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Original Article

Formulation and Evaluation of Phytosomes of Hydroalcoholic Extract of *Adiantum capillus-veneris* for Antimicrobial Activity

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ABSTRACT

Background: Natural plant extracts and phytoconstituents have high *in vitro* bioactivity but low *in vivo* bioactivity because of poor lipid solubility, multiple ring large molecules, or digestion in the gut. Polyphenolic phytoconstituents were complexed with phospholipids, mainly phosphatidylcholine, which molecularly attaches components to one another to produce novel drug delivery systems like phytosomes. The important constituents of the herbal extract are designed to protect from being disrupted by gastric juices and gut bacteria, as phytosomes have the ability to cross the bio-membranes that which are lipid enriched and boost bioavailability. **Objectives:** The current work is aimed to formulate and evaluate phytosomes from *Adiantum capillus-veneris (*ACV) hydroalcoholic extract for antimicrobial activity. **Materials and Methods:** Antisolvent precipitation method was chosen on the basis of the solubility of hydroalcoholic extract in dichloromethane. The prepared trial batches of the phytosomes complex were characterized by using particle size, zeta potential, drug entrapment efficiency, drug content, and *in vitro* drug release study. The optimized batch of phytosomes was characterized for compatibility studies by using Infrared Spectrometry (IR), Transmission Electron Microscopy (TSM), Differential Scanning Calorimetry (DSC), and further for stability studies. **Results:** The results revealed that ACV hydroalcoholic extract and phosphatidylcholine were found to be connected by a non-covalent bond and did not generate a new molecule in the ACV hydroalcoholic extract-phosphatidylcholine complex and showed enhanced *in vitro* antimicrobial activity as compared to the hydroalcoholic extract. **Conclusion:** The complex's dissolution profile was found to be improved. As a result, the phospholipid complex of ACV extract can therefore be concluded to have the potential to enhance bioavailability.

Keywords: *Adiantum capillus-veneris* (ACV), Hydroalcoholic extract, Phytosomes, H-ACV extract phytosomes, Antimicrobial activity.

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INTRODUCTION

Plants are one of the most important sources of medication. The medicinal plant is the world's earliest type of medicine. Plants have been used by all communities throughout the heritage, and plant-based traditional medicine is used by the majority of people in impoverished nations for their primary health care needs. In Indian medical systems like Ayurveda, Unani, and Siddha as well as numerous other regional systems like Chinese and Tibetan medicine, traditional medicinal plants have an ancient legacy of use. Traditional medicine practices vary from country to country and are influenced by culture and philosophy. The World Health Organization (WHO) estimates that approximately 80% of people worldwide rely on natural products for their health since it is widely believed that herbal medications have no side effects, are affordable, and are easily accessible.[1]

Herbal medicine is a prevalent part of Ayurveda, homeopathic, Native American Indian medicine, naturopathic, and traditional oriental, and is a key component in all indigenous peoples' traditions. It's also estimated that roughly 74 percent of the 119 pharmaceutical drugs derived from plants are utilized in contemporary medicine in methods that are strongly tied to their traditional applications as plant medicines by local communities. Traditional medicines have a promising future as an accessible source of effective medicinal ingredients to treat a wide range of illnesses in underdeveloped countries like India. Herbal

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medications are presently in high demand for primary health care in the poor globe.

Pteridophytes are a substantial contributor to the diversity of plant life on earth and are a foremost element of numerous plant communities, particularly in tropical and temperate regions. Pteridophytes don't have seeds or blooms and instead rely on spores to reproduce. Adiantum, commonly known as maiden hair ferns, is a genus of about 200 fern species in the Pteridaceae family; however, some of the other researchers put them in its own family called Adiantaceae. It is widely spread throughout the world, from cool temperate zones to hot tropical regions. Pteridophytes have received little research and are regarded as the plant kingdom's least economically important group. It has been shown that Pteridophytes are immune to microbial diseases, which could be one of the key causes of their evolutionary success and the reality that they have lived for over 350 million years. Pteridophytes are found in the Himalayas, Jammu and Kashmir , Western Ghats, and Vindhya, as well as hilly portions of Bihar, Madhya Pradesh, and Orissa, and the Aravalli, primarily in Mount Abu, Rajasthan.[1,2]

The genus is significant in ethnomedicine and is commonly referred to as "Hansraj". *Adiantum capillus-veneris* L. (ACV) has been employed in the Ayurvedic System of Medicine which belongs to the Adiantaceae family. In traditional medicine, the herb has been used as a diuretic, demulcent, emollient, purgative, stimulant, and tonic in the treatment of colds, fevers, coughs, and bronchial problems. In addition to skin problems, spleen tumors, and liver tumors, it is used as a general tonic and hair tonic.[2-4]

The leaves of *Adiantum capillus-veneris* show presence of different phytoconstituents like oleananes, alicyclic, carbohydrates, carotenoids, phenylpropanoids, flavonoids, and triterpenoids.[4] Polyphenolic phytoconstituents were complexed with phospholipids, mainly soya lecithin, which molecularly bind components to one another to create novel drug delivery systems such as phytosomes. Phytosomes that incorporate phospholipids into standardized extracts are greatly improving absorption and usage. Phytosomes are improved herbal products produced by binding individual components of herbal extracts to phosphaditylcholine, developing into a product that is more easily absorbed and gives better effects than standard herbal extracts. Because many phytoconstituents have many rings, they cannot be taken into the bloodstream through simple diffusion from the colon. Furthermore, due to the lipoidal nature of the small intestine, several herbal phytoconstituents are weakly miscible with oils and other lipoidal drugs and frequently are not absorbed. The effectiveness of any herbal product is determined by the amount of active ingredients delivered.[5-7]

MATERIALS AND METHODS

Quercetin was procured from Sigma Eldrich, Mumbai, India. Tetracycline disc 10 mcg was procured from Himedia, India. All other substances were analytical grade.

Plant material

The whole plant was procured from Shyam Sunder Ayurvedic, Hyderabad. The plant material was authenticated and identified by depositing the herbarium sheet of the plant specimens to the Botanical Survey of India, Koregaon road, Pune, under Specimen no: 1.

Extraction procedure

The dried leaves of *Adiantum capillus-veneris* (ACV) were crushed to obtain a homogeneous powder. The powder sample was extracted with hydroalcoholic solvent by using a Soxhlet extraction method. The hydroalcoholic solvent was prepared by using 70% ethanol and 30% water. The organoleptic characters, percentage yield of extract, and solubility of ACV extract were determined in different solvents to finalize the solvent for the preparation of phytosomes. The extract was stored in the refrigerator for further use.

Preliminary phytochemical screening of extract

The hydroalcoholic extracts were carried out for preliminary phytochemical investigation to validate the extraction of important constituents like alkaloids, flavonoids, glycosides, saponins, and steroids.[8-10]

High Performance Thin Layer Chromatography

The purified hydroalcoholic extract was exhaustively studied for its chemical constituents. Depending upon the nature of the chemical constituents, a suitable mobile phase was selected for developing the HPTLC plate. During the mobile phase selection, importance was given to the active constituents. [8-10]

Procedure

The hydroalcoholic extract was purified using the solvent toluene to isolate quercetin. Furthermore, the isolated quercetin (i.e., purified extract) was applied as a 6 mm long band to a precoated silica gel $60F_{254}$ aluminum plate (10 cm x 10 cm and 0.2 mm thick) using a Linomat 5 applicator CAMAG in conjunction with a 100 µL syringe. A CAMAG double trough chamber (20 x 10 cm) saturated with mobile phase for 15 min was used to perform ascending development using toluene mobile phase: Methanol: ethyl acetate: formic acid (5:2:4:0.5).

The volume applied on every track was 4 µL. The plate was permitted to run about 80 mm from the application point. After development, a drier was used to dry the plates. Using CAMAG TLC Scanner-3 operated and the win CATS software V, the densitometric scanning was carried out at 268 nm for standard

quercetin and sample. The slit size was 5 x 0.45 mm and the scanning speed was 20 mm/s.

The standard sample of 10 mg of quercetin was dissolved into a few ml of methanol in a 10 mL volumetric flask. The final solution obtained was sonicated in an ultrasonic water bath for 10 min, and then methanol was added to get the volume up to the mark.

Preparation of standard solution

The standard sample of 10 mg of quercetin was dissolved into a few ml of methanol in a 10 mL volumetric flask. The final solution obtained was sonicated in an ultrasonic water bath for 10 min, and then methanol was added to get the volume up to the mark.

Preparation of sample solution

The sample solution of 25 mg of purified hydroalcoholic extract was dissolved into 10 mL of methanol in a 10 mL volumetric flask. The final solution obtained was sonicated in an ultrasonic water bath for 10 min, and then methanol was added to get the volume up to the mark.

Selection of sampling wavelength for analysis

The CAMAG LINOMAT-V automatic sample applicator was used to apply the standard stock solution and the extract test solution on a TLC plate. The plate was then run and chromatographed for 15 min in a twin trough glass chamber which was saturated with the mobile phase. The plate was removed after chromatographic development and allowed to air dry. The separated bands on the TLC plate were scanned over wavelength 200-400 nm. The 268 nm wavelength was selected for the densitometric analysis of separated bands.

Preparations of hydroalcoholic *Adiantum capillus-veneris* extract phytosomes (H-ACV extract phytosomes).

Selection of working method by trial batches

The antisolvent precipitation method was used to fabricate phytosome complex from plant extract in the trial batches of 1:1, 1:2, 1:3, 1:4, 1:5, and 2:1 molar ratios (F1- F6) as shown in Table 1. Anti-solvent precipitation method was used on the basis of the solubility of plant extract in a solvent.

Antisolvent precipitation technique

The precise quantity of H-ACV extract and phosphaditylcholine were dissolved in dichloromethane. The physical mixture was refluxed for 2 hr at a temperature not exceeding 60°C to get a concentrate. Antisolvent i.e, water was mixed purposely while stirring to create the precipitate, which was then filtered, and collected and stored overnight in vacuum desiccators. The powdered physical mixture complex was filled in amber colored glass bottle container and kept in the refrigerator. [11-15]

Evaluations of H-ACV extract phytosomes

The formulated phytosomes were subjected to evaluation of various parameters as per standard procedures.

Determination of particle size and polydispersity index

The average diameter of the hydroalcoholic extract-phospholipid complex was determined using a Nanophox (Sympatec GmbH, Germany) at a fixed dispersion angle of 90° at 25°C. Polydispersity Index PDI is a parameter used to measure the width of particle distribution which was obtained at an angle of 90°.

Determination of entrapment efficiency

The entrapment efficiency of H-ACV extract -phosphadithylcholine complex was utilized to estimate by

Table 1: Composition of different formulations and Particle size of trial batches.

Sl. No.	Phytosomes	Phytosome (Molar Ratio)	Drug	Phospholipid	Solvents	Particle Size (nm)	PDI
1	F1	1 : 1	H-ACV extract	Phosphatidylcholine	Dichloromethane + Water	187.1 nm	0.2421
2	F2	1 : 2	H-ACV extract	Phosphatidylcholine	Dichloromethane + Water	432.1 nm	0.3528
3	F3	1 : 3	H-ACV extract	Phosphatidylcholine	Dichloromethane + Water	444.4 nm	0.2683
4	F4	1 : 4	H-ACV extract	Phosphatidylcholine	Dichloromethane + Water	550.2 nm	0.2815
5	F5	1 : 5	H-ACV extract	Phosphatidylcholine	Dichloromethane + Water	763.2 nm	0.3478
6	F6	2 : 1	H-ACV extract	Phosphatidylcholine	Dichloromethane + Water	1045.2 nm	0.2928

centrifugation method. Methanol was used to dilute the phytosome which was subsequently centrifuged using high-speed cooling centrifuge machine at 7,000 rpm for 20 min at -4°C. A UV-visible spectrophotometer at 256 nm was used to measure the amount of free extract and collect the supernatant for hydroalcoholic extract respectively. The following formula was used to determine Entrapment efficiency.

$$\text{Entrapment efficiency} = \frac{(\text{Total amount of drug}) - (\text{Amount of free drug})}{(\text{Total amount of drug})} \times 100$$

Determination of drug content

The phytosome complex was prepared by mixing 1 mL of the complex in 10 mL methanol for determining the drug content. The drug content was measured by using UV-spectrophotometer at 256 nm absorbance subsequent to appropriate dilution.

In vitro drug release studies

The *in vitro* drug release was measured using a treated cellophane membrane affixed to one end of an open tube holding the drug. Phytosomes of H-ACV extract equivalent to 5.5 mg quercetin was loaded. The dialysis tube was suspended in a 500 mL beaker with 250mL of phosphate buffer pH 6.8 and the solution was stirred at 100 rpm at 37±0.5°C. Further 1 mL sample of was taken out at definite time intervals and equivalent volumes of fresh phosphate buffer solution were added. All samples solution were filtered, diluted, and subjected to UV-spectrophotometer analysis.

Selection of optimized batch of phytosomes

An optimized formulation was selected on the premise of predetermined criteria like minimum particle size, maximum entrapment efficiency, high drug content, and good *in vitro* drug release. [16,17]

Characterization of an optimized batch of phytosomes

Stability study

Zeta potential is the most important factor affecting the physical stability of phytosomes.

Measurement of Zeta potential (ZP)

The stability increases with increasing electrostatic repulsion between the particles. Zeta potential measurement of the optimized phytosome was measured using the Zetasizer Nano ZS90 (Malvern Instruments Ltd., Malvern, UK). The sample was diluted with water to 10 mL; a further 5 mL of this diluted sample was placed in a cuvette to determine the Zeta potential.

Morphology study

Particle size distribution and surface morphology of the complexes have been studied using Transmission electron microscopy.

Transmission Electron Microscopy (TEM)

The TEM study investigates the enclosed components where the drug is entrapped and its distribution within the phospholipid lattice. Digital images of phytosome complex of hydroalcoholic extract of *Adiantum capillus-veneris* were taken by random scanning of the stub at 27800x, 4640x, and 60,000x magnifications.

Compatibility studies

To determine and anticipate any physicochemical interactions between purified hydroalcoholic extract and phosphatidylcholine in a formulation the following parameters were used.

FTIR spectroscopy

The interactions of molecules involved in the formulation elements were investigated in order to generate the FTIR scans of pure hydroalcoholic extract, phosphatidylcholine, phytosomes, and physical mixture on an FTIR spectrophotometer (Bruker Alpha FTIR). The samples were previously dried to eliminate the influence of residual moisture and individual spectra were recorded for pure hydroalcoholic extract, phosphatidylcholine, phytosomes and physical mixture and finally for optimzed formulation in the 4000 to 1000 cm^{-1} wavelength range.

Differential Scanning Calorimetry (DSC)

DSC is a thermoanalytical method in which the difference between the amounts of heat required to raise the temperature of a sample and reference is determined as a function of temperature. The end result of DSC experimentation is a curve of heat flux versus temperature. In DSC the pure drug, which is crystalline in nature, shows a sharp peak as a high melting point in the DSC thermogram while the final product phytosome displays a broader peak, which signifies the loss of crystallinity and low melting point than that of pure drug. Hydroalcoholic extract and Phytosomes were heated in an aluminum crimp cell at a rate of 10°C/min from 0 to 400°C in the atmosphere of nitrogen (TA Instruments, USA, model DSC Q10 V24.4 Build 116). Using an analyzer, the peak transition onset temperatures were recorded.

In vitro antibacterial activity

Agar well diffusion method [18-22]

All glasswares and petri plates were disinfected using dry heat in a 160°C oven for an hour and a half. In distilled water, nutrient agar was made. In sterile petri plates, the nutritional agar was aseptically inserted and permitted to be set at room temperature. All of the petri plates were poured with 0.1mL of the standardized culture in an aseptic way. A sterile cork borer was used to bore the 7mm holes aseptically. The agar plugs were gently removed such that the surrounding medium was not disturbed. The holes were completely filled with the chosen extract and incubated for 48 hr at 37°C. The antibacterial activity of the Petri plates was then assessed, and the zone of inhibition was calculated.

Working procedure

The nutritional agar medium is laid out in layers of roughly 4mm thickness on the plates. The hydroalcoholic extract (100-1000 ppm) and Phytosomes of H-ACV extract (50-200 ppm) were prepared in solvent DMSO. The extracts were read out in their entirety. Using a wooden applicator, dip a sterile, non-toxic cotton swab in prepared inoculums and then rotate firmly against the upper inside wall of the test tube. Using the swab, streak the entire agar surface of the plate 2–3 times, spinning the plate at 600 angles between each streaking. The inoculum was allowed to dry for 5–15 min with the lid in place. The plate is properly bored with borer and the disc is applied to standard drug. Tetracycline (10mcg/disc) was used as a standard antibiotic for the activity that was most resistant in both Gram-positive bacteria (*Staphylococcus aureus*) and Gram-negative bacteria (*Escherichia coli*).

Stability Studies

The H-ACV extract phytosomes were subjected to stability conditions by the guidelines framed by ICH. The optimized formulation was sealed at 5°C ± 3°C, 30°C ± 2°C/65% RH, and 40°C ± 2°C/75% RH for a time period of one month. Phytosomes were removed periodically and evaluated for physical characterization. The physical stability of the developed formulation was studied by using parameters such as Entrapment Efficiency. [23]

RESULTS

The present study was intended to formulate phytosomes using hydroalcoholic extract of ACV and evaluate its antimicrobial activity.

Characterization of ACV Extract

Organoleptic characteristics

The dark brown colour was obtained for ACV hydroalcoholic extract with an absorption wavelength of 765 nm. Percentage yield was 7.4% w/w.

Solubility of ACV Extract

The ACV Extract was soluble in Dichloromethane.

Preliminary phytochemical screening

H-ACV extract confirmed the presence of alkaloids, phenols, tannins, flavonoids, steroids, carbohydrates and terpenoids.

High-Performance Thin Layer Chromatography

HPTLC fingerprint scanned at different wavelengths for standard quercetin, hydroalcoholic crude extract, and purified hydroalcoholic extract showed the presence of different secondary metabolites with different concentrations from results obtained. The band R_f value obtained for standard quercetin was 0.89, for hydroalcoholic crude extract 0.76 and 0.89 for purified hydroalcoholic extract. The quercetin content in H-ACV extract was found to be 13.1µg/ml.

Evaluation parameters for trial batches

The formulated phytosomes of H-ACV extract were further evaluated for particle size, polydispersity index, drug entrapment efficiency, drug content, and *in vitro* drug release.

Particle size

The mean particle size for F1- F6 batches were found to be in the range of 187-1045 nm. As seen in Table 1. The formulated phytosomes of H-ACV extract had an average PDI value ≤ 0.3 indicating a homogenous distribution of Phytosomes as seen in Table 1.

Drug entrapment efficiency

Entrapment efficiency was found to be between 97-98.4%. It is defined as the fraction of the drug absorbed into formulations relative to the total amount of drug used. Estimation of entrapment efficiency is a vital factor with respect to Phytosomes. As summarized in Table 2 formulations F2 and F6 showed minimum entrapment efficiency. Similarly, formulations F1 and F5 showed the highest entrapment efficiency. So the highest entrapment efficiency was recorded to be 90.12% for batch F1 followed by 72.39% for batch F2 respectively. The entrapment efficiency of the total 6 batches is mentioned in Table 2.

Drug content

The drug content of all optimized phytosomes loaded with PC batches F1-F6 was found to be in the range of 90.12–58.80 in the respective batches. In batches, F1 and F2 drug content was found to be the highest as the entrapment efficiency was the highest. Hence, depending on the particle size, entrapment efficiency, and drug content, formulation F1 was chosen as the optimized batch and was selected for further study due to its minimum particle size of 187.1 nm and high % entrapment efficiency of 98.4%. The drug content of a total of six batches is shown in Table 2.

In vitro drug release

The *in vitro* release profiles of H-ACV extract Phytosomes were investigated in the Phosphate buffer of pH 6.8 for the duration of 8 hr. Surprisingly, the results give some interesting insights about the drug release profile and Kinetics. In the first two hours of administration, there is only 35% of drug release observed. Between the 3rd and 6th hr, the release is seen constant from 35 to 71%. Later on within the last hour i.e. 7th and 8th, we were able to see the drastic major release of drugs. Finally, we were able to achieve > 90% drug release from the formulation. The results of *in vitro* drug release studies of pure ACV extract and phytosomes of ACV extract made by the antisolvent precipitation method (F1 to F6). Pure drug evidenced only 69.24% in 8 hr, indicating less solubility of quercetin. However, there is the enhancement of

drug dissolution when it is made into phytosomes. Formulation F1 exhibited the highest percentage at 71.1% in 360 min and 91.49%. Hence, this was considered a promising formulation and was further investigated for drug-excipient interaction studies.

F1 showed lesser particle size with the highest entrapment efficiency and highest drug content. Formulation F1 showed lesser particle size with the highest entrapment efficiency and highest drug content as shown in Figure 1. Amongst the trial batches, the batch chosen with the size was based on entrapment efficiency as that is the major parameter for final formulation.

Evaluation parameters of optimized batch

Physical study

Zeta potential: The zeta potential value is shown in Table 3. A zeta potential value of the optimized formulation is negative, indicating a stable formulation.

Morphology study

Transmission electron microscopy

Samples were diluted with methanol and sonicated for 10 min. A drop of Phytosome was applied to a carbon-glazed grid, allowed to dry into a thin film, and then images of Phytosomes were captured using TEM. The Phytosomes were scanned in the range of 100- 300 nm. The TEM view of an optimized batch of ACV hydroalcoholic extract showed sphere-shaped vesicles as seen in Figure 2.

Compatibility studies

The compatibility studies of ACV Extract were studied by using FTIR Spectroscopy and Differential Scanning Calorimetry (DSC).

Table 2: **Entrapment Efficiency and Drug content of Trial Batches.**

Sl. No.	Phytosomes	Phytosomes trail batches	% Entrapment Efficiency	Drug content
1	F1	1 : 1	98.4%	90.12%
2	F2	1 : 2	97.44%	72.39%
3	F3	1 : 3	98.08%	68.48%
4	F4	1 : 4	98.16%	68.09%
5	F5	1 : 5	98.2%	64.40%
6	F6	2 : 1	97%	58.80%

Table 3: **Zeta Potential of optimized H-ACV extracts Phytosomes.**

Sl. No.	Phytosomes	Batch	Zeta Potential mV
1	F1	1:1	-17.22 mV

Table 4: **Zone of inhibition of H-ACV extract and Zone of inhibition of optimized H-ACV extract phytosomes.**

Sl. No.	Concentration (ppm)	*S. aureas* (mm)	*E. coli* (mm)
1	Standard (Tetracycline 10 mcg)	23	22
2	100	11	12
3	250	15	13
4	500	16	18
5	700	17	15
6	800	18	16
7	900	19	16
8	1000	21	18
Zone of inhibition of optimized H-ACV extract phytosomes			
9	Standard (Tetracycline 10 mcg)		24
10	50	14	12
11	100	16	14
12	150	18	16

Zone of inhibition of H-ACV extract

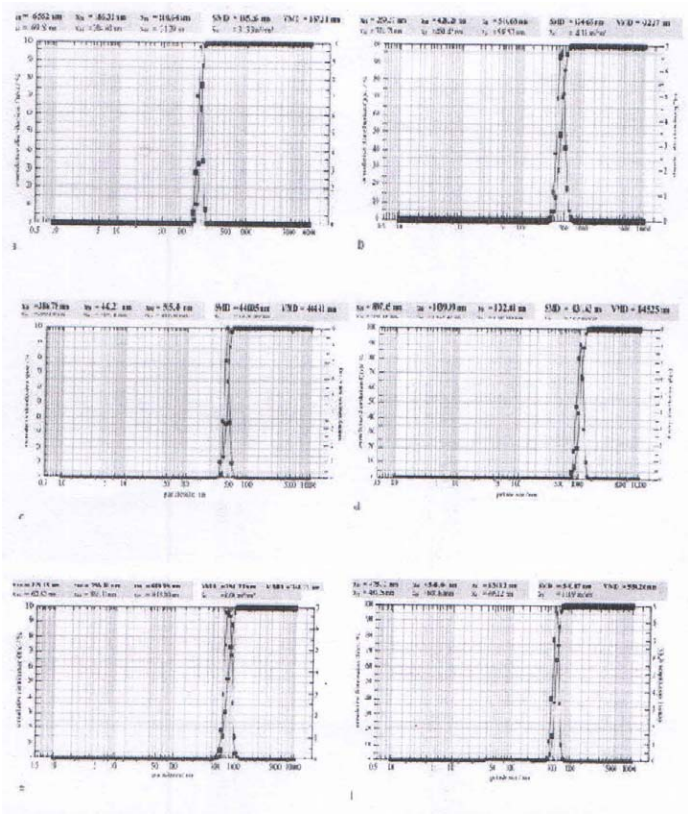

Figure 1: Particle size of trial batches of H-ACV extract phytosomes a. Batch 1: 1, b. Batch 2:1, c. Batch 1:3,d. Batch 1:4, e. Batch 1:5, and f. Batch 2:1

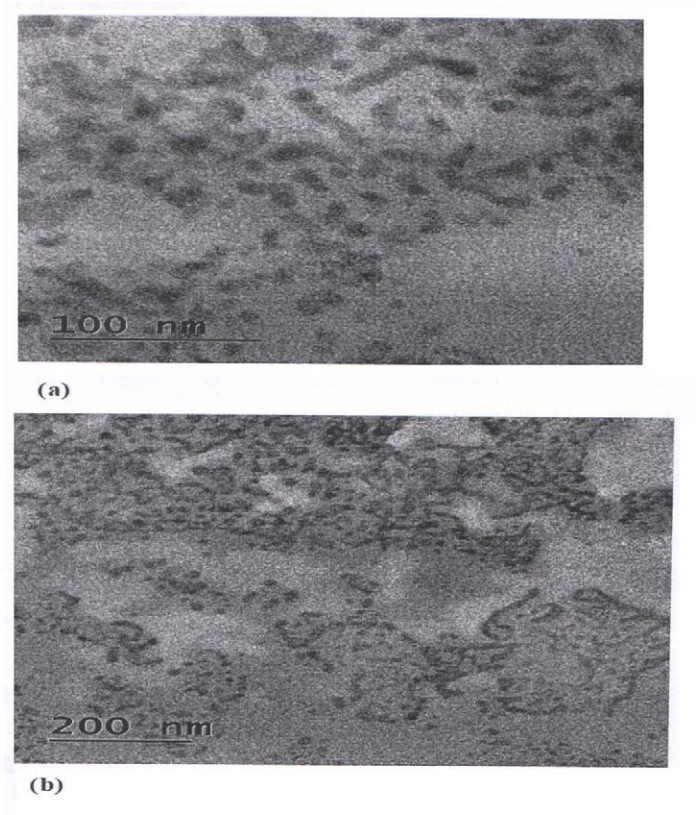

Figure 2: TEM of optimized batch H-ACV extract at (a) 100 nm and (2) 200 nm.

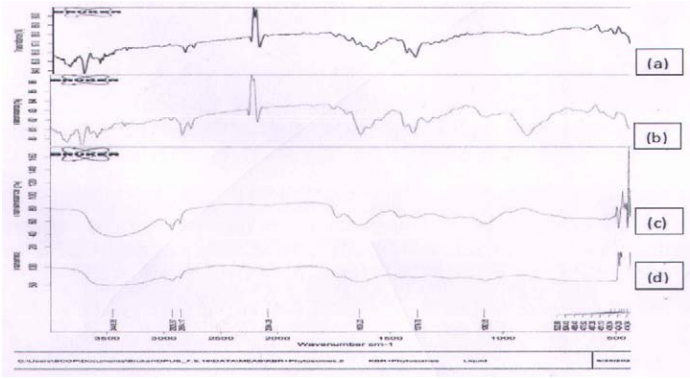

Figure 3: (FT-IR Spectra of (a) Hydroalcoholic Extract of ACV (b) Phosphotidylcholine (c) H-ACV extract Phytosomes (d) Physical mixture.

FTIR Spectroscopy

The compatibility between the hydroalcoholic extract and phosphatidylcholine was assessed using the FTIR peak matching method as depicted in Figure 3. The FTIR spectroscopy of Phytosome spectra revealed a shift of the -OH group to a lower frequency (3790 cm^{-1} to 3423.74 cm^{-1}) as compared to the hydro-alcoholic extract of ACV, indicating a significant configuration of hydrogen bonding between the hydroxyl group of Phosphatidylcholine (PC) and extract phytoconstituents in phytosome form. The band of the choline N-(CH_3) group in phosphatidylcholine spectra is shifted to a higher frequency in Phytosome spectra (1053-1071 cm^{-1}) with the decrease in intensity showing an interaction between PC and extract constituent at the level of choline moiety.

Differential scanning calorimetry

The possibility of any interaction between the drug and polymers was assessed by carrying out DSC. The physical state of hydroalcoholic extract and phosphatidylcholine was investigated since it would have a significant impact on the *in vitro* and *in vivo* release characteristics.

Table 5: Entrapment Efficiency of optimized H-ACV extract phytosomes.

Sl. No.	Stability Conditions	Time (days)	Entrapment Efficiency of H-ACV Phytosomes
1	5 ± 3°C, 30°C ± 2°C/ 65% RH, 40°C ± 2°C/75% RH	0	98.4%
		7	98.2%
		14	97.6%
		21	97.5%
		30	97.2%

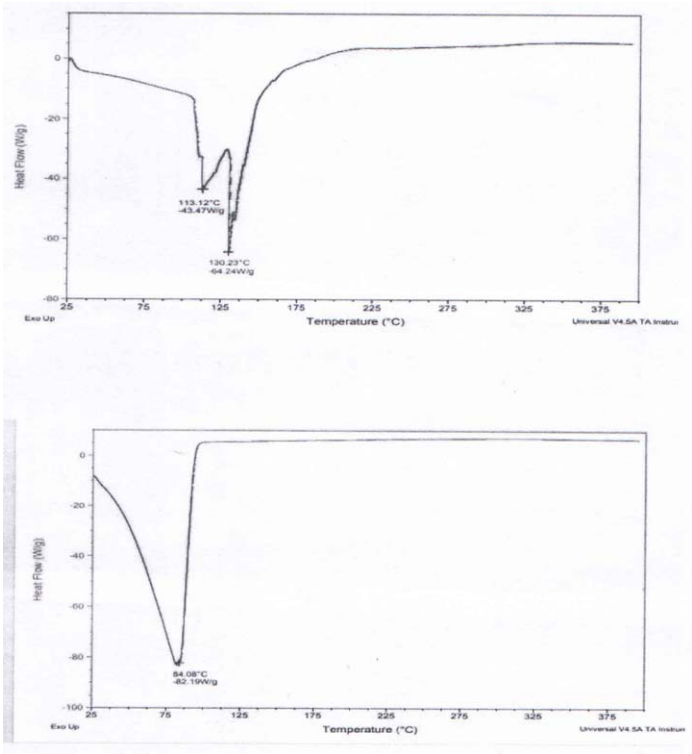

Figure 4: (a) DSC curve for H-ACV extract (b) DSC curve for H-ACV extract Phytosome.

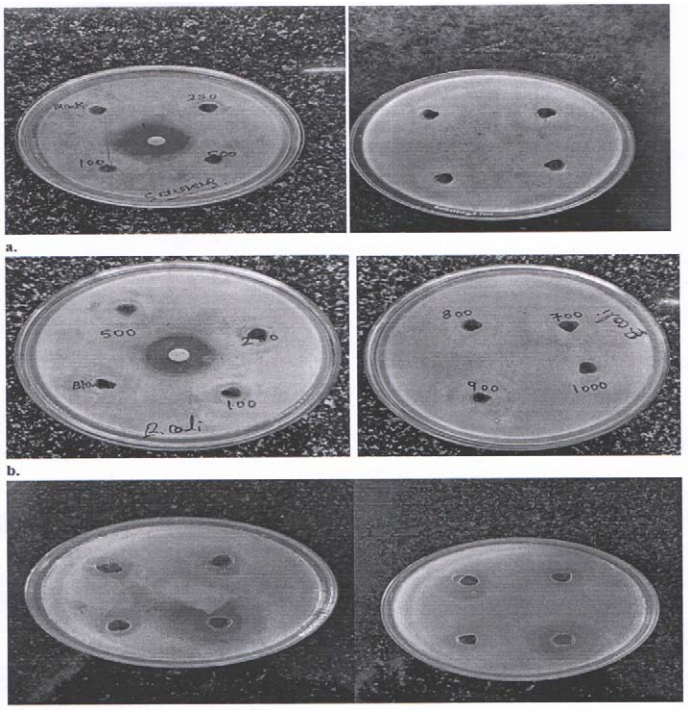

Figure 5: (a) Antibacterial activity of H-ACV extract (100- 1000ppm) against *S. aureus* ; b) Antibacterial activity of H-ACV extract (100- 1000ppm) against *E. coli* ; c) Antibacterial activity of H-ACV extracts phytosomes (50-200ppm) against *S.aureus* and *E. coli*.

Pure H-ACV extract showed an endothermic peaks at temperatures of 113°C and 130°C as seen in Figure 4. The endothermic peak of optimized batch 1:1 was seen at 84°C. Therefore, it was revealed that the shift of endothermic peak at 25-30°C suggest the interaction of ACV Extract with Phosphatidylcholine and account for enhanced entrapment.

In vitro antimicrobial activity of hydroalcoholic extract and H-ACV extract phytosomes

The hydroalcoholic extract of leaves of *Adiantum capillus-veneris* with different concentrations were examined for antibacterial activity against the Gram-positive bacteria (*Staphylococcus aureus*) and Gram-negative bacteria (*Escherichia coli*) as shown in Table 4. The antibacterial sensitivity of the crude hydroalcoholic extract was determined by quantifying the diameter of the clear zone in cultures in petri plates.

The antibacterial activity of these extracts could be related owing to the presence of secondary metabolites such as alkaloids, flavonoids, phenolic compounds, terpenoids, and tannins. The results found that there was no intense inhibition seen in extracts. The results found that an increase in the concentration of extract also increases the zone of inhibition but with less intensity as seen in Figure 5.

In vitro antibacterial activity of H-ACV extract phytosomes

The Phytosomes of hydroalcoholic extract *Adiantum capillus-veneris* with different concentrations were examined

for antibacterial activity against the Gram-positive bacteria (*Staphylococcus aureus*) and Gram-negative bacteria (*Escherichia coli*) as shown in Table 4. The antibacterial sensitivity of the crude hydroalcoholic extract was determined by quantifying the diameter of the clear zone in cultures in petri plates.

The antibacterial activity of the phytosomes was carried out as there was no intense zone of inhibition seen in the hydroalcoholic extract. There was a significantly intense zone of inhibition as compared to the hydroalcoholic extract as shown in Figure 5. The antibacterial activity of Phytosomes was enhanced due to the less particle size and better penetration of drugs in the cell walls of bacteria.

Stability Studies

The optimized formulation was sealed and stored at 5°C ± 3°C, 30°C ± 2°C/ 65% RH and 40°C ± 2°C/75% RH for a time period of 30 Days. The Phytosomes were removed periodically and evaluated for physical characterization. The physical stability of the developed formulation was studied by using the parameter entrapment efficiency. The results of the stability study are depicted in Table 5. From the results, it is evident that in the formulation no significant change was observed in entrapment efficiency either stored in the refrigerator or a room temperature. This implies that there is no degradation of the formulation during storage.

DISCUSSION

In the present study, the percentage yield of ACV leaves in a hydroalcoholic extract with a ratio of 70:30 solvent was reported maximum as 7.4% w/w which indicates good results of percentage yield. In the HPTLC densitogram the quercetin content in the H-ACV extract was found to be 13mcg/mL. Six formulations (F1-F6) of H-ACV phytosomes were prepared in which F1 was chosen as an optimized batch due to its lesser particle size, high entrapment efficiency, and high drug content. The prepared F1 formulation of ACV hydroalcoholic extract and phosphatidylcholine were found to be connected by a non-covalent bond and does not form a new compound in the hydroalcoholic extract-phosphatidylcholine complex and showed enhanced *in vitro* antimicrobial activity as compared to the hydroalcoholic extract.[24] As a result, the prepared formulation F1 may be a promising candidate as an antimicrobial drug. Thus, current research work on this medicinal plant could serve as the baseline for further investigations for the discovery of new compounds and more investigations into their biological activities.

CONCLUSION

In the current study, ACV extracts of phospholipid complex were prepared using an antisolvent precipitation method and evaluated using various physiochemical parameters. The investigation of physiochemical parameters revealed that H-ACV extract formed complexes with phosphatidylcholine that had higher bioavailability. The complex's formation was confirmed by IR, DSC, and TEM studies. The complex's dissolution profile was found to be improved. As a result, the phospholipid complex of H-ACV extract can therefore be concluded to have the potential to enhance bioavailability.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

ACV: *Adiantum capillus-veneris*; **H-ACV Extract phytosomes:** Hydroalcoholic extract of *Adiantum capillus-veneri* Phytosomes.

SUMMARY

The leaves of the plant *Adiatum capillus-veneris* belonging to the family Pteridaceae were used and investigated for phytochemical screening, formulation, evaluation of phytosomes, and antimicrobial activity for hydroalcoholic extracts. Six formulations were prepared from which F1 showed significant results for antimicrobial activity.

REFERENCES

1. Vadi R, Manisha V, Swati K. Hansraj (*Adiantum capillus-veneris* Linn.): a systematic review on its ethnobotany, phytochemical and pharmacological profile. Int J Ayurveda Pharm Res. 2017;5(6):5-21.
2. Shaikh SS, Bamer AH, Jamkhande PG. Plant profile, Phytochemistry and pharmacological activity of Plant *Adiantum capillus-veneris* Linn. Hansraj. 2021;3(5):13.
3. Ranjan Vadi, Vats M. Pharmacognostical and physico-chemical standardization of the whole plant of *Adiantum capillus-veneris* Linn. Int J Pharm Sci Res. 2016;7(2):773-82.
4. Dehdari Saher, Hajimehdipoor H. Medicinal properties of *Adiantum capillus-veneris* Linn. Iran J Public Health. 2018;47(2):188-97. PMID 29445628.
5. Singh RP, Narke R. Preparation and evaluation of phytosomes of lawsone. Int J Pharm Sci Res. 2015;6(12):5217-26.
6. K S S, Nesalin J AJ, Mani T T. Preparation and evaluation of curcumin phytosomes by rotary evaporation method. Int J Pharm Biomed Eng. 2019;6(1):29-34. doi: 10.14445/23942576/IJPBE-V6I1P104.
7. Dhase AS, Saboo SS. Preparation and evaluation of phytosomes containing methanolic Extract of Leaves of *Aegle marmelos*. Int J Pharm Tech Research. 2015;8(6):231-40.
8. O A T Ebuehi, N A Okorie. Phytochemical screening and quantification of flavonoids from leaf extract of *Jatropha curcas* Linn. Nig Q J Hosp Med. 2009;19(4):200-5.
9. Gaurav Mahesh Doshi, Hemant Devidas Une. Quantification of quercetin and rutin from *Benincasa hispida* seeds and *Carissa congesta* roots by High-performance Thin Layer Chromatography and High-performance Liquid Chromatography. Pharmacognosy Res. 2016;8(1):37-42.
10. Shivraj Hariram Nile and Se Won Park. HPTLC Analysis, Antioxidant and antigout activity of Indian Plants. Iran J Pharm Res. 2014;13(2):531-9.
11. Pande SD, Wagh AS, Bhagure LB, Patil SG, Deshmukh AR. Preparation and evaluation of phytosomes of Pomegranate peels. Rese Jour Pharm Technol. 2015;8(4):416. doi: 10.5958/0974-360X.2015.00070.0.
12. Kumar A, Kumar B, Singh SK, Kaur B, Singh S. A review on phytosomes: novel approach for herbal phytochemicals. Asian J Pharm Clin Res. 2017;10(10):41. doi: 10.22159/ajpcr.2017.v10i10.20424.
13. Patel J, Patel R, Khambholja K, Patel N. An overview of phytosomes as an advanced herbal drug delivery system. Asian J Pharm Sci. 2009;10.

14. Chouhan A, Shah SK, Tyagi CK, Budholiya P, Wazid A, Khan F. Formulation, development and evaluation of phytosomes of *Swertia perennis* [journal]. AJPER JOURNAL. 2021;10(2):55-66.
15. Barani M, Sangiovanni E, Angarano M, Rajizadeh MA, Mehrabani M, Piazza S, *et al*. Phytosomes as innovative delivery systems for phytochemicals: A comprehensive review of literature. Int J Nanomedicine. 2021;16:6983-7022.
16. Udapurkar PP, Bhusnure OG, Kamble SR. Diosmin phytosomes: development, optimization and physicochemical characterization. IJPER. 2018;52(4):29-36.
17. Rani A, Kumar S, Khar RK. *Casuarina equisetifolia* extract loaded phytosomes: optimization, characterization and *in vivo* evaluation of antidiabetic and antihyperlipidemic activities in Wistar rats. Drug Deliv Lett. 2019;9(2):116-33.
18. Chime SA, Akpa PA, Onyishi IV, Ezenduka DE. Phytosomes enhanced the antibacterial and antifungal properties of *Lantana camara*; innovare. J Ayurvedic Sci. 2020;8(1):5.
19. Nguyen TLA, Bhattacharya D. Antimicrobial activity of quercetin: an approach to its mechanistic principle. Molecules. 2022;27(8):2494.
20. Correia D, matias H. Isolation, modeling and phytosome forms of antimicrobial and antiproliferative compounds from *Plectranthus* spp.*ectranthus* spp. Universidad de Alcalá; 2016. Available from: http://purl.org/dc/dcmitype/Text [cited Jul 27 2022]. Available from: https://dialnet.unirioja.es/servlet/tesis?codigo=181992.
21. Sahibzada MUK, Sadiq A, Faidah HS, Khurram M, Amin MU, Haseeb A, *et al*. Berberine nanoparticles with enhanced *in vitro* bioavailability: characterization and antimicrobial activity. Drug Des Dev Ther. 2018;12:303-12.
22. Suzilla WY, Izzati A, Isha I, Zalina A, Rajaletchumy VK. Formulation and evaluation of antimicrobial herbosomal gel from *Quercus infectoria* extract. IOP Conf Ser Mater Sci Eng. 2020;736(2):022030.
23. Andleeb M, Shoaib Khan HM, Daniyal M. Development, characterization and stability evaluation of topical gel loaded with ethosomes containing *Achillea millefolium* L. extract. Front Pharmacol. 2021;12:603227.
24. Kadu AS, Phytosome AM. A novel approach to enhance the bioavailability of phytoconstituent. Asian J Pharm. 2017;11(2):453-61.

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