

“Development And Characterization of a Phytosome-Based Delivery System of Tephrosia Pul Seed Extract and Its Antibacterial Activity”

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Abstract

Aim:

To develop and characterize a phytosome-based delivery system of *Tephrosiapurpurea* seed extract and to evaluate its antibacterial activity. **Materials and Methods:** Seeds of *Tephrosiapurpurea* were collected and extracted using the Soxhlet method with methanol following defatting with petroleum ether. The extract was subjected to solubility studies, preliminary phytochemical screening, and quantitative estimation of total phenolic and flavonoid content. Phytosomes were prepared using a thin-film hydration method and characterized for physical appearance, particle size, zeta potential, and surface morphology using SEM. Stability studies were performed under ICH guidelines. Antibacterial activity was evaluated against *Escherichia coli* and *Staphylococcus aureus* using the agar well diffusion method. **Results:** The methanolic extract showed higher yield (2.94%) compared to petroleum ether extract (1.64%) and contained प्रमुख phytoconstituents such as flavonoids, phenolics, alkaloids, and glycosides. Total phenolic and flavonoid contents were found to be 25.45 mg/g GAE and 24.9 mg/g RE, respectively. Among the formulations, F1 exhibited the smallest particle size (190.96 nm) and highest zeta potential (-42.9 mV), indicating good stability, which was confirmed over 90 days. The phytosome formulation showed enhanced antibacterial activity compared to the crude extract, with maximum zones of inhibition at 1.5 mg/ml concentration against both test organisms. **Conclusion:** The study demonstrates that phytosome encapsulation significantly improves the physicochemical properties, stability, and antibacterial efficacy of *Tephrosiapurpurea* seed extract. This approach offers a promising strategy for enhancing the therapeutic potential of plant-based formulations in pharmaceutical applications.

Keywords: *Tephrosiapurpurea*, Phytosomes, Antibacterial activity, Phytochemical screening, Total phenolic content.

I. Introduction

Tephrosiapurpurea Linn are Thila (in Gujarati), Sarponkh (in Hindi), Vempali (in Telugu). This drug is also not official in Ayurvedic Pharmacopoeia (Khatri et al, 2009). It is one of the excellent plants gifted by the nature for human beings, and is composed of all the essential constituents that are required for normal and good human health. Leaves of *T. purpurea* are taken as emetic in the form of leaf juice or decoction. This along with sugar is also used in jaundice. *T. purpurea*, commonly known in Sanskrit as Sharapunkha, is a highly branched, suberect, herbaceous perennial herb. (Chopra et al, 2011). According to Ayurveda literature, this plant has also been given the name of “wranvishapaka” which means that it has the property of healing all types of wounds (Deshpande et al., 2003). It is an important component of some preparations such as Tephroli and Yakrifit used for liver disorders. In Ayurvedic system of medicine, various parts of this plant are used as remedy for impotency, asthma, diarrhea, gonorrhea, rheumatism, ulcer and urinary disorders. The plant has been claimed to cure diseases of kidney, liver spleen, heart and blood (Kirtikar & Basu, 1918). A promising method to increase the effectiveness of phytochemicals for treating wounds is to incorporate them into nano-drug delivery systems (NDDS). Phytosomes are phyto-phospholipid complexes, obtained from the interaction between phospholipids and naturally occurring active phytochemicals. (Bombardelli & Patri, 1991). From an economic point of view, phospholipids have a dual role as a phytochemical-complexing agent as well as a vesicle-forming unit during the preparation of phytosomes. Moreover, phytosomes show

superior drug encapsulation, enhanced bioavailability, and better stability profile (due to chemical interaction between the polar head of the amphiphilic molecule and phytochemical) associated with improved pharmacological and pharmacokinetic features.³⁴ Beneficially, enhanced bioavailability due to a higher absorption rate could result in a lower dosage of active ingredients needed to exert a biological effect (Barani et al., 2021). The study successfully demonstrated that phytosome-based delivery of *Tephrosiapurpurea* seed extract significantly improves its physicochemical properties, stability, and antibacterial activity.

II. Materials and methods

2.1 Plant collection

Seeds of *Tephrosiapurpurea* (Pul) were collected in the required quantity (500 g) from a natural habitat/local agricultural area during the appropriate harvesting season. The plant material was authenticated by a qualified botanist, and voucher specimen was preserved for future reference (Krakowska et al., 2022).

2.2 Extraction process

The dried seeds of *Tephrosiapurpurea* were first cleaned, shade-dried, and coarsely powdered using mechanical grinder to increase the surface area for extraction. About 500 mg of the powdered seeds was initially defatted by extraction with 500 mg of petroleum ether in Soxhlet apparatus for 4–6 hours to remove non-polar components such as fats and oils. (Krakowska et al., 2022).

2.3 Solubility study of the solubility of the *Tephrosiapurpurea* seed extract

The solubility of the *Tephrosiapurpurea* seed extract was evaluated in various solvents to determine its suitability for formulation and delivery. The solutions were observed for complete or partial solubility, turbidity, precipitation, or formation of residue (Yadav et al., 2014; Zhou et al., 2015).

2.4 Phytochemical screening of the plant extract

The crude seed extract of *Tephrosiapurpurea* was subjected to a comprehensive series of qualitative phytochemical tests to determine the presence of various bioactive constituents, including alkaloids, glycosides, carbohydrates, flavonoids, tannins, phenolic compounds, saponins, triterpenoids, and steroids (Goswami & Singh, 2018).

2.3 Determination of Total Phenolic Content

The total phenolic content of *Tephrosiapurpurea* seed extract was determined using the Folin-Ciocalteu method, a widely accepted and reliable colorimetric assay for phenolic

estimation. The total phenolic content was expressed as milligrams of gallic acid equivalents (GAE) per gram of dried extract (Goswami & Singh, 2018).

2.4 Determination of Total Flavonoid Content (TFC)

The total flavonoid content of the *Tephrosiapurpurea* seed extract was estimated using a colorimetric aluminum chloride method with minor modifications. The total flavonoid content was expressed as milligrams of rutin equivalents per gram of dried extract (Matić et al., 2017).

2.5 Preparation of extract loaded phytosomes

Tephrosiapurpurea seed extract-loaded phytosomes were prepared using a modified film hydration method. Soya lecithin, cholesterol, and stearic acid were dissolved in a chloroform–methanol mixture to form a homogeneous lipid phase, followed by solvent evaporation under reduced pressure to obtain a thin lipid film. The film was hydrated with phosphate-buffered saline (pH 7.4) containing the plant extract under continuous stirring, facilitating phytosome formation. Sorbitol was added as a cryoprotectant, and the formulation was sonicated to reduce particle size and achieve uniform dispersion. The prepared phytosomes were then stored for further characterization (Bala et al., 2022).

2.6 Characterization of extract loaded phytosomes

2.6.1 Physical appearance of extracts loaded Phytosomes

The physical appearance of the *Tephrosiapurpurea* seed extract-loaded phytosomes was evaluated by visual inspection. The formulation was examined for color, clarity, uniformity, presence of aggregates, precipitation, and phase separation (Sasongko et al., 2019).

2.6.2 Particle size

Particle size was determined using a Malvern Zetasizer (Malvern Instruments, UK) based on the dynamic light scattering (DLS) technique. The instrument determined the mean particle size (Z-average) and size distribution profile, and values were recorded after averaging multiple runs to ensure accuracy (Jain et al., 2013).

2.6.3 Zeta potential

Zeta potential of the *Tephrosiapurpurea* seed extract-loaded phytosomes was measured using a Malvern Zetasizer based on electrophoretic light scattering to determine the surface charge and colloidal stability of the dispersion (Bhattacharjee, 2016).

2.6.4 Scanning Electron Microscopic (SEM)

The surface morphology of the optimized phytosomes loaded with *Tephrosiapurpurea* seed

extract was examined using scanning electron microscopy (SEM). During SEM, a focused electron beam scanned the coated sample, causing emission of secondary electrons from the surface, which were collected by detectors to generate high-resolution images of the particle morphology and surface structure (Ali et al., 2023).

2.6.5 Stability study

Stability studies were conducted to evaluate the effect of various environmental storage conditions on the physical and chemical integrity of the *Tephrosiapurpurea* seed extract-loaded phytosome formulation. At predetermined time intervals (for example, initial, 15, 30, 45, 60, and 90 days or as per study design), samples were withdrawn from

each storage condition and evaluated for changes in key quality attributes (Cha et al., 2011).

2.7 Anti-bacterial activity of extract-loaded phytosomes by Well diffusion assay

The antibacterial activity of *Tephrosiapurpurea* extract-loaded phytosomes was evaluated using the agar well diffusion method. Sterile nutrient agar plates were inoculated with standardized cultures of *Staphylococcus aureus* and *Escherichia coli*. Wells were aseptically prepared, and the phytosome formulation along with appropriate controls was introduced. After incubation at 37 °C for 18–24 h, zones of inhibition were observed around the wells, indicating antibacterial activity of the formulation (Tamilselvi et al., 2021).

III. Results

3.1 Percentage Yield

Plant name	Solvent	Color of extract	Theoretical weight	Yield (gm)	Yield (gm)
<i>Tephrosiapurpurea</i>	Petroleum	Brownish yellow	250	8.20	1.64%
<i>Tephrosiapurpure</i>	Methanol	Brownish	250	13.23	2.94

3.2 Solubility study

Extract	Solvents	Observation/Inference
<i>Tephrosiapurpurea</i>	Distilled water	Very slightly soluble
	Methanol	Freely soluble
	Petroleum ether	Freely soluble
	Acetone	Soluble
	Chloroform	Soluble

3.3 Preliminary Phytochemical study

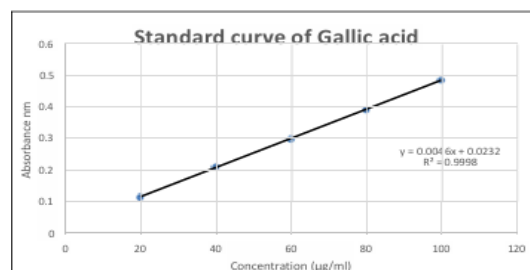
Table 2: Phytochemical testing of the extract of methanol

Sr.no	Experiment	Presence or absence of phytochemical test	
		Pet. Ether extract	Methanolic extract
1.	Alkaloids	Present	Present
2.	Glycoside	Present	Present
3.	Carbohydrates	Present	Present
4.	Flavonoids	Present	Present
5.	Tannin and Phenolic Compounds	Present	Present
6.	Saponin	Absent	Absent
7.	Test for Triterpenoids and Steroids	Present	Present

3.4 Total Phenolic content (TPC) estimation

Table 3: Standard table for Gallic acid-

S. No.	Concentration (µg/ml)	Absorbance
1.	20	0.114
2.	40	0.211
3.	60	0.298
4.	80	0.391
5.	100	0.485



Total 4: Total Phenolic Content in extract of *Guazuma ulmifolia*

3.5 Total Flavonoids content (TFC) estimation

Table 5: Standard table for Rutin

S.No.	Concentration (µg/ml)	Absorbance
1.	20	0.117
2.	40	0.220
3.	60	0.302
4.	80	0.400
5.	100	0.489

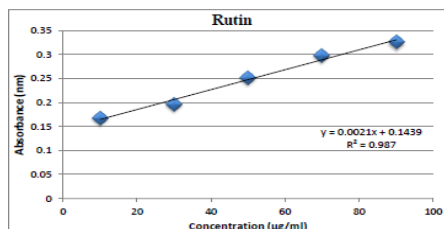


Table 6: Total Flavonoid Content

S. No.	Absorbance(nm)	TFC in mg/gm equivalent of Rutin
1.	0.117	24.9 mg/gm
2.	0.128	
3.	0.136	

S. No	Absorbance	TPC in mg/gm equivalent of Gallic Acid
1.	0.115	25.45 mg/gm
2.	0.125	
3.	0.137	

3.6 Evaluation parameter of phytosomes

3.6.1 Physical Appearance

Table 7: Physical Appearance phytosomes formulation

Parameter	Observation
Color	Light to dark brown
Odor Nutty	Nutty, slightly sweet, and aromatic
Appearance	Slightly opaque dispersion
State	Liquid

3.6.2 Particle size

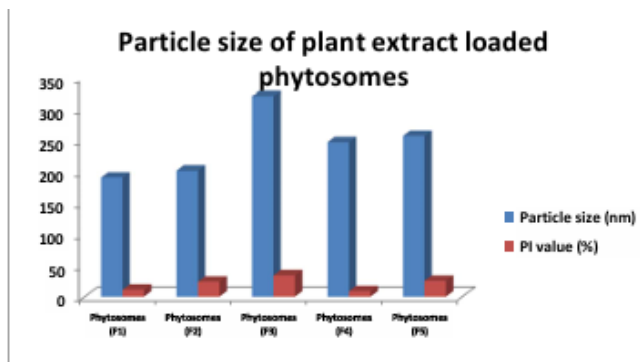


Figure 1: Graphical representation of Particle size of Phytosomes

Table 8: Particle size of plant extract loaded phytosomes

S. No	Formulation	Particle size (nm)	PI value (%)
1.	Phytosomes (F1)	190.96	11.3
2.	Phytosomes (F2)	202.1	24.03
3.	Phytosomes (F3)	321.7	34.3

4.	Phytosomes (F4)	247.8	8.9
5.	Phytosomes (F5)	257.9	25.3

3.6.3 Zeta potential

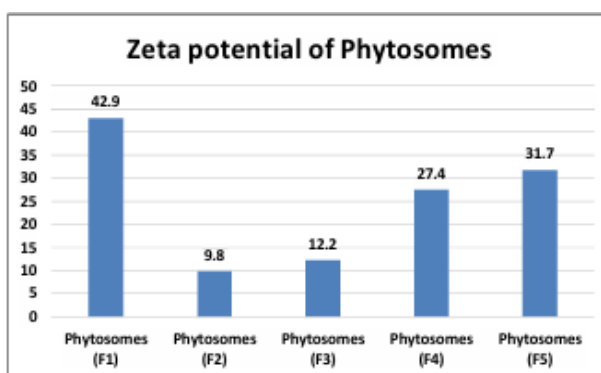


Figure 2: Graphical representation of Zeta potential of Phytosomes

Table 9: Zeta potential of Phytosomes formulation

S. No	Formulation	Particle size (nm)
1.	Phytosomes (F1)	-42.9 mV
2.	Phytosomes (F2)	-9.8 mV
3.	Phytosomes (F3)	-12.2 mV
4.	Phytosomes (F4)	-27.4 m V
5.	Phytosomes (F5)	-31.7 m V

3.6.4 SEM analysis of Optimized formulation

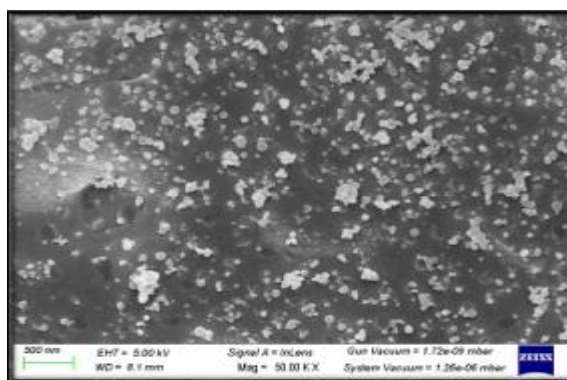


Figure 3: Scanning electron microscope (SEM) and microscopy image

3.7 Stability study

Table 10: Stability Study of phytosome formulation (ICH Q1A guidelines)

S. No	Time (Days)	25 0 C±2 0 C and 60 ± 5% RH		40 0 C±2 0 C and 70 ±5% RH	
		Particle size	State	Partcile Size	State
1.	0	190.96 nm	Liquid	190.96 nm	Liquid
2.	30	189.90 nm	Liquid	192.93 nm	Liquid
3.	45	193.87 nm	Liquid	194.89 nm	Liquid
4.	60	196.92 nm	Liquid	195.91 nm	Liquid
5.	90	194.88 nm	Liquid	191.87 nm	Liquid

3.8 Results of antimicrobial activity of phytosomes formulation

3.8.1 Antimicrobial activity of phytosomes

Table 11: Antimicrobial activity of phytosomes formulation against E.coli and S. aureus

S. no	Sample Name	Zone of Inhibition (mm) of <i>E. coli</i>	Zone of Inhibition (mm) of <i>S. aureus</i>
1.	Extract	2.0	3.1
2.	Phytosome Formulation (1mg/ml)	4.0	5.2
3.	Phytosome Formulation (1.5 mg/ml)	6.2	7.0

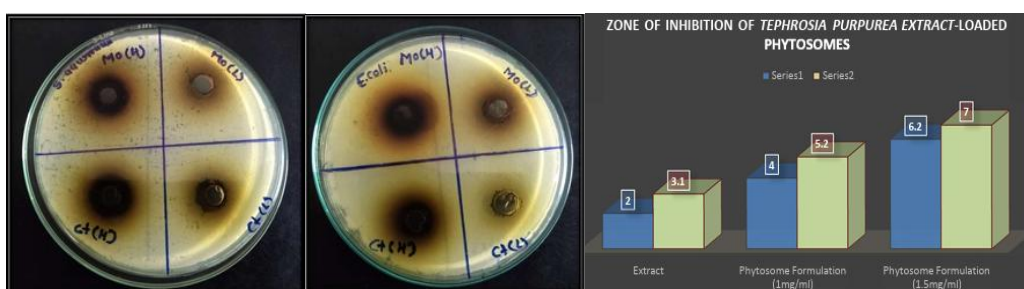


Figure 3: Photograph showing zone of inhibition of plant extract loaded phytosomes gel against A: *Staphylococcus aureus*, B: *Escherichia coli* C: *Tephrosiapurpurea* extract-loaded phytosomes against different microorganisms

IV. Discussion

The investigation focused on developing a phytosome-based delivery system for *Tephrosiapurpurea* seed extract, assessing its antibacterial properties. Using 500 g of seeds, methanol extraction yielded a higher percentage of bioactive constituents compared to petroleum ether. The extract demonstrated good solubility in organic solvents and contained significant secondary metabolites, contributing to its antioxidant and therapeutic potential. Ethanol proved to be a more effective solvent than petroleum ether, extracting 2.94% compared to 1.64%. Its solubility profile indicated a range of polar and moderately polar bioactive constituents, with good solubility in methanol, acetone, and chloroform, but poor in water. Preliminary phytochemical screening revealed key secondary metabolites, including alkaloids, flavonoids, glycosides, phenolic compounds, tannins, and triterpenoids. Quantitative analysis indicated notable total phenolic content at 25.45 mg/g and flavonoid content at 24.9 mg/g, highlighting the extract's strong antioxidant and therapeutic potential. Five phytosome formulations were tested, with formulation F1 emerging as the most optimized due to its small particle size (190.96 nm), low polydispersity index, and excellent stability (zeta potential of -42.9 mV). Stability studies confirmed F1's viability over 90 days.

Antimicrobial testing indicated that the phytosome formulation significantly enhanced its antibacterial activity against *Escherichia coli* and *Staphylococcus aureus* at a concentration of 1.5 mg/ml. The study concluded that phytosome encapsulation markedly improves the extract's physicochemical properties, stability, and antibacterial efficacy, positioning it as a promising drug delivery system for future pharmaceutical applications.

V. Conclusion

This study showed the effective creation of a phytosome delivery system for *Tephrosiapurpurea* seed extract, which enhanced its antibacterial properties. The methanolic extract exhibited a higher yield and contained important phytoconstituents such as flavonoids, phenolics, alkaloids, and glycosides. The optimized phytosome formulation (F1) demonstrated favorable physicochemical characteristics, including small particle size, low polydispersity index, and high zeta potential, indicating good stability. Stability studies confirmed the formulation's robustness under various storage conditions. Notably, the phytosome formulation significantly improved antibacterial activity against *Escherichia coli* and *Staphylococcus aureus* compared to the crude extract. Overall, the findings suggest that phytosome technology can enhance the stability, bioavailability, and therapeutic

efficacy of plant extracts, presenting a promising method for future pharmaceutical and antimicrobial uses.

References

- [1]. Ali, A., Zhang, N., & Santos, R. M. (2023). Mineral characterization using scanning electron microscopy (SEM): a review of the fundamentals, advancements, and research directions. *Applied Sciences*, 13(23), 12600.
- [2]. BalaYadav, R., Pathak, D. P., Varshney, R., & Arora, R. (2022). Design and optimization of a novel herbosomal-loaded PEG–poloxamer topical formulation for the treatment of cold injuries: A quality-by-design approach. *Drug Delivery and Translational Research*, 12(11), 2793-2823.
- [3]. Barani, M., Sangiovanni, E., Angarano, M., Rajizadeh, M. A., Mehrabani, M., Piazza, S., ... & Nematollahi, M. H. (2021). Phytosomes as innovative delivery systems for phytochemicals: A comprehensive review of literature. *International journal of nanomedicine*, 6983-7022.
- [4]. Bhattacharjee, S. (2016). DLS and zeta potential—what they are and what they are not?. *Journal of controlled release*, 235, 337-351.
- [5]. Bombardelli, E., & Patri, G. F. (1991). *U.S. Patent No. 5,043,323*. Washington, DC: U.S. Patent and Trademark Office.
- [6]. Cha, J., Gilmor, T., Lane, P., & Ranweiler, J. S. (2011). Stability studies. In *Separation science and technology* (Vol. 10, pp. 459-505). Academic Press.
- [7]. Chopra, H. K., & Nanda, N. C. (2012). *Textbook of cardiology (a clinical & historical perspective)*. JP Medical Ltd.
- [8]. Deshpande, S. S., Shah, G. B., & Parmar, N. S. (2003). Antiulcer activity of *Tephrosiapurpurea* in rats. *Indian journal of Pharmacology*, 35(3), 168-172.
- [9]. Goswami, S., & Singh, R. P. (2018). Quantitative Estimation of Phytoconstituents and in vitro Anthelmintic Assessment of leaf Extracts of *Tagetes erecta* Linn. *Research Journal of Pharmacy and Technology*, 11(5), 2058-2063.
- [10]. Jain, A., Metzger, M. J., & Glasser, B. J. (2013). Effect of particle size distribution on segregation in vibrated systems. *Powder technology*, 237, 543-553.
- [11]. Khatri, A., Garg, A., & Agrawal, S. S. (2009). Evaluation of hepatoprotective activity of aerial parts of *Tephrosiapurpurea* L. and stem bark of *Tecomella undulata*. *Journal of ethnopharmacology*, 122(1), 1-5.
- [12]. Kirtikar, K. R., & Basu, B. D. (1918). *Indian medicinal plants* (Vol. 2). publisher not identified Basu, BhuwaneśwariĀśrama.
- [13]. Krakowska-Sieprawska, A., Kiełbasa, A., Rafińska, K., Ligor, M., & Buszewski, B. (2022). Modern methods of pre-treatment of plant material for the extraction of bioactive compounds. *Molecules*, 27(3), 730.
- [14]. Kupina, S., Fields, C., Roman, M. C., & Brunelle, S. L. (2018). Determination of total phenolic content using the Folin-C assay: single-laboratory validation, first action 2017.13. *Journal of AOAC international*, 101(5), 1466-1472.
- [15]. Matic, P., Sabljic, M., & Jakobek, L. (2017). Validation of spectrophotometric methods for the determination of total polyphenol and total flavonoid content. *Journal of AOAC International*, 100(6), 1795-1803.
- [16]. Sasongko, R. E., Surini, S., & Saputri, F. C. (2019). Formulation and characterization of bitter melon extract (*Momordica charantia*) loaded phytosomes. *Pharmacognosy Journal*, 11(6).
- [17]. Tamilselvi, E., Karuppaiah, A., Shyamala, G., Shobana, S., Thangaraj, P., Hariharan, S., & Sankar, V. (2021). Exploring combined herbal extract-loaded phytoniosomes for antimalarial and antibacterial activity against methicillin-resistant *Staphylococcus aureus*. *3 Biotech*, 11(4), 177.
- [18]. Yadav, M., Chatterji, S., Gupta, S. K., & Watal, G. (2014). Preliminary phytochemical screening of six medicinal plants used in traditional medicine. *Int J Pharm PharmSci*, 6(5), 539-42.
- [19]. Zhou, J. X. G. Y. X., Xie, G., & Yan, X. (2011). Encyclopedia of traditional Chinese medicines. *Isolat Compound AB*, 1, 455.