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GC-MS and FTIR screening of ethanol extract of fruits of *Rivina humilis* L

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Abstract
The present study was aimed at identifying the phytocomponents and functional groups present in fruit of *Rivina humilis* using Gas chromatography-Mass Spectroscopy (GC-MS) and Fourier Transform Infrared Spectroscopy (FT-IR). GC-MS analysis showed 20 phytochemical compounds such as 1-P-Methen-8-Ylacetate, Henicosylformate, Citronella, Hexadecanoic acid, methyl ester (CAS, Neryl propionate, Hexadecanoic acid, -d-Nerolidol, Octadec-9-Enoic acid, Ocadecanoic acid, Zonarone 1-Eicosanol etc. The result of the FTIR spectroscopic studies revealed the presence of 18 functional groups such as Hydroxy groups, Alcohol, phenol, Alkanes, Alkene, Amino acid, Nitro, aromatic, aliphatic amines, Primary/secondary amines, Alkyl halide, halogen etc. *Rivinahumilis* fruit contains various bioactive compounds which have are used to cure various diseases.

Keywords: *Rivina humilis*, GC-MS, FTIR, phytocomponents, functional groups, hexadecanoic

Introduction

Plants are one of most important sources of medicines and most of the modern drugs used today are derived plant products [1]. Plant and products are commonly used element in the practice of ethno medicine this is depend on the reality that plants and their products are easily available, cheap and effective [2]. Plants have been ad significant source of medicines with qualifies for thousands of years. Plants are used medicinally in various countries and they are the source of many potent and powerful drugs. Mainly on traditional remedies such as herbs for their history, they have been used as popular folk medicines [3]. Phytochemicals are the chemicals extracted from plants. These organic chemicals are classified as primary or secondary constituents, hinging on their role in plant metabolism. Primary constituents include the common sugars, amino acids, chlorophyll etc. Secondary constituents are the remaining plant chemicals such as alkaloids (derived from amino acids), terpenes (a group of lipids) and phenolics (derived from carbohydrate) [4]. Today a number of chemicals obtained from plants are used as essential drugs in more countries in the world [5]. Secondary metabolites from plants are referred to as phytochemicals which are naturally occurring and biologically active compounds that have the potential to prevent diseases. Estimation of the phytochemical constituents of medicinal plants is considered to be the main step in medicinal plants research [6]. Phytochemicals which are the requisite antibacterial activities are reported for the treatment of bacterial infections [7]. GC/MS can also be used in airport security to detect substances in luggage or on human beings. GC/MS is a combination of two different analytic techniques. Gas Chromatography (GC) and Mass Spectrometry (MS), used to analyze biochemical and organic samples. GC can separate semi-volatile and volatile compounds present in sample with great resolution, but it cannot identify them. Application of GC-MS is to monitor and clean environment, security, food, beverage and perfume analysis [8]. GC/MS plays an essential role in the phytochemical analysis and chemotaxonomic studies of medicinal plants containing biologically active compound [9]. In recent years GC/MS studies have been increasingly applied for the analysis of medicinal plants as this technique has proved to be a useful method for the analysis of non - polar compounds and volatile essential oil, fatty acids, lipids and alkaloids [10]. Gas chromatography separates the constituents by mass spectrometry which helps to determine the molecular weight of these compounds [11]. The GC also separates volatile components in a sample while MS fragments the compounds and identifies them on the basis of their mass. Separation of GC is based on their boiling point where the substances with maximum boiling pint come out later and those with minimum point come out first. In GC/MS analysis the percent area represents the percentage wise amount of the respective compounds [11]. Due to its simplicity of operation, speed, versality and reproducibility, as a number of samples can be analysed simultaneously on a single run using only a small amount of sample [12, 13]. FTIR is a technique to identify their functional groups in organic molecules depending on their vibrating modes at different wave numbers. The bands correspond to polysacharide, polyphosphate groups; carbohydrate functional groups can be identified. The characteristic of chemical bond can be analyzed by wavelength of light absorbed by interpreting the infrared absorption spectrum, the chemical molecule can be identified [14]. Infrared spectroscopy is a standard method of analytical pharmacy and chemistry which provides the images of vibration of atoms of compound. Therefore, it is also referred to as vibrational spectroscopy. IR spectrum is obtained by passing infrared radiation through the testsample and determining the amount of fraction of the incident radiationis absorbed at a particular frequency. Jean Fourier demonstrated Fourier transformation which is a mathematical operation, converts the frequency domain into time domain [15]. Our present work was aimed to identify the possible phytochemical compounds present in the ethanolic extract of *R. humilis* using GC/MS along with its functional groups using FTIR.

Materials and Methodology

Collection of plant sample

Rivina humilis was collected from Nagercoil, Kanyakumari district of Tamil Nadu, India and authenticated by Botanist Dr. R. Murugan, BSI, Southern Circle, Kovai. A voucher specimen was deposited in the Department of Botany and Research Centre, S.T. Hindu College, Nagercoil (Herbarium Code No: STHC-12).

Plant sample extraction

2gm of air dried powder of fruit sample was extracted with 50ml of ethanol, with gentle stirring for 72 hrs. The sample was kept in dark for 72h with intermittent shaking. After incubation, the solution was filtered was collected (Crude extracts). It was then transferred to glass vials and kept at 4°C before use [16].

Gas chromatography-mass spectrometry (GC-MS)

10g of powdered fruit sample was soaked with 30ml ethanol overnight and filtered through ash less filter paper with anhydrous sodium sulphate (2g), to remove residual water from the sample before soxhlet extraction. The extract is concentrated to 1ml by bubbling nitrogen into the solution. The extract contained both polar and non-polar phyto-components. 1µl of the ethanolic extract of *R. humilis* fruit was employed for GC-MS analysis. The Clarus 500 GC used in the analysis, employed a fused silica column packed with Elite-1 (100% dimethyl poly siloxane. 30nm × 0.25nm ID × 1µm df) and the components were separated using Helium as carrier gas at a constant flow of 1ml/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 min GC extraction process, the oven was maintained at a temperature of 110°C, with 2 min holding. The injector temperature was set at 250°C (mass analyzer). 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 min.

FTIR spectrum analysis

The extract of powder samples were mixed with KBr pellet, using a mortar and pestle compressed in a thin pellet. The samples were loaded into FTIR spectroscopic and the spectroscopic results were recorded on a FTIR spectrometer, the scan range was between 4000 to 400 cm^{-1}.

Identification of phytocomponents

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 component stored in NIST library. The name, retention time, and peak area percentage of the components of the test materials were ascertained [16].

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 in to FTIR, the functional groups of the components were separated based on its peaks ratio [16].

Results and Discussion

GC-MS is one of the best methods to identify the bioactive compounds, 20 compounds were identified from the ethanolic extract of fruit of *Rivina humilis*. The identification of the phytochemical compounds ware confirmed based on the name, peak area, retention time presented in Figure 1, and Table 1.

The GC-MS analysis showed 20 phytochemical compounds of which 17 compounds were reported to possess various bioactivities. The presence of phytochemicals represented 1-P-Mente-8-Ylaceate, Heneicosylformate, Tricyclo [3.3.1.1 (3.7)] decane, 1, 3-dimethyl, Citronella, Hexadecanoic acid, methylester (CAS), Neryl propionate, Hexadecanoic acid, d-Nerolidol, 9-Octadecenoic acid (Z)-2 hydroxy-1-(-hydro), carbamic acid, Diphenyl-3, 7-Dime, 4-Isopropenyl-4, 7-Dimethyl-1-Oxa, Octadec-9-Enoic acid, Octadeconoic acid, Neryl propionate, Zonarone, Dotriacontane (CAS) h-Dotriacontane, 2, 4A, 8, 8-Tetra methyl-decahydro, 2, 6, 10, 14, 18-pentamethyl-2,6,10,14,18,2,6,10,14,18, 1-Eicosanol.

Study showed that the ethanolic extract of *R. humilis* root was subjected to GC-MS analysis and 30 phytocompounds were identified namely; d-Glycero-d-ido-heptose, Boldione, 1-Hexadecanol, sucrose, Dimethyl phthalate, 3,7,11,15.19-pentaoxa-2,20-disilaheneicosane, 2,2,20,20-tetramethyl, melezitose, Myo-Inositol,4-c-methyl, Tetradecanoic acid, caffeine, 1, 2-Benzendicarboxylic acid, ethyl ester, dasycarpidan-1-methanol, acetate(ester), 1H-Indene, 2-butyl-3-hexyl, Dasycarpidan-methanol, acetate (ester), Heptadecanoic acid, 9.12-Octadecadienoic acid (z, z)-g-octadecenoic acid, (E), octadecanoic acid, Hexadecanoic acid, 1-(hydroxymethyl)-1, 2-ethanediyl ester, Isopropyl linoleate, 9, 12, 15-octadecatrienoic acid, 2, 3-dihydroxypropyl ester, (z, z, z)-2, 2-Dimethyl-6-methylene-1-[3, 5-dihydroxy-1-pentenyl]cyclohexan-1-perhydrol, Glycidyloleate, 3, 9 - Methano-10H - Furo [3, 2 - d] azonine-10, 11-dione, 9-[2-(dimethylamino)-3-methoxyphenyl]decahydro-2, 6-dimethyl-, [2R-(2R*, 3R*, 3aS*, 9R*, 10aR*)]-, 5, 8, 11-Eicosatrienoic acid, (z)-, TMS derivative, cyclohexane [17, 18].

GC/MS analysis of ethanol extract of *Rivina humilis* leaves revealed the existence of 2-Butenoic acid, butyl ester (3.851), Hexadecanoic acid, ethyl ester (9.479),3,7,11,15-Tetramethyl-2-hexadecen-1-ol(8.795),

Cyclopropaneoctanoic acid, 2-[[2-[(2-ethylcyclopropyl) methyl] cyclopropyl] (10.478), 9, 12, 15-Octadecatrienoic acid, 2-(acetyloxy)-1-[(acetyloxy)methyl] ethyl ester, (Z, Z, Z)- (11.267), Stigmasterol (22.787), Cholestane, 1-vinyl-1-hydroxy (22.208), 9β, 10α-Androst-4-en-3-one, 4-bromo-17β- hydroxy, acetate(23.155), 4, 8, 12, 16-Tetramethylheptadecan-4-olide (11.688), Ethanol, 2-(9-octadecenyloxy) (13.161), γ-Tocopherol (19.684), 4-(1, 5-Dihydroxy-2, 6, 6-trimethylcyclohex-2- enyl) but-3-en-2-one(8.164),3,7,11,15-Tetramethyl-2-hexadecen-1-ol 8.795), 2-(Toluen-4-sulfonyl)-2, 3-dihydro-1H- isoquinolin-4-one (9.952), 1-Monolinoleoylglycerol trimethylsilyl ether (20.42), Phytol (8.585), 2-(Toluen-4-sulfonyl)-2,3-dihydro-1H-isoquinolin-4-on(9.952), Cyclopropaneoctanoic acid, 2-[[2-[(2-ethylcyclopropyl) methyl] cyclopropyl] methyl]-,methyl ester (10.47) [19]. Whole plant extract in *R.humilis* showed the presence of 3-(pro-2-enoloxy)dodecane, Benzene, 14-Dichloro, 1H-Indene, 1-methylene, 3-

Tetradecane (E), Myo-Inositol, 4-c-methyl, caffeine, n-Hexadecanoic acid, octadecanoic acid, cyclohexane, 1,3,4-trimethyl-2-octadecyl,cis-13-octadecanoic acid, 9-Hexadeconoic acid, 9-octadecenyl ester, (z, z)-, Hexadeconoic acid, 1-(hydroxymethyl)-1,2-ethanediyl ester, 7, 8-Epoxylanostan-11-ol, 3-acetoxy, oleic acid eicosyl ester [20].

The GC-MS analysis of ethanol flower extract of *Litsea floribunda* has revealed the presence of nine compounds and most of the reported. There are cis- sabinol, 5-methylene-3-cyclooctene-1,2-di,1,2-benzoldi carbonsaeure, Pentadecanoic acid, Tetratriacontane, Hexadecanoic acid, 1, 2-Benzenedicarboxylic acid, dibutyl ester, Octadecanoic acid, stearic acid [21].

The Gas chromatography and mass spectroscopy analysis in the whole dry powdered material of ethanol extracts on *R. hypocrateriformis* (Desr.) Choisy. explicate the existence of seven phytocompounds such as Oleic acid (RT:13.44), 3,7, 11, 15-Tetramethyl-2-hexadecen-l-ol (RT: 13.98), Cyclo propane butryic acid 2-(2-noncyclopropyl) methyl ester (RT: 14.28), Oleic acid, eicosyl ester (RT: 14.04), E-10-Pentadecenol (14.52), 9-Octadecenoic acid (Z)-2, 3-dihydroxypropyl ester (RT: 17.39) and Phytol (RT: 17.73) [22].

GC-MS determination of bioactive constituents of the ethanolic extract of *Phoebe wightii* (stem) revealed the 1-methyl -3-deuteroxy quinazoline-2, 4(1H, 3H) dione, 14,15-Dimethylbenzo[s]picene, Propanoic acid, 2-(3- acetoxy-4,4, 14-trimethylandrost-8-en-17-yl), 2-(3-acetoxy-4, 4, 10, 13, 14-pentamethyl2,3,4,5,6,7,10,11,12,13,14,15,16,17 tetradecahydro-1H-cyclopenta[a] phenanthren-17-yl)-Propioni, 2,4(1H)-Cyclo-3, 4-secoakuammilan-16-carboxylic acid, 17-hyroxy-10-methoxy-, methyl ester, (16R)-(CAS), 1'"-Trimethylsilyl-3-bromo[1-[4-(2-phenyl-1, 4-dihexyl phenyl)phenyl]]benzene, Spiro[2H-1-benzopyran-2,2'-[2H]indole], 1', 3'-dihydro-8-methoxy- 3', 3'-dimethyl-6-nitro-1-pheny, l-(CAS) Cyclodecasiloxane, eicosamethyl-Octasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-trans, trans-1-(3,4-Dimethoxyphenyl)-2-methyl-3-ethyl-5,6-ethoxyindan, Cyclohexane, 1,1'-dodecylidenebis[4-methyl-Cyclohexane, 1,1'-dodecylidenebis[4-methyl- (CAS) 1-Tetradecanol (CAS) Cycloheptane, methyl-1-Dodecanol (CAS)revealed the presence of various compounds with corresponding peaks at different retention time [23].

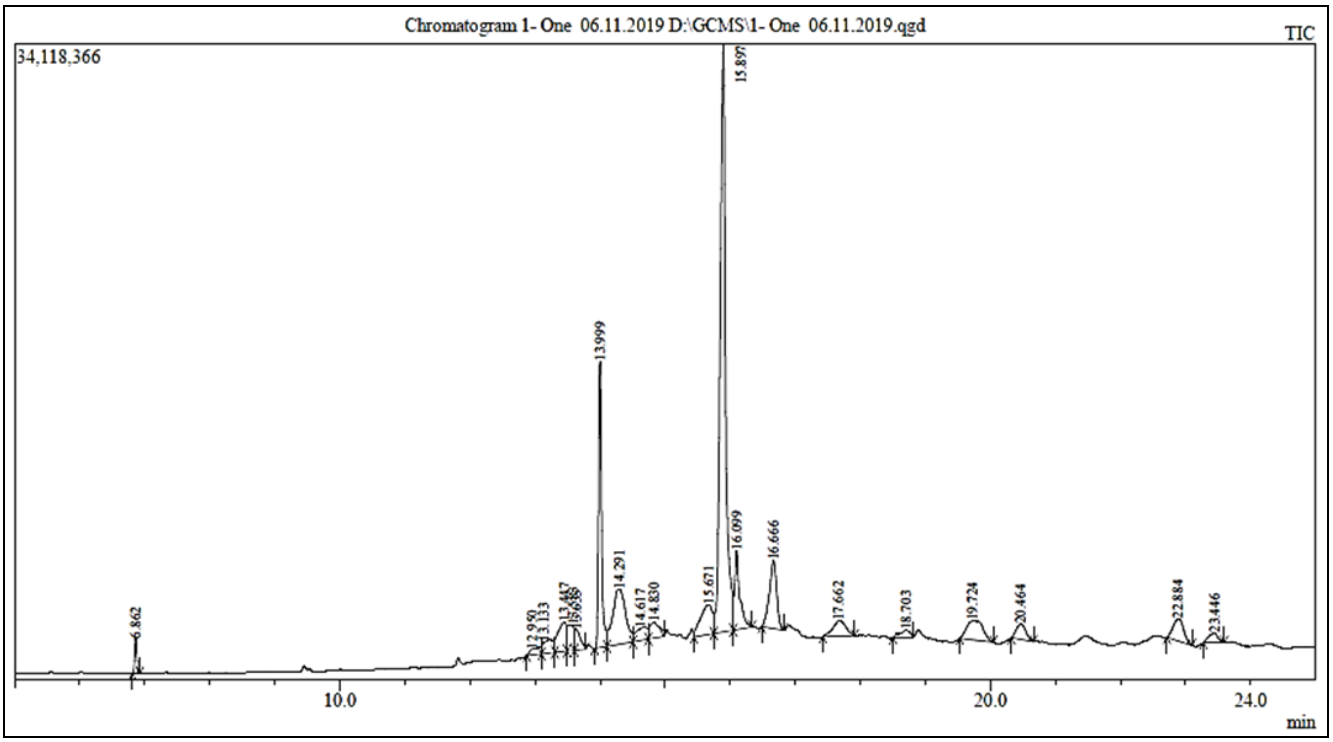

Fig 1: GC-MS analysis in the fruit sample of *Rivina humilis*

Table 1: GC-MS analysis in the fruit sample of *R. humilis*

S. No	Compound Name	RT	Area %	Biological activity
1	1-P-Menthen-8-Ylaceate	6.862	0.89	Antimicrobial
2	Heneicosylformate	12.950	0.85	Bio control activity
3	Tricyclo [3.3.1.1(3.7)] decane, 1, 3-dimethyl-	13.133	1.75	Antimicrobial
4	Citronella	13.447	2.98	Antimicrobial
5	Hexadecanoic acid, methyl ester (CAS)	13.583	1.97	Antioxidant, Antimicrobial decrease blood cholesterol, anti-inflammatory
6	Neryl propionate	13.633	1.42	Antimicrobial
7	Hexadecanoic acid	13.999	10.60	Anti-inflammatory, anticancer, antioxidant
8	d-Nerolidol	14.291	8.70	Antimicrobial, anti-biofilm, antioxidant, anti-parasitic, Skin-repellent, anti-inflammatory and anticancer
9	9-Octadecenoic acid (z)-2 hydroxy-1-1(-hydro)	14.617	1.81	No activity
10	Carbamic acid, Diphenyl-3, 7, Dime	14.830	1.93	No activity
11	4-Isopropenyl- 4, 7-Dimethyl-1-Oxa	15.691	4.37	Antibacterial activity
12	Octadec-9-Enoic acid	15.897	38.92	Antihypertensive, Increase HDL and Decrease LDL.
13	Octadecanoic acid	16.099	4.87	Antifungal, Antitumor, antibacterial
14	Neryl propionate	16.666	6.07	Antimicrobial

15	Zonarone	17.662	2.45	Phosphatase inhibition, antimetastatic, anti-inflammatory, hypercholesterolemic, Transferase stimulant
16	Dotriacontane (CAS) n-Dotriacontane	18.703	0.96	Antimicrobial, antioxidant, antispasmodic
17.	2, 4A, 8, 8-Tetra methyl-decahydro	19.724	3.84	Antibacterial
18	2, 6, 10, 14, 18-Pentamethyl-2, 6, 10, 14, 18	20.464	1.76	No activity
19	2, 6, 10, 14, 18 - Penta methyl-2, 6, 10, 14, 18	20.464	1.76	No activity
20	1-Eicosanol	23.446	1.07	Antimicrobial

Ethanolic extract of *Cyamposis tetragonoloba* fruit showed 34 phytochemical constituents [24], 8 Phytocomponents reported in ethanolic extract of *Terminalia bellerica* fruit and it contain significant quantities of antioxidants are believed to have health benefits by counteracting oxidative stress thus reducing the risks of chronic diseases [25, 26] reported 58 compounds in the methanol extract of *Xylopia ethiopica* fruits. Methanolic extract of red grapes pulp contain 61 compounds [27]. Fifteen compound reported in Aqueous extract of *Carica papaya* fruit [28]. 13 phytoconstituents reported in methanolic extract of *Capparis decidua* fruit [29]. In petroleum ether *Aegle marmelos* fruit extract 21 compounds were identified as compared to the methanol and ethanol extract [30].

From the Figure-2 and Table-2, the results of FT-IR spectroscopic analysis of ethanolic fruit extract revealed the presence of 18 functional groups. The peak at 3939.60 cm^{-1} revealed the presence of hydroxyl group. The peak at 3868.64 and 1685.75 revealed the presence of hydroxyl compounds. The peak at 3466.67, 3073.42, 2785.64 and 1685.75 revealed the presence of alcohol and phenol, alkenes, alkanes and amino acids. The peak at 1570.05, 1483.09 and 1336.93 refers to the presence of Nitro and aromatic, aromatic and Nitro compounds. The peak of 857.23, 821.82, 757.20 and 682.96 indicate the primary/secondary amines, amines, aromatic compound and halogen. The peak at 615.86 and 547.49 cm^{-1} denotes the alkyl halide and halogen, alkyl halide.

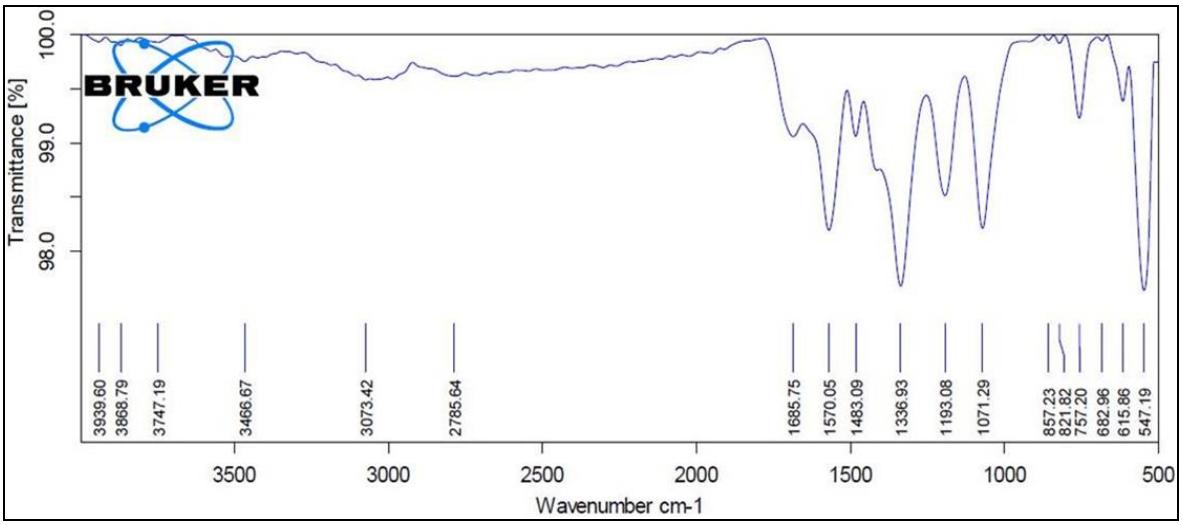

Fig 2: Absorption spectra of fruit sample of *R. humilis*

Table 2: FT-IR Peak value and functional groups of fruit sample of *Rivina humilis*

Sl. No.	Peak Value	Functional Group
1	3939.60	Hydroxy group
2	3868.79	Hydroxy group
3	3747.19	Hydroxy group
4	3466.67	Alcohol, phenol
5	3073.42	Alkene
6	2785.64	Alkanes
7	1685.75	Amino acid
8	1570.05	Nitro, aromatic
9	1483.09	Aromatic
10	1336.93	Nitro
11	1193.08	Aliphatic amines
12	1071.29	Aliphatic amines
13	857.23	Primary/Secondary amines
14	821.82	Amines
15	757.20	Aromatic compound
16	682.96	Halogen
17	615.86	Alkyl halide
18	547.19	Halogen, alkyl halide

[16] Reported that the presence of arenes, alcohols, phenols, carboxylic acids, ethers, aromatics, aryl ketone, alkenes, saturated aldehyde and phenols might be responsible for various medicinal properties of the *Xylopiaa ethiopica* fruit. 12 functional groups reported in methanolic extract of red *Vitis vinifera* pulp [28].

The ethanolic extract of *Rivea hypocrateriformis* is passed into the FTIR spectroscopy and the functional groups of the

components are separated based on the peak ratio. The results of FTIR analysis confirm the presence of functional groups such as hydroxyl compound, methyl group, cyclo alkane, carbonyl compound, sulphur compound, alkyl ketone, amino acids, sulphones compound, halogen compound and alkyl halide showing the frequency range such as 3266.14, 2916.35, 2848.51, 1607.80, 1374.30, 1315.30, 1241.56, 1025.79, 500.42 and 484.22cm-¹ respectively. Based on the functional group analysis, it is evident that *R. hypocrateriformis* plant does not consist of any toxic compound [23, 31].

Conclusion

The present study identified 20 bioactive compounds in the ethanol extract of *R. humilis* fruits using GC/MS and 18 peak values reported in FTIR study. *R. humilis* contain more bioactive components that posses antimicrobial, antioxidant, anti- inflammatory activities.

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