Formulation and Evaluation of Phytosome containing *Trigonella foenum graecum* Extract for *in vitro* Antioxidant and Anti-inflammatory Potential

Nayak Smita S., Patil Akshay K.*, Patil Shreya, Deshpaik Saba and Gudasi Sachin
Department of Pharmacognosy, KLE College of Pharmacy, Belagavi, KLE Academy of Higher Education and Research, Belagavi, Karnataka, 590010, INDIA
*pakshay1577@gmail.com

Abstract
The objective of current research work was to formulate Trigonella foenum graecum extract loaded phytosome and to evaluate its in vitro antioxidant and anti-inflammatory potential. The plant material was analysed for its quality and subjected for extraction. The extract was subjected for phytochemical investigations. Compatibility of drug and excipients was confirmed by FTIR. Phytosomes were prepared by thin film hydration method. The phytosomes were analysed for particle size and polydispersability index. The optimized formulation (TFG4) was subjected for in vitro antioxidant and anti-inflammatory assays.

All physicochemical parameters were found to be within limit. The phytochemical investigation confirmed the presence of alkaloids, glycosides, steroids, terpenoids and phenols. The compatibility study confirms the compatibility of excipients and drug. Batch 4 (TFG4) exhibited smaller particle size of 244 nm and PDI of 0.5. With an IC_{50} value of 54.17 µg/ml, it is proven to have beneficial effects as antioxidant which was compared with standard ascorbic acid with an IC_{50} of 34.50µg/ml. In vitro anti-inflammatory activity showed an IC_{50} value of 56.68 µg/ml for sample and 28.63 µg/ml for standard. It can be concluded that phytosomes loaded with Trigonella foenum graecum exhibited promising antioxidant and anti-inflammatory potential and can be used as treatment model with better therapeutic efficiency.

Keywords: Phytosomes, *Trigonella foenum graecum*, Antioxidant, Anti-inflammatory, Extraction.

Introduction

Plants-based products play a multifaceted role in many aspects of human life. In addition to the three fundamental requirements of human life, health is also a critical need that is highly dependent on natural resources [24]. The earliest civilizations held medicinal plants in high esteem and utilized them to alleviate human diseases[5]. The global demand for herbal medicinal products, health-related products, pharmaceuticals, nutritional supplements and cosmetics is on the rise due to the increasing acceptance of these products, which are generally considered as of highest quality due to their lack of adverse effects and affordability[29]. The majority of the global population, particularly in the major developing nations, adopts natural products-based medicine as their primary healthcare option due to their increased cultural acceptance and compatibility with human physiology, as well as their low side effects[22].

Trigonella foenum graecum (fenugreek) belongs to fabaceae family and has recently garnered the attention of researchers from around the world due to its potential medicinal properties[36]. It has been extensively studied and found to be beneficial for both health and for treatment of diseases. Numerous studies have demonstrated its effectiveness[27]. The key chemical components of seeds are alkaloids, steroidal saponins, mucilage and fibres[10]. Fenugreek is one of the world's oldest ancient medicinal herbs with the seeds and leaves being used to treat a variety of diseases[35]. The plant has traditionally employed in the treatment of a variety of ailments including diabetes, hypertension, wound healing, inflammation and gastrointestinal disorders[31].

Although phytocompounds offer therapeutic potential, their use is limited due to poor solubility, stability, bioavailability and gastrointestinal degradation, all of which result in poor therapeutic properties [23]. Traditional dosage forms have been linked to issues such as low solubility and permeability, poor bioavailability, breakdown by gastrointestinal enzymes, food interactions and toxicity. To overcome these restrictions, novel lipid-based nanoformulation has sparked substantial interest in drug delivery strategies[25]. In order to address the challenges, a variety of novel drug delivery systems (NDDS) have been developed. These NDDS have been found to improve the solubility of the drug to increase its bioavailability, to reduce the dose and side effects, to provide control release characteristics, to enhance the stability of components within the body fluid and gastric environment and to enhance the therapeutic activity[21].

Phytosomes are of novel dosage form that has recently been introduced for improving the bioavailability and therapeutic effect of herbal drugs[33]. It is a nano-vesicular drug delivery system in which lipid components bind to phytoconstituents. It improves absorption and bioavailability of herbal extracts through pharmacodynamic and pharmacokinetic parameter[12]. The development of new drug delivery mechanisms such as phytosomes could be beneficial in increasing the therapeutic effectiveness of drugs[19].

Hence, an attempt has been made in the current work to formulate *Trigonella foenum-graceum* extract loaded

phytosomes and to evaluate *in vitro* antioxidant and anti-inflammatory potential.

Material and Methods

Collection and processing of raw materials: The seeds of *Trigonella foenum-graecum* L were procured from Shri BM Kankanawadi Ayurveda Mahavidyalaya, Postgraduate Studies and Research Centre, Belagavi, Karnataka, India. All other chemicals were obtained from KLE College of Pharmacy, Belagavi.

Pharmacognostical study

a) **Organoleptic evaluation:** The dried seeds of *Trigonella foenum-graecum* were subjected to the organoleptic evaluation. Characters such as shape, size, colour, odour, taste were evaluated.
b) **Physicochemical evaluation:** Physicochemical analysis of coarsely powdered raw material including moisture content, ash value and extractive value was evaluated[16,17].

Extraction: Maceration followed by Soxhlet extraction was used to carry out the extraction. A precise weighed quantity of dried raw material (100gm) was initially kept for 24-hour maceration process using ethanol and water (70:30 v/v) as a solvent. Marc from maceration was further subjected for Soxhlet apparatus. The filtrates were combined and concentrated using rotary evaporator [1]. The extract obtained was then stored in sealed container until further use.

Phytochemical screening: In order to identify the presence of different phytoconstituents, the resultant extract was subjected for qualitative phytochemical tests.[15,17]

Pre-formulation study: The physical and chemical interactions between drug and excipients can be analysed using the Fourier Transform Infrared (FT-IR) approach. The compatibility of the drug and excipients was tested for crude extract, excipients and their physical mixtures[2].

Preparation of phytosome: The phytosomes were formulated using a thin film hydration process with varying molar ratios of soy lecithin and cholesterol. The molar ratios are depicted in table 1. Soya lecithin and cholesterol were precisely weighed and dissolved in 10 ml of chloroform and drug was dissolved in 10 ml of methanol and sonicated for 10 min using sonicator. Solution from both flasks was combined and subjected to evaporation using rotary evaporator (IKA-10) at 180 rpm to remove organic solvents. After the solvent has completely evaporated, a thin film had developed on the inner wall of RBF. This film was kept in refrigerator for 24 hours, it was then hydrated using ethanol: water (1:1) using rotary evaporator. Sonication was done to reduce the particle sizes. The prepared phytosomes were stored in amber coloured glass container until further use[6,7,20]. The formulation are depicted in table 1.

Characterization of prepared phytosomes: The prepared batches of *Trigonella foenum-graecum* phytosomes were subjected to particle size and polydispersability index analysis. The Nanotrac instrument was used to analyse the particle size and polydispersity index (PDI) of prepared phytosome batches. The particle size of a phytosomes was determined by diluting 1 mL up to 10 mL with millipore water[26].

Biological assays: Based on characterization parameters, optimized batches of prepared phytosomes were subjected for *in vitro* anti-oxidant and anti-inflammatory assays using standard procedures.

Antioxidant activity: The DPPH free radical scavenging test was utilized to assess the *in vitro* antioxidant activity of the prepared phytosomes. In a test tube, 3 ml of standard DPPH reagent (0.1 mmol/L in methanol) and 1 ml of test solution were added. Similar to that, the control was made by mixing methanol into the DPPH solution. The test tubes containing the solutions were securely sealed and left to remain in dark at room temperature for 30 minutes.

Absorbance was measured at 517 nm after 30 minutes. The concentration at which DPPH radicals were scavenged by 50% and values extrapolated from linear regression analysis were used to determine the IC_{50} value[11,24].

$$Radical\ scavenging\ activity(\%) = \frac{Abs\ Control - Abs\ sample}{Abs\ control} \times 100$$

Anti- inflammatory activity: Egg albumin method was used to evaluate anti-inflammatory activity. The reaction solution (5 ml) contained 0.2 ml fresh hen's egg albumin, 2.8 ml PBS (pH 6.4) and 2 ml of different sample concentrations (20, 40, 60, 80, 100 µg/ml). A same amount of double-distilled water was utilized as the control.

Table 1
Preparation of *Trigonella foenum-graecum* loaded phytosomes

Formulations	Extract (mg)	Cholesterol (mg)	Soya lecithin (mg)	Ratio
TFG 1	50	25	50	1:0.5:1
TFG 2	50	50	75	1:1:1.5
TFG 3	50	75	100	1:1.5:2
TFG 4	50	100	125	1:2:2.5

The mixture was then incubated at (37°C) in a BOD incubator for 15 minutes and then heated at 70°C for 5 minutes. After cooling, absorbance at 660 nm was measured using a vehicle as a blank. The percentage inhibition was calculated by following formula[4,8]:

$$Percent\ Inhibition(\%) = \frac{Abs\ Control - Abs\ sample}{Abs\ control} \times 100$$

Comparative study: The comparative study of previously reported formulations containing *Trigonella foenum graecum* was also made as shown in table 9.

Results

The freshly collected *Trigonella foenum-graecum* (Fenugreek seeds) was subjected to pharmacognostical study, organoleptic study, physicochemical evaluation followed by phytochemical screening. The organoleptic characters such shape, size, colour, odour and taste were studied and results are depicted in table 2.

Physicochemical parameters were evaluated by standard procedures. The results were compared with standard limits. Table 3 depicts the physicochemical values of raw materials.

The raw material was subjected for extraction and the yield of resultant extract was found to be 13.6 %. The extract was subjected for phytochemical analysis. Table 4 represents phytochemical analysis. From the results, plants showed the presence of alkaloid, steroid (triterpenoid), flavonoid, saponin, glycosides.

The FTIR technique was used to assess drug and excipient compatibility. The compatibility between extract, soya lecithin, cholesterol and physical mixture was evaluated. Table 5 represents FTIR data. The thin film hydration process was used for preparation of phytosomes. Four batches containing different concentration of drug and excipients were prepared. Figure 1 represents *Trigonella foenum-graecum* loaded phytosomes.

Characterization of phytosomes: The prepared batches of phytosomes were evaluated for particle size and polydispersability index.

a) **Particle size and Polydispersability index:** The particle size and PDI of the prepared phytosomes were assessed. The results are depicted in table 6.

Table 2
Organoleptic characters of *Trigonella foenum-graecum*

S.N.	Characters	Observation
1.	Shape	Rhomboidal, smooth
2.	Size	5-8mm long and 1-2 mm thick
3.	Colour	Light to dark yellow brown
4.	Odour	Characteristic
5.	Taste	Mucilaginous and slightly bitter

Figure 1: *Trigonella foenum-graecum* loaded phytosomes batches

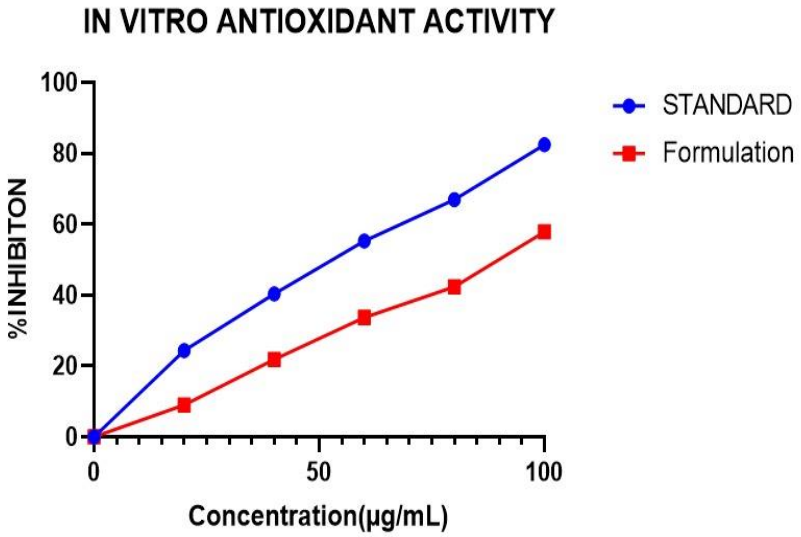

Figure 2: *In vitro* antioxidant activity

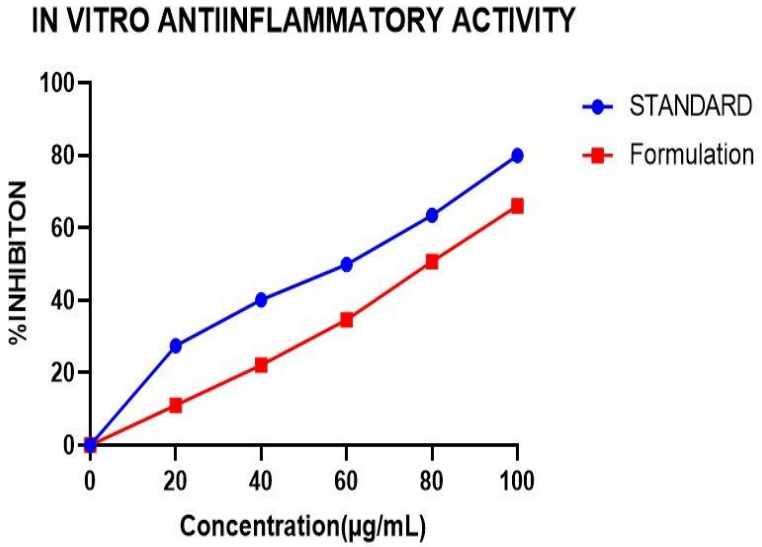

Figure 3: *In-vitro* anti-inflammatory activity

Biological assays: Based on characterization parameters, optimized batch of prepared phytosomes was subjected for *in-vitro* antioxidant and anti-inflammatory assays.

a) Antioxidant activity: The DPPH free radical scavenging assay was used to evaluate the anti-oxidant activity of prepared phytosomes *in vitro*. Ascorbic acid was utilized as the standard. Different concentrations of samples were used for the study. The percent inhibition was calculated and IC_{50} was estimated. The results are represented in table 7 and figure 2.

b) Anti- inflammatory activity: *In vitro* anti-inflammatory potential was assessed using the egg albumin method. Diclofenac sodium was used as standard. As a control, normal distilled water was used. At 660nm, the absorbance was measured. The percent inhibition was calculated and it is represented in table 8 and figure 3.

Comparison with previously reported formulations of *Trigonella foenum graecum*: Table 9 represents comparison with other previously published *Trigonella foenum graecum* formulations.

Marketed phytosomes: Some herbal based phytosomes are available in markets. Table 10 depicts the marketed phytosomes[21].

Discussion

There is an upsurge in demand for herbal-based drugs due to the wide range of advantages connected with these therapies such as fewer side effects and higher therapeutic efficiency. But, main drawback in herbal products is low bioavailability due to poor solubility of phytocomponents. Now-a-days researchers are focussing on development of new lipid-based drug delivery systems for enhanced bioavailability. The combination of herbal drugs and new drug delivery approach

will serve as the bench-marker in achieving greater therapeutic efficiencies of herbal products.

Basically, *Trigonella foenum graecum* is one of the medicinally important herbs which is used since ancient times. The therapeutic effects of *Trigonella foenum graecum* were hindered due to poor solubility of active chemical constituents, this scenario of poor bioavailability requires the development of lipid based nano formulation. Initially, raw materials were subjected for standardization (LOD, ash value and extractive value) and all the parameters were found to be within limits. Extraction was carried out by maceration and Soxhlet extractor. Phytochemical screening was done to confirm the presence of plant metabolites. FTIR technique was used for compatibility study. Phytosomes were prepared by thin film hydration technique. Particle sized and PDI were evaluated. The resultant formulation was subjected for *in vitro* antioxidant and anti-inflammatory activity.

Table 3
Physicochemical parameters *Trigonella foenum-graecum*

S.N.	Physicochemical Parameters	Test	Result (%w/w)	Standard value (%w/w)
1	Moisture content	LOD	9	NMT 12
2	Ash value	Total ash	4.5	NMT 5
		Acid insoluble ash	0.7	NMT 5
3	Extractive values	Water	9.6	-
		Alcohol	9.5	NLT 5
		ether	5.%	-

Table 4
Phytochemical screening of *Trigonella foenum-graecum* extract

Phytochemical tests	Observation	Inference
Alkaloid test		
Dragendorff's test	Orange- brown ppt	+
Wageners test	Reddish-brown ppt	+
Mayer's test	Gives ppt	+
Steroid (triterpenoid)		
Salkowski test	Chloroform layer red colour	+
Flavonoid		
Shinoda test	Pink colour	+
FeCl3 test	Bluish black colour	+
Glycosides		
Legals test	Pink colour	+
Keller killiani test	Reddish brown colour junction	+
Saponin		
Foam test	Foam formation	+

Table 5
Pre-formulation by FTIR

Group	Soya lecithin	Cholesterol	Extract	Physical Mixture
C=C	1615.45	1465.96	1559.51	1581
C=O	1734.08		1603.35	1715.76
C-H	-	2848.98	2809.44	2850.91
-OH	-	2932.89	3229.94	2930.00-2924.21

Table 6
Particle Size and PDI

Formulation	Particle size (nm)	PDI
TFG1	353.2	0.3
TFG2	262.1	0.6
TFG3	261.2	0.8
TFG4	244.1	0.5

Table 7
***In vitro* anti-oxidant activity**

S.N.	Conc. µg/ml	Absorbance	Percent Inhibition	IC_{50} µg/ml
Standard (Ascorbic acid)				
1.	20	2.461	24.37001	34.50
2.	40	1.941	40.35034	
3.	60	1.456	55.25507	
4.	80	1.075	66.96374	
5.	100	0.572	82.42163	
Sample				
1.	20	2.961	9.004302	54.17
2.	40	2.541	21.91149	
3.	60	2.156	33.74309	
4.	80	1.875	42.37861	
5.	100	1.372	57.83651	

Table 8
***In-vitro* anti-inflammatory potential**

S.N.	Conc. µg/ml	Absorbance nm	Percentage inhibition	IC_{50} µg/ml
Standard (Diclofenac)				
1.	20	0.5239	27.43767	28.63
2.	40	0.432	40.1662	
3.	60	0.362	49.8615	
4.	80	0.264	63.4349	
5.	100	0.145	79.9169	
Sample				
1.	20	0.642	11.08033	56.68
2.	40	0.562	22.16066	
3.	60	0.472	34.62604	
4.	80	0.356	50.69252	
5.	100	0.245	66.06648	

Table 9
Comparative study

Formulation	Applications	Limitations
Powder	Nutraceuticals	Lack of standardization of raw materials, Low bioavailability[28]
Herbal gel	Antifungal	Lack of physicochemical analysis of raw materials[18]
Gel	Anti-inflammatory	Lack of quality assessment[9]
Tablets	Anti-diabetic	Lack of physicochemical evaluation and Compatibility study[13]
Self-Emulsifying Drug Delivery System	Antioxidant	Lack of quality assessment[32]
Cream	Moisturising cream	lack of Compatibility study[16]
Capsule	Anti-diabetic	Lack of quality assessment[14]
Effervescent tablet	antidiabetic	Lack of Compatibility study and quality assessment[3]
Cream	Anti-inflammatory	lack of Compatibility study[30]
Hair Gel	Nourishment and Hair Growth	Lack of quality assessment[34]

Table 10
Marketed phytosomes

S.N.	Phytosomes	Manufacturer	Uses
1.	Ginkgoselect® Phytosome	Herbal Factors	Anti-aging
2.	Ginseng Phytosome	Indena	Nutraceutical
3.	Ginseng Phytosome	Natural Factors	Immunomodulator
4.	Centevita®	Indena	Wound healing
5.	Casperome® Phytosome	Indena	Joint health
6.	Quercefit™ Phytosome	Indena	Antioxidant activity
7.	Polinacea Phytosome	Indena	Immunity booster
8.	Anthocyanose Phytosome	Indena	Antioxidant
9.	Naringin Phytosome	Indena	Anti-oxidant,
10.	Curcumin Phytosome	Indena	Anti-inflammatory

The *in vitro* biological activities of prepared *Trigonella foenum graecum* loaded phytosomes showed promising antioxidant and anti-inflammatory potential. It can be concluded that the potential drawbacks associated with herbal medicines in terms of bioavailability due to poor solubility can be overlooked by theses lipid-based nano formulations. Further, *in vivo* can be planned to support the study.

Conclusion

The current effort will act as a bridge between traditional therapies and novel drug delivery systems, enabling novel drug delivery systems to deliver herbal phytocompounds with improved therapeutic efficacy. According to the results, it can be concluded that the prepared phytosomes will be promising option for therapeutic potential of *Trigonella foenum graecum* as many herbal products and their derivatives show limitations in terms of poor bioavailability and that can be overlooked by novel drug delivery systems.

Acknowledgement

The authors would like to thank Principal, KLE College of Pharmacy, Belagavi for providing necessary facilities.

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(Received 30th May 2024, accepted 31st July 2024)