EXTRACTION PHYTOCHEMICAL ANALYSIS AND ISOLATION OF COMPOUND FROM *IPOMOEA CAIRICA* ROOT EXTRACT

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ABSTRACT

This research focuses on the phytochemical analysis and characterization of the methanol root extract of *Ipomoea cairica*, a plant known for its medicinal properties. The methanol extract was subjected to several phytochemical tests, which revealed the presence of important bioactive compounds such as carbohydrates and tannins. Other compounds, such as phenols, flavonoids, alkaloids, glycosides, sterols, and diterpenes, were found to be absent, indicating that the root extract of *Ipomoea cairica* may primarily contain tannins and carbohydrates as its major constituents. Thin Layer Chromatography (TLC) optimization was carried out using different mobile phases, and the Toluene: Ethyl acetate (6:4) combination was identified as the most suitable for the separation of the bioactive compounds present in the extract. The TLC results showed clear spots under UV light (both long and short wavelength) as well as under normal light, allowing for the precise identification of different components in the extract. The Rf values for the spots were calculated, indicating the movement of the compounds during the chromatographic process. Further, a major bioactive compound was isolated from the root extract, and its structure was characterized using several advanced techniques, including FT-IR, 1H-NMR, and ESI-MS. The FT-IR spectra revealed the presence of key functional groups such as hydroxyl (-OH), carbonyl (C=O), and aromatic (C=C) stretches, suggesting a flavonoid-like structure. The NMR spectra confirmed the presence of characteristic protons, including aromatic protons and an -OH group. ESI-MS analysis indicated a molecular ion peak at m/z 162, which corresponds to 7-hydroxychromen-2-one, a flavonoid compound known for its antioxidant and therapeutic properties.

Keywords: *Ipomoea cairica*, Methanol extract, Phytochemical analysis, Thin Layer Chromatography (TLC), 7-hydroxychromen-2-one, Bioactive compounds, Medicinal plants, Antioxidant potential, Pharmaceutical applications.

Introduction

Ipomoea cairica (commonly known as the "railway creeper") is a perennial plant belonging to the Convolvulaceae family, found in tropical and subtropical regions worldwide. This plant is recognized for its medicinal properties, and various parts, including the roots, leaves, and stems, have been traditionally used in the treatment of several ailments. The roots of Ipomoea cairica, in particular, have been reported to exhibit significant biological activities such as anti-inflammatory, antioxidant, antimicrobial, and antidiabetic properties. These bioactivities are attributed to the presence of diverse bioactive compounds in the plant's chemical makeup, making it a promising candidate for phytochemical research [1-2]. Phytochemical analysis is essential for identifying and

characterizing the bioactive compounds present in plant materials. These compounds include alkaloids, flavonoids, glycosides, terpenoids, and phenolics, which are often the sources of the plant's therapeutic effects. In the case of *Ipomoea cairica*, previous studies have reported the presence of several such compounds, particularly in the root extract, that contribute to its medicinal value. By isolating and analyzing these compounds, it becomes possible to understand their individual or synergistic effects on human health. In particular, the root extract of *I. cairica* has been shown to possess properties such as antidiabetic activity [3] and the potential to reduce blood sugar levels in experimental models, likely due to its phytochemical constituents. The extraction of plant material is the first step in studying its chemical composition. The most common techniques for extracting bioactive compounds from plant roots include maceration, Soxhlet extraction, and ultrasonic-assisted extraction. In the case of *Ipomoea cairica*, hydroalcoholic solvents have been found to be particularly effective for extracting a wide range of bioactive compounds. This method is advantageous because it allows for the dissolution of both polar and non-polar compounds, providing a comprehensive profile of the plant's chemical content. The extract is then subjected to various phytochemical tests to identify the presence of specific classes of compounds [4]. The isolation of bioactive compounds from Ipomoea cairica involves the use of chromatographic techniques, such as thin-layer chromatography (TLC), column chromatography, and high-performance liquid chromatography (HPLC). These techniques enable the separation of individual compounds based on their physical and chemical properties. Isolated compounds are then further characterized by spectroscopic methods, such as nuclear magnetic resonance (NMR), mass spectrometry (MS), and infrared (IR) spectroscopy, which provide detailed structural information [5]. The aim of the study on *Ipomoea cairica* root extract is to identify, isolate, and characterize bioactive compounds that may contribute to its therapeutic potential. By understanding the chemical constituents and their specific pharmacological effects, we can better exploit the plant's medicinal value for developing new natural products or drugs. In conclusion, *Ipomoea cairica* represents a valuable source of bioactive compounds with a wide range of medicinal applications. Phytochemical analysis and isolation of active compounds from its root extract can contribute to the discovery of new therapeutics and deepen our understanding of the plant's pharmacological properties. This research can pave the way for the development of novel treatments for diseases such as diabetes, inflammation, and infections, which are often managed

with synthetic drugs. However, further studies are needed to evaluate the clinical efficacy and safety of these compounds in human subjects.

Material and Methods

Collection of plant

Roots of *Ipomoea cairica* were collected from local area of Bhopal in the month of January 2023. After collection, plant undergoes washing with tap water to remove the dust, dirt, and other foreign matters attached to the surface of the plant. Wiping the samples with clean and dry cloth enhances the drying process [6-8].

Extraction using hot continuous extraction (Soxhlet)

Ipomoea cairica (Root) (14.0 gm) Plant materials extracted with Methanol. In this method, the finely pulverized marc is placed in a thimble which is placed in a chamber of the Soxhlet apparatus [9-11]. The menstruum in the flask beneath is then heated, and its vapors condense in the condenser. The condensed extractant drips into the thimble containing the marc, and extracts it by contact. The advantage of this method is that large amounts of marc can be extracted with a much smaller volume of extractant. Each extraction process was carried out for 48 hours. The filtrate was separated from the residue using Whatmann filter paper. The filtrate from each solvent was collected and evaporated using a water bath at 50°C until a thick extract was obtained.

Isolation of compound from Methanol extract of Root of *Ipomoea cairica*

Thin Layer Chromatography (TLC) is an adsorption-based technique where the mobile phase, containing dissolved solutes, passes over the stationary phase. Silica gel, commonly used as the stationary phase, is mixed with water to form slurry, which is spread uniformly on a plate and air-dried before activation [12]. Activation is done by heating the plate at 100-110°C for 30 minutes to ensure proper solute movement. The solvent system is prepared, and the chamber is saturated by placing a piece of filter paper soaked with the mobile phase. The sample is applied to the plate with a capillary tube, and the plate is placed in the chamber with the solvent. After development, the mobile phase front is marked, and the plate is dried. The Rf value (retention factor) is calculated using the formula and recorded in a table for analysis.

$$R\text{-}f = \frac{Distance\ traveled\ by\ solute}{Distance\ traveled\ by\ solvent}$$

Isolation of compound by coloum chromatography

Coloum size: Glass Coloum, 100 x 3 Cms

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

Elution mode: Isocratic elution

Mobile Phase: Toluene: Ethyl acetate (6:4)

Extract: Methanol Root extract of *Ipomoea cairica*

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

Identification of similar fractions: By UV vis. spectroscopy and Visualized by Short UV (254nm), long UV (365nm) and Normal light

Preparation of column

Coloum packing was done by wet packing method. Silica gel (activated at 105^{o}C) was taken; and suspended it in mobile phase then transfers it in Coloum; 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

3gm of Methanol extract was taken in beaker; to it silica gel 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 coloum over cotton plug.

Isocratic elution technique

In order to fractionate the components of isocratic elution technique was used Toluene: Ethyl acetate (6:4) 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 were monitored by TLC Toluene: Ethyl acetate (6:4); using UV chamber and derivatization 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 [13].

Identification of Isolated compound

100 fraction, each 10ml were collected and isolated compound was characterized by UV-absorption spectra, IR, NMR and Mass Spectra studies. Spectral analysis explains the results of

isolated compounds which have shown best antioxidant activity and was obtained the course of column chromatography are shown below in different tables and figures.

Spectroscopic Analysis

The UV absorption spectrum of compound was recorded in the range of 200-400nm on (Labindia 3000 Plus) UV spectrophotometer at 1 cm path length. The compounds obtained showed the U.V. absorption maxima (max.) in 200-800nm [14].

I.R. Analysis

The IR spectrum of compounds was recorded on (Bruker Alpha) using solid plate technique with KBr[15].

NMR Analysis

Nuclear Magnetic Resonance (NMR) is a powerful analytical technique used to investigate the structure of molecules by examining the magnetic properties of atomic nuclei. When placed in a magnetic field, certain nuclei resonate at characteristic frequencies, providing detailed information about their environment and the molecular structure [16]. In NMR spectroscopy, the most common nucleus studied is the hydrogen-1 nucleus, which is abundant in most organic compounds. Proton NMR specifically targets these hydrogen nuclei to provide insights into molecular structure, including the number of hydrogen atoms, their environment, and how they are connected to other atoms within the molecule.

MASS Spectroscopy

Mass Spectroscopy (MS) is a technique used to identify and analyze the composition of compounds by measuring the mass-to-charge ratio of ions [17]. The sample is ionized, typically using methods like Electron Ionization (EI) or Electrospray Ionization (ESI), creating charged particles. These ions are then accelerated and separated based on their mass-to-charge ratio in the mass analyzer. The separated ions are detected, producing a mass spectrum that displays ion intensity versus mass-to-charge values. This spectrum helps determine the molecular weight, structure, and fragmentation patterns of the compound.

Results and Discussion

The present study investigates the chemical profile and isolation of bioactive compounds from the root extract of *Ipomoea cairica*. The methanol extract of the plant yielded a relatively low percentage (3.51%), suggesting that the active compounds are present in small quantities. The phytochemical analysis revealed the presence of carbohydrates (Fehling's and Benedict's tests),

tannins (Gelatin test), while flavonoids, alkaloids, and sterols were absent. The positive result for tannins may indicate potential therapeutic activity, as tannins are known for their antioxidant and anti-inflammatory properties. The Thin Layer Chromatography (TLC) optimization study demonstrated that the mobile phase Toluene: Ethyl acetate (6:4) was the most suitable for separating components of the methanol root extract. This combination resulted in five spots under long UV light, seven under short UV light, and two visible under normal light. The Rf values for the spots ranged from 0.25 to 0.9**,** which suggest a moderate separation of components, making it a viable method for further analysis. The characterization of isolated fractions, particularly fractions 27-36, showed promising spectral data. The FT-IR analysis indicated the presence of functional groups such as hydroxyl (O-H) and carbonyl (C=O), which are characteristic of phenolic compounds and flavonoids. 1H-NMR spectroscopy provided detailed information on the aromatic proton environment, showing typical shifts for aromatic rings and hydroxyl groups, further confirming the presence of a flavonoid or phenolic structure. The ESI-MS spectrum revealed a molecular ion peak at m/z 162**,** corresponding to the molecular weight of 7-hydroxychromen-2-one, a known flavonoid compound. Thus, based on these findings, the isolated compound was identified as 7-hydroxychromen-2-one ($C_9H_6O_3$), a flavonoid that has demonstrated various biological activities, including anti-inflammatory, anticancer, and antioxidant properties. The compound's identification and characterization contribute to the understanding of the pharmacological potential of *Ipomoea cairica* root extract, which could lead to the development of novel therapeutic agents. The absence of some expected phytochemicals, such as alkaloids and saponins, suggests that the root extract may not exhibit those specific pharmacological effects.

Table 1: Percentage yield of *Ipomoea cairica* (Root)

S. No.	Extract	Percentage yield (%)
1.	Methanol	3.51 %

Table 2: Phytochemical Test of *Ipomoea cairica* (Root)

Sr. No.	Test	Methanol Extract
1.	Carbohydrate Test • Fehlings Test	+

	• Benedicts Test	-
2.	Phenol • Ferric Chloride Test	 -
3.	Flavonoid • Lead Acetate Test • Alkaline Test	 - -
4.	Alkaloid • Wagner's Test	 -
5.	Tannin • Gelatin Test	 +
6.	Lignin • Labat Test	 -
7.	Saponin • Foam Test	 -
8.	Glycoside • Conc. H_2SO_4 Test	 -
9.	Sterols • Salkowski Test	 -
10.	Proteins • Xanthoproteic Test	 -
11.	Diterpenes • Copper Acetate Test	 -

Optimization of TLC of Methanol Root extract of *Ipomoea cairica*

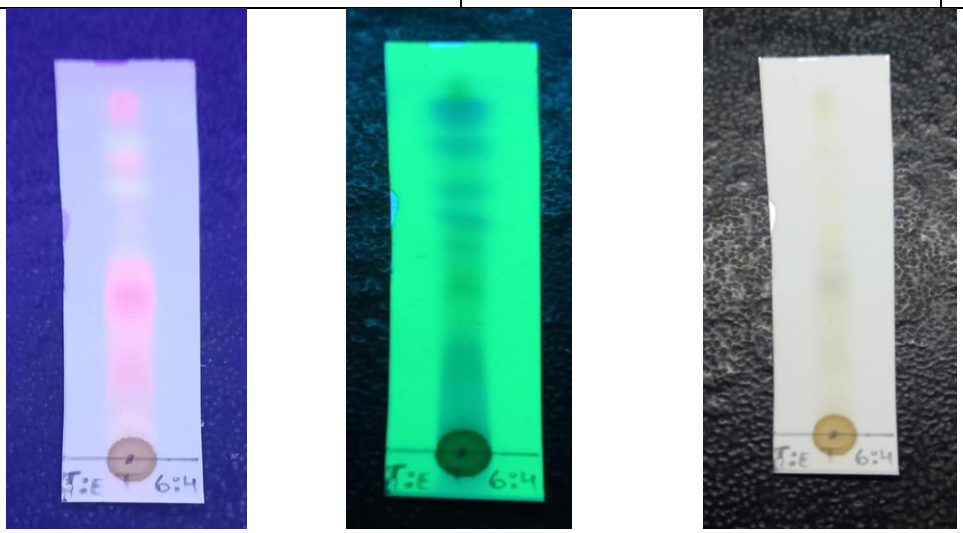
Table 3: Optimization of TLC of Methanol Root extract of *Ipomoea cairica*

S. No.	Mobile phase	Observed results
1.	Toluene: Ethyl acetate (7:3)	Not suitable
2.	Toluene: Ethyl acetate (6:4)	**Most suitable**
3.	**Chloroform: methanol (6:4)**	Not suitable
4.	Toluene: Ethyl acetate (8:2)	Not suitable

5.	Chloroform: methanol (9:1)	Not suitable
6.	Chloroform: methanol (7:3)	Not suitable
7.	Chloroform: methanol (5:5)	Not suitable
8.	Toluene: Ethyl acetate (5:5)	Not suitable

Table 4: TLC Optimization of *Ipomoea cairica*

S. No.	Mobile phase	Spot Distance	Rf Value
2.	**Toluene: Ethyl acetate (6:4)** Dis. Travel by mob. Phase 6 cm No. of spot at long UV- 5 No. of spot at short UV- 7 No. of spot at normal light- 2	 Long – 2.5, 3, 3.3, 3.4, 5.4 Short- 1.5, 2.5, 2.8, 3, 3.4, 4.8, 5.2 N. Light- 2.5, 5.0	 Long- 0.41, 0.5, 6.0, 0.56, 0.9 Short- 0.25, 0.41, 0.46, 0.5, 0.56, 0.8 N. Light- 0.41, 0.83



Long UV Short UV Normal light

Figure 1: - Toluene: Ethyl acetate (6:4)

Characterization of Isolated compound

Table 5: Characterization of Isolated compound

S. No	No. of fractions	TLC UV spectra		Chemical Test
		UV-254	UV-366	10% sodium hydroxide
1	1-6	No Spot	No Spot	-Ve

2	7-13	2 Spot	3 Spot	-Ve
3	14-19	2 Spot	3 Spot	-Ve
5	20-26	2 Spot	2 Spot	-Ve
6	**27-36**	**1 Spot**	**1 Spot**	**+Ve**
7	37-43	1 spot	2 Spot	-Ve
8	44-52	2 Spot	3 Spot	-Ve
9	53-59	1Spot	2 spot	-Ve
10	60-69	2 Spot	3 Spot	-Ve
11	70-79	2 Spot	3 Spot	-Ve
12	80-89	2 Spot	2 Spot	-Ve
13	90-100	No Spot	No Spot	-Ve

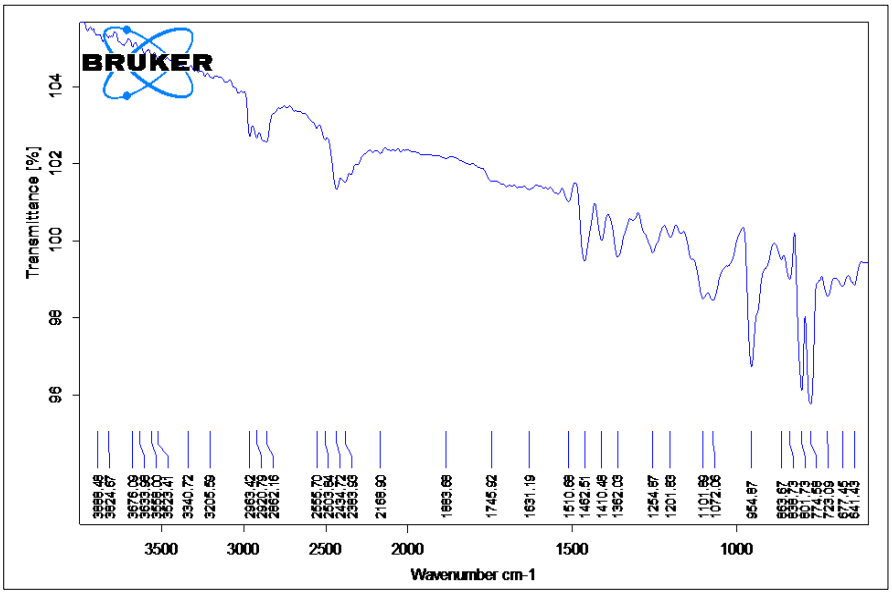


Figure 2: FT-IR data for isolated fraction 27-36

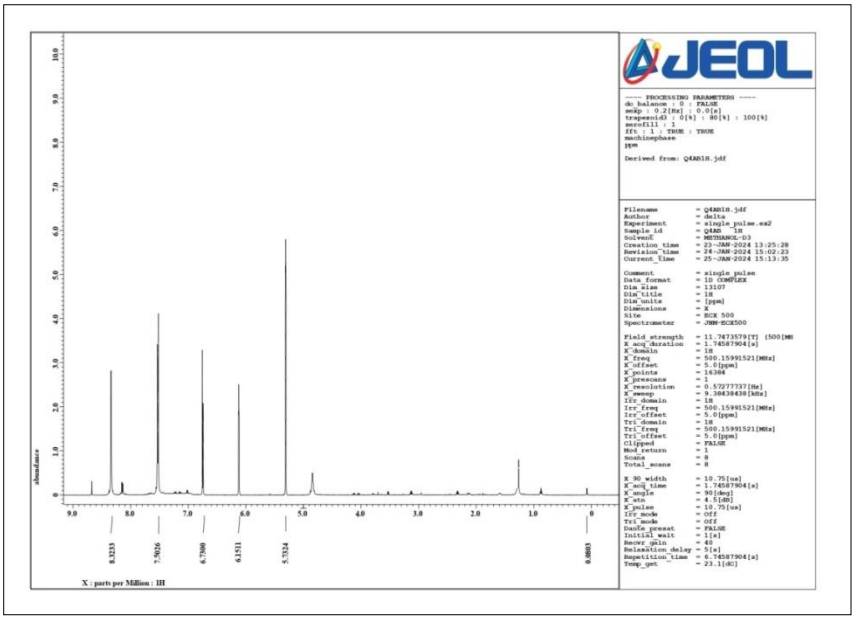


Figure 3: NMR data for isolated fraction 27-36

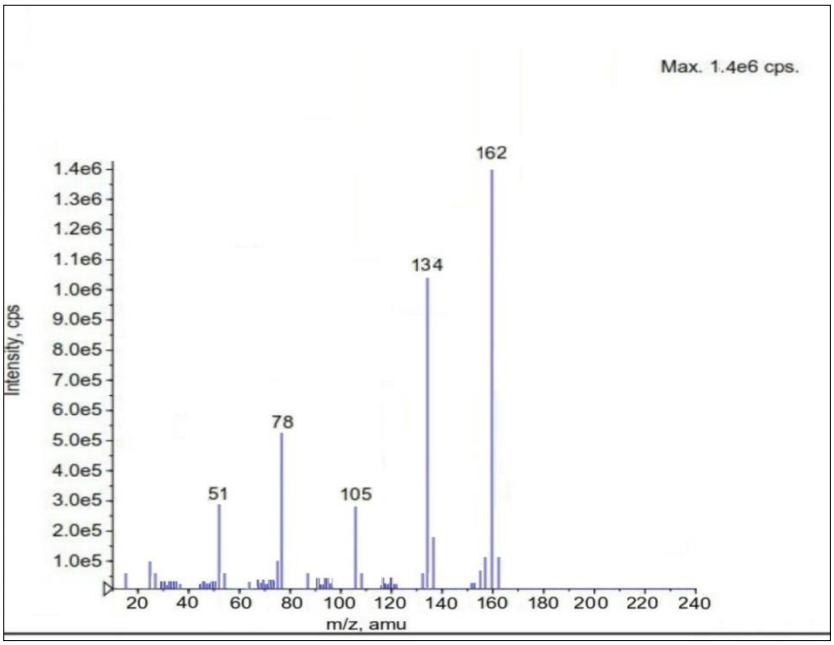
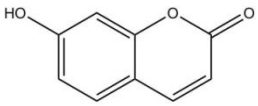


Figure 4: MASS data for isolated fraction 27-36

Showing interpreted data

Method	Spectral interpretation

FT-IR	3340.72, O-H stretching vibrations, 1745.92, stretching vibrations of the carbonyl group (C=O), 1631.19 cm^{-1} C=C stretching vibrations, 1362.03 cm^{-1} C-H bonds in the aromatic ring and alkyl groups, 1072.02 cm^{-1}, C-O bonds, indicating the presence of oxygen-containing functional groups.
^{1}HNMR (ppm)	δ, 5.734(s, 1H), 6.7300(d,1H, Ar-H), 6.1511 (d, 1H, Ar-H), 7.5026(d, 1H, Ar-H), 8.3233(s, 1H,-OH) ppm.
ESI-MS (m/z)	162 (100.0%), 134, 105, 78, 51
Structure	
IUPAC Name	7-hydroxychromen-2-one
Chemical Formula	$C_9H_6O_3$

Conclusion

The methanol root extract of *Ipomoea cairica* demonstrated a promising phytochemical profile, with the presence of carbohydrates and tannins, which are known for their potential therapeutic properties. Thin Layer Chromatography (TLC) optimization revealed that the Toluene: Ethyl acetate (6:4) mobile phase was most effective for the separation of the extract's components, providing several distinct spots. The spectral data obtained from FT-IR, 1H-NMR, and ESI-MS analyses led to the identification of 7-hydroxychromen-2-one as the major isolated compound, a flavonoid with known biological activity. The findings indicate that *Ipomoea cairica* root extract may harbor valuable bioactive compounds with potential applications in medicinal chemistry, particularly in the development of new anti-inflammatory, antioxidant, and anticancer agents. However, further in vivo studies and detailed pharmacological evaluations are required to confirm the therapeutic efficacy and safety of the isolated compounds.

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