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Research Paper

Formulation And Evaluation of Nano-Phytosomes of Ethanolic Extract Seed of *Vitis Vinifera (Vitaceae)*

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ABSTRACT

Traditional approach is the one of the emerging field of biomedical sciences. In this Research work, we have evaluated the traditional approaches of the ancient healer plant Vitis Vinifera belonging to Vitaceae family. The seed of Vitis Vinifera was subjected to extraction process, phytochemical analysis, formulation and evaluation of nano-phytosome its characterization. (1) The scientific data shows that seed extracts contain flavonoids, tannins saponins and carbohydrates etc., Nano-phytosome was prepared by Mechanical Dispersion Method. The formulation was evaluated for drug content, FTIR and Entrapment efficiency. Recently, Nanophytosome methodology has been fruitfully applied over several well-known natural drugs such as ginseng, green tea hawthorn, olive oil and grape seed etc. Indeed, phytosome are superior to conventional drug delivery systems in terms of the pharmacodynamic and pharmacokinetic properties.(2) This research, we discusses the various aspects, components, methods of preparation of phytosome and their marketed formulations, therapeutic applications along with the recent research work reported on this technology..

INTRODUCTION

Herbal formulation as a primary health care system and is tremendously increase Day by Day due to toxicity and adverse reaction associated with modern allopathic medication. The implementation of novel approaches to traditional medicines will results in increased bioavailability, decreased toxicity sustained release action & protection from GI degradation which cannot be achieved by using conventional drug delivery system because of large molecular size, poor solubility and degradation of herbal medicine in India. (3) From the last century, there are valuable

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criteria has been kept it up and centre on the event of Novel drug delivery system (NDDS) for herbal medicines. Preparations of plants or parts of them were widely used in popular medicine since ancient times and till today the use of phytomedicines is widespread in most of the world's population. The term 'Phyto' means plant while 'Some' means cell-like. Phytosome is vesicular drug delivery system in which phytoconstituents of herb extract surround and bound by lipid [4] ***Vitis vinifera*** thrives in temperate climates with hot, dry summers and mild, wet winters, preferring full sunlight and temperatures between 25°C and 30°C (77°F - 86°F). It grows to 15–30 feet (4.5–9 meters), favours well-drained, calcareous soils, and is sensitive to frost. It is cultivated in India, Africa, Egypt, Morocco, and occasionally in England. ***Vitis vinifera*** seeds are small, oval-shaped, about 1 to 1.5 cm long, with a hard outer coat. They require cold stratification to break dormancy and germinate.[5] However, grapevines are mostly propagated through cuttings to preserve desired traits. **Antioxidant Source:** Grapes are rich in antioxidants like resveratrol and quercetin, which may help protect against cell damage and chronic diseases. **Heart Health** Antioxidants in grapes may contribute to heart health by promoting healthy blood vessel function and reducing inflammation**. Cancer Prevention** Some studies suggest that compounds in grapes may have anti-cancer properties.

MATERIALS AND METHODS

COLLECTION OF PLANT MATERIAL

The fresh fruit of ***Vitis vinifera*** was bought from Surulipatti village garden in Theni district in the month of July. After collection of fruits, the fruit pulp was removed and the seeds are dried in shade for 2 weeks. After drying the seeds are powered by mixer grinder**.**

EXTRACTION OF THE PLANT MATERIAL:

The powdered grape seeds approximately weighing about 129.8 gm were extracted with 500ml of ethanol using Soxhlet apparatus. The ethanol, approximately 500ml was taken in the round bottomed flask and heated using heating mantle and the temperature was maintained at 700C. The ethanol evaporates and again condensed to moisten the powder kept in thimble which leads to the extraction of phytoconstituents. [6]

PRELIMINARY PHYTOCHEMICAL SCREENING

Ethanolic extracts of *vitis vinifera* were subjected to qualitative chemical analysis. The various chemical tests were performed on the extracts for the identification of flavonoids, steroids, terpenoids, tannins, saponins, anthocyanidins, emodin, alkaloids and phenol. [7]

PREPARATION AND EVALUATION OF NANO PHYTOSOMES

PHYTOSOMES FORMULATION BY MECHANICAL DISPERSION METHOD

In this method cholesterol and soya lecithin shall be dissolved in ether. It is act as an oil phase. Then the extracted plant medicaments dissolved in phosphate buffer pH 6.8. The aqueous medium is placed under magnetic stirrer for 5- 10 minutes. After the complete dissolution of medicaments, the oil phase added into an aqueous medium by drop wise. Then it is allowed to magnetic stirrer for 1 hour with 1000rpm. Then the product will be collected.Prepared phytosomal suspension shall be centrifuged at 8,000 rpm for 40 minutes at 4^0 C. The precipitate was diluted with distilled water for evaluation studies. [8]

FOURIER TRANSFORM – INFRA RED SPECTROSCOPY (FT-IR),

FT-IR evaluation was performed to study the compatibility between the phytoconstituent and phospholipid and to confirm phytosome formation. The analysis is based on the absorption of infrared radiation by different functional groups at characteristic wavenumbers. Samples of pure phytoconstituent, phospholipid, and the prepared phytosome formulation were analyzed using the KBr pellet method. About 1–2 mg of each sample was mixed with dry potassium bromide and compressed into a transparent pellet using a hydraulic press. [9]The FT-IR spectra were recorded in the range of 4000–400 cm^{-1} using an FT-IR spectrophotometer after recording a background spectrum with KBr. The spectrum of the phytosome formulation showed shifting and broadening of characteristic peaks compared to the pure phytoconstituent and phospholipid, indicating molecular interaction between functional groups such as hydroxyl, carbonyl, and phosphate groups. These spectral changes confirmed the successful formation of the phytosome complex and demonstrated drug–phospholipid compatibility. [10]

Entrapment efficiency determination:

The entrapment efficiency of *Vitis vinifera* seed extract–loaded phytosomes prepared by mechanical dispersion using phosphatidylcholine was determined by an indirect centrifugation method. A known volume of the phytosomal suspension was diluted with phosphate buffer (pH 6.8) and centrifuged at high speed to separate the phytosome-entrapped extract from the unentrapped free extract. After centrifugation, the supernatant containing the free drug was carefully collected and analyzed using UV–visible spectrophotometry at a suitable wavelength corresponding to the characteristic polyphenolic constituents of grape seed extract, based on a previously prepared calibration curve. The total amount of extract initially added during formulation was considered as the theoretical drug content, and the entrapment efficiency was calculated by subtracting the amount of free drug present in the supernatant from the total drug content and expressing the result as a percentage. This indirect method is widely reported for evaluating phytosomal and phyto-phospholipid complexes due to its accuracy and reproducibility. (11)

Drug content determination:

The drug content of the *Vitis vinifera* seed extract phytosome formulation was determined to evaluate the actual amount of extract present in the prepared phytosomes. An accurately weighed quantity of the phytosomal formulation was dissolved in an appropriate solvent such as methanol or phosphate buffer to ensure complete disruption of the phospholipid–phytoconstituent complex and release of the entrapped extract. The solution was then filtered to remove undissolved phospholipid material, and the filtrate was analyzed by UV–visible spectrophotometry at the characteristic absorbance maximum of grape seed polyphenols using a standard calibration curve. The drug content was calculated as the percentage of extract present relative to the total weight of the phytosomal formulation, and this method is commonly employed in phytosome studies to assess formulation uniformity and drug loading. (12)

RESULTS AND DISCUSSION

MACROSCOPIC SEED OF *VITIS VINIFERA*

Fig.1 Length and Width of The Seed of *Vitis Vinifera*

Colour: purple
Taste: sweet
Odour: pleasent
Shape: Ovoid
Size: 3.0-7.0 mm in length, 2.8-4.0 mm in width

MICROSCOPIC ANALYSIS SEED OF *VITIS VINIFERA*

Microscopy of grape seeds (*Vitis vinifera*) reveals a pear-shaped, trigonal structure comprising an outer cuticle, an epidermis, and two inner integuments surrounding the embryo and endosperm. Anatomical studies show that seed color changes from green to dark brown during ripening, accompanied by phenolic oxidation and increased hardening. Advanced techniques like NMR and scanning electron microscopy (SEM) are used to analyze these structures.

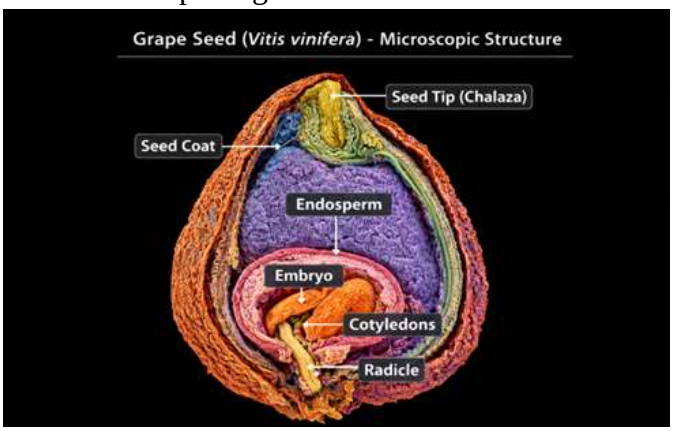

Figure- 2 T.S Of the Seed Of *Vitis Vinifera*

- **Seed Coat Structure:** The seed coat is composed of a cuticle and epidermis, with two integuments protecting the inner tissues.
- **Transverse Section (Histology):** A transverse section reveals the outer cuticle, epidermis, hard seed coat, internal endosperm, and central embryo.
- **Internal Morphology:** Ruminations (folds) of the endosperm are a key, identifiable feature.
- **Developmental Changes:** As the seed matures, it changes from green to dark brown, involving the hardening of the seed coat and oxidation of phenolics.
- **Imaging Techniques:** Scanning Electron Microscopy (SEM) is used to observe surface morphology, such as the chalaza (dorsal side) and ventral infolds. NMR microimaging has been used to study internal structures like the endosperm and embryo non-invasively.

PREPARATION OF EXTRACTION:

Seed of *vitis vinifera* was extract by using ethanolic solvent by soxhlet extraction method. After extraction the decant subject evaporation and hot plate by rotator evaporator, until it get conc. macerate kept in desiccators for complete removal of moisture from the content The products were stored at -80 ˚c for further use. The percentage yield of extract of *vitis vinifera* was found to be 16 %w/w.

PRELIMINARY PHYTOCHEMICAL STUDIES:

Phytochemical analysis was performed on the ethanolic extracts of *Vitis vinifera*. Ethanolic extract contains carbohydrates, proteins and aminoacids, flavonoids, phenolic compounds, phytosterols and tannins.

Tab 1 Phytochemicals Screening Of Vitis Vinifera

S.NO	TEST	RESULTS
1.	**TESTS FOR CARBOHYDRATES**	
	a.Molisch's test	+
	b.iodine test	_
	c.Beniticas test	+
2.	**TESTS FOR ALKALOIDS**	
	a.Mayer's test	-
3.	**TEST FOR PHENOLIC COMPOUNDS**	
	a.Ferric chloride test	+
4.	**TEST FOR PROANTHOCYANIDINS**	+
5.	**TEST FOR FLAVONOIDS**	
	a.Alkaline reagent test	+
6.	**TEST FOR TANNINS**	
	a.lead acetate test	+
7.	**TEST FOR VOLATILE OILS**	-
8.	**TEST FOR SAPONINS**	
	a.Forth test	+
9.	**TEST FOR PHYTOSTEROLS**	
	b.Salkowski reactions	+

(+) indicates positive reaction (-) indicates negative reaction

FORMULATION AND EVALUATION OF NANO PHYTOSOME OF *VITIS VINIFERA*

In this method cholesterol and soya lecithin shall be dissolved in ether. It is act as an oil phase. Then the extracted plant medicaments dissolved in phosphate buffer pH 6.8. The aqueous medium is placed under magnetic stirrer for 5- 10 minutes. After the complete dissolution of medicaments, the oil phase added into an aqueous medium by drop wise. Then it is allowed to magnetic stirrer for 1 hour with 1000rpm**.**

Tab 2 Formulations Of Phytosome Ingredents

INGREDIENTS	F1	F2	F3	F4
DRUG	500mg	500mg	500mg	500mg
SOYA LECITHIN	500mg	1000mg	1500mg	2000mg
CHOLESTEROL	200mg	200mg	200mg	200mg
ETHER	10ml	10ml	10ml	10ml
PHOSPHATE BUFFER pH $^{6.8}$	10ml	10ml	10ml	10ml

EVALUATION OF NANO PHYTOSOME

1. Determination of Drug Content:

Drug content of phytosome complex was determined by dissolving accurately weighed 10mg of complex in 10 ml methanol. After suitable dilution absorbance was determined by UV –Spectrophotometer at 286nm and drug content was determined.

2. Entrapment Efficiency (EE):

Vitis vinifera phytosomes were centrifuged at 12000 rpm for 45 min using a Remi centrifuge to separate phytosomes from unentrapped drug. Concentration of the free drug as the supernatant was determined by measuring absorbance at 286nm using UV-Visible spectrophotometer.

Tab 3 Values for Drug Content & Entrapment Efficiency

DRUG CONTENT (%w/w)	ENTRAPMENT EFFICIENCY(%w/w)
77.5 %	**99.92 %**

3. Fourier Transform – Infra Red Spectroscopy (FT-IR)

FTIR spectroscopy was performed to study the functional groups present in the phytosome formulation and to confirm the interaction between the phytoconstituent and phospholipid.The FTIR spectrum of the phytosome formulation showed characteristic absorption peaks at different wave numbers. A broad peak observed in the region of **3400–3300 cm^{-1}** corresponds to **O–H stretching vibrations**, indicating the presence of hydroxyl groups, which are commonly found in plant polyphenols and flavonoids.The absorption band around **2920–2850 cm^{-1}** is attributed to **C–H stretching vibrations** of aliphatic chains, confirming the presence of phospholipid components.A prominent peak observed near **1730–1700 cm^{-1}** represents **C=O stretching vibrations**, which may be due to ester or carbonyl functional groups of phospholipids.The peak in the region of **1650–1600 cm^{-1}** corresponds to **C=C stretching or amide bond vibrations**, indicating possible interaction between the phytoconstituent and phospholipid.Absorption bands in the region of **1250–1050 cm^{-1}** are assigned to **P=O and P–O–C stretching vibrations**, which are characteristic of phosphatidylcholine, confirming the formation of phytosome complex.The presence of all major functional group peaks with slight

shifts in wave numbers suggests **successful complexation of the phytoconstituent with phospholipids** without any chemical degradation. Hence, the FTIR study confirms the **formation and stability of the phytosome formulation**.

Broad region: 3811–3336 cm^{-1}

- These peaks correspond to **O–H stretching vibrations**
- Indicate presence of:
  - Phenolic compounds
  - Alcoholic –OH groups
  - Hydrogen bonding

- Multiple peaks instead of one broad band suggest **strong hydrogen bonding**
- This confirms **interaction between phytoconstituent and phospholipid**, not just physical mixing

Aliphatic stretching region: 2928 & 2849 cm^{-1}

Observed peaks:

2927.94cm^{-1}
2848.86 cm^{-1}

Meaning:

- Asymmetric and symmetric **C–H stretching**
- Comes from **long fatty acid chains of phosphatidylcholine**

Extra point (often missed):

- Presence of both peaks confirms **intact phospholipid bilayer**
- No peak disappearance → **no chemical degradation**

✔ Confirms **structural stability of phytosome**

Strong ester carbonyl peak: 1737.86 cm^{-1}

Meaning:

- **C=O stretching vibration**
- Characteristic of **ester linkage** in phospholipids

Extra interpretation:

- Slight shift from pure phospholipid value indicates **interaction with phytoconstituent**
- Not a new peak → interaction is **non-covalent**

✔ Confirms **phytosome formation without chemical incompatibility**

Fingerprint region :

1454–1226 cm^{-1}

Observed peaks:

1454.33cm^{-1}
1367.53cm^{-1}
1226.73 cm^{-1}

Meaning:

- C–H bending
- P=O stretching
- P–O–C vibrations

Extra detail (very important):

- This region is **unique for each compound**
- Matching of this region confirms **successful complexation**
- Any major change here would indicate incompatibility (which is NOT seen)

✔ Strong confirmation of **phytosome stability.**

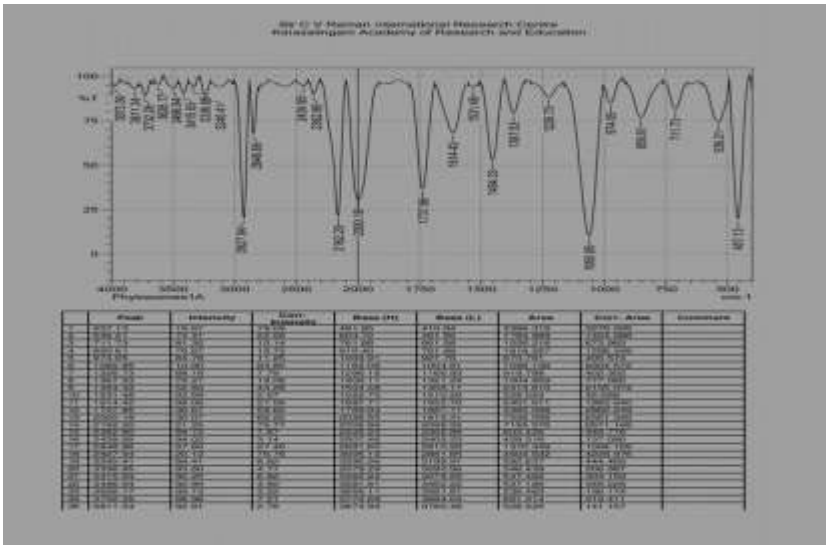

Figure 3 Ft-Ir Vitis Vinifera Nano-Phytosome

CONCLUSION

The present study successfully formulated nano phytoseme using *vitis vinifera* seed extract. Evaluation parameter such as drug content and entrapment efficiency confirmed effective incorporation of the extract into nano phytosome system. FT-IR analysis demonstrated compatability between the extract and excipients, indicating successfully phytosomes formation. Overall, the result confirm that the nano phytosome were well formulated and suitable for further pharmacological studies.

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