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FORMULATION DEVELOPMENT AND EVALUATION OF PHYTOSOMES OF HERBAL EXTRACT FOR EFFECTIVE TREATMENT OF HEPATIC DISEASE

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ABSTRACT

Objective: Carriers like phytosomes are promising systems to optimize oral absorption of encapsulated extracts. The aim of the present study is to develop phytosomes of herbal flower extract of *Delonix regia* and characterized by physicochemical method. **Methods:** This work prepared phytosomes containing bioactive phenolic extracts extract of *Delonix regia* and were characterised by various parameters like drug content, solubility studies, particle size determination, infrared absorption (FTIR), entrapment efficiency, *in vitro* release and stability studies etc. **Result:** These studies indicated the successful formation of vesicular drug-phospholipids complex. The apparent solubility, the in vitro dissolution studies indicated a significant improvement in the aqueous solubility, the drug release. When the regression coefficient values of drug loaded phytosomes were compared, it was observed that 'r^2' values of Korsmeyer Peppas was maximum *i.e.* 0.983 hence indicating drug release from formulations was found to follow Korsmeyer Peppas kinetics. **Conclusion:** The results of the study revealed that the phospholipid complex may be considered as a promising drug delivery system that improves the absorption and bioavailability of plant constituents.

KEYWORDS: *Delonix regia,* Characterization, Drug-phospholipids complex, Phytosome,

INTRODUCTION

In the past few decades, appreciable attention has been focused on the evolution of a completely unique drug delivery system (NDDS) for natural herb medicine. Conservative indefinite quantity forms, as well as prolonged-release indefinite quantity forms, they are unable to satisfy for each holding the drug element at a definite rate as per directed by the

necessities of the body, at some point of the amount of treatment, additionally as guiding the phytoconstituents to their desired target site to get an utmost therapeutic response. In phytoformulation analysis, developing nano-sized indefinite quantity forms (polymeric nanoparticles and nanocapsules, liposomes, solid lipoid nanoparticles, phytosomes, and nanoemulsion) contains a variety of benefits for natural herb medicine, as well as improvement of solubility and bioavailability, protection from toxicity, improvement of medical specialty activity, improvement of stability, rising tissue scavenger cell distribution, sustained delivery, and protection from physical and chemical degradation.[1]

Synthetic drugs exploited in the treatment of liver diseases are incompetent and may sometimes lead to serious side-effects. In this context, herbal therapy has emerged as a proficient approach with good values in treating hepatic diseases.[2,3] Developing a satisfactory herbal therapy to treat severe liver diseases requires systematic investigation of properties such as antiviral action (hepatitis b, hepatitis c), anti-hepatotoxicity (antioxidants), stimulation of liver regeneration and choleretic activity.

Delonix regia (Bojer ex Hook) Raffin (Poinciana regia, Royal Poinciana, Gul mohar, Flame tree or Flamboyant; Fabaceae-Caesalpinioideae) is a large ornamental tree with fern-like bipinnately compound leaves and attractive red peacock flowers and native to Madagascar.[4,5] The flowers, leaves and barks contain most of the active constituents. The flowers possess insecticidal,[6] antifertility, wound healing,[7] antifeedant,[8] anthelmintic activities.[9] and also inhibit the malaria parasite in humans.[10] Phytosomes are defined as “phyto” means plants and “some” means cell-like. The phytosome (technology was developed by Indena s.p.a of Italy), are used to enhance the bioavailability of phytomedicines by incorporating phospholipids into standardized plant extract.[11] It is novel drug delivery system in which hydrophilic choline moiety (head) binds to phytoconstituents (polar) and lipophilic phosphatidyl moiety surrounds choline bound phytoconstituents or form outer layer, hence water soluble phytoconstituents become lipid soluble.[12] Phytosomes contains naturally occurring phospholipid, phosphatidylcholine (PC) like soylecithin. It is also a cellular component which is biodegradable and has reported hepatoprotective activity.

Phytosomes technology has been effectively used to enhance the bioavailability of many popular herbal extract. In this study, phytosome-loaded with flower extraxt of *Delonix regia* was prepared and evaluated for effective treatment of hepatic disease.

MATERIALS AND METHODS

Plant materials

The flowers of *Delonix regia* were collected from local area of Bhopal in the month of January, 2020.

Chemicals

Chemicals were obtained from Rankem Laboratory Chemicals Pvt. Ltd., Haryana, India, Himedia Laboratories Pvt. Ltd, Mumbai, India and Loba Chemie, Mumbai, India. All solvents used were of analytical grade.

Defatting of plant material

Flowers of *Delonix regia* were shade dried at room temperature. The shade dried plant material was coarsely powdered and subjected to extraction by maceration. The extraction was continued till the defatting of the material had taken place.

Extraction by maceration process

46.5 gm of dried powdered flowers of *Delonix regia* has been extracted with hydroalcoholic solvent (methanol: water: 80:20) using maceration method for 48 hrs, filtered and dried using vacuum evaporator at 40°C and stored in well closed container for further use.

Determination of percentage yield

The percentage yield was obtained using this formula W2-W1/W0× 100. Where W2 is the weight of the extract and the container, W1 the weight of the container alone and W0 the weight of the initial dried sample.

Phytochemical analysis

Hydroalcoholic extract was analyzed for its phytoconstituents such as saponins, anthraquinone glycosides, phyto steroids, tannins, flavonoids, carbohydrates, triterpenoids, polyphenol and alkaloids.[13,14]

Quantitative estimation of bioactive compounds

Determination of total polyphenol content

The total polyphenol content of the fractions was determined in triplicate according to the Folin-Ciocalteau spectrophotometric method,[15] using gallic acid as a standard. 10 mg gallic acid was dissolved in 10 ml methanol, various aliquots of 5- 25µg/ml was prepared in methanol. 10mg of dried extracts were dissolved in 10 ml methanol and filtered. Two ml

(1mg/ml) of this solution was used for the estimation of phenol. 2 ml of extract or standard was mixed with 1 ml of Folin-Ciocalteu reagent (previously diluted with distilled water 1:10 v/v) and 1 ml (7.5g/l) of sodium carbonate. The mixture was vortexed for 15s and allowed to stand for 15 min for colour development. The absorbance was measured at 765 nm using a spectrophotometer. The results were calculated using the standard curve of gallic acid with known concentrations (5 to 25 µg/ ml), and they were expressed as mg of gallic acid (GAE)/g.

Total flavonoids content estimation

The total flavonoid content of the flower extract was determined by aluminum chloride colorimetric assay. 10 mg of dried extracts were dissolved in 10 ml methanol and filtered. Three ml (1mg/ml) of this solution was used for the estimation of flavonoids. For the preparation of standard 10 mg quercetin was dissolved in 10 ml methanol, and various aliquots of 5- 25µg/ml were prepared in methanol. 1 ml of 2% $AlCl_3$ methanolic solution was added to 3 ml of extract or standard and allowed to stand for 15 min at room temperature; absorbance was measured at 420 nm. Total flavonoids content was determined as mg quercitin equivalent per gram of sample with the help of calibration curve of quercetin.[16]

Formulation development of phytosomes

Preparation of phytosomes

The complex was prepared with phospholipids: Cholesterol and *Delonix regia* in the ratio of 1:0.5:0.5, 1:1:0.5, 2:0.5:0.5, 2:0.5:0.5 respectively. Weight amount of extract and phospholipids and cholesterol were placed in a 100ml round-bottom flask and 25ml of dichloromethane was added as reaction medium. The mixture was refluxed and the reaction temperature of the complex was controlled to 50°C for 3 h. The resultant clear mixture was evaporated and 20 ml of n-hexane was added to it with stirring. The precipitated was filtered and dried under vacuum to remove the traces amount of solvents. The dried residues were gathered and placed in desiccators overnight and stored at room temperature in an amber colored glass bottle.

Table 1: Different formulations of phytosomes.

Formulation	Ratio of phospholipids and cholesterol	Extract concentration (%)	Dichloromethane Concentration
Optimization of Phospholipids and Cholesterol			
F1	1:0.5	0.5	25
F2	1:1.0	0.5	25
F3	2:0.5	0.5	25
F4	2:1	0.5	25
Optimization of Drug Concentration			
F5	2:0.5	0.5	25
F6	2:0.5	1.0	25
F7	2:0.5	1.5	25
F8	2:0.5	2.0	25
Optimization of solvent concentration			
F9	2:1	0.5	10
F10	2:1	0.5	25
F11	2:1	0.5	50
F12	2:1	0.5	75

Characterization

Microscopic observation of prepared phytosomes

An optical microscope (cippon, Japan) with a camera attachment (Minolta) was used to observe the shape of the optimized phytosome formulation.

Drug Excipient compatibility study by FT-IR

IR spectra of physical mixture of drug and excipients were recorded by ATR (Attenuated total reflection) techniques using Fourier transform infrared spectrophotometer. A base line correction was made and the sample was directely mounted in IR compartment and scanned at wavelengths 4000 cm^{-1} to 400 cm^{-1}.

Entrapment efficiency

Phytosome preparation was taken and subjected to centrifugation using cooling centrifuge (Remi) at 12000 rpm for an hour. The clear supernatant was siphoned off carefully to separate the non entrapped flavonoids and the absorbance of supernatant for non entrapped *Delonix regia* was recorded at λ_{max} 420.0 nm using UV/visible spectrophotometer (Labindia 3000+). Amount of flavonoids in supernatant and sediment gave a total amount of *Delonix regia* in 1 ml dispersion.[17,18] The percent entrapment was calculated by following formula:

$$\text{Percent Entrapment} = \frac{\text{Amount of drug in sediment}}{\text{Total amount of drug added}} \text{X } 100$$

Particle size and size distribution

The particle size, size distribution and zeta potential of optimized phytosomes formulation were determined by dynamic light scattering (DLS) using a computerized inspection system (Malvern Zetamaster ZEM 5002, Malvern, UK). The electric potential of the phytosomes, including its Stern layer (zeta potential) was determined by injecting the diluted system into a zeta potential measurement cell.[17,18]

Transmission electron microscopy

Surface morphology was determined by TEM, for TEM a drop of the sample was placed on a carbon-coated copper grid and after 15 min it was negatively stained with 1% aqueous solution of phosphotungustic acid. The grid was allowed to air dry thoroughly and samples were viewed on a transmission electron microscopy (TEM Hitachi, H-7500 Tokyo, Japan).[17,18]

In vitro dissolution rate studies

In vitro drug release of the sample was carried out using USP- type I dissolution apparatus (Basket type). The dissolution medium, 900 ml 0.1N HCl was placed into the dissolution flask maintaining the temperature of 37 ± 0.5^0C and 75 rpm. 10 mg of prepared phytosomes was placed in each basket of dissolution apparatus. The apparatus was allowed to run for 8 hours. Sample measuring 3 ml were withdrawn after every interval (30 min, 1 hrs, 2 hrs, 4 hrs, 6 hrs, 8 hrs, and 12 hrs.) up to 12 hours using 10 ml pipette. The fresh dissolution medium (37^0C) was replaced every time with the same quantity of the sample and takes the absorbance at 286.0 nm using spectroscopy.[17,18]

Release kinetics study

To study the release kinetics of drug from the polymeric nanoparticles loaded in hydrogel, the data obtained from the *in vitro* release study were analysed using various kinetic models to describe the mechanism of drug release from the hydrogels.[19]

In order to investigate the mode of release from the polymeric nanoparticles loaded in hydrogel, the release data were analyzed with the following mathematical models.

$Qt = K_0 t$ (Zero Order Kinetics)

$\text{Log } (Q_t / Q_0) = - K_1 t / 2.303$ (First order Kinetics)

$Qt = K_{KP} t^n$ (Korsmeyer and Peppas equation)

$Qt = K_H t^{1/2}$ (Higuchi's equation)

Where, Qt is the percent of drug released at time't', K_0, K_1, K_{HC}, K_{KP} and K_H are the coefficients of Zero order, First order, Korsmeyer-Peppas and Higuchi's equations.

Stability studies of optimize phytosomes formulation

The prepared phytosomes subjected to stability studies at 40±2°C/75±5% RH and 30±2°C/60±5% RH as per ICH guidelines for a period of 3 months. Samples were withdrawn at 1 month time intervals and evaluated for physical appearance and drug content.[17,18]

RESULTS AND DISCUSSION

The percentage yield flowers extract of *Delonix regia* was found 5.687 percentage for petroleum ether extract and for hydroalcoholic extract it was found to be 7.524 percentage. The outcomes of the results are discussed in the table 2.

Table 2: % yield of flowers extract of *delonix regia.*

S. No.	Solvents	% Yield
1	Pet ether	5.687
2.	Hydroalcoholic	7.524

Taken a small amount of the dried extracts and subjected to the phytochemical screening test and the outcomes of the results are discussed in the table 3. From the results obtained it is clear that the hydrolacoholic extract shows the occurrence of alkaloids, flavonoids, phenols. proteins, carbohydrates and saponins.

Table 3: Phytochemical screening of extract *of delonix regia.*

S. No.	Constituents	Hydroalcoholic extract
1.	**Alkaloids** Wagner's Test Hager's test	 -ve +ve
2.	**Glycosides** Legal's test	 -ve
3.	**Flavonoids** Lead acetate Alkaline test	 +ve +ve
4.	**Phenolics** Ferric Chloride Test	 +ve
5.	**Proteins** Xanthoproteic test	 +ve
6.	**Carbohydrates** Fehling's test	 +ve
7.	**Saponins** Froth Test Foam test	 +ve +ve

8.	**Diterpins** Copper acetate test	-ve
9.	**Tannins** Gelatin Test	-ve

The content of total phenolic compounds (TPC) content was expressed as mg/100mg of gallic acid equivalent of dry extract sample using the equation obtained from the calibration curve: Y = 0.042X-0.002, R^2= 0.999, where X is the gallic acid equivalent (GAE) and Y is the absorbance. The total phenolic content was found to be 0.875 mg/100mg. The outcomes of the results are discussed in the table 6.

Table 4: Preparation of calibration curve of gallic acid.

S. No.	Concentration (µg/ml)	Absorbance
0	0	0
1	5	0.194
2	10	0.422
3	15	0.637
4	20	0.848
5	25	1.035

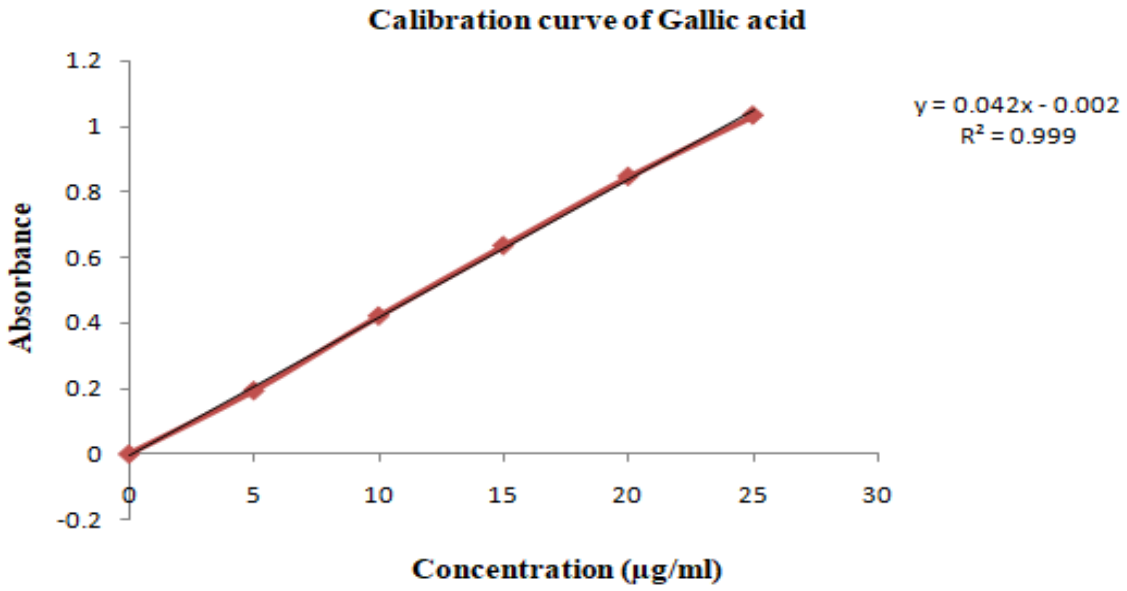

Figure 1: Graph of calibration curve of gallic acid.

The content of total flavonoid content (TFC) content was expressed as mg/100mg of quercetin equivalent of dry extract sample using the equation obtained from the calibration curve: Y = 0.06X+0.019, R^2= 0.999, where X is the quercetin equivalent (QE) and Y is the absorbance. The total flavonoid content was found to be 1.024 mg/100mg. The outcomes of the results are discussed in the table 6.

Table 5: Preparation of calibration curve of quercetin.

S. No.	Concentration(µg/ml)	Absorbance
0	0	0
1	5	0.352
2	10	0.61
3	15	0.917
4	20	1.215
5	25	1.521

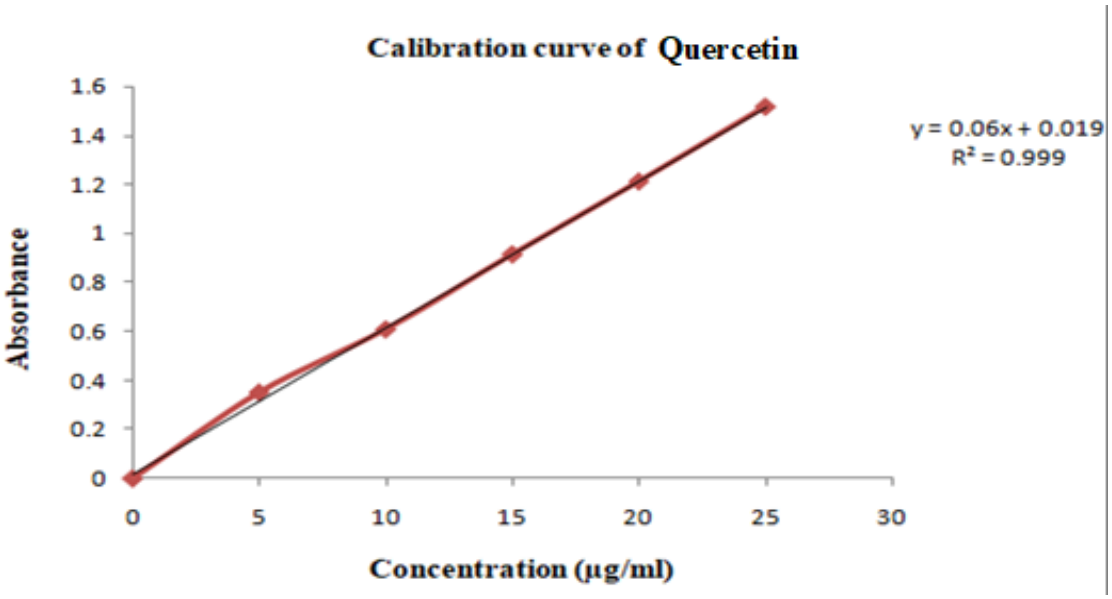

Figure 2: Graph of calibration curve of quercetin.

Table 6: Total phenolic and total flavonoid content of *delonix regia* extract.

S. No.	Extract	Total Phenol (mg/100mg)	Total flavonoid (mg/100mg)
1.	Hydroalcoholic *extract*	0.875	1.024

The spectrum of extract and phytosomes was authenticated by FTIR spectroscopy. The presences of characteristic peaks associated with specific structural characteristics of the drug molecule were noted. The appearance or disappearance of peaks and/or the shift of their positions are often indications of interactions such as hydrogen bonding. The IR spectra of extract shows stretching vibrations at 1572.3103 cm-^{1} attributed predominantly to the overlapping stretching vibrations of alkenes (C=C) and carbonyl (C=O) character. Infrared of extract show stretching vibration at 2907.1644cm^{-1} due to O-H groups, C=C aromatic stretching vibration at 1444.5606cm^{-1}. When the data obtained from FTIR spectra was compared with the spectra studied it was observed that there are similar peaks for functional groups in phytosomes.

TEMs are capable of imaging at a significantly higher resolution than light microscopes, owing to the small de Broglie wavelength of electrons. This enables the instrument's user to examine fine detail even as small as a single column of atoms, which is thousands of times smaller than the smallest resolvable object in a light microscope. TEM forms a major analysis method in a range of scientific fields, in both physical and biological sciences. TEM characterization revealed that the phytosomes are spherical in shape, however, some variation in size distribution was observed in the TEM image, which might be attributed to an uncontrolled charge neutralization process involved between oppositely charged chains occurring during the formation of phytosomes.

Percentage entrapment efficiency of prepared drug loaded phytosomes was found in range of 55.56±0.45 to 75.65±0.47 percentage. The outcomes of the results are discussed in the table 7. The maximum percentage yield and entrapment efficiency was found in formulation F10 and it was considered as optimized formulation. The optimized formulation F10 among other batches was subjected to further studies.

Table 7: Particle Size and Entrapment efficiency of drug loaded phytosomes.

Formulation Code	Particle size (nm)	Entrapment Efficiency (%)
F1	366.25	55.56±0.45
F2	355.52	62.23±0.23
F3	289.23	65.65±0.41
F4	354.12	62.23±0.65
F5	265.58	67.12±0.74
F6	289.74	62.23±0.32
F7	256.19	58.85±0.41
F8	231.32	61.25±0.35
F9	312.36	65.45±0.65
F10	212.45	75.65±0.47
F11	289.74	69.98±0.85
F12	298.78	65.45±0.32

*Average of three determinations (n=3 ±SD)

Further optimized formulation F10 selected on the basis of *in vitro* studies further *in vitro* release kinetics studies was carried out. It can be observed from the data that cumulative % of drug release against time represents that drug release from drug loaded phytosomes is perfectly following release model as the drug release profile is very closest to trend line or regression line and the highest value of coefficient of correlation values (R^2) was in the range of 0.911-0.983.

Table 8: *In-vitro* drug release data for optimized formulation F10.

Time (h)	Square Root of Time(h)$^{1/2}$	Log Time	Cumulative* % Drug Release	Log Cumulative % Drug Release	Cumulative % Drug Remaining	Log Cumulative % Drug Remaining
0.5	0.707	-0.301	12.25	1.088	87.75	1.943
1	1	0	23.32	1.368	76.68	1.885
2	1.414	0.301	39.98	1.602	60.02	1.778
4	2	0.602	55.65	1.745	44.35	1.647
6	2.449	0.778	69.98	1.845	30.02	1.477
8	2.828	0.903	85.56	1.932	14.44	1.160
12	3.464	1.079	98.89	1.995	1.11	0.045

Table 9: Regression analysis data of optimized formulation F10.

Batch	Zero order	First order	Higuchi	Korsmeyer peppas
	R^2	R^2	R^2	R^2
F10	0.938	0.911	0.939	0.983

When the regression coefficient values of drug loaded phytosomes were compared, it was observed that 'r^2' values of Korsmeyer Peppas was maximum *i.e.* 0.983 hence indicating drug release from formulations was found to follow Korsmeyer Peppas kinetics.

Results of stability studies clearly indicated that optimized batches of phytosomes were stable over the chosen temperature and humidity conditions up to 3 months as were found no significant variation in physical appearance and % drug content.

The results showed that phytosomes were successfully prepared and tested, given that this small particle size is important for oral absorption. The dissolution rate is largely influenced by the crystal morphology and the wettability of the solids,[20] and the improved dissolution rate of drug loaded phytosomes may be explained by the improved solubility, and the partially disrupted crystalline phase (amorphous form) in the prepared complex. The relatively higher amorphous state of the phytosome and their increased water-solubility may have a positive impact on the cumulative release of the drug.[21]

CONCLUSION

Phytosome is a nanovesicle that combines plant extracts and phospholipids to produce more soluble fat complex and provide better absorption. The results showed that F3 was the best formula with good physicochemical stability and physicochemical properties. The result of the study revealed that the phospholipid complex may be considered as a promising drug delivery system that improves the absorption and bioavailability of plant constituents.

Additionally, the results suggested that the polyphenolic extracts in phytosomal formulation were an interesting dosage form for nutraceutical purposes.

REFERENCES

1. Sarangi MK, Padhi S. Novel herbal drug delivery system: An overview. Archives Med Health Sci, 2019; 6(1): 103-132.
2. Handa SS. Plants as drugs. The Eastern Pharmacist, 1991; 34: 79-85.
3. Trease GE, Evans WC. Pharmacognosy. London, Balliere Tindall Press, 1983; 56-57.
4. Vargas AMM, Garcia CA, Reis EM, et al. NaOH-activated carbon from flamboyant (*Delonix regia*) pods: Optimization of preparation conditions using central composite rotatable design. Chem Eng J, 2010; 162: 43-50.
5. Indian Medicinal Plants-An Illustrated Dictionary. Springer-Verlag Berlin-Heidelberg, 2007; 205-206.
6. Baskaran V, Narayansamy P. Traditional Pest control. Caterpillar Publications, 1995; 190.
7. De Groot H. Reactive oxygen species in tissue injury. Hepatogastroenterology, 1994; 41(2): 328-332.
8. Deepa B, Remadevi OK. Larvicidal activity of the flowers of *Delonix regia* (Bojer Ex Hook.) Rafin. (Fabales: Fabaceae) against the Teak defoliator, *Hyblaea puera* Cramer. Current Biotica, 2011; 5(2): 237-240.
9. Gupta RK, Chandra S. Chemical investigation of *Delonix regia* flowers. Ind J Pharm, 1971; 33(4): 74-75.
10. Carter R, Diggs CL. Plamodia of rodents. In: Parasitic Protozoa. London: Academic Press, 1977; 359-465.
11. Bombardelli E, Spelta M. Phospholipid-polyphenol complex a new concept in skin care ingredients. Cosmet Toiletries, 1991; 106: 69–76.
12. Bombardelli E, Mustich G. Bilobalide phospholipid complex, their uses and formulation containing them. U.S. Patent EPO- 275005. 1991.
13. Kokate CK, Purohit AP, Gokhale SB. Pharmacognosy, 47th Edn, Nirali Prakashan Publication, India, 2011.
14. Sumathy V, Jothy Lachumy S, Zuraini Z, et al. *In vitro* bioactivity and phytochemical screening of *Musa acuminata* flower. Pharmacologyonline, 2011; 2: 118-27.

15. Singleton, V L., Orthofer R, Lamuela-Raventos, RM. Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. Oxidants and Antioxidants, 1999; 299: 152-178.
16. Zhishen J, Mengcheng T, Jianming W. The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. Food Chem, 1999; 64: 555-9.
17. Matias, D.; Roque, L.; Simões, M.d.F.; Diaz-Lanza, A.; Rijo, P.; Reis, C.P. *Plectranthus madagascariensi*s phytosomes: Formulation optimization. Biomed. Biopharm. Res, 2015; 12: 223–231.
18. Abd El-Fattah, A.I.; Fathy, M.M.; Ali, Z.Y.; El-Garawany, A.A.; Mohamed, E.K. Enhanced therapeutic benefit of quercetin-loaded phytosome nanoparticles in ovariectomized rats. Chem. Biol. Interact, 2017; 271: 30–38.
19. Sabri, H. S., Alia, W. K., Abdullahb, B. H., Al-Anic, W. M. Formulation design and evaluation of anti-microbial activity of emulgel containing essential oil of *Myrtus communis* L. Inter J Pharm Sci Rev Res, 40: 271–277.
20. Semalty A, Semalty M, Singh D, Rawat MS. Phytophospholipid complex of catechin in value added herbal drug delivery. J Incl Phenom Macrocycl Chem, 2012; 73(1-4): 377-86.
21. Vora AK, Londhe VY, Pandita NS. Preparation and characterization of standardized pomegranate extract phospholipid complex as an effective drug delivery tool. J Adv Pharm Tech Res, 2015; 6(2): 75-80.