

“Pharmacognostical Evaluation and Development of a Phytochemical-Based Drug Delivery System for Anticancer Therapy”

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ABSTRACT

Plant-based bioactive compounds have attracted considerable attention in recent years because of their therapeutic potential and lower side-effect profile compared to synthetic drugs. Among these compounds, lupeol is one of the most studied flavonoids due to its antioxidant and anticancer properties. The present work was carried out to isolate lupeol from the roots of *Phoenix sylvestris* and to formulate it into microspheres for improved delivery.

Roots of *Phoenix sylvestris* were collected, dried, and subjected to pharmacognostical and phytochemical investigation. The powdered roots were extracted successively using petroleum ether, chloroform, and methanol. Preliminary phytochemical screening showed the presence of flavonoids, phenolics, terpenoids, lupeol and other secondary metabolites. Among the extracts, the methanolic extract showed a higher concentration of bioactive compounds.

Isolation of phytoconstituents was performed by preparative thin-layer chromatography, and the isolated compounds were characterized by UV, IR, and NMR spectroscopy. Lupeol was identified as one of the major triterpenoid constituents. Further, lupeol-loaded microspheres were prepared by the solvent evaporation method using Eudragit as the polymer.

The prepared microspheres were evaluated for their physical characteristics, entrapment efficiency, and stability. The findings of the study indicate that the roots of *Phoenix sylvestris* can serve as a useful source of lupeol, and its microsphere formulation may be beneficial for improving therapeutic effectiveness in anticancer treatment.

KEYWORDS

Phoenix sylvestris, Lupeol, Phytochemical investigation, Microspheres, Anticancer activity.

INTRODUCTION

Cancer is one of the major health problems worldwide and remains a leading cause of death. Although several treatment options such as chemotherapy, radiotherapy, and surgery are available, their limitations like toxicity, drug resistance, and side effects often reduce treatment effectiveness. Because of this, there is growing interest in plant-based medicines as supportive or alternative therapeutic options.

Medicinal plants have always been an important source of bioactive compounds. Many modern drugs have originated from natural sources. Among these, flavonoids are widely recognized for their biological activities such as antioxidant, anti-inflammatory, antimicrobial, and anticancer effects. Lupeol is one such flavonoid that has shown promising activity against different cancer cell lines by affecting cell growth and inducing apoptosis.

The genus *Phoenix* belongs to the family Arecaceae and includes several species of medicinal and nutritional importance. *Phoenix dactylifera* is widely known for its edible fruits, while *Phoenix sylvestris*, commonly called wild date palm, is distributed in different parts of India. Different parts of the plant have been traditionally used for medicinal purposes, but root-based studies remain comparatively less explored.

Previous reports have shown that *Phoenix* species contain flavonoids, phenolic compounds, tannins, and triterpenoids, which may contribute to their biological activities. However, there is limited information regarding isolation of lupeol specifically from root material and its formulation into a suitable delivery system.

One of the major challenges with lupeol is its poor water solubility and low bioavailability. To overcome this problem, formulation approaches such as microspheres can be used. Microspheres help in controlling drug release, improving stability, and enhancing therapeutic action.

The present study was therefore planned to isolate lupeol from the roots of *Phoenix sylvestris*, characterize it using spectroscopic techniques, and formulate it into microspheres for possible anticancer application.

MATERIALS AND METHODS

Collection of Plant Material

Roots of *Phoenix sylvestris* and *Phoenix dactylifera* were collected and washed properly to remove soil and foreign matter. The roots were shade-dried and powdered using a grinder. The powdered material was stored in airtight containers until further use.

Extraction Procedure

About 500 g of dried root powder was subjected to Soxhlet extraction using petroleum ether, chloroform, and methanol in increasing order of polarity. Each extract was concentrated separately and dried for further analysis.

Pharmacognostical Studies

The roots were examined for morphological characteristics such as color, texture, and odor. Microscopic studies were carried out by preparing transverse sections and staining with suitable reagents. Powder microscopy was also performed to identify important cellular structures.

Physicochemical Evaluation

Moisture content was determined by loss on drying method at 105°C until constant weight was obtained. Total ash value was determined by incinerating the powdered drug in a silica crucible at 450°C.

Phytochemical Screening

All extracts were subjected to qualitative chemical tests for identification of alkaloids, flavonoids, tannins, phenolic compounds,

carbohydrates, proteins, terpenoids, and saponins using standard procedures.

Isolation of Lupeol

TLC and preparative TLC were carried out for separation of phytoconstituents. Suitable solvent systems were optimized to obtain clear separation. The isolated spots were collected and purified.

Spectral Analysis

The isolated compound was characterized by UV-visible spectroscopy, IR spectroscopy, and NMR spectroscopy to confirm its structure.

Preparation of Microspheres

Lupeol-loaded microspheres were prepared by

solvent evaporation method. The isolated lupeol and Eudragit polymer were dissolved in methanol to form the organic phase. This was added slowly to an aqueous phase containing 0.2% polyvinyl alcohol with continuous stirring at 500 rpm for 3 hours. The formed microspheres were collected, washed, and dried.

Evaluation of Microspheres

Prepared microspheres were evaluated for particle size, percentage yield, entrapment efficiency, and stability studies.

RESULT AND DISCUSSION

Phytochemical Investigation of Extracts- A, B, C
Qualitative Analysis

Sr. No	Constituents	Tests	Extract A Pet. Ether	Extract A Chloroform Me	Extract A thanol
1.	ALKALOIDS	Mayer's test	-	-	-
		Dragendoff's test	-	-	-
		Hager's test	-	-	-
		Wagner's test	-	-	-
2.	STEROLS	Liebermann's	-	-	-
		Burchard test	-	-	-
		Salkowski's -	-	-	-
3.	CARBOHYDRATES AND GLYCOSIDES	Molisch's test	-	+	+
		Fehling's test	-	+	+
		Benedict's test	-	+	+
		Borntrager's test	-	+	+
4.	FIXED OILS AND FATS	Spot test	-	-	-
		Saponification test	-	-	-
5.	PHENOLIC COMPOUND	FeCl ₃ test	-	-	+
6.	PROTEIN AND AMINOACIDS	Biuret test	-	-	-
		Ninhydrin test	-	-	-
		Xanthoprotein test	-	-	-
		Millon's test	-	-	-
7.	TRITERPENOID AND SAPONINS	Foam test	-	-	+
		Haemolysis test	-	-	+
		FeCl ₃ test	-	+	+
9.	GUMS AND MUCILAGE	Precipitation with 90% alcohol	-	-	-
		Alkaline reagent test	-	-	+

Sr. No	Constituents	Tests	Extract B Pet. Ether	Extract B Chloroform Me	Extract B thanol
1.	ALKALOIDS	Mayer's test	-	+	+
		Dragendroffs test	-	-	-
		Hager's test	-	-	+
		Wagner's test	-	-	-
2.	STEROLS	Liebermann's Burchard test	-	-	+
		Salkowski's -	-	-	+
3.	CARBOHYDRATES AND GLYCOIDES	Molisch's test	+	+	+
		Fehling's test	-	+	+
		Benedict's test	+	+	+
		Borntrager's test	-	+	+
4.	FIXED OILS AND FATS	Spot test	-	-	-
		Saponification test	-	-	-
5.	PHENOLIC COMPOUND	FeCl ₃ test	+	-	+
6.	PROTEIN AND AMINOACIDS	Biuret test	-	-	-
		Ninhydrin test	-	-	+
		Xanthoprotein test	-	-	-
		Millon's test	-	-	-
7.	TRITERPINOID AND SAPONINS	Foam test	-	+	+
		Haemolysis test	-	-	+
8.	TANNINS	Gelatin test	-	-	-
		FeCl ₃ test	-	-	-
9.	GUMS AND MUCILAGE	Precipitation with 90% alcohol	-	-	-
10.	FLAVONOIDS	Aqueous NaOH	-	-	-
		Conc. H ₂ SO ₄	-	-	-

S.No	Constituents	Tests	Compound C Pet. Ether	Compound C Chloroform	Compound C Methanol
1.	ALKALOIDS	Mayer's test	-	-	+
		Dragordraff's test	-	-	+
		Hager's test	-	-	+
		Wagner's test	-	-	+
2.	STEROLS	Libermann's	-	-	+
		Burchard test	-	-	-
		Salkowski's -	-	+	+
3.	CARBOHYDRATES AND GLYCOIDES	Molisch's test	+	-	+
		Fehling's test	+	+	+
		Benedict's test	+	-	+
		Borntrager's test	+	+	+
4.	FIXED OILS AND FATS	Spot test	-	-	-
		Saponification test	-	-	-
5.	PHENOLIC COMPOUND	FeCl ₃ test	--	-	+
6.	PROTEIN AND AMINOACIDS	Biuret test	-	-	-
		Ninhydrin test	-	-	-
		Xanthoprotein test	-	-	-
		Millon's test	-	-	-
7.	TRITERPINOID AND SAPONINS	Foam test	-	-	+
		Haemolysis test	-	-	+
8.	TANNINS	Gelatin test	-	-	-
		FeCl ₃ test	-	-	-
9.	GUMS AND MUCILAGE	Precipitation with 90% alcohol	-	-	-
10.	FLAVONOIDS	Aqueous NaOH	+	+	+
		Conc. H ₂ SO ₄	+	+	+

QUANTITATIVE ANALYSIS

Thin Layer Chromatography Extract -A

Solvent system: Toluene in ratio Ethyl Acetate (in Ratio 80 and 20) R_f - 0.57.

Extract B

Chromatographic Conditions for prep TLC:

Solvent system: Acetonitrile: Methanol: Acetic acid (80:20:0.01) R_f -0.7

Extract C

Stationary Phase: Silica gel 60 F, 254 (E Merk)
Aluminium percolated plates,

Mobile phase: Chloroform: methanol (8:2)

Visualization: scanned at 254 nm. R_f value: 0.86

3.2.2 UV Spectroscopy

Quantitative estimations of major phytoconstituents from root *Phoenix sylvestris*

For quantitative estimations, in phenolic content, in flavonoid material and in terpenoid material have been decided from methanolic extract of root of *Phoenix sylvestris*.

Each quantitative estimation was executed through the use of usual samples. outcomes of every quantitative parameter are given below.

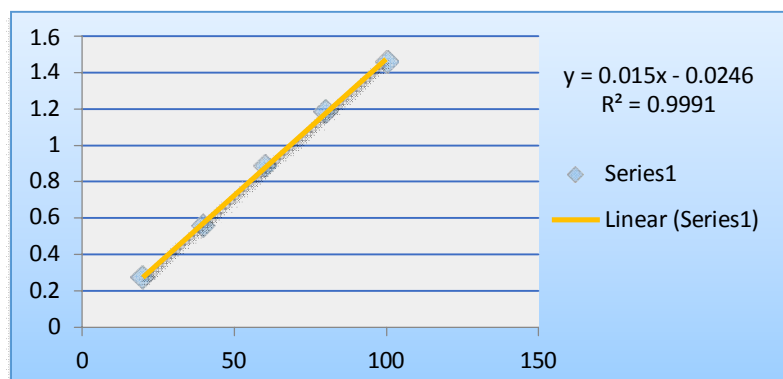
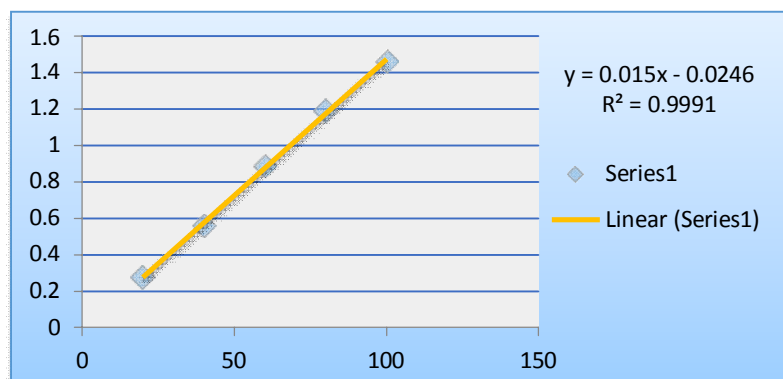


Figure no 2 Calibration curve of standard Gallic acid

Determination of total concentration of Phenolic

compound content was calculated on the basis of standard calibration curve of Gallic acid. Total Phenolic content from root of PS was calculated as 6.10 %.

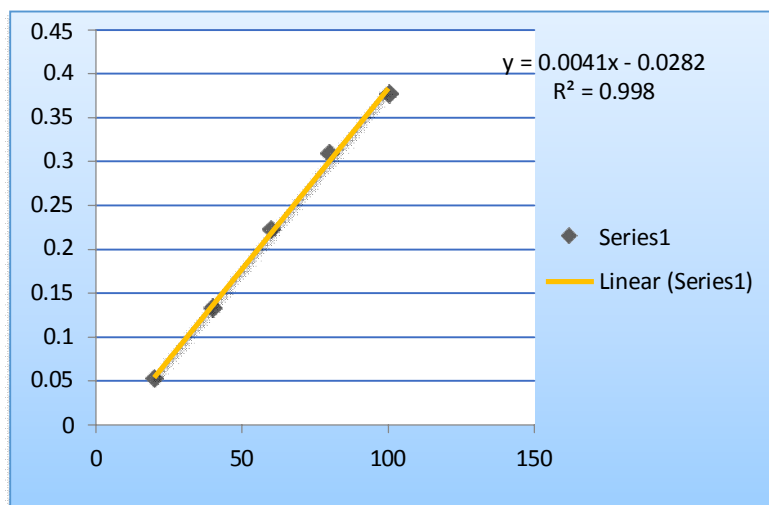


Figure no.3 Calibration Curve of standard Lupeol

Determination of total concentration of flavonoid compound content was calculated on the basis of standard calibration curve of lupeol. Total flavonoid content from root of PS was calculated as 8.02%.

Calculation of total terpenoid from root of PS

Influence utilized in calculation:

Every millilitres about .0.1Normal NaOH = 48.8 milligram triterpenoids

0.26 miligraml.0.1Normal NAOH = X milligram

triterpenoid So $X = .48.8 \times .0.36 \times 0.1 / 0.1$

$X = 17.56$ milligram triterpenoids about 5gram

Therefore, in 100 gm of drug total terpenoids was found to be 0.30% gm.

INFRARED SPECTROSCOPY-

IR spectrum of compound: IR spectrum in PerkinElmer pellets contained KBr.

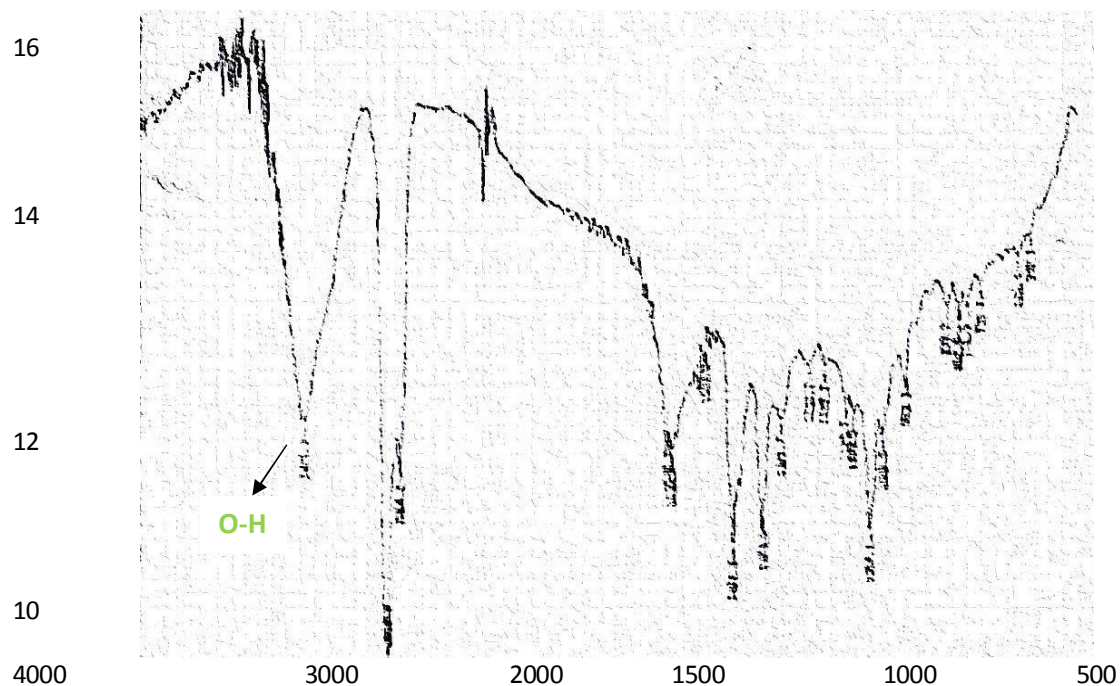
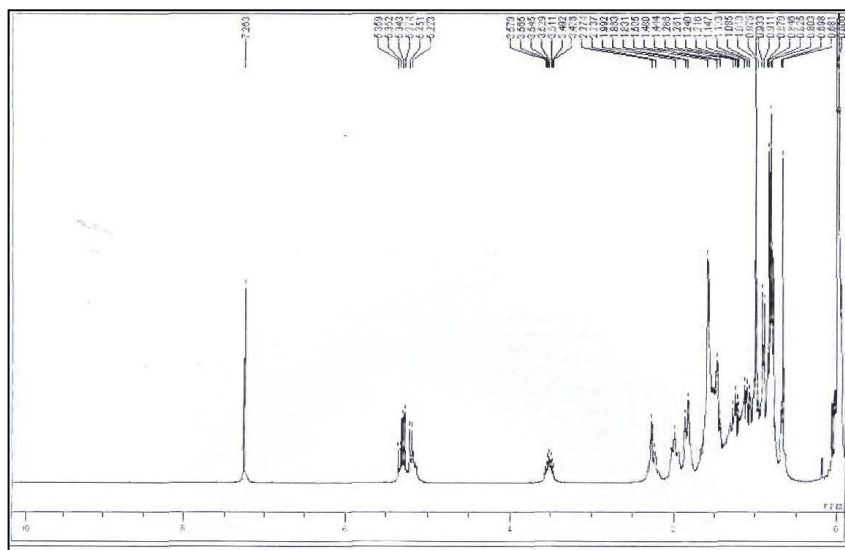


Figure no.4 IR spectrum of compound: IR spectrum in PerkinElmer pellets contained KBr

NMR SPECTROSCOPY

COPMPOUND A NMR Spectra (H1): Noted utilizing CDCl₃ about 400 Megahertz



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8

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6

5

FIGURE NO.5 COPMPOUND NMR Spectra (H1): Noted utilizing CDCl₃ about 400 Megahertz

DISCUSSION

The present work was carried out to explore the roots of *Phoenix sylvestris* for phytochemical investigation and formulation development. The study mainly focused on isolation of Lupeol and its incorporation into microspheres.

Pharmacognostical evaluation confirmed the identity of the root material by showing characteristic structures such as cork cells, cortex, vascular bundles, and central pith. These observations are useful for authentication of the crude drug.

The physicochemical parameters obtained were within acceptable limits, indicating good quality of the root material. Such parameters are important in maintaining consistency during extraction and formulation.

Among the three solvent extracts, methanol showed better extraction efficiency for polar phytoconstituents. Preliminary screening confirmed the presence of flavonoids, phenolics, and triterpenoids, which are known for their therapeutic value.

Quantitative estimation showed that the flavonoid content was comparatively higher than phenolic and triterpenoid content. This suggests that the roots of *Phoenix sylvestris* may serve as a good source of flavonoids.

TLC and preparative TLC gave satisfactory separation of compounds, and spectral studies confirmed the presence of lupeol. The UV, IR, and NMR results supported the structural identification of the isolated compound.

Microsphere preparation by solvent evaporation method was found suitable for Lupeol formulation. The prepared microspheres showed acceptable characteristics and may improve stability and release of Lupeol.

Overall, the study supports the medicinal value of *Phoenix sylvestris* roots and their possible use in anticancer therapy. Further in vitro and in vivo studies are required to confirm the biological activity.

CONCLUSION

From the present investigation, it can be concluded that the roots of *Phoenix sylvestris* contain important phytoconstituents, particularly flavonoids such as lupeol. Pharmacognostical and physicochemical studies confirmed the quality and authenticity of the plant material. Methanolic extraction was found to be more effective for isolation of bioactive compounds.

The isolated lupeol was successfully characterized by different spectroscopic methods and formulated into microspheres using Eudragit polymer. The developed microspheres showed satisfactory properties, suggesting their usefulness as a drug delivery system.

The study highlights the potential of *Phoenix sylvestris* as a natural source of anticancer agents. However, detailed pharmacological and clinical studies are needed to establish its therapeutic application.

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