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Pharmacognostical, Phytochemical and Gas Chromatography Mass Spectroscopy Profiling of *Stenosiphonium russellianum* Nees

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Stenosiphonium russellianum Nees. is rarely known species belongs to the family Acanthaceae and it is a shrub, found above 500m on slopes of mountain. It was traditionally used for wound healing in and as blood purifier. The current study designed to provide the requisite pharmacognostical and phytochemical properties of *Stenosiphonium russellianum*. Pharmacognostical studies like microscopic and macroscopic analysis of the leaves were carried out. Physiochemical parameter and preliminary phytochemical screening for secondary metabolite were also performed. Extracts were taken from nonpolar to polar solvants like hexane, diethyl ether, ethyl acetate, alcohol and water. Their extractive values are calculated. GCMS analysis of hexane, diethyl ether, ethyl acetate and ethanol extract of the leaves of *Stenosiphonium russellianum* were studied. Preliminary phytochemical evaluation showed the presence of alkaloids, phytosterols and glycosides. GCMS analysis revealed the presence compounds like lupeol, gamma sitosterol and stigmasterols. In conclusion, the information obtained from these studies can be used

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as markers in the identification and standardization of this plant as an herbal remedy and also towards further pharmacological activity estimation.

Keywords: Stenosiphonium russellianum; GCMS; pharmacognostic; phytochemical; microscopic and gcms.

1. INTRODUCTION

Stenosiphonium russellianum Nees. is a species distributed as shrub comes under the Kingdom: Plantae, Division: Tracheophyta, Class: Magnoliopsida, Order: Lamiales, Genus: Stenosiphonium, Family: Acanthaceae. Acanthaceae is dicotyledonous family consisting of 250 genera and about 2500 species. Leaves, stem, flowers and roots of most of the species of Acanthaceae has pharmacological activities such as wound healing, anticancer, antioxidant, Antifungal, antiviral, anti-inflammatory, anti-pyretic, antioxidant, insecticidal, hepatoprotective, antiplatelet and immunomodulatory. The leaves are the most often used part in the Acanthaceae family and specially used for wound healing. These pharmacological actions are produced by presence of phytochemicals like alkaloids, phytosterols, glycosides, flavonoids, phenolic compounds, triterpinoids, benzonoids and naphthoquinones present in the species of the family [1].

Stenosiphonium Nees (Acanthaceae) was one of the genera described by Nees. This genus forms a well-defined and putatively monophyletic group, distributed in southern India and Sri Lanka, which were morphologically similar to strobilanthes Blume [2]. *Stenosiphonium russellianum* is characterized by leaves with silky undersurface, stems not winged, corolla blue to violet and has tube ventrilose usually found in 500m above the slope of the hills [3]. Regional names are kal-kurinji and karumaththi-poondu. For wound healing, the leaf paste of *Stenosiphonium russellianum* is applied in the morning for two days [4]. It is also used as blood purifier [5].

As we know that plant species consumed by animals are known to have therapeutic effect on human also. For example, *Cynodon Dactylon* (scutch grass), *Abutilon indicum* (Indian mallow leaves), *Solanum trilobatum* (pea eggplant), *Hibicus rosasinensis*, these are known to produce many pharmacological activity which were consumed by animal. With all this background, we choose this plant for research. In this article you can get a broad detail regarding the monographs and phytocompounds of the species *Stenosiphonium russellianum*.

2. MATERIALS AND METHODS

2.1 Plant Material Collection and Authentication

The leaves of *Stenosiphonium russellianum* were collected from hills at village Thevanadhapettai, Gingee district, Tamil Nadu, India. The plant material was identified and authentication by Dr. N. Ayyappan, Researcher, French Institute of Pondicherry, Puducherry, India. Leaves were then washed to remove adhering material, shade dried and powdered. The powders were stored in an airtight self-sealed cover.

2.2 Pharmacognostical Studies

2.2.1 Morphological characters

The macroscopic study was conducted to aid in the identification as well as standardization of this plant species. The fresh leaves were subjected to macroscopic studies which comprised of organoleptic characters. Morphological studies of leaf such as color, size, odor, taste, surface characteristic were examined using the terms and outlined given in [6].

2.2.2 Microscopic analysis

For microscopic studies of the fresh leaves, free-hand sections of midrib with lateral extensions of lamina on either side of the leaves were taken to prepare the specimens, stained with phloroglucinol in Hcl and mounted with glycerin for microscopic evaluation [6,7]. Sections were viewed under 10x, and 45x magnifications in microscope for the identification of various regions and photographs were taken.

2.2.3 Physicochemical analysis

Physiochemical parameter such as loss on drying of leaves, loss on drying of powder, total ash, and acid insoluble ash of leaves powder of *Stenosiphonium russellianum* were performed according to the WHO guidelines on quality

control methods for medicinal plant material [8,9].

2.2.4 Fluorescence analysis

The fluorescence characters of powdered drugs of medicinal plants helps in the determination of quality and purity of test samples. To study the fluorescence behavior powder of *Stenosiponium russellianum* were leaves treated with few drops of different reagents on a clean watch glass, waited for few minutes and observed under UV Visible at 254nm [10,11].

2.2.5 Preparation of leaves extract and their extractive value

250gms of coarsely powdered drug at room temperature materials of *Stenosiphonium russellianum* is placed in a 500 ml stoppered conical flask. 500 ml of five different solvents of from highly non polar to polar (hexane, diethyl ether, ethyl acetate, ethanol and water) were poured on top until completely covered the drug material and kept aside for 7 days with periodical shaking. At the end, micelle of *Stenosiphonium russellianum* is separated from marc by filtration and then solvents are removed using distillation. Percentages of extractive value of extracts were calculated. The obtained extracts of hexane, diethyl ether, ethyl acetate, ethanol and water of *Stenosiphonium russellianum* were stored in airtight glass container for further phytochemical and pharmacological analysis [12-14].

2.2.6 Qualitative preliminary phytochemical screening

Extracts of hexane, diethyl ether, ethyl acetate, ethanol and water of *Stenosiphonium russellianum* were subjected to test for secondary metabolites like alkaloids, glycosides, phytosterols, saponins, tannins and flavonoids. The phytochemical screening for alkaloids was carried out by Mayer's test, Dragendroff's test, Hager's test and Wagner's test. Flavonoids were detected using Shinoda test, phenolic compounds by ferric chloride test, saponins by foam test, glycosides by Borntager's test and legal's test. Salkowski test and Libermann burchard's test were used to detect the presence of phytosterols in the extracts of *Stenosiphonium russellianum* [15,14,16,17].

2.3 GC-MS Analysis

The Clarus 680 GC was used in the analysis employed a fused silica column, packed with Elite-5MS (5% biphenyl 95% dimethylpolysiloxane, 30 m × 0.25 mm ID × 250µm df) and the components of extracts were separated using Helium as carrier gas at a constant flow of 1 ml/min. The injector temperature was set at 260°C during the chromatographic run. The 1µL of extracts of *S. russellianum* injected into the instrument the oven temperature was as follows: 60 °C (2 min); followed by 300 °C at the rate of 10 °C min−1; and 300 °C, where it was held for 6 min. The mass detector conditions were: transfer line temperature 240 °C; ion source temperature 240 °C; and ionization mode electron impact at 70 eV, a scan time 0.2 sec and scan interval of 0.1 sec. The fragments from 40 to 600 Da and the total run time is 32 minutes.

The spectrums of the components of different extracts (hexane extract of *Stenosiphonium russellianum* (HESR), diethylether extract of *Stenosiphonium russellianum* (DEESR), ethyl acetate extract of *Stenosiphonium russellianum* (EAESR) and ethanol extract of *Stenosiphonium russellianum* (EESR) were compared with the database of spectrum of known components stored in the GC-MS NIST (2008) library.

3. RESULTS AND DISCUSSION

Authentication and standardization are the main prerequisite steps when come for research on natural products in any system of medicine [18]. The pharmacognostic studies are the identity of crude drugs; it gives a complete characterization of the species.

Table 1. Morphological characteristics of S. russellianum

Characters	Observation
Colour	Moss green
Odour	Characteristic
Taste	Characteristic
Texture	Fine
Shape	Ovate
Veination	Arcuate
Apex	Caudate
Surface	Silky undersurface
Length	4cm-7cm
Width	2cm -5cm

3.1 Morphological Characters

Macroscopic as well as organoleptic evaluation of the leaves includes position and arrangement, size, shape, base, texture, margin, apex,

veination, colour, odour, taste of leaves were observed and listed in Table 1.

The position and arrangement, size, shape, base, texture, margin, apex, veination, colour, odour, taste of leaves were observed (Table 1).

3.2 Microscopical Studies

Transverse section of leaf midrib shows rounded shape with single layer of the adaxial and abaxial epidermis with small trichomes. Midrib parenchymatous cells are 7 layers of rounded closely arranged. Vascular bundles are C shaped open collateral. More than 16-18 xylem rows in vascular bundles. Phloem cells are present in abaxial side. Leaf lamina projection are connected with midrib (Fig. 1). Adaxial epidermis consists of uniseriate conical trichome which might be eglandular, multicellular and uniseriate unbranched. Abxial epidermis consists of unicellular conical trichome and Simple filiform trichome, these might be eglandular, multicellular and uniseriate unbranched. Epidermal layer has abundant diacytic stomata with cystoliths (Fig. 2).

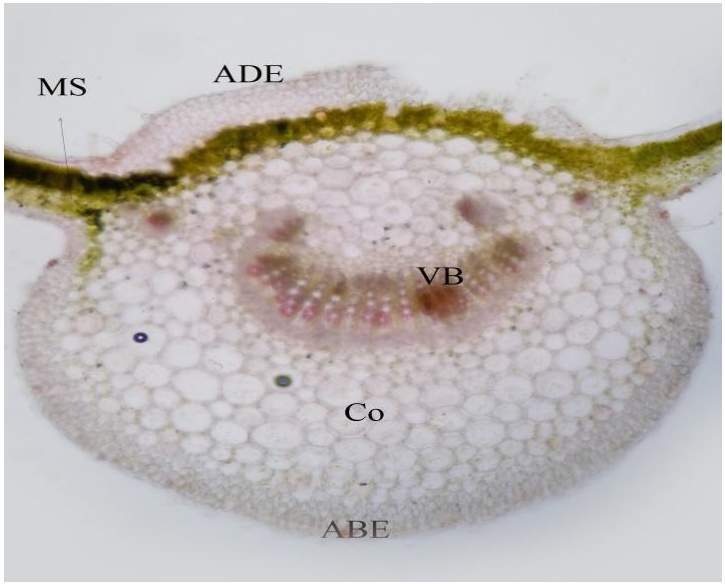

Fig. 1. ADE – Adaxial epidermis; ABE – Abaxial Epidermis; MS – Mesophyll Cells; VB – Vascular Bundels; Co – Cortex

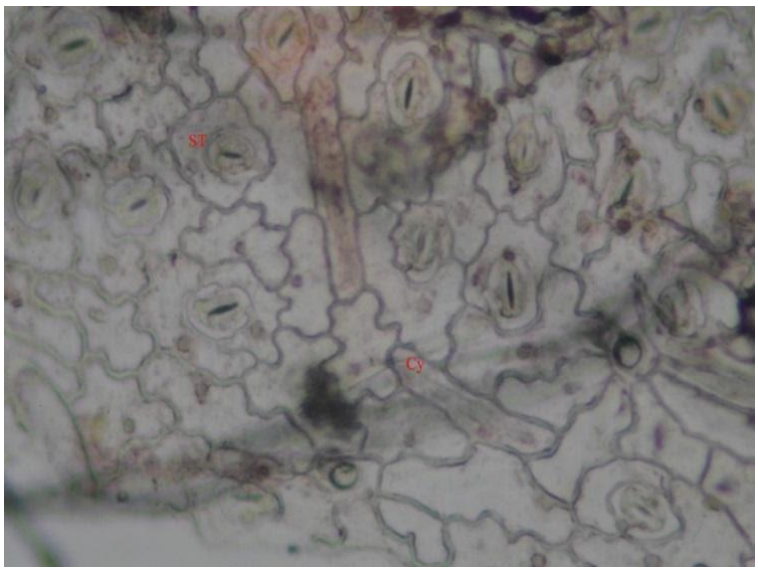

Fig. 2. ST – stomata; Cy – Cystoliths

3.3 Physicochemical Analysis

Physicochemical parameters like loss on drying of leaves, loss on drying of leaves powder, total ash and acid insoluble ash of powder were investigated and the results were interpreted for determining the values and are summarized in Table 2. Physicochemical parameters identification of a crude drug is a crucial factor for proper identification of plant.

Table 2. Physicochemical specification of leaves powder of *Stenosiphonium russellianum*

Parameter	Content (percentage by weight)
Loss on drying of powder	0.42
Total ash	11.47
Acid-insoluble ash	02.23

3.4 Fluorescence Analysis

Different reagents treated powder was observed at UV 254nm and visible light for fluorescence characteristics. Those observations are presented in Table 4.

3.5 Extractive Value

Extractive values of all the solvent extracts shown in Table 3. Ethanol soluble extractive value was found to be more than ethyl acetate soluble extractive value however it was less than aqueous soluble extractive value.

3.6 Preliminary Phytochemical Analysis

Name of the test and its inference of preliminary phytochemical screening of the extracts of leaves were interpreted in the table 5. Phytochemical investigation of extracts of *Stenosiphonium russellianum* revealed the presence of alkaloids, glycosides, flavonoids, phytosterols, phenolic compounds and tannins. Among various solvent used for extraction, ethyl acetate extracts of leaves gave maximum positive results. Alkaloids are present in ethanol and aqueous extract whereas glycosides are present only in ethyl acetate extract. Hexane and diethyl ether extracts didn't showed any positive inference in all performed test. Phytochemical analysis of the leaves extracts revealed the presence of constituents known to exhibit therapeutic as well as physiological activities.

3.7 Gas Chromatography Mass Spectroscopy (GCMS)

GCMS is one of the most precise spectroscopic analytic methods to identify various secondary metabolites present in the plant extract [5,15,6]. The crude hexane, diethyl ether, ethyl acetate and aqueous extract of *Stenosiphonium russellianum* was analyzed by GCMS to detect the phytocompounds with the help of NIST library. GCMS reported phytocompounds

Table 3. Fluorescence behavior of powdered leaf treated with different reagents

S.NO.	Testing	Visible Light	Short –UV (254nm)
1	Powder(P)	Moss green	Black
2	P + 1N NaOH in methanol	Moss green	Black
3	P + 1N HCL	Black	Orangish black
4	P + HNO3	Moss green	Yellowish black
5	P + H2SO4	Brownish black	Black
8	P + Ammonia	Moss green	Black
9	P + Acetic acid	Moss green	Yellowish black

Table 4. Extractive values of different extracts of *Stenosiphonium russellianum*

S. No	Name of the extract	Colour	Nature	% of extractive value
1	Hexane extract	Pale green to yellow	Slightly sticky	9 %
2	Ether extract	Bright green	Sticky paste	14.5%
3	Ethyl acetate extract	Dark green	Sticky paste	18%
4	Ethanol extract	Dark green	Sticky paste	22.5%
5	Water extract	Brown	Semisolid	27%

Table 5. Preliminary Qualitative phytochemical examination of *Stenosiphonium russellianum*

S:no	Phyto-constituents	Tests	Hexane extract	Ether extract	Ethyl acetate extract	Ethanol extract	Aqueous extract
1	Alkaloids	Mayer's test	-	-	+	+	+
		Dragendroff's test	-	-	+	+	+
		Hager's test	-	-	+	+	+
		Wagner'test	-	-	+	+	+
3	Glycosides	Borntager's test	-	-	+	+	-
		Legal's test	-	-	-	-	-
4	Phytosterols	Salkowski test	-	-	+	-	-
		Libermann burchard's test	-	-	+	+	-
5	Saponins	Foam test	-	-	-	-	-
6	Phenolic compound & Tannins	Ferric chloride test	-	-	+	+	+
9	Flavonoids	Shinoda test	-	-	+	-	-

Table 6. Phytoconstituents identified in the hexane extract of *Stenosiphonium russellianum* (HESR) by GC-MS peak report

Name of the Compound	Molecular Formula	Retention Time	Area%	Molecular Weight
hexadecanoic acid, ethyl ester	C18H36O2	18.005	3.415	284
9,12-octadecadienoic acid, ethyl ester	C20H36O2	19.400	2.710	308
bicyclo[4.1.0]heptane, 7-pentyl-	C12H22	19.430	4.325	166
9,12,15-octadecatrienoic acid, ethyl ester	C20H34O2	19.475	12.710	306
tetratetracontane	C34H70	26.118	15.030	478
octacosane	C28H58	24.157	2.347	394
2,6,10,14,18,22-tetracosahexaene, 2,6,10,15,19,23-hexamethyl-, (all-e)-	C30H50	24.282	5.290	410
hexatriacontane	C36H74	24.822	4.094	506
tetratriacontane	C44H90	25.468	3.313	618
octacosane	C28H58	26.818	4.345	394
pentacosane	C31H64	27.703	30.067	436
heptacosane	C27H56	28.654	5.682	380
9-octadecene, 1,1'-[1,2-ethanediylbis(oxy)]bis-, (z,z)-	C20H40O	29.449	1.753	296

of hexane extract are listed Table 6, totally of 13 peaks in chromatogram (Fig. 3) showed the presence of phytocompunds like hexadecanoic acid- ethyl ester, 9,12-octadecadienoic acid, ethyl ester, pentacosane and etc. Diethyl ether extract phytocompounds are listed in Table 7, totally of 11 peaks in chromatogram (Fig. 4) revealed the presence of compounds such as ,4-epoxynaphthalene-1(2h)-methanol, 4,5,7-tris(1,1-dimethylethyl)-3,4-dihydro, phytol and 3,7,11,15-tetramethyl-2-hexadecen-1-ol. Among all the extracts, GCMS report of ethyl acetate extract showed the presence of pharmacologically active compounds such as lupeol, gamma sitosterol and stigmasterol along with some other compounds are listed table 8 and the chromatogram showed 13 peaks with 13 compounds (Fig. 5). The chromatogram of ethanol extract (Fig. 6) has totally of 11 peaks with 11 compounds like eicosanoic acid, 22,23-dibromostigmasterol acetate and stigmastan-6,22-dien, 3,5-dedihydro with other compounds are are listed in Table 9.

Table 7. Phytoconstituents identified in the diethyl ether extract of *Stenosiphonium russellianum* (DEESR) by GC-MS peak report

Name of the compound	Molecular formula	Retention time	Area%	Molecular weight
3,7,11,15-tetramethyl-2-hexadecen-1-ol	C20H40O	16.519	6.387	296
phytol	C20H40O	19.180	3.467	296
1-hexyl-2-nitrocyclohexane	C12H23O2N	20.175	25.378	213
2,6,10,14,18,22-tetracosahexaene, 2,6,10,15,19,23-hexamethyl-, (all-e)-	C30H50	24.307	4.767	410
heptacosane	C27H56	24.487	1.874	380
tetratetracontane	C44H90	26.113	13.538	618
nonacosane	C29H60	26.828	3.381	408
pentacosane	C25H52	27.663	28.128	352
pentatriacontane	C35H72	28.684	5.551	492
heptacosane, 1-chloro-	C27H55Cl	29.874	2.711	414
,4-epoxynaphthalene-1(2h)-methanol, 4,5,7-tris(1,1-dimethylethyl)-3,4-dihydro-	C23H36O2	31.005	2.739	344

Table 8. Phytoconstituents identified in the ethyl acetate extract of *Stenosiphonium russellianum* (EAESR) by GC-MS peak report

Name of the Compound	Molecular Formula	Retention time	Area%	Molecular weight
heptacosane	C27H56	26.808	1.372	380
pentacosane	C25H52	27.658	8.009	352
stigmasterol	C29H48O	27.954	3.249	412
.gamma.-sitosterol	C29H50O	28.644	4.194	412
lupeol	C17H30O3	29.419	2.193	282
3,7,11,15-tetramethyl-2-hexadecen-1-ol	C20H40O	16.339	4.727	296
n-hexadecanoic acid	C16H32O2	18.120	17.825	256
phytol	C20H40O	18.995	5.777	296
9,12-octadecadienoic acid (z,z)-	C18H32O2	19.650	17.077	280
(z)6,(z)9-pentadecadien-1-ol	C15H28O	19.795	20.530	224
2,6,10,14,18,22-tetracosahexaene, 2,6,10,15,19,23-hexamethyl-, (all-e)-	C30H50	24.272	3.745	410
octacosane	C28H58	24.812	1.995	394
heptacosane	C28H58	26.098	6.298	394

Table 9. Phytoconstituents identified in the ethanol extract of *Stenosiphonium russellianum* (EESR) by GC-MS peak report

Name of the Compound	Molecular formula	Retention time	Area%	Molecular weight
phytol	C20H40O	19.080	23.110	296
3,7,11,15-tetramethyl-2-hexadecen-1-ol	C20H40O	16.679	1.836	296
1-octadecyne	C20H40O	16.874	3.492	296
eicosanoic acid	C20H40O2	18.590	14.311	312
(z)6,(z)9-pentadecadien-1-ol	C15H28O	20.115	32.400	224
trichloroacetic acid, tridec-2-ynyl ester	C15H23O2CL3	20.656	1.632	340
2,6,10,14,18,22-tetracosahexaene, 2,6,10,15,19,23-hexamethyl-, (all-e)-	C30H50	24.30	1.646	410
9-octadecene, 1-[3-(octadecyloxy)propoxy]-, (z)-	C39H78O2	25.788	2.281	578
stigmastan-6,22-dien, 3,5-dedihydro-	C29H46	28.144	3.381	394
22,23-dibromostigmasterol acetate	C31H50O2Br2	28.759	2.915	612

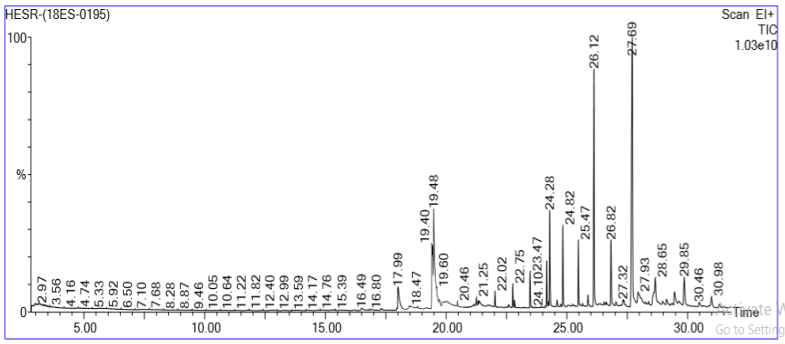

Fig. 3. GCMS chromatogram of the phytoconstituents present in the hexane extract of Stenosiphonium russellianum (HESR)

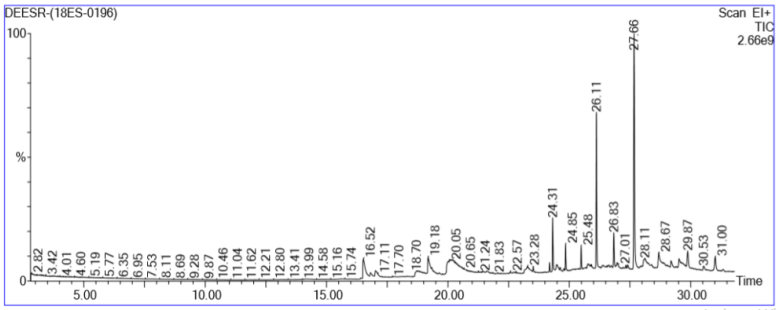

Fig. 4. GCMS chromatogram of the phytoconstituents present in the diethyl ether extract of *Stenosiphonium russellianum* (DEESR)

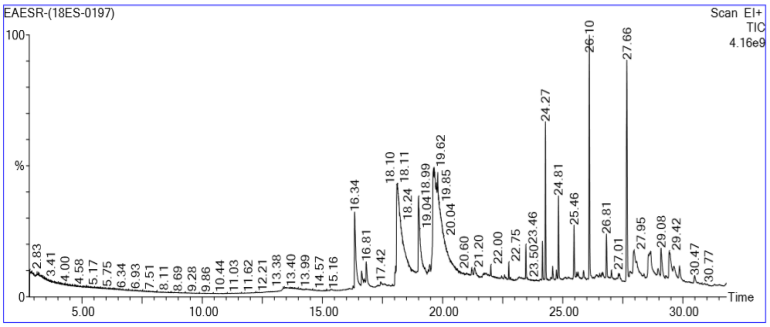

Fig. 5. GCMS chromatogram of the phytoconstituents present in the ethyl acetate extract of *Stenosiphonium russellianum* (EAESR)

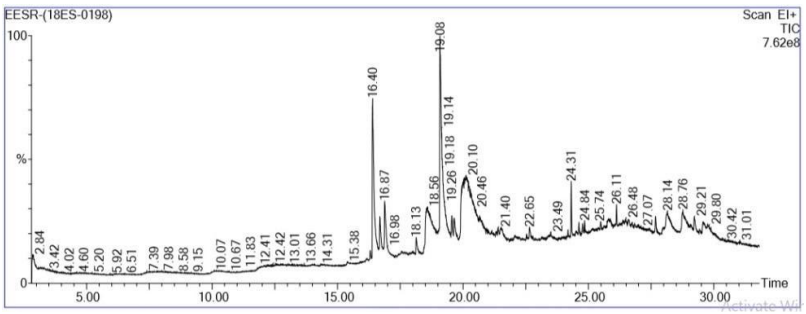

Fig. 6. GCMS chromatogram of the phytoconstituents present in the ethanol extract of *Stenosiphonium russellianum* (EESR)

4. CONCLUSION

The pharmacognostic standards for the leaves of *Stenosiphonium russellianum* are laid down for the first time. Morphological and microscopic studies of leaves will enable to identify the crude drug. Preliminary phytochemical screening as well as GCMS profiling will be useful in further research on this species like isolation of lead and determination of pharmacological activity.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

NOTE

The study highlights the efficacy of "herbal remedy" which is an ancient tradition, used in some parts of India. This ancient concept should be carefully evaluated in the light of modern medical science and can be utilized partially if found suitable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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