NPC Natural Product Communications

2018
Vol. 13
No. 9
1157 - 1160

Plants Extracts Loaded in Nanocarriers: an Emergent Formulating Approach

Anna Rita Bilia*, Vieri Piazzini, Martina Asprea, Laura Risaliti, Giulia Vanti and Maria Camilla Bergonzi

Department of Chemistry "Ugo Schiff", University of Florence, Via Ugo Schiff 6, 50019, Sesto Fiorentino, Florence, Italy

ar.bilia@unifi.it

Received: February 1st, 2018; Accepted: March 29th, 2018

Over the millennia, plants have represented for Humankind the main source of food, but also a vast resource to maintain health, for prophylactic properties or to cure human and animal diseases. Presently, between 65 and 80% of populations in developing countries use medicinal plants as therapeutic remedies for their primary healthcare and in Europe and USA there is an increasing demand of botanical products both on the form of food supplements and herbal medicinal products. Botanicals on the market are mainly based on traditional (infusions or decoctions), conventional (using organic solvents) and innovative (supercritical CO2 or subcritical water) extracts but there is an increasing demand of essential oils for aromatherapy. Conversely, the clinical use of many extracts is limited due to the need of repeated administrations or high doses because of low hydrophilicity and intrinsic dissolution rate(s), or physical/ chemical instability. Other limits are low absorption, poor pharmacokinetics and bioavailability, scarce biodistribution, first pass metabolism, trivial penetration and accumulation in the organs of the body. In the case of essential oils, the high volatility and instability are further limitations. Nowadays, the design and production of appropriate drug delivery systems, in particular nanosized ones (between 50 and 300 nm), have already entered into clinical use and can offer an advanced approach to optimized the therapeutic efficacy of extracts and essential oils. A successful drug carrier system should have optimal drug loading and release properties, a long shelf life, and exert a much higher therapeutic efficacy as well as lower side effects. Polymeric nanoparticles and lipid based-nanocarriers including micelles, vesicles, nanocochleates, micro- and nanoemulsions represent successful examples of extract nanoformulations overcoming these limitations. This review reports on some paradigmatic success stories of extract and EO nanoformulations with remarkable advantages over conventional formulations, which include increase of solubility, stability, permeation and bioavailability, sustained delivery. Paradigmatic examples include formulations of extracts from *Vitex agnus-castus*, *Sylibum marianum*, *Phyllanthus amarus*, *Ginkgo biloba*, *Panax notoginseng*, *Hypericum perforatum* and thyme essential oil.

Keywords: Plant extracts, Drug delivery systems, Nanocarriers, Micro- and nanoemulsions, Micelles and vesicles, Nanoparticles, Cyclodextrins.

Over the millennia, plants have represented for Humankind the main source of food, but also a vast resource to maintain health, for prophylactic properties or to cure human and animal diseases. Currently, numerous strong scientific evidences have confirmed traditional knowledge suggesting that the worldwide botanical cornucopia represents an eclectic collection of the most reliable early medicines. Almost every culture around the world has individually contributed to the modern knowledge of phytotherapy. Presently, between 65 and 80% of populations in developing countries use medicinal plants as therapeutic remedies for their primary healthcare [1]. Additionally, in the last decades in Europe and USA there was an increasing demand of botanical products both on the form of food supplements and herbal medicinal products because often perceived as "natural" and therefore safer products [2]. Mostly of the marketed preparations are based on extracts, including traditional (infusions or decoctions), conventional (using organic solvents) and innovative (supercritical CO2 or subcritical water) ones. Extracts are generally very complex mixtures, made of molecules with different solubility and chemical structures. It is unquestionable that extracts can have a general better therapeutic performance over single constituents because other components than active constituent(s) can modulate solubility, stability, absorption and metabolism, inhibit multi-drug resistance, resulting in a better bioavailability and efficacy when compared with isolated compounds. Additionally, in some cases, all the constituents can provide synergistic or additive activity and thus enhance the therapeutic value [3]. In truth, numerous extracts need repeated administrations or high doses to be used in clinical practice because of low hydrophilicity and intrinsic dissolution rate(s), or physical/ chemical instability. Other limits are low absorption, poor pharmacokinetics and bioavailability, scarce biodistribution, first pass metabolism, trivial penetration and accumulation in the organs of the body.

In addition to extracts, the use of essential oils (EOs) dates back thousands of years. They have been used for both religious and medicinal purposes in ancient Rome, Egypt, China and India. They are mentioned in the Bible and other ancient texts. EOs can be applied topically, used as an incense and even taken internally for therapeutic purposes. Currently, they have been rediscovered for the health and medicinal properties and largely used in aromatherapy.

Eos are volatile, limpid, and rarely coloured liquids, lipid soluble and soluble in organic solvents with a generally lower density than that of water. They can be synthesized by all plant organs, that is, buds, flowers, leaves, stems, twigs, seeds, fruits, roots, wood, or bark and are stored in secretory cells, cavities, canals, epidermis cells, or glandular trichomes. Constituents are lipophilic and highly volatile secondary plant metabolites, reaching a mass below a molecular weight of 300 Da, which can be physically separated from other plant components or membranous tissue.

Encapsulation of bioactive compounds of Eos represents a feasible and efficient approach to modulate drug release, increase the physical stability of the active substances, protect them from the interactions with the environment, decrease their volatility, enhance their bioactivity, reduce toxicity, and improve patient compliance and convenience [4].

Nowadays, the design and production of appropriate drug delivery systems, in particular nanosized ones (between 50 and 300 nm),

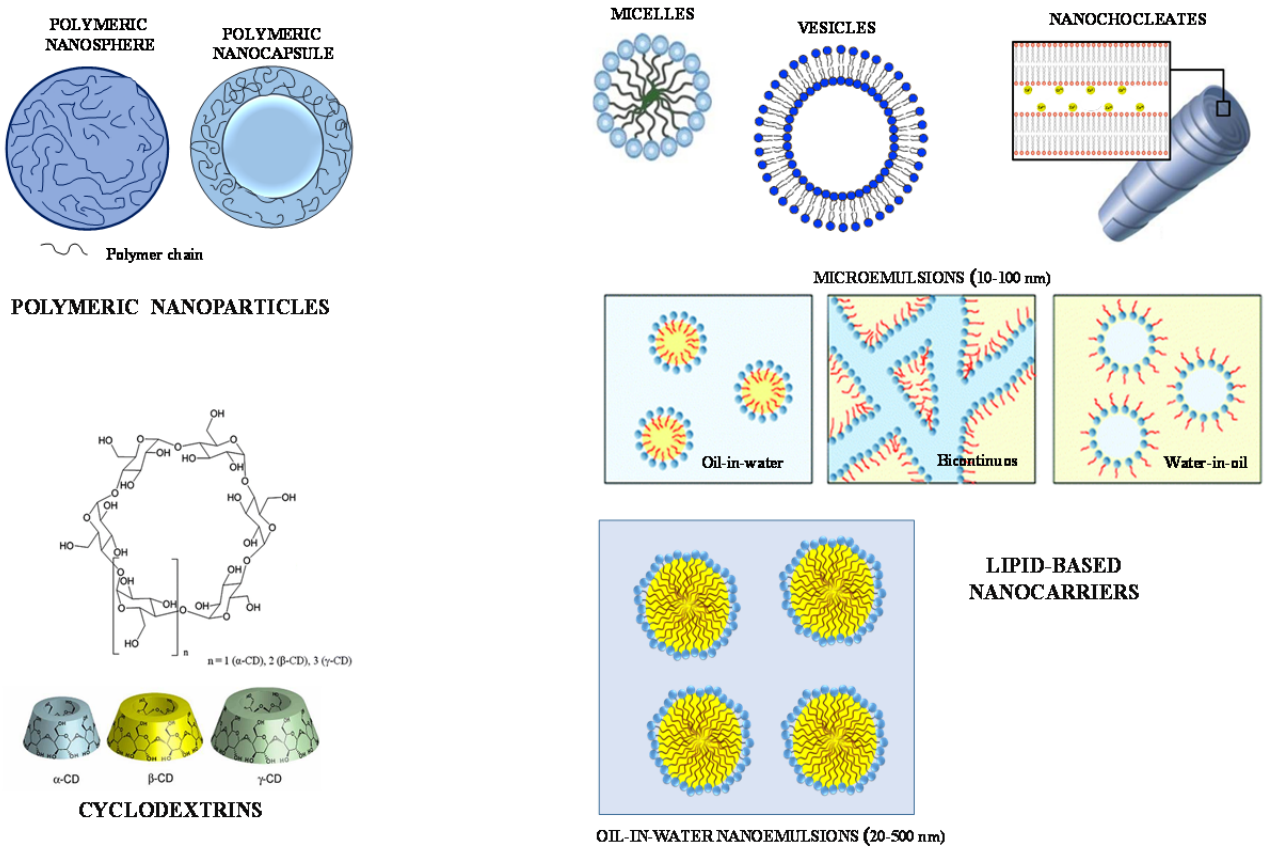

Figure 1: Polymeric nanoparticles, lipid based-nanocarriers including micelles, vesicles, nanocochleates, micro- and nanoemulsions, and cyclodextrins.

have already entered into clinical use and can offer an advanced approach to optimized the therapeutic efficacy of extracts. A successful drug carrier system should have optimal drug loading and release properties, a long shelf life, and exert a much higher therapeutic efficacy as well as lower side effects. Polymeric nanoparticles, lipid-based nanocarriers including micelles, vesicles, nanocochleates, micro- and nanoemulsions, and cyclodextrin complexes represent successful examples of extract nanoformulations overcoming these limitations. Their structures are reported in Figure 1.

Nanoparticles (NPs) include nanospheres and nanocapsules (Figure 1). Nanospheres are matrix systems in which drug is physically and uniformly dispersed, while nanocapsules are the system in which the drug is confined to a cavity surrounded by a unique polymeric membrane. Polymers used for the preparation of NPs are classified as synthetic or natural sources and biodegradable or non-biodegradable ones. Natural polymers or biopolymers may be naturally occurring materials, which are formed in nature during the life cycles of green plants, animals, bacteria and fungi, and consist of polysaccharides and proteins. Polysaccharides include compounds from plant origin (e.g., pectin, cellulose and its derivatives, starch and its derivatives, arabic gum, carrageenan, and alginate) and polysaccharides from microbial or animal origin (e.g., xanthan gum, gellan gum and chitosan).

In addition to polysaccharides, several natural proteins are very useful to produce NPs and they include albumin, gelatin, soy proteins hydrolysate, and casein. Gelatin obtained from collagen has an excellent compatibility with human tissue. Albumin is polymerised to obtain a versatile nontoxic, non-immunogenic, biocompatible and biodegradable NPs having high binding capacity of various drugs and being well tolerated without any serious side-effects [2]. In a recent publication, Bergonzi and coworkers reported the production of albumin nanoparticles (NPs) without the use of organic solvents to obtain useful drug-delivery systems to cross the blood-brain barrier. NPs were obtained by coacervation using thermal cross-linking processes [5].

Lipid-based nanocarriers include nanometric-scaled emulsions such as micro- and nanoemulsions, vesicles, micelles (Figure 1). Lipids and surfactant active agents are generally selected from a big plethora of edible constituents and Generally Recognized As Safe (GRAS) approved compounds. Vesicles, roughly divided in liposomes and niosomes are colloidal association of amphiphilic lipids that organize themselves spontaneously in bilayer vesicles and that are suitable to load hydrophilic and hydrophobic compounds [6]. Vesicles can be converted to nanocochleates which are unique lipid nanocarriers, composed of simple, naturally occurring materials such as phosphatidilcholine, cholesterol and calcium ions. They are stable phospholipid-calcium precipitate, structurally different from liposomes. Their unique structure consists of a large, continuous, solid, lipid bilayer sheet rolled up in a spiral, with no internal aqueous space. Calcium ions maintain the cochleate in its rolled form, bridging each successive layer, through ionic interaction. Nanocochleates are very attractive nanocarriers because biodegradable, nontoxic, non-immunogenic and biocompatible [7].

Micelles are self-assembling colloids, spontaneously formed under certain concentration and temperature from amphiphilic or surface-active agents (surfactants), molecules of which consist of two clearly distinct regions with opposite affinities towards a given solvent.

Microemulsions (10-100 nm) are currently defined as homogeneous thermodynamically stable transparent dispersions of two immiscible liquids stabilized by an interfacial film of surfactants. Structurally, there are three different type of microemulsions: oil-in-water (o/w), water-in-oil (w/o) and bicontinuous. They have droplet size above 100-500 nm and require very low energy to formulate emulsion, since they form spontaneously when aqueous, oil, and amphiphilic components are brought into contact, besides having a lower production cost compared to nanoemulsions. Nanoemulsions are non-equilibrium systems with a spontaneous tendency to separate into the constituent phases, prepared using lower surfactant concentrations than microemulsions. Nevertheless, nanoemulsions may possess a relatively high kinetic stability even for several years, due to their very small size, essentially the consequence of significant steric stabilization between droplets. They have droplet covering the size range of 20–500 nm and referred to as mini-emulsions, ultrafine emulsions, and submicrometer emulsions [2].

Lastly, cyclodextrins (CDs, Figure 1) are natural macrocyclic oligosaccharides well known for having toroid-shaped structures with rigid lipophilic cavities and a hydrophilic outer surface insuring good dissolution of the complex in an aqueous environment. They are able to entrap a variety of organic compounds in their cavities, which can influence the dissolution rate, the aqueous solubility of poorly water-soluble substances and their stability in the presence of light, heat and oxidising conditions, as well as modify physico-chemical properties of drugs. The major advantages of the use of CD-complexation are protection of the active ingredients against oxidation, light induced reactions, decomposition and thermal decomposition, loss by evaporation and sublimation, and elimination or reduction of undesired tastes/odours. They also can reduce or prevent gastric-intestinal irritation (mainly due to anti-inflammatory drugs) or ocular disturbances, prevent drug-drug or drug-additive interactions, or even to convert oils and liquid drugs into microcrystalline or amorphous powders and to reduce microbiological contamination, fibres, or the elimination of other undesired components and hygroscopicity. Moreover, formation of inclusion complex increases the guest's *in vivo* stability against hydrolysis, oxidation, decomposition, and dehydration, consequently increasing bioavailability and bioefficacy. There are three main types of CDs: α-, β-, and γ-cyclodextrins, corresponding to 6, 7, and 8 glucopyranose units linked by α-(1,4) bonds, respectively. The dimensions of the internal cavity are 0.5–0.8 nm and are crucial for the "encapsulation" of guest molecules. An increasing number of CDs derivatives is also available, randomly methylated-CDs, hydroxypropyl-CDs, and low methylated-CDs [8].

Currently, nanocarriers have an enormous impact in clinic, significantly improving the performance of drugs in terms of efficacy, safety, and patient compliance. This review reports on some paradigmatic success stories of extract and EO nanoformulations with remarkable advantages over conventional formulations, which include increase of solubility, stability, permeation and bioavailability, sustained delivery. Recently, Piazzini and coworkers [9,10] focused the studies on the development of innovative oil in water nanoemulsions to improve the solubility and the oral absorption of *Vitex agnus-castus* and *Silybum marianum* extracts. Therapeutic indications of *Vitex agnus-castus* are premenstrual syndrome including symptoms such as mastodyna or mastalgia and menstrual cycle disorders. *Silybum marianum*, well known as hepatoprotector, has a broad spectrum of pharmacological activities. Physical characterization showed the nanoscopic dimension of dispersed droplets (from 10 to 20 nm); further investigations showed that the solubility of the extracts was ameliorated considerably respect to water (about 10 times in the case of *Vitex agnus-castus* and about 100 times in the case of *Silybum marianum*). The oral absorption of extract-loaded microemulsions was tested *in vitro* using different models such as parallel artificial membrane permeability assay (PAMPA) and Caco-2 cell line. These studies clearly demonstrated that developed drug delivery systems increased the permeation of the main constituents of the extracts compared to the aqueous solutions. Thus, microemulsions represent potential candidates to improve the therapeutic effects of *Vitex agnus-castus* and *Silybum marianum*.

Deepa and coworkers [11] investigated a nanoemulsified ethanolic extract of *Phyllanthus amarus*, traditionally used as hepatoprotective remedy. Limits of the clinical use is the requirement of a large quantity of herbal extract to maintain a longer treatment duration. The study proved that the nanoencapsuled *P. amarus* extract (NPA), with a dose of 20 mg/kg body wt., showed better hepatoprotective activity than the extract itself (100 mg/kg body weight). Nanoencapsulation was obtained by emulsifying the extract with sodium alginate and Tween 80® to realize a core shell solidified by calcium chloride solution. Furthermore, nanoencapsulation was useful both to perform an intestine sustained release and to facilitate its maximum absorption. The aforementioned efficacy was tested comparing the two products on induced hepatotoxic male rats by oral administration. These biochemical assessments were supported by rat biopsy examinations and the dose oral toxicity was proved to be safe. Based on total phenol content the loading efficiency of nanocapsules was 89% (pH 7.0) and the optimum ratio plant extract: olive oil was 2:18 (mg/mL). Several tests have proven a spherical morphology, a mean particle diameter of 213 nm and the stability of all the loaded constituents. In conclusion, the nanoemulsification method may be applied for poor water-soluble ethanolic herbal extracts to reduce the dosage and time.

In the study by Han and coworkers [12] an injectable nanoparticulate system based on monomethoxy polyethylene glycol, poly lactide-co-glycolide and monomethoxy polyethyleneglycol (mPEG-PLGA-mPEG) was formulated. The nanoparticulate (PELGE) was used to co-encapsulate four active components (ginkgolides A, B, C and bilobalide) of *Ginkgo biloba* extract in order to obtain their sustained release. Drug loaded nanoparticles were prepared with 10% PEG(2000) modified PLGA by a co-precipitation method. The encapsulation efficiency of the total ginkgo terpenes in the optimal formulation was 78.84±2.06% with a loading dose of 11.90±0.31mg/150mg PELGE. The particles exhibited a spherical shape with a mean diameter of 123.3±44.0nm and zeta potential of -30.86±2.49mV. Sustained and synchronized release of the four components from PELGE nanoparticles was observed both *in vitro* and *in vivo*. It was due to the long circulation of PEGylated nanoparticles and to the slow degradation of PLGA. The half-life time of the four terpenoid compounds was also significantly improved after their loading into PELGE nanoparticles. The results indicate that a PELGE nanoparticle is a promising carrier system for sustained and synchronized release.

Zhang and coworkers [13] developed novel *Panax notoginseng* saponins (PNS) loaded core-shell hybrid liposomal vesicles (HLV) to improve the limited bioavailability and to enhance both its protective effects and its *in-vivo* oral administration. PNS loaded in conventional PLGA nanoparticles and PNS loaded in liposomes were formulated and compared with the innovative PNS-HLV made of mPEG-PLGA using a water-in-oil-in-water double emulsion solvent evaporation method. Morphology, particle size, zeta potential, encapsulation efficiency (EE%), stability of all the nanocarriers and *in vitro* release studies were performed. PNS-HLV carriers had the best performance, they were stable for at least 12

months at 4°C. Satisfactory improvements in the encapsulation efficacy of notoginsenoside R1, ginsenoside Rb1, and ginsenoside Rg1 were also found and the greatest controlled drug release profiles were exhibited from PNS-HLV. Secondly, to evaluate its oral treatment potential, they researchers compared the effect of PNS-HLV and the other formulations on global cerebral ischemia/reperfusion and acute myocardial ischemia injury in rats. PNS-HLV was able to significantly inhibit the edema of brain and reduce the infarct volume better than the other formulations.

Bergonzi and coworkers [14] used octanoyl-6-O-ascorbic acid (ASC8) a synthetic surfactant, to protect all the characteristic constituents of St. John's wort (SJW), from oxidation processes. Given the intrinsic ability of ASC8 to form micelles, aqueous solubility of the constituents has also been increased. Thermal and photostability of SJW commercial dried extract in the presence of ASC8 were evaluated according to the ICH test conditions. ASC8 increased stability of the constituents of the extract, in particular naphtodianthrones. A very high increment of shelf life of phloroglucinols was also evidenced in the accelerated test. In addition, ASC8 improved the solubility of total phloroglucinols. ASC8 could represent an appropriate and auxiliary excipient to improve the technological and biopharmaceutical characteristics of SJW extract.

In another study, Isacchi and coworkers [15] evaluated the optimisation of biopharmaceutical properties of a dried commercial extract of SJW employing the *in vivo* forced swimming test (FST). Three new dosage forms containing β-cyclodextrin and surfactants (sodium dodecyl sulphate, SDS, and ASC8) were compared in the FST with the commercial extract. The commercial extract showed antidepressant activity in mice after 60 min at a dosage of 100 mg/kg. The same antidepressant activity appeared in 30 min with a micellar solution of SDS containing the same quantity of extract (100 mg/kg), while with micelles of ASC8 the effect appeared at 15 min and with a dosage of 30 mg/kg. In the case of β-cyclodextrin the best results were obtained at 30 min, administering 60 mg/kg of the extract. Finally, the influence of the formulations on the water solubility of the constituents of the extract is reported. The tensides dramatically enhanced solubility, in particular that of the more lipophilic compounds, in the case of β-cyclodextrin this effect was very pronounced for flavonoids and biapigenin, lower for hypericins and practically insignificant for hyperforins.

A recent review on nanoencapsulation of Eos in drug delivery systems has been proposed for their capability of decreasing volatility, improving the stability, water solubility, and efficacy of essential oil-based formulations, by maintenance of therapeutic efficacy. Two categories of nanocarriers have been reported, polymeric nanoparticulate formulations, extensively studied with significant improvement of the essential oil antimicrobial activity, and lipid carriers, including liposomes, solid lipid nanoparticles, nanostructured lipid particles, and nano- and microemulsions. Furthermore, molecular complexes such as cyclodextrin inclusion complexes also represent a valid strategy to increase water solubility and stability and bioavailability and decrease volatility of Eos [4].

Recently, Asprea and coworkers [7] developed and characterized innovative nanocarriers, the nanocochleates, to increase the stability and to preserve the anti-oxidative properties of thyme essential oil. Thyme essential oil is composed mainly of phenolic compounds such as thymol and carvacrol and it represents a paradigmatic essential oil with huge antioxidants, antiradicals and antimicrobial properties. Transmission electron microscope characterization evidenced the cigar-like structure of nanocochleates. Particle size of nanochochleates was 210 nm, the encapsulation efficiency resulted 46% for thymol and 51% for carvacrol, using 1 mg/mL of essential oil. Anti-oxidative activity was investigated as DPPH radicals scavenging capacity of thyme essential oil, proving that nanocochleates were able to preserve the anti-oxidative activity of the essential oil.

References

[1] Cameron A, Ewen M, Auton M, Abegunde D. (**2011**) The world medicines situation 2011: Medicines prices, availability and affordability. WHO (World Health Organization). Geneva, Switzerland.

[2] Bilia AR, Piazzini V, Guccione C, Risaliti L, Asprea M, Capecchi G, Bergonzi MC. (**2017**) Improving on nature: the role of nanomedicine in the development of clinical natural drugs. *Planta Medica*, ***83***, 366-381.

[3] Bilia AR. (**2014**) Science meets regulation. *Journal of Ethnopharmacology*, ***158***, 487-494.

[4] Bilia AR, Guccione C, Isacchi B, Righeschi C, Firenzuoli F, Bergonzi MC. (**2014**) Essential oils loaded in nanosystems: a developing strategy for a successful therapeutic approach. *Evidence-Based Complementary and Alternative Medicine,* 651593. doi: 10.1155/2014/651593.

[5] Bergonzi MC, Guccione C, Grossi C, Piazzini V, Torracchi A, Luccarini I, Casamenti F, Bilia AR. (**2016**) Albumin Nanoparticles for Brain Delivery: A Comparison of Chemical versus Thermal Methods and in vivo Behaviour. *ChemMedChem, **11***, 1840-1849.

[6] Bilia AR, Bergonzi MC, Guccione C, Manconi M, Fadda AM, Sinico C. (**2016**) Vesicles and micelles: two versatile vectors for the delivery of natural products. *Journal of Drug Delivery Science and Technology*, ***32***, 241-255.

[7] Asprea M, Leto I, Bergonzi MC, Bilia AR. (**2017**) Thyme essential oil loaded in nanocochleates: Encapsulation efficiency, *in vitro* release study and antioxidant activity. *LWT-Food Science and Technology*, ***77***, 497-502.

[8] Bergonzi MC, Bilia AR. (**2015**) Cyclodextrins supramolecular complexes with botanical compounds. In *Cyclodextrins: Synthesis, Chemical Applications and Role in Drug Delivery*'. Francis G. Ramirez (Ed). Nova Publishers, 400 Oser Avenue, Suite 1600, Hauppauge, NY, USA.

[9] Piazzini V, Monteforte E, Luceri C, Bigagli E, Bilia AR, Bergonzi MC. (**2017**) Nanoemulsion for improving solubility and permeability of *Vitex agnus-castus* extract: formulation and in vitro evaluation using PAMPA and Caco-2 approaches. *Drug delivery*, ***24***, 380-390.

[10] Piazzini V, Rosseti C, Bigagli E, Luceri C, Bilia AR, Bergonzi MC. (**2017**) Prediction of permeation and cellular transport of *Silybum marianum* extract formulated in a nanoemulsion by using PAMPA and Caco-2 Cell models. *Planta Medica*, ***83***, 1184-1193

[11] Deepa V, Sridhar R, Goparaju A, Reddy PN, Murthy PB. (**2012**) Nanoemulsified ethanolic extract of *Pyllanthus amarus* Schum & Thonn ameliorates CCl_4 induced hepatotoxicity in Wistar rats. *Indian Journal of Experimental Biol*ogy, ***50***, 785-794.

[12] Han L, Fu Y, Cole AJ, Liu J, Wang J. (**2012**) Co-encapsulation and sustained-release of four components in ginkgo terpenes from injectable PELGE nanoparticles. *Fitoterapia*, ***83***, 721-731.

[13] Zhang J, Han X, Li X, Luo Y, Zhao H, Yang M, Ni B, Liao Z. (**2012**) Core-shell hybrid liposomal vesicles loaded with *Panax notoginseng*: preparation, characterization and protective effects on global cerebral ischemia/reperfusion injury and acute myocardial ischemia in rats. *International Journal of Nanomedicine*, ***7***, 4299-4310.

[14] Bergonzi MC, Isacchi B, Bilia AR. (**2013**) Octanoyl-6-O-ascorbic acid: an efficient antioxidant with potent solubilizing properties the case of active constituents from Saint John's wort. *Journal of Drug Delivery Science and Technology*, ***23***, 505-509.

[15] Isacchi B, Galeotti N, Bergonzi MC, Ghelardini C, Bilia AR, Vincieri FF. (**2009**) Pharmacological in vivo test to evaluate the bioavailability of some St John's wort innovative oral preparations. *Phytotherapy Research*, ***23***, 197-205.