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Research

Formulation and evaluation of liposomes specific delivery of herbal medicine

Dr. G. Praveen Kumar[1*], Musthafa Hussain[2]

[1]Principal, Professor & HOD, Department of Pharmaceutics, Sahasra Institute of Pharmaceutical Sciences, Hanumakonda 506007, Telangana, India.
[2]M.Pharm Final Year Student, Department of Pharmaceutics, Sahasra Institute of Pharmaceutical Sciences, Hanumakonda 506007, Telangana, India.

*Author for Correspondence: G. Praveen Kumar
Email: *prof.dr.gunn@gmail.com

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Abstract

Worldwide, more than 80,000 plant species are utilized in herbal therapy. The vesicular structure of liposomes is formed of one or more bilayers enclosing an equal number of water-filled compartments. Made from cholesterol and naturally occurring, harmless phospholipids, liposomes are tiny synthetic vesicles with a spherical form. To boost its oral bioavailability, a formulation of P. amarus lipid-derived carrier system (liposomes) with strong wound-healing properties needed to be developed. P. amarus leaves were successively extracted using Soxhlet method, producing 3.26±0.24% hexane extract, 6.48±0.65% ethyl acetate extract, and 12.57±1.28% methanol extract. P. amarus leaves included carbohydrates, alkaloids, flavonoids, glycosides, tannins, and saponins, as shown by the methanolic extract. Phyllanthus amarus was found to be 1.28 ± 0.02 mg/mL soluble in distilled water, but at 250C, it was found to be 15.36 ± 0.31 mg/mL soluble in methanol, with polyethylene glycols having a higher solubility. Phyllanthus amarus has a maximum absorption (max) at 329 nm. In FTIR study, suggested that Phyllanthus amarus and the other excipients in the formulations did not interact chemically. After analysis, it was discovered that the liposome formulations' total drug content ranged from 76.38±0.95 to 86.32±0.51%. It was discovered that the cumulative % drug release for formulations PAL1, PAL2, and PAL3 was 102.43±1.28 in 20 hours, 92.46±1.68 in 22 hours, and 72.43±1.06 in 24 hours, respectively. Based on the conducted experiment, it was determined that cholesterol, soy lecithin, and stabilizers such as stearylamine and diethyl phosphate were appropriate carriers for the Phyllanthus amarus liposome synthesis.

Keywords: Phyllanthus amarus, Liposomes, Specific Delivery, Herbal Medicine.

INTRODUCTION

Herbal therapy has been the mainstay of medical practice since prehistoric times. Herbal medicine is still quite popular in this new period and is being accepted exceptionally well in both developed and developing nations for a number of reasons.[1]

To start, compared to synthetic drugs, herbs usually have far less side effects (Tyler, 2003). In contrast to the side effects associated with high dosages of a single agent that acts on a single site, the cumulative effect of these drugs is remarkable because they operate through many pathways activated by different chemical components that impact different receptor sites. For instance, when Serenia repens Batr., or saw palmetto, is taken to treat benign prostatic hyperplasia, it shares many of finasteride's beneficial properties without any of the drug's bad ones.[2]

The fact that phytomedicines have advantageous qualities absent from synthetic medications is another argument in favour of their use. In terms of beneficial immunomodulatory effects, no synthetic medicine now exists that can compare to echinacea (Tyler, 1999). At this time, self-selected drugs for the common cold and influenza only treat the symptoms, not the underlying infection. Panax species contain a complex combination of ginsenosides, which no synthetic adaptogenic drug on the market today can match.[3]

Biological background of herbal drugs

Worldwide, more than 80,000 plant species are utilized in herbal therapy. Currently, over 25% of prescription medications supplied in the US are made from plants. In addition to being widely used as therapeutic items in underdeveloped nations, botanical products are quickly making their way into the "Complementary and alternative system of medicines" (CAM), an integrative health care system in industrialized nations.

As a natural aspect of their metabolic processes, all plants create chemical compounds. The two main groups into which they fall are primary and secondary metabolites. Fats, carbohydrates, and amino acids are examples of primary metabolites, while phenols, lignans, flavonoids, and alkaloids are examples of secondary metabolites. Secondary metabolites are considerably more complex molecules that serve various purposes in the plants that produce them. While all plants contain primary metabolites, the spectrum of secondary metabolites is considerably more limited, with some being unique to a single species or even a single genus. Secondary metabolites serve a wide range of purposes. These byproducts of secondary metabolism are the active ingredients in several pharmaceuticals, including quinine (found in Cinchona), morphine (found in Papaver), and codeine (found in Poppy), among others.[4]

Novel Drug Delivery System

When discussing methods, formulations, technologies, and systems for the safe and effective transportation of pharmaceutical compounds throughout the body, the term "novel drug delivery system" (NDDS) is used interchangeably. One alternative to the standard method of medication distribution is the non-drug delivery system, or NDDS. When compared to traditional dose forms, NDDS are much superior due to their combination of advanced methodology and dosage form.

Delivering a therapeutic dose of medication to the right place in the body at the right time and keeping that concentration stable are the goals of NDDS. NDDS integrating field of polymer science with pharmaceuticals and molecular biology[5].

LIPOSOMES

The vesicular structure of liposomes is formed of one or more bilayers enclosing an equal number of water-filled compartments. Made from cholesterol and naturally occurring, harmless phospholipids, liposomes are tiny synthetic vesicles with a spherical form. In addition to being biocompatible, liposomes' size, hydrophobic and hydrophilic properties make them an attractive drug delivery mechanism. Peptides, proteins, hormones, enzymes, antibiotics, anti-fungal, and anti-cancer agents7 were included within the liquid interior of the spherical shell.[6]

What a liposome can do depends heavily on its size, lipid makeup, surface charge, and manufacturing method. The components used for the bilayer dictate its 'rigidity' or 'fluidity,' in addition to its charge. Saturated phospholipids with long acyl chains, like dipalmitoyl phosphatidyl choline, create a bilayer structure that is stiff and mostly impermeable, in contrast to unsaturated phosphatidylcholine species in eggs and soybeans, which form a bilayer structure that is much more permeable but less stable.[7]

Phospholipids spontaneously form closed structures when hydrated in water, according to research. These vesicles can transport lipid or water-based medications; the exact type depends on the number of phospholipid bilayers. Because of their thermodynamic phase properties and self-assembling properties, lipides influence the entropically concentrated packing of their hydrophobic sections into spherical bilayers. In water, lipids exhibit both hydrophobic and hydrophilic properties; this property is known as amphipathicity. These strata are formally known as lamellae.[8]

Liposomes can range in size from three micrometres down to thirty nanometers. They consist of a lipid bilayer or more enclosing aqueous units, and their polar head groups face the interior and outer aqueous phases, respectively. Polar lipids have the ability to self-assemble into a diverse array of colloidal particles, as opposed to the usual bilayer topologies that are dictated by factors such as molecule shape, temperature, and environmental and preparation conditions.[9]

AIM, OBJECTIVE

To boost its oral bioavailability, a formulation of P. amarus lipid-derived carrier system (liposomes) with strong wound-healing properties needed to be developed.

Randomized means that no criteria are used; instead, the research is conducted arbitrarily each time a specimen becomes available. Phylogenetically or taxonomically, in which species are chosen based on a specific chemical category of compounds within a genus or family. Ethnopharmacological: a method where plants are chosen according to how a particular ethnic group uses them for medicinal purposes. There is another aspect with which everyone agrees. If the selection of plants is made on the grounds of their traditional use, the chance of research success is greater (Trotter et al., 1982; Elisabetsky and Wannmacher., 1993).

MATERIALS & METHODS

Materials

The materials used in the present investigation were either AR/LR grade or the best possible pharma grade.

S.No	Ingredients	Manufactures
1	Soybean lecithin	Lipoid Pvt. Ltd., Mumbai.
2	Cholesterol	Lipoid Pvt. Ltd., Mumbai.
3	Dicetyl phosphate	Sigma Aldrich, Mumbai.
4	Stearylamine	Sigma Aldrich, Mumbai.
5	Ammonium sulphate	Triveni chemicals, Mumbai.
6	Chloroform	Fisher scientific, Mumbai.
7	Sodium hydroxide	Merck chemicals, Mumbai.
8	Methanol	Merck chemicals, Mumbai
9	Sodium lauryl sulphate	Merck chemicals, Mumbai
10	Isopropyl alcohol	Merck chemicals, Mumbai.

Equipments and Instruments

1. UV- Visible spectrophotometer (PerkinElmer's)
2. Infra Red spectroscopy (Bruker)
3. Rotary vacuum evaporator (Buchi)
4. Homogenizer (Panda)
5. Peristaltic pump (Electro lab)
6. Electronic balance (Sartorius)
7. Centrifuge (Remi Instruments)
8. Bath Sonicator
9. Electronic Microscope (Motic)
10. Magnetic stirrer (Remi Instruments)
11. Zeta Sizer version 6.00(Malvern)
12. Scanning Electron Microscopy(Field Instruments)
13. Soxhlet extraction

Phyllanthus amarus Schum. & Thonn.

Taxonomical classification
Kingdom : Plantae
Division : Magnoliophyta
Class : Magnoliopsida
Order : Euphorbiales
Family : Euphorbiaceae
Genus : Phyllanthus

Species : amarus
Botanical Name : Phyllanthus amarus Schum. & Thonn.

Vernacular names
Tamil : Keelanelli (Keezhanelli)
Hindi : Bhuyiavla, Jangli amla
Bengali : Bhuiamala, Sadahazurmani
Telugu : Nela uirika, Nelavusari
Kanada : Nela – nelli, Kirunelli

Flowering and Fruiting: Throughout the year.
Parts used: Root, bark, fruits, leaves, seeds.
Traditional Uses

1. Gallstones, Kidney Diseases, Kidney Stones.
2. Aperitif, Cold, Constipation, Fever, Flu, Laxative, Stomach Ache, Typhoid
3. Joint Ache, Antispasmodic, Bladder Diseases, Cystitis, Diabetes, Diuretic, Fever, Gallbladder Diseases, Gallstone, Hepatitis, Hydropsy, Kidney Trouble, Kidney Stones, Liver, Prostate and Urinary Diseases.
4. Stomach Ache,, Carminative, Colic, Digestive, Diuretic, Fever, Malaria, Stomachic, Tenesmu.
5. Anemia, Asthma, Bronchitis, Cough, Diuretic, Dysentery, Gonorrhea, Hepatitis, Jaundice, Thirst, Tuberculosis, Abdomen Tumor.
6. Cough, Gonorrhea, Stomachache.
7. Caterpillersting, Dermatosis, Diarrhoea, Diuretic, Itch, Miscarriage, Pesticide, Renosis, Syphilis, Vertigo.
8. Dysentery, Itch, Rectitis, Vaginitis.
9. Diuretic, Hepatitis, Gallstone, Kidney stones.
10. Blennorrhagia, Diabetes, Diarrhoea, Diuretic, Dropsy, Dysentery, Dyspepsia, Emmenagogue, Fever, Gallstone, Gonorrhea, Kidney Stones, Malaria, Tonic.

Collection of the plants

In October 2010, leaves belonging to Phyllanthus amarus were gathered from the Paderu region in Visakhapatnam District, Andhra Pradesh. Andhra University taxonomist Dr. M. Venkaiah verified the identity of the aforementioned plant, and the voucher specimen (BG/PMK/PA-10-10) was placed in the university college of pharmaceutical sciences' herbarium.

RESULTS & DISCUSSIONS

Percentage Yield of extracts

The yield and % yield of different extracts of the selected plant is given below.

Table 1: yield and % yield of P. amarus aerial part extracts

Sl.No.	Type of extract	Yield (gm)	% Yield
1	Hexane extract	126.35±10.35	3.26±0.24
2	Ethylacetate extract	264.51±12.46	6.48±0.65
3	Methanol extract	428.95±15.86	12.57±1.28

Sterols, lignans, terpenoids, flavonoids, tannins, and saponins were found in the hexane extract using qualitative phytochemical screening. Alkaloids, carbohydrates, flavonoids, tannins, triterpenes, sterols, and saponins were all detected in the ethyl acetate extract of P. amarus leaves. P. amarus leaves included carbohydrates, alkaloids, flavonoids, glycosides, tannins, and saponins, as shown by the methanolic extract. Table 3.02 listed the various phytoconstituents and their respective abundances.

Table 2: Nature of phytoconstituents present in hexane, ethylacetate and methanol extracts of P.amarus leaves

Sl.No.	Phytochemicals	Hexane extract	Ethyl acetate extract	Methanol extract
1	Sterols	+	+	-
2	Lignans	+	-	-

3	Carbohydrates	-	+	+
4	Terpenoids	+	+	+
5	Glycosides	-	-	+
6	Saponins	+	+	+
7	Flavonoids	+	+	+
8	Tannins	+	+	+
9	Alkaloids	+	+	+

+ Presence - Absence

P. amarus leaves were successively extracted using Soxhlet method, producing 3.26±0.24% hexane extract, 6.48±0.65% ethyl acetate extract, and 12.57±1.28% methanol extract. Using hexane, ethyl acetate, and methanolic extracts of P. amarus leaves, qualitative phytochemical screening identified the following compounds: sterols, lignans, terpenoids, carbohydrates, glycosides, alkaloids, tannins, flavonoids, and saponins. The results of these laboratory tests may reveal the presence of lignans such as phyllanthin, hypophyllanthin, neoxanthin, and phyltetralin; flavonoids such as rutin, quercetin; tannins such as gallic acid, ellagic acid, and geraniin; sterols such as β-sitosterol, stigmasterol, and amarosterol A & B; alkaloids like securinol, epibubbialine; terpenes such as lupeol, phyllantheol, oleanolic acid, and ursolic acid, which have been reported from the Phyllanthus genus in the literature.

While it is generally accepted that medicinal herbs and their products carry less danger than synthetic medications, they are not totally immune to toxic or other unfavourable effects. Nonetheless, there are difficulties specific to medicinal herbs. When signs of safety concern appear, limitations include the general lack of toxicological knowledge on herbs, the underreporting of adverse events, and the quality of the reported information can pose problems.

Melting Point

The capillary fusion method was used to determine the drug's melting point. It was discovered that the drug's observed melting point fell within the reference value range. The table displays the values. Using the glass capillary method and melting point equipment, the melting point of Phyllanthus amarus was determined to be 96.85±0.27 °C. This value was compared to the standard melting point of Phyllanthus amarus, which is described in literature to be 96.67-97.03 °C.

Table 3: Melting Point of Phyllanthus amarus

Apparatus	Observed value	Reference Value
Melting point apparatus	96.85±0.27 ^{0}C	96.67-97.03 °C

Solubility studies

Phyllanthus amarus solubility investigations were conducted using the equilibrium solubility method in several solvents utilizing a cyclone mixer (REMI CM 101, India). The drug solubility tests in the buffer media and various solvents are displayed in the table. Phyllanthus amarus was found to be 1.28 ± 0.02 mg/mL soluble in distilled water, but at 250C, it was found to be 15.36 ± 0.31 mg/mL soluble in methanol, with polyethylene glycols having a higher solubility. The medication is soluble in methanol and nearly insoluble in water, according to the results of the solubility research. Phyllanthus amarus is said to dissolve in water at a rate of 0.8 mg/mL.

Table 4: solubility determination in different mediums

S. No	Medium	Solubility determination Concentration (mg/mL)
1	Methanol	15.36±0.31
2	Dichloromethane	5.79±3.45
3	Ethanol	12.39±1.25
4	Chloroform	6.43±0.83
5	0.1 N HCl pH 1.2	8.37±0.07
6	Buffer pH 6.8	2.35±0.023
7	Buffer pH 7.4	1.26±0.053
8	water	In soluble

Ultraviolet (UV) spectrum

An analytical method must be developed for preformulation investigations in order to assess a drug's potency. A well-tolerated piperazine derivative called Phyllanthus amarus is used to treat this illness, providing relief from painful and incapacitating symptoms. Phyllanthus amarus is a promising anti-anginal treatment, acting on a different mode of action than medicines used to treat the same ailment. The FDA first gave it approval in 2006.[10] Figure shows that UV absorption spectra of Phyllanthus amarus were obtained in methanol and buffers containing 1% sodium lauryl sulphate (SLS). It was shown that in methanol and buffer containing 1% SLS, Phyllanthus amarus has a maximum absorption (max) at 329 nm.

Physicochemical properties of the therapeutic molecule were first determined, and only then did formulation development start. Drug concentrations in formulations and human body sample formulations can now be determined analytically. During the formulation phase, these investigations help identify potential roadblocks and determine how to overcome them. Both the finished pharmaceutical goods and active medicinal components can be produced with the use of preformulation research.[11]

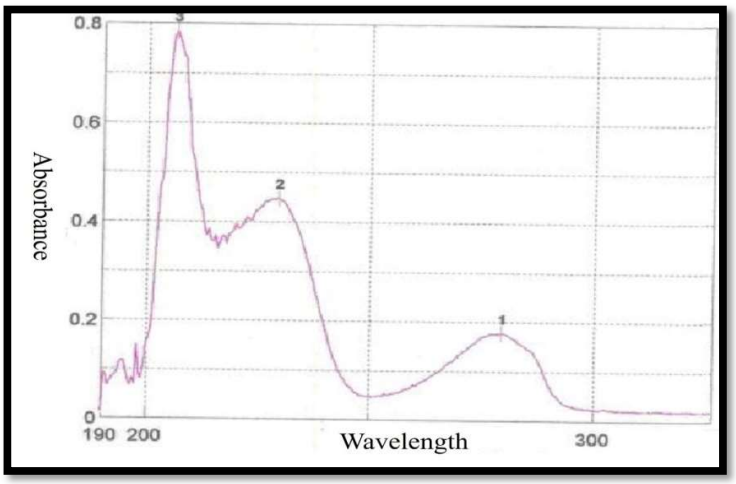

Fig 1: Determination of λmax by Uv Visible spectrophotometer

Analytical method

UV spectrometric analysis Phyllanthus amarus

As seen in the images, a standard calibration plot has been created in a number of solutions. At 329 nm in 50% v/v aqueous methanolic solution and 329 nm in 10% v/v methanolic 0.1 N HCl and 10% v/v methanolic 0.2 M trisodium buffer pH 6.8, spectroscopic detection was performed. To find the concentrations of various formulations and release samples, a mathematical transformation proportional to the UV absorbance of matching concentrations was established. A standard plot's linearity is its capacity to use the absorbance value to directly compute the concentrations of a given sample. Correlation coefficients and linear equations are presented for a range of concentrations of Phyllanthus amarus. The same observed absorption peaks at 329 nm were found in the UV absorption spectrum of Phyllanthus amarus. The analytical wavelength chosen for additional research is 329 nm, which is the wavelength of maximum absorption.[12]

Drug-Excipients Interaction Studies

To investigate the relationship between the medication and excipients, FT-IR tests were conducted on pure Phyllanthus amarus, cholesterol, and soy lecithin as well as Phyllanthus amarus + cholesterol + soy lecithin. The primary peaks and patterns of the spectra were comparable in both the pure drug and the combination that had the greatest number of excipients and the drug, according to IR spectral analysis. This suggested that Phyllanthus amarus and the other excipients in the formulations did not interact chemically. The figure displays the spectrum data.[13]

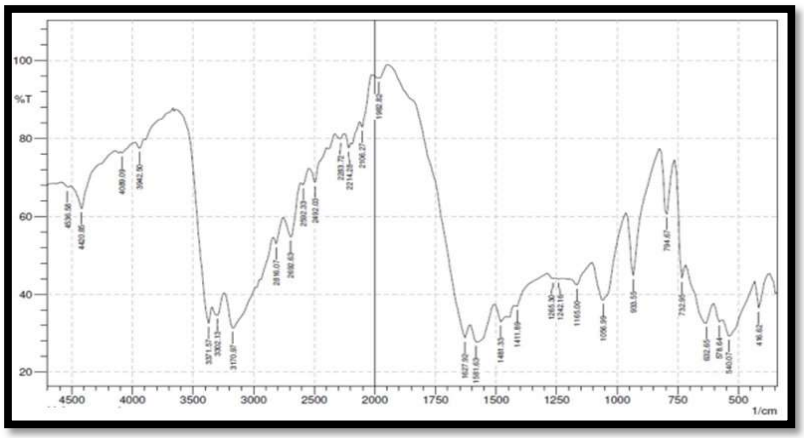

Fig 2 : FTIR structure of Pure ethanolic extract of Phyllanthus amarus

FTIR spectrophotometer was used to determine medication, lipid, and cholesterol compatibility. Phyllanthus amarus FTIR spectrum displayed the distinctive peaks of the drug structure in figure 1. Pure drug (Phyllanthus amarus) exhibited the bond variations at 3372 cm^{-1} N-H stretching, 1582 cm^{-1} Amino N-H bending, 1466 cm^{-1} CH3 bending alkanes, 1057 cm^{-1} C-N Stretching, 957 cm^{-1} Alkene C-H bending, FTIR spectrum of cholesterol showed the characterstic peaks at 3421 cm^{-1} N-H stretching, 1466 cm^{-1} CH3 bending alkanes, 1057 cm^{-1} C-N Stretching, 955 cm^{-1} Alkene C-H bending, the FTIR spectrum of soya lecithin showed the characterstic peaks at 3379 cm^{-1} N-H stretching, 1620 cm^{-1} Amino N-H bending, 1464 cm^{-1} CH3 bending alkanes, 1104 cm^{-1} C-N Stretching, 864 cm^{-1} Alkene C-H bending, the physical mixture of Phyllanthus amarus, cholesterol and soyalecithi showed the characterstic peaks at 3372 cm^{-1} N-H stretching, 1582 cm^{-1} Amino N-H bending, 1466 cm^{-1} CH3 bending alkanes, 1057 cm^{-1} C-N Stretching and 957 cm^{-1} Alkene C-H bending. This fact verified that no chemical interaction between the drug, lipid and surfactant was observed.

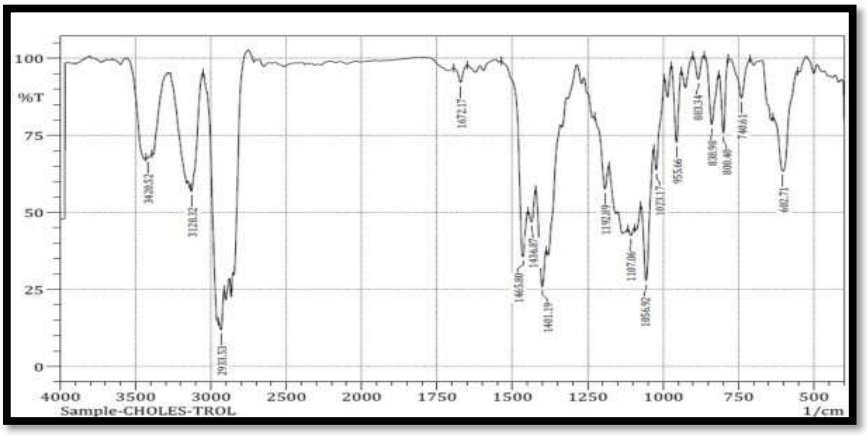

Fig 3 : FTIR spectrum of Cholesterol

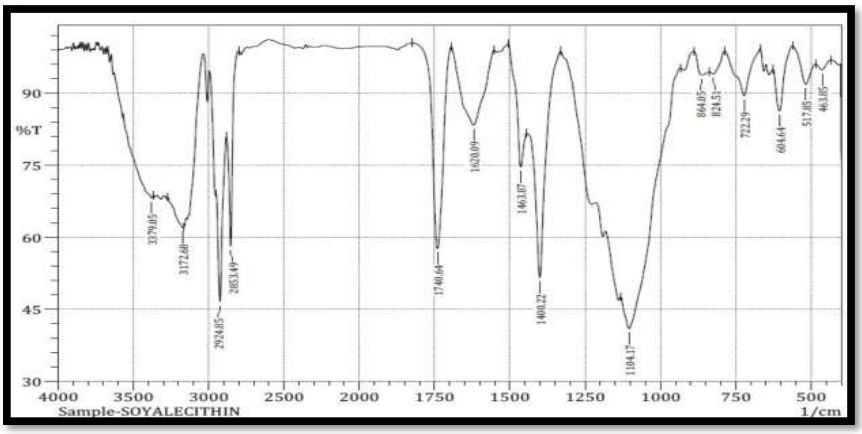

Fig 4 : FTIR spectrum of Soya Lecithin

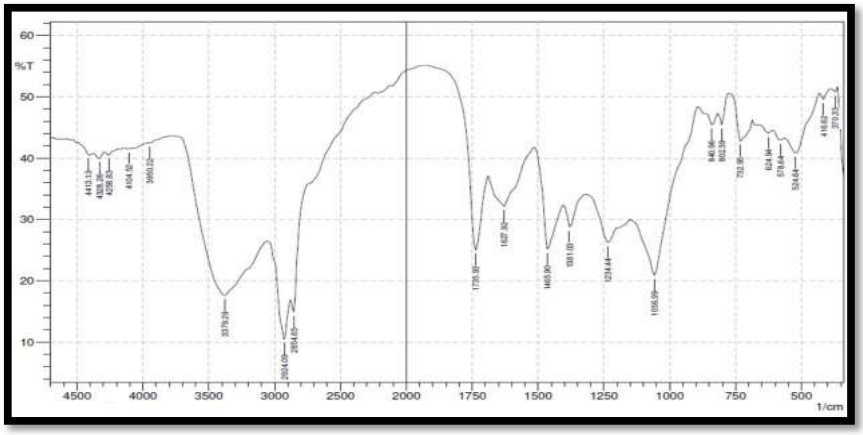

Fig 5: FTIR spectrum of Physical mixture of Phyllanthus amarus + Cholesterol + Soya Lecithin

Determination of entrapment efficiency and drug content

After analysis, it was discovered that the liposome formulations' total drug content ranged from 76.38±0.95 to 86.32±0.51%. The liposome formulations' entrapment efficiencies ranged from 65.48±1.54% to 92.54±0.69%. Phyllanthus amarus's high lipophilicity led to a high drug entrapment efficiency in triglyceride nanoparticles. This could be as a result of the glyceride's long-chain fatty acids, which enhance the space available for lipophilic medications.

The less ordered lipid matrix created imperfections leading to void spaces in which drug molecules could be entrapped. In this method of preparation, drug was dissolved in molten lipid at temperature above the melting point of lipid and there was no drug leakage or precipitation of drug during the preparation. High encapsulation efficiency of drug in lipid nanoparticles can cause high amount of drug to pass through the lymphatic transport, which in turn bypasses the first pass metabolism.

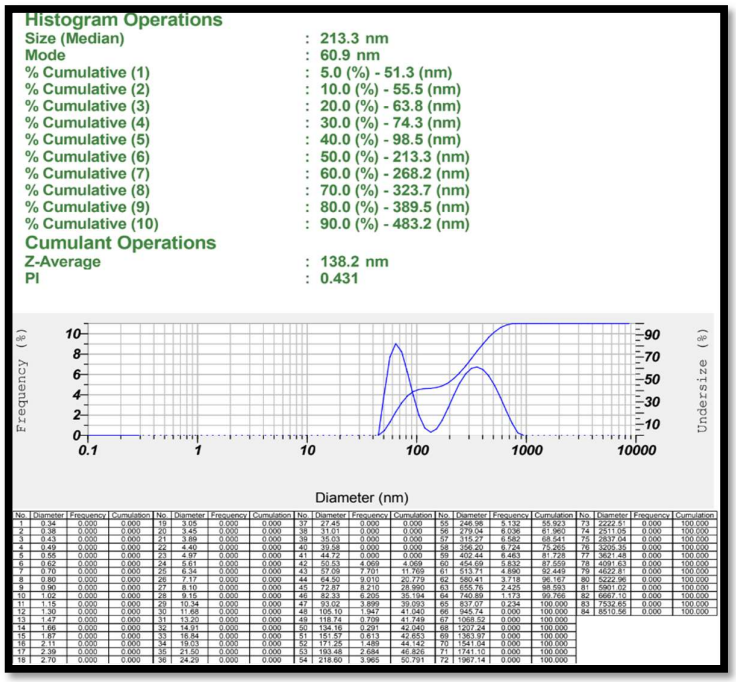

Fig 6 : Particle size distribution of Phyllanthus amarus optimized liposomal formulation

Morphology of Liposomes using Scanning electron microscopic

Using SEM, the surface morphology of liposomal formulation was examined at 10 k, 15 k, and 20 k magnification times. When the particle size grew as a result of the lyophilization process, the particles had a smooth surface and a spherical form, which enhanced the agglomeration phenomena (Fig 7).

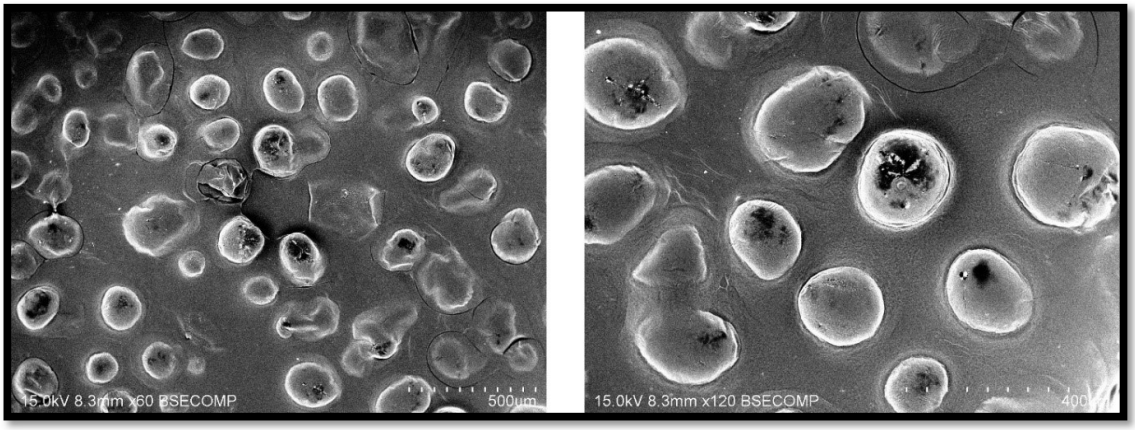

Fig 7: SEM image of optimized Liposomal formulation with different magnification

In vitro drug release studies

The drug release from the produced Phyllanthus amarus liposomes was assessed by in vitro release tests.

Table 5: displayed the findings of the in vitro release tests for each formulation.

Time (h)	PAL 1	PAL 2	PAL 3	PAL 4	PAL 5	PAL 6
0	0	0	0	0	0	0
0.5	5.36±0.02	6.59±0.35	1.35±0.11	8.59±0.36	10.24±1.26	14.53±1.26
1	8.95±0.11	12.46±0.16	3.64±0.013	17.46±0.38	23.51±2.34	26.59±1.48
2	19.86±0.24	20.35±0.42	8.76±0.04	26.59±1.24	30.42±2.15	38.79±1.49
4	32.46±0.31	31.42±0.18	15.94±0.06	38.46±1.31	42.18±2.61	46.58±2.31
6	45.87±0.13	39.76±1.23	22.61±0.12	45.76±1.05	56.79±2.08	62.13±2.06

8	59.82±0.25	48.75±1.05	28.76±0.021	59.86±1.42	68.94±2.46	73.02±2.58
10	72.43±0.26	56.31±1.42	34.12±0.37	66.48±1.36	86.53±2.35	80.67±2.47
12	85.64±1.54	63.02±1.26	42.35±1.05	72.43±1.52	99.57±2.84	88.49±2.39
16	91.25±1.03	71.49±1.34	52.46±1.04	80.46±1.42		95.43±2.51
20	102.43±1.28	80.59±1.05	60.42±1.03	89.76±1.32		102.43±2.43
22		92.46±1.68	68.79±1.42	101.42±1.48		
24			72.43±1.06			

All the values expressed as mean± standard deviation, n = 3

Phyllanthus amarus liposomes were made with a variable ratio of soy lecithin to cholesterol using the physical dispersion method. Various formulations were compared in terms of cumulative percentage medication release. It was discovered that the cumulative % drug release for formulations PAL1, PAL2, and PAL3 was 102.43±1.28 in 20 hours, 92.46±1.68 in 22 hours, and 72.43±1.06 in 24 hours, respectively. Because the formulation PAL1 contains less soy lecithin than formulations PAL2 and PAL3, it releases more quickly. After 22 hours, the cumulative percentage medication release of formulations PAL4 was determined to be 101.42±1.48. Furthermore, after 12 and 20 hours, it was discovered that the cumulative percentage drug release of formulations PAL5 and PAL6 was 99.57±2.84 and 102.43±2.43, respectively. Compared to formulations PAL4 and PAL6, formulation PAL5 exhibits a faster release. The release of the medication decreased as soy lecithin content increased. The medication released from the produced liposomes, PAL 1 through PAL 6, was sustained. In both preparation methods, the drug release increased when the ratio of soy lecithin was increased. The formulations F1, F2, and F3 as well as F4, F5, and F6 in a 24-hour period are displayed in Figure No.

Stability data

The Phyllanthus amarus Liposomes were stored for 60 days at 40 degrees Celsius and room temperature before their stability was assessed. The sample tests were established in relation to the storage duration. It was discovered that the liposomes kept at 40 degrees Celsius were stable for 60 days. Table No. presented the results. At 4oC storage, all of the Phyllanthus amarus liposome formulations showed a fair amount of stability. The original drug levels entrapped in liposomes had very little drug leakage %, and one month later, there was no discernible variation in the amount of medication retained in the vesicle from the amount just after synthesis. Yet, all of the Phyllanthus amarus liposome formulations were unstable when stored at 25°C±2°C. Furthermore, investigations on drug entrapment revealed that higher temperatures led to greater leakage. This could be because to the lipid bilayer's increased fluidity at higher temperatures, which causes more drug leakage.

Stability Parameters		**Intial**	**30 Days**	**60 days**
At 4°C±2ºC 60 ± 5%RH	%EE	92.54±0.69	89.67±0.21	86.49±0.11
	Size	205.31±4.85	206.49±5.64	208.46±4.52
	%CDR	72.43±1.06	74.36±0.11	76.89±0.37
At 25°C±2ºC 60 ± 5%RH	%EE	92.54±0.69	86.53±0.24	80.74±0.49
	Size	205.31±4.85	214.57±3.59	225.36±5.84
	%CDR	72.43±1.06	78.64±0.25	83.54±1.23

After being stored at 4ºC and 25ºC±2ºC for a month, the morphological characteristics of optimized Phyllanthus amarus liposomes (PAL 3) showed no distinctive changes. After a month of storage at 25ºC±2ºC, the optimized Phyllanthus amarus liposomes (PAL 3) formulations exhibited a modest increase in size; however, there were no changes seen for the same formulation when stored at 4ºC. 4°C proved to be a more stable storage setting than any other. This could lower the stability because of their high temperature. However, Phyllanthus amarus liposomes (PAL 3) and other formulations with a higher amount of soy lecithin demonstrated superior stability in both storage conditions.

CONCLUSION

Based on the conducted experiment, it was determined that cholesterol, soy lecithin, and stabilizers such as stearylamine and diethyl phosphate were appropriate carriers for the Phyllanthus amarus liposome synthesis. Even though the in-vitro dissolving profile, release kinetics, and stability investigations provided early evidence supporting the acceptability of these formulations, an extensive experiment will still be necessary based on animal research. The real mechanism of action for this type of dosage form can then be found.

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