IJBPAS, November, Special Issue, 2021, 10(11): 31-42
ISSN: 2277–4998

International Journal of Biology, Pharmacy and Allied Sciences (IJBPAS)
'A Bridge Between Laboratory and Reader'
www.ijbpas.com

PHYTOCHEMICAL SCREENING, EXTRACTION AND ISOLATION OF NEW COMPOUND FROM THE *SANSEVIERIA ROXBURGHIANA* LEAF EXTRACT

R. SHANMUGAPRIYA AND D. VIJI SARAL ELEZABETH*

PG& Research Department of Chemistry, Nehru Memorial College of (Autonomous), (*Affiliated to Bharathidasan University*), Puthanampati, Trichy -621007, Tamilnadu, India

***Corresponding Author: E Mail: Dr. Viji Saral Elezabeth. D: azahirhussainchem@yahoo.co.in**

Received 19th July 2021; Revised 20th Aug. 2021; Accepted 29th Sept. 2021; Available online 1st Nov. 2021

https://doi.org/10.31032/IJBPAS/2021/10.11.1003

ABSTRACT

Different solvent soluble fractions of *Sansevieria roxburghiana* (Agavaceae) leaves were tested for preliminary phytochemical analysis and antibacterial exploration against clinically significant bacterial strains in this study. The existence of different primary and secondary plant metabolites such as alkaloids, flavonoids, saponins, steroids, terpenoids, tannins, Resin, carbohydrate, and phenols was confirmed by qualitative analysis of the selected portions. Using column chromatography, the novel 2-(4-hydroxy-3-methoxyphenyl)-5-methoxy-4H-furo[2,3-h] chromen-4-one was extracted from the methanol extract. HNMR, CNMR, LC/Ms, and IR analyses were used to confirm the structure of the molecule.

Keywords: *Sansevieria roxburghiana,* Phytochemical, Isolation, NMR, Mass, Characterization.

INTRODUCTION

Sansevieria roxburghiana (S. roxburghiana) is a hemp species with concave, short petioled leaves that are transversely banded with light and dark green, as well as linearly striated with whitish to light green and dark green striations **[1]**. This plant is utilised for decorative purposes and has long rhizomes with a quick rate of growth and long fibrous roots **[2]**. Sansvieria has around 70 species, including *Sansevieria trifasciata, Sansevieria ehrenbergii, Sansevieria guineensis, Sansevieria longiflora, Sansevieria zeylanica*, and others. Medicinal plants remain an essential medicinal assistance in the treatment of

human illnesses. This herb has been utilised for a variety of therapeutic uses in India for centuries **[3, 4]**. The whole plant has been used in the treatment of glandular enlargement and rheumatism as a cardiotonic, expectorant, febrifuge, purgative, tonic, and tonic. In STZ (streptozotocin) induced diabetic mice, recent investigations on *S. roxburghiana* rhizome exhibited outstanding anti-diabetic potential. Infected wounds, cuts, and grazes are treated using leaf sap. **[5-7]**.

In cancer treatment, a variety of plants and plant-derived compounds are used. Breast cancer is treated with taxol, while leukaemia is treated with vinca alkaloids. Due to the existence of chemopreventive chemicals, these compounds are effective as anticancer agents **[8]**. Chemotherapy and cytotoxic medications, on the other hand, are fundamentally damaging **[9]**. Some herbal medications, such as paclitaxel, etoposide, and vincristine, are used to treat cancer in this age, but they are quite expensive due to the scarcity of plants and the small number of active constituents. (example, 500 mg vincristine from 5 kg vinca). With this background in mind, *S. roxburghiana* was chosen for anticancer activity testing because it has been shown to have cytotoxicity in a brine shrimp lethality assay **[10]** as well as anticancer activity in mice against Ehrlich ascites carcinoma (EAC) **[11]**.

Sansevieria species have traditionally been used to treat cancer, particularly abdominal malignancies. **[12]** *S. roxburghiana* is a rhizomatous herb in the Asparagaceae family that is used to make Murva, an Ayurvedic medicine. Leaves are 8–9 in a tuft, up to 90 cm long and 2.5 cm wide, hard and cross-striped in the centre, and have a rigid spine-like tip. Flowers in fascicles of 3–6 on long racemes are greenish to white tinted with violet. Perianth tube can be up to 1 cm long, with narrow lobes. Fruit is a berry that is globose in shape. *S. roxburghiana* is an attractive plant native to India, tropical Africa, Indonesia, and Sri Lanka that blooms from January to June. The whole plant is said to be helpful for a variety of maladies, including heart disease, fever, itching, cough, indigestion, and rheumatism. Mucilaginous rhizomes are used to treat colds, earaches, and chronic coughs.

Alkaloids, sugars, flavonoids, phenols, glycosides, proteins, anthocyanin and betacyanin, steroids, and saponins were found in previous studies of the plant. Palmitic acid, isorhamnetin-3-O—D-glucopyran oside, gallic acid, 6,4-dihydroxy-3-propen chalcones, bis (2-ethylhexyl) phthalate, bupanidrine, caftaric

acid, diisobutyl phthalate, and 4-propenoxy-7-hydroxy anthocyanins were discovered in an ethanolic extract of *S. roxbughiana* *S.roxburghiana* rhizomes have been shown to have anti-diabetic, analgesic, antibacterial, and antioxidant properties. It also shown antitumor efficacy in mice **[4]** and cytotoxicity in a brine shrimp lethality experiment **[13-14]**.

So far, there have been no investigations into the isolation of new phytochemicals in this plant. The current work aims to address this gap by isolating the active phytochemical and evaluating the phytochemical screening analysis of *S. roxburghiana*.

MATERIALS AND METHODS

Chemicals. All the chemicals used in this investigation were of analytical grade and were obtained from Sigma Chemicals.

Plant collection and identification

The leaves of *S. roxburghiana* were collected form cholan nagar, Trichy local area, Tamilnadu, India. The plant part was authenticated by state Horticulture Farm, Mudhalaipatti, Trichy, Tamilnadu. The leaves are washed and air-dried, ground in to fine powder for phytochemical analysis.

Sample Preparation

Fresh samples are washed and dried in the open air using distilled water. With a laboratory pestle and mortar and an electric grinder, the dried plant material (leaves) is ground into a fine powder, then placed in a clean sample container, labelled, and kept for future use. The classic extraction method was used, which entailed soaking powdered components in solvent in order to increase polarity. The cold soaking procedure was used to extract 1 kilogramme of powdered material. As the polarity of the solvents grew, the ground plant material was soaked in non-polar, medium polar, and polar solvents, in that order. The dried and powdered leaves of *S. roxburghiana* were used. In a 5 litter Erlenmeyer flask, the sample was soaked in hexane at a 1:3 ratio for 72 hours at room temperature. The resulting hexane solution was then filtered using filter paper, and the residue was extracted for 72 hours with new hexane before being filtered one more. The extract was combined and concentrated using a rotary evaporator (type Heidolph Laborota 4000 efficient) at reduced pressure to get hexane crude extract. The leftovers were re-extracted using a similar process with dichloromethane, chloroform, ethyl acetate, chloroform, and methanol crude extracts to obtain dichloromethane, ethyl acetate, chloroform, and methanol crude extracts. At the end of the extraction process, the dry weight and yield of each crude extract were determined. However, dichloromethane extract was used in the investigation.

Phytochemical analysis

Standard protocols were followed for preliminary phytochemical investigation to identify key types of secondary metabolites **[15, 16]**.

Compound Purification

Column Chromatography

The 70% ethanol extract from *S. roxburghiana* plants was chromatographed on a silica gel (60120 mesh) column and eluted with various percentages of solvent combinations containing petroleum ether, chloroform, and methanol. Compound purification was carried out using the fraction eluted with n-hexane:ethyl acetate (3: 2), which has a yellow colour. A vacuum evaporator was used to collect the eluting solvent and concentrate it. TLC was used to establish the purity of the final active compound. (methanol:acetonitrile:formic acid (80:20:0.1% (v/v).) with 0.47 Rf (*S. roxburghiana*) value. The final eluent was recrystallized by Ethanol (Room Temperature). Melting point of this compound is 70-72°C.

Compound characterization

Purity analysis

For purity testing, the physiologically active extract fraction was employed. Reversed-phase high-performance liquid chromatography was used to determine the purity of (RP-HPLC-Shimadzu LC-10 system, Shimadzu Co., Kyoto, Japan). The C18 column (1004.60mm 2.6 m, 100) was utilised for separation, and the column temperature was kept at 35oC. For HPLC analysis, gradient elution was utilised. A-water + 0.1 percent formic acid; B-methanol:acetonitrile:formic acid (80:20:0.1 percent (v/v)). With a flow rate of 1 mL/min, the gradient elution was begun at 90:10 (A:B) and adjusted to 10:90 (A:B) for a total run time of 18 minutes. The injection volume was ten litres, and the detecting wavelength was 280 nm.

Fourier-transformed infrared (FTIR) spectra

FTIR analysis was used to determine the functional group present in the molecule. The optimised extract was mixed with KBr powder to make a 1 percent (w/v) slurry concentration, and the KBr pellet was made by pressing 5.5 tonnes for 3 minutes. The observations were then made on a JASCO FT / IR-6300 instrument (JASCO Corporation, Tokyo, Japan) with a resolution of 4 cm1, and the spectra were recorded over the 400–4,000 cm1 IR spectrum.

NMR analysis

Nuclear Magnetic Resonance (NMR) spectroscopy was used to confirm the structure of the molecule (Bruker BioSpin, Rheinstetten, Germany). The spectra were acquired using a 1H NMR

spectrometer operating at 400 MHz with DMSO as the solvent of choice. The spectra were acquired using a 13C NMR spectrometer operating at 100 MHz with DMSO as the solvent of choice.

LC-MS/MS analysis

LC-MS/MS analysis was used to determine the molecular weight of the molecule. 0.5 percent (v/v) acetic acid (A) and 100 percent methanol were utilised as solvents (B). The isocratic elution went like this: I 55 percent solvent A from 0 to 10 minutes, (ii) 65 percent from 11 to 20 minutes, and (iii) 35 percent from 21 to 30 minutes. The column temperature was kept at 30 °C and the PDA detector (UPLC LG 500 nm) was monitored at 340 nm. With a mass range of 150 m/z to 1000 m/z, a capillary voltage of 3.50 kV, a cone voltage of 30 V, an extractor voltage of 3V, a gas flow of 30 L/Hr, and a collision gas flow of 0.18 mL/Min, the mass spectrometer (MS) was operated in positive ionisation mode.

RESULT AND DISCUSSION

Preliminary Photochemical screening

The presence of bioactive secondary metabolites such as alkaloids, flavonoids, saponins, steroids, terpenoids, tannins, Resin, carbohydrate, and phenols was discovered in the leaf portions of *S. roxburghiana* **(Table 1)**. When comparing methanol extract to acetone, chloroform, and ether, phytochemical screening found that methanol extract had a higher concentration of alkaloids. Ethanol and ether contain moderate amounts of flavonoids, while ethanol and methanol have moderate amounts of saponins. Steroids were found in higher concentrations in methanol, chloroform, and ether, and in a moderate amount in acetone; terpenoids were found in chloroform but not in the other extracts. Acetone and methanol have a lot of tannins, while ethanol and chloroform have a lot of them. Phenols are exclusively found in large concentrations in methanol fractions, while quinones are found in moderate amounts in methanol, chloroform, and ether. Alkaloids, Flavonoids, Saponins, Steroids, Terpenoids, Tannins, Resin, Carbohydrate, and Phenols were found in abundance in *S. roxburghiana* leaf extracts. All of them have the potential to improve one's health, at least in certain conditions.

Purification new compound

The component 1 was obtained from *S. roxburghiana* methanol leaf crude extract. The yellow colour extract obtained from a 3: 2 solvent ratio of n-hexane to ethyl acetate was concentrated and submitted to TLC analysis to confirm the single fraction chemical. The fraction was TLC-analyzed in an 80:20:0.1 percent (v/v) mixture of methanol, acetonitrile, and formic acid. It depicts a single compound under UV light with an Rf value of 0.47. **(Figure 1)**. This indicates that the TLC shows a pure chemical, which was given

the designation Compound 1 with a concentration of 10.2 mg.

Structure Elucidation

The compound 1 was isolated from methanol leaf extract of *S. roxburghiana.* It was eluted with n-hexane: ethyl acetate (3: 2) with its physical appearance as light yellow colour powder.

IR (υmax, cm^{-1}, KBr):3435 (OH, stretching) 2924 (O-CH_3), 1635 (C=O stretching) **(Figure 2)**: ^{1}H NMR (400 MHz, DMSO): δ 3.82 (6H, s, two methyl group), 6.39 (1H, s), 6.71 (1H, dd, *J* = 1.8, 1-ethylene), 6.74 (1H, dd, *J* = 8.4, benzofuran), 6.97 (1H, t, *1*-benzene), 7.25 (1H, dd, *J* = 1.7Hz, 1-benznene), 7.59 (1H, dd, *J* = 1.8, benzofuran), 7.98 (1H, dd, *J* = 8.4, 1-benzene) **(Figure 3)**. ^{13}CNMR (400 MHz, $CDCl_3$): 182 (1-carbonyl), 164 (ethylene), 161 (1-benzene), 156 (1-benznene), 153 ((1-benznene)), 149 (1-benznene), 147(1-benznene), 144 (benzofuran) ,124 (1-benznene), 119 (1-benznene), 115 (1-benznene), 113 (1-benznene), 106 (1-benznene), 104 (1-benznene), 103 (benzofuran), 103 (ethylnene), 95 (1-benzene), 55 (aliphatic C), 53 (aliphatic C) (Figure 4). LC-MS (ESI) *m/z* (% of relative abundance) calculated for $C_{19}H_{14}O_6$: 338.31, Found $C_{19}H_{14}O_6$ $^{+1}$:339.42 [M+1] **(Figure 5)**. The purity of the isolated compound was confirmed by HPLC with RT 8.47 **(Figure 6)**. The complete assignments of IR, NMR, Mass data of compound 1 was confirmed as a 2-(4-hydroxy-3-methoxyphenyl)-5-methoxy-4H-furo[2,3-h] chromen-4-one **(Figure 7)**. From the report of PubChem data base and literature survey for this compound 1, revealed that the 2-(4-hydroxy-3-methoxyphenyl)-5-methoxy-4H-furo[2,3-h] chromen-4-one (IUPAC Name of Compound 1) was not reported anywhere.

Table 1: Preliminary Phytochemical screening analysis of *sansevieria roxburghiana*

Phytochemicals	PET Ether	Chloroform	Acetone	Ethanol	Methanol
Alkaloids	High	-	High	High	Very high
Flavonoids	-	-	Slightly present	Very high	Very High
Saponins	-	-	-	High	High
Steroids	Slightly present	Slightly present	High	High	High
Terpenoids	-	-	Slightly present	Slightly present	Slightly present
Tannins	High	High	High	High	High
Resin	High	-	High	-	-
Phenols	Slightly present	-	-	High	Very High
Carbohydrate	-	Slightly present	Slightly present	Slightly present	High

Figure 1: TLC Image of isolated 2-(4-hydroxy-3-methoxyphenyl)-5-methoxy-4H-furo[2,3-h] chromen-4-one from *sansevieria roxburghiana*

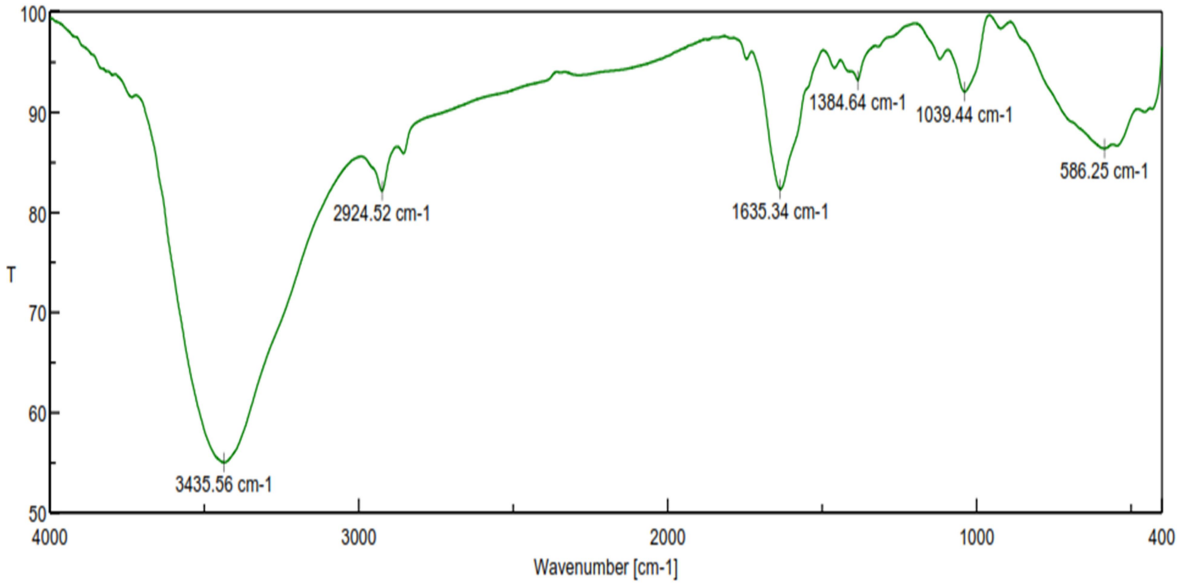

Figure 2: IR analysis of the isolated 2-(4-hydroxy-3-methoxyphenyl)-5-methoxy-4H-furo[2,3-h] chromen-4-one from *sansevieria roxburghiana*

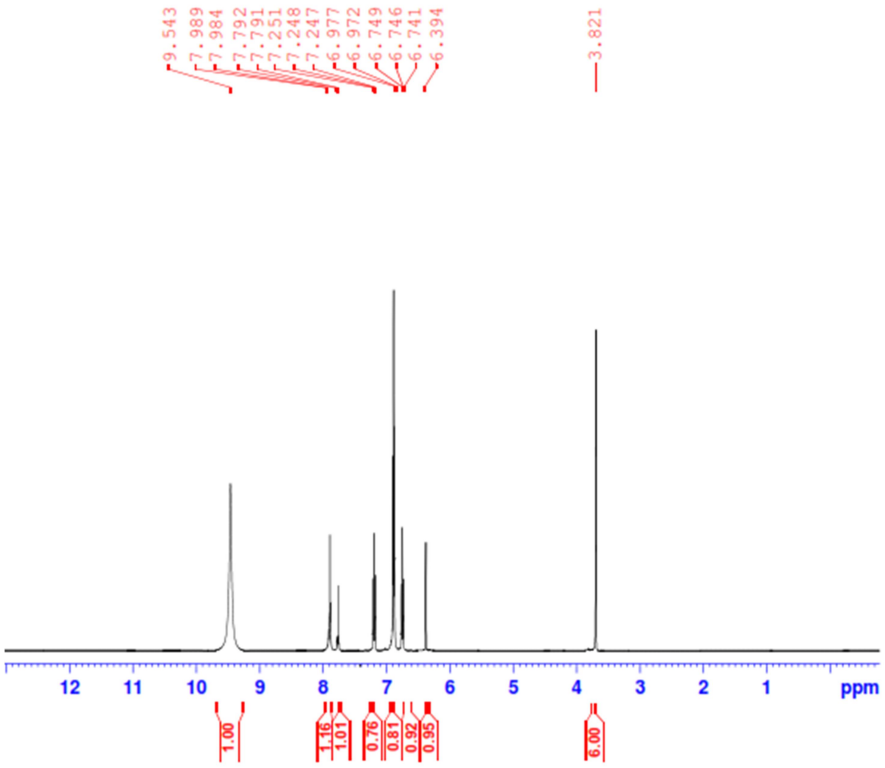

Figure 3: [1]H-NMR analysis of isolated 2-(4-hydroxy-3-methoxyphenyl)-5-methoxy-4H-furo[2,3-h] chromen-4-one from *sansevieria roxburghiana*

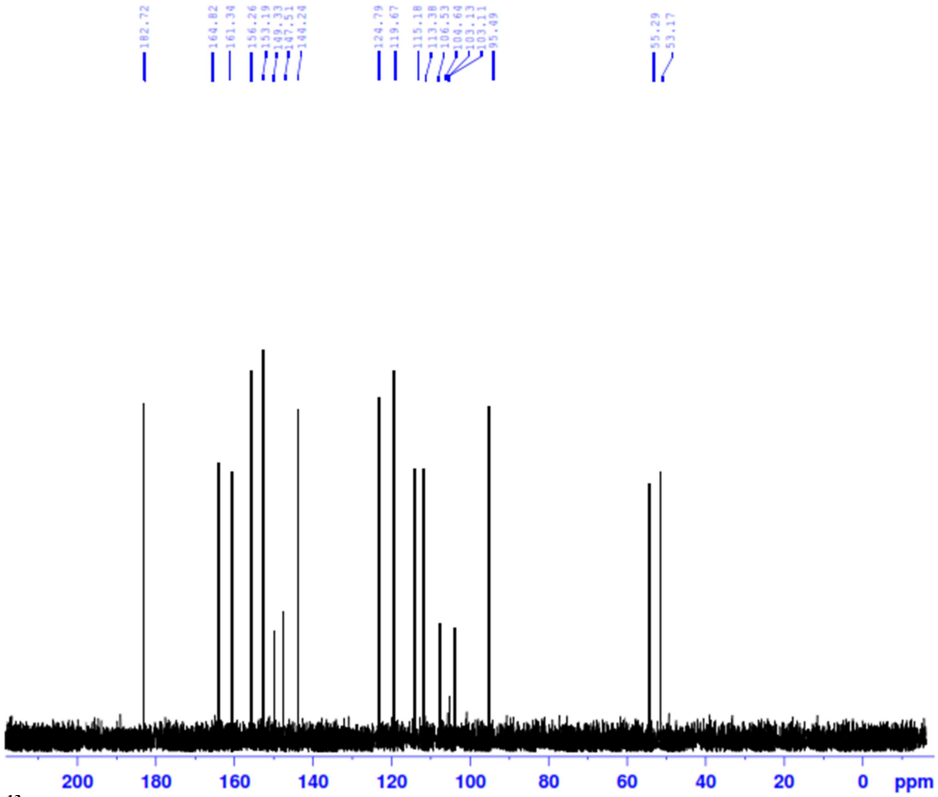

Figure 4: [13]C-NMR analysis of isolated 2-(4-hydroxy-3-methoxyphenyl)-5-methoxy-4H-furo[2,3-h] chromen-4-one from *sansevieria roxburghiana*

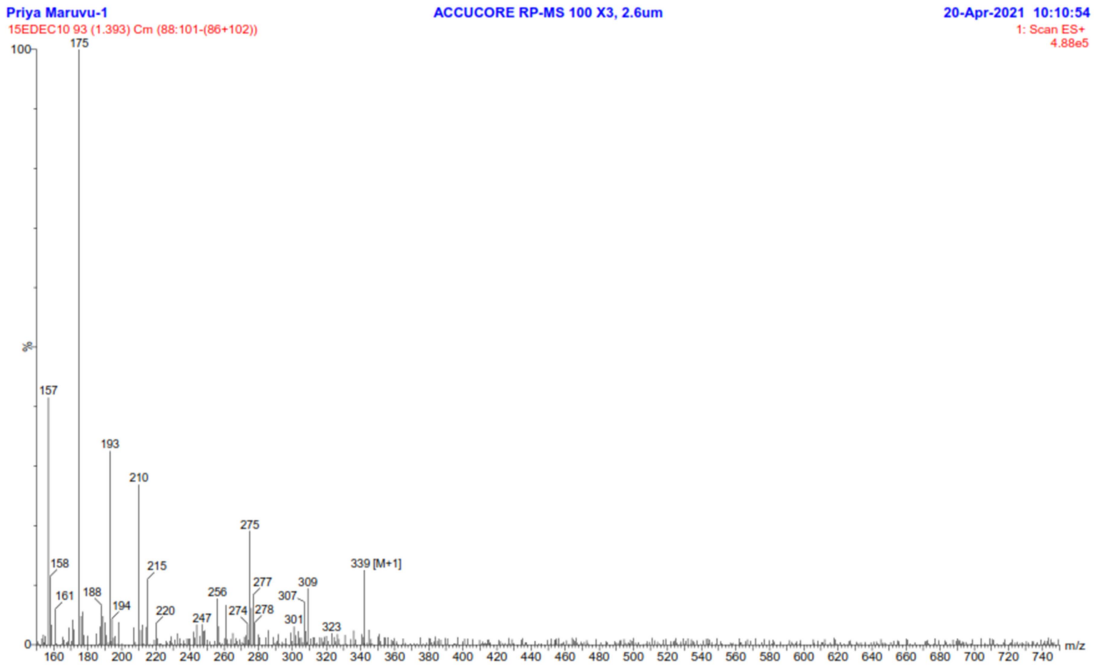

Figure 5: LC/MS analysis of the isolated 2-(4-hydroxy-3-methoxyphenyl)-5-methoxy-4H-furo[2,3-h] chromen-4-one from *sansevieria roxburghiana*

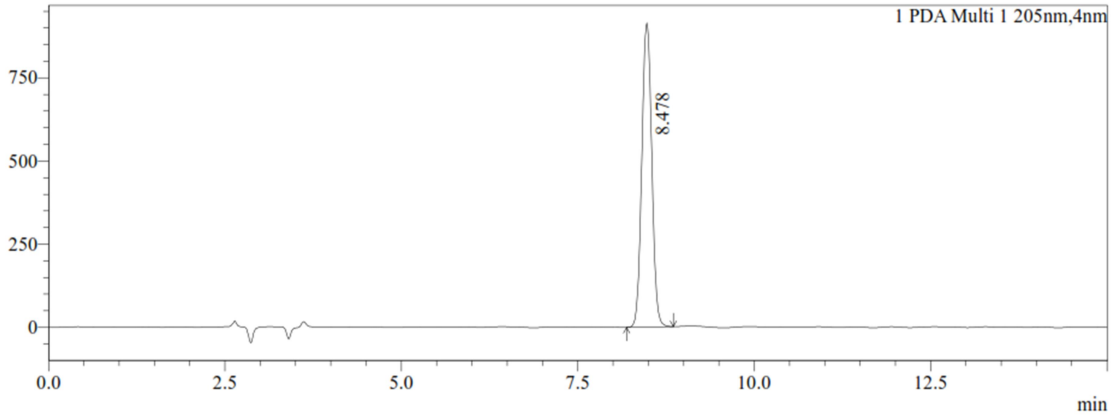

Figure 6: HPLC analysis of the isolated 2-(4-hydroxy-3-methoxyphenyl)-5-methoxy-4H-furo[2,3-h] chromen-4-one from *sansevieria roxburghiana*

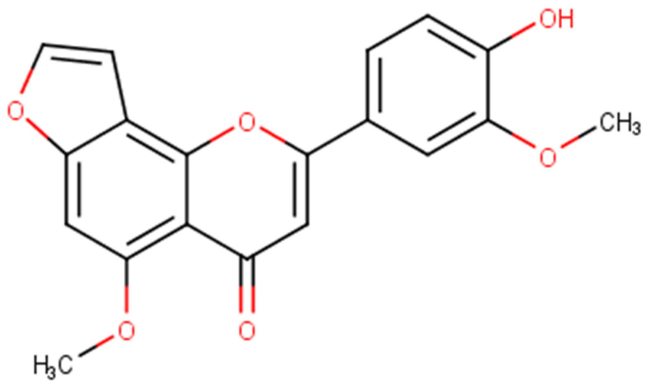

Figure 7: 2-(4-hydroxy-3-methoxyphenyl)-5-methoxy-4H-furo[2,3-h]chromen-4-one

CONCLUSION

The existence of crucial phytochemical analyses in the *sansevieria roxburghiana*. leaves was discovered in this study and could be used for therapeutic purposes. These findings confirm the plant's medical usage while also revealing the possibility that it could be a source of secondary metabolites. The structure of the pure and unique 2-(4-hydroxy-3-methoxyphenyl)-5-methoxy-4H-furo[2,3-h]chromen-4-one) chemical was derived from this crude extract using chromatographic and spectroscopic methods. The current investigation showed good results; nonetheless, only pharmacology can determine the efficacy of the products.

Conflict of Interest: Nil

REFERENCES

[1] Aneesh TP, Mohamed H, Sonal SM, Manjusree M, Deepa TV. International Market scenario of Traditional Indian Herbal drugs-India declining, 2009; 184-190.

[2] Yogita Sardessai, Gauri Pai Angle, Arun Joshi, Sonia Carvalho, Maya bhobe. Anti-microbial Activity of Methanolic Extract of the Rhizomes of Costus igneus. J Pharm Chem Biol Sci, 2014; 2(3): 176-185.

[3] Pallab K. Haldar, Biswakanth Kar, Sanjib Bhattacharya, Asis Bala R, Suresh Kumar B. Antidiabetic activity and modulation of antioxidant status by *sansevieria roxburghiana* rhizome instrepto-zotocin-induced diabetic rats. Diabetologia croatica, 2010; 39-4.

[4] D. Jeya Sheela, S. Jeeva, I. M. Ramzan Shamila, N. C. J. Packia Lekshmi and J. Raja Brindha. Antimicrobial activity and phytochemical analysis of *Sanseiveria roxburghiana* leaf. Asian Journal of Plant Science and Research, 2012; 2(1): 41-44.

[5] Bharathi.P, Elavarasi.N and Mohanasundaram.S (2012). Studies on rate of Biodegradation of Vegetable oil (Cocunut oil) by using *Pseudomonas sp.* International Journal of Environmental Biology, Vol 2, Issue 2, 12-19.

[6] Prakash O, Kumar A, Kumar P, Ajeet. Anticancer potential of plants and natural products: A Review. Am J Pharmacol Sci 2013;1:104-15.

[7] Alan NH. The Use of Herbs and Natural Plant Compounds in the Fight Against Cancer. Cancer support association, Information, Wellness and Healing. Available from: http://www.canceractive.com/cancer-active-page-link.aspx?n=1415. [Last accessed 2016 Jul 21].

[8] Mohanasundaram.S, N.Rangarajan, V.Sampath, K.Porkodi, M. Pennarasi (2021). GC-MS and HPLC analysis of Antigly-cogenolytic and Glycogenic compounds in Kaempferol 3 – O – gentiobioside containing Senna alata L leaves in experimental rats. Translational Metabolic Syndrome Research., 4(2021): 10-17.

[9] M.A. Thakar, S. Saurabh Jha, K. Phasinam *et al*., X ray diffraction (XRD) analysis and evaluation of antioxidant activity of copper oxide nanoparticles synthesized from leaf extract of Cissus vitiginea, Materials Today: Proceedings, https://doi.org/10.1016/j.matpr.2021.05.410.

[10] Mohanasundaram. S, N. Rangarajan, V. Sampath, K. Porkodi, M.V. Dass Prakash, N. Monicka (2021). GC-MS Identification of Anti-infla-mmatory and Anticancer Metab-olites in Edible Milky White Mushroom (Calocybe indica) against Human Breast Cancer (MCF-7) Cells. Res J Pharm and Tech., 14(8):4300-4306.

[11] Victor Arokia Doss, Prasad Maddisetty, Mohanasundaram.S (2016). Biological active compounds with various medicinal values of Strychnos Nux-vomica – A Pharmacological summary. J Global Trends Pharm Sci 7(1): 3044-3047.

[12] Deepa P, Kaleena PK, Valivittan K, Girish Kumar CP. Phytochemical screening and antimicrobial activity of *Sansevieria roxburghiana* Schult. and Schult. f. Middle East J Sci Res 2011; 10: 512-8.

[13] Arun J, Maya B, Gauri PA. Phytochemical investigation of the rhizomes of *Sansevieria roxburghiana*. Am J Pharm Health Res 2013; 1: 16-26.

[14] Deepa P, Kaleena PK, Valivittan K. Antioxidant potential of Sansevieria roxburghiana Schult. and Schult. f. Asian J Pharm Clin Res 2012; 5: 166-9.

[15] Mohanasundaram S, Victor Arokia Doss, Prasad Maddisetty, Magesh R, Sivakumar K and Subathra M (2019). Pharmacological analysis of hydroethanolic extract of Senna alata (L.) for in vitro free radical scavenging and cytotoxic activities against HepG2 cancer cell line. Pak. J. Pharm. Sci., Vol.32, No.3, May 2019, pp. 931-934.

[16] Sharma, A., Raja, M. K. M. M., & Manne, R. (2021). Evaluation of Physicochemical Properties and Chemical Constituents of Rasam, a Traditional South Indian Soup. Journal of Pharmaceutical Research International, 18-25.