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Identification of Bioactive Compounds in the Leaf Extracts of *Brassica oleracea Var. Botrytis L* by FTIR and GCMS analysis

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Abstract: The present study designed to identify the functional bioactive compounds in the ethanol leaf extract of *Brassica oleracea var.botrytis L.* using FTIR and GCMS. FTIR was performed by using Thermo Scientific Nicolet Is 10 Spectrometer and the characteristic peaks were detected. The FTIR spectroscopic studies revealed the presents of phenolic compounds and alcohol, aromatic compounds, aldehyde and saturated aliphatic, alkanes, and alkenes. The phytochemical constituents screened by GC-MS method and the compound detection employed the NIST Ver.2.0 year 2005 library. The results of GCMS analysis provide different peaks determining the presence of thirty phytochemical compounds in the extracts. The results of present study generated the FTIR and GCMS spectrum profile for the medicinally important vegetable plant, *Brassica oleracea var.botrytis* leaf extract having various bioactive compounds.

Keywords: Bioactive compounds, *Brassica oleracea*, FTIR, GCMS

1. INTRODUCTION

Phytochemicals are bioactive compounds of plants that have been associated in the protection of human health. FTIR 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 of 400-4000^{cm-1}. For most common plant compounds, the spectrum of an unknown can be identified by comparison to a library of known compounds (Griffiths and Haseth, 1986).

Gas chromatography Mass Spectrometry is a very compatible and one of the best methods in identify the pure compounds present at less than 1µg 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 (Hites, 1997).

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 functional bioactive compounds which increasing interest among researchers (Roberts and Xia, 1995; Alagammal *et al*., 2011; Bharathi *et al*., 2012; Sumathi and Uthayakumari, 2014; Nithyadevi and Sivakumar, 2015).

B. oleracea L. is a small sized bush or shrub, commonly growing to about 2 to 4 feet short and much branched. It is commonly called Cauliflower, one of the widely consumed white coloured flower with green leaf vegetable has a characteristic pungent odor because of sulphated compounds. This vegetable has a strong antioxidant property that can prevent oxidative stress (Blomhoff, 2005).

Hence the present investigation was aimed to identify the functional groups present in ethanol extract of *B. oleracea* leaf with the aid of FT-IR and GC-MS analytical techniques, which may provide an insight in its use of traditional medicine.

2. MATERIALS AND METHODS

2.1 Collection methods of leaves: *B.oleracea L.* plant leaves collected from the weekly market, Muthur, Tirupur District, South Tamilnadu, India. The healthy fresh leaves collected thoroughly washed with distilled water and kept in shade at room temperature about one week to dry. Then made into powder with the help of a Pulveriser and sieved. The Dried powdered samples were Soxhlet extracted with ethanol until the solvent was colourless. The extracts were filtered and concentrated under reduced pressure in a rotary evaporator. The dried extracts were kept in the refrigerator at 4·C until use.

2.2 Fourier Transfer Infrared Spectrometry (FT-IR): *B. oleracea* ethanol leaf extract was subjected to FT-IR, Thermo Science Nicolet iS10 was conducted using the database of Aldrich Condensed Phase Sample Library and Sigma Biological Sample Library. The region carried 4000 cm-1 to 400^{cm-1}.

2.3 Identification of functional groups in FT-IR: The FTIR spectrum was used to identify the functional groups of the active components present in plant sample based on the beaks 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.

2.4 Gas Chromatography and Mass Spectrometry (GC-MS): Ethanol extract of leaves of *B. oleracea* was subjected to GC-MS analysis. Extracts were dissolved in high-performance liquid chromatography (HPLC)-grade ethanol and subjected to Thermo MS DSQ II. Helium was used as the carrier gas at a flow rate of 1.0 mL (Micro Litre)/min.

The temperature was programmed at oven temperature 70˚C raised to 260˚C at 6˚C /min 1µl of plant extract was injected with a Hamilton syringe into the GC-MS manually.

2.5 Identification of components in GCMS: Interpretation of mass spectrum obtained from GC-MS was conducted using the database of National Institute of Standard and Technology (NIST). The spectrum of the unknown component was compared with the spectra of the known components of the test materials were ascertained.

3. RESULTS

3.1 FTIR study: The results of FT-IR spectroscopic analysis revealed the presence of alcohols, phenols, alkanes, alkenes, aromatic compounds, and aldehydes (Fig-1 and Table-1).

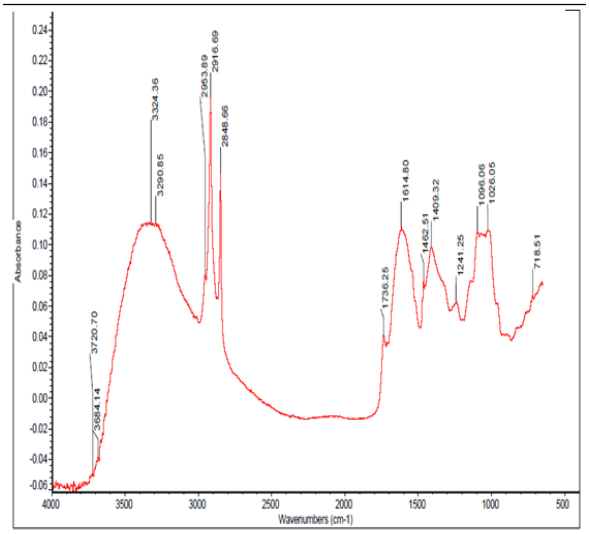

Fig. 1: FT-IR Spectrum for ethanolic extract of *B. oleracea*

The absorption at 3324.36cm^{-1}due to H-bonding O-H stretching of alcohol and phenolic compounds that present in the extract. 3290.85cm^{-1} stretching of H-bonding O-H phenol and alcohol with normal group; the bond at 2916.69cm^{-1} showed C-H alkanes stretch; the band at 2848.66cm^{-1} C-H, alkanes and the band at 1736.25cm^{-1} showed C=C, stretch aldehyde and aliphatic; 1614.80cm^{-1} is C=C, aromatic and Carboxylic groups stretch; the band 1409.32cm^{-1} is showed C-O, Acid group stretch; the band at 1096.06cm^{-1} C-H; alkanes the band at 1026.05cm^{-1} is the C-H alcohol and amines; the band at 718.51cm^{-1} is the bend, Alkenes and aromatics shows the chemical structure.

Table 1: FT-IR peak values and functional groups of *B. oleracea* leaf

Peak No.	Group frequency (cm-1)	Origin	Functional groups
1	3324.36	O-H	Phenolic compounds and Alcohol
2	3290.85	C-H	Alcohol
3	2916.69	C-H	Alkanes
4	2848.66	C-H	Alkanes
5	1736.25	C=C	Aldehyde and saturated aliphatic
6	1614.80	C=O	Aromatic and Carboxylic groups
7	1409.32	C-O	Acid group
8	1096.06	C-H	Alkanes
9	1026.05	C-O	Alcohol
10	718.51	C-H	Alkene and aromatics

3.2 Gas Chromatography- Mass Spectrometry analysis: GC-MS is one of the best methods to identify the bioactive compounds of non-polar components and fatty acids and lipids. Thirty one compounds were identified from the ethanolic extract of *B. Oleracea* leaves. The identification of the phytochemical compounds was confirmed based on the retention time, peak area, molecular weight, molecular formula were presented in Table 2 and Fig 2.

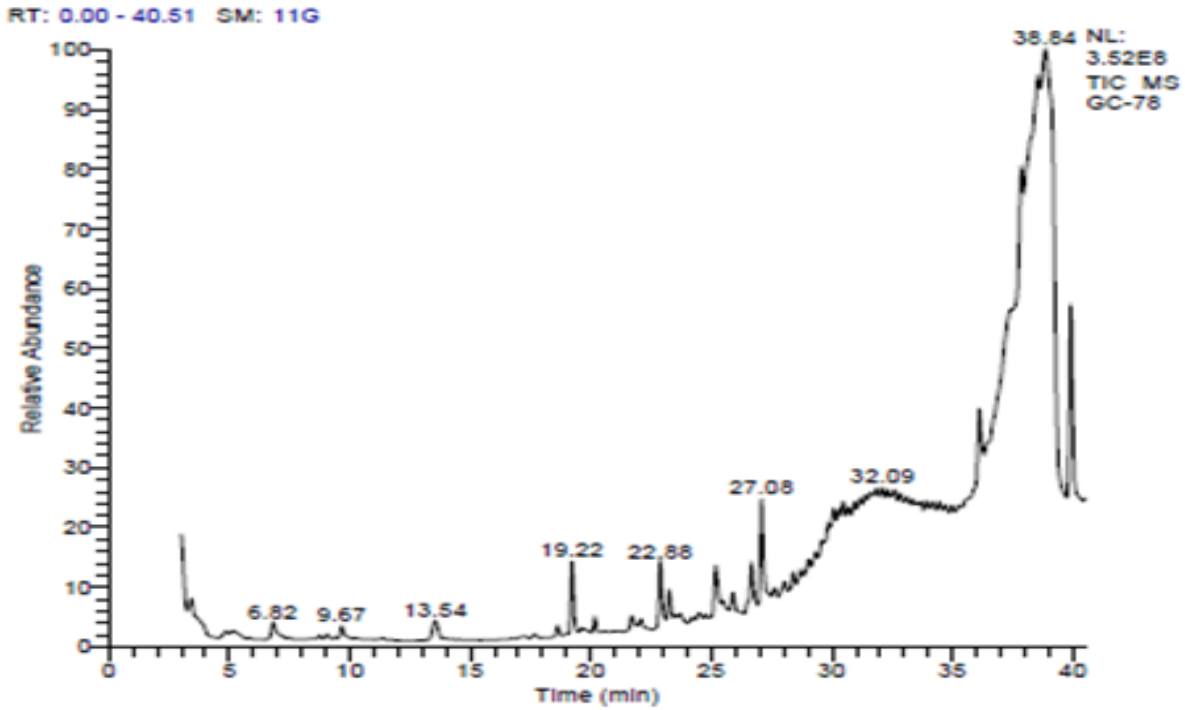

Fig. 2: GC-MS Chromatogram of ethanolic extract from *B. oleracea* leaf

The GC-MS analysis of *B. oleracea* leaf extract revealed the presence of phytochemicals represented 2,5,8-Tris (diethylamino) benzo [1,2-d:3,4-d':5,6-d''] tristhiazole (68.50%); Tetrakis (Dimethy-lsilyl-carbodiimide) (5.45%); Ethyl linoleate (2.94 %);2-Octen-I-oI (CAS) (2.1%); 2-(2,4,6-trimethylphenyl)pyridine (2.09%); Docosane (1.96%); Hexadeconic acid, ethyl ester (1.92%); 1-[Bis(methylthio)methylene]-2-(4-(4-methoxyphenyl)-1,3-butadienyl) cyclo-prophene (1.79%); 5-amino-3H-pyrrole (1.68%); Ethyl linoleate (1.47%); c-Linolenic acid, methyl ester (1.05%).

Table 2: Phytoconstituents identified in the ethanolic leaf extract of *B. oleracea* by GC-MS analysis

Compound name	RT	Area %	M. W	M. Formula
2-Octen-1-o1 (CAS)	25.17	2.10	128	$C_8H_{16}O$
Bicyclo [6,1,0]nonane, 9-(-methylethylidene)-	25.90	0.49	164	$C_{12}H_{20}$
1-Dodecene	6.82	0.72	168	$C_{12}H_{24}$
2-(4'=Chlorophenyl)-1-azacyclopent-1-ene	4.87	0.31	179	$C_{10}H_{101}N$
Methyl 2-Formylfuran-5-ethonate	3.44	0.53	180	$C_9H_8O_4$
2-(2,4,6-trimethylphenyl)pyridine	30.06	2.09	197	$C_{14}H_{15}N$
1-Tetradecanol (CAS)	9.67	0.38	214	$C_{14}H_{30}O$
(IR,2R,4S,5S)-2-(tert-Butyldimethylsilyloxy)-4-hydroxy-8-ox abicyclo[3,2,1] oct-6-en-3-one	31.88	0.34	270	$C_{13}H_{22}O_4Si$
Hexadecanoic acid, methyl ester (CAS)	21.68	0.47	270	$C_{17}H_{34}O_2$
Methyl	3.87	0.38	282	$C_{15}H_{22}O_5$
Hexadeconic acid, ethyl ester	22.38	1.92	284	$C_{18}H_{36}O_2$
3-(2-hydroxyanilino)thiochromone –oxide	5.16	0.47	285	$C_{15}H_{11}NO_3S$
c-Linolenic acid, methyl ester	23.25	1.05	292	$C_{19}H_{32}O_2$
Ethyl linoleate	26.67	1.47	308	$C_{20}H_{36}O_2$
Ethyl linoleate	27.08	2.94	308	$C_{20}H_{36}O_2$
Docosane	36.11	1.96	310	$C_{22}H_{46}$
1-[[Bis(methylthio)methylene]-2-(4-(4-methoxyphenyl)-1,3-butadienyl)cycloprophene	19.22	1.79	346	$C_{19}H_{22}O_2S_2$
2,6-Dibromo-4-[(tert-butyl)azo]-N-isopropylaniline	31.60	0.57	375	$C_{13}H_{19}Br_2N_3$
Dimethyl 4-amino-5-oxo-5H-dibenzo[c,f]-2H-chromen-2-3-dicarboxylate	30.46	0.85	377	$C_{21}H_{15}NO_6$
4,4'-Isopropylidene-(2-Cyclohexylphenol)	29.57	0.30	392	$C_{27}H_{36}O_2$
Tetrakis (Dimethylsilylcarbodiimide)	39.90	5.45	392	$C_{12}H_{24}N_8Si_4$
2-(4-Methoxyphenyl)-6-methyl-4-(phenylselanyl)[1,8] naphthyridine	13.54	0.84	406	$C_{22}H_{18}N_2OSe$
4-N-(3'-Iodophenyl)amino-6,7-dimethoxyquinazoline	20.15	0.34	407	$C_{16}H_{141}N_3O_2$
8-Chlorobicyclo[5.1.0]ocy-1(8)-ene trap with Diphenylisobenofuran	28.02	0.38	412	$C_{28}H_{25}C_1O$
2-(3-Bromophynyl)-3,3-diphenyl-4-ethoxycarbonyl-5-amino-3H-pyrrole	37.29	1.68	460	$C_{25}H_{21}BrN_2$
2,5,8-Tris(diethylamino)benzo[1,2-d:3,4-d':5,6-d"]tristhiazole	38.86	68.50	462	$C_{21}H_{30}N_6S_3$
Methyl 2-[2-(N-(4-methylphenylsulfonyl)-4-(cyclohexyl)thiazol-5-carboxylate	24.47	0.35	470	$C_{24}H_{26}N_2O_4S_2$
3a-chloro-4,4-dimethyl-3a-nitro-5a-cholestane	22.05	0.30	479	$C_{29}H_{50}C_1NO_2$
1,7-dibromo-11,12-bis(methoxycarbonyl)-4,4-dimethyl-3,5,9-trioxa-4-silatricyclo[5,3,2,0(2,8)]dodec-11-ene hydroisoquinolin -5-yl)naphthalene	30.91	0.37	484	$C_{12}H_{18}Br_2O_7$
Methy-1-4a)-5,5-dimethyl-3a-hydroxy-11-oxa-2-oxotricyclo(7,2,1,0)dodecane-1a- carboxylate	3.87	0.38	282	$C_{15}H_{22}O_5$
(1R,3R,5P) and (1R,3R,5M)-Hydroxymethyl-5'-Isopropoxy-4'methoxcy-1'-(N-benzyl-6-hydroxy-8-Isopropoxy-1,3-dimethyl-1,2,3,4-tetra hydroisoquiolin-5-yl)naphthalene	23.68	0.67	569	$C_{36}H_{43}NO_5$

4. DISCUSSION

FT-IR spectral analysis is 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, amides, and aromatic compounds in the molecules. Such functional groups can identified by their absorption bands (Manfred et al., 1997). In the present analysis, the crude ethanolic extract of *B. oleracea* leaf was subjected to FT-IR analysis, the functional group of the components were separated based on its peak ratio (3324.36 $^{cm-1}$ to 718.51 $^{cm-1}$) and chemical compounds were identified. It can be confirmed that presence of alcohols, phenolic compounds, carboxylic acids, aromatics, alkanes, alkenes, aldehydes, and amides were noticed.

GC-MS is a valuable tool for reliable identification of bioactive compounds and also can identify pure compounds present at less than 1ml in biological specimens (Liebler *et al*., 1996; Johnson *et al*., 2011). In 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). In the present work, thirty compounds were isolated from the ethanolic extract of *B. oleracea* leaf. According to Duke's ethabotanical and phytochemistry database (Duke's, 1998) the identified compounds possess many biological properties.

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