between the molecule and the environment. It also stabilizes volatile molecules, shielding them from oxidative degradation, evaporation and photo-degradation (Jain, 2003) [20]. Nano carriers in addition can increase the antimicrobial potential of bioactive compounds like essential oils by increasing cellular interactions between them and the microbes as a result of the very small size that

Enhances cellular uptake. The review was aimed at proposing a new way of combating antimicrobial resistance.

Essential Oils Mechanism of Action

Essential oils have a very close interaction with the phospholipids of cell membrane due to the short extension of their carbon chains as well as high hydrophobicity of their constituents. Terpenes and their derivatives are able to rupture the lipid in the cell membranes of microorganisms, upsetting the flow of external membrane, including the mitochondrial membrane, breaking and consequently killing the pathogens Lv *et al.* (2011) [47], (Solorzano-Santos and Miranda-Novales, 2012 [41]; Devi *et al.,* 2010) [10]. Their metabolites are able to cross the cell membrane, binding and inhibiting specific proteins. There been report of their attachment to or insert into DNA or RNA. Furthermore, essential oils constituents' show synergy, working together and interacting by different mechanisms not yet fully explained. This results in significant decrease of the potential development of resistance by microorganisms (Bassole and Juliani, 2012 [7]; Efferth and Koch, 2011) [13], great advantages essential oils have over commercial antibiotics in the fight against infectious microorganisms. The different combinations of active compounds that make up these essentials oil makes it difficult for the microbe to develop resistance quickly to them all. Therefore, the microbe will be completely inhibited or killed before they devise means/change their genetic make up to escape or negate the actions or effects of the essential oils High concentration essential oils do not pose any great health risk to humans due to high rate at which the body metabolizes them eliminating them through various means.

In addition, essential oils can be used in combination with commercial antimicrobial agents to improve their activity and decrease resistance to them. As was reported by Effert and Koch (2011) [13], the nanocarriers gives protection to the active compounds of the essential oil from enzymatic degradation by the pathogen; modification of transport across enabling the evasion of myriad multi drug resistance mechanisms based on expulsion of antibiotic molecules that is efflux-mediated. Essential oils phytochemicals have been shown to be able to target organisms in multiple ways, and this can be used to explain the synergism with commercial antibiotics as observed in a number of studies. It showed outstanding enhanced antibiotic activities of these drugs against even resistant strains of microorganisms. This results in decrease in the overall intake of drugs with consequent reduction of adverse effects, making treatment safer (Hemaiswarya *et al*., 2008 [18]; Langeveld *et al.,* 2014) [24]. Example is seen in the experiments reported by Ahmad *et al.* (2013) [2] who combined thymol and carvacrol, together with fluconazole. They were able to restore fluconazole susceptibility in resistant clinical strains of *Candida.*

Nanoencapsulated essential oils with enhanced antimicrobial activity

Thyme essential oils

Thyme essential oil has been encapsulated in a chitosan-benzoic acid nanogel, a potent antifungal, antiviral and antibacterial agent. Its antimicrobial activity has been studied against a substantial pathogenic microorganism such as *Salmonella typhemurium, Yersinia enterocolitica, Shigella flexneri, Listeria monocytogenes, Shigella sonnei, Salmonella cholereasuis and Aspergillus niger* and also clinical strains of Staphylococcus Enterococcus, Escherichia and Pseudomonas genus Sienkiewicz *et al.* (2012) [39], with good results. However, essentials are typically volatile compound with easy degradability at room temperature. For that reason, different techniques or approaches for increasing their stability and activity are imperative. Chitosan-benzoic acid nanogel was used by Seyede *et al.,* (2015) [48] to provide stability, increase availability and activity of thyme oil. They are self-assembling polymers, with chitosan having antimicrobial activity of its own as was shown by Eaton *et al.* (2008) [12], and Eldin *et al.* (2008) [14]. The minimum inhibitory concentrations of nanogel encapsulated thyme oil that was able to completely inhibit the growth of the fungi *Aspergillus flavus* under sealed condition was 300 ml/L, and a higher level of 500ml/L was required under non-sealed, compared to the free thyme essential oil that was only able to delay growth of the fungi at 400 ml/L and 1000 ml/L of sealed and non-sealed conditions respectively. They discovered that the encapsulation of the free essential oils of thyme in chitosan-benzoic acid nanogels, led to a marked increase in their half-life and antifungal properties.

In a similar study by Wattanasatcha *et al.* (2012) [45] created a Zein colloidal nanoparticles film coated with sodium caseinate (SC), which also served as an emulsifier, loaded with thymol. Zein (a maize protein) nanoparticles were employed as reinforcement in the film matrix. Thymol is among the major compounds found in many essential oils like Thyme, Oregano essential oils, with greater antimicrobial properties than most other constituents. It was shown to be active against both gram positive and negative bacteria in a number of researches that has led to its use in many area of application such as the food, agriculture and pharmaceuticals Sivropoulou *et al.* (1996) [40]. The aim of this work was therefore to use this essential oil in a novel fabrication of nanosphere films, proposed to be used as active packaging material, to confer it with antimicrobial activity against Escherichia coli and Salmonella. The nanosphere film showed improved physical and mechanical properties that could make it an excellent packaging material for protecting and preventing microbial infection. The film showed a two phase drug release kinetic which implies its possible use as sustained drug release material. This sustained release ability is thought to be as a result of the Zein-SC nanoparticles embedded in the matrix

Mentha piperita essential oils

In a similar work, Beyki *et al.* (2014) [8] were able to demonstrate an enhanced antimicrobial activity of *Mentha piperita* essential oils encapsulated in chitosan-cinnamic acid nanogels against pathogenic *Aspergillus flavus.* They evaluated the minimum inhibitory concentrations (MIC) of both the encapsulated and free essential oil against *Aspergillus flavus* under sealed and non-sealed conditions. Their findings were that the encapsulated oil had an MIC OF 500 ppm while the free had 2100 ppm under sealed condition. The nanogel was able to protect the essential oils from environmental factors which led to increased stability and its performance as an antifungal was also greatly enhanced. Furthermore, under non-sealed condition the encapsulated oil was able to inhibit the fungi *Aspergillus flavus* at 800 ppm, while the free oil could not completely inhibit the fungi even at high concentration of up to 3000 ppm. These obtained results further highlights the role nanogels and other nano carriers can play in enhancing antimicrobial activities of essential oils.

Zataria multiflora essential oils

The effectiveness of nanoencapsulated *Zataria multiflora* essential oil (ZEO) in chitosan nano particles was investigated by Mohammadi *et al.* (2015) [28]. The essential oil was encapsulated in order to improve its stability and antifungal activity against an isolate of *Butyris cinerea*, the pathogen that causes gray mould disease. Using ionic gelation technique, they encapsulated the essential oil into chitosan nano particles with an average size of 125-175 nm. This was confirmed using transmission electron microscopy. The encapsulation efficiency and loading capacity of the chitosan nano particles were determined by finding out the percentage of the essential oil in the ZEO loaded nano particle. They did this by finding the concentration of ZEO after lyses of the encapsulated nano particles using hydrochloric acid solution and alcohol, as was described before Keawchaoon and Yoksan (2011) [21]. The maximum encapsulating and loading efficiency were gotten at the weight ration of CS to ZEO of 1:0.25, which translated to 45.24% and 9.05%, respectively. They recorded significant increase in the antifungal activity at a concentration of 1500 ppm in vivo experiments, compared to the free ZEO.

***Eucalyptus staigeriana* essential oil (ESO)**

The essential oil of eucalyptus staigeriana has been shown to have good antimicrobial properties by a number of scientists. The eucalyptus extracts, including its essential oils, have at present been approved to be used as food additives, and in cosmetic formulations. The essential oil however show characteristic high volatility and are non-stable like other essential oils Gilles *et al.* (2010) [17]. So despite their great antimicrobial potential, it has found limited use in the pharmaceutical industries. With the advent of nanoencapsulation technology, the bioactive components of the essential oil can now be stabilized physically and protected against the environment or physiological conditions of the body. Their nano size will also help in improving their availability and antimicrobial activity. According to Herculano *et al.* (2015) [19], they experimentally showed increased bioactivity and stability of nano encapsulated essential oil of *Eucalyptus staigeriana*. They used cashew gum as the wall material, which is a polysaccharide gotten from the plant *Anacardium accidentale* exudates. It has a similar structural property to gum Arabic frequently used Mothé and Rao (2000) [29]. It is hydrophilic and therefore can act as a stabilizer, emulsifier and an adhesive. It has been proposed to be used as substitute for the more costly gum Arabic.

The nanoparticles of the cashew gum were loaded with the essential oil using spray dry technique previously described by Paula *et al.* (2010) [33]. Minimum bactericidal concentration of the loaded nanoparticles was determined using the procedure established by Sahm and Washington (1991) [36], against gram positive pathogenic *Listeria monocytogenes* and gram negative equally pathogenic *Salmonella enteritides*. The free essential oil and cashew gum solutions were used as controls. Particle size distribution, loading and encapsulation efficiency, zeta potential were all determined (Paula *et al.*, 2011) [34]. Herculano *et al.* (2015) [19] discovered that the nano particle had a more effective bactericidal action on the gram positive than to the gram negative bacteria. They attributed this result to probably be as a result of the synergistic effect between the cashew gum and essential oil. The nanoparticles had negative charges on their surfaces and a size range of 27.70 nm to 432.67 nm. The loading capacity was between 4.76% and 7.12% with encapsulation efficiency ranging from 24.89% to 26.80%. The stability of the nanoparticle depended upon the CS: ESO ratio when stored within one year. Higher CG proportion in the matrix gave more stable complex with 8% decrease of initial oil content.

Savory Essential Oil (SEO)

In another recent study, Atef *et al.* (2015) [3] conducted experiments to determine the concentration of savory essential oil that could be integrated into an agar based nanocomposite films. Their aim was to prepare an active packaging film that will have a longer protection against food spoiling microbes, environmental degradation and in essence its shelf life. Savory essential oil is obtained from *Satureja hortensis*, an herb that is also referred to as summer savory. It is an aromatic herb widely distributed that has found great application in culinary, but also has well known medicinal benefits. This is attributed to its high carvacrol content (> 55%), β-cymene (12.30) and γ-terpinene (20.94%) among other compounds Tozlu *et al.* (2011) [43].

Nanotechnology has opened up a new era of abundant possibilities, including its use in improving biopolymer films properties at a reasonable cost-price with good efficiency (Espitia *et al.,* 2012) [15]. In this work, the agar nanocomposite films were reinforced with cellulose nanoparticles so as to enhance its properties. These novel biopolymer films are been proposed to be used as replacements of plastic packaging materials of petroleum origin (Rhim, 2011) [35]. Also, Atef *et al.* (2015) [3] were able to evaluate the effects of incorporating savory essential oil into the cellulose nanoparticle reinforced agar-based composites on their antimicrobial properties by testing it against gram positive bacteria *(Staphylococcus aureus, Listeria monocytogenes,* and *Bacillus cereus*) and gram negative bacteria (*Escherichia coli*). They found out that the gram positive bacteria were more susceptible to the nanocomposite film containing the essential oil compared to the gram negative. This implies that incorporating savory essential oil into the nanocomposites films to be used as active food packaging will increase food safety and shelf life.

Melaleuca alternifolia Essential Oil

Another example of enhanced antimicrobial activity was demonstrated by Flores *et al.* (2013) [16], where polymeric nanocapsules and nanoemulsions containing *Melaleuca alternifolia* essential oil (also known as tea tree oil) were tested against pathogenic fungi *Trichophyton rubrum*. The fungus is a dermatophyte that infects the human nails. Two distinct sets were used in-vitro evaluation; one set used nail powder that was first infected with the fungi and then treated using the free essential oil, nanoemulsions and nanocapsule containing the essential oil. They were able to confirm that the cells were viable after 7 and 14 days. They plate counts obtained were 2.37, 1.45 and 1.0 log $cfuml^{-1}$ for the free oil, nanoemulsion and nanocapsule respectively. The other set had nail fragments infected with the fungus and then treated with the formulations as in the first model. Measuring the diameter of the fungal colony was used to obtain a 2.88 ± 02.08 mm^2 (nanocapsule incorporated with essential oil), 14.59 ± 2.01 mm^2 (nanoemulsion incorporated with essential oil), 40.98 ± 2.76 mm^2 (emulsion) and 38.72 ± 1.22 mm^2 (untreated nail). At the end of the experiments, they found out that the two (nanocapsule and nanoemulsions) had increased antimicrobial activity compared to the free essential oil. This could be attributed to larger surface area of nano material (as a result of their very small size), which probably enable a greater interactions between the active compounds and cell surfaces of the microorganisms (Lboutounne *et al.*, 2002) [25]. But the polymeric nanocapsule had a much higher efficiency in inhibiting the fungal colony from growing (Flores *et al.* (2013) [16]. This could be as a result of more interaction between the hydrophilic end of the polymer and the lipid layer of the microbial cell membrane.

Carvacrol-Oregano essential oils

Carvacrol is one of the major and vital constituents that contribute greatly to the antimicrobial activities observed in most essential oils like oregano and thyme essential oils. Carvacrol has a broad spectrum of antimicrobial activity that has generated a lot interest in its potential use as an alternative to commercial antimicrobials. A lot of researchers have reported its ability to inhibit the continual growth of biofilms and also prevent biofilm formation (Lambert *et al.,* 2001) [23]. Among such work is that of Nostro *et al.* (2009) [31], that were able to show remarkable decrease in biofilm biomass of *Staphylococcus aureus* and *Staphylococcus epidermis*, by modifying the impenetrable matrix of the biofilm making it accessible to antimicrobials, changing of cellular characteristic as well as reducing the number of cells within the biofilm that could proliferate. Biofilms are a thin layer of slimy sticky substances that can be produced by some bacteria and other types of microorganisms. They form a community of different microbe protected by the robust slimy layer that can attach to virtually all kinds of surfaces. This biofilm formation ability has substantially increase pathogenicity of the microbes, and treating or getting rid of them is has become even more challenging that the free unattached pathogens. A lot of medical devices based infections are as a result of this microbial biofilms. The remarkable resistance showed by cells in the biofilm towards antimicrobials and the difficulty experienced in destruction of biofilm-based infections have been attributed to a number of reasons, for example, high number of metabolically active and developing cells at the biofilm surface and low or no development in the inside resulting to a subpopulation of persister cells (Balaban *et al.*, 2004 [5]; Keren *et al*., 2004) [22]. The cells within the biofilm are protected by an extracellular polymeric substance (EPS) matrix that cannot be engulfed by the host defence phagocytic cells. It contains many enzymes that inactive antimicrobials when they come in contact with it. In addition, they have efflux pumps they use to flush out antimicrobials (Bagge *et al*., 2004) [4]. They bacteria inside the biofilm have also been found to constantly mutate, giving rise to antibiotic resistant strains (Driffield *et al*., 2008) [11].

In an interesting study Carvacrol was encapsulated into a nano carrier (PLGA) for drug delivery application against microbial films. Their aim was to design a polymeric nanocapsule loaded with Carvacrol and test it against preformed biofilm of Staphylococcus epidermis for antimicrobial activity. They highlighted the effect such a material had on the matrix by observing changes in the properties of the biofilm matrix. The elasticity and mechanically stability of the strong biofilm layers reduced, and this could possible allow for the penetration of active substances or antimicrobials into the innermost parts of the biofilm where persistent cells reside. The nanocapsule can be used in combination with other antimicrobial agents for a more concise and effective eradication or prevention of biofilm forming microorganisms and their biofilms (Li *et al.,* 2012) [28]. There was also a study carried out by Santos *et al.* (2015) [27], were they prepared and characterized Carvacrol Beta - Cyclodextrin inclusion complexes and investigated their effects on the antimicrobial activity against two bacteria, *Escherichia coli* k (12) and *Salmonella enterica* serova typhemurium LTS. The entrapment efficiency was good (83.79 ± 2.89% & 91.31 ±.41%), and there was increase in the antimicrobial activity of the encapsulated carvacrol compared with the free. This was indicated by the minimum inhibition concentration of 300-400 µg/ml for the encapsulated as compared to that of free that was greater than 1000 µg/ml.

Lantana camara essential oil

Lantana camara is an infamous tropical flowering plant that has been largely used in folk medicine. It has been shown to have diverse applications that include its use as an antimicrobial agent (Sharma *et al.,* 2005) [38]. Lantana camara essential oil has germacrene - D, E - caryophyllene, bicyclogermacrae and α - humulene as its major constituent (Barreto *et al*., 2010) [6]; Passos *et al.,* 2012) [32]. Although, Naz and Bano (2013) [30] in their study screened the plants extract and found that it has about 53 and 41% total phenol and flavonoids respectively. They also evaluated the plants extract using different solvents for its antimicrobial activity and found out that the methanol extract had a greater activity against gram positive and negative organisms (*Staphylococcus aureus, Pseudomonas aeruginosa, Aspergillus fumigates and Aspergillus flavus*). In another study by Deena and Thoppil (2000) [9] showed wide range of antimicrobial activity with the highest inhibition seen for *Candida albicans Aspergillus F solium* and *Pseudomonas aeruginosa*. It was also shown to have a remarkable inhibition of multi-drug resistant clinical strains of microorganism, which are clinically important as there is no single available antimicrobial agent that can inhibit these resistant strains, making treatment of such infections more challenging. The organisms tested were *Escherichia coli, Pseudomonas aeruginosa, Vibrio cholareae* and *Staphylococcus aureus* (Barreto *et al*. 2010) [6].

Recently, Verma and Balasubramanian (2014) [44] incorporated essential oil of *Lantana camara* in a polymeric nanocomposite membrane to be used as a pulsatile medium of drug delivery. Polyacrylonite (PAN) is a polymer with unique properties and has found applications in a great number of areas including medical and drug delivery. Its surface is electrostatic which attracts a lot of microbes and over dust particles, resulting in the membrane getting contaminated and finally malfunctioning. This necessitates the need for the surface to be functionalized using different types of antimicrobial agents like peptides, antibiotics. In this study, they used the essential oil to enhance antimicrobial activity of the membrane and in addition deliver the Eos in a pulsatile or controlled released manner. They immobilized the essential oil on the PAN membrane and showed it to have exceptional antibacterial activity against *E coli* and *Bacillus subtilis* (7-10 nm zone of inhibition). Its potential as a system that could be used for pulsatile drug delivery was also confirmed.

Conclusion

In this review, enhanced antimicrobial activity of essential oils through the use of nanotechnology especially nanoencapsulation was highlighted and its great potential as a solution to the ever growing global problem of emergence of multi drug resistant microbes. The possibility for new and excellent compounds like essential oils and other plant extracts that could be used as novel antimicrobial compounds together with the innovative nanotechnology approaches is enormous. So even though essential oils are not without their limitations, nanotechnology can be used to overcome such constraints.

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