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Original Article

Exploration of Phytopharmacognostical Study of *Ipomoea obscura* (Linn.) Ker Gawl

Poonam Shinde*, Rajendra Bhambar, Pankaj Patil, Khanderao Jadhav, Prashant Malpure

ABSTRACT

Background: *Ipomoea obscura*, also known as "Lakshmanvel" in India, is a member of the Convolvulaceae family. Early studies were mostly concerned with early phytochemical analysis, with only a few focusing on detailed phytochemical analysis. **Objectives:** The aim of this study was to identify the phytochemical and pharmacognostic characteristics of *I. obscura* leaves. **Materials and Procedures:** The methanolic extracts were separated into flavonoids and phytosterol by column chromatography, which were subsequently examined by spectroscopy and HPTLC methods. **Results:** According to this research's findings, *I. obscura* leaves may be a good source of phytochemicals such flavonoids (Kaempferol and quercetin), terpenids (β-sitosterol and lupeol), which were identified by HPTLC fingerprinting, FTIR NMR, and UV spectroscopy. **Conclusion:** The spectroscopical data and HPTLC fingerprinting profile for the plant of interest would hopefully aid herbal medicine researchers in developing additional herbal medications that require the specific ingredient or extract.

Keywords: Microscopical study, Phytochemical investigation, Quantitative estimation, isolation, Characterization, HPTLC fingerprinting.

Poonam Shinde*, Rajendra Bhambar, Pankaj Patil, Khanderao Jadhav, Prashant Malpure

Department of Pharmacognosy, MGV's Pharmacy College, Panchvati, Nashik, Maharashtra, INDIA.

Correspondence

Mrs. Poonam Ramesh Shinde

Research Scholar, Department of Pharmacognosy, MGV's Pharmacy College, Panchvati, Nashik-422003, Maharashtra, INDIA.
Email id: poonam.shindeprs@gmail.com
ORCID iD: 0000-0002-2388-9151

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INTRODUCTION

Ipomoea obscura (L.), commonly described to this in Ayurveda as "Laksmana," is an herb in the Convolvulaceae family. It's a little climber with small cordate leaves with apex that's acuminate. Five fully united petals make up the corolla. In disturbed environments, the plant grows on fences or as a low ground cover as a substrate. It's a slender, twinning perennial herb that grows in grasslands, hedges, and wastelands all over India, up to a height of 3000 feet. As a climber with lovely flowers, it also has decorative value.[1] This plant's medicinal benefits have been found by Ayurveda, and it is used to treat diarrhoea, open sores, and pustules. Ulcers, haemorrhoids, and swellings are treated with a leaf paste. Fruits and seeds are being used as purifying agents, to help with difficult breathing, to relieve pain, and also to improve vision.[2] To treat colds, asthma, and dry cough, gingely oil is combined with the fresh plant extract.By widening blood vessels, the plant extract relieves pain in the body. Many siddha medications contain plant extracts.[3]

Other phytochemicals found in this plant were flavonoids, steroids, essential oil, and phenolic acids.[4] This plant contains indole alkaloids like Ipobscurine A, Ipobscurine B, Ipobscurine C, and Ipobscurine D as well as tropane alkaloid including Calysteginine B-1,2, 3 and 4. Calysteginine C-1;[5-6] Ipobscurine-A, C, and D are significant pharmacologically active macrolactum type indole alkaloids found in *Ipomoea obscura*.

The anti-inflammatory and anti-angiogenic effects of these indole alkaloids have been documented.[7] Bulnesene (23.8%), -humulene (13.7%), and seychellene were the three primary components of the essential oil (11.2 percent). Other trace amounts of -guaiene (8.3%), -caryophyllene (7.1%), -terpinene (4.2%), -hexadecanoic acid (3.0%), and -elemene were present (2.7 percent). The oil was found to have many components of the sesquiterpene hydrocarbon class (78.4%).[8]

Ipomoea obscura extracts have been shown to exhibit anti-inflammatory, nephroprotective, anti-angiogenic, and immunological modulatory properties.[9] *Ipomoea obscura* whole plant extracts have high antioxidant activity against a variety of free radicals.[10] *Ipomoea obscura* methanol extracts have been demonstrated to have good cytotoxic and moderate antibacterial properties.[11]

The literature review revealed that the separation and characterization of phytoconstituents from the plant had not been investigated, thus the investigations were carried out in the current study.

MATERIALS AND METHODS

Plant Material Authentication and Collection

Ipomoea obscura leaves were collected from several locations in the Nashik region in Maharashtra, India.

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Standard literature was used to confirm plant species identification. Dr.Shimpi, Taxonomist, Head of Botany Department, G. E. Society's NSC Science College, Nashik, India, validated the plant sample. The voucher specimen No. 508 for *Ipomoea obscura* (L.) Ker Gawl was preserved in the college herbarium (NSCSC/PCG/2017/12) for future reference. To remove any dust, the leaves were rinsed with tap water and then dried in the shade. With the use of a grinder mixture, the dried leaves material was ground into a fine powder. The powdered leaves were kept in an airtight container for further usage.

Microscopical Examination

The exterior morphology was investigated using the methods described in the literature. A basic microscope was used to conduct the macroscopic research. Leaves, flowers, and seeds were studied for colour, odour, taste, size, and morphology.[12]

Microscopic Analysis

Thin leaf fragments were prepared for microscopic examination. The thin slices were washed in water and dyed with safranin, haematoxylin, picric acid, and dil. liquid iodine before mounting in glycerine for clarity and confirmation of magnifications. (10X, 45X).[13-14]

Physicochemical Investigation

It is impossible to overestimate the relevance of physicochemical standards in establishing the quality, purity, and adulteration of a given crude medicine. The amount of care used in collecting and preparing a medicine for market, as well as the amount of foreign matter in a natural drug, are reflected in the ash values. Traces of organic matter that ordinarily interfere with analytical determination were removed by determining the ash value. In general, ash from incineration comprises phosphates and carbonates, as well as calcium, potassium, sodium, and magnesium in the form of silicates. The extractive value, moisture content, and total, acid-insoluble, water soluble and sulphated ash values were assessed using Indian Pharmacopoeial techniques.[15-16]

Methodology of Extraction

250 g of powdered plant material was packaged in white cotton bags and extracted in a Soxhlet device using several solvents in a sequential manner. Petroleum ether (60-80°C), chloroform, and methanol were utilised as extraction solvents. In an oven below 50°C, the powdered material was dried before extracting with the next solvent. These consecutive extracts were subjected to extractive values and preliminary phytochemical analyses.[17]

Phytoconstituents: A Qualitative and Quantitative Analysis

To confirm the existence of phytoconstituents, protein, alkaloids, phenolic compound, terpenoid, glycosides, phytosterols and flavonoids and other substances, preliminary phytochemical analyses on successive extracts were carried out in this work. The approach was also used to calculate the total phenolic and flavonoid content of the extracts. Total phenol and total flavonoid concentrations were calculated in milligrams of gallic acid equivalents per gram of extract and miligrames of rutin equivalents per gram of extract, respectively, using the mean of three readings.[15]

Isolation and Characterization of Phytoconstituents from *Ipomoea obscura* Leaf;[18-19]

Before being extracted (1 kg) in a Soxhlet extractor for 18–20 hr with 4.5 L methanol, the fresh whole plant was dried in the shade, ground up, and sieved No. 20. The extracts were dried using a rotary evaporator at a controlled temperature between 40–50°C and lower pressure. It dried to a 655 g solid residue that was dark yellowish green. The unsaponifiable material was isolated using the n-hexane fraction after the methanolic extract was partitioned in n-hexane and water for 3 hr (240 g). The aqueous layer then treated three times (100 mL) with carbon tetrachloride that before bottom layer of carbon mono tetrachloride was removed and concentrated (35 g) (80 g).

Isolation and Characterization of Triterpenoids from *I. obscura*

The already obtained n-hexane soluble fraction was separated using column chromatography and fine column silica. After that, n-hexane, ratios of n-hexane and ethyl acetate, ethyl acetate-methanol, and finally methanol were used to elute the column. The polarity was progressively enhanced by gradually raising the polar solvent quantities. 30 50 mL chunks in total were gathered and sorted. All fractions were monitored using TLC spraying with VS/AS reagent. To elute the fractions, n-hexane and chloroform were combined (no.16-24). 15 sub-fractions were once more gathered. Before being columned, sub-fractions 5–10 underwent preparative thin layer chromatography using n-hexane–chloroform. (Plate thickness: 0.5 mm; stationary phase: GF254; mobile phase: 95:5) Sub-fraction's preparative TLC provided compound IO1 (15mg).

Using column chromatography with n-hexane and rising acetone proportions, the soluble component of carbon tetrachloride was segregated to produce 15 fractions (25 ml each). 13 sub-fractions were recovered after the elution of the fraction (fractions numbers 4-6) using a methanol-chloroform mixture. The formation of the compound IO2 (92 mg) using preparative TLC (sub fraction no.5-9). [Plate thickness: 0.5 mm; stationary phase: F_{254} silica gel; mobile phase: 95:5 toluene\ ethyl acetate]

Isolation and Characterisation of Flavonoids from *I. obscura*

After partitioning the crude MIO extract, the n-hexane fraction (45.5 g), dichloromethane fraction (25.4 g), and Ethyl acetate fraction (48.7 g) were obtained. To get 15 fractions, the ethyl acetate fraction (40.0 g) was separated using silica column chromatography eluting with mixes of n-hexane/Ethyl acetate (100:0-0:100 v/v), Ethyl acetate/Methanol(100:0-0:100 v/v), and Ethyl acetate/Methanol (100:0-0:100 v/v) (Fr. A to Fr. O). Fraction D (14.1 g) was separated into 5 sub-fractions (D1–D5) on a silica gel CC using a 9:1 v/v combination of n-hexane and Ethyl acetate. CC split fraction D4 (1.1 g) into 3 sub-fractions (D4.1 to D4.3) using Ethyl acetate. Recrystallization purified fraction D4.1 (361.2 mg) to get compound IO3 (211.7 mg). CC purified fraction D4. 2 (188.9 mg) by using Chloroform/Methanol (5:1 v/v) to produce compound IO4 (17.4 mg). The thin layer chromatography (TLC) was used to examine all of the obtained fractions, and the fractions with identical Rf values were merged. Recrystallization was used to purify the principal portions. Spectroscopic methods (UV, FTIR, and NMR spectroscopy) were used to characterise all of the isolated chemicals.[20-22]

HPTLC fingerprinting analysis;[23-25]

The research's main objective was to evaluate and enhance the HPTLC fingerprint technique for standardization crude drugs. The sample was applied with a microlitre syringe using a Camaglinomat IV applicator with a band width of 7 mm to precoated silica gel plates 60 F_{254} [10 cm × 10 cm, 0.2 mm thickness, E. Merck]. The plates were created in a solvent system and then saturated with the solvent for 20 min in a Camag twin through glass chamber. They were separated by 8 centimetres. After scanning on a Camag TLC Scanner in absorbance at 254, 365, and

740 nm with wincats software 4.03, TLC plates were air dried. The HPTLC profile were summarised in Table 1-3.

RESULTS

Macroscopic Characteristics

It's a little climbing vine with alternately arranged short cordate leaves with whole margins and acuminate apex. Flowers are solitary or in cymes, with peduncles as long as the petioles. The corolla is made up of five fully united white petals with a scarlet core. The corolla tube's neck has five stamens joined to it. With two locules and numerous seeds, the ovary is superior. At maturity, the fruit is a sub-globose, glabrous capsule.

Microscopic Study

Leaf microscopy: Transverse section of the epidermis reveals uniformity, with somewhat larger cells on the dorsal side due to noticeably higher anticlinal walls. The paracytic stomata are on the same level as the neighbouring cells, and the guard-cells have discernible outer cuticular ledges. About half of the height of the chlorenchyma is made up of the dorsiventral region of the mesophyll, which is comprised of two or three layers of palisade parenchyma and four to six rows of spongy parenchyma. There were discovered to be 2 - 3 layers on the dorsal side and 8 to 10 layers on the adaxial side of the angular collenchyma that makes up the epidermis. Several ovate, ring-shaped vascular bundles that was ancillary with fascicular cambium and cross the ground parenchyma. Four cells with thick cytoplasm, a visible nucleus, and the ability to secrete lipophilic substances are found in a number of secretory ducts with uniseriate epithelium that are frequently aligned by the arterial bundles (Figure 1 A)

Microscopy of the stem: The epidermis of juvenile branches is thicker, with sclerotized outer cell walls that are robust and hairy, like a piece of glands, as in the leaf. Collenchyma is a big clump of cells found beneath the epidermis. Cells of the epidermis with thin beaded walls, stomata are tiny, round, and have a prominent stomatal slit bordered by 2-4 paracytic cells. Mucilage inclusions are found in epidermal stem cells. The epidermis has a thick layer of hair on it. Pith occupies a substantial percentage of the stem's central region, while hypodermis exists outside of it. Pith cells in the shape of barrels store a lot of food. Vascular bundles are arranged in rays that are parallel to each other (Figure 1 B).

Table 1: HPTLC profile *of I. obscura extract* with standard lupeol.

Sample preparation	10mg sample dissolved in 10ml chloroform
Standard preparation	5mg lupeol in 5ml chloroform
Stationary phase	Silica Gel G (HPTLC precoated plates)
Size of plate	10 × 10
Mobile phase	Benzene: Ethyl acetate (9:1)
Spraying reagent	Libermann- Burched reagent
Detection	Scanned at 540nm (after derivatization)

Table 2: HPTLC profile of *I. obscura* extract with standard β-sitosterol.

Sample preparation	10mg sample dissolved in 10ml chloroform
Standard preparation	5mg β-sitosterol in 5ml chloroform
Stationary phase	Silica Gel G (HPTLC precoated plates)
Size of plate	10 × 10
Mobile phase	Benzene: Ethyl acetate (9:1)
Spraying reagent	Libermann- Burched reagent
Detection	Scanned at 540nm (after derivatization)

Table 3: HPTLC profile of *I. obscura* extract with standard kaempferol and quercetin.

Sample preparation	10mg sample dissolved in 10ml methanol
Standard preparation	5mg Quercetin and Kaempfrol sonicated in 5ml methanol respectively.
Stationary phase	Silica Gel G (HPTLC precoated plates)
Size of plate	10 × 20
Mobile phase	Tolune: Acetone: Formic acid(7:3:0.2)
Detection	Scanned at 254 nm

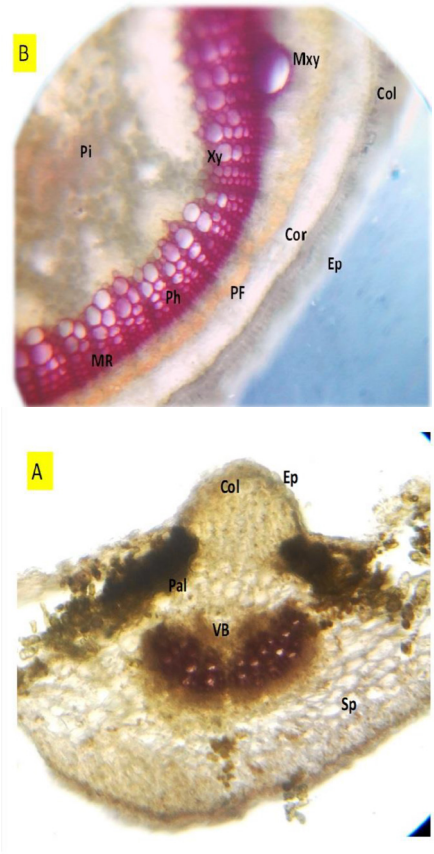

Figure 1 A and B: Transverse section of *Ipomoea obscura* leaf (A) and stem (B).

Uep: Upper epidermis, PF: Pericyclic fiber, Pal: Palisade tissue, VB: vascular bundle, Mxy: Metaxylem, Col: collenchyma, Cal oxa: Calcium oxalate crystals, C tri: covering trichome, Spa: Spongy parenchyma, Lep: Lower epidermis. Cor: Cortex, Xy: Xylem, Pi: Pith

Table 4: Physicochemical analysis of *I. obscura*.

Physical parameter	*I. obscura* (% w/w)
Total ash	8.46± 0.16
Acid insoluble ash	1.1±0.22
Water soluble ash	3.3±0.49
Sulphated ash	8.56 ± 0.02
Moisture content	4.60 ± 0.01
Alcohol soluble extractive	15.54 ± 0.23
Water soluble extractive	11.23 ± 0.46

Physiochemical Assessment

Table 4 shows the physicochemical parameters of the *I. obscura* plant that were analysed. The drying loss of leaf dry powder was 4.6 ± 0.01%. Total ash was 8.56 ± 0.02 %, water soluble ash was 3.3 ± 0.49%, and acid insoluble ash was 1.1 ± 0.22%. The sulphated ash content was 8.56 percent with a margin of error of 0.02 percent. Methanol solvent extracts had the highest soluble extractive value, which was 15.74 % in leaves. Petroleum ether extracts had the lowest soluble extractive value of 7.11 %. The extractive value in water was 11.75 % w/w.

Methodology of Extraction

The extractive value was determined to be highest when extraction was done with methanol, chloroform, and lowest when extraction was done with petroleum ether, i.e. 15.25 %, 9.34 %, and 5.82 %, respectively. Table 5 shows the results. In *Ipomoea obscura*, maximal extraction with methanol has also been recorded.

Quantitative and qualitative investigation of phytoconstituents

The qualitative phytochemical analysis of the plant extract as shown Table 6 revealed the presence of steroids, saponins, alkaloids, glycosides, terpenoids, and flavonoids. The total phenolic component concentration was calculated from the equation for such gallic acid calibration graph. The Folin-Ciocalteu reagent was used to estimate the extract's total phenolic content. The total phenolic content of leaf methanolic extract is

Table 5: Result of extraction of *I. obscura* leaves with different solvent

Sl. No.	Extraction solvent	Color	% w/w
1.	Petroleum ether	Yellowish green	15.25
2.	Chloroform	Pale green	9..34%
3.	Methanol	Dark green	5.82 %

Table 6: Preliminary phytochemical investigation.

Constituents	*Ipomoea obscura*		
	P.E.	CHCl3	MeOH
Alkaloid		+	+
Steroids		–	+
Triterpenoids	+	-	-
Coumarin	-	+	+
Flavonoids	-	-	+
Phenolics	-	+	+
Glycoside	+	+	+
Carbohydrates	-	-	+
Saponin	-	-	+
Fatty acids	-	-	+

Table 7: Absorbance observed in estimation of total phenolic content.

Sl. No.	Conc. (µg/ml)	Absorbance (nm)			Mean ± SEM
		I	II	III	
		Standard (Gallic acid)			
1	20	0.0750	0.0744	0.0748	0.0746 ± 0.0002
2	40	0.1536	0.1532	0.1553	0.1534 ± 0.0002
3	60	0.2481	0.2484	0.2492	0.2486 ± 0.0001
4	80	0.3172	0.3170	0.3164	0.3168 ± 0.0002
5	100	0.3685	0.3790	0.3780	0.3787 ± 0.0001
		Samples			
MIB	400	0.31740	0.31570	0.31876	0.3172 ± 0.0008

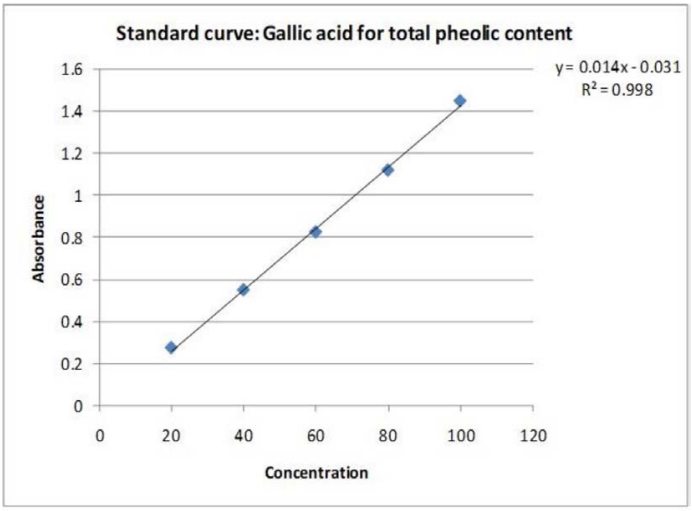

Figure 2: Showing calibration curve of Gallic acid.

Table 8: Absorbance observed in estimation of total flavonoids content.

Sl. No.	Conc. (µg/ml)	Absorbance (nm)			Mean ± SEM
		I	II	III	
		Standard (Rutin)			
1	20	0.1945	0.1953	0.1949	0.195 ± 0.0002
2	40	0.3888	0.3893	0.3891	0.389 ± 0.0001
3	60	0.5825	0.5826	0.5831	0.583 ± 0.0002
4	80	0.7781	0.7786	0.7788	0.779 ± 0.0002
5	100	0.9327	0.9329	0.9321	0.933 ± 0.0002
		Samples			
MIB	400	0.2730	0.2740	0.2731	0.2735± 0.0004

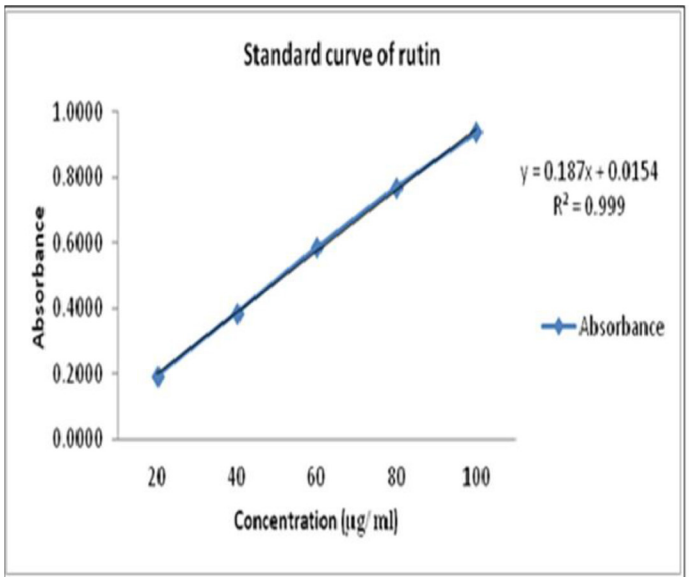

Figure 3: Showing calibration curve of rutin.

5.56 percent (Table 7 and Figure 2). The total concentration of flavonoids was determined using the calibration. The total flavonoid content of the methanolic extract of *I. obscura* leaves was examined using an aluminium chloride colorimetric method and a calibration curve of rutin at various concentrations, and it was discovered to be 0.34 percent (Table 8 and Figure 3).

Isolation and Characterization of *I. obscura* Leaves

Isolation and characterization of terpenoid from I. obscura leaves

Compound IO1: The Lieberman-Burchard steroid test was passed by compound IO1. Crystals of compound IO1 with a melting point of 214–215 degrees Celsius were isolated as the substance. The IR spectra revealed the presence of such -OH group at 3303 cm^{-1}, C-O at 1732 cm^{-1}, and 1637 cm^{-1} (Figure 4). The 1H NMR for -CH3 protons showed signals at (ppm) 0.83, 1.02, 0.79, and 1.70. The carbonyl proton was discovered as a broad singlet at 5.11, whereas the olefinic resonance was discovered as a singlet at 4.56. In Figure 5, A comparison of the spectroscopy data from compound IO1 with the literature led to the discovery that it is lupeol.

Compound IO2: The Lieberman-Burchard steroid test was passed by compound IO2. With a melting point of 147°C, the compound IO2 was isolated as colourless crystals. The IR spectrum confirmed the existence of the hydroxyl group at 3433.06 cm^{-1} and alkene (C=C) at 1639 cm^{-1}

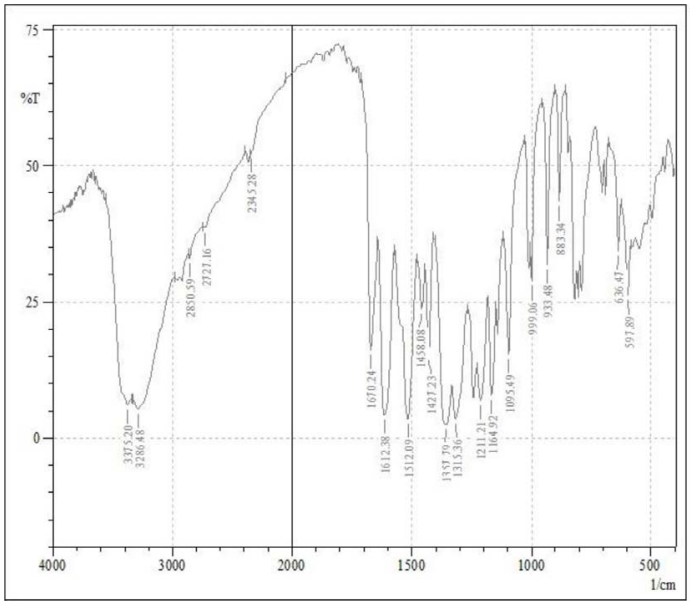

Figure 4: FI-IR of compound IO1.

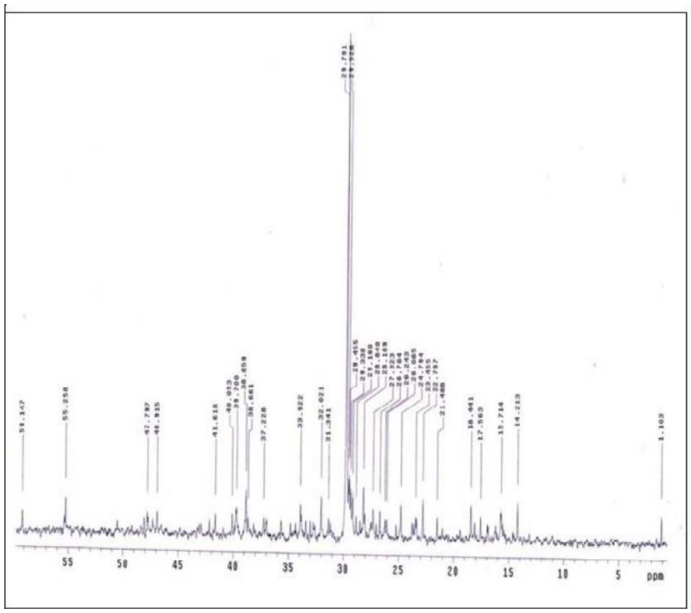

Figure 5: ^{13}C NMR spectra of compound IO1.

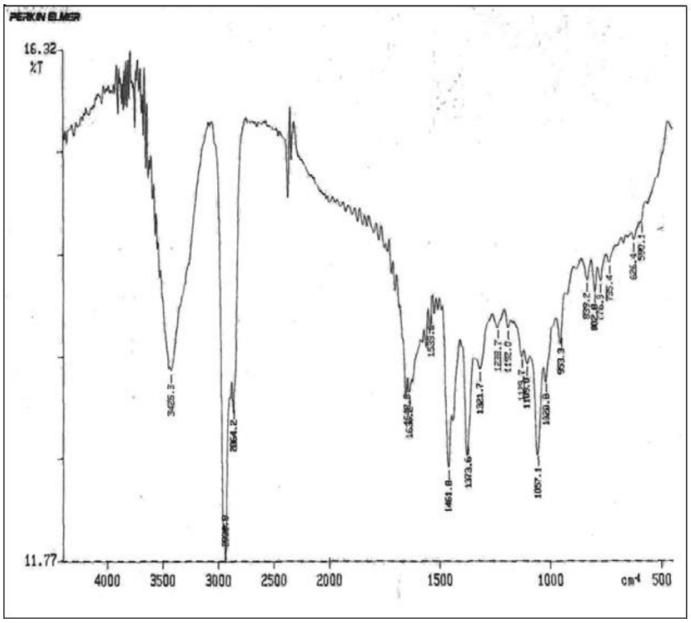

Figure 6: FT-IR spectra of compound IO2.

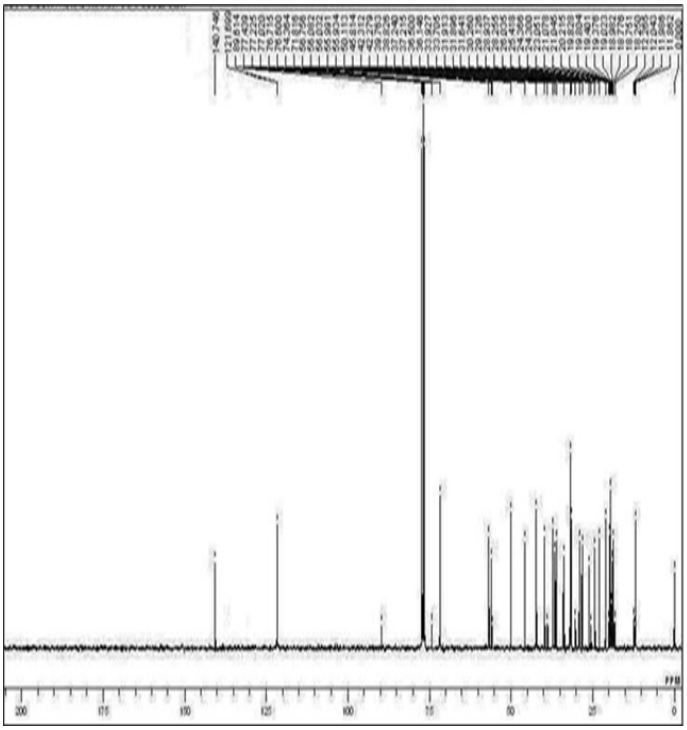

Figure 7: ^{1}H NMR spectra of compound IO2.

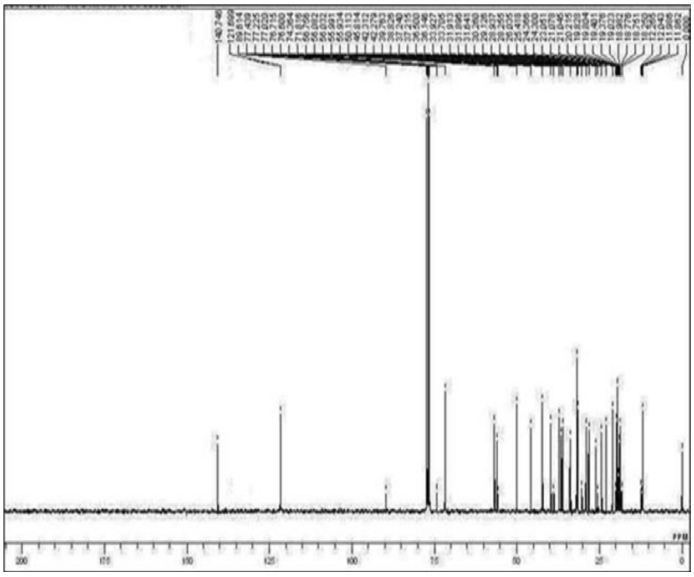

Figure 8: [13] CNMR spectra of compound IO2.

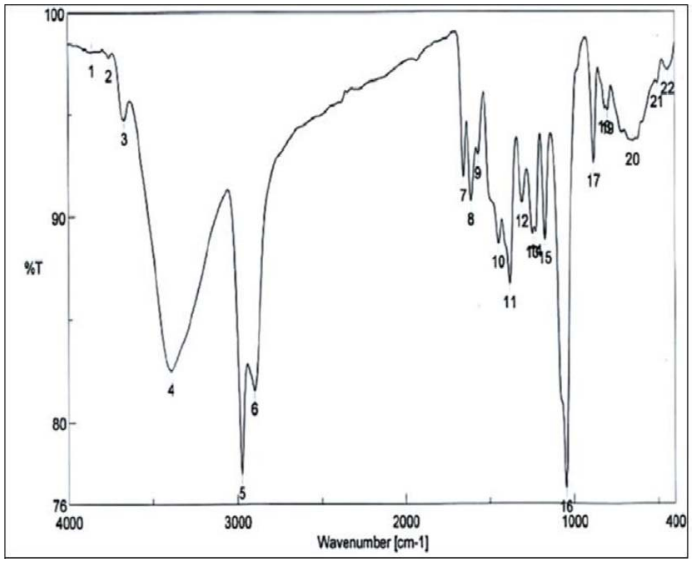

Figure 10: [1]H –NMR spectra of compound IO3.

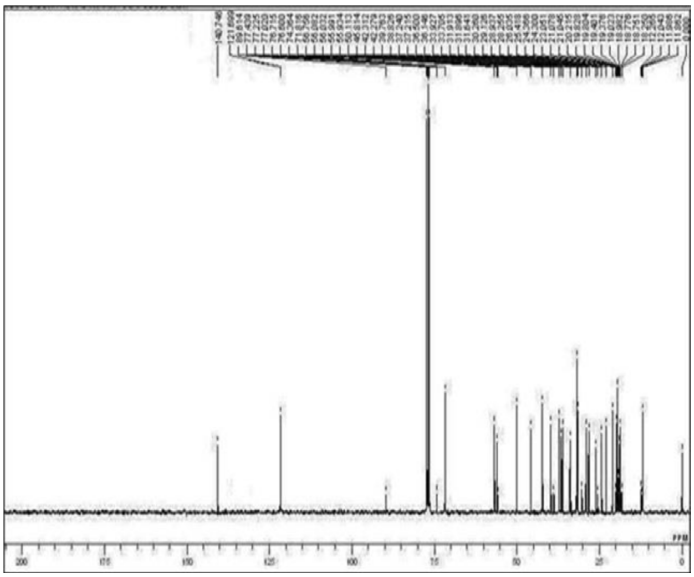

Figure 9: FT-IR spectra of compound IO3.

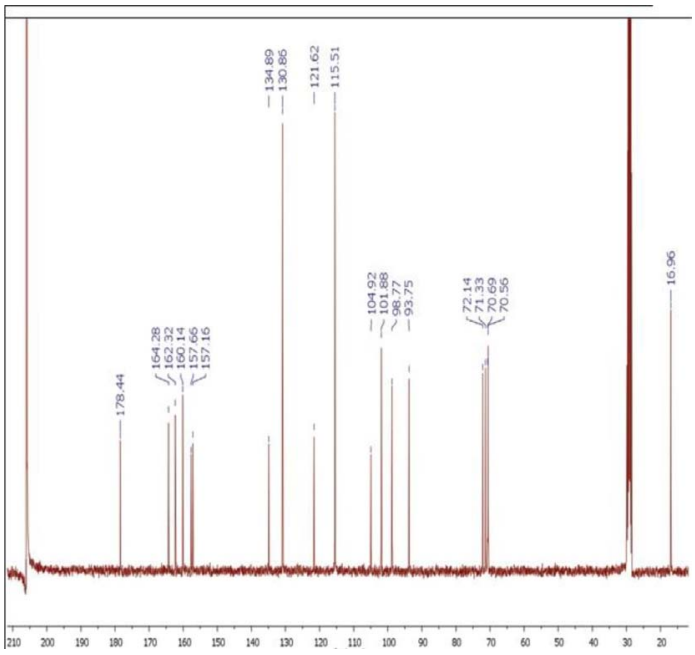

Figure 11: [13]C –NMR spectra of compound IO3.

(Figure 6). The 1H NMR (Figure 7) showed indications for methyl protons at 1.25, 1.01, 0.88, and 0.93. The carbonyl proton was discovered as a broad singlet at 5.01, whereas the olefin resonance was discovered as a multiplet at 3.52. The 13C NMR peaks at 140.09 and 121.4 proved that an olefin bond existed between C-5 and C-6 (Figure8). Following a comparison of the produced spectrum data with published data, compound IO2 was determined to be a β- sitosterol.

Isolation and characterization of flavonoids from *I. obscura* leaves

Compound IO3: Compound IO3 was created as an amorphous, yellow powder. Two sizable peaks of absorption at 265 and 365 nm were visible in the compound's UV spectra. These absorption peaks are typical of flavonols, which also exhibit significant peaks in the 200–400 nm range. Band I (between 300 and 380 nm) and band II (between 400 and 450 nm) refer to these two peaks (240-280 nm). Band I deals with the B-(the ring's cinnamoyl system's) absorption, while Band II deals with the A-(the ring's ring's) absorption (benzoyl system).

The presence of hydroxyl and carbonyl functional groups was detected at 3253 and 1651 cm^{-1}, C-H stretching at 2956 cm^{-1}, C=C olefin ring at 1361 cm^{-1}, asymmetric C-O-C stretching at 1171 cm^{-1}, and substituted benzene at 828 cm^{-1}, according to IR measurements (Figure 9). The 1H NMR spectrum of 1 revealed four aromatic hydrogen signals comprising six protons (Figure 10). Two protons at 7.83 ppm (2H, dd, J = 8.6, 2.9 Hz, H-2 and H-6), and two protons at 7.04 ppm (2H, dd, J = 8.6, 2.9 Hz, H-3and H-5), were assigned symmetric patterns with replacement at 1 and 4 places. H-8 and H-6 were ascribed to the last two aromatic proton signals at 6.43 ppm (1H, d, J = 2.3 Hz) and 6.25 ppm (1H, d, J= 1.8 Hz)

Accordingly, trihydroxyl substitutions at C-5 (161.4 ppm), C-7 (164.2 ppm), and C-4 (157.4 ppm) in the flavone skeleton were predicted for this

molecule. There were 19 carbon signals seen in the 13C NMR spectra (Figure 11). H-6 (6.24 ppm)/C-6 (98.5 ppm), H-8 (6.45 ppm)/C-8 (92.5 ppm), H-2 or H-6 (7.85 ppm)/C-2 and or C-6 (131.4 ppm), and H-3 or H-5 (7.05 ppm)/C-3 and or C-5 were the six methines that were found to be aromatic in IO3 (115.1 ppm). We identified this isolated substance IO3 as kaempferol-3-O-rhamnoside by comparing the spectrum data of the isolated compound with published research.

Compound IO4: Compound IO4 was isolated as a yellow amorphous powder. Two distinct high absorption peaks at 256 and 372 nm were visible in the compound IO4's UV spectra. IR absorption bands at 3296 cm^{-1}, 1659 cm^{-1}, 1596 cm^{-1}, and 1169 cm^{-1}, respectively, confirmed

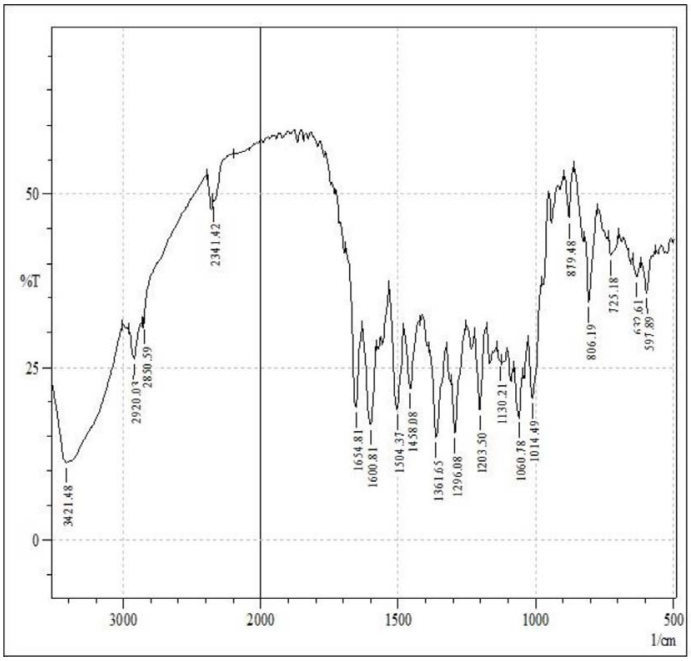

Figure 12: FT-IR spectra of compound IO4.

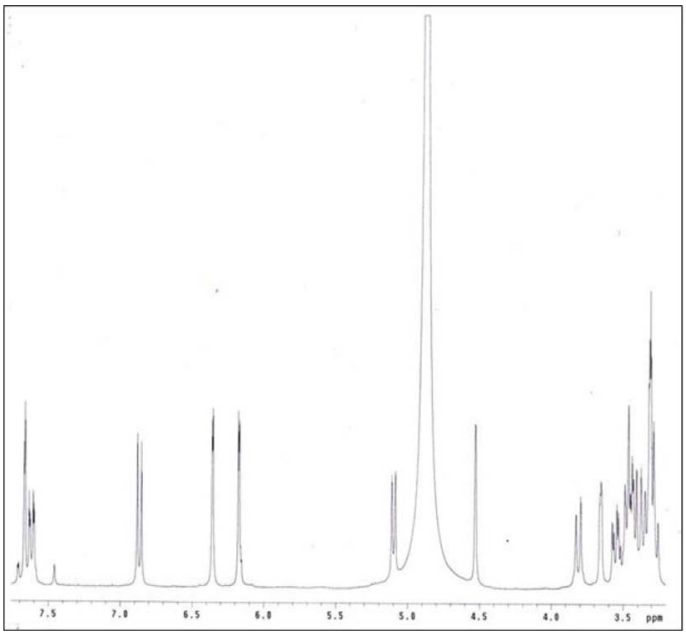

Figure 13: ^{1}H NMR spectra of compound IO4.

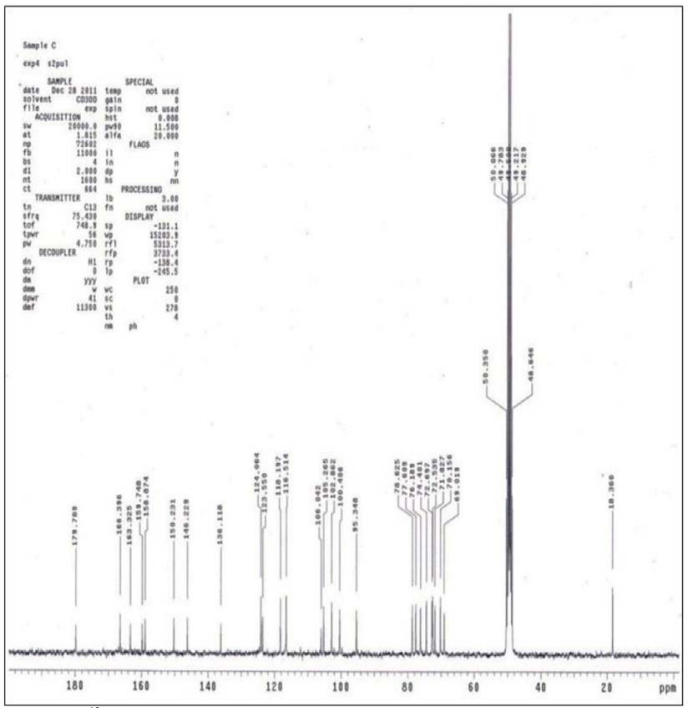

Figure 14: ^{13}C NMR spectra of compound IO4.

the presence of hydroxyl, carbonyl, aromatic ring, and ether groups (Figure 12). In Figure 13, the molecule IO4's ^{1}H- and ^{13}C-NMR spectra, aromatic systems showed resonances. The presence of hydroxyl groups at C-4, C-6, and C-3 and C-4 of the B ring corresponds to the existence of a strong singlet signal at 4. 87 in the ^{1}H-NMR spectrums (Figure 15). Another broad singlet is seen at position 12. This may indicate that OH is present at the C-3 position next the carboxylic group. The existence of 15 aromatic carbon signals could be seen in the 13C NMR spectra (Figure 14). The presence of carbonyl carbon was suggested by the signal at 179. The structure of compound IO4 was predominantly identified as a Quercetin based on the obtained spectral data and comparison with the data provided in the literature.

HPTLC Fingerprinting

HPTLC fingerprinting of I. obscura with lupeol

The resolution of the chosen mobile phase was excellent. HPTLC chromatogram of standard lupeol revealed a single peak (Figure 15 and 16). After the chromatogram was developed, the plate was derivatized with vanillin-H_2SO_4, which produced a violet colour spot in both the standard and the n-hexane fraction of IO. The plate was then scanned at 540 nanometers. The R_f value of the chemical found in the n-hexane fraction of IO (0.52) was virtually identical to that of the standard (0.53) (Table 9). When the R_f values of the IO extract are compared to the standard, HPTLC finger printing confirms the presence of Lupeol.

HPTLC fingerprinting of CCl4 extract with β-sitosterol

The resolution of the chosen mobile phase was excellent. HPTLC chromatogram of standard -sitosterol revealed a single peak (Figure 17 and 18). After the chromatogram was developed, the plate was derivatized with the visualizing agent vanillin- H_2SO_4, which revealed a pink colour spot in both the standard and extract. The plate was then scanned at 540 nanometers. The Rf value of the chemical found in the CCl4 fraction of IO (0.39) was almost identical to that of the standard (0.39). (Table 10). When the Rf values of the CCl4 fraction are compared to the standard, it indicates the existence of β -sitosterol in the IO extract.

HPTLC fingerprinting of fraction D of IO with standard Quercetin and Kaempferol

Various solvent compositions with varying polarities were used to optimise the mobile phase. In the fraction D of the mobile phase Tolune:

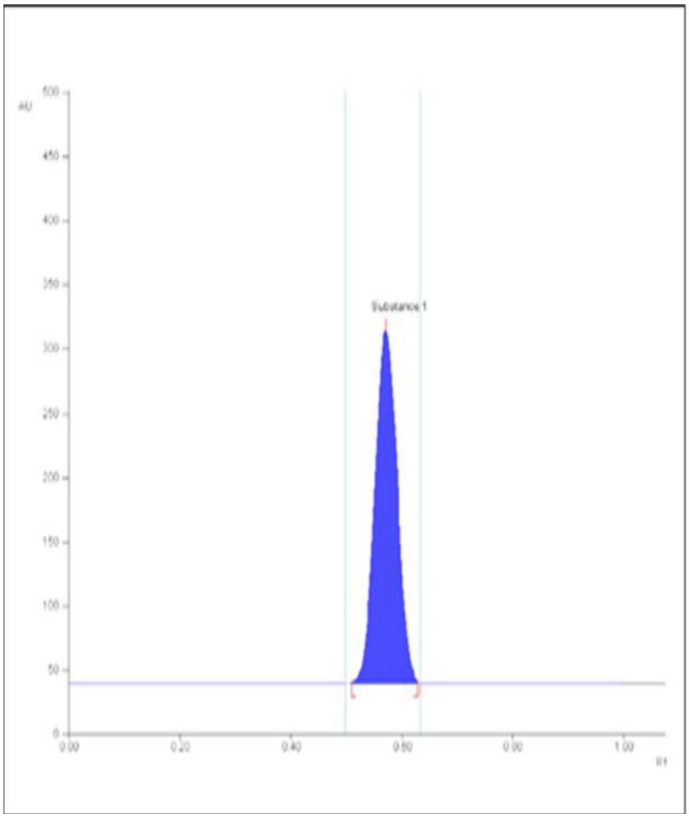

Figure 15: HPTLC chromatogram of standard Lupeol.

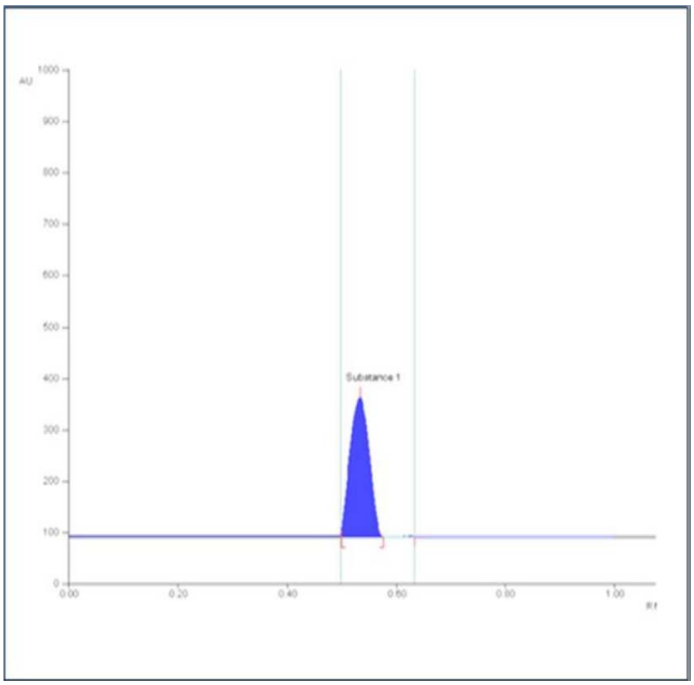

Figure 16: HPTLC chromatogram of n-hexane fraction of *I. obscura*.

Table 9: Showing max Rf, AUC and % peak area of lupeol and fraction of IO scanned at 540 nm.

Track	Sample	Start R_f	Max R_f	End R_f	AUC	% peak area
1	L1	0.50	0.53	0.57	6397.2	100
2	L2	0.50	0.53	0.57	9534.1	100
3	IOF2	0.48	0.52	0.57	6435.2	94.3
4	IOF2	0.49	0.52	0.57	6123.2	96.7

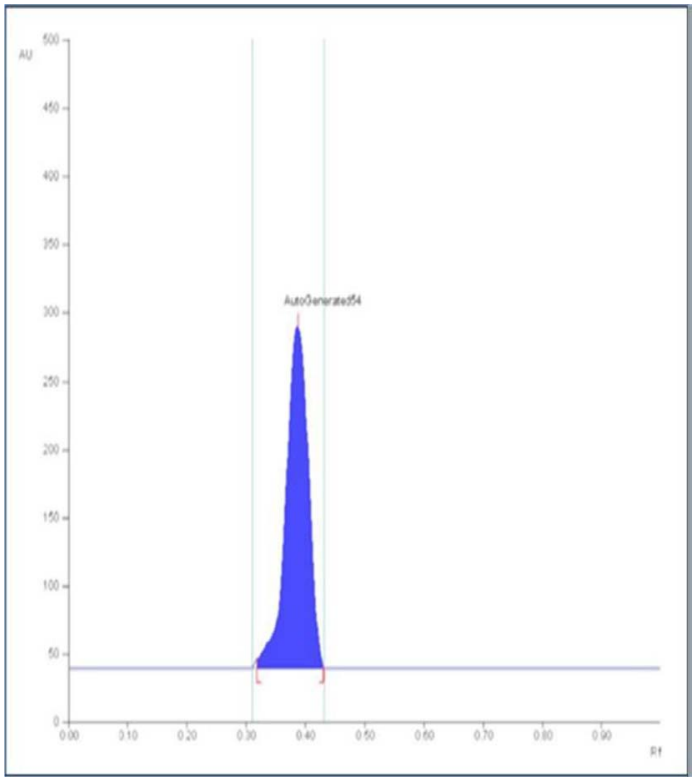

Figure 17: HPTLC chromatogram of standard β-sitostrol.

Acetone: Formic acid, standard quercetin and kaempferol display a single peak (Figures 19, 20, and 21). (7:3:0.2). The plate was scanned at 254 nm after development. The chemicals found in the D fraction of *I. obscura* had similar Rf (0.42 and 0.63) values (Table 11), and fluorescence indicated the presence of quercetin and kaempferol.

CONCLUSION

The *Ipomoea obscura* extract contains a variety of phytochemicals such as reducing sugars, glycosides, phenolic compounds, flavonoids, steroids, and alkaloids, according to the phytochemical study. The quantitative analysis showed the presence of 5.56% phenolic component and 0.34 % flavonoids. The phytochemical investigation of *Ipomoea obscura* led to the discovery of triterpenoid and flavonoid components. These chemicals were purified by crystallisation after being isolated using various scientific procedures. The chemical structure of lupeol and β-sitosterol in n-hexane fraction, quercetin and kaempferol in carbon tetrachloride fraction of methanolic extract of *Ipomoea obscura* was determined using UV-Vis, IR, ^{1}H, and ^{13}C NMR spectroscopic techniques. The above-mentioned study provides information on the identification of *I. obsura*, chemical components, and physical features. These studies assist in the identification and verification of plant material.

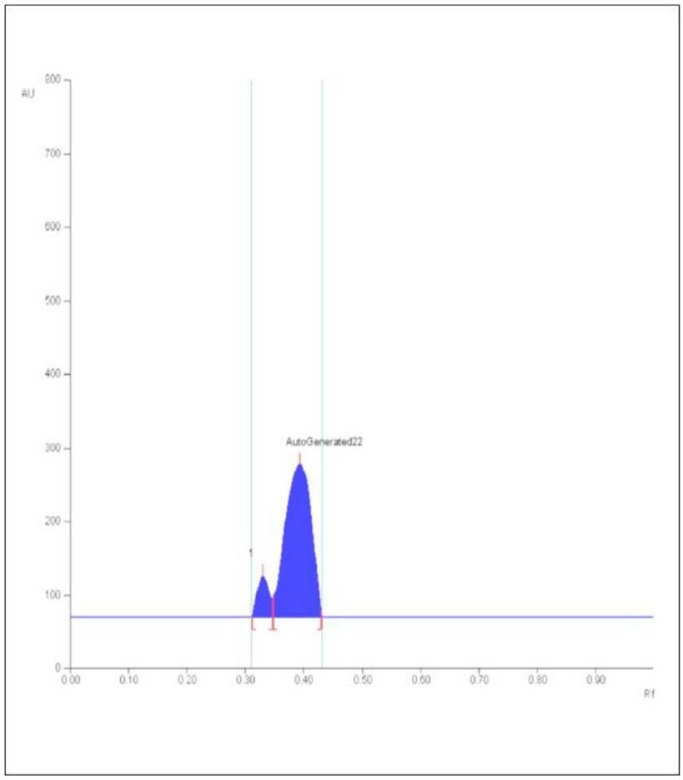

Figure 18: HPTLC chromatogram of CCl4 fraction of *I. obscura*.

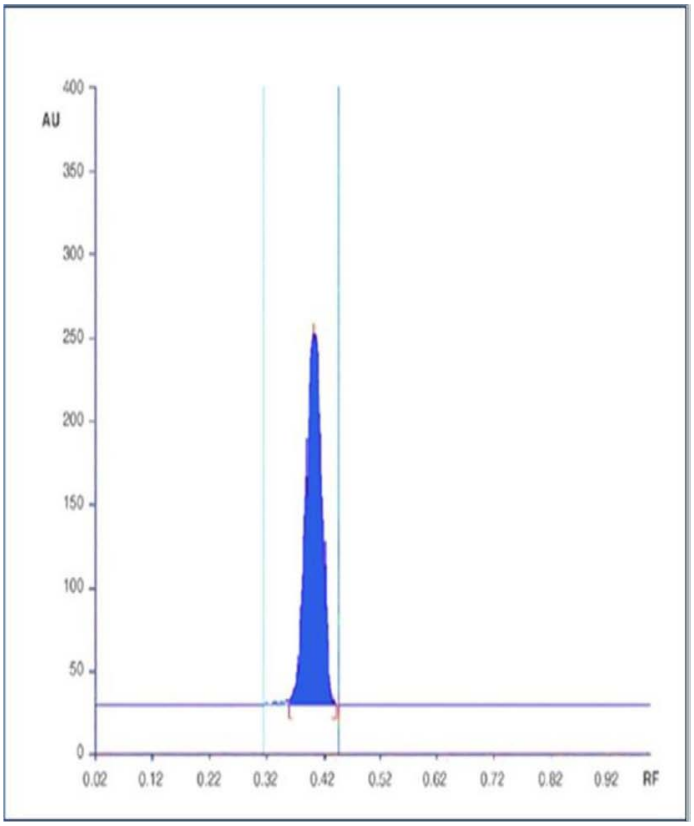

Figure 19: HPTLC chromatogram of Standard quercetin.

Table 10: Showing max Rf, AUC and % peak area of β-sitosterol and fraction of IO scanned at 540 nm.

Track	Sample	Start R_f	Max R_f	End R_f	AUC	% peak area
1	B1	0.32	0.39	0.43	6434.2	100
2	B2	0.32	0.39	0.43	6534.1	100
3	IOF2	0.32	0.39	0.43	6625.2	94.3
4	IOF2	0.35	0.39	0.43	6123.2	96.7

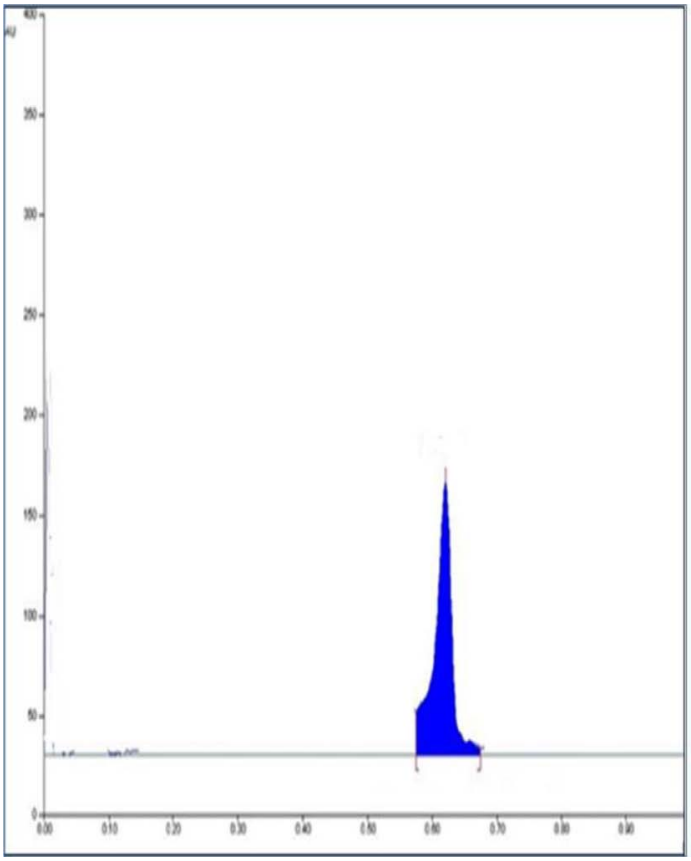

Figure 20: HPTLC chromatogram of Standard kaempferol.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest

REFERENCES

1. Eckart E. Solanaceae and Convolvulaceae – secondary metabolite. 2008;1:637.
2. Christophe W. Ethnopharmacology of medicinal plants: Asia and the Practice. Vol. 1. Humana press; 2002. p. 6928.
3. Sripathi SK, Sankari U. Ethnobotanical documentation of a few medicinal plants in the Agasthiayamalai Region of Tirunelveli District, India. Ethnobotanical Leafl. 2010;14:173-81.
4. Mungole AJ, Awati AA, Chaturvedi ZP. Preliminary Phytochemical screening of *Ipomoea obscura* (L.) – A hepatoprotective medicinal plant. Int J PharmTech Res. 2010;2(4):2307-12.
5. Asano N, Yokoyama K, Sakurai M, Ikeda K, Kizu H, Kato A, *et al*. Dihydroxynortropane alkaloids from calystegine-producing plants. Phytochemistry. 2001;57(5):721-6. doi: 10.1016/s0031-9422(01)00131-5, PMID 11397439.

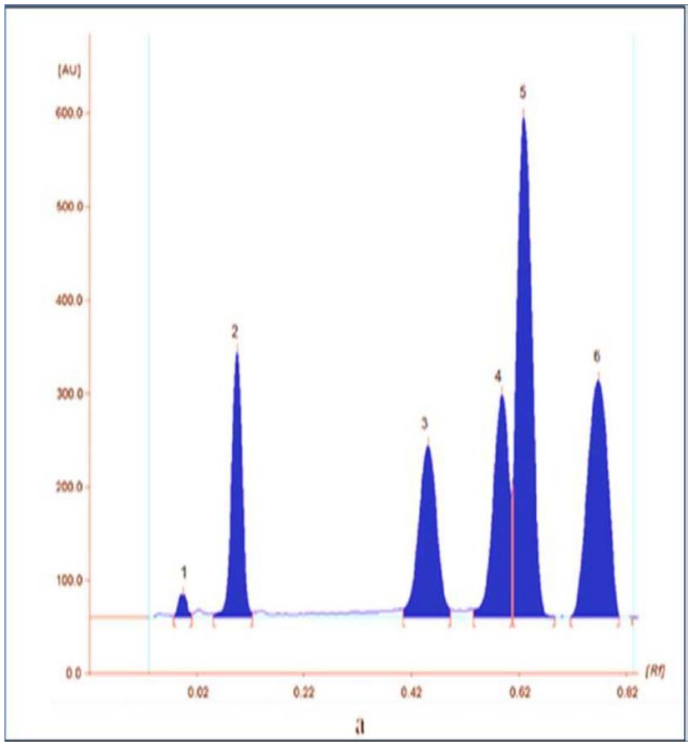


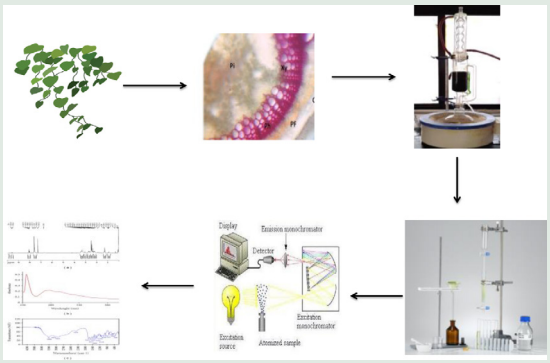
Figure 21: HPTLC chromatogram of D fraction of IO was showed presence of quercetin and kaempferol.

Table 11: Showing max Rf, AUC and % peak area of Quercetin and Kaempferol with fraction D of IO scanned at 254 nm.

Track	Sample	Start R_f	Max R_f	End R_f	AUC	% peak area
1	Q1	0.38	0.41	0.44	10164.7	96
2	Q2	0.37	0.42	0.44	11703.5	100
3	D1(Q)	0.39	0.43	0.48	2007.3	45.2
4	D2 (K)	0.60	0.63	0.66	1643.3	66.3
5	K1	0.59	0.64	0.67	10198.2	94.3
6	K2	0.60	0.65	0.68	11192.3	100

6. Eich E, Sattler HJ, Henn E. Ipobscurines, Non-ergoline type indole compounds from the seeds of *Ipomoea obscura*. Planta Med. 1986;52(6):523-4. doi: 10.1055/s-2007-969307, PMID 17345460.
7. Hamsa TP, Kuttan G. Anti-angiogenic activity of *Ipomoea obscura* extract and Ipobscurine- A. Immunopharmacol Immunotoxicol. 2011;33(3):488-97. doi: 10.3109/08923973.2010.531277, PMID 21058930.
8. Joshi RK. Sesquiterpene-rich volatile constituents of *Ipomoea obscura* (L.) Ker-Gawl. Nat Prod Res. 2015;29(20):1935-7. doi: 10.1080/14786419.2015.1010085, PMID 25675269.
9. Kiritikar KR, Basu BD. Indian medicinal plants. Vol. III. Dehradun, India: International Book Distributors; 1999. p. 1722-3.
10. Srinivasan R, Chandrasekar MN, Nanjan MJ, Suresh B. Free radical scavenging activity of *Ipomoea obscura* (L.) Ker-Gawl. J Nat Rem. 2007;7(2):184-8.
11. Srinivasan R, Rajan S, Ashok D, Kumarappan C. Cytotoxic and antimicrobial activity of *Ipomoea obscura*. Aust J Basic Appl Sci. 2014;8(1):529-32.
12. Khandelwal KR. Practical pharmacognosy. 16th ed Nirali Prakashan. Pune; 2006. p. 149-53.
13. O'Brie TP, Fader N, McCull ME. Polychromatic staining of plant cell walls by toludine blue-O. Protoplasma. 1964;59:364-73.
14. Kokate CK, Gokhale SB. Practical pharmacognosy. 12th ed. Pune: Nirali Prakashan; 2008. p. 129.
15. Khadabadi SS, Deore SL, Baviskar BA. Experimental phytopharmacognosy. 1st ed. Nirali prakashan; 2011. p. 3.2-8, 4.29-33.
16. Indian pharmacopoeia. Vol II. 4th ed. Government of India, Ministry of Health and Family Welfare, controller of publication, New Delhi. Who/PHARM/92.559/rev. 1996: A53-4.
17. Trease GE, Evans WC. Pharmacognosy. Philadelphia: Saunders Publisher; 2002. p. 42-4, 221-9, 246-9, 304-6, 331-2, 391-3.
18. Still WC, Kahn M, Mitra A. Rapid chromatographic technique for preparative separations with moderate resolution. J Org Chem. 1978;43(14):2923-25. doi: 10.1021/jo00408a041.
19. Bajpai VK, Majumder R, Park JG. Isolation and purification of plant secondary metabolites using column-chromatographic technique. Bangladesh J Pharmacol. 2016;11(4):844-8. doi: 10.3329/bjp.v11i4.28185.
20. Sharma PC, Bhatia V, Bansal N, Sharma A. A review in Bael tree. Nat Prod Radiance. 2007;6(2):171-8.
21. Yadav NP, Chanotia CS. Phytochemical and pharmacological profile of leaves of Aegle marmelos Linn. Pharmcognosy Rev. 2009;10(3):144-9.
22. Nugroho AE, Riyanto S, Sukari MA, Maeyama K. Effects of aegeline, a main alkaloid of Aegle marmelos Correa leaves, on the histamine release from mast cells. Pak J Pharm Sci. 2011;24(3):359-67. PMID 21715270.
23. Sethi PD. CBS Publishers and distributors. 1st ed. New Delhi; 1996. p. 39.
24. Gomathi D, Ravikumar G, Kalaiselvi M, Vidya B. Chandrasekar Uma, HPTLC fingerprinting analysis of Evolvulus alsinoides (L.).J Acut. Med. 2012;2:72-82.
25. Senguttuvan J, Subramaniam P. HPTLC Fingerprints of Various Secondary Metabolites in the Traditional Medicinal Herb Hypochaeris radicata L J Bot. 2016;2016:1-11. doi: 10.1155/2016/5429625.

GRAPHICAL ABSTRACT



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