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PHYTOCHEMICAL STUDY ON THE EXTRACT OF *SCOPARIA DULCIS* LINN. LEAVES

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ABSTRACT: The plant *Scoparia dulcis* Linn. belongs to the family Scrophulariaceae, is a native of tropical America, commonly called a sweet broom. The traditional healers identified its uses as anti-diabetic, antipyretic, antihypertensive, diuretic, and they have developed many promising traditional medicines and formulations from this plant. The main objective of this present study reveals about the phytoconstituents present in the leaves extract by preliminary phytochemical study, isolation of active compounds by column chromatography, and characterize the isolated compound by spectral studies like UV, IR, NMR, and MASS spectra. The preliminary phytochemical study reveals that the plant possesses more phytochemicals such as carbohydrates, alkaloids, tannins, phenols, flavonoids, vitamin C, and amino acids. The quantitative estimation shows that it consist of a considerable quantity of flavonoids, Phenols, Vitamin C. The methanol extract was prepared and applied in column chromatography to isolate the phytoconstituents. A dull white crystalline compound was isolated in the fraction of Acetone: Ethanol (60:40), (40:60) ratio. This compound was subjected to spectral studies. The spectral studies indicate the presence of –OH, C=O, and C-H groups from the NMR signal. It shows the presence of methyl proton and α $–CH_2$ proton. Based on the spectral studies, the chemical nature of the isolated compound may be a heptadeconic acid with the molecular formula CH_3-$(CH_2)_{15}$-COOH. Future studies also can go for further identification of new compounds and their medicinal values.

INTRODUCTION: Phytochemicals are the complex mixture of chemicals naturally synthesized by plants, and they are a very important base for phytotherapy and nutritional sciences. Some are completely characterized, and some are incompletely characterized and have recently been subject to scientific scrutiny [1]. Abundant of photochemical are present in plant sources without knowing their chemical nature and therapeutic values.

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Because of the growing demand for photochemical for medicines, pharmaceuticals, health and food products, nutraceuticals, cosmetics in the international market, nowadays methodologies have been developed for the isolation and characterization of phytochemicals [2].

The medicinal plant *Scoparia dulcis* L. belongs to the family Scrophulariaceae is a small herb [3]. It is growing throughout the tropic and subtropical countries and growing as wasteland herb and grows in wetland area commonly called as sweet broom having more medicinal values and used in traditional system of medicine [4]. The traditional healers have developed many promising traditional medicinal uses. In India, it is used to treat diabetes[5], in Taiwan to treat Hypertension.

In Brazil, it has been used to treat hemorrhoids and wounds, used to manage sickle-cell disease in Nigeria [6]. This plant has been shown good antioxidant [7], analgesic [8], anti-inflammatory [9], anti ulcer [10], antiurolithiatic [11], Hyperlipidemic [12] and antimicrobial properties due to the presence of the phytoconstituent Scoparinol [13]. Some other active principles like Scoparic acid, Scopadulcic acids [14], Scopadulcinol, and Scopadulin also present in this plant [15]. The antidiabetic activity of this plant has been owed to the existence of diterpene[16], triterpenes [17] and flavonoids, which have been present in the aerial part of the plant. With this background the present study has been designed to identify new phytoconstituents present in the leaf of the plant. The aim of this study is to determine the bioactive compounds present in the *Scoparia dulcis* L. leaves extract with the aid of UV-VIS, FTIR, NMR, and MASS Spectral Techniques, which may provide an insight in its use of traditional medicine.

MATERIALS AND METHODS:

Plant Materials: The plant *Scopario dulcis* L. was collected from the Palakad district in Kerala. The botanical identity has been authenticated by the Director, Botanical Survey of India, Coimbatore, No: BSI/SRC/5/23/2012-13/Tech/496. The voucher specimen has been submitted and preserved in the herbarium for future reference.

Processing of Plant Material: The leaves are collected, shade dried at room temperature and then size reduced to get course powder of desired particle size. The powdered drug was passed through mesh size 80 and stored in an air-tight container. This powdered material was subjected to successive extraction. One kg of powdered drug was extracted with methanol and water separately by cold maceration method for 7 days. The extracts are dried under reduced pressure in a rotary evaporator to get the dry extract. The yield of the dry extracts was calculated and stored in desiccators and used for further experiments[18].

Preliminary Phytochemical Analysis: The methanol and aqueous extract of the plant material was separately prepared and subjected to chemical tests for the identification of its chemical constituents. Chemical tests were carried out on the aqueous and methanol extracts and on the powdered specimens using standard procedures to identify the constituents [19].

Test for Flavanoids:

a. 5 ml of dilute ammonia solution were added to a portion of the aqueous filtrate of plant extract, followed by the addition of concentrated sulphuric acid. A yellow colouration observed in each extract indicated the presence of flavonoids. The yellow colouration disappeared on standing.

b. The extract was treated with ammonia solution. It gives red colour. The extract was treated with potassium hydroxide solution. It gives canary yellow colour.

c. A portion of the powdered plant sample was heated with 10 ml of ethyl acetate over a steam bath for 3 min. The mixture was filtered, and 4 ml of the filtrate was shaken with 1 ml of dilute ammonia solution. A yellow colouration was observed, indicating a positive test for flavonoids.

d. Shinoda test: To the ethanolic extract added and few drops of concentrated hydrochloric acid. To this add, 0.5 gm magnesium turnings were added. The pink colour indicate the presence of flavonoids.

e. Lead acetate test: To the ethanolic extract, the lead solution was added. The formation of Yellow Precipitate shows the presence of Flavonoids.

Test for Saponins: About 2 g of the powdered sample was boiled in 20 ml of distilled water in a water bath and filtered. 10ml of the filtrate was mixed with 5 ml of distilled water and shaken vigorously for a stable, persistent froth. The frothing was mixed with 3 drops of olive oil and shaken vigorously, then observed for the formation of an emulsion.

Test for Phlobatannins: Deposition of a red precipitate when an aqueous extract of plant sample was boiled with 1% aqueous hydrochloric acid was taken as evidence for the presence of phlobatinins.

Test for Steroids: Two ml of acetic anhydride was added to 0.5 g ethanol extract of each sample with

2 ml sulphuric acid. The colour changed from violet to blue or green, indicating the presence of steroids.

Test for Terpenoids: Five ml of extract was mixed in 2 ml of chloroform, and concentrated sulphuric acid 3 ml was carefully added to form a layer. The reddish-brown colour of the interface was formed to show positive results for the presence of terpenoids [20].

Fluorescence Analysis: The drug samples were treated with different chemical reagents, acids, and alkalis. The developed colours were observed under UV – Fluorescent light and documented [21].

Quantitative Phytochemical Estimation:

Estimation of Protein: The dried and powdered samples were extracted by stirring with ethanol (1:5 w/v) at 25 °C for 24 h and centrifuged at 7,000 rpm for 10 min 25. 0.25 ml of the ethanolic solution of the samples were taken. The volumes in all the tubes were made up to 1.0 ml with distilled water. 5.0 ml of alkaline copper reagent was added to each tube. Mixed well and allowed to stand for 10 min. Then 0.5 ml of Folin-Ciocalteau reagent was added. Mixed well and incubated at room temperature for 30 minutes. A reagent blank was also prepared. After 30 minutes, the blue colour developed was read at 660 nm. The results were expressed as mg/g dry matter [22].

Estimation of Total Flavonoids: The flavonoid content in the leaf extract was determined by the use of a slightly modified colorimetry method [23, 24]. A 0.5ml aliquot of appropriately (2mg/2ml) diluted sample solution was mixed with 2ml of distilled water and subsequently with 0.15ml of 5% NaNO2 solution. After 6 min, 0.15 ml of 10% $AlCl_3$ solution was added and allowed to stand for 6 min, and then 2ml of 4% NaOH solution was added to the mixture. Immediately, water was added to bring the final volume to 5ml, and then the mixture was thoroughly mixed and allowed to stand for another 15min. The absorbance of the mixture was determined at 510 nm versus water blank. The analysis was performed in triplicate, and the results were expressed as rutin equivalent. Values are means of three independent analyses ± standard deviation (n = 3) RE – Rutin equivalent. Results are given in the table.

Estimation of Total Phenols: The total phenolic content was determined according to the following method [25, 26, 27]. Ten microlitre aliquots of the extracts (2mg/2ml) were taken in test tubes and made up to the volume of 1 ml with distilled water. Then 0.5 ml of Folin-Ciocalteu phenol reagent (1:1 with water) and 2.5 ml of sodium carbonate solution (20%) were added sequentially in each tube. Soon after vortexing the reaction mixture, the test tubes were placed in the dark for 40 min, and the absorbance was recorded at 725 nm against the reagent blank. The analysis was performed in triplicate, and the results were expressed as tannic acid equivalents. The absorbance was measured for the determination of total phenolic compound in both the extract separately by using the formula;

$$C = C1 \times V/m.$$

Where; C = Total content of phenolic compounds in mg/g, in TAE (tannic acid equivalent); C1 = concentration of Tannic acid established from the calibration curve in mg/ml; V = The volume of extract in ml; M = The weight of plant extract in gm. Values are means of three independent analyses ± standard deviation (n = 3) TAE – Tannic acid equivalent. Results are given in the table.

Estimation of Total Lipid Content: Estimation of total lipid content was determined by the following method [27, 28]. About 10g of the samples was used to extract lipids with 150 ml of petroleum ether for 16 hr, at a solvent condensation rate of 2–3 drops/sec according to AACC Approved Method 30-25 with minor modifications of sample size and extraction time. The obtained extract was concentrated and evaporated at room temperature to dryness. The weight of extract gives the total lipid content, which was expressed as mg/g dry matter. Results are given in the table.

Isolation of Phytoconstituents:

Column Chromatography: Column chromatography was used to separate the compounds. For this purpose, methanol extract of the plant 5 gm sample was taken and placed in a column (column size 90cm × 2.5cm); packed with Silica Gel (100-200 mesh) are the adsorbent used to complete separation of the component of the sample and various organic solvents in various proportions were used.

Silica gel was made into homogenous suspension by shaking with petroleum ether (first eluent). The bottom of the column was plugged with a little cotton to prevent pass put, and then the silica gel suspension was poured into the column, set aside for 10 minutes, and used. Methanol extract of the sample was subjected to column chromatography over silica gel. The column was eluted with solvents of increasing polarity. They were Petroleum ether, Chloroform, Ethyl acetate, Acetone, Ethanol. The column was run with organic solvents in various proportions to isolate compounds from the plant extract [29].

Spectral Study: The isolated compound was examined under visible and UV light for proximate analysis [30]. For UV and FTIR spectrophotometer analysis, the sample was scanned in the wavelength ranging from 260-900 nm using Perkin Elmer Spectrophotometer, and the characteristic peaks were detected [31, 32]. FTIR analysis was performed using the Perkin Elmer Spectrophotometer system, which was used to detect the characteristic peaks ranging from 400-4000 cm^{-1} and their functional groups [33]. The peak values of the UV and FTIR were recorded. NMR and MASS spectral studies were also performed and recorded. Each and every analysis was repeated twice for the spectrum confirmation.

RESULTS:

Preliminary Phytochemical Screening: The preliminary phytochemical test was performed by the standard procedure, and it confirms the presence of Flavonoids, Phytosterols, Terpenoids, Phloba-tannins, and Saponins.

Fluorescent Analysis:

TABLE 1: COLOUR DEVELOPMENT OF DRUGS AT 254 & 366 NM WITH DIFFERENT REAGENTS

S. no.	Treatment	*Scoparia dulcis* L.	
		254nm	366 nm
1	Powder as such	----	-----
2	Powder + Dilute Nitric acid	Yellow	Fluorescent Yellow
3	Powder + 10% Sodium hydroxide	Brown	Orange Brown
4	Powder + 1N hydrochloric acid	Yellowish	Yellow
5	Powder + 50% Nitric acid	Light yellow	DarkYellow
6	Powder + Acetic acid	Brown	Orange
7	Powder + Picric acid	Yellow	Yelloish Orange
8	Powder + 50% Ferric chloride	Orange	Yelloish Orange
9	Powder + N/50 Iodine Solution	Brown	Redish Orange
10	Powder + 50% Sulphuric acid	Blue	Blue Violet
11	Powder + Ethanol	Light yellow	Orange

TABLE 2: ESTIMATION OF PROTEIN, LIPID, PHENOLS, FLAVONOID

Sample	Total protein (mg/g dry matter	Total Lipid (mg/g Dry matter)	Total Phenols (mg TAE/g extract)	Flavonoid content (mg RE/g)
Scoparia dulcis L.	17.23 ± 0.29	60.00 ± 0.45	70.16 ± 12.52	1.95 ± 0.030

Values represents the mean ± SD number of readings in each group = 3

Column Chromatography: By column chromatography one compound was isolated from methanol extract: A dull White crystalline compound was isolated in the fraction of Acetone: Ethanol (60:40), (40:60), & (20:80).

Spectral Studies: The isolated compound was taken for structural elucidation by spectroscopic study.

Spectrophotometric Analysis: The UV-VIS profile of plant extract was taken at the 200 to 800 nm wavelength due to the sharpness of the peaks and proper baseline. The UV-visible spectra were performed to identify the compounds containing σ-bonds, π-bonds, and lone pair of electrons, chromophores, and aromatic rings.

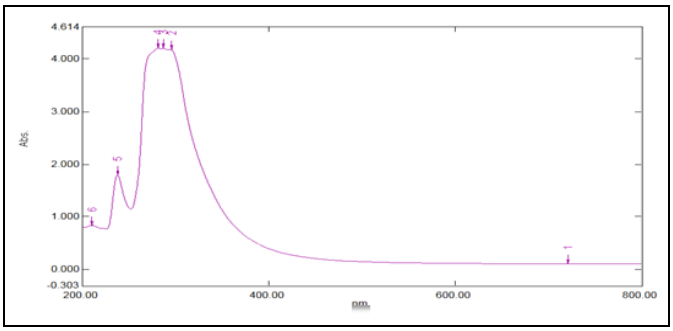


FIG. 1: UV SPECTRUM OF THE ISOLATED COMPOUND

TABLE 3: UV-VIS PEAK VALUES OF THE PLANT EXTRACT

S. no.	Wavelength	Absorbance
1	721	0.111
2	295	4.182
3	286	4.201
4	281	4.204
5	237	1.790
6	209	0.835

The FTIR spectrum was used to identify the functional groups of the active components present in the extract based on the peak's values in the region of IR radiation. When the extract was passed into the FTIR, the functional groups of the components were separated based on its peak's ratio.

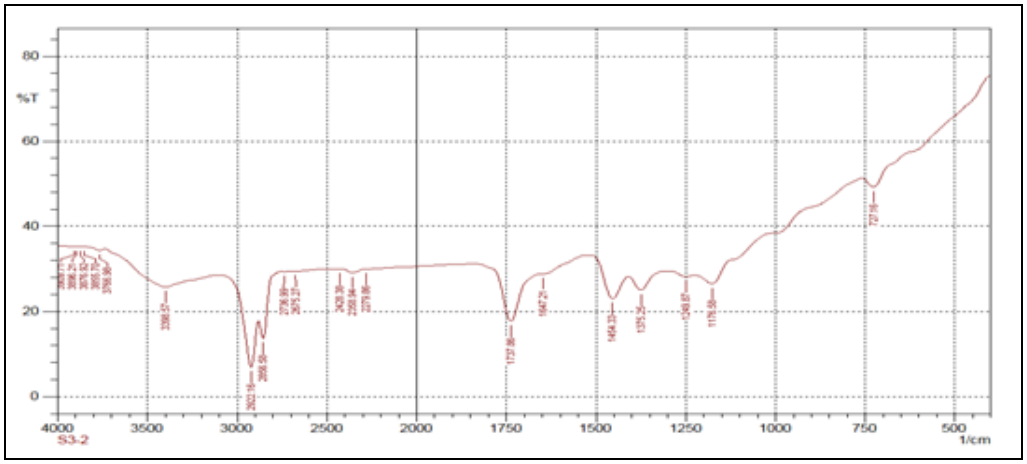


FIG. 2: IR- SPECTRUM OF THE COMPOUND

TABLE 4: FTIR FREQUENCY PEAK VALUES OF THE ISOLATED COMPOUND FROM THE LEAF EXTRACT

S. no.	Peak Values absorption	Functional groups	Compound class
1	1737	C=O Streching	¥ lactone
2	2856	C-H Stretching	alkane
3	2358	-	
4	1249	C-N	
5	1175	C-O Stretching	Tertiary alcohol
6	1375	OH- Bending	Phenol
7	1454	C-H bending	Alkane
8	2656	-	
9	2675	O-H	
10	2922	C-H Stretching	Alkane
11	3398	N-H Stretching	Aliphatic primary amine
12	3766	O-H Stretching	Alcohol

The results of FTIR analysis confirmed the presence of phenol, alkanes, alcohol, aliphatic primary amine, Tertiary alcohol.

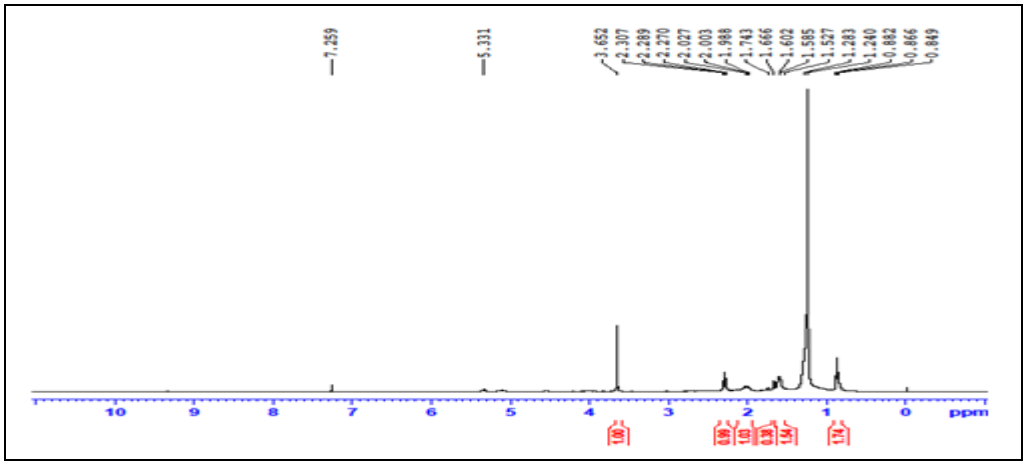


FIG. 3: NMR SPECTRUM OF THE COMPOUND

TABLE 5: NMR SPECTRUM OBTAINED CORRELATION OF ^{1}HCHEMICAL SHIFT WITH ENVIRONMENT

S. no.	Type of Hydrogen	Chemical Shift
1	0.7 to 1.3 = - CH3 saturated primary	1.74
2	1.4 to 1.7 = saturated tertiery	1.54
3	Saturated primary	0.38
4	Saturated primary	1.03
5	Saturated primary	0.98
6	Saturated primary	1.00
7	6.5 to 8 -aromatic	7.25
8	5.331 to 6.5 - vinyl	5.331
9	3.3, 3.65 – 4 = Alcohol ether	3.652
10	2.1 to 2.4 = methyl ketone	2.307

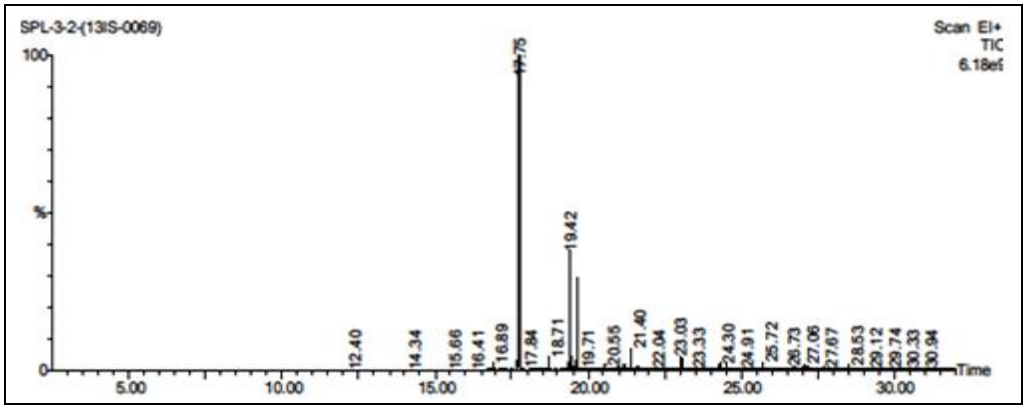
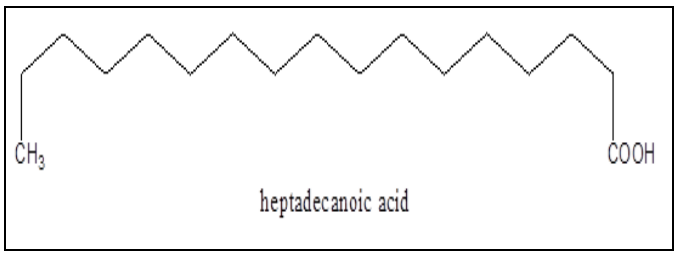


FIG. 4: MASS-SPECTRUM OF THE COMPOUND

DISCUSSION: From the spectral studies like UV, IR, NMR, and MASS, the structure of the isolated compound was identified[33]. From the UV absorption spectra, the compound showed a λmax at 286 nm. The absorption bands at 3396 cm^{-1} are due to –OH group **Fig.1**, 1737 cm^{-1} is due to C=O group at 2922, 1454, 1375 cm^{-1} are due to C-H group **Fig.2**. It shows *m/z* 270 [34,35]. Based on the peak values and absorption, all reports from different spectral in the NMR[36, 37], the signal at δ 0.86 is due to methyl protons **Fig.3**. The MASS spectra show the fragment ions from the m/z are 17.75 - OH, 19.42 - F, 18.710 $–H_2O$,19.65 -F,17.65 –OH. The broad singlet at δ 1.28 is due to long-chain methylene protons **Fig.4** [38,39]. The triplet at δ 2.89 is due to α – CH_2 protons. These studies gave an idea for the isolated compound containing the molecular formula of CH_3-$(CH_2)_{15}$-COOH, so it may be a hepta deconic acid. The proposed structure of the compound is as follows



It may be the final product of the secondary metabolite or it may be the intermediate of the compound for the production of some other phytoconstituent present in the leaf extract. The results of this study offer a platform of using *Scoparia dulcis* L. leaves as herbal alternative for various diseases, including diabetic, cardiovascular *etc*.

CONCLUSION: Natural Products are the secondary metabolites, which are very important phytochemicals used to cure many of the diseases that humans are currently fighting. It is necessary to conduct researches to find out the natural products through biotechnological methods. In this study also effort to isolate phytoconstituents present in the selected plant, and it shows more components present in the methanol extract, it confirms the presence of heptadecanoic acid by spectral studies. It may be useful for further studies to develop a new semi-synthetic phytoconstituent or develop new secondary metabolites in the same plant.

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CONFLICTS OF INTEREST: Nil

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