Pharmacognostic Approaches on Qualitative and Quantitative Phytochemical Estimation of *Ipomoea obscura* (L.) Ker-Gawl leaf extract

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ABSTRACT

Ipomoea obscura (L.) Ker-Gawl, a medicinal plant belonging to the family Convolvulaceae, has been widely used in traditional medicine for the treatment of various ailments, including inflammatory disorders, skin diseases, jaundice, and headache. Despite its ethnomedicinal importance, comprehensive information regarding its phytochemical composition remains limited. Therefore, the present study aimed to investigate the qualitative and quantitative phytochemical profile of the ethanolic leaf extract of *I. obscura* to generate baseline pharmacognostic data for its standardization and future therapeutic exploration. *I. obscura* leaves were collected and extracted by using 70% ethanol in a soxhlet apparatus. Preliminary phytochemical screening was performed using standard qualitative tests to detect major classes of secondary metabolites. Quantitative estimation of total alkaloids, flavonoids, phenolics, saponins, and steroids was carried out using spectrophotometric methods employing atropine, quercetin, gallic acid, diosgenin, and cholesterol as standards, respectively. The ethanolic leaf extract exhibited an extractive value of 10.85% w/w. Qualitative analysis revealed the presence of alkaloids, flavonoids, phenols, phytosterols, tannins, terpenoids, saponins, and glycosides, while fixed oils and fats were absent. Quantitative analysis demonstrated that the extract contained 10.34 ± 2.30 mg AE/g of alkaloids, 9.32 ± 0.64 mg QE/g of flavonoids, 5.84 ± 0.77 mg GAE/g of phenolics, 7.45 ± 0.22 mg diosgenin/g of saponins, and 2.13 ± 0.86 mg cholesterol/g of steroids. Alkaloids and flavonoids were identified as the predominant phytoconstituents. In conclusion, the phytochemical profile generated in this study provides valuable pharmacognostic standards for authentication and quality control and highlights the potential of *I. obscura* as a promising source for the development of standardized herbal formulations and future pharmacological investigations.

Keywords: *Ipomoea obscura*; Pharmacognostic evaluation; Phytochemical screening; Quantitative estimation; Flavonoids; Alkaloids.

1. INTRODUCTION

Since ancient times, plants have been the mainstay of traditional medicine, and the ethnopharmacological understanding of them continues to drive modern drug development (1). There are more than 600 species in the genus Ipomoea, which is part of the Convolvulaceae family and is found in tropical and subtropical parts of the world (2). *Ipomoea obscura* (L.) Ker-Gawl is a thin, twining herb that grows throughout South and Southeast Asia, Africa, and the Indian subcontinent. It is also referred to as the obscure morning glory or "Lakshmanvel" in India (3). The plant has significant ethnobotanical value because it has long been used in traditional medicine to treat ailments like headache, jaundice, skin issues, and inflammatory illnesses (4, 5). Both the plant's leaves and aerial parts have been used in Ayurvedic medicine, and phytochemical analyses have shown the presence of flavonoids, such as kaempferol and quercetin, phenolic acids, and tropane alkaloids compounds that are credibly associated with the plant's purported medicinal properties (6).

A wealth of medicinal agents can be found in phytochemicals, which are physiologically active secondary metabolites produced by plants as defense and signaling molecules (7). A wide range of pharmacological actions, including antioxidant, antibacterial, anti-inflammatory, anticancer, and hepatoprotective properties, have been shown by substances including alkaloids, flavonoids, terpenoids, saponins, tannins, phenolic acids, and glycosides (8, 9). One of the biggest families of small molecular secondary metabolites, flavonoids exhibits a variety of pharmacological and advantageous health effects, such as the ability to scavenge free radicals, hepatoprotective activity, and anticancer characteristics (9). While quantitative estimation offers trustworthy data on their relative concentrations-information essential for dose-response correlations and the standardization of herbal formulations qualitative phytochemical screening enables the initial identification of the classes of bioactive constituents present in a plant extract (10, 11).

The scientific literature on *Ipomoea obscura's* whole pharmacognostic profile and phytochemical composition is still limited and dispersed, despite the plant's proven ethnomedical use. According to the few research, that are currently available, *I. obscura* leaves may be a promising source of phytochemicals such as terpenoids and flavonoids (3, 6). However, the majority of the

works are narrowly focused and frequently do not provide sufficient characterization of the underlying chemical contents. The previous studies reported that leaf and root of *I. obscura* included the isolation of steroidal chemicals as well as macroscopic, microscopic, and physicochemical standards (12). More recently, Shinde et al. used spectroscopy and HPTLC fingerprinting to discover kaempferol, quercetin, β-sitosterol, and lupeol in a phytopharmacognostical investigation of *I. obscura* (3). Nevertheless, a thorough, independent pharmacognostic investigation with precise qualitative and quantitative phytochemical analysis of the leaf extract is still necessary. The need for a thorough, methodical pharmacognostic study of the plant is highlighted by this gap.

Hence, the present study was undertaken with the objective of performing both qualitative and quantitative phytochemical estimation of the leaf extract of *Ipomoea obscura* (L.) Ker-Gawl. The findings are expected to contribute baseline scientific data for the standardization of this medicinal plant, provide chemical leads for future pharmacological and drug development studies, and serve as a reference for quality control in herbal medicine practice.

2. MATERIALS AND METHODS

2.1. Collection and Authentication of Plant Material

The fresh plant of *I. obscura* was collected from the different geographical locations of Palakkad district, Kerala, India. The entire well grown plant was collected with flower during the period of September 2024 to December 2024. The collected plant was botanically identified and voucher specimen was preserved for further references.

2.2. Preparation of Plant Material

The leaves were separated from the collected plant and washed with running tap water until no trace of sand and dusts, further washed with distilled water. The leaves were left in the shade at room temperature to air dry. A sterile electrical blender was used to powder the dried leaves into a coarse powder and sieved (Mesh no. 40). After that, the powdered leaf material was stored for subsequent use in an airtight container.

2.3. Extraction

The 500gm of the powdered material of *I. obscura* leaves were extracted by using a soxhelt apparatus and continuous hot percolation procedure. The powdered material was first defatted with petroleum ether (40–60^{0}c) and then extracted with 70% v/v ethanol until the siphon tube was clear. Following extraction, it was filtered and dried in a rotary evaporator at 60°C to create

a semisolid mass. Until it is needed again, the dried extract was kept in an airtight container at 4°C.

2.4. Preliminary phytochemical analysis

The qualitative preliminary phytochemical screening was carried to identify the phytochemical profile of the *I. obscura* leaves ethanolic extract (IOLE) (13).

2.4.1. Test for alkaloids

A part of extract was diluted with dilute hydrochloric acid and filtered. Then 1ml of filtrate was mixed with 1ml of Dragendorff's reagent (Potassium bismuth iodide solution). Formation if reddish-brown precipitate indicates the presence of alkaloids (14).

2.4.2. Test for flavonoids

The extract 0.05 g was treated with few drops of 10% (w/v) sodium hydroxide solution and a few drops of conc. H_2SO_4. The formation of intense yellow color indicates the presence of flavonoids (14).

2.4.3. Test for phenols

The extract 0.05 g was treated with 3-4 drops of 5% (w/v) ferric chloride solution. Appearance of bluish black color indicates the presence of phenolic compounds (15).

2.4.4. Test for phytosterols

The extract 50 mg was treated with 1 mL of chloroform. Then 3-4 drops of Conc. H_2SO_4 was added to form a lower layer and allowed to stand. The development of a reddish brown color at the interface shows the presence of steroidal ring (14).

2.4.5. Test for tannins

The extract 5 g was mixed with 10 mL of distilled H_2O. The combined solution boiled for 5 min. The appearance of greenish precipitate up on the addition of 2 drops of 5% $FeCl_3$ shows the presence of tannins (16).

2.4.6. Test for terpenoids

The extract 0.5 g was mixed with the 2 mL of tin solution (Two or three granules of tin metal dissolved in 2ml of thionyl chloride solution). Then the test tube was warmed, formation of pink color indicates presence triterpenes.

The 0.5 g of extract was dissolved in water. 1 mL of extract solution was mixed with 3-4 drops of copper acetate solution; appearance of emerald green color indicates the presence diterpenes (14, 16).

2.4.7. Test for saponins

The 50 mg of the extract was diluted with 20 ml of distilled H_2O in a calibrated cylinder and vigorously shaken for 15 min. Formation of 1 cm and above of foam layer indicates the presence of saponins (16).

2.4.8. Test for glycosides

The 30 mg of extract was diluted with 3 mL of distilled water. Then 2 mL of glacial acetic acid and a drop of ferric chloride were added. To this mixture 1 mL of conc. H_2SO_4 added slowly. The appearance of a brown ring at the interface shows the presence of glycosides (16).

2.4.9. Fixed oils and Fats

The 50 mg of the extract was diluted with 20 ml of distilled H_2O and few drops of 0.5N alcoholic KOH and a drop of phenolphthalein was added and heated for 2 hours. Absence of soap formation indicates the absence of fixed oils and fats (16).

2.5. Quantitative phytochemical analysis

2.5.1. Estimation of total alkaloid content

To determine the total alkaloid content in the *I. obscura* leaves ethanolic extract (IOLE) was dissolved with 1mL of 2N Hcl and filtered. The filtered solution was transferred into the separating funnel, and then 5 mL of phosphate buffer and 5 mL bromocresol green solution (BCG) were added. Then this mixer was diluted with chloroform. The absorbance of the test and standard solutions were determined at 470 nm. Atropine 20-100µg/mL is used as standard. The total alkaloid content is expressed as mg of Atropine (AE)/g of extract (17).

2.5.2. Estimation of total flavonoid content

Total flavonoid content in the IOLE is estimated by $AlCl_3$ method. Quercetin was used as standard. The quercetin 1mg/mL methanolic solution is prepared and different aliquots 20-100µg/ml from this solution is prepared with methanol. 3 mL of IOLE solution (1mg/ml solution in methanol) or standard solution was added into the test tube containing 1 mL of 2% $AlCl_3$ methanolic solution and allowed to stand for 1 hour at room temperature. The absorbance of the solution measured at 420 nm. The total flavonoid content is expressed as mg of Quercetin (QE)/g of the extract (18).

2.5.3. Estimation of total phenolic content

The total phenolic content in the IOLE was determined by the Folin-Ciocalteu method. The 0.1 mL of extract (0.1 mg/mL in distilled water) was treated with 0.5 mL of Folin-Ciocalteau reagent

and 1.5 mL of 7% sodium carbonate. The combined solution is shaken well and made up to 10 mL with distilled water. Then it is incubated in dark at room temperature for 2 hrs. Then the absorbance of the test and standard is taken at 725 nm in spectrometer against a reagent blank. Gallic acid (20-100 µg/mL) was used as standard. The total phenolic content is expressed as mg of gallic acid (GAE)/g of extract (18).

2.5.4. Estimation of total saponin content

To determine the total saponin content in the IOLE 0.1 mL of the extract (0.1 mg/mL in distilled water) was diluted with 80% methanol aqueous solution. After that 1 ml of the 72% H_2So_4 was added to the side of the sample containing test tube and heated for 10 minutes in a water bath at 60^0C. Then the absorbance of test and standard solutions were determined at 544 nm against the reagent blank. The diosgenin (20-100µg/mL) was used as standard. The total saponin content in IOLE was expressed as mg of diosgenin/g (19).

2.5.5. Estimation of total steroidal content

The steroidal content in the ethanolic leaves extract of *I. obscura* was quantified by the method of Liebermann and Burchard with modifications. The extract 1 mg/mL was prepared by dissolved in chloroform, the freshly prepared Libermann-Burchard reagent (50 ml acetic anhydride and 5 ml of concentrated sulphuric acid) was mixed. Cholesterol (20-100 µg/ml) was used as standard. The absorbance of the test and standard was measured at 650 nm against a reagent blank. The total steroidal content in the extract was expressed as mg of cholesterol/g of extract (20).

3. RESULTS AND DISCUSSION

3.1. Extractive value

The cumulative extractive value of the *I. obscura* leaves ethanolic extract was calculated as 10.85% w/w. It indicates effective recovery of semi polar and polar soluble constituents. The use of 70% ethanol, a solvent that is recognized for its capacity to extract a wide range of secondary metabolites, such as flavonoids, phenolics, alkaloids, glycosides, and saponins, may be responsible for the impressive extractive yield (21). The extractive value is a critical pharmacognostic parameter for evaluating the quality, purity, and consistency of botanical raw materials. It may serve as a benchmark for the future standardization of *I. obscura* preparations.

3.2. Qualitative Phytochemical Analysis

The preliminary phytochemical study results of *I. obscura* leaves ethanolic extract revealed that the presence of alkaloids, flavonoids, phenols, phytosterols, diterpenoids and triterpenoids, saponins, and glycosides, while the fixed oils and fats were absent (Table 1). These results are confirmed the previous studies on phytochemical profile of *I. obscura* leave.

The presence of flavonoids and phenolic chemicals, which are known to neutralize reactive oxygen species and prevent oxidative stress-related cellular damage, indicates that the plant has considerable antioxidant potential. Numerous pharmacological effects of alkaloids, such as analgesic, antibacterial, and anti-inflammatory qualities, have been thoroughly established. Similarly, glycosides have been linked to cardioprotective and therapeutic properties, while saponins contribute to immunomodulatory, hypocholesterolemic, and antidiabetic activities. The presence of phytosterols and terpenoids, which have anti-inflammatory, hepatoprotective, and anticancer properties, adds to the plant's therapeutic value (22, 23).

The absence of fixed oils and fats in the extract can be explained by the preliminary defatting process using petroleum ether before ethanolic extraction. This procedure selectively removed non-polar lipid components, thereby enriching the extract with pharmacologically active polar constituents. The present findings corroborate earlier reports describing the phytochemical profile of *I. obscura*, thereby validating its ethnomedicinal applications and establishing the reliability of the extraction procedure adopted in this study.

Table 1: Preliminary Phytochemical Analysis of *I. obscura* leaves ethanolic extract

Phytoconstituents	***I. obscura* leaves extract**
Alkaloids	+
Flavonoids	+
Phenols	+
Phytosterols	+
Tanins	+
Terpenoids	++
Saponins	+
Glycosides	+
Fixed oils and Fat	-

3.3. Quantitative phytochemical analysis

Quantitative estimation demonstrated that the ethanolic leaf extract of *I. obscura* contained measurable amounts of all investigated phytoconstituents, represented in Table 2.

Table 2: Quantitative Phytochemical estimation of *I. obscura* leaves ethanolic extract

S. No.	Phytoconstituents	Total Content
1.	Alkaloid (mg of atropine (AE)/g of extract)	10.34±2.30
2.	Flavonoid (mg of quercetin (QE)/g of extract)	9.32±0.64
3.	Phenolic (mg of gallic acid (GAE)/g of extract)	5.84±0.77
4.	Saponin (mg of diosgenin/g of extract)	7.45±0.22
5.	Steroids (mg of cholesterol/g of extract)	2.13±0.86

Values are expressed as mean ± SD (Standard deviation), n=3

The total alkaloid content was found to be 10.34 ± 2.30 mg atropine equivalent (AE)/g extract, representing the highest concentration among the constituents analyzed. This finding indicates that alkaloids may constitute one of the major bioactive groups may responsible for the therapeutic effects of *I. obscura* (Figure 1).

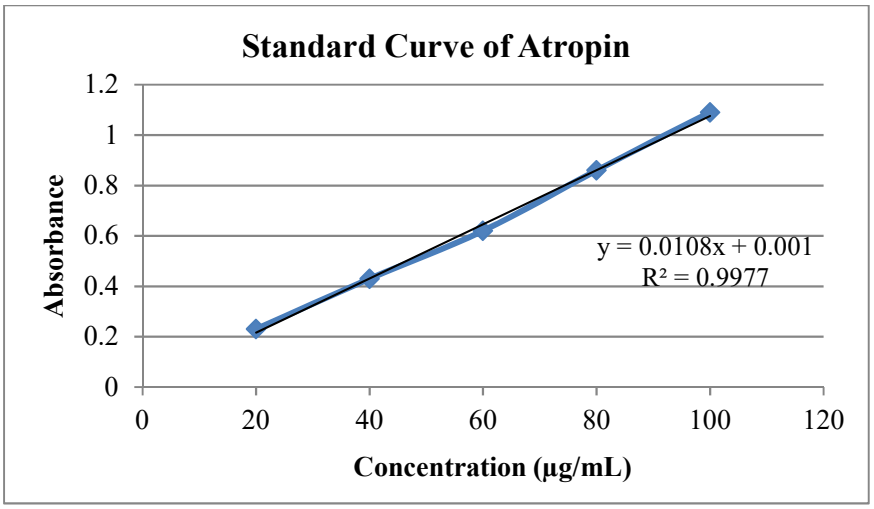

Figure 1: Standard calibration curve of atropin for the estimation of total alkaloids content in *I. obscura* leaves ethanolic extract

The total flavonoid content was determined as 9.32 ± 0.64 mg quercetin equivalent (QE)/g extract, indicating a substantial abundance of flavonoids. Flavonoids such as quercetin and kaempferol have previously been identified in *I. obscura* and are known to possess antioxidant, anti-inflammatory, and hepatoprotective activities. The relatively high flavonoid concentration observed in the present study supports the traditional use of the plant in treating inflammatory disorders and conditions associated with oxidative stress (Figure 2) (3).

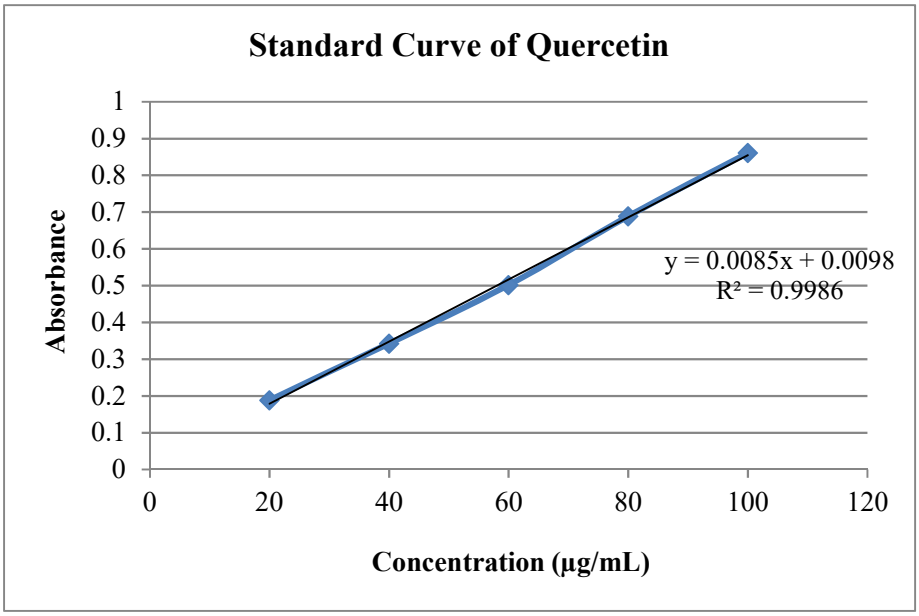

Figure 2: Standard calibration curve of quercetin for the estimation of total flavonoid content in *I. obscura* leaves ethanolic extract

The total phenolic content was estimated to be 5.84 ± 0.77 mg gallic acid equivalent (GAE)/g extract. Phenolic compounds contribute significantly to antioxidant activity through their hydrogen-donating and metal-chelating properties. Although the phenolic content was lower than that of alkaloids and flavonoids, its presence further strengthens the therapeutic potential of the extract (Figure 3) (24).

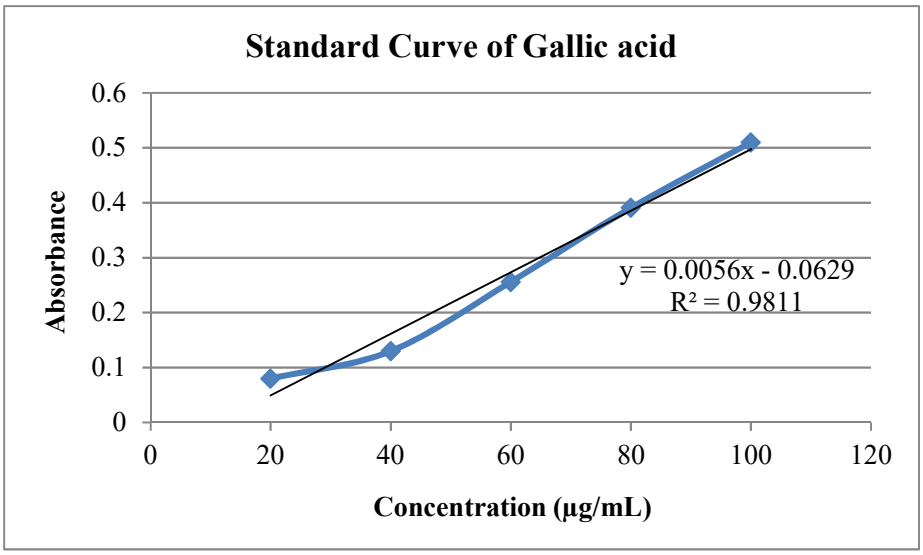

Figure 3: Standard calibration curve of gallic acid for the estimation of total phenolic content in *I. obscura* leaves ethanolic extract

The total saponin content was found to be 7.45 ± 0.22 mg diosgenin/g extract, suggesting that saponins are another important class of constituents in the plant. Saponins have been reported to exhibit hypoglycemic, immunomodulatory, and cholesterol-lowering activities, thereby providing a scientific rationale for exploring *I. obscura* in metabolic and inflammatory disorders (Figure 4) (25).

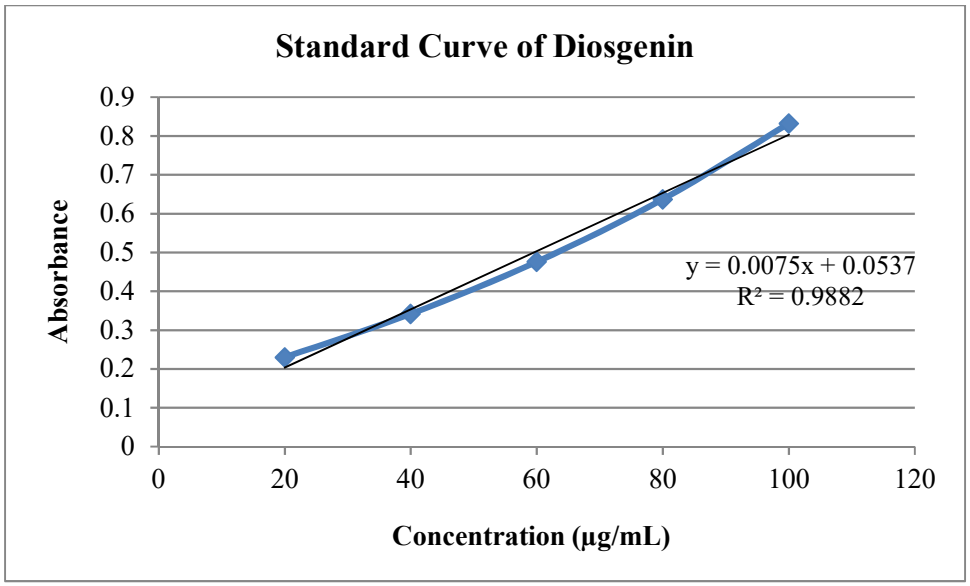

Figure 4: Standard calibration curve of diosgenin for the estimation of total saponin content in *I. obscura* leaves ethanolic extract

Steroidal constituents were quantified as 2.13 ± 0.86 mg cholesterol/g extract, representing the lowest concentration among the evaluated phytoconstituents. Nevertheless, phytosterols and related steroidal compounds contribute to anti-inflammatory activities and membrane-stabilizing actions may act synergistically with other secondary metabolites present in the extract (26) (Figure 5).

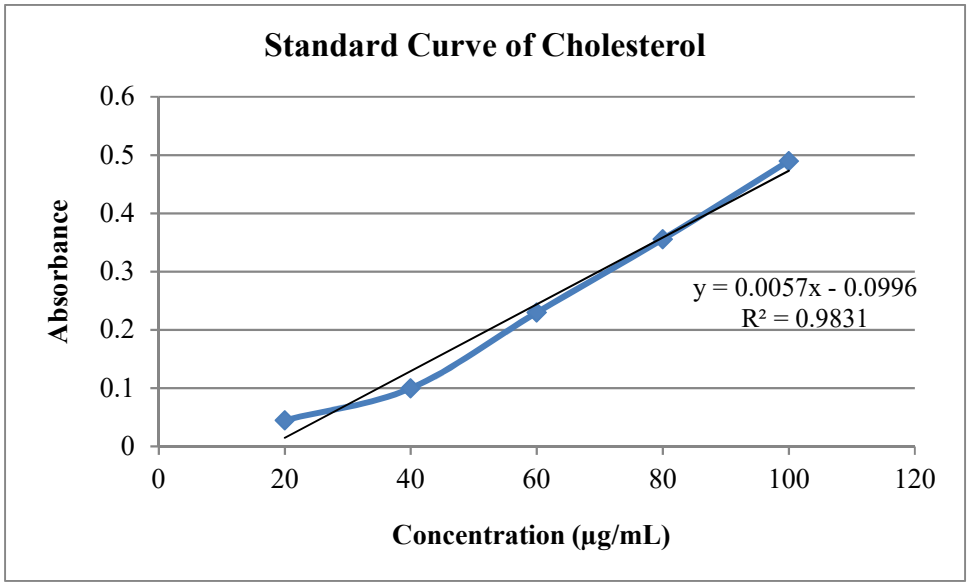

Figure 5: Standard calibration curve of cholesterol for the estimation of total steroids content in *I. obscura* leaves ethanolic extract

Overall, the quantitative analysis indicates the following order of abundance of hytoconstituents: Alkaloids > Flavonoids > Saponins > Phenolics > Steroids. This distribution highlights the richness of *I. obscura* leaves in biologically active secondary metabolites and supports its potential as a valuable source of phytotherapeutic agents.

4. Conclusion

In conclusion, the results of this study offer scientific proof of *Ipomoea obscura's* ethnopharmacological significance. The leaves have a wide range of phytochemicals with known biological actions, according to the combination of qualitative and quantitative investigations. The abundance of flavonoids and alkaloids indicates that these substances may be essential to the plant's alleged therapeutic effectiveness in conventional medical systems.

Additionally, the phytochemical profile and extractive yield produced in this study can be used as baseline pharmacognostic standards for *I. obscura* leaf preparation quality control and authentication. These findings also lay the groundwork for further research into mechanistic pharmacological studies, chromatographic fingerprinting, antioxidant and anti-diabetic activity assessment, and active principle isolation. As a result, *I. obscura* could be regarded as a viable option for the creation of new phytopharmaceutical compounds and standardized herbal formulations.

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