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Phytochemical Profiling, Standardization and Quantification of *Prunus armeniaca L.* Kernel Extract Using FTIR and HPTLC Techniques

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Abstract

The present study aimed to characterize, standardize, and quantify the major phytoconstituents of the methanolic kernel extract of *Prunus armeniaca* using Fourier Transform Infrared Spectroscopy (FTIR) and High-Performance Thin Layer Chromatography (HPTLC). The plant kernels were extracted using maceration with methanol, and the extract was subjected to phytochemical fingerprinting. FTIR analysis revealed prominent functional groups corresponding to O–H, C–H, C–O, C=C, and C≡N, confirming the presence of phenolics, flavonoids, glycosides, and cyanogenic compounds such as amygdalin. HPTLC-densitometric analysis was performed for quantification of amygdalin, and the calibration curve exhibited excellent linearity with the equation y = 127.51x + 327.67 and R^2 = 0.9963. The chromatogram overlay confirmed the presence of amygdalin by matching the extract peak with standard Rf values. The findings demonstrate that *Prunus armeniaca* kernels contain significant levels of bioactive constituents and that FTIR and HPTLC offer reliable tools for the standardization of herbal formulations.

Keywords: *FTIR, HPTLC, Phytochemicals, Plant extract, Functional groups, Bioactive compounds.*

1. Introduction

Prunus armeniaca, commonly known as apricot, is an important medicinal and edible plant species belonging to the Rosaceae family [1]. This plant is recognized globally for its delicious and commercially traded fruits [1, 2]. The kernels and seeds of the apricot are particularly noted for their medicinal applications [3].

Historically, various parts of *Prunus armeniaca* have been utilized in traditional medicine systems across different cultures [2, 4-7]. The seeds of *Prunus armeniaca*, known in Chinese medicine as Armeniacae semen amarum or Kuxingren, have a long history of use for lung and intestinal disorders [4]. They are traditionally used to alleviate coughs and asthma, as well as to lubricate the colon and ease constipation [2, 4, 8]. In Iranian traditional medicine, the plant is used to address memory loss [9]. Additionally, *Prunus armeniaca* has been used in oriental medicine to treat conditions such as leprosy, asthma, leukoderma, bronchitis, and nausea [2, 8]. Traditional applications also include treating tumors, anemia, colds, coughs, fever, laryngitis, and hemorrhages [10]. Furthermore, various Prunus species are traditionally used for the treatment of diverse disorders, highlighting their therapeutic potential [11].

1.1 Pharmacological Importance of *Prunus armeniaca*

Prunus armeniaca exhibits a wide range of pharmacological activities due to its rich phytochemical composition [4, 11-13]. The plant is abundant in glycosides, organic acids, amino acids, flavonoids, terpenes, phytosterols, phenylpropanoids, polyphenols, carotenoids, fatty acids, cyanogenic glucosides, sterol derivatives, and volatile compounds [2, 4, 14, 15]. These phytochemicals contribute to its diverse therapeutic effects, which have been extensively studied.

1.2 Key Pharmacological Activities

- **Anticancer Activity:** Numerous studies indicate that *Prunus armeniaca* possesses anticancer properties [3, 4]. The kernels and seeds, particularly due to the presence of amygdalin, have shown potential in treating liver carcinogenesis and

inhibiting cancer cell proliferation [16- 19]. Amygdalin, also known as vitamin B17, has been used as an anticancer drug and is believed to have anti-tumor effects [8, 20, 21].

- **Antioxidant Activity:** The high polyphenolic content of *Prunus armeniaca* contributes to its significant antioxidant activity [4]. This activity is crucial for preventing degenerative diseases such as cancer and cardiovascular diseases.
- **Anti-inflammatory Activity:** The plant exhibits anti-inflammatory effects [4,11, 12].
- **Antimicrobial Activity:** *Prunus armeniaca* has demonstrated antimicrobial potential, including antibacterial and antifungal properties [4, 14].
- **Hepatoprotective and Cardioprotective Effects:** The apricot has been noted for its protective effects on the liver and cardiovascular system [4, 14].
- **Antidiabetic and Neuroprotective Potentials:** Scientific literature highlights the antidiabetic and neuroprotective qualities of *Prunus armeniaca* [14].

1.3 Amygdalin and its Significance

Amygdalin is a key component found in the seeds of *Prunus armeniaca* and other Prunus species [4, 20]. It is a cyanogenic glycoside that can be converted into hydrogen cyanide in the abdomen [20, 22]. While known for its potential anticancer and antitussive properties, the release of hydrogen cyanide can also lead to metabolic poisoning [20, 22]. Therefore, potential adverse reactions and pharmacokinetic properties of amygdalin need to be carefully considered during its clinical use [4]. Research is also exploring nanotechnological concepts for targeted drug delivery of active constituents from *Prunus armeniaca* to improve precision and reduce unnecessary exposure of drugs to tissues [3].

1.4 Need for standardization of herbal extracts

The rising global acceptance of herbal drugs necessitates stringent standardization to ensure their quality, safety, and efficacy [23 24], Herbal medicines are frequently used for chronic diseases, and their increasing use has unfortunately led to instances of abuse and adulteration, causing consumer disappointment and even fatal consequences [23, 25]. Standardization addresses the inherent variability in herbal extracts stemming from genetic factors, environmental conditions, and processing methods, which can significantly affect their biochemical components. Therefore, establishing quality parameters for collection, handling, processing, and production of herbal medicines is crucial to assure biochemical consistency, optimize safety and efficacy, and facilitate their responsible integration into global healthcare systems [23, 25, 26]

1.5 Importance of FTIR + HPTLC in phytochemical profiling

The combination of HPTLC and FTIR is instrumental in identifying and characterizing phytochemical compounds within complex extracts [27- 29]. HPTLC is adept at separating various constituents, producing distinct fingerprint profiles with specific retention factor (Rf) values for different compounds [28- 30]. For instance, HPTLC analysis of Hypericum gaitii Haines can identify hypericin content by showing several peaks with distinct Rf values [28]. Similarly, in Holostemma ada-kodien, HPTLC fingerprint profiles reveal different phytoconstituents that serve as markers for drug standardization [29]. The technique has been used to detect adulteration and authenticate herbal medicines by producing clear banding patterns [28, 29]. FTIR spectroscopy complements HPTLC by identifying functional groups present in the separated compounds [30, 31]. This is achieved by analyzing the infrared transmittance data across a specific wavenumber range, typically from 500 to 4000 cm−1. For example, the FTIR spectrum of Senna auriculata flower extract showed prominent peaks indicating the presence of phenolic, aromatic, and ether functional groups [32]. Specific bands, such as those between 3000 and 3500 cm−1 for hydroxyl groups and 1200 to 1700 cm −1 for aromatic rings, are indicative of phenolic compounds [33]. This spectroscopic data can distinguish chemical categories and even subtle differences in chemical constituents among various plant taxa or geographical origins [34, 35]

The primary objective of this study is to comprehensively characterize the phytochemical profile of the plant extract through an integrated approach utilizing High-Performance Thin-Layer Chromatography (HPTLC) and Fourier Transform Infrared (FTIR) spectroscopy.

2. MATERIALS AND METHODS

2.1 Plant Material and Extraction

Kernels of *Prunus armeniaca* were collected, cleaned, dried, and pulverized. The powdered kernels were extracted by **maceration** using **methanol** as the solvent for 72 hours with intermittent shaking. The extract was filtered and concentrated under reduced pressure to obtain a dry methanolic extract.

2.2 FTIR Analysis

FTIR spectra of the extract were recorded using an FTIR spectrophotometer in the range of 4000–400 cm^{-1}. The sample was prepared using the ATR method. Spectral data were interpreted to identify characteristic functional groups based on peak positions and reported literature.

2.3 HPTLC Analysis: HPTLC method development

2.3.1 Test solutions

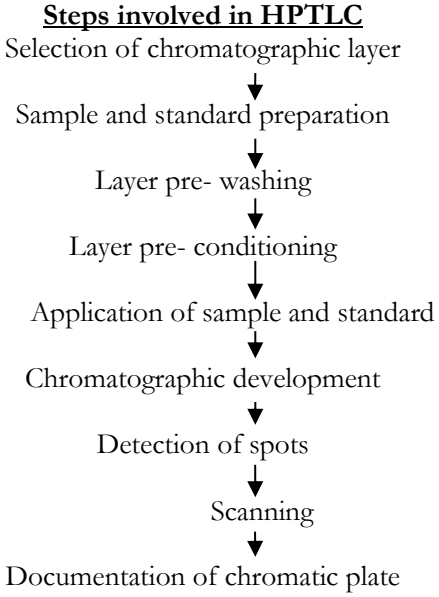
Coarsely powdered plant material (10 g) was exhaustively extracted with methanol by maceration after defatting by Pet. ether. The solvent was recovered under reduced pressure. The extract was dried, and volume was then adjusted to 25 ml with methanol in a volumetric flask.

2.3.2 Preparation of standard plot

A stock solution of 25 mg/ml concentration of the marker was prepared in water RP-HPTLC studies. The stock solution of marker was diluted with water to get six dilutions of different concentrations (5, 10, 15, 20, 25 and 30 µg/µl). A volume of 5 µl from each dilution was applied in triplicate on pre-coated TLC plate. The plate was developed in solvent system acetonitrile: water (1:1) in a chamber saturated for 10 min, to a distance of 8 cm. The developed plate was dried in a current of hot air and then scanned in TLC scanner at 210 nm [2]. The AUC of the peak corresponding to marker compound was noted in each track

2.3.3 Estimation of marker compound

Test solutions (10 µl) of methanol extract was applied, on pre-coated RP-HPTLC plate (5 × 10 cm). The plate was developed and scanned following the same procedure as used for the preparation of standard plot. The average AUC of the peak corresponding to marker compound was noted at 210 nm in the test sample, and its concentration was calculated from the standard plot.



Steps involved in TLC densitometric method development

3. RESULTS

3.1 FTIR Analysis

The FTIR spectrum of the methanolic extract showed several characteristic absorption peaks indicating the presence of major functional groups associated with phenolics, flavonoids, and cyanogenic glycosides.

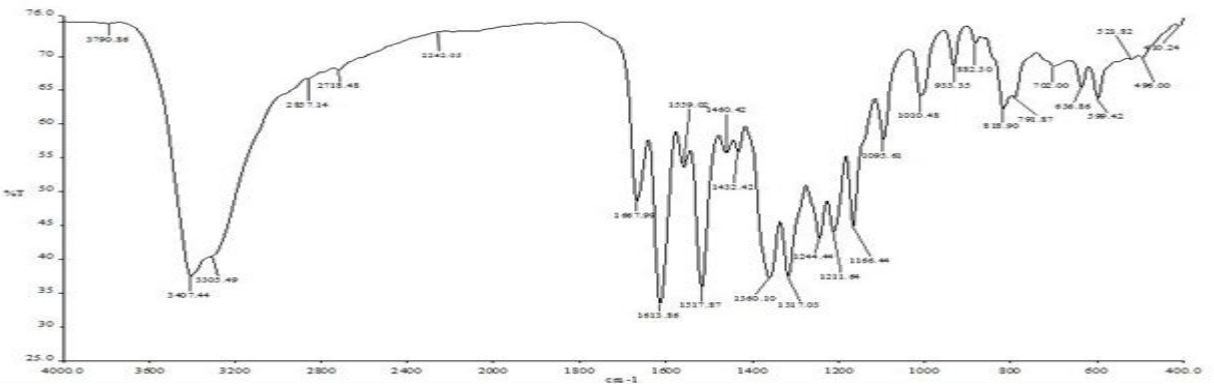


Figure 1: FTIR sepctra of methanol plant extract

Table 1: The major peaks and their tentative assignments

Functional Group	Observed Peak (cm^{-1})	Interpretation
O–H stretching (glucose moiety)	**3305.49**	Indicates presence of carbohydrates, phenolics
Aromatic C–H stretching	**2857.14**	Suggests aromatic compounds
Aliphatic C–H stretching	**2718.48**	Associated with aliphatic chains
C≡N (nitrile) group	**2242.05**	Characteristic of cyanogenic glycosides (e.g., amygdalin)
C=C stretching of benzene ring	**1667.99, 1432.42**	Indicates flavonoids and aromatic compounds
C–H bending	**1559.02**	Typical of aromatic constituents
C–O stretching of ether	**1244.44**	Suggests glycosides
Hydroxy group (O–H)	**1010.48**	Confirms carbohydrate/phenolic presence
C–H bending (aromatic)	**933.35, 882.30, 818.90, 791.87, 702.00, 636.86, 599.42**	Various aromatic substitutions

3.1.1 FTIR Interpretation

The appearance of a strong nitrile (C≡N) peak at **2242.05 cm^{-1}** confirms the presence of **cyanogenic glycosides**, supporting the occurrence of **amygdalin**, a major constituent of *Prunus* species. Broad O–H bands and aromatic C=C signals also indicate the presence of phenolics, flavonoids, and glycosidic compounds.

3.2 HPTLC- Densitometric Quantification of Amygdalin

A five-point calibration curve of standard amygdalin was developed using TLC-densitometric analysis.

Table 2: The AUC values for different concentrations

Amount (µg)	AUC
5	1012
10	1654
15	2145
20	2789
25	3541
30	4214

The calibration curve showed a linear relationship between peak area and concentration with the equation:

$y = 127.51x + 327.67$ ($R^2 = 0.9963$)

This high correlation coefficient indicates excellent linearity and suitability for quantitative estimation.

3.2.1 HPTLC Fingerprinting of Extract

The densitogram and chromatogram overlay **(Figure 2)** demonstrated multiple peaks in the plant extract. A major peak corresponding to the Rf value of standard amygdalin was observed, confirming its presence in the extract.

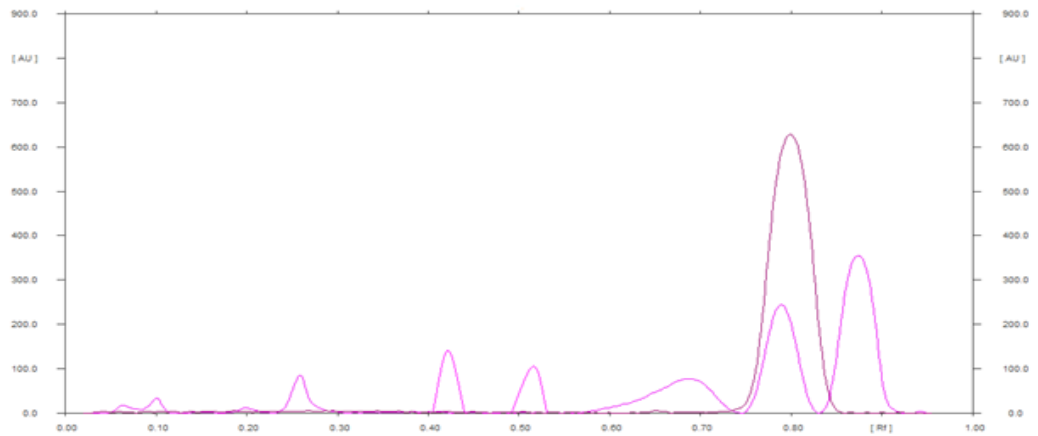


Figure 2: The thin layer chromatogram overlay of amygdalin and methanol extract of plant

The TLC/HPTLC densitometric overlay chromatogram of standard amygdalin and the methanolic plant extract was recorded at the selected detection wavelength to assess the presence of amygdalin in the extract. The extract displayed multiple phytoconstituent bands between Rf 0.05–0.90. The chromatogram displayed a well-resolved, sharp peak for standard amygdalin at an Rf value of approximately 0.78–0.80. A corresponding peak at the same Rf value was observed in the methanolic extract, confirming the presence of amygdalin in the plant material. The extract chromatogram additionally showed several other peaks at different Rf values, indicating the presence of other phytoconstituents, while no interfering peaks were detected at the amygdalin Rf. The clear overlap of peaks at identical Rf values demonstrates the specificity and selectivity of the chromatographic method and supports its suitability for the identification and subsequent quantification of amygdalin in the methanolic extract.

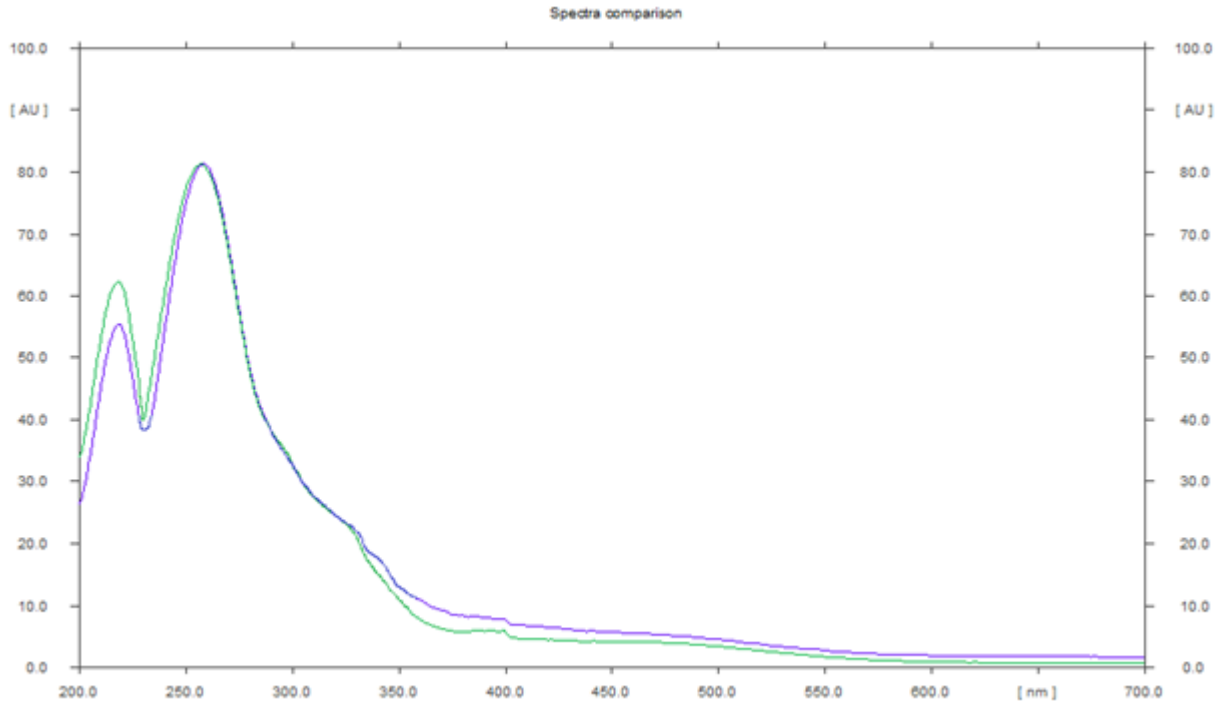


Figure 3: The thin layer UV spectra overlay of amygdalin and methanol extract of plant

The UV spectral overlay obtained from the TLC spot of standard amygdalin and the corresponding spot of the methanolic plant extract demonstrated a high degree of spectral similarity, confirming the identity of amygdalin in the extract. Both spectra showed characteristic absorption maxima in the UV region, with closely overlapping λmax values (approximately in the range of 250–270 nm), indicating comparable electronic transitions of the chromophoric groups. The near-superimposition of the spectra across the scanned wavelength range (200–700 nm) suggests that the compound present in the plant extract at the same Rf value possesses an identical UV absorption profile to that of the standard amygdalin. Minor variations in absorbance intensity may be attributed to matrix effects or co-eluting phytoconstituents but do not affect the overall spectral congruence. This spectral matching confirms the specificity of the chromatographic method and further substantiates the presence of amygdalin in the methanolic extract.

4. DISCUSSION

FTIR and HPTLC analyses collectively confirm the presence of various phytochemical groups in the methanolic extract. The FTIR spectrum revealed characteristic O–H, C–H, C–O, C=C, and C≡N functional groups, supporting the presence of key classes such as phenolics, flavonoids, glycosides, and cyanogenic compounds.The strong nitrile absorption at 2242.05 cm^{-1} is particularly significant as it is a marker for amygdalin, a cyanogenic diglucoside commonly found in *Prunus armeniaca* kernels.

The HPTLC fingerprint further validated the presence of amygdalin by comparing Rf values and peak overlap with the standard. The high linearity (R^2 = 0.9963) of the calibration plot indicates the reliability of the developed method for quantification.Multiple additional peaks in the extract suggest a rich phytochemical profile, which may contribute to the plant's reported pharmacological activities, including antioxidant, anticancer, and neuroprotective effects.

5. Conclusion

The methanolic extract of *Prunus armeniaca* kernels contains significant phytochemicals as confirmed by FTIR and HPTLC analysis. FTIR identified important functional groups such as O–H, C–H, C–O, and C≡N, indicating the presence of phenolics, flavonoids, and cyanogenic glycosides. HPTLC successfully quantified amygdalin with high precision and accuracy. Thus, FTIR and HPTLC serve as effective tools for phytochemical profiling, authentication, and standardization of *Prunus armeniaca* kernel extract.

6. Declaration of interest: None

7. References

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