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FORMULATION AND EVALUATION OF PHYTOSOMES OF PANAX GINSENG, NYMPHAEA STELLATA, MUCUNA PRURIENS, SYZYGIUM CUMINI AND AEGLE MARMELOS

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Abstract
Since the turn of the century, numerous studies have been carried out to ensure the successful distribution of these plant-derived goods. Phytosomes possess a broad range of applications and attributes such as pharmacokinetics and pharmacodynamics. By considering the medicinal uses *of Panax ginseng, Nymphaea stellata, Mucuna pruriens, Syzygium cumini and Aegle marmelos*. This study deals with formulation, evaluation of polyherbal phytoosme to increase the phytoconstituents bioavailability. The preparation & characterization were performed by standard methods. Results showed that the Polyherbal phytosomes size and entrapment efficiency 215.65nm and 74.45%. The polyherbal phytosomal formulation was observed to have λ_{max} of 276nm. Further the *In vitro* drug release study data suggested that in 12 hours 97.85% and 98.78% drug release occur in PGF2 and NSF 3. In case of MPF3, SCF3, and AMF3 about 97.65 %, 98.12% 97.14% drug release occurred in 12 hours. In case of polyherbal phytosome about 96.65% drug is released. Acoording to Regression analysis data the R^2 value for PGF2 was found to be highest for Higuchi which is 0.995. NSF3 was also found to follow Higuchi model with R^2 value of 0.990. In case of MPF3, SCF3, AMF3 the R^2 value was estimated to be 0.987, 0.975, 0.983 which is highest and found to follow Korsmeyer Peppas model. For polyherbal phytosomal gel Higuchi was observed to be followed with R^2 value of 0.979. Thus, it can be concluded that it is crucial to remember that a number of variables, such as dietary lipid consumption, can have a substantial impact on the pace, degree, and total bioavailability of medications via these systems.

Keywords: Phytososme, *Panax ginseng, Nymphaea stellata, Mucuna pruriens, Syzygium cumini and Aegle marmelos*, polyherbal

Introduction

Plants are rich in compounds that are good for your health; these are primarily secondary metabolites like flavonoids. Molecules that are polar or water soluble make up the majority of the physiologically active components of plants. The utilization of these ingredients is restricted by their toxicity and absorption issues. In addition, the gut flora and digestive secretions break down the plant extracts. Since the turn of the century, numerous studies have been carried out to ensure the successful distribution of these plant-derived goods. There are numerous ways to increase oral bioavailability, including adding solubility and bioavailability enhancers, changing the structure, and entangling the drug in lipophilic carriers. As a result, a lot of research has to be done on herbal

drug delivery systems in order to create therapeutic indices for medications (Poddar *et al*., 2020; Rungsung *et al*., 2013).

A revolutionary approach to drug distribution, the novel drug delivery system overcomes the drawbacks of the conventional drug delivery methods. Modern phytopharmaceutical research, however, can address the scientific requirements for herbal medicines to be included in novel drug delivery systems, such as solid dispersions, liposomes, solid lipid nanoparticles, nanoparticles, microemulsions, phytosomes, and so on. These requirements include pharmacokinetics, mechanism of action, site of action, accurate dose required, and so forth. Plants were the only source of medicine for humans for a very long time. Almost all medications in the past came from them (Devi *et al*., 2010; Date *et al*., 2005).

A new compound known as a phytosome is created when active pharmacological ingredients react with synthetic or natural phospholipids in a ratio of 0.5 to 5. but typically eaten in a 1:2 ratio to prepare phytosomes. The phospholipids needed to prepare phytosomes are derived from a group that includes soylecithin, which comes from the brain or dermis of cows or pigs, and phosphatidylcholine, phosphatidylethanolamine, and phosphatidylserine. The acryl group in this group can be the same or different, and it is primarily derived from palmitic, stearic, oleic, and linoleic acid (Tripathy *et al*., 2013).

Phytosomes possess a broad range of cosmetic applications and attributes such as pharmacokinetics and pharmacodynamics; nevertheless, this technology is not exclusive to polyphenolic alkaloids. Any kind of molecule can be converted in that case. Part of the phospholipid utilized in the manufacture of phytosomes is a simple and repeatable formulation for phytosomes. They significantly increase the oral phyto constituent drug's bioavailability and have its own positive physiological effects (Awasthi and Kulkarni, 2011; Kumar *et al.,* 2017).

The ability of *Panax ginseng* to modulate immunity has long been recognized. Through actions on the immune system, ginseng's roots, stems, and leaves, as well as their extracts, have been utilized to support immunological homeostasis and enhance resistance to disease or microbial attacks. The immune system is made up of many cell kinds, each of which has a distinct function. The effects of ginseng therapy vary depending on the type of immune cell. (Kiefer and Pantuso, 2003; Coleman et al., 2003).

A prominent and well-known medicinal plant, *Nymphaea stellata* Willd. (Syn. *Nymphaea nouchali* Burman f.) (Nymphaeaceae) is used extensively in the Ayurvedic and Siddha medical systems to treat a variety of conditions, including diabetes, inflammation, liver disorders, urinary disorders, menorrhagia, blenorrhagia, menstrual problems, aphrodisiac, and bitter tonic. The traditional application and experimental findings, such as the hepatoprotective, anti-inflammatory, and especially antidiabetic action, appear to be in agreement (Das *et al*., 2012).

Popular Indian medicinal plant *Mucuna pruriens* Linn. has been used for many years to treat a variety of ailments, including parkinsonism, in traditional Ayurvedic Indian medicine. Numerous functions of this plant, including antidiabetic, aphrodisiac, antineoplastic, antiepileptic, and antibacterial properties, are being investigated pharmacologically. This plant contains a diverse array of phytochemical components that have been identified (Lampariello *et al*., 2012).

Syzygium cumini has been demonstrated in preclinical research to have chemopreventive, radioprotective, and antineoplastic activities. It is also beneficial in treating diabetic mellitus, inflammation, ulcers, and diarrhea. Anthocyanins, glucoside, ellagic acid, isoquercetin, kaemferol, and myrecetin are among the many chemicals found in abundance in the plant. It is stated that the seeds contain the glycoside jambolin or antimellin and the alkaloid jambosine, which prevent starch from being converted to sugar by diastatic means (Srivastava and Chandra, 2013).

Several medications are prepared using *Aegle marmelos*. Fruit is one of the essential elements that has the most potential for disease cures among all other parts. Numerous forms of paste, powder, and pill are made from the bael plant. Bales are essential for making dasamula, chyavanprash, and other recipes. It is used to treat a variety of illnesses because of its carminative and digestive qualities. When it comes to treating chronic diarrhea, dysentery, brain tonic, and other conditions,

bael is regarded as a significant Ayurvedic medication. The root, bark, leaf, flower, and fruit of the bael plant can all be combined to create a potent mixture that is particularly helpful in treating a variety of mental illnesses. Bael fruit powder has anti-proliferative and anti-cancer properties (Sabu and Kuttan, 2004).

By considering the medicinal uses of medicinal plants. This study aims at formulating and evaluating phytosome of different plants to increase phytoconstituents efficacy.

Materials and Methods

Collection of plant material

Panax ginseng, Nymphaea stellata, Mucuna pruriens, Syzygium cumini and Aegle marmelos plant material was collected from different sources.

Extract preparation

The plant material was extracted with proper solvent and by specific method to get the extract.

Formulation of phytosomes of *Panax ginseng, Nymphaea stellata, Mucuna pruriens, Syzygium cumini* and *Aegle marmelos*

The complex was prepared with phospholipids: Cholesterol and each separated extract of *Panax ginseng*, *Nymphaea stellata*, *Mucuna pruriens*, *Syzygium cumini* and *Aegle marmelos* in the ratio of 1:0.5:1.0, 1:1:1.0, 2:0.5:1.0, 2:1.0:1.0 respectively. A 100 ml round-bottom flask was filled with the weighted amounts of each extract, phospholipids, and cholesterol, and 25 ml of dichloromethane was added as the reaction medium. The mixture was refluxed for 3 hours while maintaining a temperature of 50°C for the reaction of the complex.

After the resulting clear fluid had evaporated, 20 ml of n-hexane was stirred in. To get rid of the traces of solvents, the precipitate was filtered and dried under vacuum. The leftovers were collected, desiccated overnight, and stored in an amber-colored glass bottle at room temperature (Gnananath *et al.*, 2017).

Table 1: Different formulations of phytosomes of *Panax ginseng*

Formulation	Ratio of Phospholipids and Cholesterol (%)	Extract Concentration (%)	Dichloromethane Concentration (ml)
		Panax ginseng	
PGF1	1:0.5	1	25
PGF2	1:1	1	25
PGF3	2:0.5	1	25
PGF4	2:1.0	1	25

Table 2: Different formulations of phytosomes of *Nymphaea stellata*

Formulation	Ratio of Phospholipids and Cholesterol (%)	Extract Concentration (%)	Dichloromethane Concentration (ml)
		Nymphaea stellata	
NSF1	1:0.5	1	25
NSF2	1:1	1	25
NSF3	2:0.5	1	25
NSF4	2:1.0	1	25

Table 3: Different formulations of phytosomes of *Mucuna pruriens*

Formulation	Ratio of Phospholipids and Cholesterol (%)	Extract Concentration (%)	Dichloromethane Concentration (ml)
		Mucuna pruriens	
MPF1	1:0.5	1	25
MPF2	1:1	1	25
MPF3	2:0.5	1	25
MPF4	2:1.0	1	25

Table 4: Different formulations of phytosomes of *Syzygium cumini*

Formulation	Ratio of Phospholipids and Cholesterol (%)	Extract Concentration (%)	Dichloromethane Concentration (ml)
		Syzygium cumini	
SCF1	1:0.5	1	25
SCF2	1:1	1	25
SCF3	2:0.5	1	25
SCF4	2:1.0	1	25

Table 5: Different formulations of phytosomes of *Aegle marmelos*

Formulation	Ratio of Phospholipids and Cholesterol (%)	Extract Concentration (%)	Dichloromethane Concentration (ml)
		Aegle marmelos	
AMF1	1:0.5	1	25
AMF2	1:1	1	25
AMF3	2:0.5	1	25
AMF4	2:1.0	1	25

Table 6: Different formulations of polyherbal phytosomes of*Panax ginseng, Nymphaea stellata, Mucuna pruriens*, *Syzygium cumini* and *Aegle marmelos*

Formulation	Ratio of Phospholipids and Cholesterol (%)	Extract Concentration (%)	Dichloromethane Concentration (ml)
PF1	1:0.5	1:1:1	25
PF2	1:1	1:1:1	25
PF3	2:0.5	1:1:1	25
PF4	2:1.0	1:1:1	25

Characterization of phytosomes

Entrapment efficiency

A phytosome preparation was obtained and centrifuged for an hour at 12000 rpm using a cooling centrifuge (Remi).To separate the non-entrapped flavonoids, the clear supernatant was carefully removed. The absorbance of the clear supernatant for the non-entrapped flavonoids in *Panax ginseng, Nymphaea stellata, Mucuna pruriens, Syzygium cumini* and *Aegle marmelos* were recorded separately at max 420.0 nm using UV/visible spectrophotometer (Labindia 3000+). The amount of flavonoids in the 1 ml dispersion was calculated from the amount of flavonoids in the sediment and supernatant. The % entrapment was calculated by dividing amount of drug in sediment by total amount of drug added multiplied by 100 (Deleanu *et al*., 2023)

Particle size and size distribution

A computerised inspection system was used to measure the particle size, size distribution, and zeta potential of an improved phytosomes formulation using dynamic light scattering (DLS) (Malvern

Zetamaster ZEM 5002, Malvern, UK). By infusing the diluted system into a zeta potential measurement device, the electric potential of the phytosomes, including its Stern layer, was ascertained (Clogston and Patri, 2011).

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) (Froiio *et al*., 2019)

***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 corresponding wavelength of optimized formulations using spectroscopy (Kalita *et al*., 2013).

Results and Discussion

For each plant, about four formulations of phytosome were prepared. In evaluation of Particle size (nm) and Entrapment Efficiency (%) the PGF2 in case of Panax ginseng was estimated to be 220.15nm and 73.32% respectively.

With *Nymphaea stellate* NSF3 the particle size and entrapment efficiency was observed to be 210.41 nm and 73.32%. For *Mucuna pruriens* the entrapment efficiency and size was seen to be 74.45% and 215.52nm.

For *Syzygium cumini* the SCF3 size and entrapment efficiency was detected to be 205.65 nm and 73.32%. The *Agele marmelos* phytosome was having size and entrapment efficiency as 215.45nm and 76.65%. The Polyherbal phytosomes size and entrapment efficiency 215.65nm and 74.45%.

The entrapment efficiency declined as the lipid content increased, suggesting that the additional lipid did not aid in the entrapment of more medicines into the matrix. The extract/soy lecithin ratio increased with entrapment efficiency, which may have been caused by the higher polymer content, which was predicted to promote entrapment efficiency by giving the drug greater area to be incorporated.

It was observed that mean particle size increased with increase in phospholipid concentration. This is due to the increase in number of the polymeric chains per volume unit of solvent that leads to collisions and formation of larger nanoparticles.

The λ_{max} of *Panax ginseng and Nymphaea stellate was observed to be 294 & 224 respectively. The Mucuna pruriens, Syzygium cumini, Aegle marmelos* λ_{max} was seen to be 282nm, 228nm and 240 nm respectively.

The phytosomal formulation was observed to have λ_{max} of 276nm.

Further to construct calibration curve concentrations ranging from 5-25 µg/ml were created. The calibration curve for each phytosomal formulation was made accordingly.

The drug was entrapped close to the surface, which caused the initial burst of release, and the release diffusion from the phytosomes was responsible for the persistent release. The highest correlation coefficient indicated the best match among the kinetic models that were fitted to the data from the in vitro release investigation.

Further the *In vitro* drug release study data suggested that in 12 hours 97.85% and 98.78% drug release occur in PGF2 and NSF 3. In case of MPF3, SCF3, and AMF3 about 97.65 %, 98.12% 97.14% drug release occurred in 12 hours.

In case of polyherbal phytosome about 96.65% drug is released. Acoording to Regression analysis data the R^2 value for PGF2 was found to be highest for Higuchi which is 0.995.
NSF3 was also found to follow Higuchi model with R^2 value of 0.990. In case of MPF3, SCF3, AMF3 the R^2 value was estimated to be 0.987, 0.975, 0.983 which is highest and found to follow Korsmeyer Peppas model.
For polyherbal phytosomal gel Higuchi was observed to be followed with R^2 value of 0.979.
Two primary mechanisms are responsible for the observed increase in the relative bioavailability are (1) the formation of molecular aggregates based on phospholipids, which increases apigenin's aqueous solubility and improves intestinal absorption; and (2) the function of amphiphilic phospholipids as a vesicular carrier matrix for apigenin, which may shield it from hepatic first-pass metabolism. Although we have seen an increase in in vivo bioavailability with polyherbal phytosome, it is crucial to remember that a number of variables, such as dietary lipid consumption, can have a substantial impact on the pace, degree, and total bioavailability of medications via these systems.

Table 7: Particle size and entrapment efficiency of drug loaded phytosomes

Formulation Code	Particle size (nm)	Entrapment Efficiency (%)
	Panax ginseng	
PGF1	332.25	68.85
PGF2	**220.15**	**73.32**
PGF3	298.85	69.98
PGF4	236.65	70.12
	Nymphaea stellata	
NSF1	298.85	68.85
NSF2	265.65	71.12
NSF3	**210.41**	**73.32**
NSF4	230.14	65.54
	Mucuna pruriens	
MPF1	278.85	69.98
MPF2	265.54	65.74
MPF3	**215.52**	**74.45**
MPF4	236.65	71.12
	Syzygium cumini	
SCF1	295.65	68.85
SCF2	274.45	69.12
SCF3	**205.65**	**73.32**
SCF4	236.12	67.47
	Aegle marmelos	
AMF1	268.98	70.32
AMF2	245.65	67.74
AMF3	**215.45**	**76.65**
AMF4	230.15	73.32
	Polyherbal phytosomes	
PF1	255.65	69.98
PF2	241.12	70.12
PF3	**215.65**	**74.45**
PF4	232.12	67.12

Table 9: *In-vitro* drug release data for optimized formulation PGF2

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	18.85	1.275	81.15	1.909
1	1	0	29.98	1.477	70.02	1.845
2	1.414	0.301	36.65	1.564	63.35	1.802
4	2	0.602	58.78	1.769	41.22	1.615
6	2.449	0.778	68.85	1.838	31.15	1.493
8	2.828	0.903	81.12	1.909	18.88	1.276
12	3.464	1.079	97.85	1.991	2.15	0.332

Table 10: *In-vitro* drug release data for optimized formulation NSF3

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	23.32	1.368	76.68	1.885
1	1	0	31.15	1.493	68.85	1.838
2	1.414	0.301	38.85	1.589	61.15	1.786
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	86.65	1.938	13.35	1.125
12	3.464	1.079	98.78	1.995	1.22	0.086

Table 11: *In-vitro* drug release data for optimized formulation MPF3

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	20.15	1.304	79.85	1.902
1	1	0	29.98	1.477	70.02	1.845
2	1.414	0.301	35.65	1.552	64.35	1.809
4	2	0.602	53.32	1.727	46.68	1.669
6	2.449	0.778	67.74	1.831	32.26	1.509
8	2.828	0.903	86.65	1.938	13.35	1.125
12	3.464	1.079	97.65	1.990	2.35	0.371

Table 12: *In-vitro* drug release data for optimized formulation SCF3

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	18.85	1.275	81.15	1.909
1	1	0	26.65	1.426	73.35	1.865
2	1.414	0.301	31.15	1.493	68.85	1.838
4	2	0.602	49.98	1.699	50.02	1.699
6	2.449	0.778	62.23	1.794	37.77	1.577
8	2.828	0.903	86.65	1.938	13.35	1.125
12	3.464	1.079	98.12	1.992	1.88	0.274

Table 13: *In-vitro* drug release data for optimized formulation AMF3

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	16.65	1.221	83.35	1.921
1	1	0	22.32	1.349	77.68	1.890
2	1.414	0.301	29.98	1.477	70.02	1.845
4	2	0.602	45.65	1.659	54.35	1.735
6	2.449	0.778	64.48	1.809	35.52	1.550
8	2.828	0.903	83.32	1.921	16.68	1.222
12	3.464	1.079	97.14	1.987	2.86	0.456

Table 14: *In-vitro* drug release data for polyherbal phytosomes formulation PF3

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	23.36	1.368	76.64	1.884
1	1	0	36.65	1.564	63.35	1.802
2	1.414	0.301	41.12	1.614	58.88	1.770
4	2	0.602	55.65	1.745	44.35	1.647
6	2.449	0.778	62.23	1.794	37.77	1.577
8	2.828	0.903	81.15	1.909	18.85	1.275
12	3.464	1.079	96.65	1.985	3.35	0.525

Table 15: Regression analysis data of optimized formulations

Batch	Zero Order	First Order	Higuchi	Korsmeyer Peppas
	R^2	R^2	R^2	R^2
PGF2	0.957	0.916	**0.995**	0.993
NSF3	0.965	0.909	**0.990**	0.989
MPF3	0.962	0.940	0.985	**0.987**
SCF3	0.968	0.914	0.972	**0.975**
AMF3	0.973	0.932	0.980	**0.983**
PF3	0.969	0.911	**0.979**	0.975

Conclusion

In conclusion, in this study, the combined the extracts of *Panax ginseng, Nymphaea stellata, Mucuna pruriens, Syzygium cumini and Aegle marmelos_to form a phytosome* found to exhibit significant results. The polyherbal Phytosomes has better physical characteristics than that of all extracts phytosome.In-vitro studies revealed that phytosomes showed control release of phytoconstituents. Hence, Polherbal phytosomal formulation of this herbal drug combination can be used for clinical application to enhance the therapeutic effect.

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