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Antihyperlipidemic Activity of *Terminalia Chebula Retz* Extract-Loaded Phytosomes: Development and Characterization

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Abstract

Ethnopharmacological evidence has demonstrated that Terminalia chebula Retz is traditionally employed for the management of hepatic ailments. Presently, a significant number of prevalent diseases and nutritional disorders are managed through the utilization of natural remedies. The efficacy of herbal medications relies on the administration of a sufficient dosage of the therapeutically active component. However, there is a significant constraint in terms of their bioavailability when taken via oral or topical routes. Phytosomes are a novel class of herbal formulations that have been recently introduced. These formulations exhibit enhanced absorption properties, leading to improved bioavailability and efficacy compared to traditional phyto compounds or botanical extracts. The objective of the current investigation was to assess the qualitative and quantitative phytochemical analysis, high-performance liquid chromatography, optical microscopic research, and in vitro antioxidant properties of Terminalia chebula Retz leaves obtained from the Bhopal region of Madhya Pradesh. The hydroalcoholic extract of phytosome was prepared using a mixture of phospholipids and cholesterol. The characterization of phytosome was conducted using various analytical techniques, including Fourier-transform infrared spectroscopy, determination of entrapment efficiency, measurement of particle size and size distribution, examination under an optical microscope, high-performance liquid chromatography analysis. The concurrent utilization of phospholipids and Terminalia chebula Retz has the potential to produce a synergistic outcome. This synergistic effect can be assessed by evaluating the free radical scavenging activity using the DPPH model.

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Keywords: *Free radical scavenging activity, Phytosome, Phospholipids, and Terminalia chebula Retz*

1. Introduction

The liver, being the largest organ, is susceptible to damage from various factors such as pathogenic infections, exposure to toxic substances, and the abuse of alcohol or drugs. The liver is widely recognized for its remarkable capacity for regeneration and recuperation following injury [1].

Infrequently, the occurrence of a severe and sudden instance of liver injury can result in clinically significant syndromes that pose a hazard to life, such as jaundice, severe coagulopathy, and elevated death rates [2]. There remain medical challenges pertaining to the elucidation of pathophysiological pathways and the development of effective therapies, particularly for cases of severe hepatic injury [3].

Moreover, it is imperative to conduct additional investigations into alternative natural sources of medicinal compounds, such as Terminalia chebula Retz. (Combretaceae), that exhibit enhanced effectiveness and safety. Terminalia chebula is a perennial angiosperm tree that is native to many regions in Asia and Africa [4]. The many components of this plant, including its fruits, stem, bark, and leaves, include antioxidant, anti-inflammatory, anti-cancer, hepatoprotective, and cardiotonic properties. The antibacterial and free radical scavenging properties of the methanol extract of T. chebula have been verified. A previous investigation shown the existence of anti-hyperlipidemic action in a composite of Gaumutra extract and the aqueous extract of T. chebula fruit. Nevertheless, additional research is necessary to fully explore the potential of T. chebula extract in its anti-hyperlipidemic action. Therefore, the current study aims to evaluate the anti-hyperlipidemic effects of the methanol bark extract derived from T. chebula [4-6].

Terminalia chebula, often known as T. chebula, has been widely utilized in traditional systems of medicine such as Ayurveda, Unani, and Homoeopathy. Its therapeutic properties have garnered significant attention in the field of modern medicine. The observed health advantages can be attributed to the presence of a diverse range of phytochemicals, including polyphenols, terpenes, anthocyanins, flavonoids, alkaloids, and glycosides. Phytosomes are employed to augment the bioavailability of phytomedicines through the integration of phospholipids into standardized plant extracts [7,8].

The drug delivery system described here is characterized by the incorporation of a hydrophilic choline moiety that binds to polar phytoconstituents. This binding is then surrounded by a lipophilic phosphatidyl moiety, either forming an outer layer or directly enveloping the choline-bound phytoconstituents. As a result, the originally water-soluble phytoconstituents acquire lipid solubility. Phytosomes consist of a naturally occurring phospholipid known as phosphatidylcholine (PC), which is similar to soy lecithin. Additionally, it functions as a cellular constituent that is capable of undergoing biodegradation and has been documented to possess hepatoprotective properties. Phytosomes exhibit enhanced pharmacokinetic and pharmacological characteristics [7-9].

2. Materials And Methods

The plant material of Terminalia chebula was gathered from close by of Bhopal, Madhya Pradesh.

Chemical and Reagents

The chemicals utilized in this investigation were acquired from HiMedia Laboratories Pvt. Ltd. (Mumbai, India), Sigma Aldrich Chemical Co. (Milwaukee, WI, USA), SD Fine-Chem Chem. Ltd. (Mumbai, India), and SRL Pvt. Ltd. (Mumbai, India). All compounds utilized in this investigation were of analytical grade.

Extraction procedure

The production of Hydroalcoholic extracts from the shade dried and powdered herbs of T. chebula was carried out using the following process.

Plant material fattening

The leaves of Terminalia chebula were subjected to a shadow drying process at ambient room temperature. The plant material that had been dried in the shade was finely ground into a coarse powder and then underwent extraction using petroleum ether. The extraction process was carried out until the removal of fat from the material was completed.

Maceration-based extraction

The hydroalcoholic solvent (30:70) was used to extract the dried powdered leaves of T. chebula using a maceration procedure lasting 48 hours. The extracts were subjected to evaporation at temperatures beyond their respective boiling points, and subsequently preserved in a hermetically sealed container to

prevent any kind of contamination until their utilization. Subsequently, the percentage yields of the desiccated extracts were determined [11].

Plant extract qualitative phytochemical analysis

The T. chebula leaf extract was analyzed using standard procedures as outlined by Khandelwal and Kokate for preliminary phytochemical analysis. The sample was analyzed to determine the presence or absence of several bioactive constituents such as phenolic compounds, carbohydrates, flavonoids, glycosides, saponins, alkaloids, fats or fixed oils, protein and amino acids, and tannins [12].

Determination of Total flavonoids

The method employed for the determination of total flavonoid content was based on the procedure described by Olufunmiso et al. A volume of 1 milliliter of a methanolic solution containing 2% AlCl3 was combined with 1 milliliter of either an extract or a standard. The resulting mixture was left undisturbed for a duration of 60 minutes at ambient temperature. The absorbance of the reaction mixture was afterwards measured at a wavelength of 420 nanometers using a UV/visible spectrophotometer. The quantification of flavonoid content was performed by employing a standard graph of quercetin, and the obtained values were reported in terms of quercetin equivalent (mg/g) [13].

Free radical scavenging test using DPPH

By using a modified approach, DPPH scavenging activity was assessed. The spectrophotometer measured the DPPH scavenging activity. A 1.5 ml sample of the stock solution in 1.5 ml of methanol produced an initial absorbance. After 15 minutes, a decrease in absorbance was seen when sample extracts of various concentrations were present. After diluting 1.5 ml of the DPPH solution with methanol to make 3 ml, the absorbance was measured right away at 517 nm for the control reading. In a series of volumetric flasks, 1.5 ml of DPPH and 1.5 ml of the test sample at various concentrations were added. The final volume was then adjusted to 3 ml with methanol. Three test samples were collected, and they were all handled similarly. The mean was finally taken. Each concentration was measured using absorbance at zero time [14].

Development of phytosomes formulation

A specified quantity of extract, phospholipids, and cholesterol was introduced into a round-bottom flask with a volume of 100ml. Subsequently, a reaction medium consisting of 60ml of methanol was added. The combination underwent reflux and the reaction temperature of the complex was maintained at 50°C for a duration of 3 hours. The resulting transparent solution was subjected to evaporation, followed by the addition of 20 ml of n-hexane under continuous stirring. The precipitate was subjected to filtration and vacuum drying in order to eliminate any residual solvents present in trace amounts. The dried residues were collected and subsequently placed in desiccators for an overnight period. Following this, they were maintained at room temperature within an amber colored glass bottle, as indicated in Tables 1, 2, and 3 [15, 16].

Table 1: Different phytosome formulations phospholipid and cholesterol optimization

Batch No.	Phospholipid: Cholesterol Ratio	Drug Conc. (%)	Alcohol (mi)	% EE
F1	0.6:0.5	1.6%	60	52.50±0.21
F2	1.2:1.0	1.6%	60	65.12±0.57
F3	1.8:1.5	1.6%	60	78.80±0.41
F4	2.5:1.5	1.6%	60	67.14±0.34

Table 2: Drug Concentration Optimization

Batch No.	Phospholipid: Cholesterol Ratio	Drug Conc. (%)	Alcohol	% EE
F5	1.6:1.3	0.8	60	64.24±0.27
F6	1.6:1.3	1.6	60	66.74±0.58
F7	1.6:1.3	2.4	60	54.87±0.56

F8	1.6:1.3	3.2	60	62.24±0.67

Table 3: Alcohol Concentration Optimization

Batch No.	Phospholipid: Cholesterol Ratio	Drug Conc. (%)	Alcohol	% EE
F9	1.6:1.3	1.6%	20	52.50±0.21
F10	1.6:1.3	1.6%	40	65.12±0.57
F11	1.6:1.3	1.6%	60	78.80±0.41
F12	1.6:1.3	1.6%	80	67.14±0.34

Characterization

Analyzing the relationship between T. chebula and phospholipids

The study employed a Fourier transform infrared spectrophotometer (FT-IR Spectrometer, namely the Bruker alpha model) to investigate the interaction between T. chebula and phospholipids. The infrared spectra of Terminalia chebula extract, phospholipids, their complex, and physical combination were acquired using the KBr technique [17].

% EE

The phytosome preparation was subjected to centrifugation using a cooling centrifuge (Remi) at a speed of 12000 revolutions per minute for a duration of 30 minutes. The transparent liquid portion was extracted with caution in order to distinguish the quercetin that was not trapped, and the spectrophotometer (Labindia 3000+) was used to measure the absorbance of the non-trapped T. chebula supernatant at a wavelength of 420.0 nm. The sediment was subjected to lysis of vesicles by the addition of 1 ml of a 0.1% Triton X-100 solution. The resulting mixture was then diluted to a final volume of 100 ml using phosphate buffer saline (pH 7.4). The absorbance of the diluted solution was measured at a wavelength of 420.0 nm. The quantification of quercetin in both the supernatant and sediment fractions yielded the cumulative amount of T. chebula present in a 1 ml dispersion. The percentage of entrapment was determined using the following formula [18-20].

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

Particle size and PDI

The dynamic light scattering (DLS) technique was employed to assess the particle size, size distribution, and zeta potential of the improved phytosomes formulation. This analysis was conducted utilizing a computerized inspection system, namely the Malvern Zetamaster ZEM 5002, manufactured by Malvern in the United Kingdom. The determination of the electric potential of the phytosomes, which encompasses the Stern layer or zeta potential, was achieved through the injection of the diluted system into a zeta potential measurement cell [21, 22].

In-vitro drug release study

The in vitro drug release of the sample was conducted using a USP-type II dissolution equipment. The dissolution flask was filled with 900 ml of 0.1N hydrochloric acid (HCl) as the dissolution medium. The temperature was maintained at 37±0.50°C, and the rotational speed was set at 50 revolutions per minute (rpm). Each bowl of the dissolution equipment contained 100 mg of phytosomes. The experimental setup was permitted to operate for a duration of 10 hours. Samples of 5 mL were extracted at hourly intervals for a duration of 10 hours using a 10 mL pipette. The dissolving media at a temperature of 37°C was consistently replaced with an equal volume of the sample during each instance.

For this experiment, extract 0.5 ml of the sample and afterwards dilute it to a total volume of 10 ml. Measure the absorbance of the diluted sample at a wavelength of 420.0 nm using spectroscopy [23,24].

Preparations for T. chebula phytosome hydroalcoholic extract

This study involved the development of a complex between T. chebula and phospholipids in order to enhance the lipophilic characteristics of T. chebula. The findings indicated that the stability of the T. chebula –phospholipids complex was compromised when the ratio fell below 1. In order to optimize the formation of a phospholipid complex with T. chebula, we devised a formulation with a precise ratio of ingredients, specifically 1:1.5:1.2. This approach aims to achieve the highest complexity while minimizing the amount of phospholipid required [25].

Study with optical microscopy

The Phytosome was observed using microscopy at Cippon in Japan. A single droplet of a phytosome solution with diluted extract was carefully placed onto a glass slide. The surplus solution was removed by filtration using a filter paper, after which the slide was left to undergo the drying process. Subsequently, the sample underwent examination by optical microscopy [26].

Stability studies

The stability studies yielded conclusive results, demonstrating that the improved batches of phytosomes remained stable under the specified temperature and humidity settings for a period of three months. No major changes were seen in terms of physical appearance and the percentage of drug content [27].

3. Results and Discussion

The organoleptic evaluation encompasses the assessment of qualities pertaining to materials through the utilization of the sensory organs. Consequently, this process delineates some distinct attributes of the substance, thereby serving as an initial endeavor in ascertaining the material's identity and level of purity. Table 4 displays the outcomes of a qualitative phytochemical analysis conducted on the crude powder derived from the leaves of T. chebula.

Table 4: Phytochemical analysis of T. chebula hydroalcoholic extract

Sr. No.	Constituents and tests	*T. chebula*
1.	**Alkaloids [**Hager's test]	Negative
2.	**Flavonoids** Lead acetate Alkaline test	Positive
3.	**Phenolics** Fecl3	Negative
4.	**Proteins and Amino acids** Xanthoproteic test	Positive
5.	**Carbohydrates** Fehling's test	Positive
6.	**Saponins** Foam test	Positive
7.	**Diterpins** Copper acetate test	Positive

The quantification of flavonoids in the extracts was determined by expressing the overall quantity as a percentage of quercetin equivalent per 100 mg of dry weight of the sample. The hydroalcoholic extracts of T. chebula leaves were analyzed to determine the total flavonoid content. The results indicated that the content values were 0.982, as shown in Table 3.

Table 5: Total flavonoid content of T. chebula hydroalcoholic extract

S. No.	Sample	Total flavonoid content
1.	Extract of Terminalia chebula in alcohol	0.982

There is a growing body of research suggesting the potential utility of indigenous antioxidants in mitigating the adverse effects of oxidative stress. Moreover, there is a rising interest in exploring the beneficial biochemical properties of natural antioxidants found in herbs and medicinal plants. The antioxidant activity of the hydroalcoholic extract of T. chebula is evaluated through the assessment of its free radical scavenging activity and reducing power test. The plant extracts that were subjected to testing exhibited robust antioxidant activity.

The quantification of entrapment efficiency holds significant importance in the characterization of phytosomes. To achieve maximum encapsulation efficiency, various parameters were manipulated, encompassing the lipid concentration, drug concentration, and alcohol concentration. Table 6 displays the entrapment efficiency of all the formulations that were developed. The size of phytosomes has been found to be significantly influenced by the concentration of lipids. The formulation designated as F10 was determined to be the most optimal and was then selected for further investigation in drug release analysis, solubility assessments, and UV spectroscopy.

Table 6: Particle size and the efficacy of drug-loaded phytosomes entrapment

Batch No.	Particle size	% EE
F1	244.78±0.56	52.47±0.78
F2	240.98±0.21	62.11±0.51
F3	279.44±0.57	75.44±0.29
F4	304.12±0.45	65.28±0.51
F5	339.92±0.72	66.71±0.08
F6	284.78±0.52	66.77±0.55
F7	292.22±0.24	56.71±0.33
F8	280.77±0.89	64.33±0.64
F9	270.77±0.68	65.56±0.79
F10	226.23±0.54	78.97±0.78
F11	291.47±0.77	62.14±0.91
F12	289.66±0.56	57.64±0.99

In-vitro dissolution study

The results of the in vitro dissolution research of F10 demonstrated that the phytosomes exhibited a dissolution pattern characterized by protracted release. The analysis of the kinetic release profile of the phytosome formulation reveals that formulation F10 exhibited the most favorable characteristics, closely adhering to the pappas release profile. The phytosome formulation exhibited controlled release characteristics, with the medication being delivered over a period of 6 hours, as indicated in Table 7.

Table 7: Data on the drug's release from F10 in vitro

S. No.	Time (Hrs)	Cumulative* % Drug Release±SD	Log Cumulative % Drug Release	Cumulative % Drug Remaining	Log cumulative % Drug Remaining
1	1	13.36	1.245	87.14	1.748
2	2	24.74	1.324	74.47	1.145
3	3	34.47	1.578	66.68	1.258
4	4	44.33	1.358	56.78	1.369
5	5	61.78	1.147	39.63	1.123
6	6	70.47	1.698	30.11	1.654

The sample was subsequently subjected to optical microscopy, as depicted in Figure 1. During the examination using Optical Microscopy, the obtained phytosomes formulation was observed with clarity.

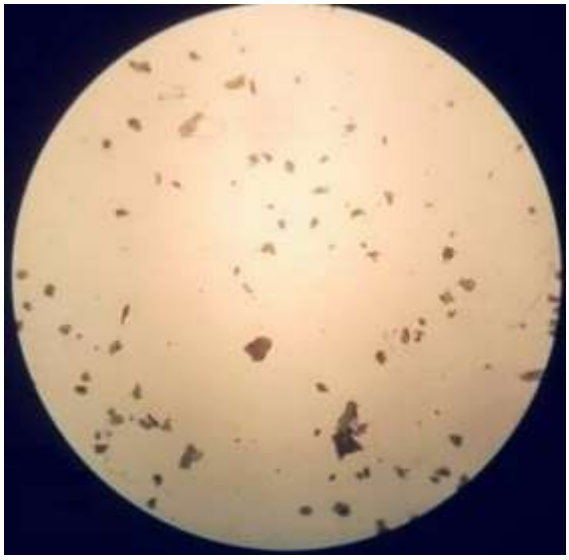

Figure 1: Analysis of the optimized formulation using optical microscopy

The stability studies yielded conclusive results, demonstrating that the optimized batches of phytosomes remained stable for a period of three months under the selected temperature and humidity settings. No major variations were seen in terms of physical appearance and the percentage of drug content.

4. Conclusion

Based on the findings of the current study, it can be inferred that the analysis of T. chebula leaves using HPLC and preliminary phytochemical investigation has resulted in the identification of a collection of benchmarks. These benchmarks can be considered crucial evidence for establishing the authenticity, as well as assessing the quality and purity of the plant material in relation to its potential applications in the future. The phytochemical investigation provided valuable insights into the various phytoconstituents found in the plant. This information is beneficial for future researchers in selecting specific extracts for further investigation aimed at isolating the active principle. Additionally, the investigation shed light on the diverse range of activities exhibited by different phytochemicals. The hydroalcoholic extract of leaves exhibited a greater concentration of flavonoids, as supported by subsequent in vitro antioxidant investigations. There exists a positive association between the potential antioxidant activity and the therapeutic efficacy in the management of liver illnesses. Additional investigation is required to separate distinct substances and evaluate their antioxidant properties in vivo, employing various mechanisms.

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None

Conflict of Interest

None

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