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DEVELOPMENT AND CHARACTERIZATION OF PHYTOSOMAL FORMULATION OF *PLUMBAGO ZEYLANICA* FOR ANTI ULCER EFFECT

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ABSTRACT

Up to 10% of people worldwide are affected by the chronic condition known as peptic ulcer**.** There are many chemical treatments for peptic ulcers, but some of them have unfavourable side effects. Herbal remedies are thought to be safe for the treatment of ulcers. One such medicinal plant is, *Plumbago zeylanica* and has been accepted in numerous traditional medical systems for the treatment of a variety of human ailments. Thus, goal of this work is formulation and evaluation of phytosome of *Plumbago zeylanica.* For that the plant material was collected and subjected to extraction by hydroalcoholic solvent. The qualitative, quantitative studies as well as preparation and evaluation of phytosome were performed by standard methods. Results showed that plant contain alkaloid, glycoside, flavonoid, phenol, protein, carbohydrate, saponin and tannin. Total phenol and flavonoid content was observed to be 1.52 mg/100mg and 1.69 mg/100mg. The *In-vitro* drug release data for optimized formulation F10 showed that at 12 hrs the drug release was found to be 98.98%. When the regression coefficient values of were compared, it was observed that 'r^2' values of Higuchi was found maximum i.e. 0.977 hence indicating drug release from formulations was found to follow Higuchi order release kinetics. Results of stability studies clearly indicates 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. From results it can be summarized that phytosomes of hydroalcoholic extract of *Plumbago zeylanica* roots can be used to treat ulcer.

Keywords: *Plumbago zeylanica,* Phytosome, Ulcer, Medicinal plants, Phytochemicals.

INTRODUCTION

A frequent gastrointestinal illness that affects many people is ulcer. Basically, it is a swollen tear in the skin or the mucous membrane lining the digestive tract. The pathogenesis of peptic ulcer disease involves a complex imbalance between gastric defensive mucosal factors like prostaglandins (PG's), gastric mucus, cellular renovation, blood flow, mucosal cell shedding, glycoproteins, mucin secretion, proliferation, and antioxidant enzymes like catalase (CA). Defensive mucosal factors include bile salts, ethanol, *Helicobacter pylori* (*H. pylori).* The location and severity of the disease can be used to classify peptic ulcers. Other factors that contribute to the development of peptic ulcers include the production of histamine, reactive

oxygen species (ROS), tumour necrosis factor- (TNF), apoptosis incidence, and bile acid secretion (Bereda, 2022; Lau *et al*., 2011; Susser, 1967).

There are many chemical treatments for peptic ulcers, but some of them have unfavourable side effects. For example, H2 antagonists can cause impotence, headaches, skin rashes, and arrhythmias, while proton pump inhibitors can unexpectedly result in hypergastrinemia and atrophic gastritis. Antacid use causes stomach distention, belching, constipation, and the possibility of an ulcer perforating. Other medications, such as anticholinergics, also cause same side effects as well as dry mouth, urine retention, impaired vision, xerostomia, and the acceleration of glaucoma (Fashner and Gitu, 2015; Melcarne *et al*., 2016).

Medicinal plants are regarded as the main source of novel pharmaceuticals due to the fact that they have less or no side effects than synthetic drugs, which are used to treat numerous ailments and are known to cause severe unwanted effects. Numerous studies are conducted in search of effective antiulcer agents of plant origin since herbal remedies are thought to be safe for the treatment of ulcers with fewer side effects, are affordable, effective, and substantially less toxic (Kuna *et al*., 2019; Gadekar *et al*., 2010; Dharmani *et al*., 2006).

One such medicinal plant is, *Plumbago zeylanica*. It is also referred to as white chitraka, is a member of the plumbaginaceae family. All of the tropical and subtropical nations of the world have it as a weed.It is often referred to as "Chitraka" and has been accepted in numerous traditional medical systems for the treatment of a variety of human ailments in the form of paste and powder. Mostly found in plants are naphthoquinones and steroidal substances. This plant's various parts are claimed to have medicinal properties that include anti-fungal, anti-tumor, heart disease, rheumatic pains, liver illnesses, fever, diabetes, and renal disease, to name a few.

But due to absorption issues of phytochemicals, their use is constrained, which eventually reduces their bioavailability. Many attempts have been made to increase the medicine's bioavailability by designing it to work with certain drug delivery systems, such as phytosomes and liposomes, which are viable choices. In contrast to typical herbal extracts, the employment of these approaches in the formulation development process may result in good bioavailability of herbal medicines. The term "phytosomes" refers to vesicles containing herbal drugs that are available in nano form. Due to the protective coating that the phytosomes produce around the drug's active ingredient, the major ingredient in herbal extract is protected from deterioration (Kumar *et al*., 2017; Tripathy *et al*., 2013). Thus, goal of this work is formulation and evaluation of phytosome of *Plumbago zeylanica*.

MATERIALS AND METHODS

Chemical and reagent

Potassium Mercuric Iodide, Potassium Iodide, Iodine, Ferric chloride, Lead acetate, Nitric acid, Copper acetate, Aluminum chloride Potassium Bismuth Iodide, Picric acid, Sodium nitropruside and Sodium hydroxide obtained from Loba Chemical Pvt Ltd (Mumbai, India). Hydrochloric acid, methanol

and ethanol were obtained from Merck Ltd, Mumbai, India. Phospholipids, Cholesterol, Quercetin and Gallic acid were purchased from Hi Media, Mumbai. All solvents and reagents were of analytical grade.

Collection of Plant material

The plants have been selected on the basis of its availability and Folk use of the plant. Roots of *Plumbago zeylanica* were collected from local area of Bhopal in the month of January, 2023.

Extraction by maceration process

50.5 gm of dried powdered roots of *Plumbago zeylanica* were coarsely powdered and subjected to extraction with petroleum ether by maceration. The extraction was continued till the defatting of the material had taken place.

Defatted dried powdered roots of *Plumbago zeylanica* has been extracted with hydroalcoholic solvent (ethanol: water: 25:75) using maceration method for 48 hrs, filtered and dried using vacuum evaporator at 40^0C.

Phytochemical examinations

Phytochemical examinations were carried out for the extract as per the standard methods (Pandey and Tripathi, 2014).

Quantitative estimation of bioactive compounds

Total Phenolic content estimation

The total phenolic content of the extract was determined by the modified folin-ciocalteu method (Parkhe and Bharti, 2019).

Total flavonoids content estimation

Determination of total flavonoids content was based on aluminium chloride method (Mishra *et al.*, 2014).

Preparation of phytosomes

The complex was prepared with phospholipids: Cholesterol and *Plumbago zeylanica* in the ratio of 1:.5:1, 1:1:1, 2:1.5:1, 2:2:1 respectively (Kidd, 2009). 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 hour at 4 (Sharma and Sahu, 2016).

The clear supernatant was siphoned off carefully to separate the non entrapped flavonoids and the absorbance of supernatant for non entrapped *Plumbago zeylanica* 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 *Plumbago zeylanica* extract in 1 ml dispersion. The percent entrapment was calculated by following formula.

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

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.

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).

***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°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 (37^0C) was replaced every time with the same quantity of the sample and takes the absorbance at 256.0 nm using spectroscopy.

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.

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

Table 2: Phytochemical screening of extract *of Plumbago zeylanica*

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

	Foam test	+ve
8.	**Diterpenes** Copper acetate test	-ve
9.	**Tannins** Gelatin Test	+ve

Table 3: Total phenolic and total flavonoid content of *Plumbago zeylanica*

S. No.	Extract	Total Phenol (mg/100mg)	Total Flavonoids (mg/100mg)
1.	Hydroalcoholic extract	1.52	1.69

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

Formulation Code	Particle size (nm)	Entrapment Efficiency (%)
F1	345.21	63.25
F2	**272.15**	**71.15**
F3	326.58	68.85
F4	312.25	64.58
F5	285.45	66.45
F6	**235.65**	**72.15**
F7	280.14	70.74
F8	290.45	69.98
F9	256.65	67.74
F10	**220.23**	**76.65**
F11	236.65	68.85
F12	258.74	67.85

Average of three determinations (n=3)

Table 5: *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	22.25	1.347	77.75	1.891
1	1	0	35.65	1.552	64.35	1.809
2	1.414	0.301	49.98	1.699	50.02	1.699
4	2	0.602	68.87	1.838	31.13	1.493
6	2.449	0.778	79.98	1.903	20.02	1.301
8	2.828	0.903	88.85	1.949	11.15	1.047
12	3.464	1.079	98.98	1.996	1.02	0.009

Table 6: Regression analysis data of optimized formulation F10

Batch	Zero Order	First Order	Higuchi	Korsmeyer Peppas
	R^2	R^2	R^2	R^2
F10	0.888	0.945	0.977	0.984

RESULTS AND DISCUSSION

The results of phytochemical screening revealed the presence of alkaloid, glycoside, flavonoid, phenol, protein, carbohydrate, saponin and tannin. Total phenol and flavonoid content was observed to be 1.52 mg/100mg and1.69 mg/100mg. The method employed for phenol and flavonoid detection was FC method and Aluminum chloride method respectively. Out of the 12 formulations the formulation F10 have lowest particle size of 220 nm and maximum entrapment efficiency of 76.65 %.

Through the use of egg membrane and a Franz diffusion cell, the in-vitro release research from the phytosomal suspension was conducted. The pH 7.4 phosphate buffer solution was employed as the diffusion medium. Cellophane membrane had been immersed in the diffusion media throughout the previous night. Egg membrane was attached between the donor and receptor compartments of a Franz diffusion cell. The samples were taken out at regular interval and absorbance was noted.

The *In-vitro* drug release data for optimized formulation F10 showed that at 12 hrs the drug release was found to be 98.98%. When the regression coefficient values of were compared, it was observed that 'r^2' values of Higuchi was found maximum i.e. 0.977 hence indicating drug release from formulations was found to follow Higuchi order release kinetics. Results of stability studies clearly indicates 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.

CONCLUSION

According to the results obtained above, phytosomes have superior physical properties to extract. According to in-vitro research, phytosomes of hydroalcoholic extract of *Plumbago zeylanica* roots exhibited the potential to treat ulcer. Due to the unique characteristics of Phytosomes, which include high stability, high carrier capacity, and prolonged drug release for up to hours, drug bioavailability can be improved, therapeutic impact can be increased, and dose frequency can be decreased. Additionally, since the dose is substantially decreased to a very small quantity and is given to the target area, there are fewer hazardous side effects.

DECLARATION OF INTEREST

The authors declare no conflicts of interests. The authors alone are responsible for the content and writing of this article.

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