International Journal of Multidisciplinary Research and Development

Volume: 2, Issue: 8, 335-340
Aug 2015
www.allsubjectjournal.com
e-ISSN: 2349-4182
p-ISSN: 2349-5979
Impact Factor: 3.762

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Preliminary Phytochemical Screening GC-MS and FT-IR Profiling of Methanolic Extract of Leaves on *Eupatorium triplinerve* Vahl

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Abstract
The GC-MS studies of the methanolic leaf extracts *Eupatorium triplinerve* Vahl. The four major compounds highest peaks area (%) (9, 12, 15-Octadecatrienoic acid (20.18), n-Hexadecanoic acid (13.73), 4H-Pyran-4-1-2, 3-dihydro-3, 5-dihydroxy-6-methyl (8.36), 2-Methoxy-4-Ethyl-6-Methyphenol) The FT-IR spectroscope studies related different characteristic peak values with many functional compounds in the extracts. FT-IR analysis of methanol leaf extract confirmed the presence of amide, alcohols, phenol, alkanes, carboxylic, aldehydes, ketones, alkenes, primary amines, aromatic, esters, alkyl halides, and aliphatic amines compounds. This showed major peak. The bioactive compounds in the methanolic leaf extract *of Eupatorium triplinerve* have been screened using this analysis. Isolation of individual components would however help to find new drugs.

Keywords: *Eupatorium triplinerve* Vahl, Phytochemical Screening, GC-MS and FT-IR analysis

1. Introduction
In recent years the use of plants in the management and treatment of diseases has gained considerable importance. Plants and fruits are considered as one of the main sources of biologically active compounds. An estimate of the World Health Organization (WHO) states that around 85 – 90% of the world's population consumes traditional herbal medicines [1]. Plants are capable of synthesizing an overwhelming variety of low-molecular weight organic compounds called secondary metabolites, usually with unique and complex structures. Many metabolites have been found to possess interesting biological activities and find applications, such as pharmaceuticals, insecticides, dyes, flavors and fragrances. *Eupatorium triplinerve* Vahl is belongs to Asteraceae family. It is a slender herb with narrow lanceolate leaves and large number of pedicelled flower-heads at the top of the branch. The methanolic extract of *Eupatorium triplinerve* is reported to have hepatoprotective effect and antioxidant effect against carbon tetracholoride induced hepatotoxicity in rats [2]. While the ethanolic extract had analgesic effect in inflammatory model of pain [3]. Antibacterial and antifungal activity [4, 5]. Antiseptic and in the treatment of various ulcers and hemorrhages [6] Although the plant is used in Ayurvedic medicine for the treatment of ailments there are no reports on the constituents that are responsible for the therapeutic effect. With this background the present study was aimed to identify the phytoconstituents present in *Eupatorium triplinerve* using GC-MS analysis.
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. The FTIR analysis of aqueous methanolic leaf extracts of *Bauhinia racemosa* for phytochemical compounds was done [7].
While studying the in vitro efficacy of bioactive extracts of 15 medicinal plants against ESLproducing multi drug resistant bacteria, detected major groups of compounds as the most active fraction of four plants extracts by infrared spectroscopy [8]. Saponins in crude dry powder of 11 plants using FTIR spectroscopy [9]. FTIR spectroscopic analysis in the powder samples of leaf, stem and root of *Eclipta alba* and *Eclipta prostrate* [10]. The functional groups in various extracts of *Aerva lanata* using spectroscopic method [11]. Screened the bioactive group of chemicals in the dry leaf powder of *Calotropis gigantean* by FTIR analysis [12]. The elements and functional groups in the ethanol extract of whole plant of *Ichnocarpus frutescens* using FTIR spectroscopic method [13]. 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 analysis the functional groups of phyto active 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 [14].

2. Materials and Method

2.1. Collection of Plant material

Healthy plants of *Eupatorium triplinerve* Vahl were collected from State Forestry Research Institute Kolapakkam, Kanchipuram District, Tamil Nadu. Deposited in the Herbarium of Department of Biotechnology, Periyar University, Salem (PU/BT/*Eupatorium triplinerve* Vahl / Voucher specimen No: 012/2010).

2.2. Plant sample extraction

2 g of air dried powder of leaf sample was extracted with 50 ml of solvents such as methanol with gentle stirring for 72 h. The sample was kept in dark for 72 h with intermittent shaking. After incubation the solution was filtered through Whatmann No. 1 filter paper and the filtrate was collected (crude extracts). It was then transferred to glass vials and kept at 4 °C before use.

2.3. GC-MS analysis

10 g of powdered leaf sample is soaked with 30 ml ethanol overnight and filtered through ash less filter paper with sodium sulphate (2 g). The extract is concentrated to 1 ml by bubbling nitrogen into the solution. The extract contained both polar and non-polar phyto components. 2µl of the methanolic extract of *Eupatorium triplinerve* Vahl was employed for GC-MS analysis 20. The Clarus 500 GC used in the analysis employed a fused silica column packed with Elite-1 [100% dimethyl poly siloxane, 30 nm × 0.25 nm ID × 1µm df and the components were separated using Helium as carrier gas ata constant flow of 1 ml/min. The 2µl sample extract injected into the instrument was detected by the Turbo gold mass detector (Perkin Elmer) with the aid of the Turbo mass 5.1 software. During the 36th minute GC extraction process, the oven was maintained at a temperature of 110 °C with 2 minutes holding. The injector temperature was set at 250 °C (mass analyser). The different parameters involved in the operation of the Clarus 500 MS, were also standardized (Inlet line temperature: 200 °C; Source temperature: 200 °C). Mass spectra were taken at 70 eV; a scan interval of 0.5 s and fragments from 45 to 450 Da. The MS detection was completed in 36 minutes.

2.4. Identification of components

Interpretation on mass spectrum GC-MS was conducted using the database of National Institute Standard and Technology (NIST) having more than 62,000 patterns. The spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST library. The name, molecular weight and structure of the components of the test materials were ascertained.

2.5 Fourier Transform Infrared Spectrophotometer (FT-IR)

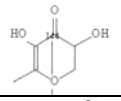
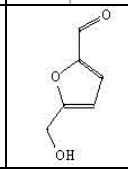
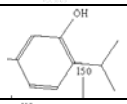
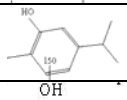
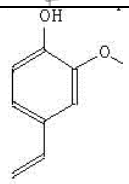
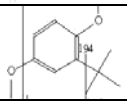
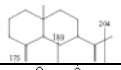
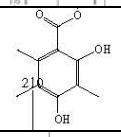
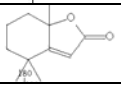
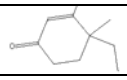
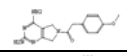
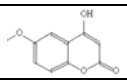
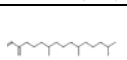
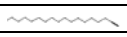
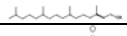
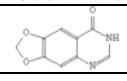
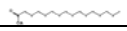
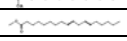
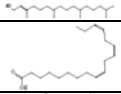
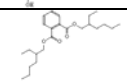
Fourier Transform Infrared Spectrophotometer (FTIR) is perhaps the most powerful tool for identifying the types of chemical bonds (functional groups) present in compounds. The wavelength of light absorbed is characteristic of the chemical bond as can be seen in the annotated spectrum. By interpreting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

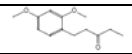
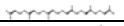
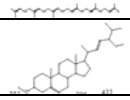
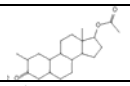
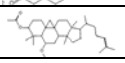
Dried powder of different solvent extracts of each plant materials were used for FTIR analysis. 10 mg of the dried extract powder was encapsulated in 100 mg of KBr pellet, in order to prepare translucent sample discs. The powdered sample of each plant specimen was loaded in FTIR spectroscope (Shimadzu, IR Affinity 1, Japan), with a Scan range from400 to 4000cm^{-1} with a resolution of 4 cm^{-1}.

3. Results and Discussion

The identified compounds of the leaves of *Eupatorium triplinerve*, their retention indices, percentage composition, chemical structure and activities are given in (Table 1). The compound prediction is based on Dr. Duke's phytochemical and Ethno botanical Databases. The results showed the presence of major compounds 9,12,15-Octadecatrienoic acid, (Z,Z,Z)-(20.18), n-Hexadecanoicacid-(13.73), 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl (8.36),2-methoxy-4-ethyl-6-methylphenol(7.22). The spectrum profile of GCMS confirmed the presence of major components with retention time (19.240, 17.435, 7.324, 13.087 (Fig.1). The individual fragmentation of the components The FT-IR spectrum of *E.triplinerve* plant leaf extract as shown in the fig. From the figure we observed different functional groups peaks with different intensity. The broad peak observed at 3366 cm^{-1} assigned to O-H of alcohols and phenol groups. The medium peak observed at 2945 cm^{-1} attributed to C-H stretching of alkanes groups. The small peaks observed at 1647 and 1452 cm^{-1} correspond to C=O group and CH_3 bending of alkene and ketones groups. The strong peak observed at 1026 cm^{-1} assigned to C-H stretching of alkenes.

Table 1: Phytocomponents identified in Methanolic leaf extract of *Eupatorium triplinerve* Vahl using GC-MS

R. Time	Name of the compound	Molecular formula	Molecular weight	Peak Area%	Structure	Activity
6.194	1-(2-FURANYL)-2-HYDROXYETHANONE	$C_6H_6O_3$	126	2.61		Antioxidant activity
7.324	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl	$C_6H_8O_4$	144	8.36		Antimicrobial,Anti inflammatory
8.925	5-Hydroxymethylfurfural	$C_6H_6O^3$	126	6.38		Antioxidant, Ant proliferative
9.391	PHENOL, 5-METHYL-2-(1-METHYLETHYL)	$C_{10}H_{14}O_2$	150	0.52		antibacterial *activity, Antioxidant*
9.546	PHENOL, 2-METHYL-5-(1-METHYLETHYL)	$C_{10}H_{14}O_4$	150	0.36		antimicrobial and antifungal *activity*
9.734	2-Methoxy-4-vinylphenol	$C_9H_{10}O_2$	150	0.33		Antioxidant Antimicrobial Anti-inflammatory
11.105	Benzene, 2-(1,1-dimethy lethyl)-1,4-dimethoxy	$C_{12}H_{18}O_2$	194	5.88		Anti-inflammatory, Analgesic activity
12.063	7-ISOPROPENYL-4A-METHYL-1-METHYLENEDECAHy	$C_{15}H_{24}O_2$	204	4.84		Antifungal activity
12.309	Benzoic acid, 2,4-dihydroxy-3,5,6-trimethyl-, methyl ester	$C_{11}H_{14}O_4$	210	0.70		structure-*activity*, ant oxidative *activity*
12.742	2(4H)-Benzofuranone, 5,6,7,7a-tetrahydro-4,4,7	$C_{11}H_{16}O_2$	180	0.08		anti-diarrhoeal *activity*, cancer cell lines
13.087	2-METHOXY-4-ETHYL-6-METHYLPHENOL	$C_{10}H_{14}O_2$	166	7.22		Antifungal *activity*
13.315	2-CYCLOHEXEN-1-ONE, 4-ETHYL-3,4-DIMETHYL	$C_{10}H_{16}O$	152	0.41		Anticancer antioxidant *activity*
14.084	6-p-Methoxyphenylacetyl-2,4-diamino-6,7	$C_{15}H_{17}N_5O_2$	299	2.34		potentiating *activity*
15.493	4-Hydroxy-6-methoxycoumarin	$C_{10}H_8O_4$	192	0.43		Antibiotic, anti H. pylori *activity*
15.890	2,6,10-TRIMETHYL,14-ETHYLENE-14-PENTADECNE	$C_{20}H_{38}$	278	1.13		Anticancer antibacterial, antifungal
16.135	1-Octadecyne	$C_{18}H_{34}$	250	0.25		Catalytic polymerization,
16.329	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	$C_{2O}H_{40}O$	296	0.71		antioxidananti-inflammatory t
16.648	6,7-Methylenedioxy-4(3H)-quinazolone	$C_9H_6N_2O_3$	190	4.77		Anticonvulsant, protozoan
17.435	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	13.73		Antibacterial
18.437	9,12-Octadecadienoic acid, methyl ester,	$C_{19}H_{34}O_2$	294	0.12		Antimicrobial
18.512	9,12,15-Octadecatrienoic acid, methyl ester,	$C_{19}H_{32}O_2$	292	0.16		Antifungal, Cancer
18.622	Phytol	$C_{20}H_{40}O$	296	0.19		Anti-proliferativ antioxidant, cancer.
19.240	9,12,15-Octadecatrienoic acid,	$C_{18}H_{30}O_2$	278	20.18		Antifungal
23.128	1,2-BENZENEDICARBOXYLIC ACID	$C_{24}H_{38}O_4$	390	1.11		Antimicrobial

24.878	1-(2,4-Dimethoxy-phenyl)-pentan-3-one	$C_{13}H_{18}O_3$	222	0.29		anti-Candida *activity* – resorbing
27.068	Squalene	$C_{30}H_5O$	410	0.17		Antioxidant, cytotoxic anti-tumor
34.033	Stigmasta-5,22-diene, 3-methoxy-, (3.beta.,22E)	$C_{30}H_{50}O$	426	0.12		spectrometric data, antimicrobial
37.448	19-D-TORULOSOL	$C_{2O}H_{33}DO_2$	307	0.05		Antioxidant antibacterial
38.953	Estran-3-one, 17-(acetyloxy)-2-methyl-, (2.alpha.,5.alpha	C21H32O3	332	0.05		Progestational,dehydrogenase
40.548	3-O-Acetyl-6-methoxy-cycloartenol	$C_{33}H_{54}O_3$	498	0.79		anti-inflammatory, anticonvulant,

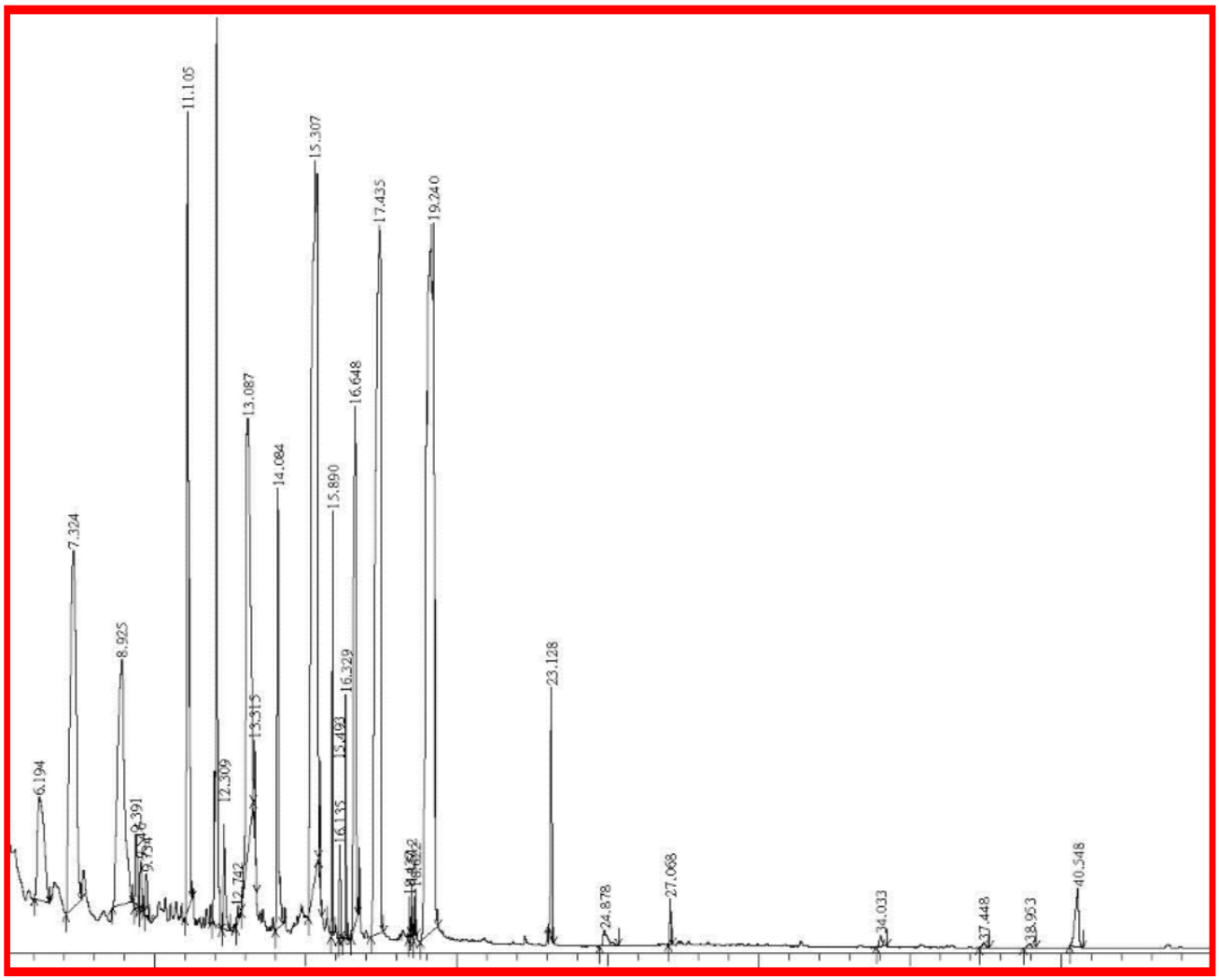


Fig 1: GC-MS Analysis of Phytocomponents in the Methanolic Extract of *Eupatorium triplinerve* Vahl

In the present study the GC-MS analysis of the methanolic extract of *Eupatorium triplinerve* showed (Fig.2) the presence of compounds. 9, 12, 15-Octadecatrienoic acid, (Z, Z, Z)-(20.18), n-Hexadecanoicacid-(13.73), 4H-Pyran-4-one, 2, 3-dihydro-3, 5-dihydroxy-6-methyl (8.36), 2-methoxy-4-ethyl-6-methylphenol (7.22) are predominant in the extracts. The four major compounds activity viz. (Antifungal, Antibacterial, Antimicrobial and Anti- inflammatory Activity). There is a growing awareness in correlating the phytochemical components and their biological activities [15,16]. *Eupatorium triplinerve* is a plant used in Ayurvedic medicine however there are no reports on the thorough phytochemical analysis of the plant. We report the presence of some of the important components resolved by GC-MS analysis and their biological activities. Thus this type of GC-MS analysis is the first step towards understanding the nature of active principles in this medicinal plant and this type of study will be helpful for further detailed study.

The GC-MS analysis of the methanolic extract of *Eupatorium triplinerve* showed the presence of ten compounds. In terms of percentage amounts hexadecanoic acid, tetradecanoic acid and octadecanoic acid were predominant in the extract. These three major compounds have all shown to have hypocholesterolemic activity, antioxidant and lubricating activity. Anticancer and ant proliferative are shown by tetradecanoic acid and 2, 6, 10,-trimethyl, 14-ethylen-14-pentadecne, while 1-hexyl-1-nitrocyclohexane and 1,14-tetradecanediol other compounds show antimicrobial and anti-inflammatory activities [17].

Fig 2: FT-IR spectrum of methonol leaf extract of *Eupatorium triplinerve* Vahl

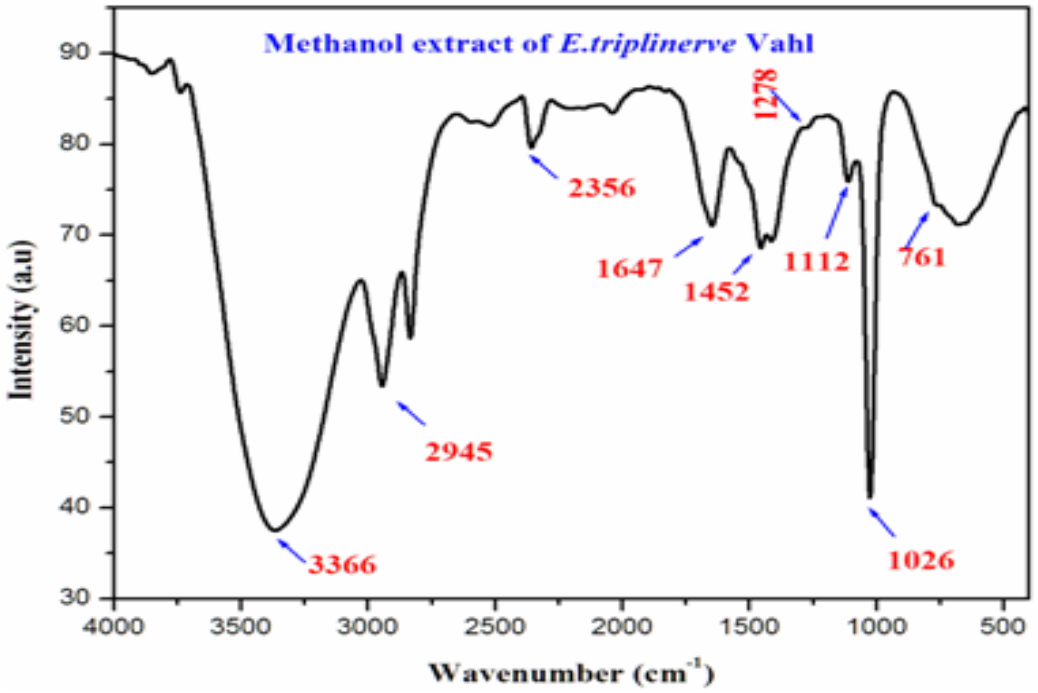


Table 2: FTIR peaks values and functional groups of Methanolic Extract of *Eupatorium triplinerve Vahl*

Extracts	Peak value	Types of stretching	Functional group
Methanol	761	C-H-bending	Alkenes
	1026	C-H- Stretching	Alkenes
	1112	C=S group	Thio
	1278	C- N group	Amines/Amides
	1452	CH_3 group	Alkane
	1647	C=O group	Ketones
	2356	C=N group	Nitriles,Alkynes
	2945	C-H Stretching	Alkanes
	3366	(OH group)	Alcohols, phenol,Amines

The FTIR and EDS spectral analysis of plant parts like leaf, stem, and root of the medicinal plants, *Eclipta alba* and *Eclipta* prostrate 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*. 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*. 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. Analyzed the methanolic leaf extract of *Ampelocissus latifolia* by FTIR and reported that the transition metal carbonyl compounds and aliphatic fluor compounds were only present in the extract. 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 has been already demonstrated.

4. Conclusion

The bioactive compounds and in the methanolic extract of *Eupatorium triplinerve* Vahl which have been screened using this analysis. Isolation of individual components would however help to new drugs.

5. Reference

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