

SCREENING AND ISOLATION OF NOVEL PHYTOCONSTITUENTS FROM THE TRUNK OF *PHOENIX SYLVESTRIS*

Dr J Geetha^{1*}, Dr S Jayaprakash², Dr S K Senthil Kumar³, B Gopika⁴, V Gopika Devi⁵, S Gopinathan⁶, E N Gowsheela⁷, D Hariharan⁸

¹ Department of Pharmacognosy, Arunai College of Pharmacy, Tiruvannamalai, Tamil Nadu, India.

² Department of Pharmaceutics, Arunai College of Pharmacy, Tiruvannamalai, Tamil Nadu, India.

³ Department of Pharmaceutics, Arunai College of Pharmacy, Tiruvannamalai, Tamil Nadu, India.

⁴ Department of Pharmacognosy, Arunai College of Pharmacy, Tiruvannamalai, Tamil Nadu, India.

⁵ Department of Pharmacognosy, Arunai College of Pharmacy, Tiruvannamalai, Tamil Nadu, India.

⁶ Department of Pharmacognosy, Arunai College of Pharmacy, Tiruvannamalai, Tamil Nadu, India.

⁷ Department of Pharmacognosy, Arunai College of Pharmacy, Tiruvannamalai, Tamil Nadu, India.

⁸ Department of Pharmacognosy, Arunai College of Pharmacy, Tiruvannamalai, Tamil Nadu, India.

*Corresponding author Dr J Geetha,

*Professor, Department of Pharmacognosy and Phytochemistry, Arunai College of Pharmacy, Tamil Nadu, India.

Tel: +918778145615 E-mail: geethammc@gmail.com

ABSTRACT

Background: Phytochemical screening is important because it helps to identify bioactive compounds present in plants, which are responsible for the medicinal and therapeutic properties. Screening of phytochemicals helps in drug discovery, supports traditional medicine validation, and guides further pharmacological studies. This study aimed to screen and isolate novel phytoconstituents from the plant trunk part.

Methodology: The extract was collected from the trunk part of *Phoenix sylvestris* using a Soxhlet apparatus. The extract was allowed to perform several preliminary tests to confirm the presence of phytoconstituents. And the extract was then subjected to column chromatography till 8 fractions were collected. Thin-layer chromatography was performed for each of the fractions, and a certain optimised fraction was then selected for further spectral analysis, including IR spectroscopy and NMR spectroscopy.

Result: The fractions 4 and 5 are optimised for IR spectroscopy, and NMR spectroscopy has been performed for the identification of structure and IUPAC name.

Conclusion: By performing NMR spectroscopy, a novel compound, Creosol, was identified from F4 and Isoquercitrin was identified from F5.

Keywords: Indian date palm, Soxhlet extraction, Phytochemical screening, Column chromatography, Spectral analysis.

INTRODUCTION

Phytochemical screening is the basis for the discovery of such phytoconstituents, which can be utilised to make medicine to cure various diseases. The study of phytoconstituents is significant because both research institutes and pharmaceutical corporations have a commercial stake in the development of novel medications for the treatment of various diseases. Medicinal plants and herbal preparations have garnering wide spread interest in scientific circles due to their consistent pharmacological activities and low cost to the general public, making them effective in the treatment of various disorders. The main purpose of the present study is phytochemical screening in the ethanolic extract of the medicinal plant *Phoenix Sylvestris* ⁽¹⁾.

Phoenix sylvestris (*sylvestris*- Latin, of the forest) is also known as silver date palm, Indian date palm, sugar date palm or wild date palm and is native to most of India, Sri Lanka, Nepal, Bhutan, Myanmar, and Bangladesh and the southern portions of Pakistan ⁽²⁾. Traditionally important and known for its nutritional values throughout the World. It is a rich source of carbohydrates, Phenols, Amino acids, Flavonoids, Tannins, Terpenoids, Dietary fibres, Essential vitamins and minerals ⁽³⁾.

Phoenix sylvestris is locally known as “Khejus” in Bangladesh and possesses various medicinal uses. The sap of the plant is nutritious, cooling and laxative, whereas the central tender part is useful to treat Gonorrhoea. Root is used in toothache, and in nervous debility and Helminthiasis. Gum is useful in diarrhoea and genitourinary disease. Fruit is tonic and restorative. Being a sedative and nervine tonic, the fruit is usually employed for relieving backache and pain in the buttocks ⁽²⁾. It is also prescribed for cough, fever, nervous debility and gonorrhoea. *Phoenix sylvestris* is reported to have analgesic and diuretic activity ⁽⁴⁾.

P. sylvestris is a member of the Arecaceae family. The plant is recognised for its anti-oxidant, cardiogenic, diuretic and antipyretic properties. As the interest in natural remedies and plant-based medicines continues to grow, the phytochemical analysis and biological screening of medicinal plants like *P. sylvestris* have become an area of significant scientific

inquiry. This study aims to explore and document the phytochemical properties of *P. sylvestris*, focusing on its potential as a source of bioactive compounds with therapeutic applications ⁽⁵⁾.



Figure 1: Indian date palm (*Phoenix Sylvestris*)

CHEMICAL CONSTITUENTS

Phoenix sylvestris is known for being a rich source of dietary fibre, essential vitamins, and minerals, further enhancing its appeal as a functional food and medicinal plant. This palm has long been recognised for its substantial nutritional and medicinal values. The fruit of this plant has high carbohydrate content, and other parts of the plant are rich in a wide range of active compounds like Phenols, amino acids, flavonoids, tannins, alkaloids, and terpenoids ⁽⁵⁾.

PHARMACOLOGICAL PROFILE

Various medicinal properties identified in *Phoenix Sylvestris* are anti-ulcer, anti-inflammatory, anti-diabetic, anti-diarrheal, anti-microbial, anti-cancer, anti-oxidant, antinociceptive, anti-mutagenic, anti-obesity, diuretic, hematopoietic, hepato-protective, haemolytic activity and improve energy and vitality. Also, to cure various ailments like abdominal complaints, fever, and loss of consciousness ⁽²⁾.

Used in folk medicine for gastrointestinal issues, respiratory infections, diabetes, and as a general tonic. The fruit is also used to treat back pain, stomach ache, toothache, headache, arthritis, pain in the buttocks, piles, nervous debility, and as a nervine tonic, restorative, and sedative in ethnomedicine. CNS depressant and anxiolytic activity ⁽⁶⁾.

MATERIALS AND METHODOLOGY

SOLVENTS AND CHEMICALS

Ethanol, Ethyl acetate, Chloroform, Benzene, Glacial acetic acid, n-butanol, Silica gel 60, Silica 120 Mesh were purchased from **Great Scientific Industries**, Tiruvannamalai 606 601.

INSTRUMENTS

IR- SHIMADZU IR- SPIRIT L, at Arunai College of Pharmacy, Tiruvannamalai.

NMR- BRUCKER AVANCE NEO 500MHZ at the Interdisciplinary Institute of Indian System of Medicine (IIISM), SRM Institute of Science and Technology (SRMIST)

AUTHENTICATION

The trunk of *Phoenix sylvestris* was collected from Edapalayam, Tiruvannamalai, on 17th June, 2025. The herbarium was prepared and authenticated by Dr J. Suresh Kumar, M.Sc., M.Phil., PhD, PGDCA., Botanist, Assistant Professor, Dept of Botany, Kalaignar Karunanidhi Government Arts College, Tiruvannamalai.

METHODOLOGY

EXTRACTION

The extraction of phytochemicals from *Phoenix sylvestris* was carried out using a Soxhlet extraction method. Plant material of *P. sylvestris* was shade-dried at room temperature. The dried material was coarsely powdered and subjected to extraction. 200 g of coarsely powdered drug of trunk was packed into a thimble and extracted with solvent Ethanol for four consecutive days. The extraction was concentrated by evaporating on a hot plate above its boiling point. The residue obtained was weighed and stored at 4°C ⁽⁷⁾.

PHYTOCHEMICAL SCREENING

Phytochemicals are chemical compounds naturally present in plants attributing to positive or negative healthy effects. The medicinal properties of the plants are determined by the phytochemical constituents. Some of the important phytochemicals present are alkaloids, flavonoids, phenolics, tannins, saponins, steroids, glycosides, and terpenes, which are distributed in the plants ⁽⁵⁾.

COLUMN CHROMATOGRAPHY AND FRACTIONATION

The fractionation of the plant residue was carried out using column chromatography. In the present study, a glass burette of 30cm long and 1.5cm diameter, having 50ml capacity, was used. Silica gel (Mesh-120) was used as a stationary phase, which was poured into the column. 1 g of the sample was loaded on top of the column. The selective solvent systems (mobile phase) used for phytoconstituents detection were Ethyl acetate, Chloroform, Ethanol: water (7:3). Elution was carried out using a gradient of solvents ⁽⁸⁾.

THIN-LAYER CHROMATOGRAPHY (TLC)

TLC was employed as a preliminary analytical method to separate and identify the different phytoconstituents present in the plant residue. The presence of different phytoconstituents in the extract of the plant *P. sylvestris* was established by TLC plates. Silica gel G was used as a stationary phase. A slurry was prepared by mixing adsorbent (silica gel G) with distilled water. Spread this slurry evenly onto a clean glass plate using a TLC spreader, ensuring no bubbles are present ⁽⁹⁾.

Allow the plate to dry in the oven at approximately 100-120°C for about 30 mins to activate the adsorbent. The selected solvent systems (mobile phase) used for the detection of phytoconstituents were n-butanol: glacial acetic acid: water (4:1:5), Benzene: ethyl acetate: ethanol (4:4:2) and n-butanol: water (1:1). By using a spotting capillary, the extract was spotted onto the silica gel plates, and the plates were developed in the development chamber. After the complete development of spots on TLC plates, the colour was noticed and a single fine spot. Rf values of the fractions were calculated, providing a basis for further chromatographic purification ⁽¹⁰⁾.

INFRARED SPECTROSCOPY

The optimised fractions were dried, and the moisture was removed by using a desiccator. IR spectroscopy was performed separately for each fraction. The powdered sample was then formed into a pellet using the pressed pellet technique. The thin pellet was placed on the sample cell, and the detector shows peak formation, hence the functional groups can be determined ⁽¹¹⁾.

RESULT AND DISCUSSION

The *Phoenix sylvestris* plant's trunk was collected and dried in shade for 2days and powdered. Then extracted with ethanol by using the Soxhlet apparatus, and the residue was collected and concentrated in hot plate shown in Figure 2. The Percentage yield of the residue was found to be **2.6% w/w**.



Figure 2: Ethanolic extraction and extract residue.

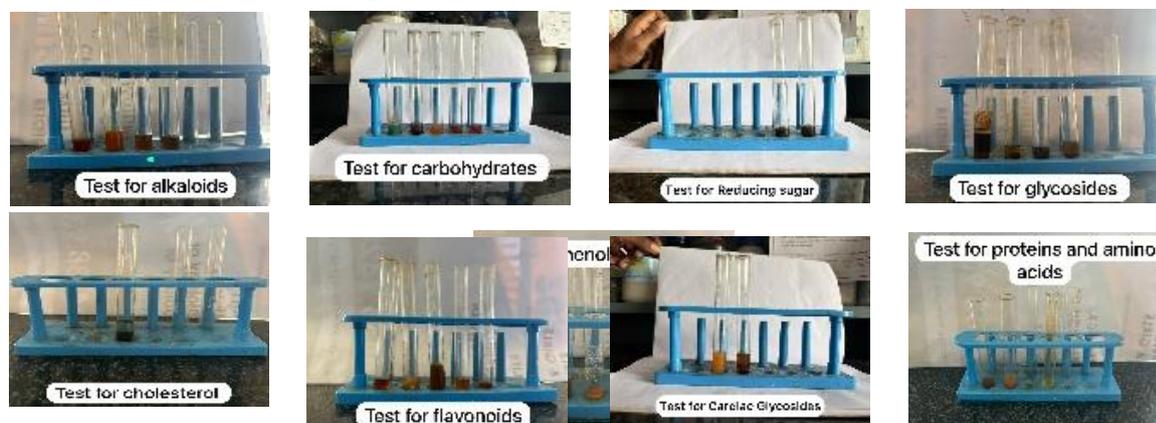


Figure 3: Phytochemical screening of the residue.

The Phytochemical screening was performed by chemical tests using the extract and respective reagents, and the inference of phytoconstituents is shown in tables 1,2 & 3.

Tables 1 & 2: shows presence of phytoconstituents

Phytoconstituents	Test	Inference
Alkaloids	Dragendroff's test	+
	Hager's test	+
	Mayer's test	+
	Picric acid test	+
Carbohydrates	Barfoed's test	+
	Molish test	+
	Salwanoff test	+
	Resorcinol test	+
	Pentose test	+
Reducing sugar	Benedicts test	+
	Fehlings test	+
Glycosides	Modified Borntrager's test	+
	Legal test	+
	Sodium hydroxide test	+
	Concentrated sulfuric acid test	+
Cholesterol	Cholesterol test	+
Phytoconstituents	Test	Inference
Flavonoids	Alkaline reagent test	+
	Lead acetate test	+
	Ferric chloride test	+
	Ammonia test	+
	Concentrated sulfuric acid test	+
Phenolic glycosides	Iodine solution test	+
	Ferric chloride test	+
	Gelatin test	+
	Ellagic acid test	+
Cardiac glycosides	Cardenolides test	+
	Bromine water test	+
Protein and amino acids	Biuret test	+
	Millons test	+
	Xanthoprotein test	+
Tannins	Lead subacetate	+
	Gelatin test	+
	Braymers test	+
	Bromine water test	+

Table 3: shows the absence of phytoconstituents.

Phytoconstituents	Test	Inference
Phlobatannins	Hydrochloric acid test	—
Saponin test	Sodium bicarbonate test	—
	Foaming test	—
Terpenoids	Terpenoid test	—
Triterpenoids	Salkowskis test	—
Quinones	Alcoholic KOH test	—
	Concentrated hydrochloric acid test	—
	Concentrated sulfuric acid test	—

The Concentrated residue was subjected to column chromatography. The column was packed with a stationary phase (Silica 120 mesh) and 1 g of residue was added on the top of the column, then different mobile phases [Ethyl acetate, chloroform, Ethanol: water (7:3)] were allowed to run within the column.



Figure 4 **Figure 5** **Figure 6** **Figure 7**
Fig 4 & 5: Elution of active compounds - Mobile phase (Ethyl acetate)
Fig 6: Elution of active compounds - Mobile phase (Chloroform)
Fig 7: Elution of active compounds - Mobile phase (Ethanol: Water) (7:3)

The fractions were separated and collected on the basis of color characterization. 8 fractions were collected separately in each beaker and shown in Table 4 and Figure 8.



Figure 8: The colour of fractions.

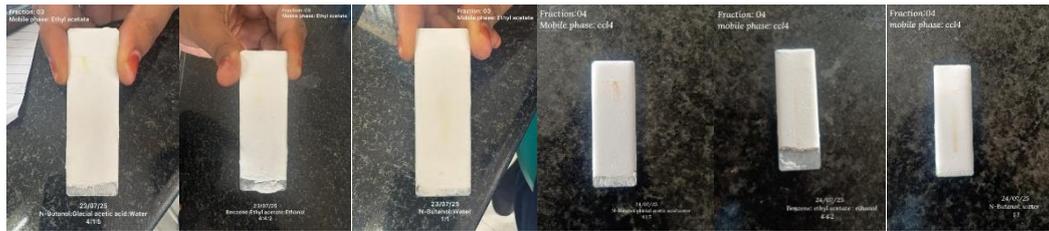
Table 4: The solvents used for the fractions and colours obtained.

FRACTIONS	COLOR
F1 (Ethyl acetate)	Yellowish brown
F2 (Ethyl acetate)	Reddish brown
F3 (Ethyl acetate)	Pale yellow
F4 (Chloroform)	Brown
F5 (Ethanol: water) (7:3)	Reddish black
F6 (Ethanol: water) (7:3)	Light brown
F7 (Ethanol: water) (7:3)	Dark red
F8 (Ethanol: water) (7:3)	Pale orange

Each fraction was spotted on the activated TLC plates with a different mobile phase, and the eluted spots can be shown in the following Figure 9 and Table 5.



TLC of Fractions 1 & 2



TLC of Fractin 3 & 4



TLC of Fractions 5 & 6

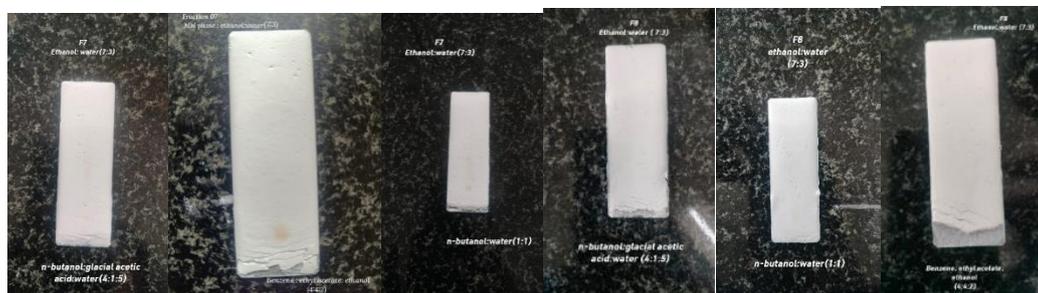


Figure 9: TLC of Fractions 7 & 8

Table 5: Retention factor of Collected fractions.

FRACTION/ EXTRACT	SOLVENT SYSTEM	Rf VALUE
F1 (Ethyl acetate)	A. n-butanol: glacial acetic acid: water (4:1:5)	0.95
	B. Benzene: ethyl acetate: ethanol (4:4:2)	0.96
	C. n-butanol: water (1:1)	0.92
F2 (Ethyl acetate)	A. n-butanol: glacial acetic acid: water (4:1:5)	0.96
	B. Benzene: ethyl acetate: ethanol (4:4:2)	0.96
	C. n-butanol: water (1:1)	0.96
F3 (Ethyl acetate)	A. n-butanol: glacial acetic acid: water (4:1:5)	0.96
	B. Benzene: ethyl acetate: ethanol (4:4:2)	0.80
	C. n-butanol: water (1:1)	0.50
F4 (Chloroform)	A. n-butanol: glacial acetic acid: water (4:1:5)	0.96
	B. Benzene: ethyl acetate: ethanol (4:4:2)	0.72
	C. n-butanol: water (1:1)	0.50
F5(Ethanol: water) (7:3)	A. n-butanol: glacial acetic acid: water (4:1:5)	0.96
	B. Benzene: ethyl acetate: ethanol (4:4:2)	0.41
	C. n-butanol: water (1:1)	0.80
F6(Ethanol: water) (7:3)	A. n-butanol: glacial acetic acid: water (4:1:5)	0.54

	B. Benzene: ethyl acetate: ethanol (4:4:2)	0.34
	C. n-butanol: water (1:1)	0.70
F7(Ethanol: water) (7:3)	A. n-butanol: glacial acetic acid: water (4:1:5)	0.96
	B. Benzene: ethyl acetate: ethanol (4:4:2)	0.23
	C. n-butanol: water (1:1)	0.61
F8(Ethanol: water) (7:3)	A. n-butanol: glacial acetic acid: water (4:1:5)	0.82
	B. Benzene: ethyl acetate: ethanol (4:4:2)	0.30
	C. n-butanol: water (1:1)	0.50

In Table 5, The R_f values for each fraction were calculated. Three different mobile phases in combination were used. The solvent system of **n-butanol: glacial acetic acid: water (4:1:5)** was selected due to the similarity R_f value of **0.96**. The fraction F4 and F5, which provides a fine single spot on the TLC plate, was optimized for IR and NMR spectroscopy. IR was performed by the KBr pellet method for Fractions 4 and 5, shown in Figure 10, and the obtained peaks for Fraction 4 and the values are shown in Figure 11 and Table 6.



Figures 10: Pellets of Fraction 4 & 5.



Figure 11: Peaks of IR spectrum of fraction 4.

Table 6: IR peak assignment table for fraction 4 ⁽¹²⁾.

S. No	Region (cm ⁻¹)	Intensity	Functional Group	Assignment
1	3410	Strong, broad	O–H stretching (hydrogen bonded)	Hydroxyl group vibration
2	1514.51	Medium	Aromatic C=C stretching	Benzene ring skeletal vibration

In Table 6, The IR spectrum of **fraction 4** shows characteristic absorption bands at **3410** and **1514.51 cm⁻¹**, corresponding to **hydroxyl group and aromatic C=C stretching vibrations**.

IR was performed by the KBr pellet method for Fraction 5, the obtained peak and the values are shown in Figure 12 and Table 7.

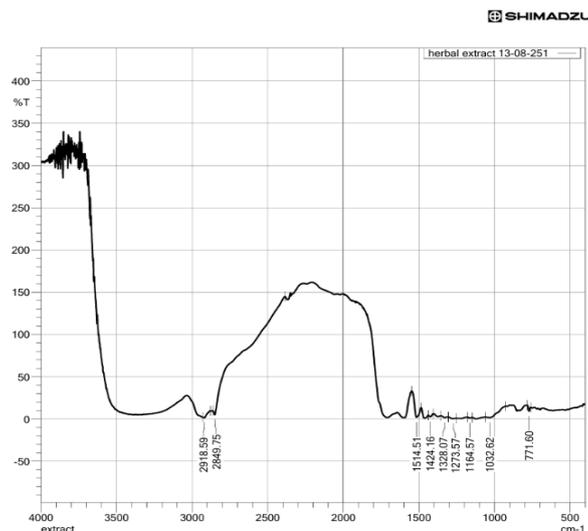


Figure 12: Peaks of IR spectrum of fraction 5.

Table 7: IR peak assignment table for fraction 5 ⁽¹²⁾.

S. No	Region (cm ⁻¹)	Intensity	Functional Group	Assignment
1	2918.59	Medium	C–H stretching (–CH ₂ / –CH ₃)	Aliphatic C–H vibration
2	2849.75	Medium	C–H stretching (symmetric)	Long alkyl chain vibration
3	1514.51	Medium	Aromatic C=C stretching	Benzene ring vibration
4	1424.16	Medium	C–H bending	Aromatic/aliphatic deformation
5	1328.07	Weak–medium	C–N stretching / O–H bending	Nitrogen or phenolic deformation
6	1273.57	Medium	C–O stretching	Phenolic / ester linkage
7	1164.57	Medium	C–O stretching	Alcohol/ether linkage
8	1032.62	Strong	C–O–C stretching	Glycosidic bond
9	771.60	Weak	C–H out-of-plane bending	Substituted aromatic ring

In Table 7, The IR spectrum of **fraction 5** exhibits characteristic absorption bands at **2918.59, 2849.75, 1514.51, 1424.16, 1328.07, 1273.57, 1164.57, 1032.62 and 771.60 cm⁻¹**, confirming the presence of **aliphatic hydrocarbons, aromatic phenolics, and glycosidic linkages** NMR was performed by using NMR- BRUCKER AVANCE NEO 500MHz for **Fraction 4**, the obtained peak, structure and the values are shown in the figure 13, 14, 15 and Table 8.

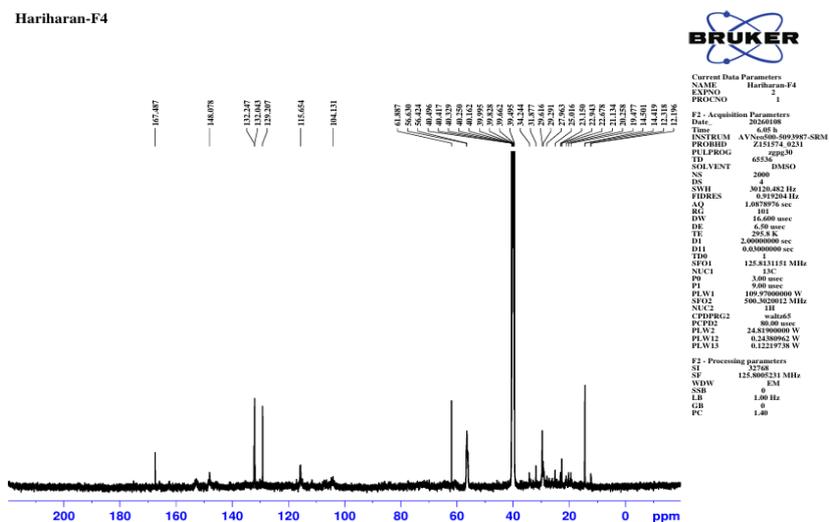


Figure 13: Peak of ¹³C NMR fraction 4.

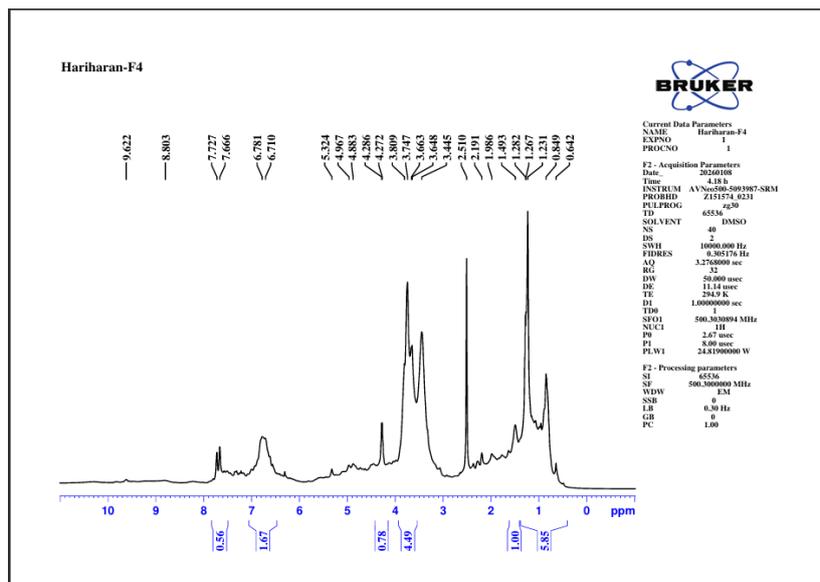


Figure 14: Peak of ¹H NMR for fraction 4.

Table 8: Spectral assignment table with Carbon Numbering⁽¹³⁾.

Carbon No.	δ ¹³ C (ppm)	δ ¹ H (ppm)	Possible compounds
C1	~155	9.62 (s)	Phenolic C–OH
C2	~148	—	Aromatic C–OCH ₃
C3	~136	—	Aromatic C–butyl
C4	~128	7.6–7.7	Aromatic CH
C5	~122	6.7–6.8	Aromatic CH
C6	~115	6.7–6.8	Aromatic CH
C7	~56	3.7–3.8 (s)	O–CH ₃
C8	~34	2.3–2.5 (m)	Benzylic CH ₂
C9	~29	1.6–1.8 (m)	CH ₂
C10	~22	1.2–1.4 (m)	CH ₂
C11	~14	0.84 (t)	CH ₃

From the figure 13, 14, 15 and Table 8 represents, The ¹H NMR spectrum (500 MHz, DMSO-d₆) displayed a characteristic downfield singlet at δ 9.62 ppm, attributable to a phenolic hydroxyl (–OH) proton engaged in hydrogen bonding. The aromatic region showed multiple resonances between δ 8.80–7.66 ppm and 6.78–6.71 ppm, consistent with a trisubstituted benzene ring. A singlet observed at δ 3.75–3.80 ppm was assigned to a methoxy (–OCH₃) group attached to an aromatic carbon. Signals in the aliphatic region between δ 2.51–1.98 ppm were indicative of benzylic methylene protons, while additional resonances at δ 1.49–0.84 ppm corresponded to the remaining methylene and terminal methyl protons of a straight-chain alkyl substituent.

The ¹³C NMR spectrum (125 MHz, DMSO-d₆) further supported this interpretation. Resonances in the range δ 148–160 ppm were assigned to oxygenated aromatic carbons, including the phenolic carbon and the methoxy-substituted aromatic carbon. Signals between δ 115–140 ppm corresponded to the remaining aromatic carbons of the substituted phenyl ring. A distinct resonance at δ ~56 ppm confirmed the presence of a methoxy carbon, while signals in the range δ 22–34 ppm were consistent with aliphatic methylene carbons of a butyl chain. The terminal methyl carbon appeared at δ ~14 ppm. Notably, no resonances were observed above δ 170 ppm, excluding the presence of carbonyl functionalities. The combined ¹H and ¹³C NMR data clearly indicate the presence of a phenolic aromatic system bearing a methoxy substituent and a linear butyl side chain. Based on chemical shift values, substitution patterns, and comparison with closely related alkyl-substituted methoxyphenols reported in the literature, the structure of compound F4 was assigned as **2-methoxy-4-butylphenol (Creosol)**.

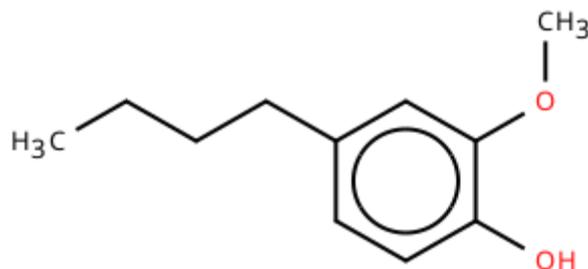


Figure 15: 2-methoxy-4-butylphenol (Creosol).

NMR was performed by using NMR- BRUCKER AVANCE NEO 500MHz for **Fraction 5**, the obtained peak, structure, and the values are shown in figure 16, 17, 18 and tables 9 & 10.

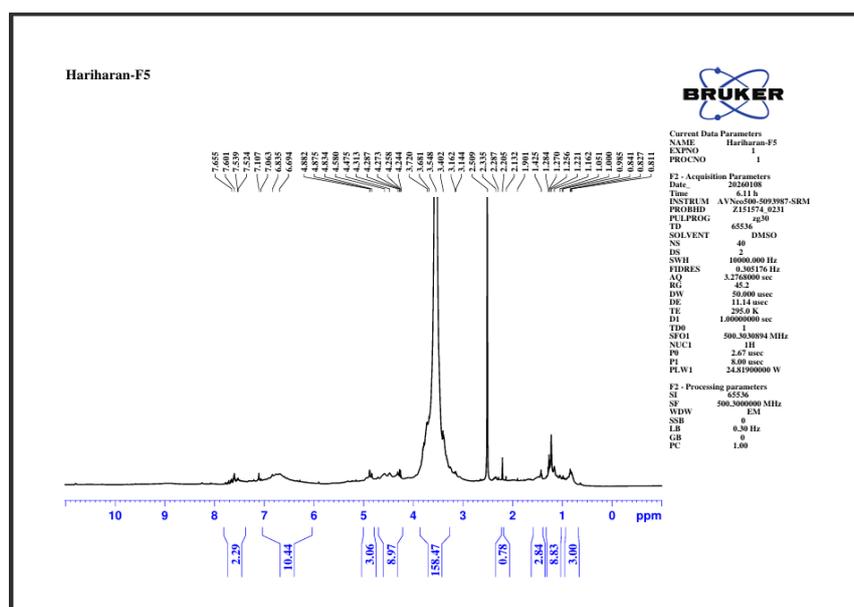
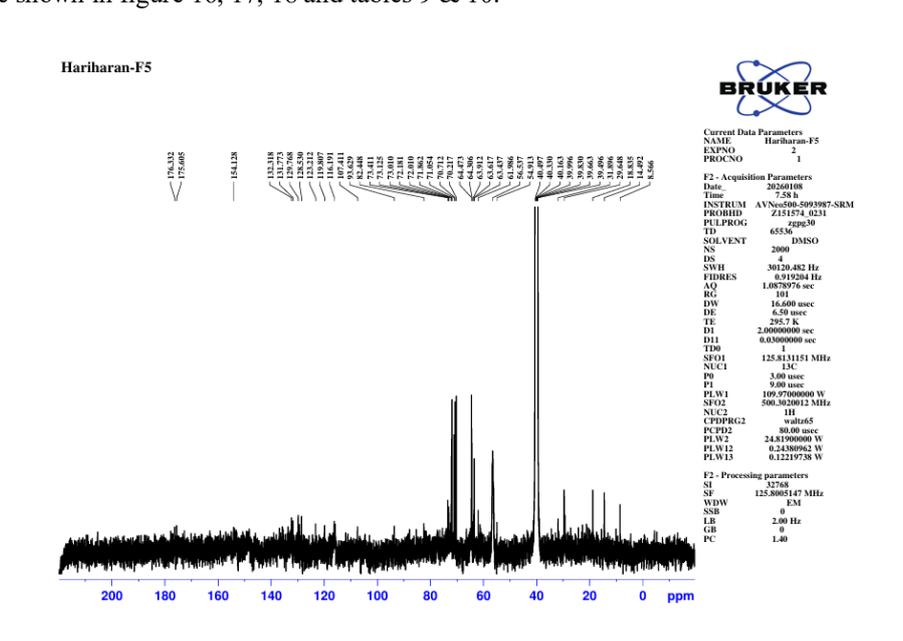


Figure 17: Peak of ¹H NMR fraction 5.

Table 9: Aglycone (Flavonol Core) ⁽¹⁴⁾.

Carbon No.	δC (ppm)	δH (ppm)	Possible compounds
C-2	~156.5	—	Oxygenated quaternary C
C-3	~133.4	—	Glycosylated C (O-linked)

C-4	~177.0	–	Carbonyl (C=O)
C-5	~161.0	–	Phenolic C–O
C-6	~98.7	6.69	Aromatic H (meta-coupled)
C-7	~164.2	–	Phenolic C–O
C-8	~93.6	6.83	Aromatic H (meta-coupled)
C-9	~156.8	–	Quaternary aromatic C
C-10	~104.5	–	Quaternary aromatic C
C-1'	~121.5	–	B-ring ipso carbon
C-2'	~116.3	7.52	Aromatic H
C-3'	~145.8	–	Phenolic C–O
C-4'	~148.7	–	Phenolic C–O
C-5'	~115.4	6.83	Aromatic H
C-6'	~121.0	7.65	Aromatic H

Table 10: Sugar Moiety (β -D-Glucopyranosyl Unit)⁽¹⁵⁾.

Carbon No.	δ C (ppm)	δ H (ppm)	Possible compounds
C-1''	~101.8	4.85	Anomeric carbon/proton
C-2''	~74.6	3.30–3.45	Glucose CH
C-3''	~77.3	3.35–3.55	Glucose CH
C-4''	~70.9	3.20–3.40	Glucose CH
C-5''	~76.8	3.25–3.45	Glucose CH
C-6''	~61.9	3.40–3.75	CH ₂ OH

From the figure 16, 17, 18 and tables 9 & 10 represents, The structure of compound F5 was elucidated based on detailed one-dimensional NMR spectroscopic analysis. The ¹H NMR spectrum (500 MHz, DMSO-d₆) displayed characteristic aromatic proton resonances in the range δ 6.69–7.65 ppm, consistent with a flavonol skeleton. The B-ring exhibited an ABX spin system, indicative of a 3',4'-dihydroxylated phenyl moiety, while two meta-coupled protons observed in the A-ring confirmed substitution at C-5 and C-7.

A series of oxygenated aliphatic proton signals between δ 3.14 and 4.88 ppm suggested the presence of a sugar unit. A distinct anomeric proton resonance at δ ~4.85 ppm appeared as a doublet with a coupling constant of approximately 7.5 Hz, diagnostic of a β -configured glucopyranosyl residue.

The ¹³C NMR spectrum (125 MHz, DMSO-d₆) showed signals corresponding to aromatic carbons between δ 107 and 133 ppm, along with downfield resonances at δ ~154–176 ppm attributable to oxygenated aromatic carbons bearing hydroxyl substituents. A characteristic flavonol carbonyl carbon was observed at δ ~177 ppm (C-4). The anomeric carbon of the sugar moiety resonated at δ ~100 ppm, while the remaining glucose carbons appeared between δ 60 and 75 ppm.

The combined NMR data are fully consistent with a flavonol O-glycoside structure. Based on these spectroscopic features and comparison with reported literature values, compound F5 was unambiguously identified as Quercetin-3-O- β -D-glucopyranoside (isoquercitrin).

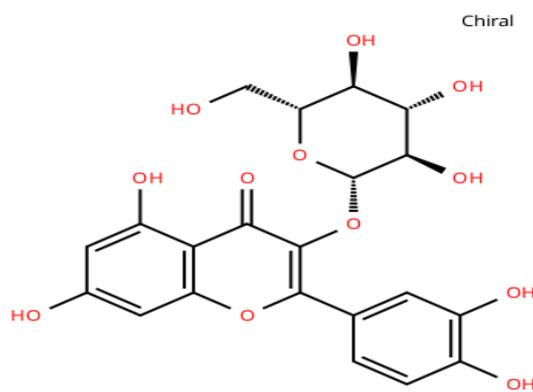


Figure 18: Quercetin-3-O- β -D-glucopyranoside (Isoquercitrin).

CONCLUSION

Phoenix sylvestris of the family Aracaceae is rich in dietary fibre, essential vitamins, minerals, phenolic compounds, amino acids, flavonoids, tannins, alkaloids, terpenoids and carbohydrates. This plant is recognised for its anti-oxidant, cardioprotective, diuretic and anti-pyretic properties. The present study aims to screen and isolate the valuable phytoconstituents from the trunk part of *Phoenix sylvestris*.

The trunk was collected, cleaned and shade-dried. The dried plant material was ground as coarse powder. The dried powder material was subjected to Soxhlet extraction. 200 g of plant powder was packed into the thimble and extracted with solvent ethanol (2L). The extraction was performed for 4 days. The extract obtained was evaporated, concentrated,

and the residue was obtained. The Percentage yield of the residue was found to be **2.6%w/w**. Which was then subjected to column chromatography for the isolation of phytoconstituents. A glass column was selected and packed with silica (mesh-120). 1 g of residue was loaded on of column. Different mobile phases (Ethyl acetate, Chloroform, Ethanol: Water 7:3) were used for the detection of phytoconstituents. Based on the colour characterisation, the fractions were separated and collected in each beaker (F1, F2, F3, F4, F5, F6, F7 and F8)

Each fraction undergone preliminary analytical method (TLC) to separate and identify different phytoconstituents present in the plant residue. The presence of different phytoconstituents in the residue was established by TLC plates. Silica gel G was used as the stationary phase. A slurry was prepared by mixing silica gel with distilled water and spreading it evenly onto the clean glass plates. The TLC plates were allowed to get activated by placing them in a hot air oven at 100-120°C for about 30 minutes to remove moisture. The mobile phases used were n-butanol: glacial acetic acid: water (4:1:5), benzene: ethyl acetate: ethanol (4:4:2), and n-butanol: water (1:1). A spot was placed on the activated TLC plates by using a capillary tube. The plates were placed into the beaker containing the mobile phase, and the phytoconstituents were allowed to elute. After the development of spots on the TLC plates, the colour was marked. Rf values of each fraction were calculated. The fraction (F4, F5), which provides a fine single spot on the TLC plate, was optimised for further studies. After development of spots on the TLC plates, Rf values of each fraction were calculated, and we optimized Fraction 4 & 5 for IR spectral analysis by the KBr pellet method. Both the optimised fractions have undergone NMR spectroscopy, was identified for Fraction 4 is 2-methoxy-4-butylphenol (Creosol), and Fraction 5 is Quercetin-3-O-β-D-glucopyranoside (isoquercitrin).

In our present study, we come to the conclusion that both fractions are spectrally analysed using IR and NMR spectroscopy. Structure and IUPAC name were identified. The compound isolated from F4 using chloroform as a mobile phase is **Creosol**, which is used as an antiseptic, antioxidant, and aromatic properties in medicinal, food and cosmetic applications and the compound isolated from F5 using ethanol: water (7:3) as a mobile phase is **Isoquercitrin**, which has anti-inflammatory property, boosts immunity, and support cardio vascular health.

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