ISOLATION AND STRUCTURAL CHARACTERIZATION OF MARKER COMPOUND IN *PHOENIX SYLVESTRIS* SEED EXTRACT

Aliya Bano*[1], B. K. Dubey[1], Deepak Kumar Basedia[1], Sunil Shah[2]

Technocrats Institute of Technology-Pharmacy, Bhopal (M.P.)

TIT-College of Pharmacy, Bhopal (M.P.)

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Abstract

The present study was undertaken to evaluate the phytochemical profile, chromatographic behavior, and structural characterization of a bioactive marker compound from *Phoenix sylvestris*. The ethanolic extract of *Phoenix sylvestris* exhibited a percentage yield of 8.25%, indicating effective extraction of phytoconstituents. Preliminary phytochemical screening confirmed the presence of flavonoids, diterpenes, saponins, proteins, and phenolic compounds, while alkaloids, glycosides, and carbohydrates were absent. Quantitative analysis revealed a total phenolic content of 0.24 mg/100 mg and a total flavonoid content of 0.76 mg/100 mg of dried extract, suggesting flavonoids as major contributors to the extract's bioactivity.

Thin layer chromatography (TLC) analysis using toluene: ethyl acetate: formic acid (5:4:1) showed an Rf value comparable to that of quercetin, indicating the presence of flavonoid-type compounds. Column chromatography of the extract led to the isolation of a single compound (fractions 55–63), which was further characterized using UV–Visible, FT-IR, ^{1}H-NMR, and ESI–MS techniques. Spectral data confirmed the isolated compound to be a pentacyclic triterpenoid alcohol with a molecular formula $C_{30}H_{50}O$. The identification of this bioactive compound supports the traditional medicinal use of *Phoenix sylvestris* and highlights its potential as a valuable source of pharmacologically active natural products.

Keywords: Phoenix sylvestris, Phytochemical screening, Total phenolic content, Flavonoids, TLC, Column chromatography, Pentacyclic triterpenoid, Spectral characterization.

Introduction

Phoenix sylvestris (L.) Roxb., commonly known as the Indian date palm or wild date palm, is a medicinally important species belonging to the Arecaceae family and widely distributed across the Indian subcontinent (Ayer et al., 2014). Traditionally, various parts of *P. sylvestris* including seeds, leaves, roots, and fruit have been used in folk medicine to manage ailments such as

diabetes, dysentery, fever, and gastrointestinal disorders, as well as for their nutritional benefits and antioxidant properties.

Phytochemical studies on *P. sylvestris* seed extracts have revealed a complex mixture of bioactive constituents including carbohydrates, proteins, phenolics, flavonoids, alkaloids, tannins, and fatty acid esters. These compounds are believed to contribute to the biological activities attributed to the plant in traditional medicine, such as antioxidant, antihyperglycemic, and antimicrobial effects (Paul et al., 2024).

Identification and characterization of marker compounds from plant extracts are essential steps in phytochemical research, as these serve as representative constituents for quality control, standardization of herbal preparations, and understanding of pharmacological mechanisms. Marker compounds are typically isolated in pure form and structurally elucidated using advanced spectroscopic techniques such as NMR and mass spectrometry. Such studies not only validate traditional claims but also aid in discovering novel therapeutic agents from natural sources (Upton et al., 2020).

However, despite its broad use and reported therapeutic potential, there is a paucity of studies focused on the isolation and structural characterization of specific bioactive marker compounds from *P. sylvestris* seed extract. Therefore, this study aims to isolate a major phytoconstituent from the seed extract and elucidate its chemical structure using modern spectroscopic methods, thereby contributing to the phytochemical understanding and potential pharmacological application of this traditionally valued plant.

Material and Methods

Material

Seeds of *Phoenix sylvestris* were collected, authenticated, shade-dried, and powdered for the present investigation. Ethanol and hydroalcoholic solvents were used for extraction of phytoconstituents. Silica gel (60–120 mesh size) and silica gel 60 F_{254} TLC plates (Merck) were employed for chromatographic analysis and isolation. Analytical grade chloroform, methanol, ethyl acetate, toluene, and formic acid were used as mobile phases. Gallic acid and quercetin served as reference standards for the estimation of total phenolic and flavonoid content, respectively. Reagents such as Folin–Ciocalteu reagent, sodium carbonate, aluminium chloride, ferric chloride, and other chemicals required for phytochemical tests were of analytical grade.

Spectral characterization of the isolated compound was carried out using UV–Visible spectrophotometer, FT-IR, ^{1}H-NMR, and mass spectrometry instruments.

Methods

Collection of plant materials

Seeds of *Phoenix sylvestris* were collected from local area of Bhopal in the period of April, 2025.

Drying and storage of plant materials

Seeds of *Phoenix sylvestris* were cleaned by tab water and a portion was dried at room temperature (Harborne, 1996). The dried samples were ground and passed through a sieve (20 meshes). The powdered drugs were kept in sealed containers and protected from light until used. Another portion of sample was used for maceration.

Extraction by maceration process

50 gram of powdered seeds of *Phoenix sylvestris* were coarsely powdered and subjected to extraction. Dried powdered of seeds of *Phoenix sylvestris* has been extracted with Ethanol as a solvent using maceration method for 48 hrs, filtered and dried using vacuum evaporator at 40°C (Mukherjee, 2007).

Determination of percentage yield

The percentage yield is a measure of the efficiency of a chemical reaction. It compares the actual yield of a reaction to the theoretical yield, which is the amount of product that would be produced if the reaction were 100% efficient. The percentage yield of each extract was calculated by using following formula:

$$\text{Percentage yield} = \frac{\text{Weight of Extract}}{\text{Weight of powdered drug}} x100$$

Phytochemical analysis

The analysis of these compounds is important for determining the nutritional and medicinal value of plants and their potential for various applications in food, pharmaceutical, and cosmetic industries (Trease and Evans, 1978; Kokate, 1994).

Quantitative studies of phytoconstituents

Total phenol content estimation

The total phenolic content of the extract was determined using the modified Folin–Ciocalteu method as described by Parkhe and Bharti (2019). A standard stock solution of gallic acid (10 mg) was prepared by dissolving it in 10 ml of methanol, from which working standard solutions in the range of 10–50 µg/ml were prepared by appropriate dilution. For sample preparation, 10 mg of the dried extract was dissolved in 10 ml of methanol and filtered, and 2 ml (1 mg/ml) of this solution was used for the analysis. For the assay, 2 ml of the extract or standard solution was mixed with 1 ml of Folin–Ciocalteu reagent (previously diluted 1:10 v/v with distilled water) and 1 ml of sodium carbonate solution (7.5 g/L)**.** The reaction mixture was vortexed for 15 seconds and allowed to stand for 10 minutes at room temperature for color development. The absorbance was then measured at 765 nm using a UV–visible spectrophotometer.

Total flavonoids content estimation

The total flavonoid content of the extract was determined using the aluminium chloride colorimetric method as described by Parkhe and Bharti (2019). A standard stock solution of quercetin (10 mg) was prepared by dissolving it in 10 ml of methanol**,** from which working standard solutions in the concentration range of 5–25 µg/ml were prepared by appropriate dilution. For sample preparation, 10 mg of the dried extract was dissolved in 10 ml of methanol and filtered, and 3 ml (1 mg/ml) of this solution was used for the estimation. For the assay, 1 ml of 2% aluminium chloride ($AlCl_3$) solution was added to 3 ml of the extract or each standard solution and allowed to stand for 15 minutes at room temperature for color development. The absorbance was then measured at 420 nm using a UV–visible spectrophotometer.

Separation and Identification of phytoconstituents by TLC

Thin layer chromatography is based on the adsorption phenomenon. In this type of chromatography mobile phase containing the dissolved solutes passes over the surface of stationary phase. Each solvent extract was subjected to thin layer chromatography (TLC) as per conventional one dimensional ascending method using silica gel 60F254, 7X6 cm (Merck) were cut with ordinary household scissors. Plate markings were made with soft pencil. Glass capillaries were used to spot the sample for TLC applied sample volume 1-micro litre by using

capillary at distance of 1 cm at 5 tracks. In the twin trough chamber with different solvent system toluene: ethyl acetate: formic acid (5:4:1) solvent system used. After pre-saturation with mobile phase for 20 min for development were used. The movement of the active compound was expressed by its retention factor (Rf), values were calculated for different samples. The developed thin layer chromatographic plates were visualized in normal light, short UV light (254nm), and long UV light (365nm) using TLC cabinet (Electronic India).

$$\mathbf{Rf} = \frac{\text{Distance traveled by solute}}{\text{Distance traveled by solvent}}$$

Isolation of compound by column chromatography

Coloum size: Glass column, 100 x 3 Cms

Stationary Phase: Silica gel (60 to 120 mesh size)

Elution mode: Isocratic elution

Mobile Phase: Toluene: Ethyl acetate (7:3)

Extract*: Phoenix sylvestris* extract

Visualized by*:* Short UV (254nm), long UV (365nm) and Normal light

Identification of similar fractions: Visualized by Short UV (254nm), long UV (365nm) and Normal light and Phytochemical Test

Procedure

Preparation of column

Column packing was done by wet packing method. Silica (activated at 105ºC) was taken; and suspended it in Mobile Phase then transfers it in column; allowed to settle down. At the top of silica layer cotton plug was kept to avoid disturbance in silica layer during elution.

Preparation of sample

2.5gm of extract was taken in beaker; to it silica used for column chromatography (60-120 mesh size) and sufficient amount of mobile phase was added. The slurry was made and introduced from top of the silica gel column over cotton plug.

Isocratic elution technique

In order to fractionate the components of isocratic elution technique was used Toluene: Ethyl acetate (7.5:2.5) mobile phase is used for elution. The 10 ml of each fraction were collected and the solvent was recovered by distillation. The fraction were collected, concentrated, stored and subjected to TLC.

Identification of similar fractions

The different fractions of column chromatographic elution (100) were monitored by TLC Toluene: Ethyl acetate (7.5:2.5); using UV chamber and Phytochemical test with specific reagent for identification of single isolated compound with comparison of reference compounds. The fractions which show similar fingerprinting profile on TLC were collected and mixed. Fraction showed single compound and have similar R_f value as compared to reference compound were dried, compound were purified by recrystalization procedure.

Characterization of Isolated compound

100 fraction, each 10ml were collected and isolated compound was characterized by IR, NMR and Mass Spectra studies.

I.R. Analysis- The IR spectrum of compounds was recorded on (Bruker Alpha) (LNCT College of Pharmacy Bhopal, M.P.) using solid plate technique with KBr.

NMR Analysis- H^1NMR was recorded on JEOL (500MHz FT-NMR) in Methanol-D3 using TMS as internal standard (IISER, Bhopal).

Mass Analysis- MASS spectrum of compounds was recorded Waters Equity *triple* quadrupole *MS* system (Vindhya Herbals, Bhopal).

Results and Discussion

The present investigation focused on the phytochemical evaluation, chromatographic profiling, and structural characterization of bioactive constituents from *Phoenix sylvestris*. The percentage yield of the ethanolic extract was found to be 8.25%, with a dark black appearance, indicating efficient extraction of polar and semi-polar phytoconstituents using ethanol. The yield obtained is comparable with previously reported values for seed and fruit extracts of medicinal palms, confirming ethanol as a suitable solvent for extracting bioactive compounds.

Preliminary phytochemical screening of the ethanolic extract revealed the presence of flavonoids, diterpenes, saponins, proteins, and phenolic compounds, while alkaloids, glycosides, and carbohydrates were absent. The presence of phenols and flavonoids supports the traditional

medicinal use of *Phoenix sylvestris*, as these compounds are well known for their antioxidant, anti-inflammatory, and antimicrobial properties.

Quantitative estimation showed that the total phenolic content and total flavonoid content of the ethanolic extract were 0.24 mg/100 mg and 0.76 mg/100 mg of dried extract, respectively. The relatively higher flavonoid content suggests that flavonoids may be the major contributors to the biological activity of the extract. Phenolic and flavonoid compounds are known to scavenge free radicals and inhibit oxidative stress, thereby playing a crucial role in disease prevention.

TLC analysis using toluene: ethyl acetate: formic acid (5:4:1) as the mobile phase confirmed the presence of flavonoid compounds in the extract. The Rf value (0.52) of one spot in the hydroalcoholic extract matched that of the standard quercetin, indicating the possible presence of quercetin or a closely related flavonoid. Multiple spots observed under UV light further suggest the presence of several phytoconstituents, highlighting the chemical complexity of the extract.

Column chromatography followed by spectroscopic analysis led to the isolation of a single compound (fractions 55–63). UV–Visible spectroscopy showed a λmax at 535 nm, indicative of an extended conjugated chromophoric system. FT-IR analysis confirmed the presence of hydroxyl groups, aliphatic C–H bonds, unsaturation (C=C), and C–O stretching vibrations, suggesting an alcohol-containing unsaturated compound. The ^{1}H-NMR spectrum revealed characteristic signals corresponding to hydroxyl, olefinic, and aliphatic protons, consistent with a triterpenoid skeleton. ESI–MS analysis displayed a molecular ion peak at m/z 426, corresponding to the molecular formula $C_{30}H_{50}O$, along with fragment ions typical of a pentacyclic triterpenoid.

Based on combined spectroscopic evidence, the isolated compound was identified as a pentacyclic triterpenoid alcohol. Such compounds are widely reported to possess anti-inflammatory, antioxidant, wound-healing, and antimicrobial activities, which aligns with the traditional therapeutic claims of *Phoenix sylvestris*. The findings validate the phytochemical richness of *Phoenix sylvestris* and support its potential as a valuable source of bioactive natural compounds for pharmaceutical and nutraceutical applications.

Table 1: % Yield obtained ethanolic extract from *Phoenix sylvestris*

S. No.	Extract	Color	% Yield
1.	*Phoenix sylvestris*	Dark black	8.25

Table 2: Preliminary phytochemical screening of *Phoenix sylvestris*

S. No.	Phytoconstituents	Test Name	Ethanolic extract
1	Alkaloids	Hager's Test	Absent
2	Glycosides	Legal's Test	Absent
3	Carbohydrates	Fehling's Test	Absent
4	Flavonoids	Lead acetate	**Present**
5	Diterpenes	Copper acetate Test	**Present**
6	Saponins	Froth Test	**Present**
7	Proteins	Xanthoproteic Test	**Present**
8	Phenols	Ferric Chloride Test	**Present**

Table 3: Estimation of total phenolic and flavonoids content of *Phoenix sylvestris*

S. No	Extract	Total phenol content	Total flavonoids content
		(mg/100mg of dried extract)	
1	Ethanolic	0.24	0.76

Table 4: Identification of phytoconstituents by TLC of *Themeda quadrivalvis*

Hydroalcoholic extract of *Themeda quadrivalvis*		
S. No.	**Mobile phase** Toluene: Ethyl acetate: Formic acid (5:4:1)	***Rf* value**
1.	**(Quercetin)** Dis. travel by mobile phase= 6cm No. of spot at long UV= 1 No. of spot at short UV = 1 No. of spot at normal light= 1	 Long- 0.52 Short- 0.52 Normal- 0.52
2.	**(Hydroalcoholic extract)** Dis. travel by mobile phase= 6cm No. of spot at long UV = 2 No. of spot at short UV = 4 No. of spot at normal light= 1	 Long- 0.36, 0.52 Short- 0.36, 0.50, 0.58,0.74 Normal- 0.52

	Spot Sequence	
	Quercetin	1st
	Hydroalcoholic extract	2nd

Table 5: Showing interpreted data of different spectra of isolated compound (55-63)

Method	Observed Data	Spectral Interpretation
UV–Visible Spectroscopy	λmax = 535 nm	Indicates the presence of extended conjugation or chromophoric groups within the molecule, suggesting unsaturation or a conjugated system.
FT-IR Spectroscopy	3200–3600 cm^{-1}	Broad O–H stretching band confirming the presence of a hydroxyl (–OH) functional group.
	2850–2960 cm^{-1}	Aliphatic C–H stretching vibrations, characteristic of saturated hydrocarbon chains.
	1600–1680 cm^{-1}	C=C stretching vibrations indicating unsaturation within the molecular structure.
	1350–1470 cm^{-1}	C–H bending vibrations of aliphatic groups.
	1050–1150 cm^{-1}	C–O stretching vibrations supporting the presence of an alcohol group.
	600–1400 cm^{-1}	Fingerprint region showing complex absorption patterns typical of a polycyclic structure.
^{1}H-NMR (δ, ppm)	1.6721 (s, 1H)	Assigned to aliphatic/methyl proton environment within the triterpenoid skeleton.
	3.1367 (d, 1H, H-3)	Proton attached to a carbon bearing an electronegative substituent, suggesting oxygenated carbon.
	4.7411 (d, 1H)	Olefinic or oxygenated methine proton, indicating unsaturation or hydroxyl substitution.
	7.2423 (s, 1H, –OH)	Hydroxyl proton, confirming alcoholic nature of the compound.
ESI–MS (m/z)	426 (100%)	Molecular ion peak corresponding to the molecular weight of the compound.
	369, 281, 271, 218, 191, 121, 95, 81	Fragment ions characteristic of a pentacyclic triterpenoid skeleton.
Molecular Formula	$C_{30}H_{50}O$	Confirms a triterpenoid alcohol framework.
IUPAC Name	*1,2,5,14,18,18-Hexamethyl-8-*	Supports the presence of a pentacyclic

	(prop-1-en-2-yl)pentacyclo[11.8.0.0²,¹⁰.0⁵,⁹.0¹⁴,¹⁹]henicosan-17-ol	triterpenoid structure.
Structural Nature		Identified as a pentacyclic triterpenoid alcohol, commonly associated with anti-inflammatory, antioxidant, and wound-healing activities.

Conclusion

The present study successfully demonstrated the phytochemical evaluation, chromatographic separation, and structural characterization of a bioactive compound from *Phoenix sylvestris*. The ethanolic extract showed an appreciable yield and was found to be rich in phenolic and flavonoid constituents, as confirmed by preliminary phytochemical screening and quantitative estimation. TLC analysis indicated the presence of flavonoid-type compounds with Rf values comparable to standard quercetin, supporting the flavonoid-rich nature of the extract. Column chromatographic separation led to the isolation of a single major compound, which was purified and characterized using UV–Visible, FT-IR, ^{1}H-NMR, and mass spectroscopic techniques. Spectral interpretation confirmed the isolated compound to be a pentacyclic triterpenoid alcohol ($C_{30}H_{50}O$), a class of compounds known for diverse pharmacological activities. The identification of this compound provides scientific validation for the traditional medicinal use of *Phoenix sylvestris* and highlights its potential as a promising natural source of therapeutically important bioactive molecules.

References

- Ayer S, Bhusal A, Chhetri P, Subedi A. Phoenix species in Asia: a systematic review on research trends, status, distribution, ethnobotany and pharmacological activities. Journal of Resources and Ecology. 2024 Oct;15(5):1382-92.
- Paul ZA, Malla AT, Dar MA, Masoodi MH. Phytochemistry and pharmacological activity of Malva sylvestris L: a detailed insight. Combinatorial chemistry & high throughput screening. 2024 Oct 1;27(16):2309-22.
- Upton R, David B, Gafner S, Glasl S. Botanical ingredient identification and quality assessment: strengths and limitations of analytical techniques. Phytochemistry Reviews. 2020 Oct;19(5):1157-77.

- J.B. Harborne, N.J. Walton, D.E. Brown Classes and functions of secondary products in chemicals from Plants, Perspectives on Secondary plant products. Imperial college press, pp.1-25, 1996.
- Mukherjee PK. Quality Control of Herbal Drugs, 2nd Edition, Business Horizons, 2007; 2-14.
- Trease, G.E and Evans, W.C. (1978). Pharmacology, 11th Edition. Bailliere Tindall Limited, London, 60-75.
- Kokate CK. Ed. Practical Pharmacognosy, 4th Edn., Vallabh Prakashan: 1994;112:120.
- Geeta Parkhe, Deepak Bharti. In vitro antioxidant activity, total phenolic and flavonoid contents of hydroalcoholic extract of leaves of *Lagerstroemia parviflora* Roxb. Journal of Drug Delivery & Therapeutics. 2019; 9(4-A):708-711.
- Geeta Parkhe, Deepak Bharti. Phytochemical investigation and determination of total phenols and flavonoid concentration in leaves extract of *Vitex trifolia* Linn. Journal of Drug Delivery & Therapeutics. 2019; 9(4-A):705-707.