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Green Synthesis of Various Nanostructures Containing Essential Oil and Silver Nanoparticles: Nanocomposites, Nanoemulsions and Nanoencapsules

Amra Bratovcic*
(Department of Physical Chemistry and Electrochemistry, Faculty of Technology, University of Tuzla, Urfeta Vejzagića 8, 75000 Tuzla, Bosnia and Herzegovina; email: amra.bratovcic@untz.ba)

ABSTRACT
This paper presents the use of essential oils (EO) for the green synthesis of nanostructures such as nanoparticles, nanoemulsions and nanoencapsules. Essential oils differ in their chemical composition depending on the type of plant, parts of the plant that are extracted, as well as the extraction method. In this work, different essential oils such as lavender oil, Litsea cubeba, Crown imperial (Fritillaria imperialis L.) and Mentha spicata L. were used for the synthesis of silver nanoparticles. The process of green synthesis of nanoparticles is simple and fast and environmentally friendly because it excludes the use of aggressive and toxic chemicals. In the green synthesis of nanoparticles, secondary metabolites from plants found in essential oils serves as reducing and capping agent. Differently prepared silver nanoparticles had a maximum UV-vis absorption peak at wavelength range from 408 - 485 nm and the average size particles range from 8 - 80 nm.

Keywords - essential oil, green synthesis, nanoencapsules, nanoemulsions, nanocomposites, silver nanoparticles

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I. INTRODUCTION

Essential oils are hydrophobic and volatile natural liquids with a low molecular weight and a strong odor extracted from vegetable parts of plants and composed of phytoconstituents which contain chemical compounds such as aldehydes, ketones, alcohols, phenols, acids, ethers, and esters. [1] They have antiviral, antimicrobial, antioxidant, anti-inflammatory, anti-allergic, and regenerative properties [2]. Essential oils are fragile by origin and unstable when exposed to extremes of heat, sunlight, or oxygen.

II. PLANT EXTRACTS AND ESSENTIAL OILS

The essential oil yield of the plant is usually very low. By applying heat and pressure, the oil contained in plant cells is released. [3] Today, lavender oil is primarily produced from lavender flowers using traditional extraction methods, namely hydrodistillation, steam distillation, and solvent extraction. These methods are simple, cheap and easy to use. However, limitations of distillation techniques include degradation of heat-labile compounds due to high operating temperature, hydrolysis of water-sensitive compounds, incomplete extraction, and the need for post-extraction treatment to remove water. Solvent extraction has a major disadvantage that solvent residues remain in the extracts. Supercritical CO_2 extraction (SCE) has been proposed as an alternative technique for obtaining lavender essential oil because it has several advantages over classical extraction methods. SCE is usually performed at low temperatures below 60 °C, whereby thermolabile compounds are retained within the extract. The use of CO_2, which is non-toxic and non-explosive and readily available as an extraction solvent, provides a solvent-free product. In particular, CO_2 shows a strong lipophilic selectivity and has the advantage that the extracts do not contain undesirable compounds such as organic and inorganic salts, sugars, amino acids and tannins. [4]

Essential oils are aromatic substances present in specific parts of the plant structure such as secretion ducts, secretory glands or resin ducts. These oils have antimicrobial properties and are enjoying increasing popularity in biomedicine. Plant extracts and essential oils have a wide variety of molecules with potential applications in various fields such as medicine, food and cosmetics. These plants derivatives are of great importance for human and animal health, including their potent anticancer, antifungal, anti-inflammatory and bactericidal effects. Given this diversity, different methods were required to optimize the extraction, purification, and characterization of each class of biomolecules.

2.1 Chemical composition of Lavender oil

Lavender is an herb native to the Mediterranean region. The oil of Lavandula *angustifolia* is referred to as true lavender oil, and it has been used for cosmetic and medicinal purposes due to its unique chemical composition, which provides both aromatic and biological effects. [5] In the essential oils are identified following compounds: aromatic hydrocarbons, alkenes, ketenes and alkaloids. [6] Lavender oil contains more than a hundred components, the most important of which are linalool, lavandulol. Lavender essential oil contains phytochemicals such as cineole (eucalyptol), fenchol, camphor, linalyl acetate. (Figure 1). [7-8] Phytochemicals are nutrients and chemicals that can be found naturally in plants. Most lavender essential oil products are made up of just 1% to 5% pure oil. [9]

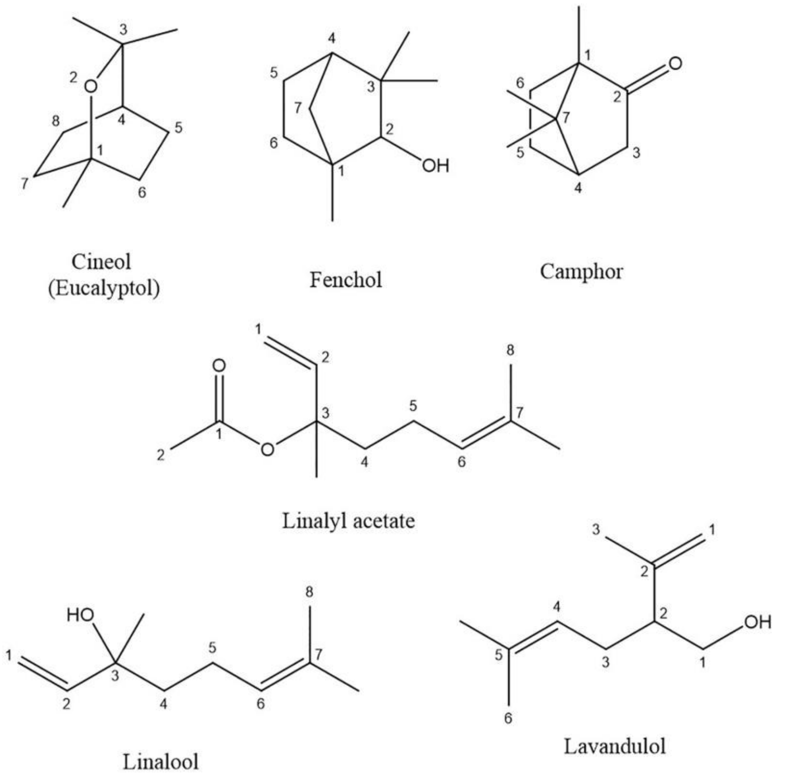

Figure 1. Phytochemicals of lavender essential oil.

These components have functional and nutraceutical properties and therefore are beneficial for human health. Moreover, lavender oil also shows antiinflammatory, antioxidant, hypnotic, antidepressant, antimicrobial, and antifibrotic properties. [10]

Lavender oil is used in food production as a natural flavor enhancer for drinks, ice cream, sweets, pastries and chewing gum and as an antioxidant that protects food from oxidative rancidity, loss of volatile compounds and unpleasant aftertaste. In addition, lavender oil is used in aromatherapy and alternative medicine. Lavender oil speeds up the rate of healing, increases the expression of collagen which keeps skin elastic and enhances the activity of proteins involved in rebuilding tissue. *Lavandula* species are used in fragrance, pharmaceutical and cosmetic industries. Physicochemical instability of lavender bioactive compounds may decrease their pharmaceutical application. Therefore, nanotechnology can be used as a way of achieving this stabilization. [11]

III. SYNTHESIS OF NANOMATERIALS BY ESSENTIAL OILS

Plant products can be used in the synthesis of nanomaterials to reduce the undesirable effects of traditional synthesis methods based on hazardous/toxic chemicals and to combine the properties of nanomaterials with those of extracts and essential oils. Vegetable oils and extracts are chemically complex, and although they have been used in the synthesis of nanomaterials, there has been limited investigation into which molecules are effective in the synthesis and stabilization of these nanostructures.

Essential oils are characterized by complex mixtures of compounds with important pharmacological properties, but they are sensitive to high temperatures, light and the presence of oxygen. Nanotechnology is then used to improve stability, ensuring quality and efficiency. Nor has it been investigated whether the molecules coating the nanomaterials derived from these extracts and essential oils could in any way enhance or limit their potential activity. [12]

Green synthesis of nanoparticles using essential oils is a quick and simple procedure which assumes an environmentally friendly preparation of particles without the use of aggressive, toxic, and expensive substances, is an alternative, more economically viable and environmentally friendly method to obtain nanostructures compared to expensive physical methods, time-consuming and harmful to the environment. Plant secondary metabolites are often used as reducing agents in "green" synthesis. [13] They are particularly promising for "green" synthesis because of low production costs, short-term synthesis, and biosafety. In vitro plant cultivation also increases the production of essential substances, since these methods produce large quantities of standardized plant material in a short time and produce the desired secondary metabolites throughout the year.

The development of an effective nanodrug to effectively fight cancer cells is based on a number of parameters such as size, composition, coating, other physico-chemical properties, perfusion properties, etc. Biocompatibility is one of the most important parameters affecting the general usability of a drug in vivo. Nanoparticles produced by the "green" synthesis method are often characterized by increased biocompatibility due to the use of natural substances with the required biological activity (noble metals, secondary metabolites, proteins), which have been successfully demonstratedin various in vitro and live studies be used. These molecules are considered promising for theranostics. The schematic synthesis of silver nanoparticles is shown in Figure 2.

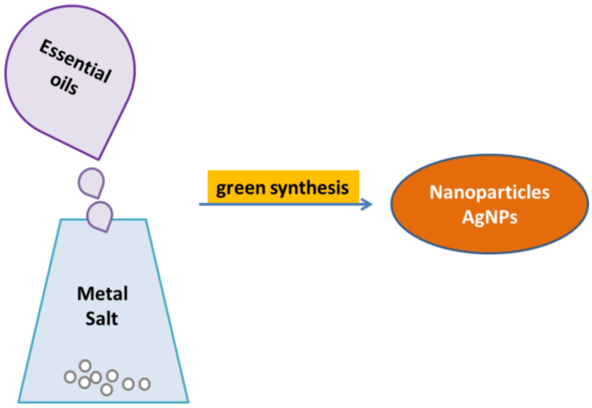

Figure 2. The schematic synthesis of silver nanoparticles

3.1 Green synthesis of various nanostructures with cytotoxic activity

Modern bionanotechnologies open up a wide range of perspectives for the development of new generations of drugs that can be used to combat diseases with societal consequences. [14] Bionanotechnological means and processes enable the creation of various nanostructures, which are effective tools for the therapy and diagnosis (theranostics) of various diseases, especially cancer. [15-16] The development of theranostic methods is based on multifunctional agents that combine diagnostic and therapeutic functions. These factors include metal nanoparticles (NPs) with localized surface plasmon resonance (LSPR) properties. The high surface chemical activity of these nanoparticles allows them to be modified with targeted drugs to deliver them to target cells, while LSPR makes these nanoparticles suitable for both detection and selective destruction of hyperthermic cells.

3.2 Lavender composite polyurethane nanofiber dressings

Sofi et al. (2019) prepared composite electrospun wound-dressing nanofibers composed of polyurethane encasing lavender oil and silver nanoparticles (Ag NPs) for wound-healing applications. Biocompatible and biodegradable

synthetic polymers can be an effective alternative in the treatment of severe wounds and burns. The ideal biomaterial must have antibacterial properties, be hydrophilic, allow for rapid healing, and control the growth of pristine epidermal cells. The abundance of silver nanoparticles (AgNPs) in the fibers reduced the fiber diameter, while increasing the concentration of lavender oil increased the diameter. AgNPs and lavender oil enhanced the hydrophilicity of the nanofibers and ensured the proliferation of chicken embryo fibroblasts grown in vitro on these fiber dressings. The antimicrobial efficacy of nanofiber dressings was tested with *E. coli* and *S. aureus* bacteria, resulting in zones of inhibition of 16.2 ± 0.8 and 5.9 ± 0.5 mm, respectively, indicating excellent bactericidal properties of the dressings. [17]
The „green“ synthesis method was used to prepare silver nanoparticles (NPs) from aqueous plant and callus extracts of the narrow-leaved lavender *Lavandula angustifolia* Mill. The surface of the small sizes of the plant extract-based NPs can be modified with protein molecules (bovine serum albumin), which makes them promising agents for cancer theranostics in vitro. [18]

Kumar et al. (2016) have prepared AgNPs using the leaf extract of lavender. The AgNPs (Ag/Ag_2O) formed had spherical form which absorbed at 440 nm with size range 10 - 80 nm. The surface-modified AgNPs showed an effective catalytic activity and antioxidant activity. [19]

3.3 *Litsea cubeba* essential oil-silver nanoparticles

An eco-friendly antimicrobial agent *Litsea cubeba* essential oil-silver nanoparticles (Lceo-AgNPs) were prepared. *Litsea cubeba* essential oil were used as a reducing and capping agent. The size of Lceo-AgNPs were 5–15 nm with the maximum UV absorption peak at 423 nm. The antibacterial activity of Lceo-AgNPs were effective against *Escherichia coli (E. coli)* and *methicillin-resistant Staphylococcus aureus (MRSA) in vitro.* The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of Lceo-AgNPs against *E. coli* and against (MRSA) are shown in table 1. *In vivo* research disclosed significant wound healing and re-epithelialization effects in the Lceo-AgNPs group compared with the self-healing group and the healing activity was better than in the sulfadiazine silver group. [20]

3.4 Crown imperial (*Fritillaria imperialis* L.) essential oil - silver nanoparticles

Green synthesis of silver nanoparticles containing Crown imperial (*Fritillaria imperialis* L.) essential oil extracted from leaves, bulbs and petals as reducing agents have been done by Zakeri et al. 2021[6]. The synthetized AgNPs were confirmed by changing the colour from pale pink to black with mostly spherical particles of 27 ± 14 nm. The maximum UV-Visible absorption peaks were at different wavelengths at 418 (bulbs), 408 (leaves) and 450 (petals) nm confirming the influence of size and shape of the particles and the surface species on position of peaks. The results are in agreement with the well-known surface plasmon resonance of silver. [13]

The antibacterial activity of the AgNPs shows a remarkable inhibition capability against four bacterial strains including *Escherichia coli* (ATCC 35218), *Salmonella typhimurium* (ATCC 14028), *Staphylococcus aureus* (ATCC 29213) and *Bacillus cereus* (ATCC 14579), with the sample obtained from petals exhibiting the strongest antibacterial effect in agreement with the obtained minimum inhibitory concentration and minimum bactericidal concentration values. The cell viability assay using Vero cell line reveals a nearly constant viability rate above 125 μg/mL of silver nanoparticles. [6]

3.5 Mentha spicata L. (M. spicata) essential oil - silver nanoparticles

Moosavy et al. 2023 have used green method for synthesis of gold NPs (AuNPs) and silver NPs (AgNPs) nanoparticles by Mentha spicata L. (M. spicata) essential oil to study their antibacterial, antioxidant, and *in vitro* cytotoxic efects. The essential oil was mixed with both Chloroauric acid ($HAuCl_4$) and aqueous silver nitrate ($AgNO_3$) solutions separately and incubated at room temperature for 24 h. Biologically active compounds (monoterpenes) determined by FTIR may assist in the formation and stabilization of both types of NPs. AgNPs exhibited better antimicrobial activity against five different bacteria, including *E. coli*, *L. monocytogenes*, S. *Typhimurium*, *S. aureus*, and *B. cereus* than AuNPs. Zones of inhibition for the AgNPs was in range 9.0-16.0 mm. They notice that by increasing of nanoparticle size, the diameter of the inhibitory zone significantly increase. [21] Table 1 shows green synthetized materials based on lavender oil and AgNPs for biomedical application.

Table 1. Green synthetized materials based on lavender oil and AgNPs for biomedical application

O/n	**Composition of synthetized material**			**Antimicrobial efficacy/ zones of inhibition**	**Anti cancer**	**Application**	**Ref.**
1	Lavender oil	AgNP	polyurethane	*E. coli* 16.2 ± 0.8 mm *S. aureus* 5.9 ± 0.5 mm	-	wound-healing	[15]
2	*Lavandula angustifolia* Mill extracts	AgNP	bovine serum albumin	-	+	cancer theranostics in vitro	[16]
3	leaf extract of lavender	AgNP	-	-	-	catalytic and antioxidant activity	[17]
4	*Litsea cubeba* EO	AgNP		*E. coli* 25 and 50 μg/ml *MRSA* 50 and 100 μg/ml	-	wound treatment	[18]
5	Crown imperial (*Fritillaria imperialis* L.) EO	AgNP	-	*Escherichia coli* *Salmonella typhimurium* *Staphylococcus aureus* *Bacillus cereus*	-	antibacterial activity	[6]
6	Mentha spicata L. (M. spicata) EO	AgNP	-	*E. coli,* *L. monocytogenes* S. *Typhimurium, S. aureus,* *B. cereus* 9.0-16.0 mm	-	antibacterial activity	[19]

*EO- essential oil
**AgNP – silver nanoparticles

Table 2 shows the average sizes and absorption peak of green synthesized silver nanoparticles (AgNPs).

Table 2. The average sizes and absorption peak of green synthesized silver nanoparticles (AgNPs)

Average size (nm)	**UV-Vis absorption peak (nm)**	**Ref.**
80	440	[19]
8-15	423	[20]
27±14	418 (bulbs) 408 (leaves) 450 (petals)	[6]
24	485	[21]

The condition of the patient and different types of wounds complicate the wound healing process. Nanotechnology could provide the physicochemical properties and specific biological reactions needed to advance this process. In this context, the use of natural biopolymers with antibacterial and anti-inflammatory properties (polysaccharides - alignate and chitosan) and nanoparticles for bandage design is suggested. [22] In addition, they would add essential oils with excellent antibacterial, antifungal, antioxidant and anti-inflammatory properties that can be enhanced by combining them with nanotechnological strategies. [23]

3.6 Nano-emulsion formulations

Ibrar et al. 2022 have shown that essential oils when used in combination with traditional drug can lead to much higher efficacies as compared to pure drugs. They have synthetized nano-emulsion (NE) composed of garlic and ginger oils (0.1 %) with antibiotic neomycin sulphate (NS) in different ratios (0.001, 0.01 and 0.1 %) and combinations. The results of physico-chemical characterization have shown that they are stable over a period of three months with droplet size (145–304 nm), zetapotential (-3.0–0.9 mV), refractive index (1.331–1.344), viscosity (1.10–1.23cP), transmittance (96–99 %). All nano-emulsions were effective against both gram positive and negative bacterial strains i.e., *B. spizizenii*, *S. aureus*, *E. coli* and *S. enterica* as compared to pure (NS) showing zone of inhibition in range of 22–30 mm and 21–19 mm, respectively. NEs were shown to be able to heal excisional wounds of rabbit skin with 86-100% wound healing within 9 days compared to NS ointment (71%). [24]

3.7 Nanoemulsions of lavender essential oil

Nanoemulsions (NEs) were formed between essential oil (*Lavandula spica*) and its major compounds camphene and α-terpinyl acetate and an aqueous solution containing 10 % of surfactant Tween 80 under constant stirring. The NEs of lavender oil and α-terpinyl acetate have similar droplet diameters and were 104.55 nm and 105.23 nm, while camphene was 117.23. The bio-products (phenolic and flavonoid compounds) as constituents of EO in high concentrations show anti-bacterial, anti-fungifungi, and anti-yeast properties. The products indicated high antioxidant activity, and the NE of lavender oil exhibited a significant scavenging capacity (IC_{50} = 261.66 mg/L). The antimicrobial activity was tested against bacteria of Gram-negative (*Salmonella typhimurium*) and Gram-positive (*Staphylococcus aureus*), fungi *Aspergillus flavus* and *Aspergillus niger,* and yeast *Candida albicans*. α-Terpinyl acetate NE was superior active (MIC = 725 and 550 mg/L against both studied gram-positive and gram-negative bacteria, respectively). Lavender oil NE exhibited the highest inhibitory action against *C. albicans* with the lowest EC_{50} value (107.70 mg/L). The antifungal data revealed that all NEs were superior active against both fungi than pure lavender oil and monoterpenes. [25]

Nanoemulsions were produced from 5% of essential oil *Lavandula dentata* Linnaueus and *Myristica fragrans* Houtt and hydrophilic and lipophilic surfactants. The droplet size of *M. fragrans* esential oil-loaded nanoemulsion (NE-MO) was 87.06 nm, its polydispersity index (PDI) was 0.17. *L. dentata* esential oil–loaded nanoemulsion (NE-LO) droplet size was 64.99 nm, its polydispersity index (PDI) was 0.26. Chemical analyses proved the greater efficacy of the NE-MO to preserve the majority compound percentages oil (L-α-pinene, (-)-β-pinene, sabinene, α-limonene, γ-terpinene) throughout the study under storage at 4 and 24 °C compared with free oil. The formulation NE-LO enabled good protection only at room temperature for α-pinene, sabinene 1.8-cineole, fenchone and camphor.[26]

3.8 Nanoencapsulation of essential oils

Essential oils are not widely used in the food industry due to some important inherent barriers such as low water solubility, bioavailability, volatility and stability in food systems. These disadvantages can be remedied by nanotechnology, i.e. nanoencapsulation. Antimicrobial foods can destroy microorganisms and inhibit their growth, improving food safety for consumers and extending food shelf life. [27]

Encapsulation is considered a sensible alternative to improve the suitability of active ingredients for food or to protect them from deteriorating environmental conditions. In general, the encapsulation is defined as a support matrix in which the bioactive substance is encapsulated, whereby the rate of release of the bioactive substance can be controlled.

Nanoencapsulation exhibits better functionality than microencapsulation in terms of higher protection, higher stability, sustained release profile and better bioavailability of bioactive compounds. By nanoencapsulation is possible to improve bioavailability of bioactive compounds, to enhancement of physical stability and long shelf life, better precision targeting, improving intercellular uptake, etc. [28]

Although EOs are characterized by high volatility and degradability, they represent promising active ingredients of biopesticides, whose properties can be improved by encapsulation within a nanostructure (nanoparticles and nanoemulsions) that will guarantee the preservation of insecticidal properties. Campolo et al. 2020 developed nano-emulsions from different commercial EOs using 15 % w/w of anise, artemisia, fennel, lavender, peppermint, rosemary, sage and four different non-ionic surfactants (5 % w/w of Tween 20, Tween 80, Span 20 or Span 80). The chemical constituents of sage, rosemary, peppermint, lavender and artemisia EOs were characterized by 50 % of oxygenated monoterpenes, while fennel and anise EOs were mainly constituted by phenylpropenes (anethol). Sonicated nano-formulations were more stable nano-emulsions than the non-sonicated ones. Emulsions produced using Tween 80 as surfactant gave the best results in terms of droplet size and polydispersity index (PDI) values which is the key parameter to consider when proposing botanical biopesticides for agricultural applications. [29]

Pereira et al. 2015 prepared the methanol extracts of *L. stoechas* ssp. *luisieri* and *L. pedunculata* were encapsulated by polymeric poly (lactic-co-glycolic) acid (PLGA) nanoparticles with a high encapsulation efficiency (>96%). This research suggested new cosmetic or dermatological formulations for the pharmaceutical industry, as anti-aging and antioxidant agents. [11]

IV. Conclusion

In this paper has been shown a new way of green synthesis of silver nanoparticles by lavender essential oil as a reductant and capping agent. It is rapid and eco-friendly method of synthesis of silver nanoparticles. The use of lavender secondary metabolites, which can reduce metal ions from their salts, is a promising and environmentally friendly way to generate nanoparticles with antibacterial and cytotoxic properties. A number of nanoparticles produced by the reduction of metal ions exhibit surface plasmon resonance properties. Nanoparticles produced in this way are characterized by increased biocompatibility due to the use of noble metals, secondary metabolites and proteins. These nanoparticles due to the high surface chemical activity allow them to be modified with targeted drugs to deliver them to target cells. In addition, lavender composites with polymers and nanoparticles with wound-healing applications were presented.

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