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PHYTOCHEMICAL SCREENING BY UV-VIS, FT-IR AND GC-MS SPECTROSCOPIC ANALYSIS OF LEAF AND CALLUS EXTRACTS OF MEDICINAL PLANTS *SIDA SCHIMPERIANA* Hochst. ex A. Rich

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ABSTRACT

In the present study an attempt has been made to establish UV-VIS, FT-IR, GC-MS profile and identify the functional components of *Sida schimperiana*. UV-VIS, FTIR, and GC-MS method was performed on a Thermo Scientific Spectrophotometer system which was used to detect the characteristic peak values and their functional groups.The fresh ethanolic extracts of *S. schimperiana* Leaves and callus. UV – VIS, FTIR and GC-MS the ethanol extract was examined under visible and UV light for the proximate analysis. The UV-VIS profile of *S. schimperiana* Leaves and callus ethanol extract showed the peaks at 643 nm, 647 nm wheels as same callus 425 nm with the absorption of 1000.20, 1000.17 and 521.43 respectively. The FTIR spectrum analysis results proved the presence of Aliphatic compounds, Amine and (amine II bands), Amides and (amine III bands), Benzene ring, Ester and lactones, Sulfides which shows major peaks at 2981.18,1641.10,1404.89, 1329.38, 1272.80, and respectively 703.70. The results of callus FTIR analysis confirmed the presence of, Aliphatic compounds, isocyanates, oximes, Primary amines, Sulfonyl chlorids, Silanes,sulfonic acids, Organo phosphorus compound, Benzenes which shows major peaks at 2986.56, 2085.12, 1640.87, 1406.40, 1394.96, 1259.78, 1066.95, 12045.99 and respectively 878.09. GC-MS analysis of ethanol extracts of *S. schimperiana* callus analysis the presence of 13 phytochemical compounds. The presence of various functional groups and phytocompounds in *S. schimperiana* callus confirm that it act as a most important source of drugs against various ailments. The results of the present study produced the UV –VIS, FTIR and GC-MS spectrum profile for the medicinally important plant *Sida schimperiana.*

KEYWORDS: *Sida schimperiana* UV- VIS, FTIR, and GC-MS Functional groups.

INTRODUCTION

Analytical technique that does not resolve the concentrations of individual metabolites but provides a snapshot of the metabolic composition of a tissue at a given time (Griffiths and De Haseth 1986). FTIR can be employed to determine the structure of unknown composition and the intensity of the absorption spectra associated with molecular composition or content of the chemical group. The FT-IR method measures the vibrations of bonds within chemical functional groups and generates a spectrum that can be regarded as a biochemical sample. At present, particularly in phytochemistry, FTIR has been exercised to identify the concrete structure of certain plant secondary metabolites (Yang and Yen 2002). (Ivanova *et al*., 2003). But, on pharmacognosy FTIR is still a new tool to characterize and identify the commercial components from the adulterant. FTIR method has been successfully utilized in the characterization of bacterial, fungal and plant species. FT-IR is one of the most widely used methods to identify the chemical constituents and elucidate the compounds structures, and has been used as a requisite method to identify medicines in Pharmacopoeia of many countries. During last few decades UV, FTIR and GC-MS were acted as powerful techniques for the identification, separation and structural determination of phytochemicals. Gas chromatography- mass spectroscopy (GC-MS) helped in identification of compounds at less than 1 mg. Commonly GC-MS applied for drug detection, environmental investigation and detection of unknown samples. GC-MS technique has been founds very effective for the separation and recognition of composite mixtures of phytochemicals. UV-Visible and FTIR can be used together or separately as conventional methods to detect phytoconstituents.

S. schimperiana Hochst. ex A. Rich. Belongs to the family *Malvaceae*. It is an annual sub shrubs live in terrestrial place. Malvaceae is a family of flowering plants containing over 200 genera with close to 2300 species. The main economic use of malvaceae plants is an a source of natural fibers, the family is also used for food, beverages timber in traditional medicine and horticulture. The largest genera are Hibiscus 300 species, dombeya 225 species, pavonia 200 species and *Sida* 200 species (Rizk and soliman 2014). *S. schimperiana* is a very important medicinal plant and used for the following diseases. Prenatal abortion, Internal worms, Amoebic dysentery, Cough, Influenza, Liver disease (Fisseha Mesfin *et al*., 2014). The plant also contains aliphatic compounds, Benzene ring, Ester and lactones, Sulfides. The present study was aimed to report the main functional components of present in the leaves

and callus of *S. schimperiana* by using FT-IR. In addition, we tried to develop a rapid, accurate and feasible analysis method to integrally reflect the fresh qualities of *S. schimperiana.*

MATERIALS AND METHODS

Collection and processing of plant material

S. schimperiana Hochst. ex A. rich. Were collected in Keeranur village of Pudukottai District, Tamil nadu. The plant specimen was identified with help of Rapinat herbarium Trichy (RTH) (Plant specimen No 29849) St. Joseph's college, (Autonomous). The plant samples leaves were washed thoroughly in running tap water to remove soil particles and adhered debris followed by sterile distilled water and callus culture sample gently removed with the help of forcipes we isolate only dried sample. The washed plants were blotted on the blotting paper and spread out at room temperature in shade. Shade dried samples were grounded to fine powder using tissue blender. The powdered samples were then stored in a refrigerator for further use.

Extraction of plant material

The powdered leaves of *S. schimperiana* were extracted using ethanol with gentle stirring for 72 h separately at room temperature. The extracts were then filtered through Whatmann No. 1 filter paper and concentrated using vacuum distillation.

UV-VIS and FTIR Spectroscopic Analysis

S. schimperiana belongs to the Malvaceae family.5 mg of callus and 5 g of leaf weighed, then callus and leaf sample grinding with mortar and pistil. Filtered with No: 1 water filter paper supernatant were collected in the Eppendrof tube. Then readings were tabulated by using UV spectrometer Instrument model: Arithmetic.

A small amount of powdered leaves was respectively placed directly on the germanium piece of the infrared spectrometer with constant pressure applied and data of infrared absorbance, collected over the wave number ranged from 4000 cm^{-1} to 400cm^{-1} and computerized for analyses by using the Omnic software (version 5.2). The reference spectra were acquired from the cleaned blank crystal prior to the presentation of each sample replicate. All spectra were collected with a resolution of 4-1 cm and to improve the signal-to-noise ratio, 256 scans were co-added and averaged (Nazneen Bobby *et al*., 2012). Samples were run in triplicate and all of them were undertaken within a day period.

GC–MS/ MS analysis

The composition of ethanol extract of *S. schimperiana* tuberous callus was analyzed by using GC-MS analysis was performed on a combined GC-MS instrument (ITQ 900 Model of Thermo Fisher Scientific make) using a HP-5 fused silica gel capillary column. The method to perform the analysis was designed for both GC and MS. 1 µL aliquot of sample was injected into the column using a PTV injector whose temperature was set at 275^{o}C. The GC program was initiated by a column temperature set at 60^{o}C for 5 min, increased to 300^{o}C at a rate of 8 C/min, held for 10 min. Helium was used as the carrier gas (1.5 mL/min). The mass spectrometer was operated in EI mode with mass source was set at 200^{o}C. The chromatogram and spectrum of the peaks were visualized. The particular compounds present in the samples were identified by matching their mass spectral fragmentation patterns of the respective peaks in the chromatogram with those stored in the National Institute of Standards and Technology Mass Spectral database (NIST-MS, 1998) library.

RESULTS AND DISCUSSION

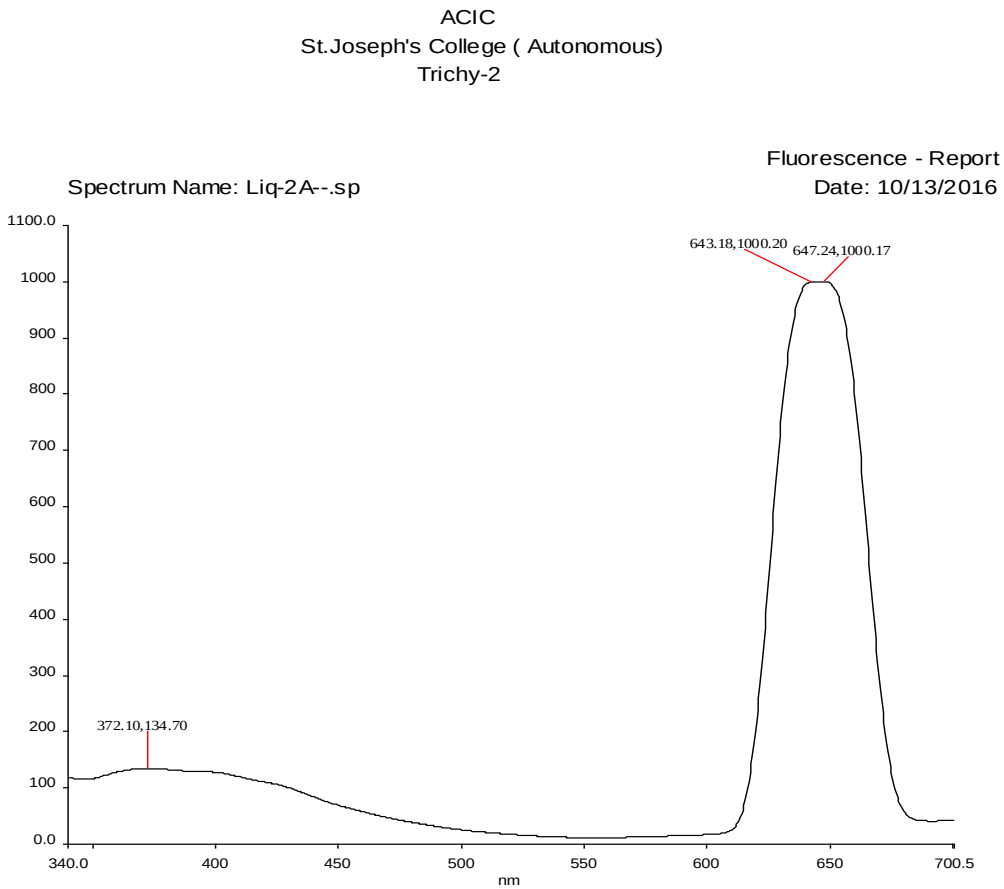
UV and FTIR analysis ethanolic extract of *Sida Schimperiana*, Leaf & Callus

A simple and sensitive ultraviolet spectrophotometric method for quantitative estimation in presence of secondary metabolites compounds. Leaf sample UV detection was performed at 425 nm and 648 nm. At the same time when it compares to the Callus sample by UV three calibration cure was performed at 372 nm, 643 nm, and 647 nm the calibration curve was plotted between resultant of absorbance of [372 nm− (643 nm), and 647 nm] and concentration of analyte The UV-V is absorption peak from 200-700 nm indicates the particles size reduction. The maximum absorption of synthesysised plant extracted is at 650 nm. (**Fig.1-2 and Table -1-2).**

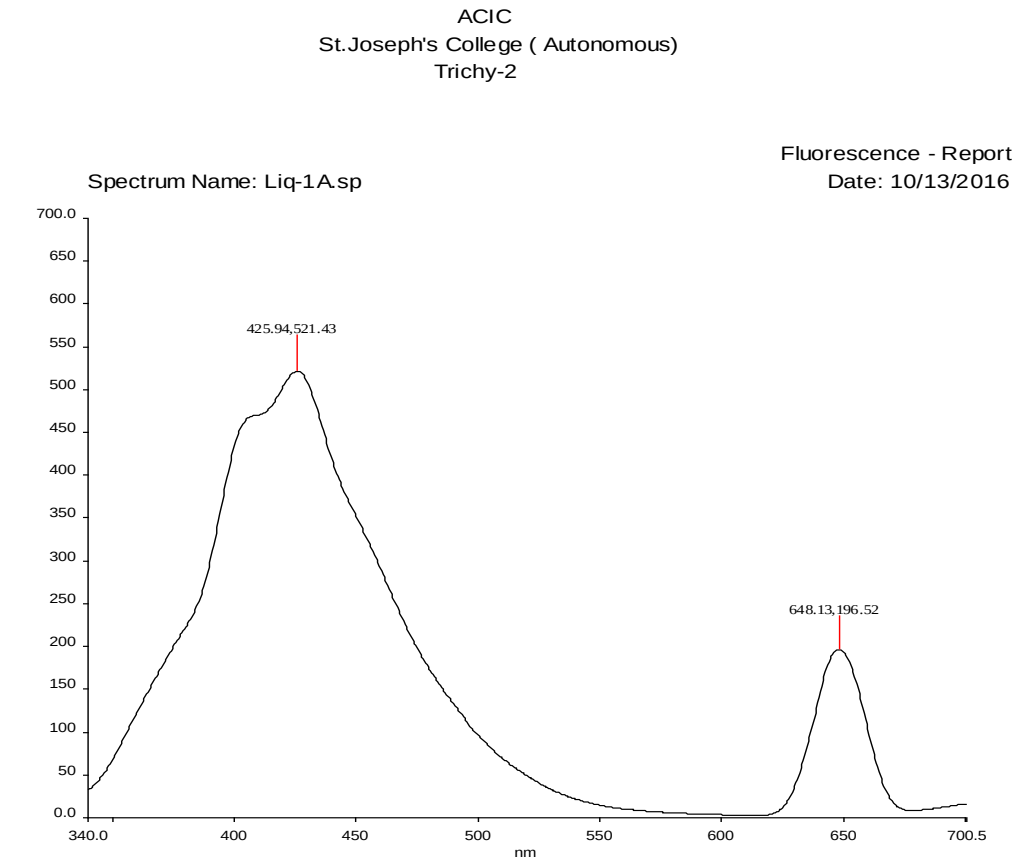
The FTIR spectrum was used to identify the functional group of the active components based on the peak value in the region of infrared radiation. The leaves powder and ethanolic extracts evaporated powder of *S. schimperiana* was passed into the FTIR and the functional groups of the components were separated based on its peak ratio. The results of *S. schimperiana* leaves FTIR analysis confirmed the presence of Primary amines and amides, aliphatic compounds, Amine and (amine II bands), Amindes and amine III band, Benzene ring, Ester and lactones, Sulfides. This shows major peaks at 3434.52, 2981.18, 1641.10, 1404.89, 1329.38, 1272.80, and respectively 703.70. **(Fig.3 and Table -3).**

The results of callus FTIR analysis confirmed the presence of, Aliphatic compounds, isocyanates, oximes, Primary amines, Sulfonyl chlorids, Silanes,sulfonic acids, Organo phosphorus compound, Benzenes which shows major peaks at 2986.56, 2085.12, 1640.87, 1406.40, 1394.96, 1259.78, 1066.95, 12045.99 and respectively 878.09. **(Fig.4 and Table - 4).**

The results pertaining to GC-MS analysis leads to the identification of number of compounds from the GC fractions of the ethanolic extract of *S. schimperiana*. These compounds were identified through mass spectrometry attached with GC. These observations may be due to the nature of biological active components and the stronger extraction capacity of ethanol could have been produced number of active constituents responsible for antibacterial activity.



Leaf analysis (Fig No:1)



Callus analysis (Fig No: 2).

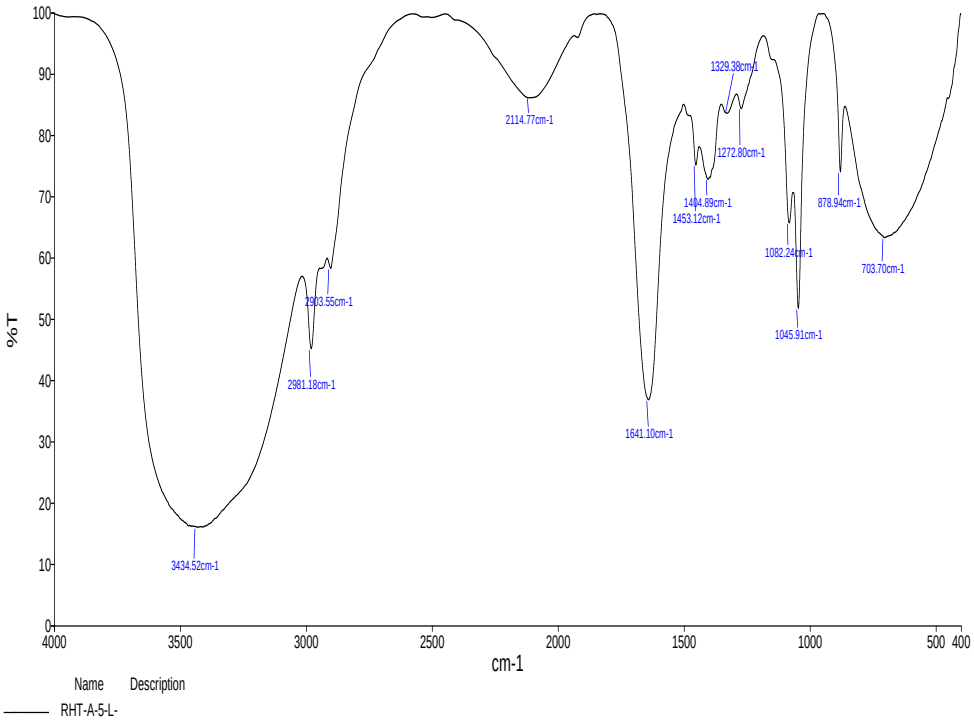


Fig. 3: FTIR Spectrum of *S. schimperiana* Hochst. ex A.Rich – Leaves.

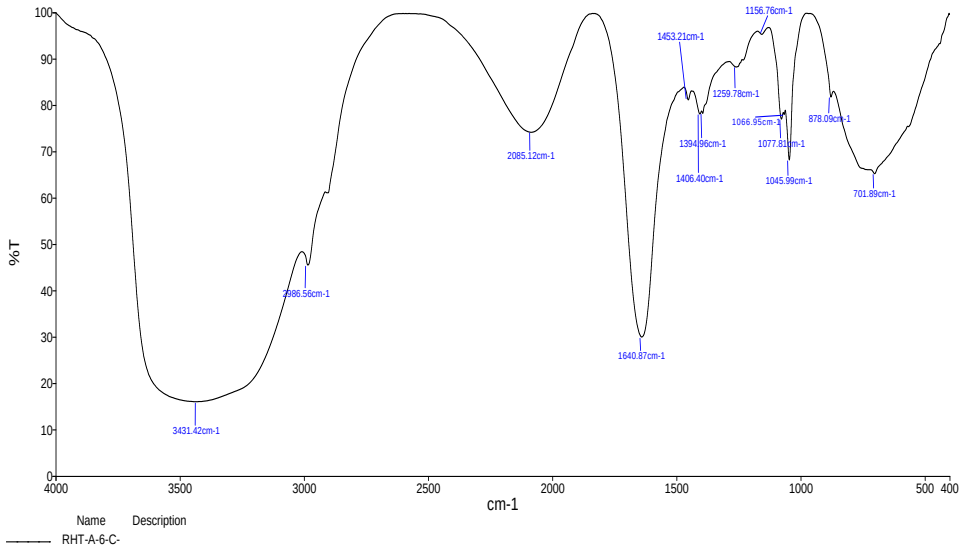


Fig. 4: FTIR Spectrum of *S. schimperiana* Hochst. ex A.Rich. – Callus.

Fig. 3: FTIR Spectrum of Ethanolic extracts of *S. schimperiana* –Leaves

Fig. 4: FTIR Spectrum of Ethanolic extracts of *S. schimperiana* –Callus

GC MC / MS Analysis

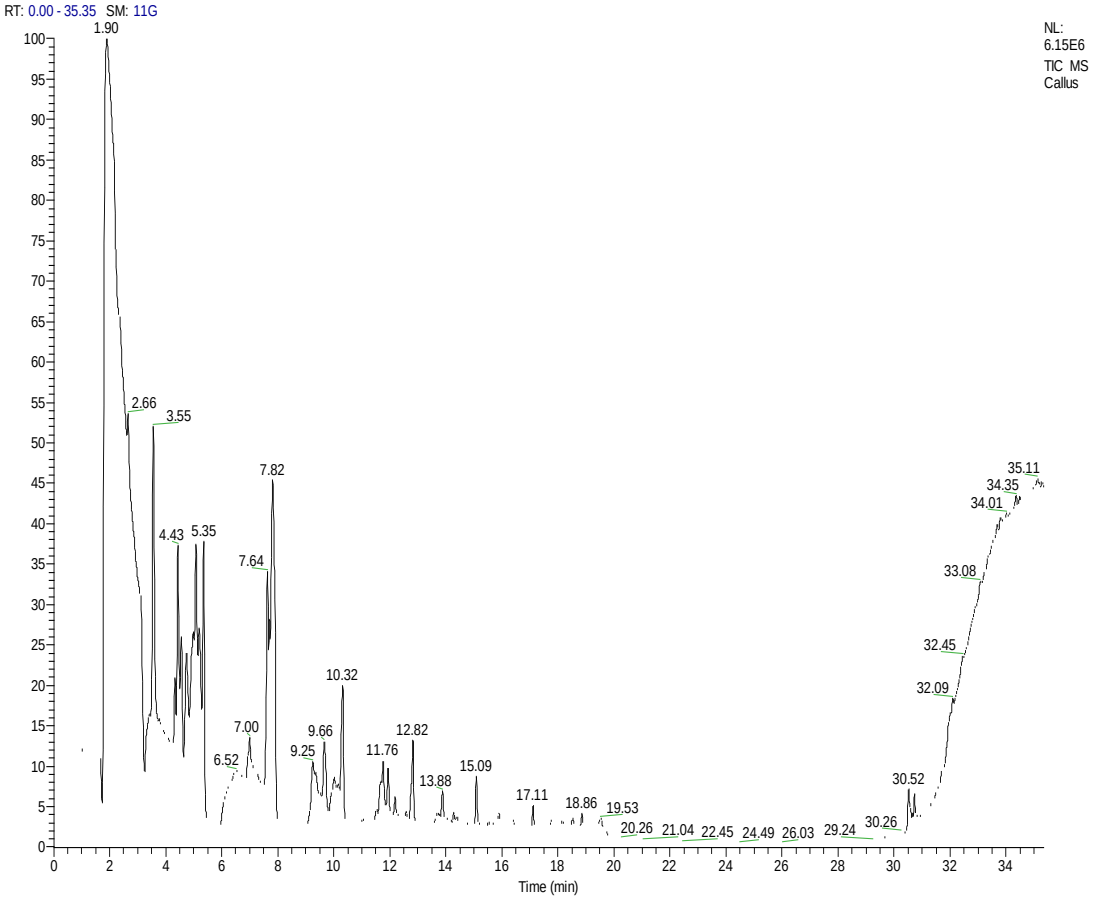


Table 1: UV - VIS Ethanolic extract of Leaf.

S.NO	Wave length	Peak value (nm)
1	400-500	425
2	600-700	648

Table 2: UV –VIS Ethanolic extract of Callus.

S. No	Wave length	Peak value (nm)
1	300-400	372
2	600-700	643,647

The UV -VIS is absorption peak from 200-700 nm indicates the particles size reduction. The maximum absorption of synthesysised plant extracted is at 650 nm.

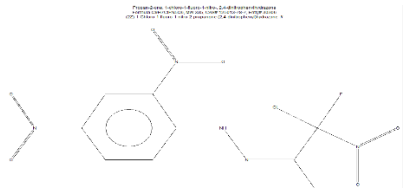
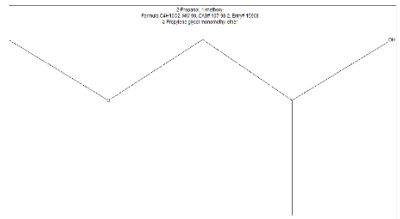
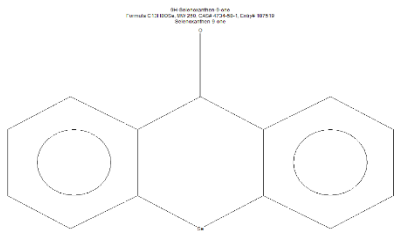
Table 3: FT-IR Peak Values and Functional groups of *S. schimperiana* Leaves powder.

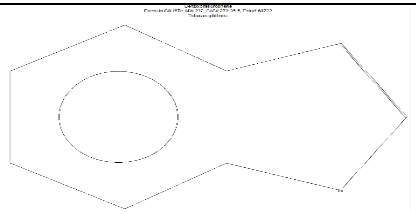
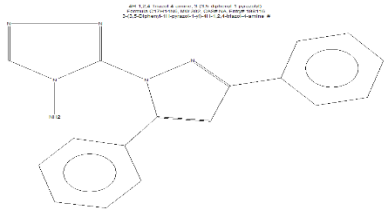
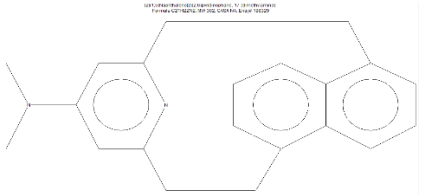
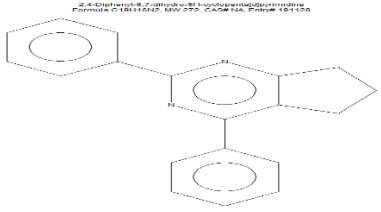
S. No	Name of the Bond	Functional Group	Stretching Frequency (cm^{-1})	Intensity
1	N-H (Stretch)	Primary amines and amides	3434.52	variable
2	-CH_3 and –CH_2 – CH Antisym and Sym (Stretch)	Aliphatic compounds	2981.18	strong
3	-CH_3 and –CH_2 – CH Antisym and sym (stretch)	Aliphatic compounds	2903.55	strong
4	C=C (Stretch) and C=O (Stretch)	Alkene (monosubst)	2114.77	Variable
5	NH_2 (stretch)and NH deformation	Amine and (amine II bands)	1641.10	strong
6	CH_3 and CH_3 antisym deformation	Alphatic compound	1453.12	Medium and weak bond
7	C-N (primary) and C-N(Stretch)	Amindes and amine III band	1404.89	Strong
8	CF_3 antisym (stretch)	Benzene ring	1329.38	strong
9	C-O-C (Stretch) and C-O-C (Stretch)	Ester and lactones	1272.80	Strong
10	Si-o-Si and Si-O-Si antisym(stretch)	Siloxanes	1082.24	Medium
11	P-O-C and antisym (stretch)	Organo phosphorus	1045.91	Medium
12	1,2,4 and CH out of plane deformation II band	Trisubst benzenes	878.94	Medium
13	C-S and C-S (stretch) strong in raman	Sulfides	703.70	strong

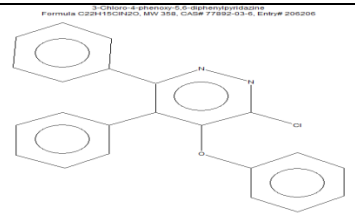
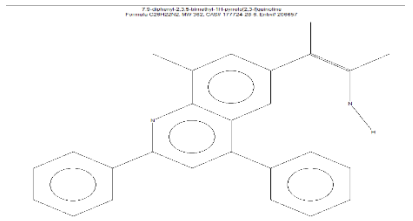
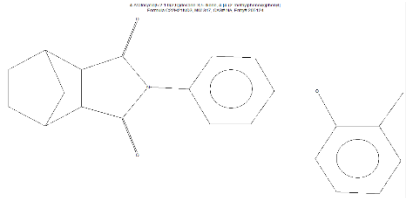
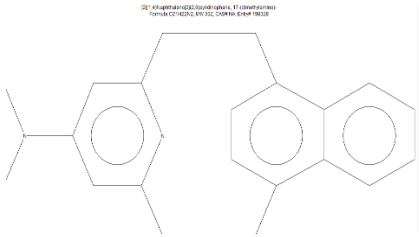
Table 4: FT-IR Peak Values and Functional groups of *S. schimperiana* Callus powder.

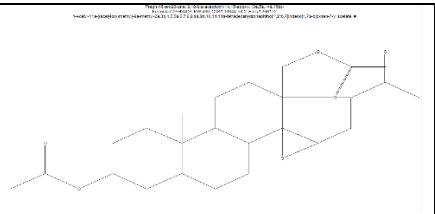
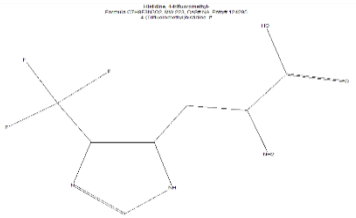
S. No	Name of the Bond	Functional Group	Stretching Frequency (cm^{-1})	Intensity
1	N-H (Stretch)	Primary amines and amides	3434.52	variable
2	-CH_3 and –CH_2 – CH Antisym and Sym (Stretch)	Aliphatic compounds	2981.18	strong
3	-CH_3 and –CH_2 – CH Antisym and sym (stretch)	Aliphatic compounds	2903.55	strong
4	C=C (Stretch) and C=O (Stretch)	Alkene (monosubst)	2114.77	Variable
5	NH_2 (stretch)and NH deformation	Amine and (amine II bands)	1641.10	strong
6	CH_3 and CH_3 antisym deformation	Alphatic compound	1453.12	Medium and weak bond
7	C-N (primary) and C-N(Stretch)	Amindes and amine III band	1404.89	Strong
8	CF_3 antisym (stretch)	Benzene ring	1329.38	strong
9	C-O-C (Stretch) and C-O-C (Stretch)	Ester and lactones	1272.80	Strong
10	Si-o-Si and Si-O-Si antisym(stretch)	Siloxanes	1082.24	Medium
11	P-O-C and antisym (stretch)	Organo phosphorus	1045.91	Medium
12	1,2,4 and CH out of plane deformation II band	Trisubst benzenes	878.94	Medium
13	C-S and C-S (stretch) strong in raman	Sulfides	703.70	strong

Table 5: Phytocomponents Identified in Ethanolic Callus extract of *S. schimperiana*. Using GC-MS/ MS.

S.No	Name	Mole.wt	Mole form	Rt	Area	Mole.str	Uses
1	Propan -2-one, 1 chloro 1 fluro, 1 – nitro	335	$C_9H_7C_1FN_{506}$	2.09	1.90		It is a potential mutagenic agent. 2, 4-Dinitrophenylhydrazine has been used as a reagent to detect the presence of aldehydes and ketones in protein carbonyls.
2	2 – propanal, 1 methoxy	90	C_4H_{1002}	2.66	2.66		In controlled human exposures, breath analysis data showed that propylene glycol mono methyl ether was rapidly excreted via the lungs.
3	9 H – Seenoxanthen - 9-one	260	$C_{13}H_{80}Se$	3.65	3.55		Selenoxanthene-9-ylidene)-piperidine and acridine and pharmaceutically acceptable salts thereof, Which exhibit useful pharmacological properties, including utility as selective 5-HT2B receptor antagonists for treatment of a disease state Which can be alleviated by treatment With a 5-HT2B receptor antagonist.
4	Benzo (b) tellurophene	232	C_8H_6Te	4.48	5.35		**Skin:** Get medical aid. Flush skin with plenty of water for at least 15 minutes while removing contaminated clothing and shoes. Wash

							clothing before reuse. **Ingestion:** If swallowed, do not induce vomiting unless directed to do so by medical personnel. Never give anything by mouth to an unconscious person. Get medical aid.
5	4 H – 1,2,4,- Triazol – 4 amine, 3 –(3,5 – Diphenyl -1 pyrazolyl)	302	$C_{17}H_{14}N_6$	7.02	7.00		pyrazole, triazole, triazine and their derivatives due to the ir wide use in medicinal chemistry. 5- 7 Hence, it was thought that the incorporation of the latter heterocyclic moieties into pyridazines moiety might modify their biological activity
6	(2) 1, 5 Napthaleno (2,6) pyridinophane, 1,7 (dimethylamino)	302	$C_{21}H_{22}N_2$	7.66	7.82		It is raw material for the production of many agrichemicals and pharmaceuticals, such as dimefox and diphenhydramine, respectively.
7	2, 4 - Diphenyl - 6, 7- dihydro – 5H – cyclopenta[d] pyrimidine	272	$C_{19}H_{16}N_2$	9.72	9.66		----
8	3- chloro -4 –	358	$C_{22}H_{15}C_1N_{20}$	10.35	10.32		-----

	phenoxy – 5, 6-diphenylpyridazine						
9	4- Azatricyclo [5.2.1.0(2,6) decane 3, 5 – dione, 4-[4-(2 methylphenoxyphenyl]	347	$C_{22}H_{21}N0_3$	13.92	11.76		-----
10	7,9- diphenyl – 2, 3, 5 trimethyl – 1H pyrrolo[2,3-F] quinolone	362	$C_{26}H_{22}N_2$	12.84	13.88		-----
11	[2](1,4) Napthaleno (2) (2, 6) pyridinophane, 1, 7 – (dimethylamine)	302	C21H22N2	15.07	15.09		-----
12	Pregn- 16 – en 20 – one 3, 18 – bis	430	$C_{25}H_{3406}$	17.17	17.11		-----

	(acetyloxy) – 14, 15 epoxy- 1 (3a, 5a, 14a, 15a)						
13	Histidine 4-trifluoromethyl -	223	$C_7H_8F_3N_{302}$	30.55	30.52		------

Among the leaves ethanolic extracts compared to the callus ethanolic extracts show the presence of maximum number of compound than other leaves solvent. Callus ethanolic extracts having high phytochemicals than other solvents (Starlin *et al*., 2012). Phytochemical screening of *S.schimperiana*. **Listed Table-4**. Spectral differences are the objective reflection of componential differences. By using FT-IR spectrum, we can confirm the functional constituent's presence in the given parts and extract, identify the medicinal materials from the adulterate and even evaluate the qualities of medicinal materials (Liu H *et al*., 2006). The results of the present study spectrum also revealed the functional constituents present in the crude powder and ethanolic extracts of *S. schimperiana*. Many researchers applied the FTIR spectrum as a tool for distinguishing closely associated plants and other organisms (Timmins EM *et al*., 1998), (Goodacre R *et al*., 2000), (Johnson *et al*., 2003), (Lamprell *et al*., 2006), (Sahoo *et al*., 2011). The results of the present study coincided with the previous observations observed by various plant biologist and taxonomist. The results of the present study developed novel phytochemical marker to identify the medicinally important plant.

The ethanolic extract of *S. schimperiana* was subjected to GC–MS analysis. Interpretation on mass spectrum GC–MS was conducted using the database of National Institute Standard and Technology (NIST). The name, molecular weight and structure of the components of the test materials were ascertained. GC-MS results shown that at 13 compounds were present in ethanolic extraction of *S. schimperiana*. The compound of *S. schimperiana* was identified through mass spectrometry attached with gas chromatography. The unknown spectrum components were compared with the known spectrum components which are stored in the (NIST) library and the data is given **Table 5**. The results pertaining to GC-MS analysis leads to the identification of number of compounds from the GC fractions of the ethanolic extract of *S. schimperiana*.

CONCLUSION

The results in the present study showed that UV-VIS, FTIR and GC-MS spectroscopy is valuable techniques to analyze the different biomolecules extracted with different samples of *S. schimperiana* is a very important medicinal plant. It is useful to prepare the phytocompond data, quantification of phytochemical compounds.

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