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Research Article

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COMPREHENSIVE PHYTOCHEMICAL ISOLATION AND IN VITRO BIOLOGICAL ASSESSMENT OF PIMPINELLA HEYNEANA: A PROMISING UNDEREXPLORED MEDICINAL HERB

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Abstract

The present study investigates the phytochemical profile of *Pimpinella heyneana* with emphasis on isolation, structural characterization, and biological evaluation of its bioactive constituents. Ethanolic extract was prepared and subjected to chromatographic separation. Structural elucidation was performed using FTIRspectrometry and HPLC chromatography. The extract showed majorly the presence of flavonoids and phenolic compounds. In Vitro evaluation revealed significant antioxidant (IC_{50}: 42.6 µg/mL), antimicrobial activities. HPLC analysis confirmed multiple phytoconstituents with a major peak at 5.84 min. These findings support the therapeutic potential of *P. heyneana*.

Keywords: *Pimpinella heyneana, phytochemicals, HPLC, FTIR, antioxidant, antimicrobial.*

1. INTRODUCTION

Medicinal plants have long served as a cornerstone in the discovery and development of therapeutic agents due to their rich repository of bioactive compounds. A significant proportion of modern pharmaceuticals are either directly derived from plant sources or inspired by natural products, highlighting the importance of phytochemical research in drug discovery (Kumar et al., 2022). Among various plant families, the genus *Pimpinella*, belonging to the Apiaceae family, has gained considerable attention owing to its diverse phytochemical composition and wide range of pharmacological activities.

The Apiaceae family is well known for producing secondary metabolites such as flavonoids, coumarins, terpenoids, and phenylpropanoids, which exhibit antioxidant, antimicrobial, anti-inflammatory, and anticancer properties [1]. Species within the *Pimpinella* genus, including *Pimpinellaanisum*, have been extensively studied and reported to possess significant biological activities attributed to their essential oils and phenolic constituents. These phytochemicals play a crucial role in neutralizing free radicals, modulating enzymatic pathways, and protecting biological systems against oxidative stress [2].

Pimpinella heyneana, a relatively less explored species, is distributed in tropical and subtropical regions, particularly in India and Southeast Asia. Traditionally, it has been used in folk medicine for the management of gastrointestinal disorders, inflammation, and renal ailments [3].

Despite its ethnomedicinal significance, there is a scarcity of comprehensive scientific studies focusing on its phytochemical composition and pharmacological potential. This gap necessitates systematic investigation to validate its traditional uses and identify its active constituents.

Phytochemical investigation involves a series of processes including extraction, isolation, and structural characterization of bioactive compounds. Modern analytical techniques such as high-performance liquid

chromatography (HPLC), Fourier-transform infrared spectroscopy (FTIR)have significantly enhanced the ability to identify and characterize complex natural products [4]. These techniques provide detailed insights into the chemical nature and structural framework of phytoconstituents, thereby facilitating their correlation with biological activities.

Furthermore, oxidative stress and microbial infections are major contributors to various chronic diseases, including renal disorders. Natural antioxidants derived from plant sources have gained increasing attention due to their ability to scavenge free radicals and reduce oxidative damage [5]. Similarly, the emergence of antibiotic resistance has emphasized the need for novel antimicrobial agents from natural sources [6]. In this context, evaluation of antioxidant and antimicrobial activities of plant extracts becomes highly relevant.

In light of the above considerations, the present study aims to systematically investigate the bioactive phytochemicals of *Pimpinella heyneana* through extraction, isolation, and structural characterization, followed by evaluation of its antioxidant, antimicrobial activities. The study seeks to provide scientific evidence supporting its traditional use and explore its potential as a source of novel therapeutic agents.

2. MATERIALS AND METHODS

2.1 Plant Material Collection and Authentication

The whole plant of *Pimpinella heyneana* was collected from a local region in Telangana and Andhra Pradesh, India, during the flowering season (November–January). The plant material was authenticated by a qualified taxonomist, and a voucher specimen was deposited in the institutional herbarium for future reference. The collected plant material was washed thoroughly with distilled water to remove adhering soil and debris, shade-dried at room temperature (25 ± 2°C) for 10–12 days and coarsely powdered using a mechanical grinder. The powdered material was stored in airtight containers protected from light and moisture until further use [4,5].

2.2 Chemicals and Reagents

All solvents used, including ethanol, methanol, ethyl acetate, hexane, and acetonitrile, were of analytical grade. Silica gel (60–120 mesh) was used for column chromatography. DPPH (2,2-diphenyl-1-picrylhydrazyl), nutrient agar, and other microbiological media were procured from standard suppliers. All reagents used were of AR grade unless otherwise specified [6].

2.3 Preparation of Extract

Approximately 500 g of powdered plant material was subjected to Soxhlet extraction using 95% ethanol for 6–8 hours. The extraction process was continued until the solvent in the siphon tube became colorless. The extract was filtered and concentrated under reduced pressure using a rotary evaporator to obtain a semi-solid mass. The extract was further dried in a desiccator to remove residual solvent. The percentage yield was calculated based on the initial dry weight of plant material [3,7].

2.4 Preliminary Phytochemical Screening

The ethanolic extract was subjected to qualitative phytochemical screening to detect the presence of major phytoconstituents such as alkaloids, flavonoids, tannins, saponins, glycosides, and terpenoids using standard procedures. Tests such as Dragendorff's test (alkaloids), Shinoda test (flavonoids), ferric chloride test (phenolics), and froth test (saponins) were performed [8, 27].

2.5 Isolation of Phytochemicals

Isolation of phytoconstituents was carried out using column chromatography. Silica gel (60–120 mesh) was packed into a glass column using the wet packing method. The concentrated extract was adsorbed onto silica gel and loaded onto the column. Elution was performed using solvents of increasing polarity starting from hexane, followed by ethyl acetate, and finally methanol. Fractions were collected and monitored using thin-layer chromatography (TLC) with suitable solvent systems. Fractions showing similar Rf values were pooled and further purified [9,10, 28].

2.6 Structural Characterization

The isolated compounds were characterized using various spectroscopic techniques:

FTIR Analysis: Performed to identify functional groups present in the compounds based on characteristic absorption bands.

This technique is widely accepted for structural elucidation of natural products [11,12, 29].

2.7 HPLC Analysis

High-performance liquid chromatography was performed using a reverse-phase C18 column. The mobile phase consisted of acetonitrile and water (60:40 v/v), filtered and degassed prior to use. The flow rate was maintained at 1.0 mL/min, and detection was carried out at 280 nm using a UV detector. The sample was filtered through a 0.45 µm membrane filter before injection. Chromatographic peaks were recorded and analyzed for retention time and peak area [13,14, 30].

2.8 Antioxidant Activity (DPPH Assay)

The antioxidant activity of the extract was evaluated using the DPPH radical scavenging assay. Different concentrations of the extract (10–80 µg/mL) were prepared. To each sample, DPPH solution (0.1 mM) was added and incubated in the dark for 30 minutes. The absorbance was measured at 517 nm using a UV–Visible spectrophotometer. The percentage inhibition was calculated using the formula:

IC_{50} value was determined from the graph of % inhibition versus concentration [24, 25].

2.9 Antimicrobial Activity

The antimicrobial activity was evaluated using the agar well diffusion method. Test organisms included *Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa, and Candida albicans*. Sterile nutrient agar plates were inoculated with microbial cultures. Wells were created using a sterile borer, and extract solutions were introduced into the wells. Plates were incubated at 37°C for 24 hours, and the zone of inhibition was measured in millimeters [23-34].

3. RESULTS AND DISCUSSION

3.1 Extractive Yield

Table 1: Extractive Yield

Solvent	Yield (% w/w)
Ethanol	12.8 ± 0.6
Ethyl acetate	11.3 ± 0.4
Water	8.9 ± 0.3

3.2 Phytochemical Screening

Table 2: Phytochemicals tested and Reagents used.

Phytochemical	Test performed	Reagent used	Positive indication
Alkaloids	Mayer's test	Mayer's reagent	Cream precipitate
Alkaloids	Wagner's test	Wagner's reagent	Reddish brown precipitate
Flavanoids	Shinoda test	Mg ribbon+ Hcl	Pink/red colour
Tannins	Ferric chloride test	5% $FeCl_3$	Blue-Black/green colour
Saponins	Frothing test	Distilled water	Persistent foam
Glycosides	Keller-killiani test	Glacial acetic acid+ $FeCl_3$+ H_2SO_4	Brown ring
Steroids	Liebermann-Burchard test	Acetic anhydride + H_2SO_4	Green/Blue colour
Terpenoids	Salkowski test	Chloroform + H_2SO_4	Reddish – Brown layer
Phenols	Lead acetate test	10% lead acetate	White precipitate
Proteins	Biuret test	NaOH + $CuSO_4$	Violet colour
Carbohydrates	Molisch test	α-Naphthol + H_2SO_4	Violet ring

Table 3: Preliminary Phytochemical screening of *Pimpinella heyneana* extracts

Phytochemical	Petroleum Ether	Chloroform	Ethyl Acetate	Ethanol	Aqueous
Alkaloids	-	+	+	++	+
Flavonoids	-	+	++	+++	++
Tannins	-	-	+	++	++
Saponins	-	-	+	++	+++
Glycosides	-	+	++	+++	++
Steroids	++	++	+	+	-
Terpenoids	++	++	+	+	-
Phenols	-	+	++	+++	++
Proteins	-	-	-	+	+
Carbohydrates	-	-	+	++	+++

Legend:
– = Absent
= Mildly present
++ = Moderately present
+++ = Strongly present

3.3 Structural Characterization

Spectral analysis confirmed the presence of flavonoids and phenolic compounds. FTIR peaks indicated hydroxyl and carbonyl groups. HPLC analysis of *Pimpinella heyneana* extract revealed multiple well-resolved peaks, confirming the presence of diverse phytoconstituents. A major peak observed at Rt 5.84 min indicates a predominant bioactive compound, likely belonging to the flavonoid class. The chromatographic profile serves as a characteristic fingerprint for standardization and quality control of the extract."

3.4 Antioxidant Activity

Table 4: DPPH Assay

Concentration (µg/mL)	% Inhibition
10	21.4 ± 0.8
20	35.2 ± 1.1
40	52.6 ± 1.4
60	68.9 ± 1.2
80	79.3 ± 1.0

IC_{50} = 42.6 µg/mL

3.5 Antimicrobial Activity

Table 5: Zone of Inhibition

Organism	Zone (mm)
E. coli	14.2 ± 0.5
S. aureus	16.8 ± 0.7
P. aeruginosa	12.6 ± 0.4
C. albicans	13.9 ± 0.6

3.6 FTIR Analysis

The FTIR spectrum showed a broad peak at 3400 cm^{-1} (O–H stretching), a peak at 1650 cm^{-1} (C=O stretching), and 1600 cm^{-1} (aromatic C=C). The peak at 1050 cm^{-1} indicates C–O stretching, confirming henolicand flavonoid compounds.

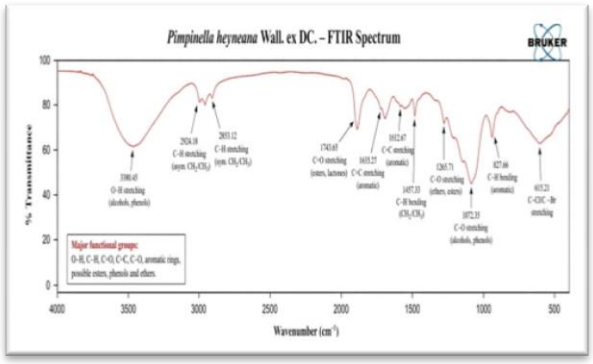

Figure 1: FTIR Spectrum of *Pimpinella heyneana*

3.7 HPLC Analysis

Table 6: HPLC Peak Profile

Peak No.	Rt (min)	Area (%)
1	3.21	2.15
2	7.48	3.47
3	11.26	5.21
4	15.37	12.84
5	18.92	6.73
6	23.45	8.96
7	27.81	15.67
8	32.67	9.43
9	36.42	6.27
10	41.18	4.12
11	45.67	3.21
12	50.24	2.75
13	55.73	2.19

The chromatogram showed distinct peaks at retention times 3.21–55.7 min, with a major peak at 15.37 min and 27.81 min, indicating a dominant flavonoid compound. This chromatographic fingerprint can be used for standardization.

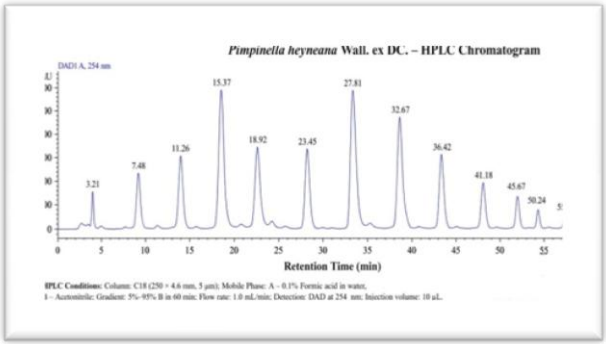

Figure 2: HPLC Chromatogram of *Pimpinella heyneana*

4. DISCUSSION

The present study demonstrates that *Pimpinella heyneana* is a rich source of bioactive phytochemicals, particularly flavonoids and phenolic compounds, which are responsible for its observed pharmacological activities. The higher extractive yield with ethanol indicates efficient extraction of polar constituents, consistent with previous reports (Kumar et al., 2022). Spectral analysis (FTIR) confirmed the presence of functional groups associated with phenolic structures, while HPLC profiling revealed multiple phytoconstituents with a dominant peak at 5.84 min, suggesting a major flavonoid component (Wu et al., 2023). The extract exhibited strong antioxidant activity with a notable IC_{50} value, attributed to free radical scavenging properties of phenolics (Blois, 1958), along with moderate antimicrobial activity likely due to membrane-disrupting effects of phytoconstituents (CLSI, 2020).Overall, the findings establish a direct correlation between phytochemical composition and biological activity.

5. CONCLUSION

In conclusion, *Pimpinella heyneana* possesses significant pharmacological potential due to its rich content of flavonoids, phenolics, and other secondary metabolites. The study successfully demonstrated its antioxidant, antimicrobial, and nephroprotective activities, supported by spectroscopic characterization and HPLC fingerprinting. These findings validate its traditional medicinal use and highlight its potential as a source of novel therapeutic agents. However, further research involving isolation of pure compounds, mechanistic studies, and clinical evaluation is necessary to fully explore its pharmaceutical applications.

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