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RESEARCH ARTICLE

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FORMULATION AND EVALUATION OF PHYTOSOMES CONTAINING GARUGA PINNATA EXTRACT FOR DIABETES TREATMENT

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ABSTRACT

Diabetes mellitus is a chronic metabolic disorder requiring long-term therapeutic management, and herbal formulations offer promising alternatives due to their safety and multitargeted action. The present study aimed to formulate and evaluate phytosomes containing hydroalcoholic extract of *Garuga pinnata* for enhanced antidiabetic activity. The hydroalcoholic extraction yielded 8.50% w/w of extract, which was subjected to preliminary phytochemical screening and quantitative estimation of alkaloids. Phytosomal formulations were prepared using the thin-film hydration method and evaluated for particle size, entrapment efficiency, in-vitro antidiabetic activity, and drug release behavior. Among twelve formulations, batch F10 exhibited the smallest particle size (228.90 nm) and highest entrapment efficiency (76.80%). In-vitro α -amylase inhibition studies demonstrated concentration-dependent inhibitory activity of the phytosomal formulation, with an IC₅₀ value of 293.92 μ g/mL compared to 99.76 μ g/mL for acarbose. The optimized formulation showed sustained drug release up to 12 hours, with release kinetics best fitting the first-order and Higuchi models, indicating diffusion-controlled release. These findings suggest that phytosomal delivery of *Garuga pinnata* extract enhances its physicochemical and biological performance, making it a promising approach for the management of diabetes mellitus.

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INTRODUCTION

Diabetes mellitus is a chronic metabolic disorder characterized by persistent hyperglycemia due to impaired insulin secretion, insulin action, or both. Long-term hyperglycemia leads to severe complications affecting the cardiovascular system, kidneys, nerves, and eyes. According to the International Diabetes Federation, the global burden of diabetes is increasing rapidly, particularly in developing countries, necessitating the development of safer and more effective therapeutic strategies with minimal side effects [1].

Medicinal plants have gained considerable attention in diabetes management due to their multitargeted action, affordability, and lower toxicity compared to synthetic drugs. Numerous plant-derived bioactive compounds such as flavonoids, phenolics, alkaloids, and terpenoids exhibit antidiabetic activity through mechanisms including enhancement of insulin secretion, improvement of insulin sensitivity, inhibition of carbohydrate-digesting enzymes, and antioxidant effects [2,3]. *Garuga pinnata* Roxb. (family *Burseraceae*) is a deciduous tree widely distributed in India and Southeast Asia and is traditionally used in the treatment of inflammatory disorders, respiratory diseases, and metabolic conditions. Phytochemical investigations of *G. pinnata* have revealed the presence of flavonoids, phenolic compounds, triterpenoids, and diarylheptanoids, which are known for their antioxidant and antidiabetic properties [4,5]. Experimental studies have demonstrated that aqueous and alcoholic extracts of

G. pinnata significantly reduce blood glucose levels, improve lipid profiles, enhance insulin secretion, and restore antioxidant enzyme levels in streptozotocin-induced diabetic animal models, supporting its potential role in diabetes management [6,7]. Despite its promising pharmacological activity, the therapeutic application of *G. pinnata* extract is limited due to poor aqueous solubility, low permeability, and limited bioavailability of its phytoconstituents. These limitations result in reduced absorption and inconsistent therapeutic outcomes [8].

Therefore, novel drug delivery approaches are required to enhance the bioavailability and efficacy of herbal extracts. Phytosome technology is an advanced lipid-based drug delivery system in which phytoconstituents are complexed with phospholipids, resulting in improved solubility, stability, and membrane permeability. Phytosomes have been reported to significantly enhance the oral bioavailability and pharmacological activity of several herbal extracts, including those with antidiabetic and antioxidant potential [9,10]. In this context, the present study focuses on the formulation and evaluation of phytosomes containing *Garuga pinnata* extract with the aim of enhancing its bioavailability and therapeutic potential for diabetes treatment. The developed phytosomal formulations were characterized for physicochemical properties and evaluated for their suitability as an effective herbal antidiabetic delivery system.

MATERIAL AND METHODS

Material

Leaves of *Garuga pinnata* were collected, authenticated, shade dried, and powdered for extraction. Hydroalcoholic solvent was used for the preparation of plant extract. Phospholipids (soya lecithin) and cholesterol were employed for the formulation of phytosomes. Acarbose was used as the standard drug for in-vitro antidiabetic activity. All chemicals and reagents used for phytochemical screening and analytical studies were of analytical grade. Double distilled/RO water was used throughout the study.

Methods

Extraction by maceration method: 50 gram of leaves of *Garuga pinnata* was exhaustively extracted with hydroalcoholic solvent (ethanol and water; 70:30) by maceration method. The extract was evaporated above their boiling points. Finally, the percentage yields were calculated of the dried extracts¹¹.

the test and standard solutions was measured at 470 nm in relation to the reagent blank. According to Shamsa *et al.*, (2008), the total alkaloid concentration was stated as mg of AE/100 mg of extract¹³.

Formulation development of phytosomes: The complex was prepared with phospholipids: Cholesterol and *Garuga pinnata* in the ratio of 1:5:1, 1:1:1, 2:1.5:1, 2:2:1 respectively¹⁴. 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.

Characterization of phytosomes

Entrapment efficiency: Phytosome preparation was taken and subjected to centrifugation using cooling centrifuge (Remi) at 12000 rpm for an hour¹⁵.

Table 1. Different formulations of phytosomes

Formulation	Ratio of Phospholipids and Cholesterol	Extract Concentration (%)	Dichloromethane Concentration
Optimization of Phospholipids and Cholesterol			
F1	1:05	1	25
F2	1:1	1	25
F3	1:1.5	1	25
F4	1:2	1	25
Optimization of Drug Concentration			
F5	1:1	0.5	25
F6	1:1	1.0	25
F7	1:1	1.5	25
F8	1:1	2.0	25
Optimization of solvent concentration			
F9	1:1	1.0	10
F10	1:1	1.0	25
F11	1:1	1.0	50
F12	1:1	1.0	75

Determination of percentage yield: By determining the percentage yield of successively extracted extracts, the study aims to optimize the extraction process and identify the most suitable solvent for obtaining maximum yield of bioactive compounds from leaves of *Garuga pinnata*. The percentage yield of yield of each extract was calculated by using formula:

$$\text{Percentage yield} = \frac{\text{Weight of extract}}{\text{Weight of powdered drug taken}} \times 100$$

Phytochemical screening: Phytochemical screening of successively extracted fractions from plant material holds significant importance in elucidating the presence of various bioactive compounds. Each solvent used in the extraction process targets different classes of phytoconstituents based on their polarity, thereby allowing for the comprehensive assessment of the plant's chemical composition. The presence of these compounds can indicate potential biological activities such as antioxidant, antimicrobial, or anti-inflammatory properties. Phytochemical examinations were carried out extracts as per following standard methods¹².

Quantitative studies of phytoconstituents

Estimation of total alkaloids content: Dissolving 1 mg of the plant extract in methanol, 1 ml of 2 N HCl was added, and the mixture was filtered. After transferring this solution to a separating funnel, 5 ml of phosphate buffer and bromocresol green solution were added. After vigorously shaking the mixture with 1, 2, 3, and 4 ml of chloroform, it was collected in a 10 ml volumetric flask and diluted with chloroform to the appropriate volume. As previously mentioned, a series of reference standard atropine solutions (40, 60, 80, 100, and 120 µg/ml) were made. Using a UV/visible spectrophotometer, the absorbance of

The clear supernatant was siphoned off carefully to separate the non entrapped flavonoids and the absorbance of supernatant for non entrapped *Garuga pinnata* extract was recorded at λ_{max} 420.0 nm using UV/visible spectrophotometer (Labindia 3000+). Sediment was treated with 1ml of 0.1 % Triton x 100 to lyse the vesicles and diluted to 100 ml with 0.1 N HCl and absorbance taken at 420.0 nm. Amount of quercetin in supernatant and sediment gave a total amount of *Garuga pinnata* extract in 1 ml dispersion. The percent entrapment was calculated by following formula.

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

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)¹⁶⁻¹⁷. 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.

In vitro anti-diabetic activity of phytosome using inhibition of alpha amylase enzyme

Preparation of standard: 10 mg acarbose was dissolved in 10 ml methanol, and various aliquots of 100- 500µg/ml were prepared in methanol.

Preparation of sample: 10 mg of phytosomewas extracted with 10 ml methanol. 500 µl of this solution was used for the estimation of enzyme inhibition.

Method: A total of 500 μ l of test samples and standard drug (100-500 μ g/ml) were added to 500 μ l of 0.20 mM phosphate buffer (pH 6.9) containing α -amylase (0.5mg/ml) solution and were incubated at 25°C for 10 min. After these, 500 μ l of a 1% starch solution in 0.02 M sodium phosphate buffer (pH 6.9) was added to each tube. The reaction mixtures were then incubated at 25°C for 10 min. The reaction was stopped with 1.0 ml of 3, 5 dinitrosalicylic acid colour reagent¹⁸. The test tubes were then incubated in a boiling water bath for 5 min, cooled to room temperature. The reaction mixture was then diluted after adding 10 ml distilled water and absorbance was measured at 540 nm. Control represent 100% enzyme activity and were conducted in similar way by replacing extract with vehicle.

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 \pm 0.5°C 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°C) was replaced every time with the same quantity of the sample and takes the absorbance at 256.0 nm using spectroscopy¹⁹.

RESULTS AND DISCUSSION

The hydroalcoholic extraction of *Garuga pinnata* yielded 8.50% w/w extract (Table 1), indicating effective recovery of phytoconstituents using a hydroalcoholic solvent system. Such solvent combinations are known to enhance extraction efficiency by solubilizing both polar and moderately non-polar bioactive compounds, which is essential for pharmacological evaluation.

Table 1. % Yield of *Garuga pinnata*

S. No.	Extract	% Yield (W/W)
1.	Hydroalcoholic	8.50

Table 2. Result of phytochemical screening of hydroalcoholic extract of *Garuga pinnata*

S. No.	Constituents	Hydroalcoholic extract
1.	Alkaloids Wagner's Test: Hager's Test:	+ve -ve
2.	Glycosides Conc. H ₂ SO ₄ Test:	-ve
3.	Flavonoids Lead acetate Test: Alkaline Reagent Test:	-ve -ve
4.	Diterpenes Copper acetate Test:	+ve
5.	Phenol Ferric Chloride Test: Folin Ciocalteu Test:	-ve -ve
6.	Proteins Xanthoproteic Test:	+ve
7.	Carbohydrate Fehling's Test:	-ve
8.	Saponins Froth Test:	-ve
9.	Tannins Gelatin Test:	-ve
10.	Sterols Salkowski's Test:	-ve

[+ve = positive; -ve = negative]

Table 3. Estimation of total alkaloids content of *Garuga pinnata*

S. No.	Extract	Total alkaloids content (mg/100mg of dried extract)
1	Hydroalcoholic	0.763

Preliminary phytochemical screening of the hydroalcoholic extract (Table 2) revealed the presence of alkaloids, diterpenes, and proteins, while glycosides, flavonoids, phenols, carbohydrates, saponins, tannins, and sterols were absent. The presence of alkaloids is particularly important, as these compounds are reported to exert antidiabetic effects by improving insulin sensitivity and regulating glucose metabolism. This observation was further supported by quantitative estimation, which showed a total alkaloid content of 0.763mg/100 mg of dried extract (Table 3), confirming the extract as a potential candidate for antidiabetic formulation development.

Table 4. Particle size and entrapment efficiency of drug loaded phytosomes

Formulation Code	Particle Size (nm)	Entrapment Efficiency (%)
F1	372.80	64.20
F2	295.40	71.85
F3	318.65	69.40
F4	348.90	66.95
F5	305.25	68.30
F6	252.10	73.85
F7	290.75	70.10
F8	304.60	66.40
F9	272.35	67.10
F10	228.90	76.80
F11	240.45	69.25
F12	258.70	70.60

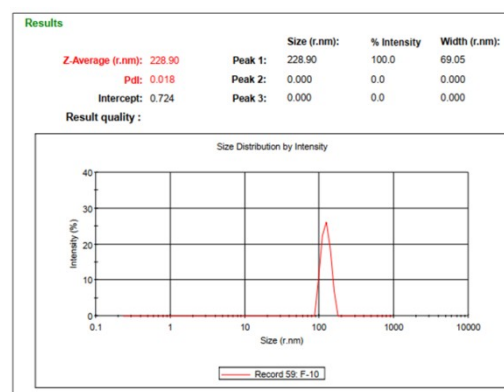


Figure 1. Particle size of optimized batch F10

Table 5. % Inhibition of acarbose and prepared phytosomes of *Garuga pinnata*

S. No.	Concentration (μ g/ml)	% Inhibition (Acarbose)	% Inhibition (Phytosomes)
1	100	48.25	28.40
2	200	62.85	41.65
3	300	71.40	52.30
4	400	82.10	60.95
5	500	91.75	69.80
	IC ₅₀ (μ g/ml)	99.76	293.92

Phytosomal formulations (F1–F12) were evaluated for particle size and entrapment efficiency (Table 4). The particle size ranged from 228.90 to 372.80 nm, indicating successful formation of nanosized vesicles. Entrapment efficiency varied between 64.20% and 76.80%, reflecting effective incorporation of phytoconstituents within the phospholipid matrix. Among all formulations, F10 demonstrated the smallest particle size (228.90 nm) and highest entrapment efficiency (76.80%), suggesting optimal formulation parameters and enhanced phytoconstituent–phospholipid interaction. The particle size distribution of the optimized batch F10 (Figure 1) further confirmed uniform vesicle formation, which is crucial for improved bioavailability and therapeutic performance. The in-vitro α -amylase inhibitory activity of *Garuga pinnata* phytosomes was evaluated and compared with the standard antidiabetic drug acarbose (Table 5).

Table 6. 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	22.36	1.349	77.64	1.890
1	1	0	32.23	1.508	67.77	1.831
2	1.414	0.301	49.98	1.699	50.02	1.699
4	2	0.602	68.36	1.835	31.64	1.500
6	2.449	0.778	89.98	1.954	10.02	1.001
8	2.828	0.903	96.65	1.985	3.35	0.525
12	3.464	1.079	99.45	1.998	0.55	-0.260

Table 7. Regression analysis data of optimized formulation F10

Batch	Zero Order	First Order	Higuchi	Korsmeyer Peppas
	R ²	R ²	R ²	R ²
F10	0.8472	0.9861	0.9499	0.8888

The phytosomal formulation showed concentration-dependent inhibition, achieving 69.80% inhibition at 500 µg/mL, while acarbose exhibited 91.75% inhibition at the same concentration. The IC₅₀ value of the phytosomal formulation (293.92 µg/mL) was higher than that of acarbose (99.76 µg/mL), indicating moderate but significant antidiabetic activity. The enhanced inhibitory activity compared to crude extracts may be attributed to improved solubility and absorption of bioactive constituents through phytosomal encapsulation. In-vitro drug release studies of the optimized formulation F10 (Table 6) demonstrated a sustained and controlled release pattern, with 99.45% cumulative drug release over 12 hours. The initial release phase may be due to surface-associated drug, followed by a gradual release governed by diffusion from the phytosomal matrix, highlighting the suitability of the formulation for prolonged therapeutic action. Release kinetics analysis (Table 7) revealed that the drug release from formulation F10 best followed first-order kinetics (R² = 0.9861), indicating concentration-dependent release behavior. A high correlation with the Higuchi model (R² = 0.9499) suggested diffusion-controlled drug release, while the Korsmeyer–Peppas model (R² = 0.8888) indicated a non-Fickian diffusion mechanism. These findings collectively confirm the controlled release characteristics of the optimized phytosomal formulation. The study demonstrates that phytosomal formulation of *Garuga pinnata* extract significantly enhances physicochemical properties, improves biological activity, and provides sustained drug release, supporting its potential application in diabetes management.

CONCLUSION

The present study successfully demonstrated the formulation and evaluation of phytosomes containing *Garuga pinnata* hydroalcoholic extract for potential antidiabetic application. The extract showed a satisfactory percentage yield and the presence of important bioactive constituents, particularly alkaloids and diterpenes, which are known to contribute to antidiabetic activity. Phytosome formulations were effectively developed, exhibiting particle sizes in the nanometer range and good entrapment efficiency, with formulation F10 identified as the optimized batch based on minimum particle size and maximum entrapment efficiency. The in-vitro antidiabetic study revealed a concentration-dependent inhibitory effect, confirming the biological potential of the formulated phytosomes, although lower than the standard acarbose. The optimized phytosomal formulation showed sustained drug release up to 12 hours, and release kinetic analysis indicated that the formulation followed first-order kinetics with a significant contribution of diffusion-controlled release.

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