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Formulation and Evaluation of Phytosomes Loaded with *Pithecellobium Bijeninum* Leaf Extract

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ABSTRACT

The objective of the present study was to prepare phytosomal formulation loaded with aqueous extract of Pithecellobium bijeninum leaves. The extraction of leaf powder was done by maceration technique using 90:10 ethanol-water mixture with an extraction yield of 6.12%. The extract was found to contain alkaloids, and flavonoids and the total phenolic content was found to be 23.2 ± 0.827 %w/w. Phytosomes of the extract were prepared by solvent evaporation method using lecithin as the lipid molecule. The particle size of the phytosomes was from 468 nm to 1827 nm in size with a polydispersity index varying between 0.178 - 0.464. The phytosomes were visible as rigid, almost spherical vesicles in SEM image. The surface of the phytosome vesicles was found to be regular and smooth. The point prediction suggested level 3 (0.3 g) of lipid concentration and level 2 (0.2 g) of extract concentration to present the lowest particle size. Sharp and distinct endothermic peaks in DSC revealed the formulation of stable phytosomes due to molecular interactions between the extract and lecithin. The phytosomes were found to possess good antioxidant action against DPPH radical in the in vitro scavenging assay. The IC50 value of the extract against DPPH was found to be 48.24 µg and that of the phytosomal formulation loaded with the extract (F4) was obtained to be 48.90 µg.

Key words: Phytosome, extraction, lecithin, Pithecellobium, antioxidant

1. INTRODUCTION

There is strong evidence that many dangerous pathophysiological processes, such as cancer, diabetes and cardiovascular and neurodegenerative diseases, are associated with the accumulation of free radicals. A free radical is an atom or molecule that has an unpaired electron and is therefore unstable. Antioxidants (Free radical scavengers) are substances that prevent and stabilize the damage caused by free radicals by supplying electrons from antioxidants to these damage cells. Antioxidants also turn free radicals into waste by-products, which are eliminated from the body.[1]

India is among the biggest producer of herbal medicines. Be that as it may, because of complex nature of herbal extract, expansive dosages, poor bioavailability and dose frequency, there use in present day therapeutic framework is restricted. The projected research work will be useful in developing new delivery system, which will assume to conquer these issues related with conventional herbal extract.[2,3] The requirements of novel drug delivery system of herbal extracts than conventional herbal extracts are as follows.

- To enhance the bio-availability of herbal extracts.
- To decrease the dose of herbal extracts.

Phytosomes are known contain the bioactive phytoconstituents of herb extract bounded by lipids and are developed by incorporating standardized plant extract or water-soluble bioactive plant constituent into phospholipids to make lipid compatible molecular complex called phytosomes and so progress their absorption and bioavailability.[4]

Over the last few years an increasing interest has been gaining momentum among the pharmaceutical scientists to incorporate the herbal extracts or phytoconstituents in phytosomes for improving the bioavailability as well as patient compliance. In 2019 more than 15 research articles for phytosomal formulation of ingredients like diosgenin, curcumin, quercetin, chrysin, Brassica nigra extract, Diospyros kaki extract etc have been published in peer review journals. The following year 2020 witnessed an increase in the research in phytosomes with several modified formulations containing phytosomes. Some noteworthy work included Icariin phytosomes with improved anticancer action, nano-formulations containing vasaka phytosomes for improved bioavailability and Centella asiatica phytosomes with improved cognitive performance. In 2021, the trend continued and surface modification of the phytosomes was also witnessed in research work. Snake venom functionalized quercetin loaded phytosomes was highlight of the most prominent research work on phytosomes.[5-14]

Literature also points out several other researches works on phytosomes which have been published in journals not in high index databases. This signifies the importance of phytosomes in developing optimized formulation of standardized extracts or phytoconstituents.

Pithecellobium bijeninum has been known to contain flavonoids and phenolic compounds that act as strong antioxidants owing to their free radical scavenging potential. The plant is used in the form of oil, gum, or extracts for cure of several diseases. No standardized delivery system for oral administration of the plant extract has been reported. Hence it was envisioned to formulate the phytosomal system as an efficient method for oral delivery of Pithecellobium bijeninum leaf hydroalcoholic extract and assess its antioxidant potential.

2. MATERIAL AND METHODS

2.1 Collection and Preparation of Plant Material

The dried leaves of Pithecellobium bijeninum were collected from the tree growing alongside the Upper-lake, Bhopal. The procured leaves were washed, dried in shade and powdered using a blender at low speed. The leaf powder was stored in air tight container until taken for use.

2.2 Physicochemical Evaluation of Plant Material

2.2.1 Determination of Foreign Matter

Accurately weighed plant material was spread in a thin layer and inspected with the help of magnifying glass. Any foreign matter was removed manually. The leaves were reweighed and the percentage of foreign matter with respect to the air-dried drug was determined.[15]

2.3 Determination of Ash Values[16]

2.3.1 Total Ash

The finely ground plant material was accurately weighed (4 g) and placed in a previously ignited and tarred silica crucible. The sample was ignited by gradual increase in temperature to 250°C until it was white, indicating absence of carbon. It was then cooled in a desiccator and weighed. The percentage of ash with respect to the air-dried drug was calculated.

2.3.2 Acid-insoluble Ash

25 mL of concentrated HCl was added to the crucible containing total ash. The beaker was covered with watch glass and boiled gently for 5 min. The watch glass was rinsed with 5 mL of hot water and the rinsing was added to the crucible. The insoluble matter was collected on an ashless filter paper and washed with hot water until the filtrate became neutral. The filter paper containing the insoluble matter was transferred to the silica crucible, dried on a hot plate and ignited to constant weight. The residue was allowed to cool in desiccator for 30 min and weighed without delay. The percentage of ash with reference to the air-dried drug was calculated.

2.3.3 Water-soluble Ash

To the crucible containing the total ash, 25 mL of distilled water was added and boiled for 5 min. The insoluble matter was collected in a sintered glass crucible or on an ashless filter paper, washed with hot water and ignited in a crucible for 15 min, at temperature not exceeding 450°C. The ash obtained was weighed and this weight was subtracted from the weight of total ash. The percentage of ash with reference to air dried drug was calculated.

2.3.4 Determination of Loss on Drying[16]

Accurately weighed powdered plant material was placed in a dry and tarred flat weighing bottle. The sample was dried in an oven at 100-105°C. The operation was repeated until two consecutive weighing that did not differ by more than 5 mg. The loss of weight in mg per g of air-dried material was calculated.

2.3.5 Alcohol Soluble Extractive Value[16]

About 5 g of powdered plant material was weighed and macerated with 100 mL of the ethanol in a glass stoppered Erlenmeyer flask for 24 h with frequent shaking during the first 6 h and allowing to settle for 18 h. It was filtered rapidly and 25 mL of the filtrate was transferred in to a tarred petri dish and evaporated

to dryness on a water bath. The residue was dried at 105°C for 6 h and cooled in desiccator for 30 min then weighed. The content of alcohol soluble extractable matter in mg per g of air-dried material was calculated.

2.3.6 Water Soluble Extractive Value[16]

About 5 g of powdered plant material was weighed and macerated with 100 mL of the distilled water in a glass stoppered Erlenmeyer flask for 24 h with frequent shaking during the first 6 h and allowing to settle for 18 h. It was filtered rapidly and 25 mL of the filtrate was transferred in to a tarred petri dish and evaporated to dryness on a water bath. The residue was dried at 105°C for 6 h and cooled in desiccator for 30 min then weighed. The content of alcohol soluble extractable matter in mg per g of air-dried material was calculated.

2.3.7 Extraction of Plant Material[17]

An accurately weighed quantity of 50 g of powdered plant material was taken in a 2 L glass jar. The jar was filled with 1000 mL of 90:10 mixture of ethanol-water and macerated for 24 h with intermittent shaking for first 6 h and allowing standing for 18 h. The macerate was filtered and the filtrate was evaporated on water bath. The thick syrupy residue obtained was subjected to rotary evaporation to remove all solvent. The dried extract was kept in desiccator until used for various tests.

2.3.8 Preliminary Qualitative Phytochemical Screening[18]

The extract was screened for phytoconstituent classes present using various qualitative tests for alkaloids, glycosides, tannins, phenolics, flavonoids, proteins and sterols.

2.3.9 Total Phenolic Content19

One gram of the nutraceutical powder was added to 15 ml of methanol (50% v/v in water) and extracted by maceration for 2h, then filtered and made up the volume with methanol (50% v/v in water) in volumetric flask upto 100 ml. One ml aliquot of the sample was taken in a test tube and diluted with 10 ml of distilled water. Then 1.5 ml Folin Ciocalteu's reagent was added and allowed to incubate at room temperature for 5 min. Four ml of 20% (w/v) Na2CO3 was added, adjusted with distilled water up to the mark of 25 ml, agitated and left to stand for 30 min at room temperature. Absorbance of the sample was measured at 765 nm against blank, i.e., distilled water.

Standard solutions of gallic acid (10-100 ppm) were similarly treated to plot the analytical curve. The control solution contained 200 µL of methanol and suitable reagents, and it was prepared and incubated under the same conditions as the rest of the samples.

2.4 Preparation and Optimization of Phytosomes by Solvent Evaporation Method[20]

A general factorial approach with three levels of lipid concentration and two levels of extract concentration was used to study the effect of extract concentration and lipid concentration on the particle size obtained for the phytosomes.

The specific amount of leaf extract of Pithecellobium bijeninum and soya lecithin (Table 1) were taken into a 100 mL round bottom flask and refluxed with 30 mL of ethanol at a temperature 40 – 50°C for 4 h. The mixture was concentrated to 5-10 ml to obtain the sticky precipitate which was lyophilized to obtain the phytosomes. The dried phytosomes complex was placed in amber colored glass bottle and stored in refrigerator.

Table 1: Batch processing for phytosome preparation

Formulation Code	Lecithin	Extract	Ethanol (mL)
F1	0.2	0.2	30
F2	0.1	0.2	30
F3	0.1	0.1	30
F4	0.3	0.2	30
F5	0.3	0.1	30
‘F6	0.2	0.1	30

2.5 Evaluation of Phytosomes[20]

2.5.1 Visualization

Visualization of phytosomes was accomplished by utilizing scanning electron microscopy. Scanning electron microscopy has been utilized to decide particle size estimate appropriation and surface morphology of the complex. The samples were sputter-covered with gold/palladium for 120 s at 14 mA under argon air for auxiliary electron emissive SEM (Hitachi-S 3400N) and watched for morphology at voltage of 15.0 kV.

2.5.2 Particle Size and Size Distribution

The particle size (z-average) and size distribution of the prepared phytosomes was calculated from the auto correlation function of the intensity of light scattered from the particles expecting a circular type of particles using Malvern Zeta sizer.

2.5.3 Antioxidant Action

The antioxidant action of the synthesized compounds was determined using 2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay.[22]

The free radical scavenging activity of the phytosomes was measured in terms of radical scavenging ability using the stable radical DPPH. The test samples (100 μL, 100-500 µg/mL) were prepared in DMSO and were mixed with 1.0 mL of DPPH solution and filled up with methanol to a final volume of 4 mL. Absorbance of the resulting solution was measured at 517 nm in a visible spectrophotometer. Ascorbic acid was used as the reference compound. Lower absorbance of the reaction mixture indicated higher free radical scavenging activity. Radical scavenging activity was expressed as the inhibition percentage of free radical by the sample and was calculated using the following formula:

$$\% \ inhibition = \frac{(Ao - At)}{Ao} X\ 100$$

where Ao is the absorbance of the control (blank, without sample) and At is the absorbance in the presence of the test samples. All tests were performed in triplicate and the results were expressed as mean values ± standard deviations.

3. RESULTS AND DISCUSSION

3.1 Physicochemical Investigation

The preliminary investigations of the leaf, was performed according to the methods reported in previous sections and the results obtained thereof are presented in Table 2.

Table 2: Results of preliminary investigation of the leaf

Plant Part	Parameter	Value Obtained (%)
Pithecellobium bijeninum leaf Powder	Foreign Matter	1.2
	Total Ash	8.1
	Water Soluble Ash	4.5
	Acid Insoluble Ash	3.2
	Ethanolic Soluble extractives	9.4
	Water Soluble Extractives	6.6
	Loss on Drying	1.78

3.2 Extraction and Phytochemical Screening

The extract was dark brown in color and obtained in a yield of 6.12 %. The phytochemical screening revealed the presence of alkaloids and flavonoids in the extract (Table 3).

Table 3: Qualitative phytochemical screening

Chemical Tests	Results
Mayer's reagent	+
Wagner's reagent	+
Dragendorff's reagent	+
Froth test	-
Bontrager's Test	-
Gelatin Solution	-
Alkaline reagent test	-
Vanillin HCl test	-
Shinoda test	-
Alkaline reagent test	+
Zinc HCl reduction test	-
Ninhydrin Test	-
Salkowski Test	-

3.3 Total Phenolic Content

Standard curve of gallic acid was calculated and plotted in distilled water for determining absorption data. From this Beer's law range and regression coefficient is determined. The linear equation of gallic acid was found to be y = 0.0046x + 0.0024 (Figure 1). The total phenolic content in extracts, expressed as percent w/w.

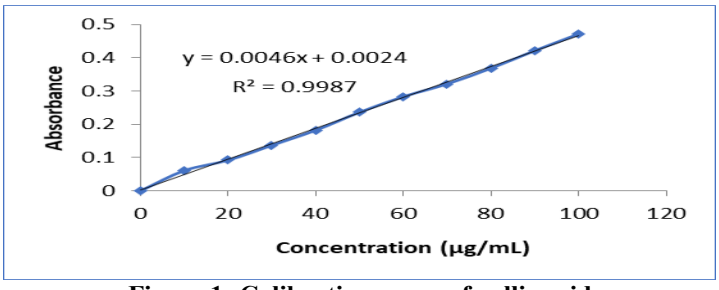

Figure 1: Calibration curve of gallic acid

The total phenolic content in the aqueous extract of Pithecellobium bijeninum was found to be 23.2 ± 0.827 %w/w.

3.4 Preparation of Phytosomes

The phytosomes loaded with Pithecellobium bijeninum leaf extract were prepared using solvent evaporation method. In this technique, the phytoconstituents or extract and the lipid (lecithin) are kept in a flask containing organic solvent. This reaction mixture is kept at an optimum temperature usually 40°C for specific time period to attain maximum drug entrapment in the phytosomes formed. The organic solvent is then removed using rotary evaporator.

3.5 Optimization of the Phytosomes

Particle size was used as the response factor to assess the best combination of the lipid and extract in preparing phytosomes. The objective was to achieve the minimum possible size. The particle size of the six prepared formulations was obtained and the formulation with the lowest particle size was considered optimum.

The particle size and size distribution for each batch of phytosomes was determined using zeta sizer. The formulations ranged from 468 nm to 1827 nm in size with a polydispersity index varying between 0.178 - 0.464 (Table 4).

Table 4: Particle size and size distribution of various batches of phytosomes

Formulation Code	Particle Size (r.nm)	Polydisperisty Index (PDI)
F 1	617	0.217
F 2	1211	0.286
F 3	1256	0.431
F 4	468	0.178
F 5	953	0.464
F 6	1827	0.392

It was evident from the results of the particle size that the amount of lipid and extract had a significant effect on the particle size of the phytosome. The phytosomes prepared with lower lipid were found to be of higher sizes whereas those with higher concentration of the lipid were small in size.

3.6 Evaluation of Phytosomes

3.6.1 Surface Morphology (Visualization)

The selected phytosomes with lowest particle size were analyzed under and electron microscope. The particles were visible as rigid, almost spherical vesicles in SEM image. The surface of the phytosome vesicles was found to be regular and smooth.

3.6.2 Antioxidant Study

The phytosomes were studied for their antioxidant action against DPPH radical by colorimetric assay. The absorbance of control (DPPH + methanol) as well as various concentration of the test solution was measured at 517 nm using UV-visible spectrophotometer and the % DPPH inhibition was measured.

The thermogram of soya lecithin gives distinct peak at 57°C indicating melting (Figure 6.4a). Sharp endothermal peak was found in the thermogram of the phytosome at 257°C.

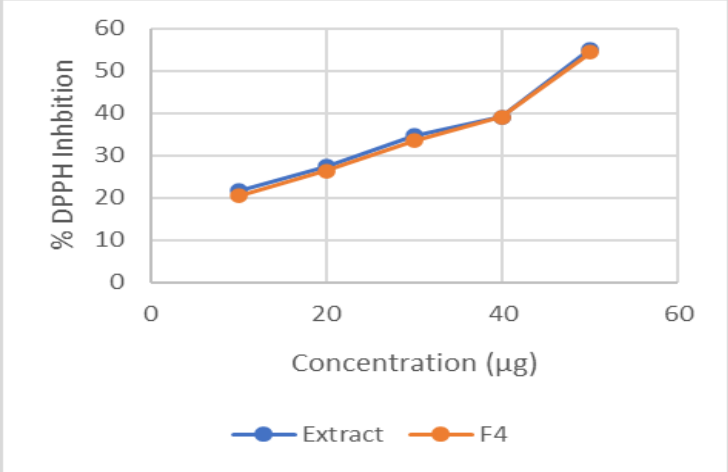

Figure 2: % DPPH Scavenging by test compounds

The IC50 value of extract and phytosome (F4) in inhibition DPPH radical was calculated from the graph (Figure 2). The phytosomes F4 (IC50 48.90 µg) were found to possess good antioxidant action against the free radical almost equivalent to the pure extract (IC50 48.24 µg).

4. CONCLUSION

The study presented in this thesis reveals the excellent potential of phytosome based drug delivery system for anti-oxidant action of plant extract. We can conclude that phytosome based formulation could be a valuable approach to improve the

therapeutic efficacy, to reduce dose and improvement in dosage regimen for plant extracts and phytoconstituents.

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