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Review

On the use of nanotechnology-based strategies for association of complex matrices from plant extracts

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ABSTRACT

Depending on the method of extraction, plant extracts can contain an enormous variety of active molecules, such as phenolic compounds, essential oils, alkaloids, among others. In many cases, from a pharmacological point of view, it is interesting to work with crude extract or fractions instead of a single isolated compound. This could be due to multi-targeting effect of the extract, lack of knowledge of the active compounds, synergistic effect of the extract compounds, among others. In any case, in order to achieve a final product some issues must be overcome, including poor stability, solvent toxicity, and low solubility of the bioactive compound. Recently many nanotechnology-based strategies have been proposed as an alternative to solve these problems, especially liposomes, nanoemulsions and nanoparticles. In this sense, the present work aims to review the main nanotechnological approaches used for association of different plant extracts and the main achievements from using these technologies.

Introduction

Mankind always had a close relationship with plants, using them for thousands of years as source of potential medicines to relieve both physical and spiritual pain. Throughout the centuries, the therapies have evolved and the use of complexes mixtures as plant extracts has been systematically replaced by therapies using a single isolated compound. Nevertheless, the plants are still a major source of bioactive compounds in modern medicine (Rates, 2001).

Ideally a single, pure and isolated compound should be used in the development of a formulation. Nevertheless, this is not always the most viable or successful approach. Among the reasons of using complex mixtures, emphasis could be given to the synergism with different compounds, the loss of activity after isolation, chemical instability, difficulty in the purification and the possibility to act in multiple targets at once (Gertsch, 2011; Kim et al., 2008; Rando et al., 2009). However, seldom a plant extract itself can be used as a final product. As for pharmaceutical dosage forms, some technological process must be performed in order to obtain a final medicine that can assure quality, safety and efficacy (Prista et al., 2011).

In the past years, the number of publications relating to the use of nanotechnology-based systems association plant extract has been growing. There is already a well-documented literature about the benefits of associating isolated compounds to nanotechnological drug delivery systems and extending these studies to more complexes matrices, such as plant extracts, was just a matter of time. There is a clear division among the works relating to plant extract and nanotechnology and two well defined groups can be distinguished. The first considers the use of plant extract for the formation of metallic nanoparticles (Mittal et al., 2013) and the second, the use of nanotechnology-based systems to improve biopharmaceutical and technological properties of plant extracts (Ajazuddin and Saraf, 2010; Bonifacio et al., 2014). The later will be the focus of this review.

Many nanostructures have been proposed for drug delivery, each one having their own advantages and drawbacks. This manuscript revises the use of lipid- and polymer-based nanostructures in the association of different extract, establishing a relation between the type of nanosystems and its preparation method to the different plant extracts and most abundant compounds.

Nanosystems: characterization and preparation techniques

So far, the association of plant extracts has been described for liposomes, nanoemulsions and nanoparticles (either lipid or

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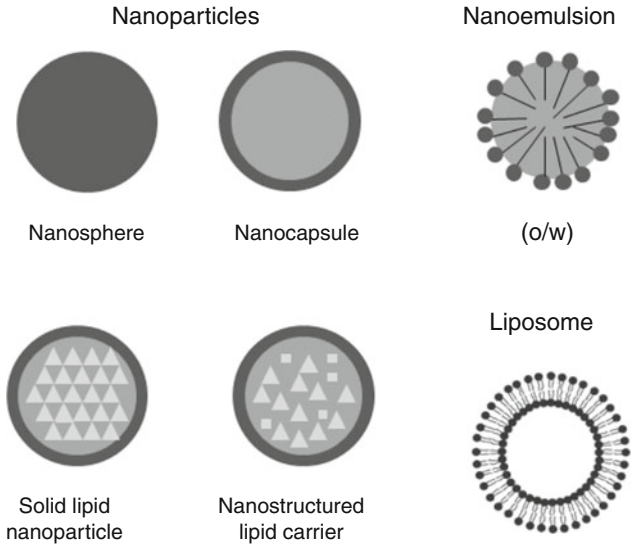

Fig. 1. Different architectures of nanosystems used to improve biopharmaceutical properties of plant extracts.

polymer-based nanoparticles) (Fig. 1). Factors such as polarity of active compound, solubility, presence of organic solvent and volatility must be taken into consideration when selecting the nanosystem and its preparation technique. For example, essential oils are rich in lipophilic compounds and extremely volatile molecules. In this sense, lipid-based systems prepared at low temperatures and without solvent evaporation after the addition of the oils, such as liposome or nanoemulsion are more likely to be successful.

Regardless the type of nanosystem or preparation technique, there are some basic characterizations of the system. The basic physicochemical parameters are average size, surface charge and association efficiency.

Determination of the size of colloidal structures can be done by several techniques such as transmission electron microscopy, laser diffraction, and small angle X-ray. Among them, dynamic light scattering offers several advantages such as wide range of measurement, simplicity and reliability (Berne and Pecora, 2003). It is a non-destructive technique that requires a small amount of sample (normally diluted). Nevertheless, for polydispersed samples the fluctuations of angle-dependency of scattered light may lead to unrealistic results. As alternative, the use of CONTIN algorithm for dynamic light scattering or nanoparticle tracking analysis is recommended, being the later also used to determine the concentration of nanoparticles (Filipe et al., 2010). Reduction of size results in the increase of surface free energy and, in this way, the preparation method must be chosen wisely (Shaw, 1992). The reduction of interfacial tension also decreases the amount of energy required for lowering the average size, as describe by the Laplace equation (Butt et al., 2003). In the nanometric world, some forces that play a major role in the macroscopic world are minimized. The Brownian motion, for example, is more significant than the gravity. In a similar way, the reduction of size can alter some physicochemical properties of bulk material (Hiemenz, 1997). Perhaps the most emblematic is the increase in the solubility. Due to the high surface area of nanostructured colloids, the solubility of lipophilic compound is increased, as dictated by the Kelvin equation (Butt et al., 2003; Cosgrove, 2010). Ultimately, this can result in loss of the active compound associated and increase in size due to the Ostwald ripening (Butt et al., 2003; Myers, 1999).

Size, however, seldom comes as a single value but rather a representative value of a distribution that can be narrow, moderate or broad. In this sense, the polydispersity index is a mathematical approach to measure the width of the particle size distribution, being the square of the division of standard deviation by mean diameter for dynamic light scattering. This property varies from 0 to 1, where lower values indicate narrower distribution (Berne and Pecora, 2003). Many factors affect the polydispesity of a system. For freshly prepared formulations, the manufacturing technique as well as type and amount of constituents plays a major role. Change of polydispersity through time is often associated to stability issues (Washington, 1992).

Table 1
Amount of extract (dry weight) usually associated to each nanotechnology-based system.

System	Amount of extract (%)
Nanoemulsion	0.5–20
Nanoparticle	0.5–2.0
Liposome	0.1–2.0

Estimation of the surface charge is important to predict colloidal stability and adequability of route of administration. According to the DLVO theory, colloidal stability is basically dependent on steric hindrance and the balance of attractive/repulsive forces (Hunter, 1989, 2001). In this way, keeping the surface charge far from neutrality is a way to favor the system stability. In the same way, the module and intensity of surface charge may result in undesired interactions with body fluids and tissues (Peeters et al., 2005; Rabinovich-Guilatt et al., 2004). For example, depending on the charge, molecules associated to nanosystem may permeate more or less in the skin layers (Gillet et al., 2011; Ogiso et al., 2001; Venuganti and Perumal, 2009). So, a given charge may be required depending on route of administration. The surface charge is easily estimated based on the quantification of the zeta potential, the difference of potential on the slipping plane of the colloid (Birdi, 2009; Butt et al., 2003). This determination of zeta potential is done by electrophoretic and electroacoustic methods, specially evaluating the electrophoretic mobility of the particles (Hunter, 1989).

Thus, zeta potential can be modulated by a judicious selection of materials that would be on the interface of nanostructure-continuous phase, such as surfactants, and compounds that would be adsorbed on the surface of the structures (e.g. polymers or compounds found in the plant extract) (Zorzi et al., 2011, 2015).

Another important factor is the determination of the association efficiency, *i.e.* the amount of active compound associated with the colloidal structure. This is a tricky task, however, because most extracts are not fully characterized and/or a marker is used to represent the total content. The best way to proceed is determining the dry residue and quantify the amount of marker (s) in respect to the dry residue, even if the extract is not used in its dried from. A judicious selection of the marker must be done and the method should be previously validated. Recently, Nemitz et al. presented a validated method to quantify isoflavone aglicone fraction from *Glycine max* ethanolic extract that was incorporated into nanoemulsion (Nemitz et al., 2015). Three relevant isoflavones, daidzein, glycitein, and genistein, could be simultaneously quantified and the method was validated to different matrices (nanoparticles, porcine skin, and esophagic mucosa). Similarly, Dias et al. have validated a head-space GC–MS method for the quantification of *Copaifera multijuga* essential oil in nanoemulsions (Dias et al., 2012). HPLC-UV validation of *Achyrocline satureioides* hydroethanolic extracts association to nanoemulsions was also recently reported. Three flavonoids with reported biological activity could be simultaneously determined (quercetin, 3-O-methylquercetin, and luteolin) (Bidone et al., 2014a).

Overall, the amount of extract that can be incorporated into nanosystem varies with its type (Table 1). Nanoemulsions are

the systems that can very often incorporate higher amounts of extract (especially when the active oil is used as the oil core of nanoemulsion), but may exhibit important instability once they are not thermodynamically stable systems. Nanoparticles can be made using different material, yielding different responses. Polymeric nanoparticles are made using polymers, lipid nanoparticle and nanostructured lipid carriers are made using lipid whereas nanocapsules are made using a composition of polymer and lipids.

Liposomes

Liposomes are vesicular structures composed of amphiphilic molecules, such as phospholipids. Their size can range from a few nanometers to several micrometers, being both preparation technique and components important to define their final physicochemical properties such surface charge, size, and stability (Gregoriadis, 2007b). Depending on the size and number of layers, they can be classically divided into four groups: small unilamellar vesicles (SUV), large unilamellar vesicles (LUV), giant unilamellar vesicles (GUV), and multilamellar vesicles (MLV) (Gregoriadis, 2007a). The polar groups of phospholips (or other component) are lined toward the exterior and inner cavity, where hydrophilic compounds can be adsorbed. On the other hand, lipophilic drugs can be solubilized within the bilayer among the hydrophobic alkyl chains (Choi and Maibach, 2005; Kaur et al., 2004). Liposomes are useful vehicles for topical drug delivery once they can provide prolonged and controlled action after application and also prevent enzymatic degradation (Fenske et al., 2008; Mainardes et al., 2005). The most common and simplest preparation technique is film hydration, where a thin lipid film is hydrated with the desired solution. Alternatively there are ethanol injection and reverse phase evaporation, the later recognized as having superior entrapment of hydrophilic substances in the vesicle cavity. Regardless the technique of choice, a second step of sizing down the liposomes and decrease the size distribution may be required, such as membrane extrusion, ultrasound, high pressure homogenization, freeze and thaw, among others.

Nanoemulsions

Nanoemulsions are dispersions of two immiscible liquids stabilized by a surfactant or surfactant system. Their physical stability can be substantially improved by selecting suitable emulsifiers that are capable of surrounding the dispersed droplets in such way as to reduce interfacial tension or increase droplet-droplet repulsion (Solans et al., 2005; Tadros et al., 2004). Depending on the concentrations of oil/water/emulsifier and the efficiency of the emulsification equipment used to obtain a reduced droplet size, the final emulsion may have different characteristics. In this context, the most studied nanoemulsion systems for pharmacological applications are oil-in-water (o/w) nanoemulsion (Tamilvanan, 2004). Spontaneous emulsification and high pressure homogenization are the most used techniques when associating plant extracts to nanoemulsion, but other may also be found such as phase inversion temperature.

Nanoparticles

Nanoparticles are structures with size between 1 nm and 1 μm, mostly of polymeric or lipidic nature and, depending on the end of use, may or may not include an active molecule (Rao and Geckeler, 2011; Sahoo et al., 2007). In this manuscript the term *polymeric nanoparticle* will be used to describe a matricial structure where the active molecule can be associated into the matrix and/or adsorbed on the surface. On the other hand, the term *nanocapsules* will represent structures with an oil core (that may or not be the active compound) surrounded by a polymeric shell. In this way, the active compound can be entrapped in the polymer/oil core or be adsorbed on the surface of the nanocapsule. There are many techniques that can be used to prepare polymeric nanoparticles and nanocapsules depending on the nature of the polymer. Ionic gelation and nanoprecipitation are suitable for hydrophilic polymers whereas for hydrophobic polymers the technique of choice is solvent displacement followed by nanoprecipitation of the pre-formed polymer using water miscible organic solvents (*e.g.* ethanol and acetone).

Lipid-based nanoparticles can be divided in two groups: solid lipid nanoparticles and nanostructured lipid carrier. Solid lipid nanoparticles are nanostructures composed of solid lipids at room and body temperature (Souto et al., 2007; Souto and Mueller, 2008). Depending on the fabrication technique employed, the use of a surfactant or co-surfactant can be required in order to stabilize the system (Muchow et al., 2008). Even though a very attractive system, solid lipid nanoparticles may exhibit several issues that will limit their application. Problems such as low loading capacity and the potential expulsion of the drug during storage are known drawbacks. Even lipophilic molecules can be expelled from the nanoparticle structure due to rearrangement of lipids (Pardeike et al., 2009). This can be in part overcome by the incorporation of liquid lipids (or mixture of different lipids) to create a matrix that is as disorganized as possible. These systems are so-called *nanostructured lipid carriers* (Fang et al., 2013).

Plant extracts

For the sake of simplicity, in this review "plant extract" will be considered as a complex matrix obtained from a plant that may or may not contain residual solvent. There is a vast set of techniques suitable for plant extraction involving different parts of the plant and main targeted compounds. Depending on the technique and protocol, the extraction may favor a given group of secondary metabolites over another. Fractioning of the extract may occur getting rid of undesired compounds. Nevertheless, this review will only take into consideration extracts associated with nanosystems that are still a complex matrix (*i.e.* two or more compounds). In this way, depending on the lipophilicity and the nanostructure of choice, the extract compounds can be basically found in three different places: (i) solubilized in the external aqueous phase; (ii) adsorbed onto the surface of the carrier, and (iii) entrapped in to the carrier.

Aqueous extracts

Aqueous extracts are those in which only water is used as extraction solvent. There are several reasons why to choose an aqueous extraction including price, easiness in handling the process, necessity of avoid a given class of substances, safety and environmental concerns. The majority of the compound extracted, such as aminoacids, sugars, mucilages, heterosides, saponins, and alkaloids in the salt form, will have a hydrophilic nature (Simões et al., 2001). Box 1 shows some of the relevant applications of nanosystem for aqueous extracts.

The association of aqueous extracts to nanostructures can be done for two main reasons: improve the stability and improve the solubility of the extract. Once most of the nanosystems are designed to associate lipophilic drugs, there are a few options left for hydrophilic compounds. Liposomes can technically accommodate hydrophilic compounds inside the vesicle, but the loading depends much of the preparation technique. Anthocyanin-rich acid extract of *Hibiscus sabdariffa* was associated to soy lecithin liposomes with high association efficiency (around 70%) (Gibis et al., 2014). This probably happened due to the interactions between the flavylium

Box 1: Representative examples of aqueous extracts associated to different nanosystems.

Plant	Part used	Active compound	Activity	System	Reference
White tea	Leaves	Phenolic compounds (catechins)	Antioxidant	Nanoparticle (PECL, alginate, Pluronic® F-127)	Sanna et al. (2015)
Phoenix dactylifera	Pit	Phenolic compounds	–	Nanocapsule (whey protein)	Bagheri et al. (2013)
Centella asiatica	Dry leaves	Asiaticoside Madecassoside Asiatic acid Madecassic acid	Antioxidant	Nanoparticle (gelatin)	Kwon et al. (2012)
Camellia sinensis	Leaves	Phenolic compounds (epigallocatechin, epigallocatechin-3-gallate, epicatechin, epicatechin-3-gallate)	Antioxidant	Nanoemulsion (lecithin, palm or sunflower oil, Tween® 20 or 80)	Gadkari and Balaraman (2015)
Hibiscus sabdariffa	Flowers	Phenolic compounds (anthocyanins)	Antioxidant	Liposome (lecithin)	Gibis et al. (2014)

Pluronic® F-68, polyoxyethylene-polyoxypropylene block copolymer; PECL, poly(epsilon-caprolactone); Pluronic® F-127, polyoxyethylene-polyoxypropylene block copolymer; Tween® 20, polyoxyethylene sorbitan monolaurate; Tween® 80, polyoxyethylene sorbitan monooleate.

cation of anthocyanins with the negative phophospholipids present in the lecithin. Lecithin is a complex mixture of lipids and due to the presence of acid phospholipids (such as phosphatidylserine and phosphatidylinositol) can interact with positively charged compounds. In this way, the authors proceed with a chitosan coating for these liposomes. Chitosan is a heteropolymer derived from chitin deacetylation, and consists of several units of *N*-acetylglucosamine and *N*-glucosamine (Aranaz et al., 2009; Dash et al., 2011; Garcia-Fuentes and Alonso, 2012). Due to the presence of several primary amine groups it can also interact with lecithin-based liposomes (Agnihotri et al., 2004; Menchicchi et al., 2014). A second cover with pectin could also be added in order to have a final negative zeta potential. Overall, the ratio between lecithin and extract proved to be critical to avoid aggregation. To improve stability a second polymeric layer, in this case of pectin, can be added to avoid aggregation and increase in size.

Gelatin nanoparticles associating *Centella asiatica* aqueous extract was proposed as skin-protective agent, due to the presence of asiaticoside, madecassoside, asiatic acid and madecassic acid (Kwon et al., 2012). Nanoparticles had superior protective effect on UVA-induced matrix metalloproteinase (MMP-1) and tyrosinase-inhibitory activity with respect to free extract. Once the authors did not use any crosslinker or denaturation, it is reasonable to think that gelatin actually acts stabilizing the extract. The spontaneous formation of nanoparticles from aqueous extracts, especially upon cooling, has already been reported for black tea extract, for example (Gröning and Breitkreutz, 1994; Gröning et al., 2002). A far less usual material for pharmaceutical application, whey protein nanocapsules could associate date palm aqueous extract (*Phoenix dactylifera*) with an encapsulation efficiency of 70–78% (Bagheri et al., 2013). The nanocapsules were prepared by coacervation (using NaCl and ethanol) and stability by heat, yielding a formation around 90% with size ranging from 100 to 160 nm. The encapsulation of this phenolic compounds-rich extract may found in food industry an interesting application.

Green tea (*Camellia sinensis*) aqueous extract is rich in catechins, especially epigallocatechin-3-gallate, epigallocatechin, epicatechin-3-gallate, and epicatechin. Generally recognized by its antioxidant activity, the green tea extract was associated to alginate and poly(epsilon-caprolactone) (PECL) nanoparticles. As expected, low entrapment of the active compounds, expressed by terms of epigallocatechin-3-gallate and epicatechin-3-gallate, was found (around 30%). Nevertheless, the association of catechins to the polymeric matrix could modulate their release where 25% was found after 2 h in comparison to nearly 100% release from free extract (Sanna et al., 2015). Control of kinetic release of antioxidant is generally recognized as the main reason for the superior activity of nanoencapsulated antioxidants (Bidone et al., 2014b; Coradini et al., 2014). Nevertheless, not always an increase in the antioxidant activity can be observed. Gadkari and Balaraman (2015) used nanoemulsion to associate aqueous extracts of green tea and no difference in the antioxidant activity (ferric reducing antioxidant potential) was observed between the free extract and the formulations. Even though the authors did not provide the values of association efficiency of the active compounds (catechins), this result is not far from expected. Due to the high lipophilicity of the nanoemulsion oil core, a low association of catechins is expected, especially because the extract used was aqueous.

Alcoholic extracts

Ethanol is one of the most used organic solvents for extract preparation. The ability to solubilize a wide variety of substances, low price, relatively low toxicity and the fact of being an environment-friendly solvent are among the most attractive properties that make ethanolic extracts popular. Depending on the preparation method, there is no need of previous removal of ethanol. Taking solvent displacement technique as an example, the ethanolic extract can be added to the organic phase and the solvent removed at the end of the nanoparticle/nanoemulsion formation. Box 2 exemplifies some relevant formulations using alcoholic extracts.

As previously stated, the biological activity of antioxidant-rich extracts can be improved upon association to nanostructures. Phenolic compounds of *Orthosiphon stamineus* (Aisha et al., 2014), *Fraxinus angustifolia* (Moulaoui et al., 2015), *Curcuma longa* (Kaur and Saraf, 2011), *Cuscuta chinensis* (Yen et al., 2008) and *Vitis vinifera* (Spigno et al., 2013) were associated to liposomes, nanoemulsions and nanoparticles.

Liposomal formulation significantly improved the solubility of *Orthosiphon stamineus* ethanolic extracts (Aisha et al., 2014). Entrapment efficiency of the markers rosmarinic acid, 3-hydroxy-5,6,7,4-tetramethoxyflavone, sinensetin and eupatorin varies from 30 to 55%. Release studies from liposomes showed improvement in DPPH scavenging effect (around 50%) in comparison to free extract. Ethosomes (liposomes with permeation enhancers, such as ethanol) favor the wound healing properties of *Fraxinus angustifolia* (Moulaoui et al., 2015). Quercetin, catechin, rutin and tannic acid are the main components of its ethanolic extract and higher level of cell uptake in comparison to free extract. For this reason the formulation was the only able to protect in vitro human keratinocytes from H_2O_2 damages.

Box 2: Representative examples of ethanolic extracts associated to different nanosystems.

Plant	Part used	Active compound	Activity	System	Reference
Curcuma longa	Dry rhizomes	Phenolic compounds (curcumin)	Antioxidant	Liposome (PC, cholesterol) Ethosome (PC) Tranferosome (lecithin:sodium deoxycholate)	Kaur and Saraf (2011)
Fraxinus angustifolia	Leaves and barks	Phenolic compounds (quercetin, catechin, rutin and tannic acid)	Antioxidant	Ethosome (lecithin)	Moulaoui et al. (2015)
Orthosiphon stamineus	Leaves	Phenolic compounds (rosmarinic acid, eupatorin)	Antioxidant	Liposome (lecithin)	Aisha et al. (2014)
Phytolacca decandra	Roots	Triterpenes	Antineoplasic	Nanoparticle (PLGA)	Das et al. (2012)
Barbera red-grape (*Vitis vinifera*)	Fruits	Phenolic compounds	Antioxidant	Nanoemulsion (lecithin, peanut or sunflower oil, maltodextrins)	Spigno et al. (2013)
Cuscuta chinensis	Seeds	Phenolic compounds (quercetin, kaempferol)	Hepatotoxicity	Nanoparticle (Pluronic® F-68)	Yen et al. (2008)

PC, phosphatydil choline; PLGA, poly(lactic-co-glycolic acid).

Liposomes, ethosomes and transferosomes (a special type of liposome with a membrane softening agent) were prepared for loading *Curcuma longa* (Kaur and Saraf, 2011). The size varied with the extract loading (0.5–2.0%) and the extract association efficiency and hydration level of skin was as followed: transferosome > ethosomes > liposomes.

Stabilization of nanoparticles formed form *Cuscuta chinensis* ethanolic extract was performed in order to improve the antioxidant activity (Yen et al., 2008). This extract is rich in quercetin, kaempferol and their glycosides and was proposed for acetaminophen hepatotoxicity management. The authors concluded that after oral administration in Wistar rats, the nanoparticles required a dose 5-times lower than the free extract to achieve the same effect.

Phytolacca decandra extract-loaded polymeric nanoparticles of poly(lactic-co-glycolic acid (PLGA) were studied as an alternative in the intervention against induced lung adenocarcinoma in mice and on A549 cells (Das et al., 2012). The major component was identified as a derivative of betulinic acid and had entrapment efficiency around 80%. Nanoencapsulation showed preventive effects concerning toxicity biomarkers (reactive oxygen species generation, CYP1A1, caspase 3 among others) and DNA fragmentation in higher extended in comparison to free extract.

Hydroalcoholic extracts

Hydroethanolic mixtures are able to extract more lipophilic compounds than aqueous one due to the lower dielectric constant of ethanol. In this way, higher amounts of extract, expressed in terms of dry weight, can be incorporated with better entrapment. In Box 3, many applications of hydroalcoholic extracts associated to liposomes, nanoemulsions and nanoparticles can be observed.

As mentioned before, nanoencapsulation can bring several advantages for the administration of a plant extract. Among them

Box 3: List of some relevant hydroalcoholic extracts incorporated to different nanosystems.

Plant	Solvent	Part used	Active compound	Activity	Systems	Reference
Panax quinquefolius	0–90% ethanol	Dry roots	Saponin (Ginsenoside)	Antioxidant	Liposome (DSPE-PEG2000)	Tsai et al. (2012)
Polygonum aviculare	50% ethanol	–	Phenolic compounds (quercetin and myricetin)	Antiaging (antioxidant)	Liposome (DOPC and cholesterol)	Kwon et al. (2015)
Phyllanthus urinaria	30% ethanol	–	Phenolic compounds	Skin antiaging	Nanoemulsion (Tween® 80, Span® 80)	Mahdi et al. (2011)
Vellozia squamata	70% ethanol	Leaves	Phenolic compounds	Cosmetic	Nanoemulsion (Babaçu oil, Span® 80 PEG-40)	Quintão et al. (2013)
Glycine max	60% ethanol	Beans	Isoflavone aglycones	Phytoestrogenic	Nanoemulsion (lecithin, Tween® 80, MCT)	Nemitz et al. (2015)
Achyrocline satureioides	80% ethanol	Flowers	Phenolic compound (3-O-methylquercetin)	Antiviral	Nanoemulsion (lecithin, MCT)	Bidone et al. (2014a,b)
			Phenolic compound (quercetin)	Topical antioxidant	Nanoemulsion (lecithin, ODD)	Zorzi (2007)
					Nanoparticle (PECL, Span® 80, Pluronic® F-68)	Carvalho et al. (2008)
Ziziphus jujuba	40–100% ethanol	Fruit	Phenolic compound (unspecific)	Antioxidant	Nanoparticle (chitosan)	Han et al. (2015)
Salvia officinalis	60% ethanol	Leaf	Phenolic compounds	Antioxidant (mercury poisoning)	Nanoparticle (PLGA, Pluronic® F-68)	Kahil and Salvia (2013)
Picrorhiza kurrooa	80% methanol	Root and rhyzome	Iridoids (Picroside I and II)	Hepatoprotection	Nanoparticle (PLA, Pluronic® F-68)	Jia et al. (2015)

DOPC, 1,2-dioleoyl-*sn*-glycero-3-phosphocholine; Pluronic® F-68, polyoxyethylene-polyoxypropylene block copolymer; MCT, medium chain triglycerides; ODD, octyl dodecanol; PECL, poly(epsilon-caprolactone); PEG, polyethylene glycol; PLA, poly(lactic acid); PLGA, poly(lactic-co-glycolic acid); Span® 80, sorbitan monooleate; Tween® 80, polyoxyethylene sorbitan monooleate.

there is the possibility to provide drug sustained release in a way to decrease side effect and decrease dosage. *Ziziphus jujuba* fruits have a low shelf-life due to the fast oxidation of its phenolic compounds (Han et al., 2015). In this way, the encapsulation of previously optimized hydroethanolic extract (40–100% ethanol) in chitosan nanoparticles increased the stability of the phenolic compounds in respect to free extract (DPPH antioxidant activity). Regardless the plant part, seed or pulp, high entrapment efficiency was observed (80–90%).

PLGA nanoparticles were developed to associate methanolic extract of *Picrorhiza kurrooa*, a Himalayan-native plant (Jia et al., 2015). This extract is rich in the iridoids picroside I and II, hepatoprotective compounds with poor aqueous solubility and intestinal absorption. Extract-loaded nanoparticles had up to 67% association efficiency of the active compound and were able to sustain the release up to 200 h. The intermediate association efficiency found may be attributed to the use of methanol, which has a higher dielectric constant than ethanol, with consequent high hydrophilicity of the extract. This was ultimately resulting in low affinity to the hydrophobic PLGA (Rowe et al., 2003). Depending on the amount of ethanol, hydroalcoholic extracts of *Salvia officinalis* may be rich in phenolic compounds such as rosmarinic, caffeic and salvianolic acids. In this way, 60% ethanol extract was associated to PLGA nanoparticles in order to increase the antioxidant activity against mercury-induced neurotoxicity (Kahil and Salvia, 2013). The nanoparticles were able to reduce the formation of oxygen reactive species and the alterations in cortex of albino rats after neurotoxicity induced by methylmercury. This effect was superior to non-treated control and nonencapsulated extract. Nanoparticles were also able to improve pharmacokinetic parameters, such as half-life, which was increased up to 10-fold after intravenous administration. Liposomes were used as carriers for commercial ginseng extract (*Panax quinquefolius*) in order to enhance the intracellular antioxidant activity (Tsai et al., 2012). These liposomes were able to increase membrane potential after H_2O_2 damage and decrease apoptosis. The use for food product is also interesting once grape marc extract, for example, was successfully associated to nanoemulsion and helped to reduce the oxidation of hazelnut paste (Spigno et al., 2013).

Oil-in-water nanoemulsions are also a good alternative for hydroalcoholic extracts. Bidone et al. and Zorzi et al. have proposed water in oil nanoemulsion to associate ethanolic extract from *Achyrocline satureioides* (Bidone et al., 2014b; Zorzi, 2007). The first study described the development of topical nanoemulsion for the delivery of the antioxidant quercetin whereas the second addressed the topical delivery of 3-*O*-methylquercetin as topical antiviral against HSV-1 (Bidone et al., 2015). Association with the nanoemulsion improved the antioxidant effect of the free extract, probably due to the controlled release of flavonoids (Bidone et al., 2014b; Zorzi, 2007). However, the higher activity of the extract nanoemulsion in comparison to quercetin nanoemulsion was attributed to the higher skin retention of the flavonoid using the formulation with extract (Zorzi, 2007). The use of nanoemulsion substantially increased 3-*O*-methylquercetin permeation (almost 5-fold). Previous technological treatment of the extract, such as freeze drying and spray-drying influenced in the total amount of flavonoids and their release from nanoemulsions (Bidone et al., 2014b). A few other reports concerning the use of nanoemulsion to encapsulate *Vellozia squamata* and *Phyllanthus urinaria* were proof-of-concept and did not bring results regarding the biological activity of the formulations (Mahdi et al., 2011; Quintão et al., 2013).

Essential oils

Essential oils are a mix of several lipophilic compounds characterized by high vapor pressure. The composition varies but the majority of essential oils are composed of terpenoids and phenylpropanoids. Especial attention must be given to the preparation technique when associating essential oils to nanostructures. As previously described, many preparation techniques require the use of high temperature or organic solvents (that must be eliminated). Due to the high vapor pressure of the essential oils they can also be removed from the formulation in this step. To circumvent this problem a few solutions can be made such as adding a miscible fixed oil or using nanosystems/techniques that involve low temperature and no evaporation of organic solvent after addition of the essential oils.

Many essential oils present antimicrobial/antiparasitic/insecticidal effect. In this sense, many formulations will have this objective. As can be observed in Box 4, liposomes, nanoparticles, nanoemulsions and nanocapsules have been proposed to incorporate essential, each one with its own advantages and drawbacks.

Nanoemulsions can associate the highest amount of oil (up to 20%). *Melaleuca alternifolia* essential oil showed lower minimum inhibitory concentration against *Saccharomyces cerevisiae*, *Escherichia coli*, and *Lactobacillus delbrueckii*, after incorporation to nanoemulsion (5%). In combination to fruit juice, the product shelf life could be extended due to the low proliferation of microorganism and maintenance of organoleptic properties (Donsì et al., 2011). Later this systems were optimized but, instead of associating the whole essential oil, isolated volatile compounds were used (carvacrol, limonene and cinnamaldehyde), where the surfactants had significant effect over the physicochemical properties of the nanoemulsion and its antimicrobial activity (Donsì et al., 2012). Similarly, association of basil oil (*Ocimum basilicum* – 88% estragole) in to nanoemulsion had a dose-dependent effect over *E. coli* viability, where nanoemulsion (0.6% of oil) could kill 100% of bacteria after 45 min (Ghosh et al., 2013).

Another important application of essential oils, especially those incorporated to nanoemulsion, is the effect over insects. Nanoemulsions of eucalypt oil (6% of eucalypt oil with 17.73% eucalyptol) were tested for larvicidal activity against *Culex quinquefasciatus*. Eucalypt oil had its larvicidal activity against mosquito significantly improved when used as nanoemulsion. The oil was stabilized with polysorbate 80 and exhibit 98% mortality after 4 h incubation with 250 ppm of oil. For the same concentration, classic emulsions only achieved this value after 24 h of exposure. Even a concentration as low as 100 ppm could result in expressive mortality of larves after 6 h incubation (around 85%, versus 20% for emulsion at same concentration and incubation time) (Sugumar et al., 2014). Similar formulations were tested against the red flour beetle (*Tribolium castaneum*) however with higher content of eucalyptol in the extract (66%) (Pant et al., 2014). This formulation was able to kill 85% of insect using 900 ppm. Nevertheless, this effect decreased 25% after two months. In order to overcome this drawback, the authors associated to the formulation the aqueous extract of *Jatropha curcas* and *Pongamia glabra*, which main compounds are phorbol esters and karinjin, respectively. This not only increased the insecticidal effect (95% vs.85%) but the effect was stable through time. In this way, after the use of the aqueous extract the IC_{50} of the mixture of extracts dropped from 5.4872 mg/l to 0.1646 mg/l. Sakulku et al. (2009) also studies the effect of nanoencapsulated essential oil over insects. In this case, the mosquito repellent activity of citronella oil (*Cymbopogon nardus*) was evaluated. This terpene rich oil (41% D-limonene, 40% citronellal) could have a controlled release of 40–80% after 48 h when in nanoemulsions. The repellent effect was proportional to the viscosity of the formulation, showing a lower kinetic release parameter.

As for microorganisms and insects, the antiparasitic effect of essential oil-loaded nanoemulsion has been studied. Trypanocidal activity of andiroba (*Carapa guianensis*) and aroeira (*Schinus molle*)

Box 4: Different essential oil associated to nanosystems. HD, hydrodistillation; SC, supercritical.

Plant	Obtention	Part used	Active compound	Activity	System	Reference
Origanum vulgare	–[a]	Leaves	–	–	Nanoparticle (chitosan)	Hosseini et al. (2013)
Lippia sidoides	–[a]	–	Terpenes (thymol)	–	Nanoparticle (chitosan, cashew gum)	Abreu et al. (2012)
					Nanoparticle (alginate, cashew gum)	de Oliveira et al. (2014)
Eucalyptus camaldulensis	HD	Leaves	Terpenes (eucalyptol)	Microbicide	Liposome[a] (phosphatidyl-choline, cholesterol)	Moghimipour et al. (2012)
Artemisia arborescens	HD	Aerial parts	Terpenes (camphor, β-thujone and chamazulene)	Antiviral	Liposome[a] (phosphatidyl-choline, cholesterol, stearylamine)	Sinico et al. (2005)
Atractylodes macrocephala	SC CO_2	Rhizomes	Sesquiterpenes (Atractylone)	–	Liposome[a] (lecithin and cholesterol)	Wen et al. (2010)
Schinus molle	HD	Woods	Essential oil (not specified)	Trypanocidal activity	Nanoemulsion (Tween® 80, Span® 80)	Baldissera et al. (2013)
Carapa guianensis	–[a]	Seeds	Essential oil (not specified)	Trypanocidal activity	Nanoemulsion (Tween® 80, Span® 80)	Baldissera et al. (2013)
Eucalyptus globulus	HD[a]	–	Eucalyptol	Insecticidal	Nanoemulsion (Tween® 80)	Sugumar et al. (2014)
Ocimum basilicum	–[a]	Leaves	Phenylpropene (estragole)	Microbicide	Nanoemulsion (Tween® 80)	Ghosh et al. (2013)
Eucalyptus globulus + *Jatropha curcas* + *Pongamia glabra*	HD[a] + Aqueous + Aqueous	–	Eucalyptol Phorbol esters Karingin	Insecticidal	Nanoemulsion (Tween® 80)	Pant et al. (2014)
Stenachaenium megapotamicum	HD	Flowers and leaves	Terpenes (fokienol, thymol and others)	Microbicide	Nanoemulsion (Tween® 80)	Danielli et al. (2013)
Copaifera multijuga	Direct	Trunks	Diterpenic acids and sequiterpenes (β-caryophyllene)	Topical anti-inflammatory	Nanoemulsion (Tween® 80, Span® 80)	Dias et al. (2012) Dias et al. (2014)
Cymbopogon nardus	–[a]	Leaves	Terpenes (limone and citonellal)	Repelent (insect)	Nanoemulsion (glycerol, cetearyl alcohol/cocoyl glucoside)	Sakulku et al. (2009)
Melaleuca alternifolia	–[a]	Leaves	Terpenes (unspecified)	Microbicide	Nanoemulsion (lecithin, palm or sunflower oil)	Donsì et al. (2011)
	HD[a]	Leaves	Terpenes (terpinen-4-ol, and terpinenes)	–	Nanoemulsion Nanocapsule (lecithin, Tween® 80, Span®® 80, PECL)	Flores et al. (2011)
Jasmine	–[a]	–[a]	Terpenes (linalool)	–	Nanocapsule (gelatin, arabic gum, Tween® 80, Span® 80)	Lv et al. (2014)

[a]Commercial product. Span® 80, sorbitan monooleate; Tween® 80, polyoxyethylene sorbitan monooleate.

essential oils associated to nanoemulsions was tested using pure oil (Baldissera et al., 2013). A dose-dependent reduction in the number of parasites was observed after 1 h, regardless the association to the nanoemulsion. Nevertheless, the mortality was more pronounced for nanoemulsion that could even kill 100% of trypanosomes after 1 h whereas free oil required up to 6 h for the same effect.

Even though high pressure homogenization and ultrasonication are the most diffused methods for essential oil nanoemulsion preparation, there are a few reports concerning spontaneous emulsification and phase inversion temperature. Dias et al. (2012, 2014) compared high pressure homogenization and spontaneous emulsification in order to prepare cobaiba oil nanoemulsion. Copaiba oil (*Copaifera multijuga*) is an oleoresin that can be directed collect from the tree trunk. The oil is a complex mixture of terpenes, being β-caryophyllene the most abundant one, and chemical marker for the oil. Spontaneous emulsification requires a step of solvent removal by reduced pressure that can eventually remove some components of the volatile essential oil. Because of its partial resinous nature, the loss during preparation was minimal, being the final oil content higher than 90%. Nevertheless, this is not always the case, as observed for *Stenachaenium megapotamicum* essential oil nanoemulsion (Danielli et al., 2013). This fokienol and thymol-rich oil was associated to nanoemulsions intended to be used against dermatophytes. However, a considerable loss of 22% of the oil was observed probably related with the choice of preparation method.

There are some reports about the use of liposomes to associate essential oils. Nevertheless, the amount of essential oil associated (0.15–1.25%) is substantially lower than for nanoemulsions (up to 20%). Low amount of essential oil usually results in high association efficiency, as for *Eucalyptus camaldulensis*, where 0.25% of oil resulted in 95% association into lecithin/cholesterol liposomes (Moghimipour et al., 2012). The antiviral activity of *Artemisia arborescens* essential oil liposome was investigated in Vero cells infected with HSV-1 (Sinico et al., 2005). For that either multilamellar or small unilamellar liposomes were prepared using phospholipids with different transition temperatures. The higher effect of multilamellar liposomes may be attributed to the higher association efficiency (mainly of β-thujone, camphor and chamazulene) and higher stability in comparison to small unilamellar vesicles. This effect was also observed for hydrogenated phosphatidylcholine (higher transition temperature) in comparison to phosphatidylcholine (lower transition temperature) due to lower leakage of essential oil. Nevertheless, a tendency toward instability could be seen regardless the composition and liposome structuration. Wen et al. studied the factors that influenced the association efficiency and drug loading of *Atractylodes macrocephala* essential

Box 5: Different organic or unspecified extracts associated to nanosystems.

Plant	Solvent	Part used	Active compound	Activity	System	Reference
Diospyros kaki	Ethylacetate	Leaves	Phenolic compound (quercetin)	Cardiac and vascular conditions	Nanoemulsion (Cremophor® EL, Labrafil® M1944 CS and Transcutol® P)	Li et al. (2011)
Manilkara subsericea	Hexane fraction of ethanolic	Fruits	α-and β-amyrin acetate	Insecticidal	Nanoemulsion (Tween®80, Span®80, ODM)	Fernandes et al. (2014)
Pterocaulon balansae	*n*-Hexane	Leaves	Coumarins (esculin)	Antifungal	Nanoemulsion (lecithin, MCT)	Vianna et al. (2011)
Curcuma comosa	*n*-Hexane	Rhizomes	Diarylheptanoid	Phytoestrogenic	Nanoemulsion (Tween®® 60, PEG 1000, olive oil)	Su et al. (2013)
Passiflora serratodigitata	Fraction of hydroethanolic (60% ethanol)	Leaves	–	Antiulcerogenic	Nanocapsule (PECL, MCT, Span® 80)	Strasser et al. (2014)
Ginkgo biloba	–[a]	Leaves	Terpenes (ginkgolides A, B, C and bilobalide)	–	Nanoparticle (mPEG–PLGA–mPEG)	Han et al. (2012)
Green tea	–[a]	Leaves	Catechins	Microbicide	Nanostructured lipid carrier (cetyl palmitate, glyceryl stearate, grape seed/St. John's wort/sea buckthorn oil.	Manea et al. (2014)
				Hypocholesterolemic	Nanoemulsion (cholesterol, cetylphosphate MCT)	Kim et al. (2012)

[a] Commercial extract.

Cremophor® EL; polyethoxylated castor oil; Labrafil® M1944 CS, oleoyl macrogol-6 glyceride; MCT, medium chain triglycerides; ODM, octyldodecyl myristate; PECL, poly(epsilon-caprolactone); PEG, polyethylene glycol; PLGA, poly(lactic-co-glycolic acid); Span® 80, sorbitan monooleate; Transcutol® P, diethylene glycol monoethyl ether; Tween®, polyoxyethylene sorbitan monostearate (60) or monooleate (80).

oil into liposomes prepared by rapid expansion of supercritical solutions (Wen et al., 2010). The key factors found were pressure, temperature and amount of co-solvent (ethanol). Nevertheless, even with the process optimization, a significant loss of essential oil was observed over time.

Due to the high volatility, the amount of essential oil can eventually be reduced over time. For that, nanocapsules can be an alternative to avoid oil loss though time. After incubation at 37 °C the oil content of *Melaleuca alternifolia* reduced 60% in nanoemulsion and 40% in nanocapsules (Flores et al., 2011). In a similar way, Lv et al. have proposed gelatin/arabic gum nanocapsules as a heat-resistant system for essential oil association (jasmine essential oil) (Lv et al., 2014). In the case of indol; however, a reduction of 95% in the nanocapsules could be observed after 5 h at high temperature (80 °C).

There are few reports concerning the association of essential oil to polymeric nanoparticles. In this sense, chitosan nanoparticles were used, as proposed by Hosseini et al. (2013) to associate *Origanum vulgare* essential oil. However, this approach resulted in low association efficiency (5–25%) and loading content around 1.3–2%, as determined by UV–VIS spectroscopy. In a similar way, the association efficiency of chitosan/cashew gum (Abreu et al., 2012) and alginate/cashew gum nanoparticles (de Oliveira et al., 2014) was not very high, being around 40–70% and 21–48%, respectively.

Other extracts

Extracts prepared with solvents with lower dielectric constant are also common and present a wide variety of secondary metabolites. Ethyl ether and dichloromethane are used for extraction of lipophilic substances such as lipid, essential oils, waxes and alkaloid in free base form. Also, mixture of different solvent may allow an optimal extraction of the active compounds. Currently our research group studies the application of the coumarin rich *n*-hexane extract of *Pterocaulon balansae*, especially for antifungal and antiparasitic applications. This lipophilic extract is associated with nanoemulsion in order to increase the solubility and activity of such coumarins (Panatieri, 2015; Vianna et al., 2011). Box 5 shows different solvent, mainly with higher lipophilicity, and their incorporation into nanosystems.

Similar to essential oils, hexane extracts show a high lipophilicity making them suitable to be associated into systems with high oil content. Nanoemulsions allow high association efficiency of the active compound. *Curcuma comosa n*-hexane extract is rich in diarylheptanoid phytoestrogens. The use of nanoemulsions showed important association efficiency of the active compound (around 75%) and an increase in 10-fold in the intestinal absorption in Wistar rats (Su et al., 2013).

Ethyl acetate fractions from *Passiflora serratodigitata* hydroethanolic extract (60% ethanol), as well as the hydroalcoholic extract itself, were associated to PECL nanocapsules in order to improve the antiulcerative effect (Strasser et al., 2014). Even though promising, the lack of proper experimental control and statistical analysis makes difficult to conclude the real effects of encapsulation. Further fractioning showed that the ethylacetate fraction was the most active. Unfortunately, the authors did not make a characterization of the extract and its fraction, only expressing the total flavonoid content using quercetin as standard. Once the flavonoid content of original hydroethanolic extract and ethylacetate were the same, the explanation for this activity is yet to be better understood.

Hexane-soluble fraction from ethanolic crude extract from *Manilkara subsericea* fruits was formulated in nanoemulsion aimed against cotton pest (*Dysdercus peruvianus*) (Fernandes et al., 2014). This apolar fraction was rich in triterpenes, mainly α-and β-amyrin acetate. The formulation was able to induce mortality in 25% of *D. peruvianus*. Even though the insecticidal activity was low, no effect against acetylcholinesterase or mortality in mice induced highlighting to a possible low toxicity. No comparison with the free extract was made.

Some studies often use commercially available extract and not always the extraction method and all the technology behind its development is know. Co-encapsulation of four components in ginkgo terpenes was performed using injectable PELGE (mPEG–PLGA–mPEG) nanoparticles (Han et al., 2012). Ginkgolides A, B, C and bilobalide from a marketed extract had association

efficiency around 80%. In this way, a controlled release of the four components was observed both in vivo and in vitro, showing a cumulative release of 60% after 24 h. Concerning commercial green tea extract, both hypocholesterolemic (Kim et al., 2012) and antibactericidal (Manea et al., 2014) were tested using nanoemulsion and nanostructured lipid carriers, respectively (Kim et al., 2012). For its hypercholesteremic activity, only LDL receptor expression was higher for the nanosystems in comparison to free extract. Antimicrobial activity suggests that green tea extract nanoemulsion could be utilized not only as antioxidant but also as a valuable natural source of antimicrobial agent.

Final considerations

The use of nanonotechnology-based systems has been growing in the past years and is relatively new, as confirmed by the recent literature. Based on the results presented here, there are clearly two major advantages of using nanocarriers to associate complex extracts, based on the biological responses: (i) increasing the antioxidant effect due to the controlled kinetic release of antioxidant, and (ii) increase the activity against microorganism, parasites and insects. The use of systems such as liposomes, nanoparticles and nanoemulsions can bring other benefits and still has a bright path ahead. Nevertheless, this path is long and depends on the advances of knowledge on the phytochemical composition and biological activity of the medicinal plant extracts, as well as on the physicochemical characterization of the nanostructured delivery systems containing these complexes matrices.

Author contributions

GZ searched the literature in different databases, collected the main data, and drafted the first version of the manuscript. All authors suggested the outline of the article, and participated in the selection of the articles used in this review. All authors participated in the writing, editing, and revising the final version of the article.

Conflicts of interest

The authors declare no conflicts of interest.

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