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Design and Development of Herbosomes: A Nove Strategy to Overcome the Bioavailability Hurdle of Quercetin

Nandkishor S. Talware[1,2*], Remeth J. Dias[3], V. Rama Mohan Gupta[4]

1.Research Scholar, Department of Pharmaceutical Sciences, Jawaharlal Nehru Technical University, (JNTU) Hyderabad, Telangana, India -500 085.

2.Shri Vile Parle Kelawani Mandal's Institute of Pharmacy, Dhule, Maharashtra, India -424 001.

3.Department of Pharmacy, Government Polytechnic, Vidyanager, Karad-Masur Road, Karad, Dist. Satara, Maharashtra, India-415 124

4.Pulla Reddy Institute of Pharmacy, Annaram, Medhak (Dt), Telangana, India -502 313.

ABSTRACT

Alike many allopathic lipophilic drug molecules, these natural phyto-constituents are potent, lipophilic, but pose problems like poor aqueous solubility, slower drug release profile, and, reduced bioavailability leading to inferior therapeutic efficacy. This creates scope as well as a challenge for researchers to overcome the above-mentioned problems while developing a formulation for poorly aqueous soluble phytoconstituents. Herbosome is one of the efficient techniques to improve these problems. Herbosome /Phytosome is nothing but the combination of liposome with phytoconstituents forming H-bond anchored amphiphilic drug-phospholipids complexes. Quercetin i.e. 2-(3,4-dihydroxyphenyl)-5,7-dihydroxy-4H-1-benzopyran-4-one is a polyphenolic flavonoid with potent and diverse biological effects like anti-inflammatory, anti-proliferative, and anti-mutagenic. However, like many other potent drugs, its usage is limited due to its poor aqueous solubility. To overcome this problem, an ameliorated complex of phospholipids loaded with quercetin was developed to improve its aqueous solubility for better absorption. Thus quercetin encapsulated in herbosomes/phytosomes was assessed for the phospholipid complex formation, appearance, surface, and porosity evaluation using different physicochemical tests like FT-IR, DSC, XRPD, 1H-NMR, SEM, and solubility studies. Apart from this anti-oxidant activity of quercetin was evaluated in vitro. The surface characteristics of herbosomes appeared to be flocculent and permeable with a coarse surface in SEM studies whereas FTIR, DSC, 1H-NMR, and XRPD data, confirmed the formation of the phospholipids complex. There were 12 folds improvement in aqueous solubility of per se quercetin and quercetin encapsulated in herbosome (i.e. from 3.44 µg/ml to 36.81 µg/ ml). On the other hand, the results of in vitro anti-oxidant activity of phytosomic quercetin showed no significant statistical difference compared to per se quercetin thus indicating no adverse effects of complexation on quercetin's availability for anti-oxidant activity. Further, we prepared tard-gelatin capsules containing phytosomic quercetin and evaluated them for drug release, drug content, and solubility studies like dissolution, disintegration, drug content, and stability studies. The results for the evaluation of the kinetics of drug release were in line with the Korsmeyer Peppas model. The drug stability studies did not affect the drug's organoleptic properties, disintegration time, drug content, and in-vitro drug release of the formulation.

Keywords: Quercetin, Aqueous solubility, Herbosome, Drug release, and in vitro study.

*Corresponding Author Email: nandkishorwani@rediffmail.com

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INTRODUCTION

Phyto-active constituent quercetin

In today's era, plants, herbal, and natural medicines gaining more and more importance as a valuable source of treating many diseases, and some have already been established as the standard line of treatment for many ailments. Many developing countries, mostly Asiatic and African countries have been using and still using these natural medicines effectively as a primary line of treatment for various diseases, disorders, and ailments as a part of their tradition and culture. Whereas developed countries now have accepted these natural remedies as alternative medicines as they have greater efficacy along with lesser or no toxic side effects, are very cost-effective as compared to modern allopathic, and are available not only at the drug store but also at doorsteps, in supermarkets, and food stores. [1] These days, intensive investigation of plant-based natural medicines for isolation, identification, of potent individual phytoconstituents, and their subsequent evaluation for various biological and pharmacological activities have become the preferred area for their preclinical and clinical research. [2] Quercetin i.e. 2-(3, 4-dihydroxyphenyl)-5, 7-dihydroxy-4H-1-benzopyran-4-one is one of them. Quercetin is a flavonol, which is one of the six sub-categories of flavonoids. This is a polyphenolic flavonoid derived from various plants and vegetable sources, such as shallots, berries, tomatoes, capers, dill, onions rapes, cilantro, apples, tea, and lovageas well as in many flowers, seeds, barks, leaves, and nuts. Quercetin is yellow colored lipophilic compound soluble in lipids and alcohol but poorly soluble and insoluble in hot water and cold water respectively. The name quercetin originated from flavonol containing plant called "Quercetum" also known as Oak Forest. Quercetin is phytochemical and not found in the human body i.e. it is not an endogenous compound. [3]

The International Union Pure and Applied Chemistry (IUPAC) name, the chemical formula, and the molecular weight for Quercetin is as follows: 2-dihydroxy phenyl)-3,5,7-trihydroxychromen-4-one, C15H10O7, and 302.236 g/mol respectively. [4]

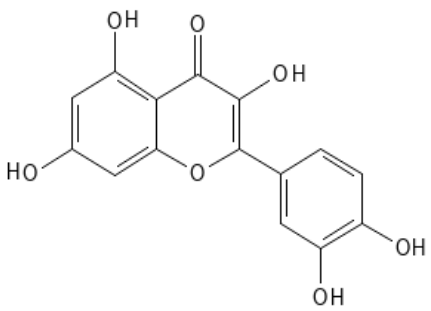

Figure 1: Chemical structure of Quercetin

Quercetin is one of the significantly potent molecules of natural origin that possess diverse pharmacological activities, such as anticancer, antiviral, anti-allergic, and anti-arthritis, and also used in the treatment of multiple disorders and diseases like metabolic, inflammatory, cardiovascular, and eye. [5] Quercetin is extensively studied in cancer treatment and prophylaxis. [6–8] It is well documented for its psychostimulant activity as well as for its action against capillary permeability, lipid peroxidation, mitochondrial biogenesis, and platelet aggregation. [5,9]

Sources of Quercetin and Its Pharmacological Activity

Many plants, grains, vegetables, and fruits are major sources of quercetin. The plants' species, including Momordica charantia, Santalum album, Cuscuta reflexa, Withania somnifera, Psoralea corylifolia,Calamus scipionum, Emblica officinalis, Foeniculum vulgare, Mangifera indica, Daucus carota, Ocimum sanctum, Curcuma domestica valeton, Swertia chirayita, Solanum nigrum, Coriandrum sativum, Asparagus officinalis, Glycyrrhiza glabra, Morua alba, Camellia sinensis, Prunus domestica, Allium fistulosum, A. cepa, Centella asiatica, Hypericum hircinum, H. perforatum, Vaccinium oxycoccus, Nasturtium officinale, Apium graveolens, Brassica oleracea var. italica, B. oleracea var. sabellica, Lactuca sativa, Capparis spinosa, P. avmm, Solanum Lycopersicum, Malus domestica, and Moringa oleifera. [5,10]

Antioxidant Activity

Quercetin is one of the major flavonols and contributes to a large portion of total flavonoids found, consisting of five classes of hydroxyl groups, 3, 5, 7, 3’, and 4' of the basic flavonol skeleton. [11] Some studies have shown that quercetin and its hydroxyl glycosylated derivatives have a structural contribution toward its antioxidant and anti-inflammatory activity. [12] The structural modification of quercetin at 3 hydroxyl position has shown a reduction in antioxidant activity as the total activity gradually decreased from quercetin > tamarixetin = isorhamnetin > quercetin-3-0-glucuronide > isorhamnetin-3-0- glucoside > quercetin-3, 5, 7, 3’, 4’-pentamethyl ether > quercetin-3,4' -di-glucoside. [13]

Quercetin modulate oxidative stress and antioxidant enzymes in turn helping prevent many cancers, such as cervical, lung, breast, prostate, liver, and colon cancers. [14] Sharmila et al. documented that quercetin not only facilitated to increase in the levels of antioxidant enzymes but also boosted the apoptosis proteins in animals infected with prostate cancer. In addition to this, they also found that quercetin controlled the expression of many protein factors like androgen receptors (AR), protein kinase B (AKT), insulin-like growth factor receptor 1 (IGFIR), and cell proliferation and anti-apoptotic proteins that usually shown to be increased in cancer. [15,16]. Quercetin effectively exerts cardioprotective action by downgrading the damage and injury to

myocardial cells by lowering ROS, increasing TNF-α, and preventing the overload of Ca2+ ions. [17–19] Quercetin as a highly effective antioxidant has also been shown to counter oxidative stress in rats with streptozotocin-induced diabetes mellitus, where the administered dose levels of quercetin dose were 25-50 mg/kg. [20]

Moreover, the ability of quercetin to form stable chelated complexes with toxic metal ions such as cadmium as quercetin-cadmium complexes. These complexes reduce oxidative stress by regulating the oxidant antioxidant equilibrium. [21] Quercetin has been reported to cure multiple other damaged tissue conditions by regulating oxidative factors like radiation-related brain damage in rats, oxidative damage caused by acrylamide, neurodegenerative disorders, oxidative stress induced by cadmium fluoride, and nerve damage in diabetic rat retinas. [22]

Antiviral Activity

Quercetin illustrates the broad range of antiviral activity as it effectively counteracts the human T-lymphotropic virus and a mosquito-borne disease Japanese encephalitis caused by the Japanese encephalitis virus (JEV). [23] Quercetin is active against the dengue virus type-2 and hepatitis C virus by suppressing the nonstructural protein 3-protease activity. [24,25] Other examples of quercetin formulations like quercetin-3-O-P-D-glucuronide, quercetin 7-rhamnoside, and quercetin-enriched lecithin, that are active against are influenza-A virus and porcine epidemic diarrhea virus respectively. [26]

Antimicrobial Activity

The potent broad-spectrum bacteriostatic activity of quercetin is studied against many strains of bacteria, such as Gram-positive like S. aureus, Staphylococcus epidermidis, Micrococcus luteus and Gram-negative like Helicobacter pylori, Pseudomonas aeruginosa, Yersinia enterocolitica, Salmonella enterica serotype Typhimurium, Campylobacter jejuni, and P. fluorescens. [5,27] Some other pieces of evidence suggest that quercetin is active against Shigella flexeneri NCIM5265 and Lactobacillus casei var Shirota in a study reported by Jaisinghani et. al. and in addition quercetin was found to have powerful antifungal activities against Cryptococcus neoformans and Aspergillus niger Candida albicans. [28]

Antiprotozoal Activity

Quercetin is a broad-spectrum antimicrobial agent active against many bacteria, and viruses as well as various protozoan parasites, like Toxoplasma, Theileria, Babesia, Trypanosoma, and Leishmania. It has potent growth inhibitory efficacy against Trypanosoma brucei rhodesiense, Leishmania donovani, T. cruzi, and T.brucei brucei parasites in vitro and in vivo. [5, 29] The potent leishmanicidal and trypanocidal activity in vitro are also reported, with an IC50 of 1.0 µg/mL and

8.3 µg/mL, respectively. Whereas in one of the in vivo studies only quercetin was shown to have inhibitory activity against the L. donovani among the other six flavonoids used. [30] In another study, Weisset al. showed that quercetin possesses remarkable inhibitory activity against Toxoplasma gondii by ceasing the heat shock protein 90 (hsp90), hsp27, and hsp70 production, interfering in the development of bradyzoite. [31] The anti-malarial activity against chloroquine-resistant (7G8), and chloroquine-sensitive (3D7) strains of Plasmodium falciparum documented by Lehane and Saliba as an antiplasmodial agent. [32]

Anti-Inflammatory Effects of Quercetin

Quercetin is a potent and long-acting anti-inflammatory agent. [9,12] As many studies suggested that quercetin inhibits the production of inflammatory agents in various cells like lipopolysaccharide (LPS)- mediated tumor necrosis factor-a (TNF-α) in macrophages and the development of IL-8 induced LPS in lung A549 cells. In addition, quercetin also plays role in protecting against neuronal cell death by apoptosis resulting from microglial activation by hindering Interleukin (IL)-lα and TNF-α levels stimulated by LPS-induced mRNA. [9, 33] Apart from this quercetin suppress and/or regulates the synthesis of multiple other factors involved in inflammation like cyclooxygenase (COX), lipoxygenase (LOX), tyrosine phosphorylationphosphatidylinositol-3-Kinase (PI3K)-(p85)mediated by Syk- and Src, Toll-like Receptor 4 (TLR4)/MyD88/PI3 K complex formation, hindering the subsequent signaling pathway in RAW264.7cells. [34] Quercetin is possibly involved in preventing the discharge of pro-inflammatory factors like cytokines, histamine, and tryptase from human umbilical cord blood-derived mast cells; and also possibly reserving the calcium influx and Phospho-protein kinase C (PKC). [35,36] Quercetin plays a significant role in the inflammatory process by upregulating the gene expression of interferon-γ (IFN-γ) in T helper cell-1 (Th-1) and down-regulating IL-4 in Th-2 by affecting the normal peripheral blood mononuclear cells (PBMC). [9,37]

Quercetin has proven itself as active against many inflammation-causing factors like nuclear factor-kappa B (NF-kB), activator protein 1(AP-l) active nitric oxide synthase, (NOS),COX-2, reactive C-protein (CRP) expression, and mitogen-activated protein kinase (MAPK). [38] But due to its poor absorption through the upper skin layer, quercetin and its glycoside derivatives rendered ineffectual in countering topical inflammation. Whereas, in one study the structurally modified potent topical anti-inflammatory derivative of quercetin i.e. pentamethyl ether has shown higher penetration and absorption through the rat skin surface. [39] In addition to the prevention of the release of several pro-inflammatory factors like TNF-α caused by bacterial LPS in macrophages, IL-IP, iNOS, and TNF-α secretion in RAW2647 cells, quercetin was also reported to have anti-

inflammatory activity against intracellular cell adhesion molecule (ICAM-1) expression, E-selection in human umbilical vein endothelial cells and cytokine-stimulated vascular cell adhesion molecules (VCAM-1). [40] In one of the studies carried out in NASH mice, quercetin and rutin significantly reduced the formation of inflammatory markers like IL-6andTNF-α. [9, 41]

Hepatoprotective and Antihypertensive Activities

The hepatoprotective activity of quercetin studied in D-galactosamine- and LPS-treated rats. [42] Another study carried out by Liu et.al, mentioned the possibility of quercetin being an appropriate hepatoprotective natural product in treating ethanol-induced oxidative damage in rat hepatocytes. [43] The antihypertensive activity of quercetin in spontaneously hypertensive rats carried out by Duarte et al. showed that chronicle administration of quercetin results in dose-dependent and potential reduction in rat blood pressure. [44]

Keiko Morikawa *et al.,* 2003, examined the effect of quercetin on the inflammatory response induced by carrageenan in the rat. The Air pouches were induced subcutaneously on the backs of rats and injected with carrageenan. The rats were treated with either vehicle or quercetin at a dose of 10 mg/kg one hour before carrageenan challenge. Forty-eight hour after carrageenan challenge, the air pouches were removed and analyzed and found that the volume, protein amounts and cell counts in the exudation obtained from the quercetin-treated animals were significantly reduced compared to those from vehicle treated animals. Osonga *et al.,* 2018, documented that quercetin derivatives e.g., quercetin 4′,5-diphosphate (QDP), quercetin 5′-sulfonic acid (QSA), and quercetin 3′,4′,3,5,7-pentaphosphate (QPP) resulted in highly biocompatible, soluble, and potent antibacterial activity with 100% inhibition of Listeria monocytogenes, Aeromonas hydrophila, and Pseudomonas aeruginosa. Ademosum *et al.,* 2016, studied the in vitro inhibition of acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) activities, inhibition of Fe(2+)-induced lipid peroxidation in rat's brain homogenates, radicals scavenging, and Fe(2+)-chelating abilities of the flavonoids were investigated with concentration of the sample ranging from 0.06 to 0.6 mM, and concluded that Quercetin had significantly higher AChE and BChE inhibitory abilities than rutin. Quercetin also had stronger inhibition of Fe (2+)-induced lipid peroxidation in rat's brain homogenates. Similarly, quercetin had higher radical scavenging and stronger Fe(2+)-chelating ability than rutin. S.H. Hakkinen *et al.,* 1999, analyzed the amounts of quercetin in 25 edible berries by an optimized RP-HPLC method with UV detection and identified with diode array and electrospray ionization mass spectrometry detection. And found that Quercetin was found in all berries, the contents being highest in bog whortleberry (158 mg/kg), lingonberry (74 and 146 mg/kg), cranberry (83 and 121 mg/kg), chokeberry (89 mg/kg), sweet rowan (85 mg/kg),

rowanberry (63 mg/kg), sea buckthorn berry (62 mg/kg), and crowberry (53 and 56 mg/kg). Gibellini *et al*., 2011, reported that quercetin is considered to be a strong anticancer candidate due to its chemoprotective activity through metastasis and apoptosis against tumor cell lines. Sahyon *et al.*, 2019, studied and reported the combination effect of sulfamethoxazole with quercetin against S. aureus, and quercetin has been shown to reduce the side effects of sulfamethoxazole while improving its bactericidal efficacy, indicating the importance of this combination therapy for the treatment of human clinical cases. Maiti *et al.*, 2005 developed the quercetin phospholipid complex in carbon tetrachloride to overcome the absorption of herbal formulation and it exerted better therapeutic efficacy to induced acute liver injury in rats. Qu *et al.*, 2019, reported the synergetic effect of quercetin-tetracycline combination treatment against multi-drug resistant (MDR) E. coli by disrupting the bacterial cell envelope, thus improving its permeability and cell lysis.

The present study deals with the development of quercetin-phospholipid complex (quercetin Herbosome) with an aim of improving the aqueous solubility of quercetin for better absorption through the GI tract, which might result in improved bioavailability. The prepared complex was characterized for various physico-chemical parameters.

MATERIALS AND METHOD

Quercetin was procured Yucca phytochemicals, Mumbai. Di-Stearoyl-Phosphatidyl-Choline (DSPC) was procured from Lipoid, Germany. Ethanol, n-hexane, Methanol, n-octanol, Dichloromethane, were purchased from Fine Chem Industries, Mumbai whereas DPPH from HiMedia Lab. Mumbai. All the chemicals and reagents used in this experiment were of Analytical grade.

Preparation of Quercetin-loaded herbosomes

The thin-film hydration/solvent evaporation technique was used to prepare herbosomes (Ruozi et al. 2005). Using a stable molar ratio, phospholipids, cholesterol, and quercetin were dissolved in 25 mL of a ethanol-chloroform combination (3:2). To get rid of any remaining solvent and also to create a film, the mixtures were evaporated in a rotary evaporator (Heidolph 591-01610-00 Hei-CHILL 350 Chiller for Hei-VAP Rotary Evaporators) for 15 minutes at a speed of 45-70 rpm and a temperature of 40-60°C. At room temperature, which was above the lipid transition temperature, the film was hydrated with phosphate buffer (pH 7.4) for 1 hour. After being homogenized with a probe sonicator (FS-500, Frontline), n-hexane was added to precipitate out quercetin herbosomes. Further the herbosome complex was filtered, dried and stored in an air tight container until further usage.

Characterization of Phytosome

Particle size, particle size distribution and Zeta potential. [1]

The particle size, polydispersity index (PDI) and zeta potential were calculated by the dynamic diffraction method using Malvern Zetasizer, UK. The quercetin phytosome suspension was mixed with double distilled water and then analysis was performed at 25^{o}C.

Determination of %Yield [1]

Determination of %Yield of herbosome was calculated by using the following formula.

% Yield = (Practical Yield/ Theoretical Yield) x 100

Fourier transform infrared spectroscopy (FTIR)

FTIR spectra of Quercetin, DSPC, physical mixture containing quercetin & DSPC, and Quercetin Herbosome were recorded by Fourier Transform Infrared Spectroscopy (Perkin Elmer Fourier Transform Infrared Spectrophotometer, Perkin Elmer, UK). Samples were analyzed in the 4000 to 400 cm^{-1} spectrum [1].

Differential scanning calorimetry (DSC)

DSC thermograms of Quercetin, DSPC, physical mixture containing quercetin & DSPC, and Quercetin Herbosomes were recorded using a differential scanning calorimeter (2910 Modulated DSC, TA Instruments, US). The thermal behavior was investigated by heating 2.0 ± 0.2 mg of each individual sample sealed in an aluminum pan under flow of nitrogen gas. The studies were performed over the temperature range 0-300°C with a scanning rate of 10°C/min [1].

X-Ray Diffraction

The crystalline state of drug (quercetin) in different samples was analyzed with X-ray powder diffraction (XRPD). The X-ray diffraction patterns were recorded using a Bruker Axs-D8 Discover Powder X-ray diffractometer.

Scanning Electron Microscopy (SEM)

Scanning electron microscopy study was performed to examine the surface morphology, size and shape of quercetin and the complex. SEM imaging of the samples were performed using a Scanning Electron Microscope [1].

Encapsulation efficiency

By quantifying the quantity of quercetin trapped in the produced herbosomes, the degree of encapsulation was assessed. Using a REMI-C24 cooling centrifuge, 10 ml of the herbosomes vesicular suspension was put in a centrifuge tube and spun at 5000 rpm for 10 min. The supernatant was collected after centrifugation and put through a Whatman filter with a pore size of 45 m. The amount of free quercetin was then measured spectrophotometrically at 256 nm after

being appropriately diluted. The calibration curve method was used to calculate the amount of quercetin, and a series of quercetin solutions at various concentrations were used to create the calibration plot. The following equation has been used to determine the encapsulation efficiency.

$$EE\ \% = W_{(Added\ drug)} - W_{(free\ drug)} / W_{(Added\ drug)} \times 100$$

Where, $W_{(Added\ drug)}$ is the total amount of drug added during the preparation of herbosomes, $W_{(free\ drug)}$ is the amount of free drug. [1]

Solubility studies

By adding extra quercetin and quercetin phytosomes to 5 ml of water or n-octanol in a glass container at room temperature, the apparent solubility investigation was conducted (25-30oC). To get rid of extra quercetin, the liquids were stirred for 24 hours. After that, they were centrifuged for 20 minutes at 1000 rpm. The supernatant was filtered using a membrane filter with a mesh size of 0.45 m, and then samples were quantified using a UV spectrophotometer at 517 nm by diluting 1 ml of the filtrate with 9 ml of distilled water or n-octanol.

In vitro release studies [1]

In vitro quercetin release from quercetin herbosmes was performed using the dialysis bag method. Artificial gastric environment using 0.1 M HCl with pH 1.2 and intestinal environment using phosphate buffer solution (PBS) with pH 6.8 and 7.4 without enzymes were used as dissolution medium. The dialysis membranes (Himedia© LA 387) with a molecular weight cut-off of 12-14 kilo Dalton were used to hold quercetin herbosmes. The dialysis membranes were soaked in double distilled water for 12 hr prior to use. Quercetin herbosmes was redispersed in double distilled water, and 1 ml of suspension was added to the membranes, which were tightly bundled at the two ends. The bags were placed in 100 ml of the dissolution media in a beaker. The beaker was shaken in a magnetic stirrer (Remi, India) at speed of 60 rpm and temperature 37±1°C. Aliquots of sample were collected periodically and refilled with a fresh dissolution medium for maintaining the sink condition. The amount of drug released from quercetin herbosmes was analyzed UV spectrophotometer at 517 nm. All the operations were carried out in triplicate.

Dissolution efficiency (DE) [1]

The % Dissolution efficiency of quercetin, DSPC, physical mixture containing quercetin & DSPC, and quercetin herbosome was determined in different medias at different time intervals according to the following equation:

$$\frac{\int_0^t y.dt \ .\ 100}{y_{100}.t}$$

Where y is the percentage of drug dissolved at time (t). Dissolution efficiencies were determined in HCl at pH 1.2 and phosphate buffer at pH 6.8 and pH 7.4. The area under the curve (AUC) was calculated at each time point by the trapezoidal rule.

Anti-Oxidant Activity [1]

The free radical scavenging activity of quercetin and quercetin herbosomes were measured and compared with the activity of Butylated hydroxyanisole (BHA) for free radical-scavenging ability using the stable free radical 2, 2-diphenyl-1-picrylhydrazyl (DPPH). The free radical scavenging activities of quercetin, quercetin herbosomes and BHA were monitored spectrophotometrically and measured by decrease in the absorbance of methanolic solution of 0.1 mM solution of DPPH. 1.5 ml of DPPH solution was added to 3.5 ml methanolic solution of quercetin, quercetin complex and BHA with different concentration ranging from 20-250 µg/ml. After 30 mins, the absorbance of samples were measured at 517 nm. Lower absorbance of the reaction mixture depict higher free radical scavenging activity. The percentage of capability to scavenge the DPPH free radical was calculated according to the following formula:

$$\text{DPPH Scavenged (\%)} = [(A_{control} - A_{test}) / A_{control} \times 100]$$

Where, $A_{control}$ = Absorbance of the control

A_{test} = Absorbance of test.

RESULTS AND DISCUSSION

Preparation of Quercetin loaded phytosomes

Determination of quercetin in Quercetin-phospholipid complex

Formula Optimization

Different Phytosome complexes containing different molar ratios of Quercetin: phosphatidylcholine were prepared and evaluated as shown in table 1.

Table 1: Formula Optimization

Formulation	Molar ratio (Quercetin: PC)	Average Particle size (nm)	PDI	Zeta potential(mV)	% Yield
F1	0.5:1	251.23 ± 2.32	0.302 ± 0.019	-35.8 ± 1.6	92.15
F2	1:1	217.90 ± 1.45	0.251 ± 0.031	-39.2 ± 1.2	95.91
F3	1:0.5	230.69 ± 2.19	0.283 ± 0.033	-31.5 ± 2.4	93.65

Particle size, particle size distribution and Zeta potential-

The Phytosomal formulation with smaller Particle size, zeta potential <±30 mV and greater percentage yield is desirable. Based on obtained results, the F2 formula with the optimal zeta potential and Polydispersity index (PDI) was chosen to prepare the quercetin phytosome.

Infrared Absorption

IR spectroscopy was used in order to investigate the possible interaction between quercetin and PC in the phytosome. In IR spectrum of phosphatidylcholine, the distinctive C-H stretching band of long fatty acid chain at 2918 cm^{-1} and 2850 cm^{-1}, C=O stretching band at 1738 cm^{-1}, P=O stretching band at 1236 cm^{-1}, P-O-C stretching at 1091 cm^{-1} and N^{+} $(CH_3)_3$ stretching band at 970 cm^{-1} can be seen.

The primary characteristic bands for the O-H stretching at 3325 cm^{-1}, C=O stretching band at 1614 cm^{-1} and benzene ring vibrations near 1522 cm^{-1} were observed in quercetin molecule. In case of complex, FTIR showed the significant changes in the spectrum and the absorption peaks of hydroxyl and keto (C=O) groups of the quercetin have been shifted to higher wave number, while the P=O absorption band of the phosphatidylcholine considerably broadened.

The spectrum of the physical mixture was moderately different from that of the Quercetin loaded phytosome and displayed the same vibrational frequencies as of the individual components and seemed to be only a sum of both the components. Thus, the spectroscopic changes showed that, the shifting of hydroxyl and keto group frequencies of quercetin from their original position accounts for the interaction of quercetin to polar end of the phosphatidylcholine.

Differential scanning calorimetry

Differential scanning calorimetry (DSC) is a quick and accurate method for detecting drug-excipient compatibility and obtaining the most information about the possible interactions. In thermograms, an interaction is concluded by elimination of endothermic peaks, appearance of fresh peaks and changes in different parameters of thermogram such as peak shape, its onset, peak melting point / temperature and relative peak area or enthalpy.

Phosphatidylcholine showed two major endothermic peaks at 83.21°C and 107.90°C as well as a smaller peak at 64.45°C. The first peak of Phosphatidylcholine is mild peak at 64.45°C, which is possibly due to the heat-induced movement of phospholipids polar head group. The second peak at 83.21°C is very sharp and it appears as a result of phase transition from gel to liquid crystalline form. During this phase, the non-polar hydrocarbon tail of phospholipid may be melted, resulting in a sharp peak. This melting could have occurred in two stages which subsequently resulted in another peak at 107.90°C which is comparatively less sharper.

The DSC curve for Quercetin showed a sharp endothermic peak at 122.38°C. On the other hand, a complete disappearance of the melting endothermic peaks of the individual components (quercetin, Phosphatidylcholine) with reduced enthalpy and melting points was observed for the Phytosomes . In comparison with free quercetin, it showed a significant reduction in the enthalpy of 71.48 J/g and the lowest melting point at 60.02°C indicating the formation of the Quercetin-PC complex.

Reduction in enthalpy and melting point explains for increased solubility and reduced crystallinity of the drugs. This phenomenon can be implicit the interactions between component and the phospholipid in the complex system and can be considered as sign of complex formation.

X-ray Powder Diffractometry

In the X-ray diffractogram, quercetin showed intense diffraction peaks for crystallinity at a diffraction angle of 2θ indicating that the drug is present in a crystalline form. The Phosphatidylcholine showed a single diffraction peak. Drug amorphization was induced by complex formation where X-ray diffraction patterns of quercetin-PC complex were characterized only by large diffraction peaks in which it was not possible to distinguish the characteristic peaks of the drug. The results, indicate that quercetin is no longer present in a crystalline state and its phospholipid complex is in an amorphous form. Thus, X-ray diffractogram data supports the DSC findings which indicated the reduced crystallinity of drug in the prepared nanophytosome by showing lower values of melting points and enthalpy. As the amorphous form of the drug is absorbed better than their crystalline form, the quercetin loaded nanophytosome may serve as a potential approach for effective drug delivery of their parental analogue.

Proton nuclear magnetic resonance spectroscopy

The ^{1}H-NMR spectrum of quercetin showed characteristics H-shift described: ^{1}H-NMR (500 MHz, DMSO-d6), δ10.77 (s, 1H, 7-OH); δ9.57 (s, 1H, 3-OH); δ9.32 (s, 1H, 3'-OH); δ9.28 (s, 1H, 4'-OH). The spe $^{(c)}$ of Phosphatidylcholine showed characteristics H-shift ^{1}H-NMR (500 MHz, DMSO-d6), δ3.17 (s, 9H). When the chemical shifts of quercetin were compared to that of phytosome complex, the key differences were seen in both the downfield aromatic region ($\delta > 7$) and the up field region ($\delta < 4$). The N-methyl groups of phytosomes were observed at δ3.32 whereas Phosphatidylcholine showed this group at δ 3.17. The change in proton signals in the aromatic region indicates the formation of molecular aggregates with quercetin. The charged head of the Phosphatidylcholine molecule was involved in weak intermolecular interaction such as hydrogen bond and ion-dipole interaction with the phenolic (7-OH, 3-OH, 3'-OH, 4'-OH). This was supported by the decreased intensity of these proton signals and their characteristic downfield chemical shifts. This showed the embedding of quercetin in the Phosphatidylcholine and hence confirmed the formation of the phytosome complex.

Scanning Electron Microscopy

The scanning electron micrographs of quercetin and the quercetin loaded nanophytosome are given. The quercetin was characterized as needle-like crystals with an apparently smooth surface, regular shape and smaller size. On the other hand, the phospholipid complex showed a drastic

change in the morphology and shape of particles, exhibiting a fluffy, porous and rough surface, indicating an apparent interaction in the solid-state that may have resulted in improved solubility and enhanced dissolution rate when compared to pure drug.

Encapsulation efficiency

The percentage of content was estimated as the difference between the initial quantity and the unentrapped quantity of drug in supernatant with respect to the total quantity of drug incorporated in phytosomal complex and was found to be 87.6 ± 0.4%.

Solubility Study

Quercetin is extremely hydrophobic in nature (log P = 1.82 ± 0.32) and slightly soluble in aqueous medium. Its poor aqueous solubility causes poor absorption or permeation through the intestinal epithelial cells of the gastrointestinal tract leading to low bioavailability of the quercetin drug. The prepared nanophytosomes improved the aqueous solubility of quercetin (3.44 µg /ml) by 12 folds (36.81 µg/ml) as shown in Table 2. Phosphatidylcholine, as an amphiphilic surfactant, could increase the solubility of the drug by the action of wetting, dispersion and micellisation. Micellisation is an significant approach able of solubilizing a hydrophobic drug in a hydrophilic environment consisting of biodegradable drug carrier micelle and a hydrophobic drug, in which the drug is compatible and not strongly bonded to the polymeric drug carrier micelle. The quercetin-PC complex showed improved solubility due to its amphiphilicity, which in turn may show the improved absorption across the biological barriers resulting in improved bioavailability of the quercetin.

Table 2: Solubility Study (Water/ n-Octanol) at 25 °C

Sample	Aqueous Solubility	n-Octanol Solubility
1.Quercetin	3.44 µg/ ml	52.17 µg/ ml
2. Quercetin loaded nanophytosome	36.81 µg/ ml	51.92 µg/ ml

Anti-Oxidant Activity

Quercetin and its phytosome showed 90.76 %, 89.82 % inhibition of DPPH at a concentration of 20 µg/ml respectively and 90.82 %, 90.70 % at a concentration of 200 µg/ml respectively. There was no statistical difference ($n = 6$, $p<0.01$) in the % inhibition of DPPH between the quercetin and its nanophytosome, at various concentrations ranging from 20-250 µg/ml (Figure). As a result, the antioxidant activity of the quercetin remains unchanged even after the quercetin- PC complex formation and it can be concluded that the process of complexation did not significantly affect the antioxidant activity of quercetin.

Stability Study

Stability Study as per ICH Guideline Stability study of hard gelatin capsule containing Powder mixture of Quercetin phytosome was done to see the effect of temperature and humidity on capsules during the storage time. Capsules were evaluated periodically for Organoleptic properties, disintegration time, drug content and in-vitro drug release. Stability study results show that there was no significant change in Organoleptic properties like shape, colour, disintegration time, drug content and in-vitro drug release of the formulation for Short term Stability Study and Accelerated stability studies shown in Table 3 and Table 4respectively.

Table 3: Short term Stability Study

Time interval (months)	Shape	colour	disintegration time (min)	% drug content	% drug release
0	Cylinder with hemispherical ends	Pale Yellow	4.5±0.471	96.97±0.633	99.04±0.318
1	Cylinder with hemispherical ends	Pale Yellow	4.8±0.523	96.15±0.461	98.87±0.530
2	Cylinder with hemispherical ends	Pale Yellow	4.8±0.279	95.97±0.674	98.09±0.581
3	Cylinder with hemispherical ends	Pale Yellow	5±0.322	95.91±0.396	98.09±0.424
6	Cylinder with hemispherical ends	Pale Yellow	5.3±0.691	95.04±0.510	97.83±0.267

Table 4: Accelerated stability studies

Time interval (months)	Shape	colour	disintegration time (min)	% drug content	% drug release
0	Cylinder with hemispherical ends	Pale Yellow	4.5±0.471	96.97±0.633	99.04±0.318
1	Cylinder with hemispherical ends	Pale Yellow	4.9±0.510	96.05 ±0.53	98.87±0.489
2	Cylinder with hemispherical ends	Pale Yellow	4.9± 0.396	95.97±0.581	98.09±0.
3	Cylinder with hemispherical ends	Pale Yellow	5.2±0. 691	95.91±0.279	98.09±0.424
6	Cylinder with hemispherical ends	Pale Yellow	5.2±0. 322	95.04±0.523	97.83±0.267

DISCUSSION

Quercetin is one of the most significant bioflavonoid compounds, found in a variety of foods including apples, berries, Brassica vegetables, capers, grapes, onions, shallots, tea, and tomatoes, as well as many seeds, nuts, flowers, barks, and leaves. It has unique biological properties that may improve mental and physical performance and reduce infection risk. These properties form the basis for potential benefits to overall health and disease resistance, including anti-carcinogenic,

antiviral, anti-inflammatory, antiviral, antioxidant, and psychostimulant activities, as well as the ability to inhibit lipid peroxidation, platelet aggregation and capillary permeability, and to stimulate mitochondrial biogenesis.

Quercetin shows a strong antioxidant activity by maintaining oxidative balance and Its application in the medicinal field has shown potential to improve human health due to its antioxidant activity in vivo. However, its application in the pharmaceutical field is limited by its low absorption into the body based on its poor solubility, low bioavailability, poor permeability, and instability. Phyto-phospholipid complexes also known as phytosomes have emerged as a promising strategy to enhance the bioavailability of active constituents. Phytosomes are advanced forms of herbal products that are better absorbed, utilized and as a result produce better results than conventional herbal extracts. It is a patented technology developed by Indena, enhancing the bioavailability of phytoconstituent. Phytosomes involve incorporation of water soluble phytoconstituent into phospholipids, which forms lipid compatible molecular complexes and so vastly improve their absorption and bioavailability. Furthermore, the production of complexes can protect phytoconstituent from destruction by external forces, such as hydrolysis, photolysis, and oxidation. The Phytosomal preparation of Quercetin containing different molar ratios of Quercetin: phosphatidylcholine were prepared and evaluated. On basis of Particle size, particle size distribution, % yield and Zeta potential Quercetin: phosphatidylcholine in 1:1 ratio was chosen to prepare the quercetin phytosome.

IR spectroscopy was used in order to investigate the possible interaction between quercetin and PC in the phytosome. The spectrum of the physical mixture was moderately different from that of the Quercetin loaded phytosome and displayed the same vibrational frequencies as of the individual components and seemed to be only a sum of both the components. Thus, this showed that, the shifting of hydroxyl and keto group frequencies of quercetin from their original position accounts for the interaction of quercetin to polar end of the Phosphatidylcholine.

In DSC thermograms, an interaction is concluded by elimination of endothermic peaks, appearance of fresh peaks and changes in different parameters of thermogram such as peak shape, its onset, peak melting point / temperature and relative peak area or enthalpy and confirms that they are compatible with each other. The results from X-ray diffractogram, indicate that quercetin is no longer present in a crystalline state and its phospholipid complex is in an amorphous form. Thus, X-ray diffractogram data supports the DSC findings which indicated the reduced crystallinity of drug in the prepared nanophytosome by showing lower values of melting points and enthalpy. The

1H-NMR spectra showed the embedding of quercetin in the Phosphatidylcholine and hence confirmed the formation of the phytosome complex.

The morphological studies were performed by SEM, the phospholipid complex showed a drastic change in the morphology and shape of particles compared to quercetin, exhibiting a fluffy, porous and rough surface, indicating an apparent interaction in the solid-state that may have resulted in improved solubility and enhanced dissolution rate when compared to pure drug. The prepared herbosomes of quercetin improved the aqueous solubility of quercetin (3.44 µg /ml) by 12 folds (36.81 µg/ml). The antioxidant activity of the quercetin remains unchanged even after the quercetin Phytosome formation and it can be concluded that the process of complexation did not significantly affect the antioxidant activity of quercetin and thus the bioactivity of the active ingredient.

Quercetin phytosomes were formulated into dosage form as hard gelatin capsules. Initially, Powder mixture containing Quercetin phytosome was prepared and evaluated for Preformulation parameters like Angle of repose, Bulk density tapped density, % compressibility and Hausner's ratio. The results indicated good micromeritic properties of Powder mixture containing Quercetin phytosome. Then powder mixture containing Quercetin phytosome was filled into the empty hard gelatin capsule shells. The hard gelatin capsules were then evaluated for dissolution studies, disintegration study, drug content, and Short term Stability Study and Accelerated stability study. All the evaluation parameters were found to be within limit. Kinetics and mechanism of drug release from Capsule was evaluated on the basis of Zero Order, First Order, Higuchi equation and Peppas model. Korsmeyer peppas model was found to be best fit with R^2 = 0.991. Short term stability study was performed for period of six months under the conditions of 25°C and 60% RH. Where as, Accelerated stability studies were performed for period of six months at 45°C and 75% RH and found that there was no significant change in Organoleptic properties like shape, colour, disintegration time, drug content and in-vitro drug release of the formulation indicating that capsules are stable for longer period of time.

CONCLUSION

According to the study's findings, herbosomes are a potentially effective medication delivery technology for increasing the bioavailability of the quercetin molecule. After forming a combination with the phospholipid, quercetin's physicochemical qualities underwent a significant alteration. In particular, the compound's increased solubility may have boosted the drug's oral absorption. Furthermore, it was observed that at all dose levels, there was no statistically significant difference in the percentage of DPPH inhibition between quercetin and its complex.

This shows that the bioactivity of quercetin was preserved after it was complexed with phospholipid and that the complex's formation did not alter or disrupt the molecular structure of the compound's active ingredient, quercetin. As these amphiphilic drug-lipid complexes have been reported to be stable and more bioavailable, the phospholipid complex of quercetin may serve as a value added herbal drug delivery system.

REFERNCES

1. Gandhi A, Dutta A, Pal A, Bakshi P., Recent Trends of Phytosomes for Delivering Herbal Extract with Improved Bioavailability. J Pharmacognosy and Phytochemistry 2012; 1(4): 6-14.
2. Yue PF, Yuan HL, Xie H. Preparation, characterization and bioavailability of ursodeoxycholic acid-phospholipid complex In vivo, Drug Dev Ind Pharm 2008; 34(7): 708-18.
3. Bombardelli E, Curri SB, Della Loggia R, Del Negro P, Tubaro A, Gariboldi P., Complex between phospholipids and vegetal derivatives of biological interest, Fitoterapia 1989; 60(1): 1-9.
4. Miean KH, Mohamed S., Flavonoid (myricetin, quercetin, kaempferol, luteolin, and apigenin) content of edible tropical plants, J Agric Food Chem 2001; 49(6): 3106-12.
5. Semalty A, Semalty M, Rawat BS, Singh D, Rawat MSM., Pharmacosomes: the lipid based novel drug delivery system, Expert Opin Drug Deliv 2009; 6(6): 599-612.
6. Abad MJ, Bermejo P, Villar A, Valverde S., Anti-inflammatory activity of two flavonoids from tanacetum microphyllum, J Nat Prod 1993; 56: 1164-7.
7. Verma AK, Johnson JA, Gould MN, Tanner MA., Inhibition of 7,12-Dimethyle anthracene and N-nitrosomethyleurea-induced rat mammary cancer by dietary flavonol quercetin, Cancer Res 1988; 48: 5754-8.
8. Lamson DW, Brignall MS., Antioxidants and cancer III: Quercetin; Altern Med Rev 2000; 5(3): 196-208.
9. Adel AKE, Amira AS, Hanan MAE, Nagwa IS. Protective effect of taurine and quercetin against renal dysfunction associated with the combined use of Gemtamycin and Diclofenac. Indian J Biochem Biophys 2008; 45: 332-40.
10. Heo HJ, Lee CY, Protective effects of quercetin and vitamin C against oxidative stress-induced neurodegeration, J Agric Food Chem 2004; 52: 7514-7.
11. Edwards RL, Lyon T, Litwin SE, Rabovsky A, Symons JD, Jalili T. Quercetin reduces blood pressure in hypertensive subjects, J Nutrition 2007; 137: 2405-11.

12. Leuner C, Dressmann J. Improving drug solubility for oral delivery using solid dispersions, Eur J Pharm Biopharm 2002; 54: 107-12.
13. Rabinow BE. Nanosuspension in drug delivery, Nat Rev Drug Discov 2004; 3: 785-96.
14. Patravale VB, Date AA, Kulkarni RM. Nanosuspensions: a promising drug delivery strategy, J Pharm Pharmacol 2004; 56: 827-40.
15. Kesisoglon F, Panmai S, Wu Y. Nanosizing oral formulation development and biopharmaceutical evaluation, Adv Drug Dev Rev 2007; 59: 63-644.
16. Nijlen TV, Brennan K, Mooter VG, Blaton N, Kinget R, Augustijns P. Improvement of the dissolution rate of artemisinin by means of supercritical fluid technology and solid dispersions, Int J Pharm 2003; 254: 173-81.
17. Nokhodchi A., The effect of type and concentration of vehicles on the dissolution rate of a poorly soluble drug (indomethacin) from liquisolid compacts, J Pharm Pharm Sci 2005; 8: 18-25.
18. Yanyu X, Yunmei S, Zhipeng C, Qineng P, The preparation of silybin-phospholipid complex and the study on its pharmacokinetics in rats. Int J Pharm 2006; 307: 77-82.
19. Imai T, Shiraishi S, Saito H, Otagiri M. Interaction of indomethacin with low molecular weight chitosan, and improvements of some pharmaceutical properties of indomethacin by low molecular weight chitosans, Int J Pharm 1991; 67: 11-20.
20. Zhang M, Li XH, Gong YD, Zhao NM, Zhang XF. Properties and biocompatibility of chitosan films modified by blending with PEG, Biomaterials 2002; 23: 2641-8.
21. Cai K, Rechtenbachb A, Haoa J, Bosserta J, Jandt KD. Polysaccharide protein surface modification of titanium via a layer-by-layer technique: characterization and cell behavior aspects, Biomaterials 2005; 26: 5960-71.
22. Maiti K, Mukherjee K, Gantait A, Saha BP, Mukherjee PK. Curcumin-phospholipid complex: Preparation, therapeutic evaluation and pharmacokinetic study in rats, Int J Pharm 2007; 330(1-2): 155-63.
23. Kalita B, Das M K, Sharma AK. Novel Phytosome Formulations in Making Herbal Extracts More Effective, Research Journal of Pharmacy and Technology 2013; 6(11):1295-1301.
24. Alam MA, Al-Jenoobi FI, Al-mohizea AM. Commercially bioavailable proprietary technologies and their marketed products, Drug Discovery Today 2013; 1-14.
25. Nikita Sanghavi, Yashwant Malode, Rashmi Srivastava. Isolation and identification of the flavonoid “quercetin” from Tridax procumbens Linn, International Journal of

Pharmaceutical Sciences and Research, 2014; 5(4)1454-1459.

26. Sivarajan VV et al. Ayurvedic drugs and their plant sources, Oxford & IBH publishing company, New Delhi 1994; 2-23 .
27. Bombardelli E. Oral compositions for the treatment of cellulite, Indena Spa, Milano, Italy 2010, Patent No. US 7691422.
28. Raju TP, Reddy MS, Reddy VP. Phytosome: A novel phyto-phospholipid carriers for herbal drug delivery, International Research Journal of Pharmacy 2011; 2(6):28-33.
29. Joshi V, Joshi RP. Some plants used in Ayurvedic & Homoeopathic Medicine, Journal of Pharmacognosy & Phytochemistry 2013; 2: 269-275.
30. Middleton E. and Kandaswami C. The impact of plant flavonoids on mammalian biology: Implications for immunity, inflammation and cancer, in the flavonoids, Advances in Research Science (Ed.) Harborne, I.R., Chapman and Hall, London 1993, 619-645.
31. Mahesh Chand Meena and Vidya Patni, Isolation and Identification of Flavonoid "Quercetin" from Citrullus colocynthis (Linn.) Schrad, Asian J Experimental Science 2008; 22(1):137-142.
32. Evans C. William. Trease and Evans Pharmacognosy, Elsevier Limited, sixteenth edition, 2009 pp. 132.
33. Lachman Leon, Lieberman A Herbert, Kanig L Joseph. The Theory and Practice of Industrial Pharmacy, Varghese publishing house, third edition, 1990, pp. 391,392.
34. Allen V Loyd Jr, Popovich G Nicholas, Ansel G Howard. Pharmaceutical Dosage Forms and Drug Delivery Systems. Lippincot Williams & Wilkins, eighth edition, 2005, 208-211.
35. Podczeck Fridrun, Jones E. Brian. “Pharmaceutical Capsules, published by pharmaceutical press, second edition, 2004, 11, 17.
36. Schulze Dietmar. Flow Properties of Powders and Bulk Solids, Research Gate, 2014: 1-21.
37. Li Xiao-yan, Chen Xi-guang, Liu Cheng-sheng, Liu Chenguang, Xue Yu-ping. Preparation of alginate-gelatin capsules and its properties, Frontiers of Materials Science in China, 2008; 2: 253-260.
38. N.M. Moursy, N.N. Afifi, D.M. Ghorab. Formulation and evaluation of sustained release floating capsules of nicardipine hydrochloride, Die Pharmazie. 2003; 58(6): 38-43.
39. Abirami A, Halith S Mohamed, Pillai KK. Formulation and evaluation of indomethacin extended release pellets, Int J Pharm Pharm Sci. 2014; 6: 247-250.
40. Pawar A Harshal and Lalitha KG. Development of an In Vitro Dissolution Method for Novel Formulation: A Systematic and Scientific Approach, Austin J Anal Pharm Chem.

2014; 1(4): 10-20.

41. Yadav Akanksha, Sahu K Atul, Singh AK, Gautam SS, Prasad Raj K. Formulation and Evaluation of Hard Vegetable Capsule for Healthy Brain Using Powder of Green Tea and Brahmi Extract, IJPPR. 2016; 6(1): 7-21.
42. Mircioiu C, Voicu V, Anuta V, Tudose A, Celia C, Paolino D, Fresta M, Sandulovici R, Mircioiu I et al. Mathematical Modeling of Release Kinetics from Supra molecular Drug Delivery Systems, Pharmaceutics. 2019; 11: 140.
43. De Pintu K, Paul Jibitesh, Dey Sanjoy K, Dinda Subas C, Rakshit Soumen. Formulation, Physico-chemical characterization and Release kinetic study of antihypertensive Transdermal Patches, Der Pharmacia Sinica, 2011; 2 (5): 98-109.
44. Dash Suvakanta, Murthy Narasima Padala, Nath Lilakanta Chowdhury P. Kinetic modeling on drug release from controlled drug delivery systems, Acta Poloniae Pharma Drug Res 2010; 67: 217-223.
45. Sowjanya N. J. Phytosome: A Novel Entity in Herbal Drug Delivery System: A review, International Journal of Pharma. Research & Development- 2010; 9: 153-164.
46. Manitha M., Srinivas P, Sadanandam, Phytosomes in Herbal Drug delivery, Journal of Natural Pharmaceuticals 2010; 1:14-18.
47. Sindhumol P G, Maria Thomas, Mohancharan P S. Phytosomes: A Novel Dosage Form for Enhancement of Bioavailability of Botanicals and Neutraceuticals, International Journal of Pharmacy and Pharmaceutical Sciences, 2010; 4: 10-14.
48. Awasthi R, Kulkarni TG, Pawar V. Phytosome : An Approach to increase the Bioavailability of Plant Extracts , Int J Pharm Pharma Sci 2011;2: 1-3.
49. Patel J, Patel R, Khambholja K, Patel N, An Overview of phytosomes as an advanced herbal drug delivery system, Asian J Pharma Sci 2009, 4(6): 363-371.
50. Chauhan S NItesh, Rajan Gowtham, Gopalakrishna B., Phytosomes: A Potential phyto-phospholipid carriers for herbal drug delivery , Journal of Pharmacy Research 2009,2(7) 1267-1270.