



Original Research Article

Phytochemical screening by FTIR spectroscopic analysis of leaf extracts of selected Indian Medicinal plants

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A B S T R A C T

Key words

Phyllanthus amarus;
Senna auriculata;
Phyllanthus maderaspatensis;
Solanum torvum;
FTIR;
Spectroscopy;
Functional groups.

The present study is aimed to analyse the petroleum ether, chloroform, ethyl acetate and methanol extracts of leaves of 4 medicinal plants such as *Phyllanthus amarus*, *Senna auriculata*, *Phyllanthus maderaspatensis* and *Solanum torvum* through FT-IR spectroscopy method. The FTIR spectroscopic studies revealed different characteristic peak values with various functional compounds in the extracts. The FTIR analysis of methanol leaf extracts of *Phyllanthus amarus*, *Senna auriculata*, *Phyllanthus maderaspatensis* and *Solanum torvum* confirmed the presence of amide, alcohols, phenols, alkanes, carboxylic acids, aldehydes, ketones, alkenes, primary amines, aromatics, esters, ethers, alkyl halides and aliphatic amines compounds, which showed major peaks. The FTIR method was performed on a spectrophotometer system, which was used to detect the characteristic peak values and their functional groups. The results of the present study generated the FTIR spectrum profile for the medicinally important plants of *Phyllanthus amarus*, *Senna auriculata*, *Phyllanthus maderaspatensis* and *Solanum torvum* can be used in the aquaculture industry.

Introduction

A large number of medicinal plants are used as alternate medicine for diseases of man and other animals since most of them are without side effects when compared with synthetic drugs. Identification of the chemical nature of phytochemical compounds present in the medicinal plants will provide some information on the different functional groups responsible for their medicinal properties. While studying the *in vitro* efficacy of bioactive extracts of 15 medicinal plants against ESβL-

producing multi drug resistant bacteria, Iqbal Ahamad *et al.* (2006) detected major groups of compounds as the most active fraction of four plants extracts by infrared spectroscopy. Ramamoorthi and Kannan (2007) screened the bioactive group of chemicals in the dry leaf powder of *Calotropis gigantea* by FTIR analysis.

Kareru *et al.* (2008) detected saponins in crude dry powder of 11 plants using FTIR spectroscopy. Muruganantham *et al.*

(2009) carried out the FTIR spectroscopic analysis in the powder samples of leaf, stem and root of *Eclipta alba* and *Eclipta prostrata*. The FTIR analysis of aqueous methanolic leaf extracts of *Bauhinia racemosa* for phytochemical compounds was done by Gauravkumar *et al.* (2010). Ragavendran *et al.* (2011) detected the functional groups in various extracts of *Aerva lanata* using spectroscopic method. Thangarajan Starlin *et al.* (2012) detected the elements and functional groups in the ethanol extract of whole plant of *Ichnocarpus frutescens* using FTIR spectroscopic method. Paraj A.Pednekar and Bhanu Raman (2013) carried out the FTIR spectroscopic analysis of methanolic leaf extract of *Ampelocissus latifolia* for antimicrobial compounds. A survey of literature revealed that the FTIR analysis of functional groups was not done so far with the medicinal plants such as *Phyllanthus amarus*, *Senna auriculata*, *Phyllanthus maderaspatensis* and *Solanum torvum*. Hence, an attempt is made in the present study to analyse the functional groups of phytoactive compounds present in the leaf extracts (in different solvents such as petroleum ether, chloroform, ethyl acetate and methanol) of the four Indian medicinal plants, *Phyllanthus amarus*, *Senna auriculata*, *Phyllanthus maderaspatensis* and *Solanum torvum* by FTIR spectroscopic analysis.

Materials and Methods

Collection of plant

Leaf samples of four medicinal plant species such as *Phyllanthus amarus*, *Phyllanthus maderaspatensis* (Family: Euphorbiaceae), *Senna auriculata* (Family: Caesalpiniaceae), *Solanum torvum* (Family: Solanaceae) were collected from Kalingarayan canal bank at Bhavani (Erode District, Tamilnadu,

India) Identification of the plant species was done with the help of Dr. R. Gopalan, Professor of Botany, Karpagam University (former Scientist, BSI, Coimbatore) Coimbatore.

Preparation of leaf extract

The shade dried leaves of each plant (at 20°C) were powdered in mechanical grinder. 20 grams of leaf powder (of each plant) was weighed, 150 ml of solvent was added and kept for 3 days. The extract was filtered using Whatman No.1 filter paper and the supernatant was collected. The residue was again extracted two times (with 3 days of interval for each extraction) and supernatants were collected. The supernatants were pooled and evaporated (at room temperature, 28 ± 1°C) until the volume was reduced to 150 ml. Extracts of the leaf powder of the four plants with 4 different solvents such as petroleum ether (PE), chloroform (CF), ethyl acetate (EA) and methanol (ME) were prepared and stored in air tight bottles for further analysis

Fourier Transform Infrared Spectrophotometer (FTIR)

Fourier Transform Infrared Spectrophotometer (FTIR) is perhaps the most powerful tool for identifying the types of chemical bonds (functional groups) present in compounds. The wavelength of light absorbed is characteristic of the chemical bond as can be seen in the annotated spectrum. By interpreting the infrared absorption spectrum, the chemical bonds in a molecule can be determined.

Dried powder of different solvent extracts of each plant materials were used for FTIR analysis. 10 mg of the dried extract powder was encapsulated in 100 mg of

KBr pellet, in order to prepare translucent sample discs. The powdered sample of each plant specimen was loaded in FTIR spectroscope (Shimadzu, IR Affinity 1, Japan), with a Scan range from 400 to 4000 cm^{-1} with a resolution of 4 cm^{-1} .

Results and Discussion

The FTIR spectrum of leaf extracts (prepared in different solvents) of *P. amarus*, *S. auriculata*, *P. maderaspatensis* and *S. torvum* are given in Fig 1 to 16. The data on the peak values and the probable functional groups (obtained by FTIR analysis) present in the leaf extracts (prepared in PE, CF, EA and ME) of *P. amarus*, *S. auriculata*, *P. maderaspatensis* and *S. torvum* are presented in Tables 1 to 4.

FTIR spectral data interpretation

Phyllanthus amarus

Petroleum ether (PE) extract

P.E extract of *P. amarus* exhibited a characteristic band at 1734 cm^{-1} indicating the presence of a pair of carbonyl (C=O) group, and at 2920 cm^{-1} for C-H group.

Chloroform (CF) extract

The characteristic absorption band were exhibited at 2912 cm^{-1} (for C-H stretching), 1492 cm^{-1} (for C-H bending) for C-H group and at 1710 cm^{-1} , 1718 cm^{-1} for carbonyl groups (C=O) were exhibited by CF extract.

Ethyl acetate (EA) extract

The EA extract showed the characteristic absorption bands were observed at 2927 cm^{-1} (for C-H stretching), 1444 cm^{-1} (for C-H bending) for C-H group and at 1714

cm^{-1} , for a carbonyl group (C=O).

Methanol (ME) extract:

The ME extract of *P. amarus* showed characteristic absorption bands at 3385 cm^{-1} for a hydroxyl (-OH) group 2929 cm^{-1} , 2343 cm^{-1} (for C-H stretching), 1382 cm^{-1} (for C-H bending), and at 1622 cm^{-1} for C=C group.

Senna auriculata:

Petroleum ether (PE) extract:

The extract of *S. auriculata* exhibited a characteristic band at 1724 cm^{-1} indicating the presence of a carbonyl (C=O) group and at 2926 cm^{-1} (for C-H stretching) and 1454 cm^{-1} for (C-H bending) for C-H group.

Chloroform (CF) extract:

The characteristic absorption bands were exhibited at 2927 cm^{-1} , (for C-H stretching), for C-H group and at 1718 cm^{-1} for carbonyl group (C=O) were exhibited by CF extract.

Ethyl acetate (EA) extract:

The EA extract exhibited the characteristic absorption bands were exhibited at 2922 cm^{-1} , (for C-H stretching), 1612 cm^{-1} for C=C group 1452 cm^{-1} (for C-H bending) for C-H group and at 1730 cm^{-1} for carbonyl group (C=O).

Methanol (ME) extract:

The ME extract of *S. auriculata* showed characteristic absorption bands at 3390 cm^{-1} and 1055 (C-O) for a hydroxyl (-OH) group 2929 cm^{-1} (for C-H stretching) and at 1627 cm^{-1} for C=C group.

Table.1 FTIR spectral peak values and functional groups obtained for the leaf extract (in different solvents) of *Phyllanthus amarus*

Extracts prepared in	Peak values	Functional groups
Petroleum ether (PE)	1734 2920	C=O carbonyl group C-H group
Chloroform (CF)	1492 1710 1718 2915	C-H bending C=O carbonyl group C=O carbonyl group C-H stretching
Ethyl acetate (EA)	1444 1714 2927	C-H bending C=O carbonyl group C-H stretching
Methanol (ME)	1382 1622 2343 2929 3385	C-H bending C=C group C-H stretching C-H stretching -OH group

Table.2 FTIR spectral peak values and functional groups obtained for the leaf extract (in different solvents) of *Senna auriculata*

Extracts prepared in	Peak values	Functional groups
Petroleum ether (PE)	1454 1724 2927	C=O carbonyl group C-H stretching C-H stretching
Chloroform (CF)	1718 2927	C=O carbonyl group C-H stretching
Ethyl acetate (EA)	1452 1612 1730 2922	C-H bending C=O group C=O carbonyl group C-H stretching
Methanol (ME)	1055 1627 2929 3390	C-O group C=C group C-H stretching -OH group

Table.3 FTIR spectral peak values and functional groups obtained for the leaf extract (in different solvents) of *Phyllanthus maderaspatensis*

Extracts prepared in	Peak values	Functional groups
Petroleum ether (PE)	1450	C-H bending
	1720	C=O carbonyl group
	2926	C-H stretching
Chloroform (CF)	1452	C-H group
	1726	C=O carbonyl group
	2926	C-H stretching
Ethyl acetate (EA)	1450	C-H group
	1716	C-H bending
	2864	C-H stretching
	2927	C-H stretching
Methanol (ME)	1056	C-O group
	1384	C-H bending
	1622	C=C group
	1707	C=O carbonyl group
	2343	C-H stretching
	2929	-OH group
	3385	-OH group

Table.4 FTIR spectral peak values and functional groups obtained for the leaf extract (in different solvents) of *Solanum torvum*

Extracts prepared in	Peak values	Functional groups
Petroleum ether (PE)	1722	C=O carbonyl group
	2856	C-H stretching
	2918	C-H stretching
Chloroform (CF)	1064	OH bending group
	1423	C-H stretching
	1703	C=O carbonyl group
	2910	C-H stretching
	3415	-OH group
Ethyl acetate (EA)	1064	OH group
	1377	C-H bending
	1450	C-H bending
	1724	C=O carbonyl group
	2858	C-H stretching
	2922	C-H stretching
Methanol (ME)	1056	C-O group
	1381	C-H bending
	1614	C=C group
	1701	C=O carbonyl group
	2860	C-H stretching
	2926	C-H stretching
	3387	-OH group

Fig.1 FTIR spectrum of PE extract of *P. amarus*

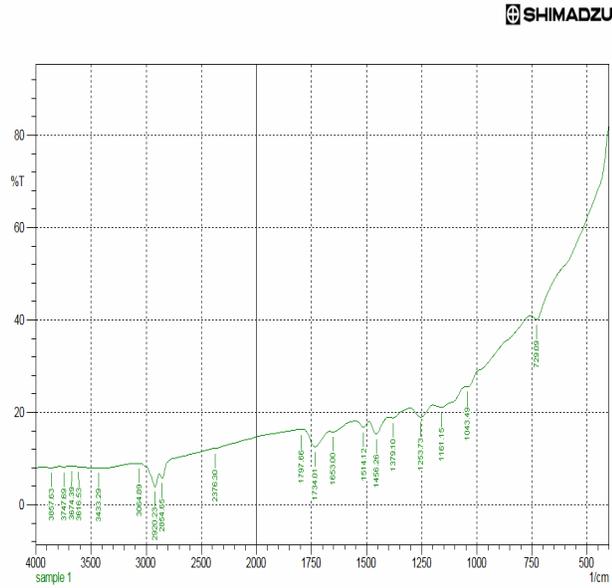


Fig.2 FTIR spectrum of CF extract of *P. amarus*

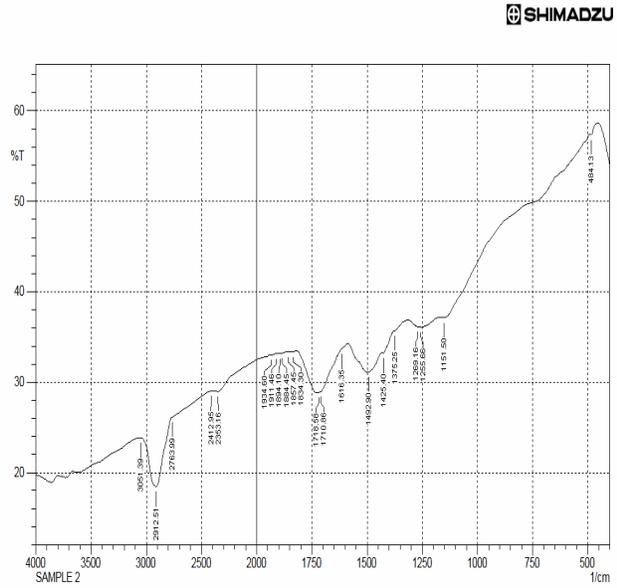


Fig.3 FTIR spectrum of EA extract of *P. amarus*

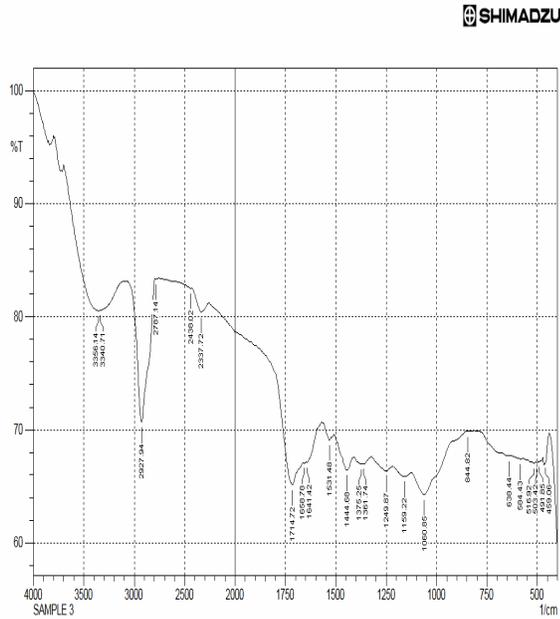


Fig.4 FTIR spectrum of ME extract of *P. amarus*

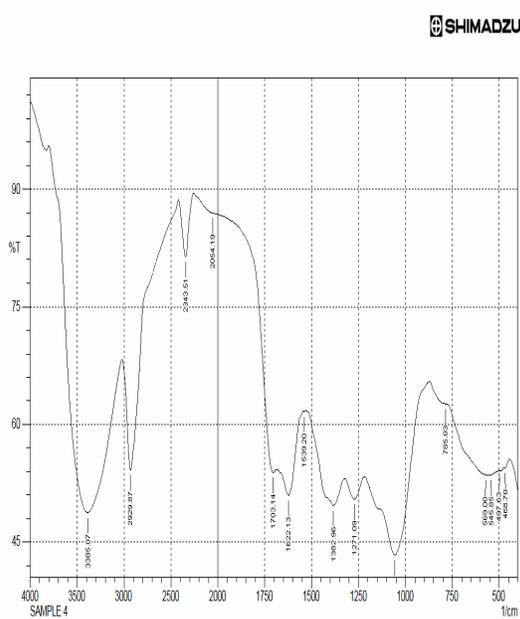


Fig.5 FTIR spectrum of PE extract of *S. auriculata*

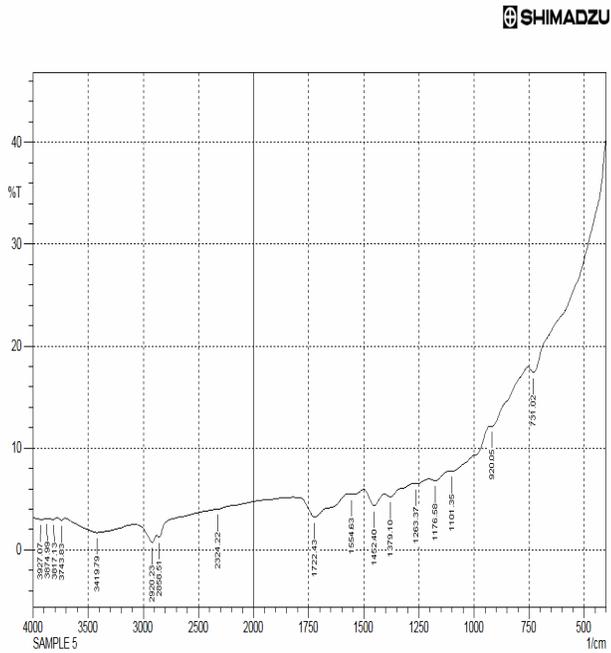


Fig.6 FTIR spectrum of CF extract of *S. auriculata*

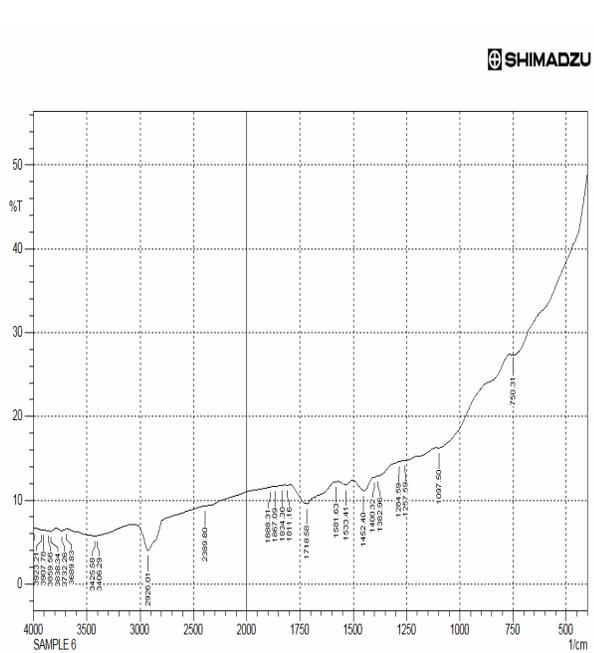


Fig.7 FTIR spectrum of EA extract of *S. auriculata*

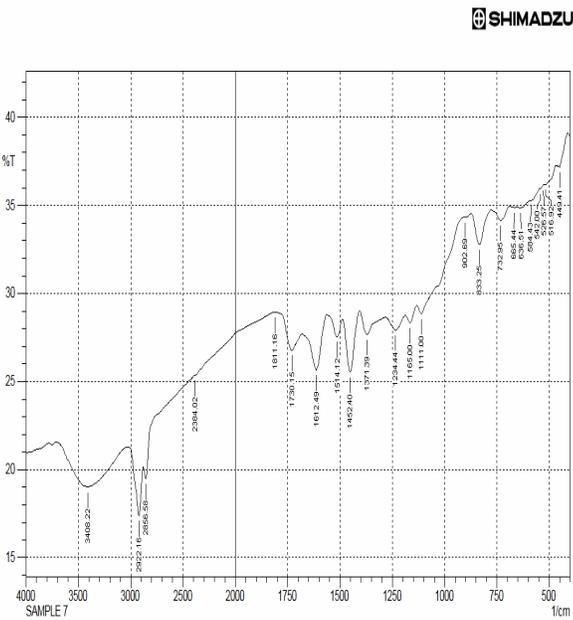


Fig.8 FTIR spectrum of ME extract of *S. auriculata*

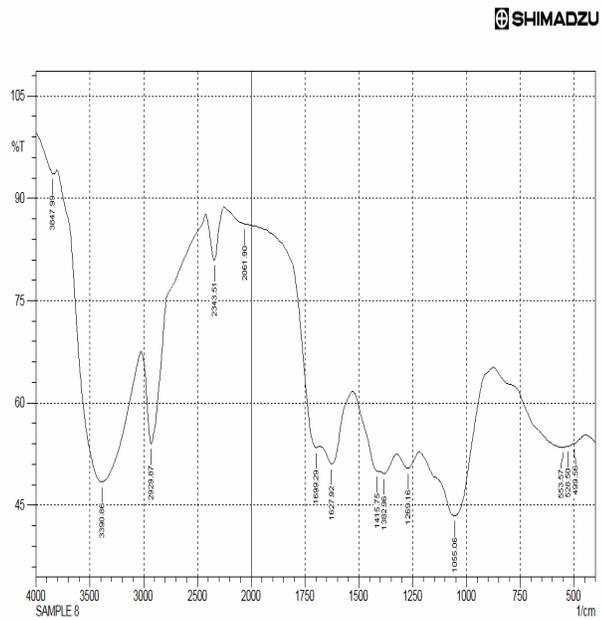


Fig.9 FTIR spectrum of PE extract of *P. maderaspatensis*

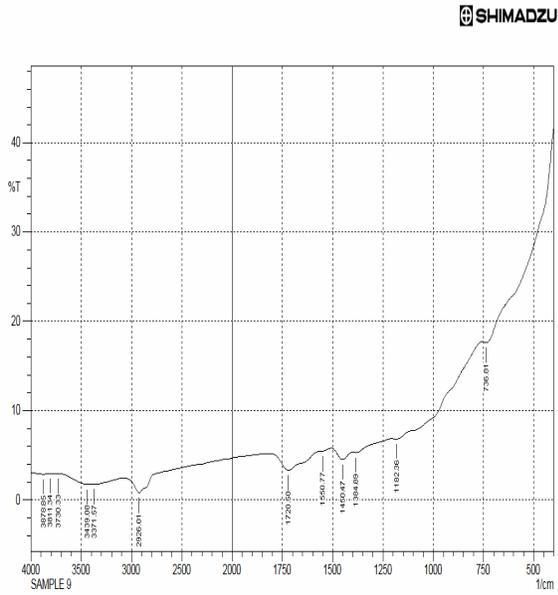


Fig.10 FTIR spectrum of CF extract of *P. maderaspatensis*

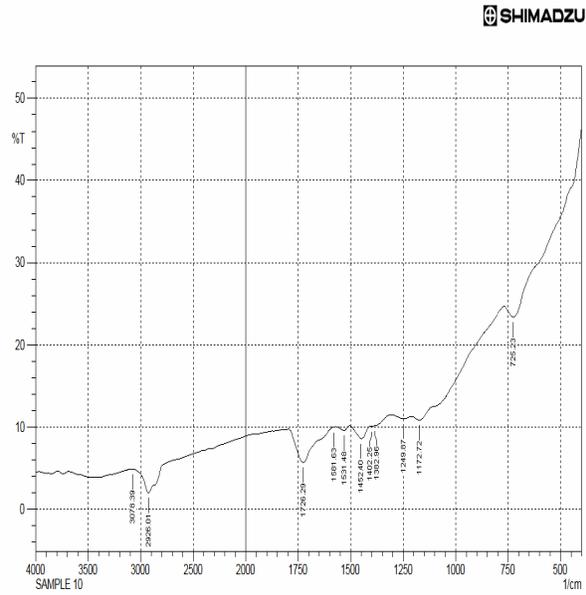


Fig.11 FTIR spectrum of EA extract of *P. maderaspatensis*

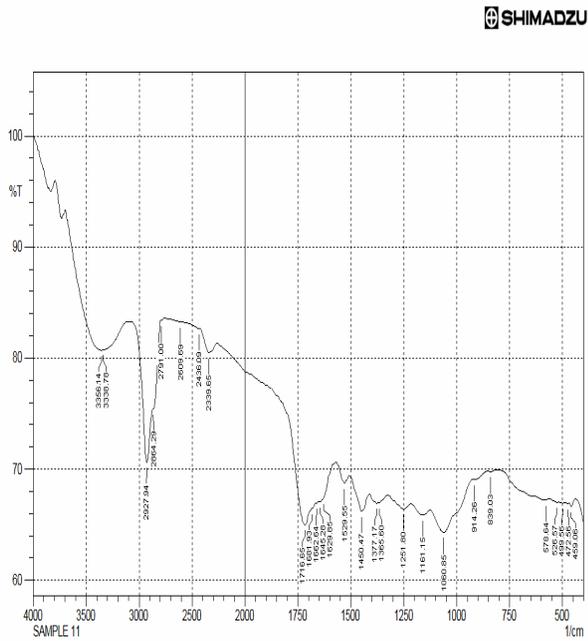


Fig.12 FTIR spectrum of ME extract of *P. maderaspatensis*

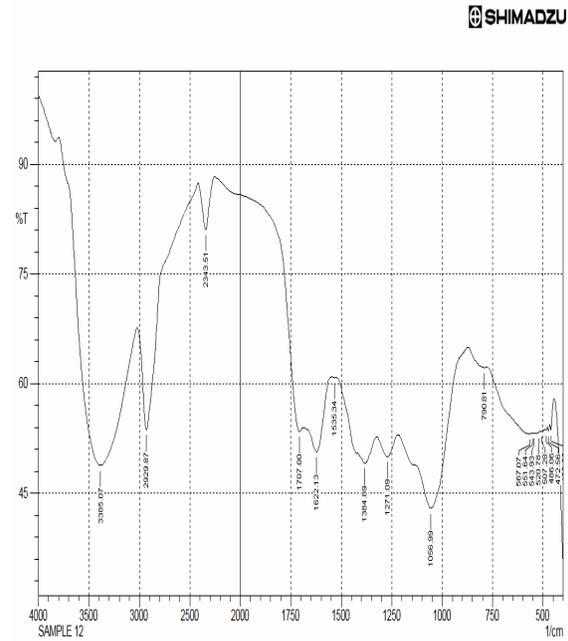


Fig.13 FTIR spectrum of PE extract of *S. torvum*

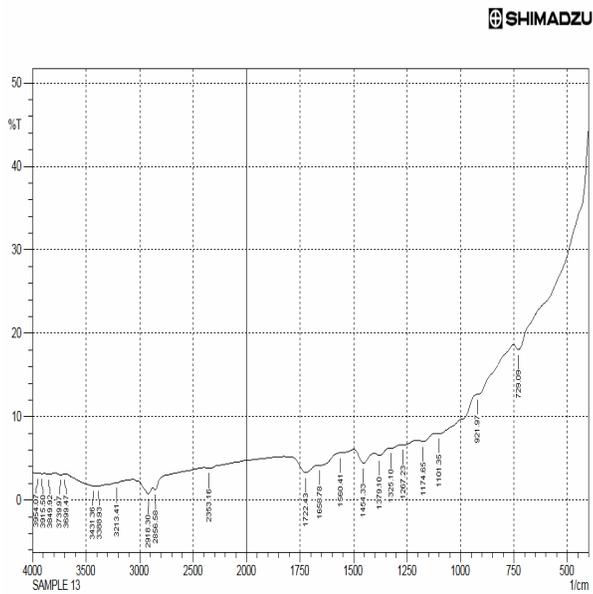


Fig.14 FTIR spectrum of CF extract of *S. torvum*

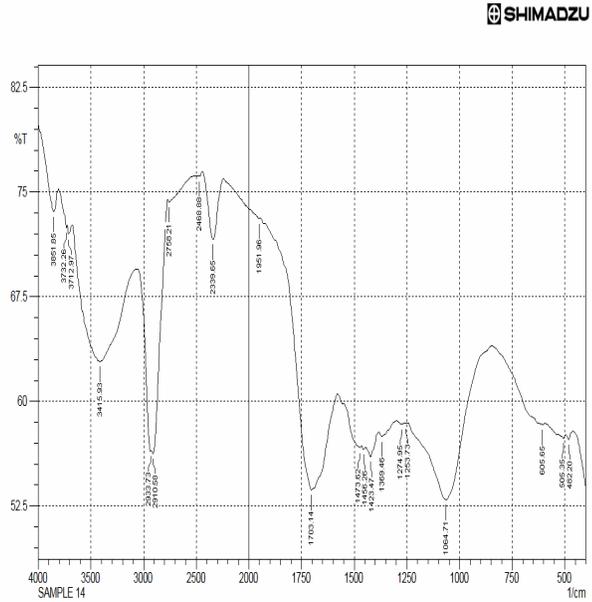


Fig.15 FTIR spectrum of EA extract of *S. torvum*

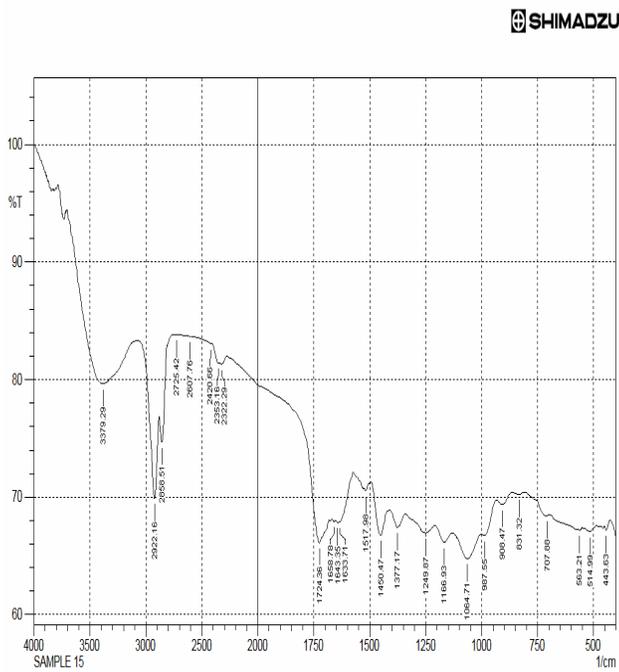
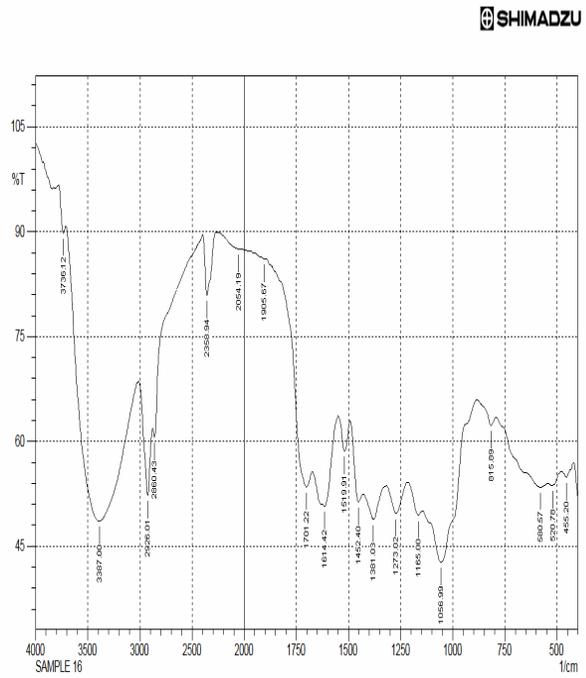


Fig.16 FTIR spectrum of ME extract of *S. torvum*



Phyllanthus maderaspatensis

Petroleum ether (PE) extract

PE extract of *P. maderaspatensis* exhibited a characteristic band at 1720 cm^{-1} indicating the presence of a carbonyl (C=O) group and at 2926 cm^{-1} (for C-H stretching) and 1450 cm^{-1} for (C-H bending) for C-H group.

Chloroform (CF) extract

The characteristic absorption bands were exhibited at 2926 cm^{-1} , (for C-H stretching), and 1452 cm^{-1} for C-H group and at 1726 cm^{-1} for carbonyl group (C=O).

Ethyl acetate (EA) extract

The EA extract showed the characteristic absorption bands at 2864 cm^{-1} , 2927 cm^{-1} , (for C-H stretching), 1450 cm^{-1} (for C-H bending) for C-H group and at 1716 cm^{-1} for carbonyl group (C=O).

Methanol (ME) extract

The ME extract of *P. maderaspatensis* showed characteristic absorption bands at 3385 cm^{-1} and 1056 cm^{-1} (C-O) for a hydroxyl (-OH) group 2929 cm^{-1} (for C-H stretching), 1384 cm^{-1} (for C-H bending), 1707 cm^{-1} , for an carbonyl group (C=O) and at 1622 cm^{-1} for C=C group.

Sollanum torvum

Petroleum ether (PE) extract:

PE extract of *S. torvum* exhibited a characteristic band at 1722 cm^{-1} indicating the presence of a carbonyl (C=O) group and at 2918 cm^{-1} and 2856 cm^{-1} (for C-H stretching) for C-H group.

Chloroform (CF) extract

The characteristic absorption bands were exhibited at 2910 cm^{-1} , (for C-H stretching), and 1423 cm^{-1} for C-H group and at 1703 cm^{-1} for carbonyl group (C=O). The absorption bands at 3415 cm^{-1} (OH) and 1064 cm^{-1} (O-H bending) are due to hydroxyl group.

Ethyl acetate (EA) extract

The EA extract of showed the characteristic absorption bands at 2922 cm^{-1} , 2850 cm^{-1} , (for C-H stretching), 1377 cm^{-1} and 1450 cm^{-1} (for C-H bending) for C-H group and at 1724 cm^{-1} for carbonyl group (C=O). The band at 1064 cm^{-1} is due to OH group.

Methanol (ME) extract

The ME extract of *S. torvum* showed characteristic absorption bands at 3387 cm^{-1} and 1056 cm^{-1} (C-O) for a hydroxyl (-OH) group 2926 cm^{-1} , 2860 cm^{-1} (for C-H stretching), 1381 cm^{-1} (for C-H bending), 1701 cm^{-1} for carbonyl group (C=O) and at 1614 cm^{-1} for C=C group.

Mueen Ahmed *et al.* (2005) showed that the leaves and latex of *Calotropis gigantea* species were found to have cardiac glycosides. The cardiac glycosides were identified as calotropogenin and calotropin. Ramamurthy and Kannan (2007) also confirmed that the leaf parts of *Caltropis gigantean* species showed the presence of cardiac glycosides such as calotropogenin and calotropin besides other organic compounds such as amino acids, chlorophyll, amides, lignins, carbohydrates and starch pertaining to a healthy plant.

Kareru *et al.* (2008), carried the spectral analysis for saponins in the crude dry

powder of 11 plants and detected that *Albizia anthelmintica*, *Senna singueana*, *Maytenus senegalensis*, *Senna didymomotrya*, *Terminalia brownii*, and *Prunus africana* were likely to be bidesmosidic, oleanane-type triterpenoids, while those detected in *Entada leptostachya* and *Rapanea rhododendroides* might be monodesmosidic saponins. Muruganantham *et al.* (2009) carried out the FTIR and EDS spectral analysis of plant parts like leaf, stem, and root of the medicinal plants, *Eclipta alba* and *Eclipta prostrata* and reported the presence of characteristic functional groups of carboxylic acids, amines, amides, sulphur derivatives, polysaccharides, nitrates, chlorates, and carbohydrate that are responsible for various medicinal properties of both herbal plants. The *Eclipta alba* contains a higher percentage of useful elements like Na, Mg, K, Ca, Cu, Zn, and Fe than *Eclipta prostrata*. In addition, *Eclipta prostrata* contains more of the toxic element Cd than *Eclipta alba* (Muruganantham *et al.*, 2009). The FTIR analysis of methanolic and aqueous leaf extracts of *Bauhinia racemosa* revealed the presence of protein, oil, fats, phenolic compounds, flavonoids, saponins, tannins and carbohydrate as major functional groups (Gaurav kumar *et al.* 2010).

Ragavendran *et al.* (2011) screened the functional groups of carboxylic acids, amines, amides, sulphur derivatives, polysaccharides, organic hydrocarbons, halogens that are responsible for various medicinal properties of *Aerva lanata*. Thangarajan Starlin *et al.* (2012), while analyzing the ethanolic extracts of *Ichnocarpus frutescens*, by FTIR, revealed functional group components of amino acids, amides, amines, carboxylic acid, carbonyl compounds, organic

hydrocarbons and halogens. Parag A Pednekar and Bhanu Raman (2013) analyzed the methanolic leaf extract of *Ampelocissus latifolia* by FTIR and reported that the transition metal carbonyl compounds and aliphatic fluoro compounds were only present in the extract.

From the results obtained in the present study, it could be concluded that the leaf extracts (in different solvents) of *Phyllanthus amarus*, *Senna auriculata*, *Phyllanthus maderaspatensis* and *Solanum torvum* with their phytoconstituents may act as source of antibiotics. The various functional groups observed in the different extracts probably indicate the presence of carbohydrates, carotenoid, glycogen, amino acids, amides, starch, calotropin, calotropogenin, phosphates, lipids, glycogen and cellulose.

Among the functional groups observed in the extracts, OH group was found to be present uniformly only in the methanol extracts of all plants. As OH group has got the ability of forming hydrogen bonding capacity, presence of OH group particularly in methanol extract of leaf of all the 4 plants probably indicates the higher potential of methanol extract towards inhibitory activity against microorganisms. Such a higher antimicrobial activity of methanol extracts of leaf of all those four plants have been already demonstrated (Ashokkumar and Ramaswamy, 2013) together with low IC₅₀ value (Ashokkumar and Ramaswamy, 2013).

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References

- Akinjogunla, N.O., Eghafona, I.O., Enabulele, C.I., Mboto, and Ogbemudia, F.O. 2010. African Journal of Pharmacy and Pharmacology. 4 (6): 402-407.
- Ashokkumar, R., and Ramaswamy, M. 2013. Comparative study on the antimicrobial activity of leaf extracts of four selected Indian medicinal plants against *Pseudomonas aeruginosa*, *Pseudomonas fluorescens*, *Penicillium chrysogenum* and *Penicillium restrictum*. Journal of Chemical, Biological and Physical Sciences. 3(2): 1376-1381.
- Ashokkumar, R., and Ramaswamy, M. 2013. Determination of DPPH free radical scavenging oxidation effects of methanolic leaf extracts of some Indian medicinal plant species. Journal of Chemical, Biological and Physical Sciences. 3(2): 1273-1278.
- Gaurav Kumar, L., Karthik and Bhaskara Rao, K.V. 2010. Phytochemical composition and *in vitro* Antimicrobial activity of *Bauhinia racemosa* Lamk (CAESALPINIACEAE). International Journal of Pharmaceutical Sciences and Research. 1(11): 51-58.
- Iqbal Ahmad and Farrukh Aqil. (2007). *In vitro* efficacy of bioactive extracts of 15 medicinal plants against ESbL-producing multidrug-resistant enteric bacteria. Microbiological Research. 162, 264 - 275.
- Kareru, P.G., Keriko, Gachanja, J.M., and Kenji, A.N. 2008. Direct detection of triterpenoid saponins in medicinal plants. African Journal of Traditional, Complementary and Alternative Medicines. 5 (1): 56 – 60.
- Mueen Ahmed, K.K., Rana, A.C., Dixit, V.K. 2005. *Calotropis* species (Asclepediaceae). A comprehensive review. Pharmacognosy Magazine. 1(2): 48–52.
- Muruganantham, S., Anbalagan, G., and Ramamurthy, N. 2009. FT-IR and SEM-EDS Comparative Analysis of Medicinal Plants, *Eclipta alba* Hassk and *Eclipta prostrata* Linn. Romanian Journal of Biophysics. 19(4): 285–294.
- Parag A. Pednekar, Bhanu Raman. 2013. Antimicrobial and Antioxidant Potential with FTIR analysis of *Ampelocissus latifolia* (roxb.) Planch. Leaves. Asian Journal of Pharmaceutical and Clinical Research. 6(1): 67-73
- Ragavendran, P., Sophia, D., Arul Raj, C., and Gopalakrishnan V.K., 2011. Functional group analysis of various extracts of *Aerva lanata* (L.) by FTIR spectrum. Pharmacologyonline. 1, 358-364.
- Ramamurthy, N., and Kennan, S., 2007. Fourier transform infrared spectroscopic analysis of a plant (*Calotropis gigantea* Linn) from an Industrial Village, Cuddalore Dt, Tamilnadu, India. Romanian Journal of Biophysics. 17 (4): 269–276.
- Redwane, A., Lazrek, H.B., Bouallam, S., Markouk, M., Amarouch, H., and Jana, M. 2002. Larvicidal activity of extracts from *Quercus iusitanica* var. *Infectoria* galls (Oliv.). Journal of Ethnopharmacology. 79, 261-263.
- Thangarajan Starlin, Paramasivam Ragavendran, Chinthamony Arul Raj, Palanisamy Chella
- Perumal and Velliyur Kanniappan Gopalakrishnan. 2012. Element and Functional Group Analysis of *Ichnocarpus frutescens* R. Br. (Apocynaceae). International Journal of Pharmacy and Pharmaceutical Sciences. 4(5): 343-345.