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Review Article

AN OVERVIEW OF DISCRETE NANOFORMULATED FLAVONOIDS AND ITS IMPLICATION IN CANCER

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ABSTRACT

The nanoformulation of phytochemical has been developed to withdraw the drawbacks of conventional phytochemical. Nanoformulations are the nanosized particles, modified in order to improve the delivery of active phytochemicals in the target, to improve bioavailability, and solubility. Early researches shows that various phytochemicals like curcumin, Hesperidin, resveratrol, ellagic acid, essential oil, Naringenin, and quercitin are highly modified to form the nanoformulated compounds to improve its bio-activities. The nanoformulated phytochemicals are syntheized by various methods such as high energy and low energy emulsification, electrostatic stabilization, emulsification/reverse salting-out, nanoprecipitation, emulsification/solvent diffusion, solvent evaporation, and multi-arm Nanoconjugates, enzyme responsive nanoconjugates, core-crosslinked nanoconjugate hydrophobic–hydrophilic nanoconjugates, and nanoconjugate-based solid dispersion. Interestingly, the encapsulation of phytochemicals with surfactants, oils, emulsifying agents, salting-out agents will promote the antioxidant, antitumor, cytotoxic agents and antimicrobial effects than a raw phytochemicals. Various studies showed that phytochemicals are nanoformulated by the nanoemulsion method are better in terms of active target drug delivery, increasing bioavailability and it also act as an effective biological and diagnosting agents. This review focus on Innovative approaches to nanoformulated phytochemicals and their biomedical applications.

Keywords: Nanoemulsion, Nanosuspension, Polymeric nanoparticle, Nanovesicle, Nano conjugation, and cancer

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INTRODUCTION

Phytochemicals are secondary metabolites produced in plants, and they have several therapeutic applications. However, they face some challenges within the biological system, such as solubility, stability, and biocompatibility. To overcome these challenges, researchers extend their thinking toward the nanoformulation of phytochemicals. The formulation of nano phytochemicals used the following methods: Nanoemulsion, nanosuspension, polymeric nanoparticle, nanovesicle, and nano conjugation. The nano-formulated phytochemicals show an increase in solubility, bioavailability, and target drug release, and enhance the antioxidant property and pharmacokinetic clearance. Therefore,nano-formulated phytochemicals are a better drug for treating various diseases than a raw phytochemical. The gradual release of curcumin from nanoparticles demonstrated by the nanoemulsion technology increased the curcumin's bioavailability [1]. The effectiveness of the curcumin nanoparticle against human laryngeal cancer has been demonstrated by extensive research [2], and nano curcumin is also beneficial against other forms of carcinoma. Using encapsulation technology Hesperidin isolated from orange peels, was tested on three cancer cell lines (HepG-2, MCF-7, and HCT-116) where in its biological effects, antioxidant capacity, and cytotoxic effectsare multifold than the conventional phytochemical [3]. The absorption in blood-brain barrier was improved and there was an increase in antioxidant activity in the drug metabolism by polymeric nano-formulated ellagic acid against Alzheimer's disease [4]. The phytochemical apigenin brings inhibition of cell proliferation by inducing apoptosis. The apigenin incorporated in whey protein capsules brings better inhibition of cell proliferation in colon cancer cell lines HT-29 and HCT-116 [5]. Another study demonstrated that polymer-encapsulated luteolin nanoparticles were tested against Tu212 and H292 cell lines and demonstrated a more potent anticancer effect than luteolin alone due to the target tumour receptor binding with the target ligand on the nanoparticle surface and demonstrating over-expressing tumour activity [6]. It also studied that Febuxostat nanosuspension prepared by lyophilization techniques exhibited an enhanced solubility and oral bioavailability [4, 7]. Nanoresveratols formulated by Cyclodextrin-based nanosponges enhance water solubility and stability, and liposome nano resveratrol showed excellent cell proliferation. Resveratrol nanosuspension increase solubility rate and antioxidant potential [7, 8]. We examined three English databases, including Web of Science, Google Scholar, and PubMed, for papers published over the last five years that discussed the various approaches used to encapsulate phytochemicals with a nanoparticle. The following keywords and terminology were used: "Nanoemulsion", "Nanosuspension", "Nano Conjugation", "Nanoprecipitation", "Nanovesicle", and "Polymeric Nanoparticle". The focus of this review is on the various methods for nanoencapsulation of phytochemicals and their biomedical applications.

Preparation of nanosphere's and nanocapsules

Over recent years, the polymeric nanoparticles are used to encapsulate the phytochemicals. Researchers due to its advanced properties attract the polymeric nano-phytochemical technique such as improve bioavailability, controlled and target release of drug, increased therapeutic application, protection of drug and other molecules against environmental factors. The two polymeric nanoparticles are nanosphere's and nanocapsule's. The nanospheres consist polymeric network with the drug adsorbed on their surface. The nanocapsule composed of drugs dissolved in oily core, which is sheltered by a polymeric material. These nanospheres and nanocapsules are produced by solvent evaporation, emulsification/solvent diffusion, nanoprecipitation, emulsification/reverse salting-out methods [10].

Solvent evaporation method

In solvent evaporation method, initially the nanospheres are produced by oil in water nanoemulsion technique by high-speed homogenization or ultra-sonication. In this, the organic phase consist of polymer and phytochemical whereas the aqueous phase consist of water and surfactant. The solvents are evaporated by stirring and pure nanospheres are collected by centrifugation process [11].

Emulsification/solvent diffusion method

In this, method the nanospheres or nanocapsules are produced by emulsification process, in which the organic phase consists of polymer, drug, and oil were as the aqueous phase consist of water and surfactant. The solution is subjected to solvent diffusion, in which the water is used as diluents, the solvents are evaporated by stirring, and the nanoparticles are collected after purification [12].

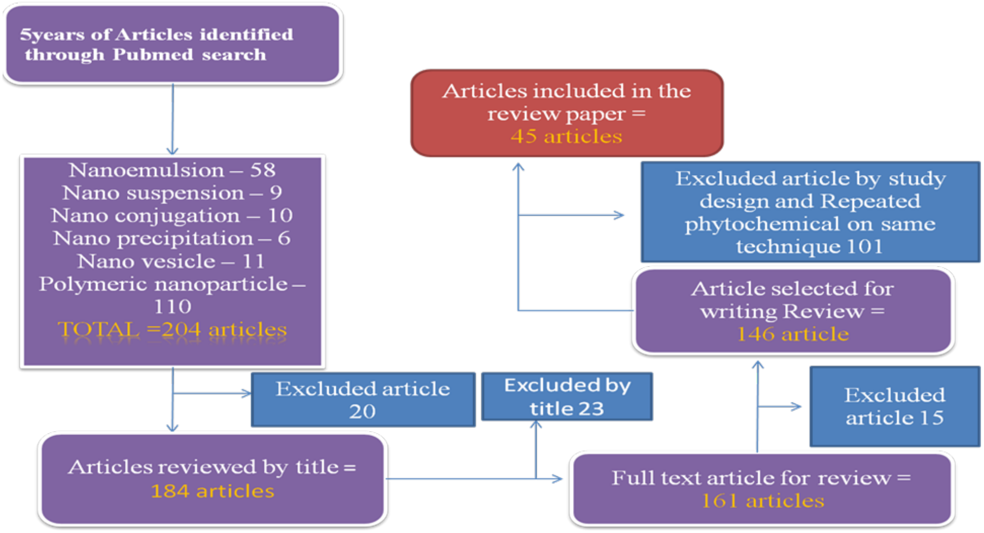

Fig. 1: The focus of this review is on the various methods for nanoencapsulation of phytochemicals and their biomedical applications

Nanoprecipitation method

In the nanoprecipitation method, to aqueous solution, the organic phase consist of polymer and drug is added slowly under continuous stirring as a result the polymer gets precipitated in the form of nanocapsules and then the solvents are evaporated and the polymeric nanoparticles are collected by filtration [13].

Emulsification/reverse salting-out method

The modifying method of emulsification/solvent diffusion is the emulsification/reverse salting-out method. The difference is the aqueous phase consists of a surfactant and salting-out agent. The commonly used salting-out agents are Magnesium chloride ($MgCl_2$), Calcium chloride ($CaCl_2$) and Magnesium acetate (Mg $(CH_3COO)_2$) [14].

Polymeric nano-phytochemicals

Polymeric-nano coumarin

Coumarin is a natural phytochemical present in seeds, leaf, root, fruit, and flowers of higher plants. The coumarin is encapsulated with a polymeric nanoparticle to increase its bioactive properties. The polymeric nanoparticle is produced by a solvent evaporation method, in which the poly (ε-caprolactone), poly (lactic acid), and poly (lactide-co-glycolide) are used as a polymer and the coumarin is encapsulated to form a polymeric-nano coumarin. The prepared polymeric nano-coumarin has the new perspective in the application of bio-imaging.

Polymeric-nano curcumin

The study showed that the polymeric nanocurcumin is synthesized by solvent diffusion method in which the poly (lactide-co-glycolide) is used as a polymer. The polymeric nanocurcumin has been studied for the curcumin release effect and its antibacterial activity. The dialysis bag method were used to study the drug release kinetics, the results exhibited that there is a sustained release of curcumin from polymeric nano-curcumin and in turn it inhibited the bacterial strains under study, Gram-negative (*Escherichia coli, Salmonella typhimurium,* and *Pseudomonas aeruginosa*) and Gram-positive (*Staphylococcus aureus, Bacillus sonorensis* and *Bacillus licheniformis*) bacteria [15]. The polymeric nano-curcumin has an increased bio-availability and solubility and thus exhibits excellent anticancer effect [16].

Polymeric-coated essential oils

The geraniol is a monoterpenoid a class of phytochemical, which is extracted from Palmarosa (*Cymbopogon martinii*). To increase the bioactive properties of geraniol it is nanoencapsulated with a polymer. The nano polymeric geraniol is formed by encapsulating the geraniol with Poly-ε-caprolactone (PCL) polymer by the nanoprecipitation method. The nano polymeric geraniol is active against *Staphylococcus aureus* and *Escherichia coli* [17]. The essential oils like Rosemary oil and Lavender oil extracted from *Rosmarinus officinalis L and Lavandula dentata L respectively are coated with a polymer* Eudragit® EPO also shows excellent antioxidant activity [18]. In the year, 2022 Bala nagamani *et al.*, studied that Ezetimibe polymeric nanoparticle has a sustained drug release and act as a suitable carrier of the drug in the drug delivery system.

Table 1: Polymer-coated phytochemicals

Phytochemical	Synthesis mechanism	Biomedical application	References
Polymeric-nano coumarin	Solvent evaporation method.	bio-imaging	[15]
polymeric nanocurcumin	solvent diffusion method	Inhibit bacterial strains. Anticancer effect. Increase bioavailability and solubility.	[16]
Polymeric-coated essential oils (geraniol, Rosemary oil and lavender oil).	Nano precipitation method	Anti-microbial and Anti-oxidant activity	[17, 18]

Preparation of nanoemulsion

In nanoemulsion technique, the nano-sized droplets get diffused in immiscible liquid results in the formation of nanoparticle. This nanoparticle will show properties appropriate for encapsulation, release and formulation. Nanoemulsion is of three types i) oil in aqueous phase, ii) aqueous in oil phase, and iii) bi-continous phase.

Compared to other method nanoemulsion technique is put forward by researchers due to the subsequent advantages: the nanoemulsion technique acquired low amount of energy, it solubilize lipophilic drug, it gives some flavor to the drug, non-toxic, small sized droplets provide large surface area which is useful for absorbtion, improve bioavailability, and it can also used as a stand-in for liposome and vesiclenanoparticles [19]. The formation of nanoemulsion require three components water, oil, and emulsifying agents. The nanoparticles are formulated by nanoemulsion technique by the process of high energy and low energy emulsification.

Quercetin nanoemulsion

Quercetin is a polyphenol, a flavonoid group of phytochemicals. The source of quercetin isacai berries, onions, broccoli, apples, olive oil and red wine, and presently as glycosides in plants [20]. The research work showed that Quercetin nanoparticle formed by nanoemulsion method is effective against rheumatoid arthritis. As a first step, the solubility of quercetin with suitable oil and surfactants were determined by vortexing the 1 gm of quercetin with 2 ml of oil and surfactant and then centrifuging it and the resulting supernatant was taken for measurement in UV-Spectrophotometer. Secondly, the quercetin nanoemulsion is synthesized by adding a weighed quantity of quercetin in the oil phase with the consequent addition of surfactants and subjected to vortexing. The resultant is slowly added to stirring aqueous phase, which results in the formation of nanoemulsion of quercetin. The effect of quercetin nanoemulsion (QCT-NE) were studied for its effectiveness in the suppression of tumor necrosis factor (TNF-α) in RAW 264.7 cell line, because tumor necrosis factor (TNF-α) involved in multiplication of fibroblast in turn activate pro-inflammatory mediators which results in arthritis and breakdown of bones. The quercetin nanoemulsion (QCT-NE) showed an effective inhibition of TNF-αwhich reduce arthritis [21]. Another study showed that quercetin nanoemulsion (QCT-NE) produced by ultrasound-based emulsification method was interconnected with human serum albumin (HSA) and holo-transferrin (HTF) which results in a formation of complex and delivered quercetin from nanoemulsion to a target protein. Thus the study showed that quercetin nanoemulsion (QCT-NE) acts as an excellent transporting agent and by regulating the caspase-3 gene expression the quercetin nanoemulsion (QCT-NE) induces apoptosis in liver cancer cell line (HePG2) [22].

Luteolin nanoemulsion

A flavonoid luteolin are obtained from celery, parsley, broccoli, onion leaves, carrots, peppers, cabbages, apple skins, etc., luteolin nanoparticle produced by an emulsion technique in which the bergamot oil is used as oil phase and cremophor EL as surfactant to load luteolin with cationic nanoemulsion(CNE1–CNE9). The ability of *in vitro* drug release and ex-vivo permeation parameters had been studied [23]. The result showed that luteolin from cationic nanoemulsion 4 (CNE4) exhibited a maximum drug release in physiological buffer which is due to good solubilization of luteolin. The formulated luteolin cationic nanoemulsion also showed good permeation ability. The cationic inducer present in nanoemulsion react with the negative charge of epidermal cells and increase the surface area. In this way, the luteolin present in LUT-cationic nanoemulsion penetrate across the stratum corneum and cationic inducer react with the negative charge of cells results in increased surface area, caused structural change and increase the solubility of luteolin. The cationic nanoemulsion 4 (CNE4) showed a maximum drug deposition in skin than other cationic nanoemulsion. The advantage of this cationic nanoemulsion is the target drug delivery to the tumor lesion avoiding the oral route of administration.

Nanoemulsion of anethole, naringenin, and taxifolin

The phytochemicals anethole, naringenin,and taxifolin present in anise seeds are extract by the maceration process. By using tween 80 as a surfactant, the bulk extract was emulsified by ultrasound homogenizer. The nanoemulsified phytochemicals are studied for its anti-microbial activity and compared with anise extract alone. The results showed that the nanoemulsified anise extract has an excellent anti-microbial effect on *Y. enterocolitica, S. aureus, B. cereus* and *E. coli* than anise extract; this is due to the size of nanoemulsified droplets and its penetration through microbial cells. These emulsified natural compounds had a wide application in food industry [24].

Nanoemulsified essential oils

Long ago, the use of essential oil is limited because of its low bioavailability and high degradation. Now a day the essential oil has been nanoemulsified to overcome these hurdles.

Nanoemulsified essential oil containing terpenoids

Generally, the Satureja essential oil has anti-oxidant and anti-microbial activity [25]. The essential oil is extracted from the plant *Satureja montana L* were subjected to nanoemulsion. The oil in water nanoemulsion technique with two different surfactant (Tween 20 and Tween80) were used for the synthesis of nanoemulsified essential oil. The volatile nature of nanoemulsified essential oil are analysed by using gas chromatography combines with mass spectroscopy. The analysis showed the presence of terpenes and terpenoid phytochemicals. The nanoemulsified essential oil is subjected to study the antibacterial effect. The study showed that lipophilic compounds like terpenes has the capacity to alter the structural and functional integrity of cell membrane, thus the nanoemulsified essential oil has a good effect against fungi, gram-negative, and gram-positive bacteria [26]. This technique has a wide application in food and pharma industry.

Nanoemulsified essential oil containing limonene and α-pinene

Essential oil extracted from *Rosmarinus officinalis*contains phytochemicals and it has anti-inflammatory, anti-atherogenic, and dyslipidemia's activity [27]. The essential oil extracted from *R. officinalis* is contained major phytochemical limonene, and α-pinene. These essential oils are nanoemulsified and introduced into triton-coconut saturated fat-induced dyslipidemia model. The study exhibited that nanoemulsified essential oil has effective anti-dyslipidemia and anti-atherogenic activity,thus the decrease lipid level reduce the risk of cardiovascular disease.

Nanoemulsified essential oil containing linalyl acetate, limonene, and α-terpineol

The major phytochemical present in the essential oil from *Citrus aurantium L. bloom* found to contain linalyl acetate, limonene, and α-terpineol, and it is nanoemulsified by ultrasonication method. The resultant nanoemulsified phytochemical is studied for its cytotoxic effects on A549 lung cancer cell line and its toxic effects are compared with normal human foreskin fibroblasts (HFF cells). The consequence of nanoemulsified essential oil showed excellent cell-dependent cytotoxic effects. The study also showed that the nanoemulsified essential oil activates the antioxidant enzymes superoxide dismutase (SOD), chloramphenicol acetyltransferase (CAT), and glutathione peroxidase (GPx) which lead to suppression of apoptosis genes [28]. Thus, the nanoemulsified essential oil is broadly used in pharmaceutical industry and it reduces side effects.

Essential oil-pectin nanoemulsion

The essential oil extracted from zataria species is nanoemulsified with pectin and studied for its anti-cancer effect on MDA-MB-231 breast cancer cells [29]. The essential oil-pectin nanoemulsion showed its antitumor activity by elevating the Reactive Oxygen Species (ROS), mitochondrial membrane potential (MMP) loss, DNA damage, and G2 and S-phase arrest in cancer cell line, which result in apoptosis. Thus, acting as a good therapeutic agent against cancer.

Nanoemulsified essential oil of plectranthus vettiveroides root

The *Plectranthus vettiveroides* are rich in diterpenoids, triterpenoids, flavones, and fatty acids. The extracted essential oil is nanoemulsified using tween80 as surfactant and lecithin emulsifier. The nanoemulsified essential oil of *P. vettiveroids* has the vasorelaxation activity via ATP sensitive^{+} Kchannel. The vasorelaxation effect was due to the involvement of endothelium-derived relaxing factors (nitric oxide, G-protein coupled muscarinic (M3) receptor) [30].

Nanoemulsified essential oil of *Heracleum persicum*

The essential oil from Heracleum persicum were extracted and nanoemulsified by ultra-sonication process. The nanoemulsified essential oil contains the phytochemicals anethole, terpinolene, ɣ-terpinene, and myrcene. The anticancer effects of nanoemulsified essential oil were studied on breast cancer cell line (MDA-MB-231). The nanoemulsified essential oil triggers the caspase-3 gene which inturn causes the apoptotis on cancer cells [31].

Nanoemulsified essential oil of *Ayapana triplinervis*

The essential oil of Ayapana triplinervis is extracted by hydro-distillation and it is nanoemulsified by low energy technique. Caryophyllene, Thymohydroquinone Dimethyl Ether in morphotype A, and morphotype B are the phytochemicals present in the nanoemulsified essential oil. The growth of *Aedes aegypti larvae, which cause dengue,* chikungunya*, etc. was controlled by using nanoemulsified essential oil of Ayapana triplinervis* [32]*. The nanoemulsion generally has good penetration activity, thus the nanoemulsified essential oil penetrate through epithelial tissues of larvae and cause distortion of anal papillae, the detaching of epithelial tissues from larval abdomen. The loss of epithelial tissue leads to decrease in mobility and respiration of larvae that showed the toxic effect of nanoemulsified essential oil of A. triplinervis on Aedes aegypti larvae.*

Resveratrol nanoemulsion

Resveratrol is a polyphenol found naturally in purple grapes, cranberries, blueberries, peanuts, rhubarb, mulberries, pines, groundnuts, and skin and seeds of grapes contains a high concentration of resveratrol. The resveratrol nanoemulsion is produced by ultrasonication process by using coconut oil as oil phase and Cremophor EL as a surfactant. The nanoemulsified resveratrol is studied for its efficient drug release, drug permeability and its effect against Alzheimer's disease [33]. Dialysis methods were used to study the release of resveratrol from nanoemulsion, and the researchers concluded that the globule size nanoemulsion showed a fine release of resveratrol and increase its solubility. The permeation of nanoemulsion resveratrol had been studied on goat nasal mucosa in phosphate buffer medium, in which the increased surface area by nanoemulsion showed an excellent paermeation of resveratrol in to nasal mucosa surface. The distribution of resveratrol in the nasal mucosa passes the blood-brain barrier and deposit at the brain and causes its efficacy against Alzheimer's disease. Another study showed that nanoemulsified resveratrol increased the water solubility and stability of resveratrol by using caprine casein (milk protein) as an emulsifying agent.

Carotenoid nanoemulsion

Carotenoids are the fat-soluble phytochemicals, they are present in all plants. Carotenoids are responsible for the pigmentation of fruits and vegetables. Carotenoids are extracted from Pomelo Leaves and its nanoemulsified form found to inhibit Melanoma Cells A375. The inhibition of cell growth is by upregulation Bax and cytochrome-C via stimulation of caspase-9,8 and 3 and downregulation cylin-dependent kinase 1 (CDK1) and cylin-dependent kinase 2 (CDK2) [34]. The decline of cylin-dependent kinase 1(CDK1) and cylin-dependent kinase 2 (CDK2) expressions subsequently culminates cell cycle arrest in G2/M phase. Thus, the study presented that the nanoemulsified carotenoids has better anti-tumor activity, than raw carotenoid.

Nanoemulsion of bioactive compounds of xoconostle" cactus

The xoconostle" cactus is a fruit, which belongs to the genus Opuntia family of cacti. Xoconostle cactus contains a phytochemicals ascorbic acid, betalains, phenols, tannins, Vitamin C, and flavonoids. The active phytochemical are extracted from xoconostle are nanoemulsified with orange oil. The nanoemulsified bioactive compounds of xoconostle cactus fruit increases the nutrient content of foods improve its shelf life. The phenolic compounds present in xonocastle fruit have the ability to decrease microorganisms [35]. Thus it is found that nano emulsification of the extract will protect the bioactive phytochemicals and make the food nutrient rich to improve consumer's health.

Table 2: Nanoemulsion phytochemical

Phytochemical	Synthesis mechanism	Biomedical application	Reference
Quercitin	Spontaneous emulsification.	➢ Suppression of TNFα in RAW264.7 cell line. ➢ Arthritis. ➢ Induce apoptosis in HepG2 Cell line.	[21, 22]
Luteolin	Cationic nanoemulsion	➢ Drug release ➢ Ex-vivo permeation	[23]
Anise seeds	Ultrasound assisted method	➢ Antimicrobial	[24]
Satureja essential oil	Hydro distillation method	➢ Anti-oxidant ➢ Anti-bacterial	[25]
Zataria EO	Water in oil nano emulsification	➢ ROS ➢ G2andS Phase arrest	[26]
Rosmarinus officinalis EO	Saturated coconut oil in water nanoemulsion.	➢ Anti-inflammatory ➢ Anti-atherogenic ➢ Dyslipidemia	[27]
Citrus aurantium EO	Ultrasonication method	➢ Cytotoxic effect on A549 cells	[28]
Plectranthus vettiveroides EO	Ultrasonication method	➢ Vaso relaxation activity	[30]
Heracleum persicum EO	Ultrasonication method	➢ Against breast cancer (MDA-MB-231)	[31]
Ayapanatriplinervis EO	Low energy nanoemulsification	➢ Control Aedes aegypti larvae	[32]
Resveratrol	Ultra sonication method	➢ Against Alzheimers disease	[33]
Carotenoid	Ultrasonication method	➢ Inhibitory effect on melanoma cells. ➢ Cyclin A and B. ➢ CDK1 andCDK2.	[34]
Xoconostle cactus	Multiple emulsion	➢ Improve shelf life of food	[35]

Nanosuspension technique

Nanosuspension is a technique in which the drug (or) phytochemicals are suspended with a stabilizer. This technique is easier than other nanoencapsulation techniques. The phytochemicals that are nanosuspended will show following properties: reduced in particle size, uncomplicated formulation, and diverse administration routes. The most commonly used stabilizers are electrostatic or steric stabilization.

Myricetin nanosuspension

Myricetin is a natural flavonoid group of phytochemicals. They are present in fruits, vegetables, nuts and beverages. The nanosuspension of myricetin had been produced by dissolving myricetin with an organic phase and then it is introduced in to a stabilizing agent and subjected to stirring, then the organic solvents are removed by rotary evaporator results in a formation of coarse suspension. Then the high-pressurehomogenizer is used to produce a nanosuspension [36]. Thus, the oral administration of myricetin nanosuspension in rats showed a gradual increase in plasma level, good absorption and increased bioavailability.

Quercitin nanosuspension

The quercitin-encapsulated nanosuspension is produced by the high pressure homogenizing technique. The stabilizers such as soya lecithin, polysorbate 80, Pluronic F68, SDS, PEG 6000, PVP K-30 are synthesized by nanoprecipitation method. The study showed that the oral route of quercitin nanosuspension in rats increased the oral bioavailability of quercitin [37]. The nanosuspended phytochemicals are active against viruses. Nanosuspended quercitin act against poliovirus, adenovirus, Epstein-Barr virus, hepatitis C virus (HCV), and respiratory syncytial virus by inhibiting heat shock proteins (Shimon Ben-Shabat *et al.*, 2020) [38].

Oleanolic acid nanosuspensions

Oleanolic acid is apentacyclic triterpenoid a group of phytochemicals that are present naturally. The nanosuspension of oleanolic acid is prepared by wet ball milling technique with sucrose ester as a stabilizer. The nanosuspension oleanolic acid showed an increased inefficacy in A549 lung cancer cell line, and elevate the bioavailability of oleanolic acid [39].

Naringenin nanosuspension

Generally, p-GP cause the outflow of drug and results in decline, the concentration of drug in cells. Vitamin E-TPGS is a good stabilizer for preparation of nanosuspension of naringenin than any other stabilizing agent. The small size of naringenin in nanosuspension increases its solubility and stability. The nanoormulated naringenin brings inhibition of p-glycoprotein (breast cancer cell line). Thus, the TPGS-naringenin inhibits p-GP and enhances the anticancer activity of naringenin nanosuspension [40].

Table 3: Nanosuspension phytochemicals

Phytochemical	Synthesis mechanism	Biomedical application	Reference
Myricetin	High pressure homogenizing technique.	➢↑Plasma level. ➢↑Bioavailability.	[36]
Quercitin	High pressure homogenizing technique.	➢↑Oral bioavailability. ➢Against polio virus, adenovirus, and HCV.	[37, 38]
Oleanolic acid	Wet ball milling technique.	➢↑Bioefficacy in A549 cell line.	[39]
Naringenin	Micellization technique.	➢Inhibit p-glycoprotein in breast cancer	[40]

Nanovesicle technique

Nanovesicle is a technique in which the phytochemicals (or) drug are entrapped by a vesicle. The vesicle may be Liposomes, Niosomes, Transfersomes, Ethosomes, Transethosomes, Phytosomes, Discomes, Pharmacosomes, Virosomes, Sphingosomes, Enzymosomes, Ufasomes, Bilosomes, Aquasomes, Emulsomes, Cubosomes, Cryptosomes, Colloidosomes, Genosomes, Photosome, Erythrosomes, and Vesosomes. Among these types, the lipid-based vesicle such as liposomes, niosomes, transfersomes, ethosomes are most commonly used nanovesicles by the researchers. Liposomes are lipid bilayer consist of phospholipid and cholesterol. Niosomes contains non-ionic surfactants and cholesterol layer, transfersomes consist of phospholipid and surfactant bilayer, and ethosomes consist of phospholipid and ethanol bilayer. Both hydrophilic and lipophilic drugs can be induced into a vesicle to form a nanovesicle drug. The formulation of phytochemicals in the form of nanovesicle becomes prominent in recent years due to its following unique properties, increased absorption and bioavailability, delivery of the drug at the target site, better pharmacokinetics activity. The characteristics of both hydrophilic and hydrophobic components of drug reduce the dosage frequency.

Saponins and flavonoids induced exosome nanovesicle

Exosomes are the extracellular nanosized lipid vesicle and has an intercellular communication. The exosomes as a phytochemical carrier act excellently against cancer cell proliferation. The phytochemicals extracted from the black bean seed by homogenization and freeze-drying technique. The exosome derived from HepG2 cell line were mixed with black bean extract and the loading capability of extract into exosome are identified by incubation and electroporation method, and it is ultra-centrifuged to collect the supernatant. The exosomal formulation of the black bean extract was studied for its anti-proliferative activity. The exosome mediated phytochemical (drug) delivery enhances the inhibition of cell growth in tumor cell lines thus the study reported that exosome nanovesicle alter the drug delivery in target and showed a promising anti-proliferative and anti-cancer activity [41].

Essential oil nanovesicle

The essential oils extracted from the aerial parts of *Origanum onites L. and Satureja thymbra L* plants are loaded into a lipid nanovesicle by lipid film hydration method. In this method, the phosphotidyl choline and cholesterol for nanovesicle formulation were selected and dissolved in dichloromethane and kept in ultrasonication. During the evaporation stage of dichloromethane the extracted essential oil is added with continous stirring, thus results in essential oil being loaded in lipid nanovesicle. The essential oil loaded nanovesicle showed an excellent antifungal and antibacterial activities by testing its inhibitory effect against bacteria *Staphylococcus aureus, Bacillus cereus, Pseudomonas aeruginosa, and Salmonella enteric and against fungi Aspergillus fumigatus, Aspergillus niger, Trichoderma viride, Penicillium verrucosum, Candida albicans, and Candida krusei* [42]. The cytotoxicity effects were also studied against HaCaT cells. Finally, the study reported that the essential oil loaded lipid nanovesicle showed a sustained release of essential oil from nanovesicle, which inhibits the food pathogens.

Table 4: Nanovesicle phytochemicals

Phytochemical	Synthesis mechanism	Biomedical application	Reference
Black bean seed (Saponins and Flavonoids).	Exosome nanovesicle	Anti-proliferative and anti-cancer activity	[41]
Origanum onites L. and Satureja thymbra L	lipid film hydration method	Antifungal and antibacterial activities	[42]

Nanoconjugation technique

Generally,the nanoconjugation technique are used to improve the bioavailability and solubility of poorly soluble phytochemicals (drug) and synthesized by different methods such as Multi-Arm Nanoconjugates, Enzyme Responsive Nanoconjugates,core-crosslinked nanoconjugate hydrophobic–hydrophilic nanoconjugates, and Nanoconjugate-Based Solid Dispersion to improve the therapeutic effects and bioavailability [43].

Curcumin nanoconjugate

Superparamagnetic Iron Oxide Nanoparticles (SPIONs) coated with raw carica papaya latex were used for the conjugation of curcumin, which showed a slow drug release, antibacterial and anti-cancer activity. The nanoconjugation of curcumin were synthesized by sonicating the latex coated SPIONs with aqua, to that the curcumin mixed with methanol was added by continuous sonication and then it is centrifuged to collect the nanoconjugates. The study showed that Bacillus subtilis and Pseudomonas aeruginosa with SPION-conjugated curcumin had an antibacterial activity than Superparamagnetic Iron Oxide Nanoparticles (SPIONs) alone [44]. The study also showed that Superparamagnetic Iron Oxide Nanoparticles (SPIONs) curcumin brings inhibition of L929 breast cancer cells in a dose dependent manner.

Elaeocarpus ganitrus nanoconjugate

The phytochemicals are extracted from the dried leaves of Elaeocarpus sphaericus, and the presence of active biocomponents in the extracts is analyzed by Gas Chromatography-Mass Spectrometry method. The extract contains the bioactive compounds like alcohols, acids, steroids, esters, hydrocarbons, amino acids and alkaloids. The resultant extracts are conjugated with the silver nanoparticle to enhance the anticancer activity on MCF-7 breast cancer cell line [45].

Table 5: Nanoconjugation-phytochemicals

Phytochemical	Synthesis mechanism	Biomedical application	Reference
Curcumin nanoconjugate	Core-crosslinked nanoconjugate	Antibacterial activity and inhibition of L929 breast cancer cell line	[44]
Elaeocarpus sphaericus	Core-crosslinked nanoconjugate	Anticancer activity on MCF-7	[45]

CONCLUSION

The nanoformulation of phytochemicals become prominent in pharmaceutical and therapeutic fields and offers several advantages such as target drug delivery, good drug carrier, improving solubility and bioavailability, sustained drug release and it also showed an excellent anticancer and antimicrobial activity compared to raw drug. Among various nanoformulation method the nanoemulsion is the most widely used method and well thought-out to be more effective. It is anticipated that further research and development will be carried out in the future regarding nanoformulation of phytochemicals.

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All the authors have contributed equally.

CONFLICT OF INTERESTS

Declared none

REFERENCES

1. Mohanty C, Sahoo SK. The *in vitro* stability and *in vivo* pharmacokinetics of curcumin prepared as an aqueous nanoparticulate formulation. Biomaterials. 2010 Sep;31(25):6597-611. doi: 10.1016/j.biomaterials.2010.04.062, PMID 20553984.
2. Lee WH, Loo CY, Young PM, Traini D, Mason RS, Rohanizadeh R. Recent advances in curcumin nanoformulation for cancer therapy. Expert Opin Drug Deliv. 2014 Aug;11(8):1183-201. doi: 10.1517/17425247.2014.916686, PMID 24857605.
3. Ahmed H, Aboul-Enein A, Abou-Elella F, Salem S, Aly H, Nassrallah A, Habiba A, Ahmed A. Nano-formulations of hesperidin and essential oil extracted from sweet orange peel: chemical properties and biological activities. Egypt J Chem. 2021. doi: 10.21608/EJCHEM.2021.84783.4139.
4. Harakeh S, Qari MH, Ramadan WS, Al Jaouni SK, Almuhayawi MS, Al Amri T. A novel nanoformulation of ellagic acid is promising in restoring oxidative homeostasis in rat brains with alzheimer's disease. Curr Drug Metab. 2021;22(4):299-307. doi: 10.2174/1389200221666201216170851, PMID 33327909.
5. Sa H, Dia VP, Baek SJ, Zhong Q. Nanoencapsulation of apigenin with whey protein isolate: physicochemical properties, *in vitro* activity against colorectal cancer cells, and bioavailability. Lebensm Wiss Technol. 2022;154. https://doi.org/10.1016/j.lwt.2021.112751
6. Lorectal cancer cells, and bioavailability. Lebensm Wiss Technol. 2022 Jan 15;154:112751. doi: 10.1016/j.lwt, PMID 34840350.
7. Nagamani DB, Reddy KB. Implementation of factorial design to optimize the formulation method of ezetimibe polymeric nanoparticle by homogenization cum ultra-sonication method. Int J App Pharm. 2022;14(2):151-9. doi: 10.22159/ijap.2022v14i2.43783.
8. Majumdar D, Jung KH, Zhang H, Nannapaneni S, Wang X, Ruhul Amin ARM. Chen1, and dong M. Shin1, 2015, luteolin nanoparticle in chemoprevention–*in vitro* and *in vivo* anticancer activity. Cancer Prev Res (Phila). 2014 Jan;7(1):65-73. doi: 10.1158/1940-6207.
9. Houacine C, Singh KK. Nano resveratrol a promising future nanonutraceutical Nanonutraceuticals. 1st ed. Vol. 2018; 2019. doi: 10.1201/9781351138949-10.
10. Zielinska A, Carreiro F, Oliveira AM, Neves A, Pires B, Venkatesh DN. Polymeric Nanoparticles: production, characterization, toxicology and ecotoxicology. Molecules. 2020 Aug 15;25(16):3731. doi: 10.3390/molecules25163731, PMID 32824172.
11. Szczech M, Szczepanowicz K. Polymeric core-shell nanoparticles prepared by spontaneous emulsification solvent evaporation and functionalized by the layer-by-layer method. Nanomaterials (Basel). 2020 Mar 10;10(3):496. doi: 10.3390/nano10030496, PMID 32164194.
12. Wang Y, Li P, Truong Dinh Tran T, Zhang J, Kong L. Manufacturing techniques and surface engineering of polymer based nanoparticles for targeted drug delivery to cancer. Nanomaterials (Basel). 2016 Feb 1;6(2):26. doi: 10.3390/nano6020026, PMID 28344283.
13. Martinez Rivas CJ, Tarhini M, Badri W, Miladi K, Greige Gerges H, Nazari QA. Nanoprecipitation process: from encapsulation to drug delivery. Int J Pharm. 2017 Oct 30;532(1):66-81. doi: 10.1016/j.ijpharm.2017.08.064, PMID 28801107.
14. Lim K, Hamid ZAA. Polymer nanoparticle carriers in drug delivery systems: research trend. In: Asiri AM, Mohammad A. editors. Applications of nanocomposite materials in drug delivery; Inamuddin. Cambridge, UK: Woodhead Publishing; 2018. p. 217-37.
15. Gao M, Long X, Du J, Teng M, Zhang W, Wang Y. Enhanced curcumin solubility and antibacterial activity by encapsulation in PLGA oily core nanocapsules. Food Funct. 2020;11(1):448-55. doi: 10.1039/c9fo00901a, PMID 31829367.
16. Illahi AF, Muhammad F, Akhtar B. Nanoformulations of nutraceuticals for cancer treatment. Crit Rev Eukaryot Gene Expr. 2019;29(5):449-60. doi: 10.1615/CritRevEukaryotGeneExpr.2019025957, PMID 32422001.
17. Jummes B, Sganzerla WG, da Rosa CG, Noronha CM, Nunes MR, Bertoldi FC. Antioxidant and antimicrobial poly-ε-caprolactone nanoparticles loaded with cymbopogon martinii essential oil. Biocatal Agric Biotechnol. 2020;23(Jan):101499. doi: 10.1016/j.bcab.2020.101499.
18. Silva Flores PG, Perez Lopez LA, Rivas Galindo VM, Paniagua Vega D, Galindo Rodriguez SA, Alvarez Roman R. Simultaneous GC-FID quantification of main components of *Rosmarinus officinalis* L. and *Lavandula dentata* essential oils in polymeric nanocapsules for antioxidant application. J Anal Methods Chem. 2019 Feb 10;2019:2837406. doi: 10.1155/2019/2837406, PMID 30881726.

19. Jaiswal M, Dudhe R, Sharma PK. Nanoemulsion: an advanced mode of drug delivery system. 3 Biotech. 2015 Apr;5(2):123-7. doi: 10.1007/s13205-014-0214-0, PMID 28324579.
20. Zang X, Cheng M, Zhang X, Chen X. Quercetin nanoformulations: a promising strategy for tumor therapy. Food Funct. 2021;12(15):6664-81. doi: 10.1039/d1fo00851j, PMID 34152346.
21. Gokhale JP, Mahajan HS, Surana SJ. Quercetin loaded nanoemulsion-based gel for rheumatoid arthritis: *in vivo* and *in vitro* studies. Biomed Pharmacother. 2019;112:108622. doi: 10.1016/j.biopha.2019.108622, PMID 30797146.
22. Ahmadi Oskooei F, Mehrzad J, Asoodeh A, Motavalizadehkakhky A. Olive oil-based quercetin nanoemulsion (QuNE)'s interactions with human serum proteins (HSA and HTF) and its anticancer activity. J Biomol Struct Dyn. 2023;41(3):778-91. doi: 10.1080/07391102.2021.2012514, PMID 34919017.
23. Altamimi MA, Hussain A, Alshehri S, Imam SS, Alnemer UA. Development and evaluations of transdermally delivered luteolin loaded cationic nanoemulsion: *in vitro* and ex vivo evaluations. Pharmaceutics. 2021;13(8):1218. doi: 10.3390/pharmaceutics13081218, PMID 34452179.
24. Ghazya OA, Fouadb MT, Saleha HH, Kholifb AE, TA Morsy. Ultrasound-assisted preparation of anise extract nanoemulsion and its bioactivity against different pathogenic bacteria. Food Chem. 2021 Mar 30;341(2):128259. doi: 10.1016/j.foodchem.2020.128259.
25. Cavar S, Maksimovic M, Solic ME, Jerkovic Mujkic A, Besta R. Chemical composition and antioxidant and antimicrobial activity of two satureja essential oils. Food Chem. 2008;111(3):648-53. doi: 10.1016/j.foodchem.2008.04.033.
26. Maccelli A, Vitanza L, Imbriano A, Fraschetti C, Antonello Filippi, Paola Goldoni, Linda Maurizi, Maria Grazia Ammendolia, Maria Elisa Crestoni, Simonetta Fornarini, Luigi Menghini, Maria Carafa, Carlotta Marianecci, Catia Longhi, Federica Rinaldi. *Satureja montana* L. essential oils: chemical profiles/phytochemical screening, antimicrobial activity and o/w nanoemulsion formulations. Pharmaceutics. 2019 Dec 19;12(1):7. doi: 10.3390/pharmaceutics12010007.
27. Santos Rodrigues AP, Faria E Souza BS, Alves Barros AS, de Oliveira Carvalho H, Lobato Duarte J, Leticia Elizandra Boettger M. The effects of rosmarinus officinalis L. essential oil and its nanoemulsion on dyslipidemic wistar rats. J Appl Biomed. 2020 Dec;18(4):126-35. doi: 10.32725/jab.2020.016, PMID 34907765.
28. Navaei Shoorvarzi SN, Shahraki F, Shafaei N, Karimi E, Oskoueian E. *Citrus aurantium L.* bloom essential oil nanoemulsion: synthesis, characterization, cytotoxicity, and its potential health impacts on mice. J Food Biochem. 2020;44(5):e13181. doi: 10.1111/jfbc.13181, PMID 32173879.
29. Salehi F, Jamali T, Kavoosi G, Ardestani SK, Vahdati SN. Stabilization of Zataria essential oil with pectin-based nanoemulsion for enhanced cytotoxicity in monolayer and spheroid drug-resistant breast cancer cell cultures and deciphering its binding mode with gDNA. Int J Biol Macromol. 2020 Dec 1;164:3645-55. doi: 10.1016/j.ijbiomac.2020.08.084, PMID 32795576.
30. Rajan Sailaja G, Venkatraman Sriramavaratharajan, Ramar Murugan, Gopal Rao Mallavarapu, David Raj Chellappan. Vasorelaxant property of *Plectranthus vettiveroides* root essential oil and its possible mechanism. J Ethnopharmacol. 2021 Jun 28;274:114048. doi: 10.1016/j.jep.2021.114048.
31. Bashlouei SG, Karimi E, Zareian M, Oskoueian E, Shakeri M. *Heracleum persicum* essential oil nanoemulsion: a nanocarrier system for the delivery of promising anticancer and antioxidant bioactive agents. Antioxidants (Basel). 2022 Apr 25;11(5):831. doi: 10.3390/antiox11050831, PMID 35624695.
32. Lobato Rodrigues ABL, Martins RL, Rabelo EM, Tomazi R, Santos LL, Brandao LB. Development of nano-emulsions based on *Ayapana triplinervis* essential oil for the control of *Aedes aegypti* larvae. PLOS ONE. 2021 Jul 9;16(7):e0254225. doi: 10.1371/journal.pone.0254225, PMID 34242328.
33. Kotta S, Mubarak Aldawsari H, Badr Eldin SM, Alhakamy NA, Md S. Coconut oil-based resveratrol nanoemulsion: optimization using response surface methodology, stability assessment and pharmacokinetic evaluation. Food Chem. 2021;357:129721. doi: 10.1016/j.foodchem.2021.129721, PMID 33866243.
34. Liu MH, Li YF, Chen BH. Inhibition of melanoma cells A375 by carotenoid extract and nanoemulsion prepared from pomelo leaves. Plants (Basel). 2021;10(10):2129. doi: 10.3390/plants10102129, PMID 34685938.
35. Fernandez Luqueno F, Medina Perez G, Perez Soto E, Espino Manzano S, Peralta Adauto L, Perez Rios S. Bioactive compounds of opuntia spp. acid fruits: micro and nano-emulsified extracts and applications in nutraceutical foods. Molecules. 2021 Oct 25;26(21):6429. doi: 10.3390/molecules26216429, PMID 34770840.
36. Hong C, Dang Y, Lin G, Yao Y, Li G, Ji G. Effects of stabilizing agents on the development of myricetin nanosuspension and its characterization: an *in vitro* and *in vivo* evaluation. Int J Pharm. 2014 Dec 30;477(1-2):251-60. doi: 10.1016/j.ijpharm.2014.10.044, PMID 25445518.
37. Sun M, Gao Y, Pei Y, Guo C, Li H, Cao F. Development of nanosuspension formulation for oral delivery of quercetin. J Biomed Nanotechnol. 2010 Aug;6(4):325-32. doi: 10.1166/jbn.2010.1133, PMID 21323105.
38. Ben Shabat S, Yarmolinsky L, Porat D, Dahan A. Antiviral effect of phytochemicals from medicinal plants: applications and drug delivery strategies. Drug Deliv Transl Res. 2020 Apr;10(2):354-67. doi: 10.1007/s13346-019-00691-6, PMID 31788762, PMCID PMC7097340.
39. Li W, Ng KY, Heng PW. Development and evaluation of optimized sucrose ester stabilized oleanolic acid nanosuspensions prepared by wet ball milling with design of experiments. Biol Pharm Bull. 2014;37(6):926-37. doi: 10.1248/bpb.b13-00864, PMID 24882406.
40. Bhia M, Motallebi M, Abadi B, Zarepour A, Pereira Silva M, Saremnejad F. Naringenin nano-delivery systems and their therapeutic applications. Pharmaceutics. 2021;13(2). doi: 10.3390/pharmaceutics13020291, PMID 33672366.
41. Donoso Quezada J, Guajardo Flores D, Gonzalez Valdez J. Enhanced exosome-mediated delivery of black bean phytochemicals (*Phaseolus vulgaris L.*) for cancer treatment applications. Biomed Pharmacother. 2020 Nov;131:110771. doi: 10.1016/j.biopha.2020.110771, PMID 33152932.
42. Vanti G, Tomou EM, Stojkovic D, Ciric A, Bilia AR, Skaltsa H. Nanovesicles loaded with origanum onites and satureja thymbra essential oils and their activity against food-borne pathogens and spoilage microorganisms. Molecules. 2021 Apr 7;26(8):2124. doi: 10.3390/molecules26082124, PMID 33917147.
43. Tran TTD, Tran PHL. Nanoconjugation and encapsulation strategies for improving drug delivery and therapeutic efficacy of poorly water-soluble drugs. Pharmaceutics. 2019;11(7). doi: 10.3390/pharmaceutics11070325, PMID 31295947.
44. Samrot AV, Saigeetha S, Mun CY, Abirami S, Purohit K, Cypriyana PJJ. Utilization of *Carica papaya* latex on coating of SPIONs for dye removal and drug delivery. Sci Rep. 2021 Dec 31;11(1):24511. doi: 10.1038/s41598-021-03328-2, PMID 34972829.
45. Mahajanakatti AB, Deepak TS, Achar RR, Pradeep S, Prasad SK, Narayanappa R. Nanoconjugate synthesis of elaeocarpus ganitrus and the assessment of its antimicrobial and antiproliferative properties. Molecules. 2022 Apr 10;27(8):2442. doi: 10.3390/molecules27082442, PMID 35458641.