Pharmaceutical Sciences 2024: Navigating the Future of Drug Discovery and Development
November 2024

PHYTOCHEMICAL PROFILE OF LEAVES OF *IPOMOEA OBSCURA*

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Abstract

This research study discusses the process of extracting plant compounds, from the leaves of *Ipomoea obscura* using water and alcohol as solvents. Initial analysis of the plant compounds revealed that the ethanol and water extracts were rich in phenols, flavonoids, tannins, alkaloids and terpenoids. The identification of these compounds was carried out using UV spectroscopy. Ethanolic extract of leaves contains more phenolic compounds and flavonoids compared to alcohol extract. The ethanolic leaf extract, from this plant displayed the concentration of compounds at 82.03± 0.088 (GAE/gDM). Flavonoids were found to be the prevalent, with a concentration of 123,83 ± 0.120 (QE mg/g DM). The LCMS method was employed to ascertain the metabolites, in both extracts and a total of 16 compounds were identified as positive.

1. Introduction

Ipomoea is a diverse genus within the family Convolvulaceae, consisting of approximately 600 to 800 species that are primarily distributed across tropical and subtropical regions worldwide. This genus includes a variety of growth forms such as herbs, shrubs, lianas, and small trees, with many species characterized by their twining or climbing habits. Commonly known as morning glories, Ipomoea species are admired for their striking funnel-shaped flowers, which display a variety of colors, including violet, blue, pink, and red. Notable members include economically important crops such as sweet potato (*Ipomoea batatas*) and water spinach (*Ipomoea aquatica*), both of which have significant culinary uses worldwide. Furthermore, numerous Ipomoea species are grown as ornamental plants for their appealing blooms.

However, some species have become invasive in various regions, posing ecological threats. The genus also holds potential for medicinal applications, with various species traditionally used in herbal remedies for ailments ranging from diabetes to inflammation.[1] *Ipomoea obscura* is a small climbing vine, characterized by its small cordate (heart-shaped) leaves with an acuminate apex. The plant produces a corolla composed of five fully fused petals, contributing to its distinct funnel-shaped

flowers. It typically grows on fences or as low ground cover in disturbed areas, where it thrives in substrates such as soil or along structures. This plant is often found in tropical and subtropical environments, where its twining or climbing growth habit allows it to spread easily across surfaces.[2] *Ipomoea obscura* has been recognized in Ayurveda for its various medicinal properties. It is effectively used to treat dysentery, and its application extends to open sores and pustules. A paste made from the leaves is applied to ulcers, haemorrhoids, and swellings to provide relief. The seeds and fruits are known to act as cleansing agents, improving difficult breathing, alleviating pain, and potentially enhancing vision. In addition to its medicinal uses, the plant holds ornamental value as a climber with attractive flowers. Furthermore, *Ipomoea obscura* is also included in lists of plants that affect the central nervous system and is actively utilized for its antioxidant properties.[3]

2. Materials and methods

2.1. Reagents and solution preparations

All chemicals used were of analytical grade reagents. Ethanol, , Gallic acid, 7% Sodium carbonate, Folin-Ciocalteu reagent (FCR), Quercetin, ferric chloride ($FeCl_3$), Potassium Chloride, Ammonium molybdate Fehling solution A Fehling solution B Magnesium turnings Methanol Sodium nitrite Aluminium Chloride Sodium hydroxide Sodium carbonate Mayer's reagent Dragendorff's reagent Wagner's reagent Acetyl chloride nitric acid Sulphuric acid Copper Sulphate Benedict's reagent Barfoed's reagent Hydrochloric acid Ammonia were obtained from Nice Chemicals pvt. Ltd

2.2. Collection of plant samples

The leaves of *Ipomoea obscura* were harvested from sites, in Malappuram, Kerala. The collected plants were authenticated by the Centre for Medicinal Plants Research, Arya Vaidya Sala, Kottakkal, Kerala. After collection, the leaves were carefully cleaned thoroughly with tap water and allowed to naturally dry under ambient room temperature. Once dried, the leaves were ground into a fine powder using a mixer. The powdered samples were securely stored in an airtight glass bottle to maintain their quality for further analysis.

2.3. Instrumentation

UV–Vis spectrophotometer (Schimadzu, Japan) have a wavelength range from 190 nm to 1100 nm, allowing for the detection of both the phenolic and flavonoid compounds, which typically absorb in the UV-visible range (200-600 nm).

2.4 Extraction

Soxhlet extraction using 200 g of *Ipomoea obscura* leaf powder was done with 95% ethanol as the solvent for 24 hours. The solvent was subsequently removed and evaporated to dryness at 42°C under reduced pressure using a rotary evaporator. The extraction process yielded an extract with a 22% (w/w) yield.[4]

2.5. Procedure for phytochemicals screening

The initial phytochemical screening of the extracted plant samples was carried out using various chromophoric reagents. The crude extract was treated with the chemical reagents in test tubes, to identify the chemical constituents present.[5] The detailed procedure for the qualitative analysis of phytochemicals in the plant samples is presented in Table 1.

Sl no	Name of phytochemical	Method of detection	Observation
1	Alkaloid	Dragendorf's Test	orange-red colour
		Mayer's Test	creamy-white precipitation
2	Flavonoids	Shinoda Test	yellow coloured precipitate
		Ethyl Acetate Test	yellow colour
3	Phenols	Ferric Chloride Test	greenish-black color
4	Terpenoids	Liebermann-Burchard's Test	violet coloured ring at the junction
5	Steroids	Salkowski Test	green fluorescence
6	Glycosides	Baljet Test	yellow to orange colour
7	Saponins	Foam Test	persistent frothing
8	Carbohydrates	Molisch's Test	Violet ring at the junction of two liquids

Table 1: Method for preliminary phytochemical screening using different chemical reagents.[5]

2.6. Estimation of total phenolic contents

The Folin-Ciocalteu (F.C.) method is used to quantify total phenols, also known as the GAE method. This method involves reacting the sample with a reagent that produces a blue color, the intensity of which is proportional to the phenolic content. The procedure measures the quantity of phenolics required to prevent oxidation of the reagent. 0.1 g plant sample was taken into a conical flask containing 10 mL of ethanol and then ultra-sonicated for 15 min of extraction time. One ml of the sample solutions of the ethanol extract and aqueous extract was blended 1 mL of two-fold diluted FCR and after 5 min, 2 mL of 7.5% Na_2CO_3 was added and the total volume of solution mixture was made with 10 mL DW. It was kept under cover of darkness for 60 min and analyzed for absorbance at 765 nm. A standard curve based on gallic acid solutions was utilized to assess the total phenolic content. The phenolic content in plant samples was quantified using a calibration curve generated from the relationship between gallic acid concentration and its absorbance. The phenolic content was reported as milligrams of gallic acid equivalent per gram of dry weight of the powdered plant sample (mg GAE/g sample), along with the ± SD (standard deviation) for three replicate measurements.[6]

2.7. Estimation of total flavonoid contents

The total flavonoid content was determined according to the aluminum chloride-colorimetric method. The $AlCl_3$ colorimetric assay functions on the principle that aluminum chloride forms stable complexes with flavonoids. This reaction occurs through the interaction between the aluminum chloride and the carbonyl group at the fourth carbon position, as well as the hydroxyl groups at either the third or fifth carbon positions of flavones and flavonols. This complexation results in a color change, which can be measured spectrophotometrically to estimate the flavonoid content.[7]

0.1 g aliquots of each plant sample were extracted following the procedure outlined in Section 2.6. 1 mL of the sample solutions of the ethanol extract and aqueous extract was mixed with, 0.3 mL of 5 percent $NaNO_2$. After a five-minute interval, 0.3 ml of 10 percent $AlCl_3$ solution was introduced, subsequently, 2 ml of 1M NaOH was added. The final volume of the solution was made upto 10 mL with distilled water. The solution mixture was then left at room temperature for 30 minutes before measuring the absorbance at 415 nm using a spectrophotometer. A standard calibration curve was similarly constructed by plotting the absorbance values against the concentrations of the quercetin standard. The results were expressed as milligrams of quercetin equivalents per gram of dry weight of the powdered plant samples (mg QE/g sample), along with the ± SD (standard deviation) for three replicate analyses.[6,8,9,11]

2.8 LC MS analysis

LC-MS is a sophisticated technique commonly used for screening bioactive secondary metabolites. It is a simple, fast, and widely accepted method for identifying bioactive molecules from crude plant extracts, requiring only a small amount of the sample. In this investigation, LC-MS was employed to detect and identify the bioactive compounds in the leave extracts of *Ipomoea obscura*. This technique allows for efficient and precise analysis, contributing significantly to the understanding and exploration of plant-based bioactive compounds**.** The chemical constituents of the ethanolic and aqueous extracts of *Ipomoea obscura* were determined using LC-MS. LC-MS analysis was performed using Mariner Bio spectrometry equipped with a binary pump. The HPLC was interfaced with a Q-TOF mass spectrometer equipped with an ESI source. Full-scan mode was conducted over an m/z range of 100 to 1200, with a source temperature set at 140°C. HPLC column Phenomenex 5µ C8, (150 × 2 mm i.d.) was used for the analysis. Solvent was methanol with 0.3% formic acid. The solvents were delivered at a flow rate of 0.1 mL/min using isocratic elution. MS spectra were obtained in positive ion mode.[11,12]

3. Results and discussion

3.1 Extraction yield

The choice of organic solvent plays an important role in the separation of specific chemicals from plant samples. The extraction yield depends on the chosen solvent as it targets different phytochemicals. It depends on their polarity, which affects the efficiency of extraction. Water and alcohol were selected as solvents for *Ipomoea obscura* leaf extraction because of their polarity to efficiently extract a variety of phytochemicals. Alcohol is particularly effective in extracting phenolic compounds, flavonoids, and other bioactive compounds, while water helps to extract more polar compounds, including certain glycosides and tannins. The present study demonstrated that the ethanolic extract of *Ipomoea obscura* had a yield of 2.05%, while the aqueous extract yielded 3.07%.

3.2. Screening of phytochemicals using reagents

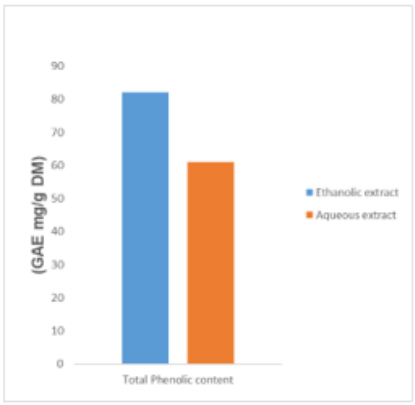
Preliminary phytochemical examination of the alcohol and aqueous extracts of *Ipomoea obscura* leaves revealed the presence of alkaloids, flavonoids, phenolic acids, terpenoids, tannins, saponins, carbohydrates, proteins and quinones. The results are summarized in Table 2. Alkaloids, glycosides and terpenoids were detected in the ethanol extract. Flavonoids, phenolics, and carbohydrates were found in both ethanol and aqueous extracts. Saponins were present in the aqueous extract. Proteins, quinones, and oxalates were absent in both extracts.

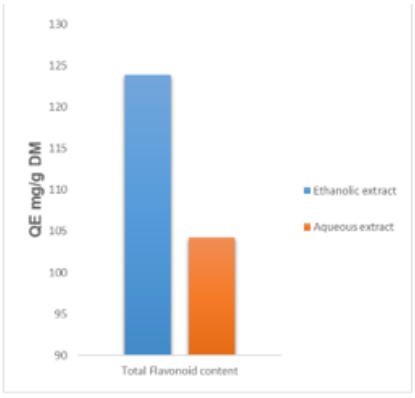
Sl. no	Chemical Test	Ethanolic extract	Aqueous extract
1	Alkaloids	+	-
2	Flavonoids	+	+
3	Phenolic compounds	+	+
4	Terpenoids	+	-
5	Steroids	+	-
6	Glycosides	+	-
7	Saponins	-	+
8	Carbohydrates	+	+

Table 2: Preliminary phytochemical screening of all extracts (+ indicates presence and - indicates absence of phytoconstituents)

3.3. Determination of total phenolic and flavonoid contents in the plant extracts

Phenolic compounds and flavonoids are natural substances known to have strong antioxidant properties. These compounds are found in different parts of plant which contributes to the health benefits of plants. Total phenolic and flavonoid contents in the leaf extracts of *Ipomoea obscura* are given in Table 2. Total phenolic contents were expressed as GAE using the standard curve equation (y = 0.0028x+ 0.0166), where the correlation coefficient (R2) was 0.9956. Ethanolic leaf extract of the plant, had the highest amount of phenolic compounds with a concentration of 82.03 ± 0.088 (GAE/gDM). The total flavonoid content of the extract was expressed as QE mg/g dry weight (DM) using the standard curve equation (y = 0.0037x - 0.0331), with the correlation coefficient (R2) 0.9943. The highest flavonoid content was observed in ethanolic extract with a concentration of 123.83 ± 0.120 (QE mg/g DM) compared with aqueous extract.





3.4 LC MS

The preliminary identification of compounds in the ethanolic and aqueous extracts was conducted through LC-MS analysis. Compounds were detected and identified based on their fragmentation patterns and by analysing their peak areas and retention times. Nine compounds were identified in the ethanolic extract of *Ipomoea obscura* leaves. The main compounds detected were 2,2-dimethyl-1,3-butanediol, bergamotol, Z-α-trans, β-sitosterol, quinic acid, 3-methoxy-2,2-dimethyloxirane, heptadecane, 2-cholestanone, 3-phenyl-, caffeic acid, quercetin, and 3,5-di-O-caffeoylquinic acid methyl ester.

Sl.No	Phytochemicals	Molecular formula	m/z (g/mol)
1	2,2-dimethyl-1,3-butanediol	$C_6H_{14}O_2$	118
2	Bergamotol, Z-α-trans-	$C_{15}H_{24}O$	220
3	Quinic acid	$C_7H_{12}O_6$	192
4	3-methoxy-2,2-dimethy loxirane	$C_5H_{10}O_2$	102
5	Heptadecane	$C_{17}H_{36}$	240
6	2-Cholestanone, 3-phenyl-	$C_{33}H_{50}O$	462
7	Caffeic acid	$C_9H_8O_4$	180
8	Quercetin	$C_{15}H_{10}O_7$	302
9	3,5-di-O-caffeoylquinic acid methyl ester	$C_{26}H_{26}O_{12}$	530

Seven compounds were identified in aqueous extract of *Ipomoea obscura* leaves. The prevailing compounds were 2,2-dimethyl-1,3-butanediol, 3-methoxy-2,2-dimethyloxirane, 3,7-dimethyl-7-octen-1-ol, Butane-1,2,3,4-tetraol, Butanoic acid, 2-oxo, cyclohexanemethanol, 4-ethenyl-α,α,4-trimethyl-3-(1-methylethenyl)-, methyl 6-deoxy-alpha-L-galactopyranoside. The identified compounds are presented in Tables 3 and 4 along with their experimental m/z values and molecular formulas.

Table 3: Compounds identified from the ethanolic extract of *Ipomoea obscura* leaves by LC-MS

Sl.NO	Phytochemicals	Molecular formula	m/z (g/mol)
1	2,2-dimethyl-1,3-butanediol	$C_6H_{14}O_2$	118
2	3-methoxy-2,2-dimethyloxirane	$C_5H_{10}O_2$	102
3	3,7-dimethyl-7-octen-1-ol	$C_{10}H_{20}O$	156
4	Butane-1,2,3,4-tetraol	$C_4H_{10}O_4$	122
5	Butanoic acid, 2-oxo	$C_4H_6O_3$	102
6	cyclohexanemethanol, 4-ethenyl-α,α,4-trimethyl-3-(1-methylethenyl)-	$C_{15}H_{26}O$	222
7	methyl 6-deoxy-alpha-L-galactopyranoside	$C_7H_{14}O_5$	178

Table 4: Compounds identified from the aqueous extract of *Ipomoea obscura* leaves by LC-MS

4. Conclusions

In summary, *Ipomoea obscura* leaf extract contains many phytochemicals, makes it a promising candidate for use as a phytotherapy with their further study. This extract is rich in phenolic compounds and flavonoids, known to have important antioxidant properties, suggests potential therapeutic benefits, particularly in the management of oxidative stress-related diseases. However, more research is needed to fully explore the medicinal properties of *Ipomoea obscura* and to establish its efficacy and safety for clinical use.

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