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FTIR and GC-MS spectral analysis of *Cucumis dipsaceous* Ex. Spach. Ehreb leaves

V Anusuba and V Priya

Abstract
The present study is aimed to identify the functional groups and phytoconstituents present in *Cucumis dipsaceous* leaf through FTIR and GC-MS spectroscopy. FTIR method was performed by using Perkin Elmer Spectrophotometer and the characteristic peaks were detected. The phytochemical constituents screened by GC-MS method and the compound detection employed. The FTIR spectroscopic studies revealed the presence of alcohols, phenols, alkanes, alkynes, alkyl halides, aldehydes, carboxylic acids, aromatics, nitro compounds and amines. The results of the GC-MS analysis provide different peaks determining the presence of 25 phytochemical compounds in the extract. The major phytoconstituents peak values are (49.648-Androstane-11,17-dione, 50.585-Squalene, 39.484-phytol) The results of the present study generated the FTIR and GC-MS spectrum profile for the medicinally important plant *Cucumis dipsaceous* leaf extract having various bioactive compounds.

Keywords: *Cucumis dipsaceous*, methanolic leaf extract, Phytoconstituents FTIR, GC-MS spectrum analysis.

1. Introduction
Cucumis dipsaceous is a deciduous large sized bush or shrub, commonly growing to about tall and much branched. It is commonly called "Hedgehog cucumber" hence the present investigation was aimed to identify the functional groups present in crude powder and phyto components present in ethanol extract of *Cucumis dipsaceous* leaf with the aid of FT-IR and GC-MS analytical techniques, which may provide an insight in its use of traditional medicine.

Fig 1: Morphology of *Cucumis dipsaceous* Ex. Spach. Ehreb.

Phytochemicals are bioactive substances of plants that have been associated in the protection of human health against chronic degenerative diseases (Fukumoto and Mazza, 2000) which do not act alone but most of the time it is in a combination of complexes (Cowan, 1999). FT-IR Spectroscopy has demonstrated to be a reliable and sensitive method for finding out the functional groups present in plant samples were determined with the help of IR region in the range of 400-4000cm-1. For most common plant compounds, the spectrum of an unknown compound can be identified by comparison to a library of known compounds. Gas Chromatography Mass Spectroscopy is a very compatible and one of the best methods to identify the pure compounds present at less than 1ng biological specimen and quantification purpose. The unknown organic compounds in a complex mixture can be determined by interpretation and also by matching the spectra with reference spectra. Within a decade there were a number of dramatic advances in analytical techniques, including FT-IR and GC-MS that were powerful tools for identification and determination of phytochemical is of increasing interest among researchers.

2. Materials and methods

Collection and processing of plant material

The plant was collected in Kallipalayam, Coimbatore, Tamilnadu, India and identified (Gamble and Fischer, 1935). The healthy and mature leaves were freshly collected and thoroughly washed with distilled water and kept in shade at room temperature for about two weeks to dry. They were made into powder with the help of a mechanical grinder and sieved. Dried and powdered samples were soxhlet extracted with ethanol until the solvent was colorless. The extracts were filtered and concentrated under reduced pressure in a rotary evaporator. The dried extracts were kept in the refrigerator at 4 ^{0}C until use. Fourier Transform Infrared Spectroscopic Analysis (FT-IR) Oven-dried leaf samples (60 ˚C) were ground into fine powder using a mortar and pestle. Two milligrams of the sample was mixed with 100 mg KBr (FT-IR grade) and then compressed to prepare a salt-disc (3 mm diameter). The disc was immediately kept in the sample holder and FT-IR spectra were recorded in the absorption range between 400 and 4000 cm-1. All investigations were carried out with a Shimadzu FT-IR spectrometer. Gas Chromatography-Mass Spectrometry analysis (GC-MS) is usually done to identify the compounds present in the plant extract thereby the medicinal uses are found which is widely used in the pharmacology for producing drugs to various diseases.

Identification of functional groups

The FTIR spectrum was used to identify the functional groups of the active components present in plant sample based on the peaks values in the region of IR radiation. When the plant extract was passed into FTIR, the functional groups of the components were separated based on its peaks ratio.

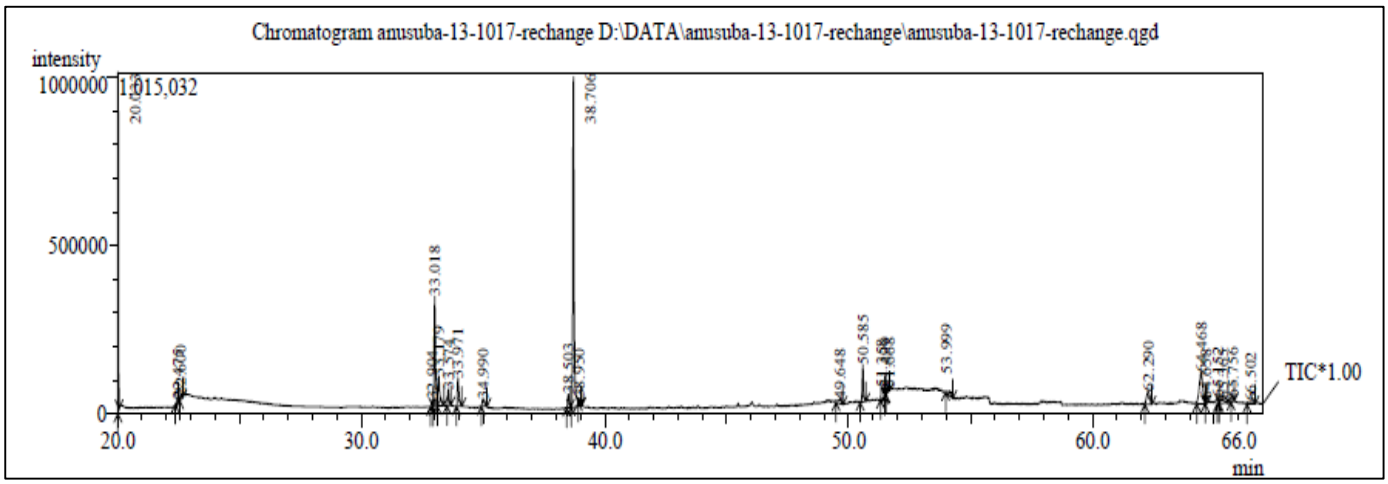
Identification of components

Interpretation of mass spectrum obtained from GC-MS was conducted using the database of National Institute Standard and Technology (NIST) having more than 82,000 patterns. The spectrum of the unknown component was compared with the spectra of the known components stored in the NIST library. The name, molecular weight, molecular formula and structure of the components of the test materials were ascertained.

3. Results

Gas Chromatography-Mass Spectrometry analysis

(GC-MS) is one of the best methods to identify the bioactive compounds of non-polar components and volatile essential oil, fatty acids and lipids. Fifty compounds were identified from the ethanolic extract of *Cucumis dipsaceous* leaves. The identification of the phytochemical compounds was confirmed based on the peak area, retention time and molecular formula were presented.



Graphical representation 1: GCMS *analysis* in *Cucumis dipsaceous.* Ex. Spach. Ehreb

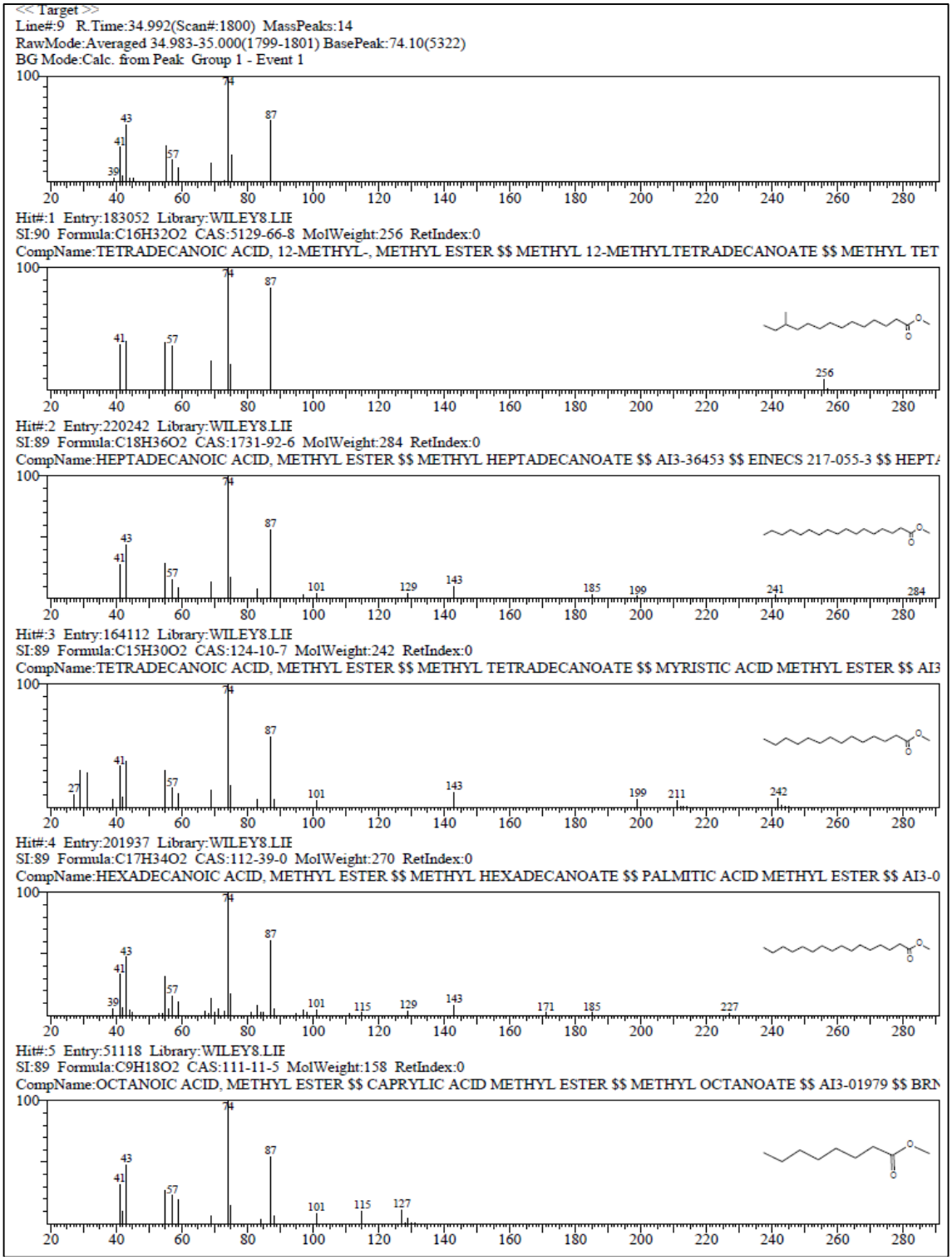
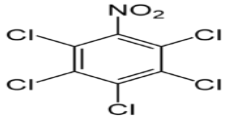
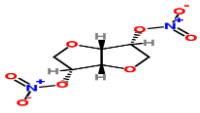
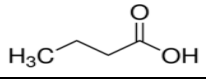
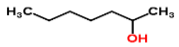
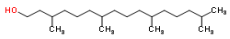
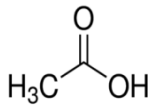
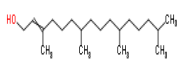
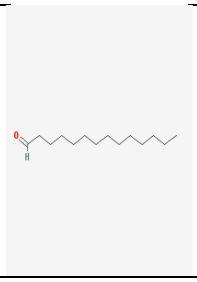
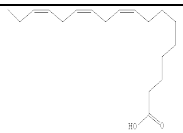
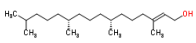
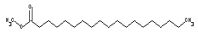
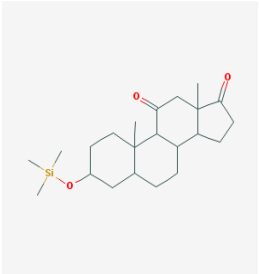
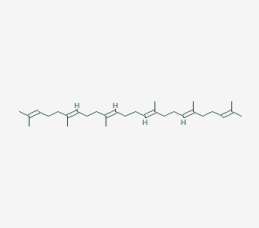
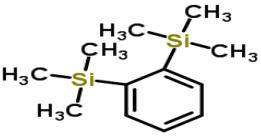
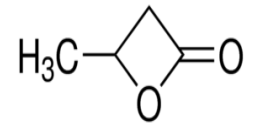
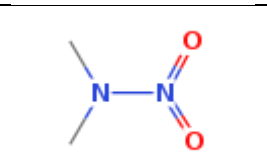
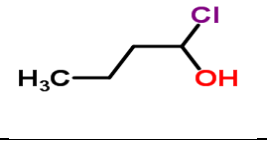
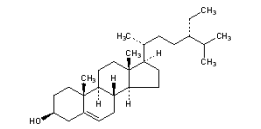
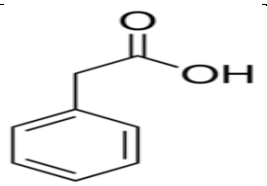
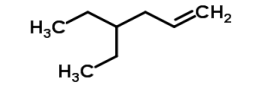
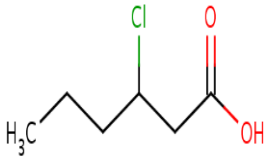
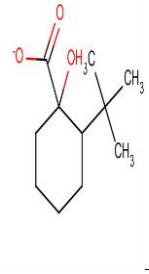
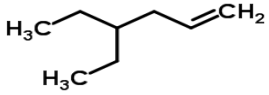


Table 1: GC-MS analysis for the *Cucumis dipsaceous*

S.NO	R.TIME	F.TIME	M.STRUCTURE	M. WGHT g/mol	M.NAME	MEDICINAL USES
1	20.332	20.017		78.114	Benzene penta nitrochoro	Pesticides designed to control insects that are harmful to man. The insects may be directly harmful, as those acting as disease vectors, or indirectly harmful, as destroyers of crops, food products, or textile fabrics.
2	22.475	22.375		675.048	diannhydromannitol)	Anticancer drugs which is tested in murines.
3	22.600	22.550		88.1051	Butanoic acid(2,methylpropanol)	It has anti carcinogenic activity. It is used as a flavor in jams, perfumes etc.

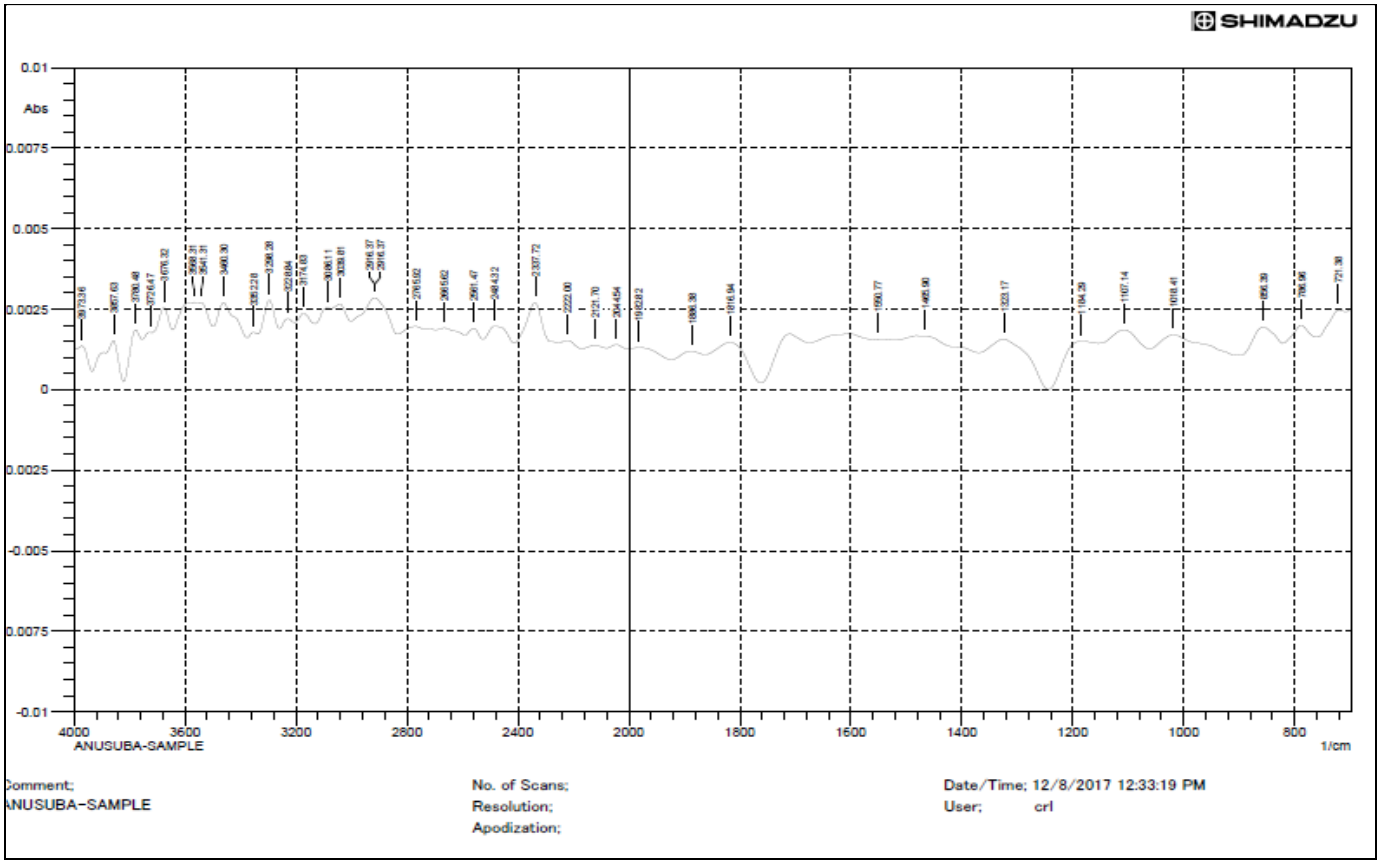
4	32.904	32.842		116.88	1-Heptanol	Heptanol also reduced left-ventricular developed pressure (LVDP), and the maximum rates of contraction and relaxation of the left ventricle; these effects were concentration dependent and reversible.
5	33.018	32.950		240.431	Hexadecen 1 ol (3,7,11,15)	Antimicrobial activity, good and natural anti-oxidant property.
6	33.179	33.125		114.023	Acetic acid, trifluoro-,	Found to be effective in preventing cataract development in severely galactosemic rats when administered as an eyedrop solution.
7	33.574	33.517		280.54	dimethyloct3,7,11,15-Tetramethyl-2-hexadecen	Antidiabetic, anti-inflammatory properties is present.
8	33.971	34.908		212.377	Tetradecanal	It is used in cosmetics such as cold creams for its emollient properties. It is also used as an intermediate in the chemical synthesis of other products such as sulphated groups.
9	34.990	38.433		228.3709	Tetradecanoic Acid,	It is used in cosmetics such as cold creams for its emollient properties. It is also used as an intermediate in the chemical synthesis of other products such as sulphated groups.
10	38.503	38.625		278.436	9,12,15-Octadecatrienoic acid	Used as an surface adhesives. Agricultural organic manures for non pesticidal items.
11	38.706	38.908		296.53	Phytol	It has antioxidant activities. It is used for preparing tablets of vitamin E.It is also showed the modulate transcription in cells via, transcription factors of PPAR alpha and retinoid x receptor (RXR)
12	38.950	49.483		298.555	Non adecanoic Acid, Methyl	It is certain insects as pheromones.

13	49.648	50.475		290.447	Androstane-11,17-dione, 3	Androsterone is a steroid metabolite derived from sex hormones, which displays weak androgenic properties. In testes is formed from progesterone Androsterone sulfate is clinically recognized as one of the major androgen metabolites found in urine, in males and females. It is made in the liver from the metabolism of testosterone. Androsterone glucuronide, a dihydrotestosterone metabolite, is present in serum Androsterone is a weak anabolic hormone.
14	50.585	51.308		410.73	Squalene	Supplementation of squalene to mice has resulted in marked increases in cellular and non-specific immune functions in a dose-dependent manner. Squalene may also act as a "sink" for highly lipophilic xenobiotics. It can be used toreduce the cholesterol and triglycerides in humans.
15	51.358	51.483		222.478	1,2-Bis(trimethylsilyl)benzene	Medical uses of the compounds, pharmaceutically acceptable salts, hydrates and the pharmaceutical composition for treating the TRPV1-mediate diseases.
16	51.499	51.533		86.09	beta.butyrolactone	β-Butyrolactone was used in the preparation of (3-O -[oligo-(3-hydroxybutyrate ester)] fluorescein, fluorescein derivative of poly(3-hydroxybutyrate) via anionic polymerization
17	51.688	53.983		31.05	methanamine, n-methyl-n-nit 2--	NCI In Vivo Anticancer Drug Screen. Data for tumor model L1210 Leukemia (intraperitoneal) in B6D2F1 (BDF1) mice
18	53.999	62.158		108.565	butanol, 1-chloro	Toxicity in Tetrahymena pyriformis
19	62.290	64.283		415.7067	2H-Pyran, 2-(7-heptadecynyloxy)tetrah y dral	Not reported.
20	64.468	64.633		414.7067	gamma.-Sitosterol	Anti diabetic ,hepatoprotectant.
21	64.658	65.133		122.123	benzeneacetic acid	Antimicrobial, antitumor, antifungal properties it is having.
22	65.152	65.217		112.213	4-hydroxy-4-ethylhex-1-ene	Analgesic, antipyretic

23	65.365	65.692		201.562	Chlorohexanoic acid, 3-fluoropheny,	Not reported
24	65.765	66.330		100.158	cyclohexanol, formate	It is used as a flavouring agents in foods.
25	66.502	66.335		246.076	4-hydroxy-4-ethylhex1ene	Not reported.

FTIR Spectrum for the *Cucumis dipsaceous*

The results of FT-IR spectroscopic analysis revealed the presence of alcohols, phenols, alkanes, alkynes, alkyl halides, aldehydes, carboxylic acids, aromatics, nitro compounds and amines). The absorption at 3201cm-1 is due to the OH stretching of Normal "polymeric" group that present in the extract. The band at 2925cm-1 is due to C-H stretching of Methylene asym./sym.; the band at 1666cm-1 showed Alkenyl C=C stretch;band at 1402cm-1 showed Phenol or tertiary alcohol, OH bend; the band at 1101cm-1 showed Aromatic C-H in plane bend; the band at 811cm-1 showed 1,4- Disubstitution (Para); the band at 649cm-1 showed Alkyne C-H bend; the band at 605cm-1 showed Aliphatic bromo compounds; the band at 464cm-1 showed Aryl disulfides (S-S stretch)



Graphical representation 2: FTIR spectral analysis of *Cucumis dipsaceous*. Ex. Spach. Ehreb.

Table 2: FTIR Spectral analysis

Peak No.	Group frequency (cm^{-1})	Origin	Functional groups
1	3201.61	O-H	Normal " polymeric" OH stretch
2	2925.81	C-H	Methylene C-H asym./sym. stretch
3	2362.64		Unknown
4	1666.38	C=C	Alkenyl C=C stretch
5	1402.15	O-H	Phenol or tertiary alcohol, OH bend
6	1101.28	C-H	Aromatic C-H in plane bend
7	811. 98	C-H	1,4- Disubstitution (Para)
8	649.97	C-H	Alkyne C-H bend
9	605.61	C-Br	Aliphatic bromo compounds
10	464.81	S-S	Aryl disulfides (S-S stretch)

4. Discussion

FTIR Spectral analysis

FT-IR spectral analysis was useful for compound identification, when run under IR region in the range of 400-4000 cm-1 there was a variation in the peaks of plant samples (Thenmozhi *et al*. 2011; Kalaiselvi *et al*. 2012). IR is used for the identification of functional groups like hydroxyl groups, amines, carboxylic acids, aromatic compounds, aliphatic bromo compounds, aryl disulfides, alkenyl groups and nitro compounds in the molecules. Such functional groups can be identified by their absorption bands (Manfred *et al*. 1997).

In the present analysis, the crude ethanolic extract of *Cucumis dipsaceous* leaf was subjected to FT-IR analysis, the functional groups of the components were separated based on its peak ratio and chemical compounds were identified. The FT-IR spectrum at 1101.28cm-1 is due to the vibration stretching for (C-H) bond of aromatic compound contains phenol, carbonyl and ether group (Silverstein and Webster, 1997). The peak at 2923.95-2926.37cm-1 assigned to the C-H stretching which means that some alkane compounds existed in rare medicinal plants (Starlin *et al*. 2012).

GC-MS analysis

GC-MS is a valuable tool for reliable identification of bioactive compounds and also can identify pure compounds present at less than 1ng in biological specimens (Liebler *et al*. 1996; Johnson *et al*. 2011). In the last few years, GC-MS has become confidently established as a key technological platform for secondary metabolites profiling in plant species (Merlin *et al*. 2009; Janakiraman *et al*. 2012). This study demonstrated the usefulness of GC-MS, not only for the determination of drugs of abuse in biological samples, for their clinical or forensic purposes, but also for physiological evaluations and development of toxicological models (Cardano *et al*. 2006; Yang *et al*. 2006; Valente *et al*. 2011).

Among the phytochemical analysis the peak values of the GC-MS analysis of the leaf extracts reveals that it has fatty acid ester and it may employed as antioxidant, antimicrobial, flavor, hypocholesterolemic agent and larvicidal activities (Falodun *et al*. 2009). 1, 2-benzenedicarboxylic acid, disoctyl ester is a plasticizer compound and acts as antimicrobial and antifouling agent. A-tocopherol and phytol has antioxidant properties and reduce the risk of prostate cancer in smokers (Heinonen *et al*. 1998).

5. Conclusion

In the present study the FTIR and GC-MS spectral analysis of *Cucumis dipsaceous* leaf extract composed of various functional groups and variety of fatty acids which are responsible for many biological activities. Thus this type of spectral analyses is the primary step towards understanding the nature of active principles in this medicinal plant which will be helpful for further detailed research.

6. Acknowledgement

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7. References

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