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Comparative Phytochemical profiling of various extracts, from different parts of *Sesuvium portulacastrum* using GCMS, FTIR and ICP AES.

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Abstract:

Sesuvium portulacastrum (Aizoaceae family) is mangrove associate known for its antimicrobial , antifungal activity.The present study focuses on comparative analysis of phytochemicals present in ***Sesuvium portulacastrum*** (Aizoaceae family) using GCMS ,ICP AES and IR spectroscopy. The work also highlights the different phytochemicals present in various parts like stem and leaves of the plants from Aizoaceae family. The IR spectroscopy elucidates different functional groups present in the phytochemicals from different parts of plant .The GCMS has identified 15,15,14 phytochemicals respectively from *Sesuvium portulacastrum* ethyl acetate leaf, petroleum ether stem and chloroform whole plant extracts. This study also is noteworthy as it uses ICP AES techniques in analyzing different elements present in *Sesuvium portulacastrum*. This work emphasizes the importance of sophisticated analytical Instrumentation, in phytochemical characterization and the technique can also reveal the difference in phytochemicals present in different parts of the same plant.

Keywords: *Sesuvium portulacastrum* ,GCMS , ICP AES ,IR spectroscopy.

Introduction:

Plants are used by human beings for food, fodder and medicine since ancient times. Medicinal plants form a large group of economically vital plants which provide the basic raw materials for native pharmaceuticals.[1] The plants that flourish in stressful coastal environment in particular mangroves, are rich in synthesis of secondary metabolites to surmount the stress.[2] Mangrove and mangrove associates contain biologically active antiviral, antibacterial and antifungal compounds. They provide a rich source of steroids, triterpenes, saponins, flavonoids, alkaloids and tannins.[3,4,5] In traditional medicines, mangrove have been used against human, animal and plant pathogen, but detailed investigations regarding the bioactive components are inadequate. [6]

Sesuvium portulacastrum (L.) L. (seapurslane) is one of the fast growing, herbaceous, perennial, dichotomous, halophyte belonging to family Aizoaceae. In India, it grows at the eastern and western coastal sides as a mangrove associate.[7] The plant is used on the Senegal coast as a haemostatic and a decoction of it is considered to be the best known antidote for stings of venomous fish. Leaves have acidulous flavour of sorrel as well as antiscorbutic[8] .The essential oil extracted from the leaves of *Sesuvium,* revealed notable antibacterial activity against both gram-positive and Gram-negative bacteria and displayed significant antifungal and antioxidant activity [9]. The plant is known to contain a polysaccharide, which showed positive activity against HIV[10]. The plant has revealed its importance in effective removal of heavy metals such as cadmium, lead and arsenic from contaminated sites [11] . *Sesuvium portulacastrum* ,molecular phylogenetic studies using 18S rRNA gene sequence has revealed, its closely reltated to *Perkesia aculata* (Cactaceae) [12].

Knowledge of the chemical constituents of plant is essential, for the discovery of therapeutic drugs as well as for finding out new sources of such economic materials as tannins, oils, gums, precursors for the synthesis of complex chemical substances [13,14].Phytochemical which possess many economical and physiological roles are widely distributed as plant constituents.

Sesuvium portulacastrum has plethora of secondary metabolites with specific pharmacological prospects and hence there is need to investigate the phytochemicals present in various plant extracts. The bioactivity of phytochemicals can vary significantly depending on, plant parts, tissue type and at times with the growth conditions , solvent used in extraction . It is very difficult to standardize any formulation if the exact composition of chemical constituents is not known. There is a need to study the various chemicals present in different parts of plant to evaluate its potential.

With the advancement in analytical technology, details about metabolites present in plants can be identified with help of instruments like GCMS and IR spectroscopy. The principle of IR spectroscopy is based on the fact that various functional groups in a chemical structure gives rise to characteristic bands both in terms of intensity and position (frequency)[15]. The present study deals with chemical identification of *Sesuvium portulacastrum* using GCMS, ICP AES and IR spectroscopy. This study gives a comprehensive result about the application of various chemical based analytical methods used for identification of various metabolites from different parts like whole plant , stem and leaves of *Sesuvium portulacastrum.*

2. Materials and Methods

2.1 Plant materials : *Sesuvium portulacastrum* was collected from Kelva beach, Thane District Maharashtra identified and authenticated .

2.2 Chemicals and Reagents: All the chemicals used were of analytical grade and were purchased from Hi Media and Merck.

2.3 Plant extract preparation: 100 g of dry leaf ,stem and whole plant of *Sesuvium portulacastrum* were extracted with ethyl acetate chloroform and petroleum ether by using soxhlet apparatus. The extraction was filtered and kept for 24 hours to evaporate. Analytical techniques like GCMS, FT-IR and ICP AES were used for further phytochemical analysis.

2.4 GCMS analysis: The GCMS analysis was performed on Thermo Scientific TSQ 8000 Gas Chromatograph - Mass Spectrometer. The MS part consists of Triple Quadrupole, is paired with the TRACE 1300 GC along with Auto-sampler for automated sample handling. It is equipped with EI Ion Source programmable to 350 ^{o} C. The Mass Range is 2.1100 amu .Gas Chromatograph Consists of Split/Splitless Injectors and multi-mode (including on-column) Programmed Temperature Vaporizing (PTV), column Temperature 400^{o}C.

2.5 IR Spectroscopy analysis: The IR Spectroscopy was performed on Perkin elmer Inc. Spectrum RX FT-IR spectrophotometer. It has an autosampler with fibre optic interfaces and range of microscope. Spectral resolution is better than 0.8 cm^{-1} It is equipped with dynascan interferometer.

2.6 ICP AES analysis: The ICP AES analysis was performed on ARCOS, Simultaneous ICP Spectrometer.It has an RF generator with a maximum of 1.6 KW , Radial plasma carries out the ionization and peristaltic pump delivers sample into analytical nebulizer.Spectrometer has a wavelength range of 130nm to 770 nm .

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Molecules from sample break into respective atoms and recombine with plasma giving its characteristic wavelength detected by CCD device

Result and Discussion:

The phytochemicals in *Sesuvium portulacastrum* were subjected to GCMS,IR ,ICP AES spectroscopy. Identification of components by GCMS was based on direct comparison of the retention times and mass spectral data with those for standard compounds from the NIST library. The NIST library is a database which contains exhaustive information about various chemical compounds. The GCMS has identified 15,15,14 phytochemicals respectively from *Sesuvium portulacastrum* ethyl acetate leaf, petroleum ether stem and chloroform whole plant extracts.

GCMS chromatogram of ethyl acetate leaf extract : The phytochemicals present in the ethyl acetate leaf extract are described in table no.1 and Figure no 1 displays the GCMS chromatogram. Tricaproin is obtained with maximum area percentage of 33.09% in ethylacetate leaf extract.

GCMS chromatogram of Petroleum ether stem extract: The phytochemicals present in the Petroleum ether stem extract are described in table no.2 and Figure no 2 displays the GCMS chromatogram.Cannabinol trifluroacetate is obtained with highest area percentage of 9.78 and Phenol is obtained with highest area percentage of 10.28%.

GCMS chromatogram of whole plant chloroform extract: The phytochemicals present in the Petroleum ether stem extract are described in table no.3 and Figure no 3 displays the GCMS chromatogram. Phenol2,4 bis phenyl ethyl is obtained at highest area percentage of 19.24%.

FTIR analysis of *Sesuvium portulacastrum* leaf: The IR spectrum is shown in figure no 4The *Sesuvium portulacastrum* leaf extract yielded maximum peak level 3356 cm^{-1} and minimum peak 779 cm^{-1}. The leaf extract yielded flavones functional group at 1637 cm^{-1} FT-IR studies confirm the presence of functional groups in leaf SeSL extract listed in table no 4.

FTIR analysis of *Sesuvium portulacastrum* whole plant:The IR spectrum is shown in figure no 5.The *Sesuvium portulacastrum* whole plant extract yielded maximum peak level 3600 cm^{-1} and minimum peak 783cm^{-1} . The leaf extract yielded flavones functional group at 1641 cm^{-1} FT-IR studies confirm the presence of functional groups in whole plant S1 extract listed in table no 5.

FTIR analysis of *Sesuvium portulacastrum* stem: The IR spectrum is shown in figure no 6.The *Sesuvium portulacastrum* stem extract yielded maximum peak level 3356 cm^{-1} and minimum peak 898 cm^{-1} . FT-IR studies confirm the presence of functional groups in stem SeSS extract listed in table no 6.

ICP AES analysis of *Sesuvium portulacastrum* : Elements present in the given sample are Cd,Cl,Co,Cr,Cu,Fe,K,La,Li,Mg,Mn,Na,Ni,P,,Pb,S,Si,Sr,Ti,V,Yb,Y,Zn,Ag, Al, B, Ba,Br, Ca.

Discussion:

Sesuvium portulacastrum has many different phytochemicals and their content varies significantly depending on the plant part and solvents used for the extraction purpose. In the present study leaf, stem and whole plant of *Sesuvium portulcastrum* was subjected to phytochemical analysis using GCMS , ICP AES, IR spectroscopy.

In *Sesuvium portulacasrum leaf, Stem and whole plant ext*racts compounds like Cyclopentasiloxane, Dibutyl phthalate are commonly found in all the plant parts. There are even certain phytochemicals like Tricaproin, Stigmasterol, Sitosterol, Cannabinol trifluroacetate which are confined to specific tissues only.

Our results are in accordance with the previous report on GCMS analysis of volatile oil from *Sesuvium portulacastrum* by Mohamed Yacoob Syed Ali which showed the presence of Hexadecanoic acid with highest peak percentage of 10.2 .[16]

The petroleum ether stem extracts of *Sesuvium portulacastrum* also contain Stigmasterol which was also reported in the previous preliminary studies performed by Amad All Azzawi et al[17] .This study gives a clear-cut particulars of the phytochemicals present in Whole plant, stem and leaves of *Sesuvium portulacastrum.* Many of the functional present in *Sesuvium portulacastrum* leaf, stem and whole plant extract are similar like aliphatic group ,alcohol group but the leaf extract contains flavones. So that compound may be phenolics. This identification is possible using IR spectroscopy as it give rise to characteristic bands both in terms of intensity and position (frequency).[15,18,19]

The emission spectroscopy uses the inductively coupled plasma to produce excited atoms and ions that emit electromagnetic radiation at wavelengths characteristic of a particular element. The intensity of this emission is indicative of the concentration of the element within the sample.

The results obtained in the above studies are noteworthy as there are many reports on preliminary phytochemical analysis of *Sesuvium portulaastrum* [9,16,17], but there are no reports on use of IR spectroscopy and ICP AES in identification of phytochemicals.

Conclusion:

Phytochemical characterization of plant extract is essential, as it helps us to determine the exact composition of metabolites / chemicals in different plant .This kind of research also helps in correlating the chemical with their biological and physiological roles. The study also highlights use of sophisticated instruments like GCMS FTIR and ICP AES in phytochemical research to have immense knowledge of the plethora of chemicals present in different parts of the same plant.

Acknowledegment:

The authors are thankful to SAIF Chandigarh and IIT Powai for providing GCMS , ICP AES facility respectively.

Table no 1 : Retention time, Area%, Molecular formula and Major peaks of chemicals detected by GCMS of *Sesuvium portulacastrum* , ethyl acetate leaf extract (easp).

Peak no.	Rt	Area %	Molecular formula	Compound
1	8.22	1.55	C10H30O5Si5	Cyclopentasiloxane
2	10.72	2.92	C14H44O6Si7	Heptasiloxane,1,1,3,3,5,5,7,7,9,9,11,11,13,13tetradeca methyl
3	13.95	3.85	C16H50O7Si8	Octasiloxane,1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15hexad ecamethy
4	14.95	4.52	C16H48O8Si8	Cyclooctasiloxane, hexadecamethyl
5	16.67	1.02	C16H48O6Si7	Heptasiloxane, hexadecamethyl
6	18.17	9.15	C16H22O4	Dibutyl phthalate
7	19.37	16.17	C19H34O2	9,12Octadecadienoicacid (Z,Z), methyl ester
8	19.97	5.85	C21H38O2	nPropyl 9,12octadecadienoate

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9	20.76	33.9	C21H38O6	Tricaproin
10	23.21	3.66	C24H38O4	Diisooctyl phthalate
11	24.25	0.53	C20H60O10Si10	Cyclodecasiloxane, eicosamethyl
12	26.66	1.70	C28H39ClO9	9Desoxy9xchloroingol 3,7,8,12tetraacetate
13	28.50	1.88	C23H48	Heptadecane, 9hexyl28
14	30.17	1.67	C26H54	Octadecane, 3ethyl5(2ethylbutyl)
15	33.07	2.56	C30H50O6	Olean12ene3,15,16,21,22,28hexol

Figure no 1: GCMS chromatogram of *Sesuvium portulacastrum* ethyl acetate leaf (EASP)

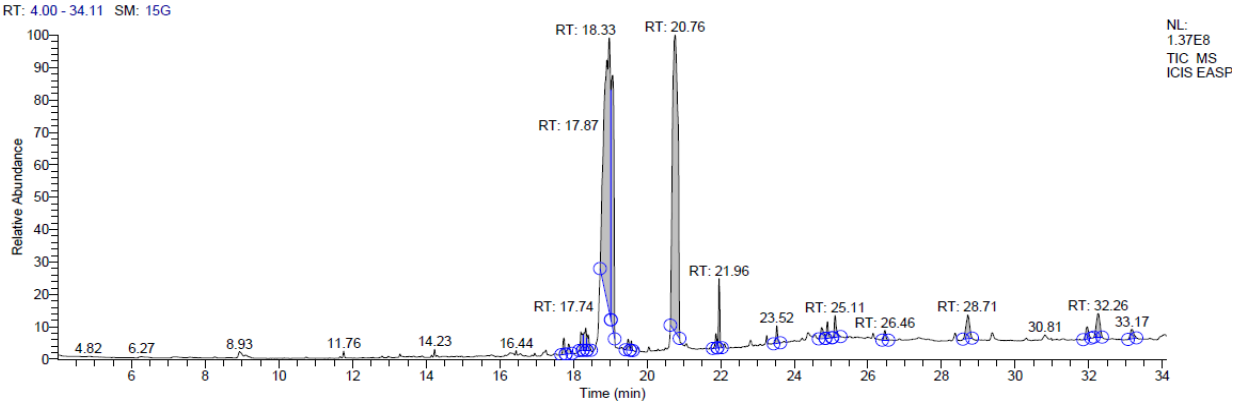

Figure no 2: GCMS chromatogram of *Sesuvium portulacastrum* petroleum ether stem(PEA4)

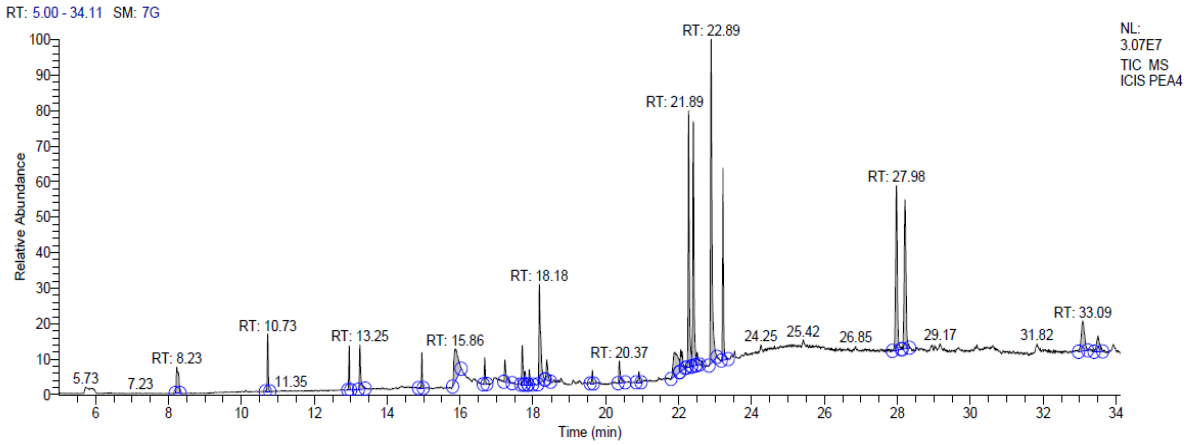

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Figure no 3: GCMS chromatogram of *Sesuvium portulacastrum chloroform wholeplant* (CLEA6)

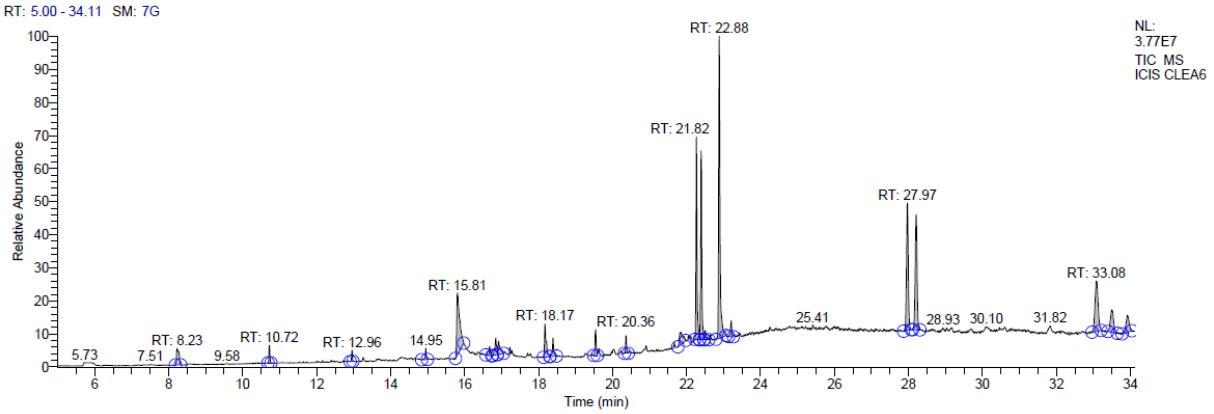

Table no2: Retention time, Area%, Molecular formula and Major peaks of chemicals detected by GCMS of *Sesuvium portulacastrum* Petroleum ether stem extract (pea4).

Peak no.	Rt	Area%	Molecular formula	Compound
1	8.23	2.03	C10H30O5Si5	Cyclopentasiloxane, decamethyl
2	10.73	1.87	C14H44O6Si7	Cyclohexasiloxane, dodecamethyl
3	13.25	2.05	C14H22O	Phenol, 2,4 bis(1,1 dimethylethyl)
4	15.86	4.38	C15H18	Azulene, 1,4dimethyl7(1methylethyl)
5	17.71	1.78	C16H22O4	Dibutyl phthalate
6	18.18	5.91	C16H22O4	Dibutyl phthalate
7	20.37	1.14	C16H34O2	Hexadecanoic acid, ethyl ester
8	21.89	3.89	C17H37N7O3	Deoxyspergualin
9	22.27	10.28	C14H22O	Phenol, 2,4 bis(1,1 dimethylethyl)
10	22.40	9.96	C14H22O	Phenol, 2,4 bis(1,1 dimethylethyl)
11	22.27	10.28	C22H22O	Phenol, 2,4bis(1phenylethyl)
12	23.22	6.48	C24H38O4	Pthalic aci di(2 propyl pentyl ester)
13	27.98	9.78	C23H25F3O3	Cannabinol, trifluoroacetate
14	28.21	9.44	C23H25F3O3	Cannabinol, trifluoroacetate
15	33.50	1.63	C29H48O	Stigmasterol

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Table no3: Retention time, Area%, Molecular formula and Major peaks of chemicals detected by GCMS of *Sesuvium portulacastrum* whole plant chloroform extract (CLEA4).

Peak no.	Rt	Area%	Molecular formula	Compound
1	8.23	1.09	C10H30O5Si5	Cyclopentasiloxane, decamethyl
2	14.95	4.08	C14H42O5Si6	Hexasiloxane, tetradecamethyl
3	15.81	8.46	C14H14O	Phenol, 2(1phenylethyl)
4	16.84	1.43	C15H18	Azulene, 1,4dimethyl7(1methylethyl)
5	18.17	2.65	C16H22O4	Dibutyl phthalate
6	18.39	1.21	C18H36O2	Hexadecanoic acid, ethyl ester
7	19.54	1.40	C20H40O	Phytol
8	20.36	0.92	C22H44O2	Eicosyl acetate
9	21.82	2.12	C22H43NO	13Docosenamide,
10	22.27	10.72	C22H22O	Phenol, 2,4bis(1phenylethyl)
11	22.88	19.16	C22H22O	Phenol, 2,4bis(1phenylethyl)
12	27.97	10.74	C23H25F3O3	Cannabinol, trifluoroacetate
13	33.08	7.41	C29H50O	çSitosterol
14	33.91	2.53	C30H50O	áAmyrin

Table no 4: FTIR Result for *Sesuvium portulacastrum* leaf (SeSL)

Wavelength in cm-1	Functional groups	Name of the Functional groups
3356	O-H	Alcohol
2924	C-H	Aliphatic
2852	C-H	Aliphatic
1637	C=O	Flavones
1099/1029	C-O streching	Alcohols/ Phenols
779	=C-H bending	(out-of-plane bending) cis –RCH=CHR

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Figure no 4: FTIR spectrum of *Sesuvium portulacastrum* leaf(SeSL)

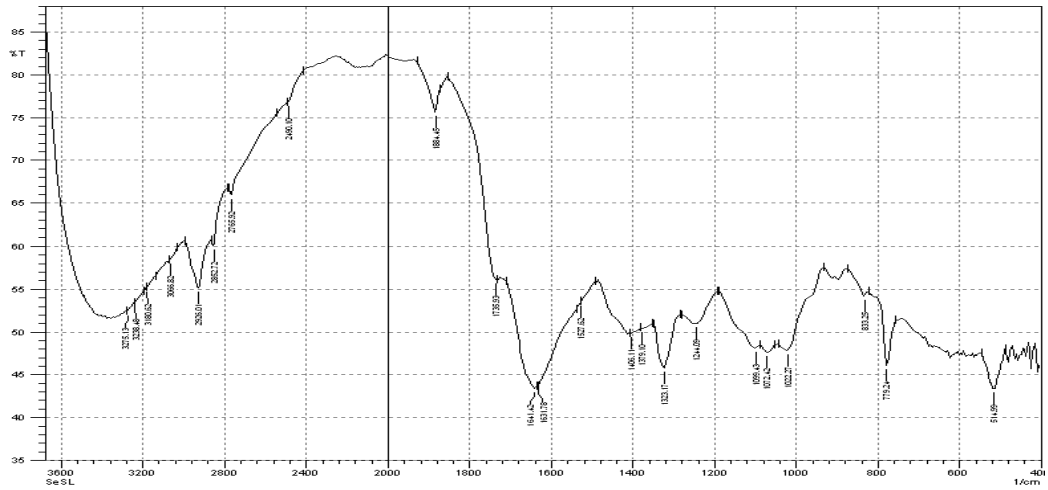

Figure no 5: FTIR spectrum of *Sesuvium portulacastrum* whole plant (S1)

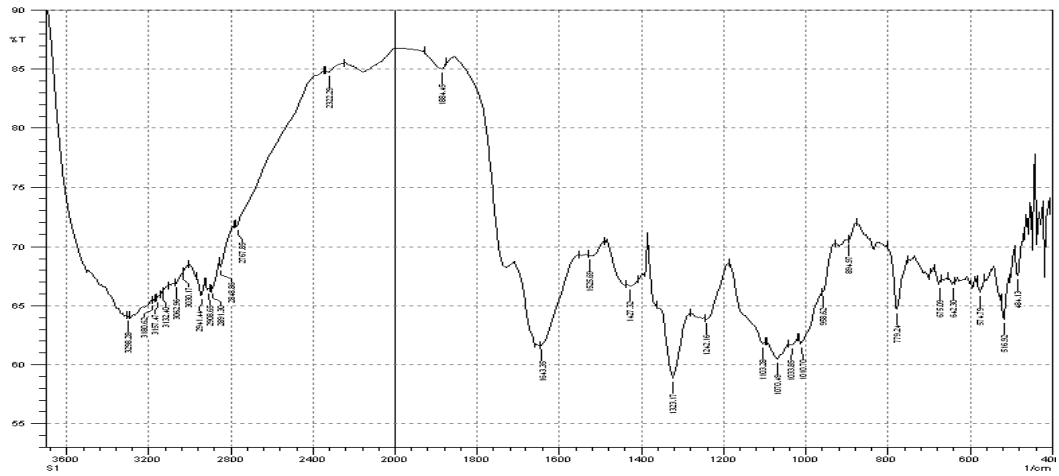

Table no 5: FTIR Result for *Sesuvium portulacastrum* whole plant (S1)

Wavelength in cm-1	Functional groups	Name of the Functional groups
3600-2400	O-H	Alcohol (very broad)
2941	C-H	Aliphatic
1641	C=C	Conjugated carbonyl (may be flavone)
1070	C-O	Alcohols/ Phenols
779	=C-H bending	(out-of-plane bending)cis –RCH=CHR

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Figure no 6: FTIR spectrum of *Sesuvium portulacastrum* stem (SeSS)

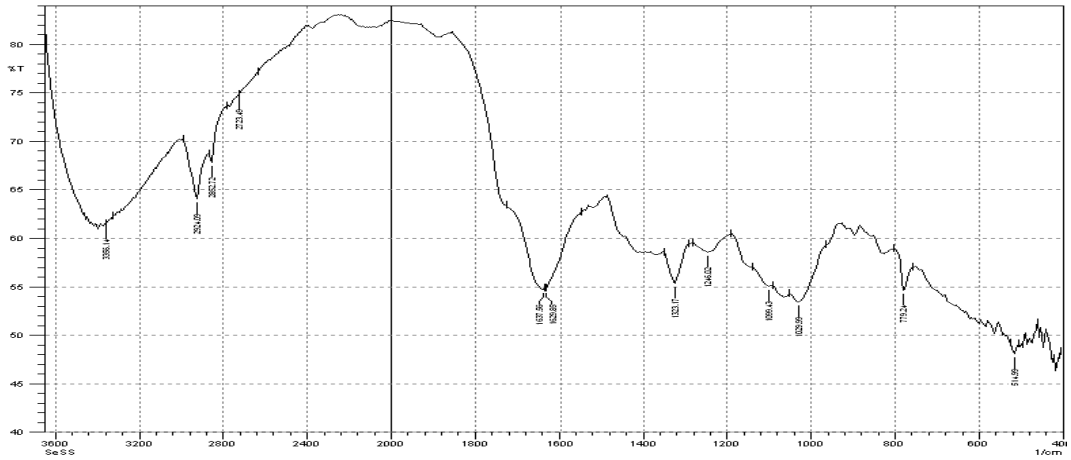

Table no 6: FTIR Result for *Sesuvium portulacastrum* stem (SeSS)

Wavelength in cm-1	Functional groups	Name of the Functional groups
3356	O-H	Alcohol
2924 .2852	C-H	Aliphatic
1629/1637	C=C	Arenes (In flavones C=O ,1637)
1099/1029	C-O stretch	Alcohols/ Phenols
898,723	=C-H bending	(out-of-plane bending) cis –RCH=CHR

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