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Original Article

GCMS and FTIR analysis on the methanolic extract of red *Vitis Vinifera* seed

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ABSTRACT

Vitis Vinifera seed is a member of vitaceae family which contains high phenolic procyanidins, Gallic acid, Catechin, Anthocyanin, Vitamin E, linoleic acid and flavonoids. Our present work aimed to identify the possible phytochemical compounds using Gas Chromatography Mass Spectroscopy along with its functional group analysis using Fourier Transform Infrared Spectrophotometer, present in the methanolic extract of Red *Vitis Vinifera* seed. From Gas Chromatography Mass Spectroscopy study we identified nearly 130 compounds of which many of the compounds identified were already proved to possess antioxidant, anti-inflammatory, free radical scavenging, Protection against membrane oxidation, antidiabetic, cardioprotective, hepatoprotective, anti-carcinogenic, anti-microbial, and anti-viral activities. As per the Fourier Transform Infrared Spectrophotometer analysis, the red *Vitis Vinifera* seed extract is rich in alkane and ester groups.

Keywords: NIST, antioxidant, spectroscopy, infrared

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INTRODUCTION

Grapes, a natural product, organically a berry fruit, of the deciduous woody vines of the blooming plant family (*Vitis)*. *Vitis Vinifera* is an individual from the *Vitaceae* family, local to southern Europe and Western Asia developed around the world. Grapes are viewed as rich wellsprings of poly-phenolic mixes, such as catechin, epicatechin, gallic acid, procyanidins and Anthocyanin. Total phenolic compounds present in red grapes seed is 62%. Grape seed has a high convergence of Vitamin E, linoleic acid, flavonoids and phenolic procyanidins. Grape seed extract is from grape seeds that are separated, dried and cleaned to create polyphenolic mixes rich concentrate that likewise has very much reported anti-cancer agent, antimicrobial and mitigating properties. It is mostly used for industrial purposes while oral grape seed extract is used in capsules or tablets. The seeds of the grape vine are used in herbal medicines, whilst fruit is consumed as a dietary supplement [1]. Grape seed extract is a rich source of antioxidants and oligomeric proanthocyanidins which possess several health benefits. It has been accounted to have a wide range of pharmacological and remedial impacts, for example, free radical scavenging, antidiabetic, cardio protective, hepato protective, anti-carcinogenic, anti-microbial, Vaso – relaxation , Protection against membrane oxidation, inhibit platelet aggregation, anti-viral activity, and metal chelating properties. *Vitis Vinifera* is used in conditions like hemorrhages, iron deficiency, skin problems, syphilis, asthma, jaundice, bronchitis, calming etc. In India, grapes are grown for table, raisin, wine and juice purpose [2].

To identify the compounds of complex mixtures, there is a need for the development of different instrumental coupling techniques. Once such technique is gas chromatography coupled with mass spectrometry (MS). GC-MS is an extremely favorable, synergistic union, as the compounds susceptible to be analyzed by GC (low-molecular-weight, medium or low polarity, in ppb-ppm concentration) are also compatible with the MS requirements. Gas chromatography can separate volatile and semi-volatile compounds with great resolution, but it fails to identify them. MS can identify and quantify the accurate amount of compounds present in samples with its structural information, but it cannot readily separate them. Application of GCMS is identification of unknown samples, drug detection, investigating explosives [3, 4].

Fourier Transform Infrared Spectrophotometer (FTIR) is 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 [5]. In recent years, FTIR becomes well-accepted method due to its ease of sample preparation, fast, need little samples size, and does not require the use of solvents which is more economical. Numerous studies have shown that there is the number of research utilising the FTIR for the analysis of bioactive compounds. These include research in food technology, pharmaceutical and medicinal field [6].

Our work aimed to identify the possible phytochemical compounds along with its functional group present in the methanolic extract of Red *Vitis Vinifera* seed using GCMS and FTIR.

MATERIALS AND METHODOLOGY

Preparation of plant materials and extract for In Vitro studies: 10 grams of the dried seed material was powdered and placed in Soxhlet extractor along with 150 ml of methanol and refluxed at 60°C for 8hrs. The methanolic extract was filtered through Whatmann No. 1 filter. The filtrate was evaporated to dryness at 80°C and stored until further analysis. For analysis, the dried material was reconstituted in 1 ml methanol, and subjected for analysis [7].

Gas Chromatography-Mass Spectrometry: The methanolic extract of the grape seeds was subjected to analysis on a GC- MS Clarus 500 Perkin Elmer system comprising a AOC-20i auto sampler and gas chromatograph interfaced to a mass spectrometer (GC- MS) instrument employing the following conditions: Restek Rtx^{R} – 5, (30 meter X 0.25 mm) (5% diphenyl / 95% dimethyl polysiloxane), running in electron impact mode at 70 eV; helium (99. 999%) was used as carrier gas at a constant flow of 1ml/min and an injection volume of 1.0 µl was employed(split ratio of 10:1); injector temperature 280 ^{0}C. The oven temperature was programmed from 40°C (isothermal for 5 min.), with an increase of 6 ^{0}C / min to 280 ^{0}C, then ending with an isothermal for 15min at 280°C. Mass spectra were taken at 70 eV; 0.5 seconds of scan interval and fragments from 40 to 550 Da. Total GC running time was 60 minutes [8].

Identification of Compounds: Interpretation of mass spectrum GC-MS was done using the database of National Institute of Standard and technology (NIST). The spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST library.

Fourier Transform Infrared Spectrophotometer (FTIR) Analysis: Fourier Transform Infrared Spectrophotometer (FTIR) is the most important and powerful tool for identifying the functional groups present in the sample. The wavelength of light absorbed is the characteristic of the chemical bond. The chemical bonds in a molecule can be determined by interpreting the infrared absorption spectrum.

Reagents required: Potassium bromide (KBr)
Control: Pong oil

Procedure: Dried powder of *Vitis Vinifera seed* was used for FTIR analysis. 10mg of the sample was encapsulated in 100mg of KBr pellet, to prepare translucent sample disc. The seed sample was loaded in FTIR spectroscope (Burker make Tensor 27 model FT-IR, 64 scans at a spectral resolution of 4 cm^{-1}).

RESULTS

Gas Chromatography Mass Spectrometry (GCMS) Analysis: The GCMS chromatogram for the methanolic extract of seeds is shown in the Figure-1 and the interpretation is given in Table-1.

Fourier Transform Infrared Spectrophotometer (FTIR) Analysis: The spectrum obtained from FTIR analysis for dry powder extract of *Vitis Vinifera* seeds are given in the Figure – 2 and their group identification is given in Table – 2.

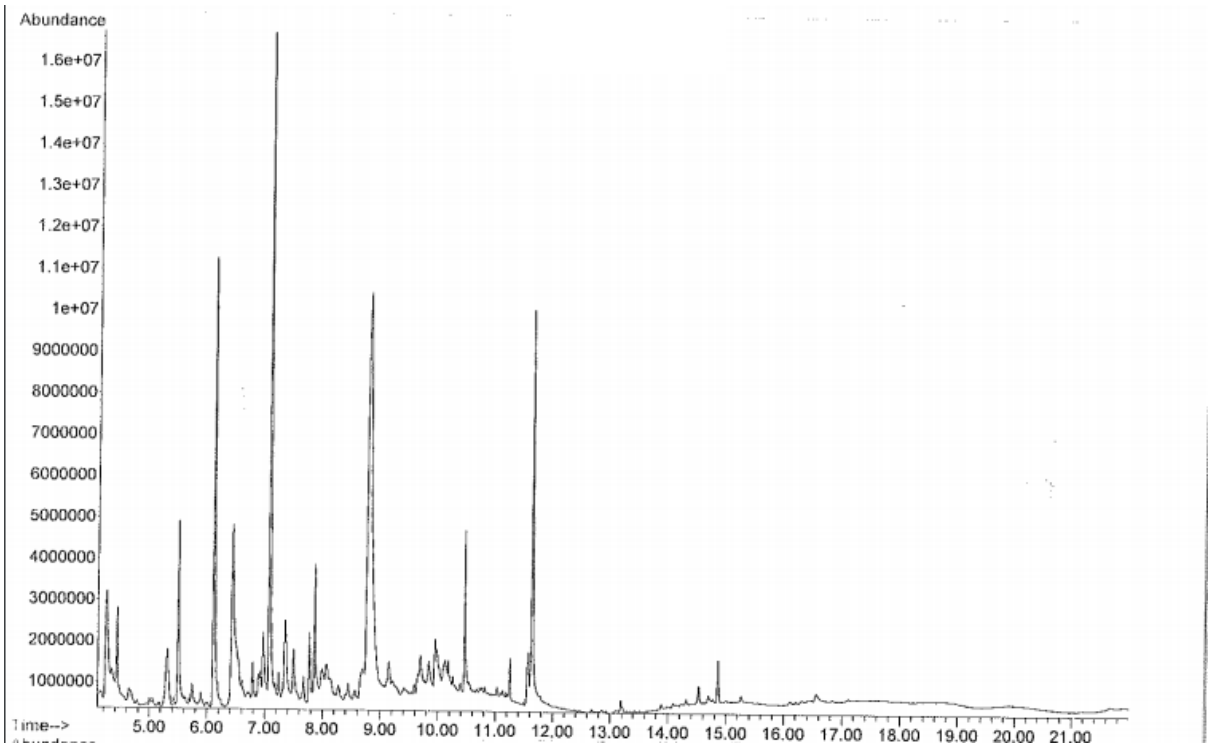

Figure – 1: GC- MS chromatogram of seeds from *Vitis Vinifera*

Figure – 1: GC- MS chromatogram of seeds from *Vitis Vinifera*

Retention time	Name of the compound	Peak area	Activity
0.243	Triethylphosphine acetaldehyde, Methylhydrazone (S) - (+) – 2-amino-3-methyl-1-butanol.	4.06	Synthesis of organic and organo metallic compounds. Acetaldehyde is used in cheese and heated milk. Used in the synthesis of Suritozole
4.428	4H-pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-3,4-difluoroanisole propane, 1-isocyanato.	1.64	In synthesis and evaluation of anticonvulsant and antimicrobial activity. Involved in the steaming process of roots. Improve properties of dual cured composite cements.
5.309	(+) -N (2) –ethyl -4 –methyl -1,2-pentanediamine. Ethyl N-tert –butylformimidate 2- hydroxyl – gamma –butyrolactone.	2.42	Used in study of human genetics. In derivatives of thienamysin. Used in pharmaceutical industries

5.500	2 – tetrazaboroline 5- ethyl- 1,4- dimethyl- thymine Thymine.	3.63	Used to study spectroscopic properties. Antidepressants Dissociate the rate of DNA replication from the culture growth rate.
5.734	Methyl 2- furoate 2- furancarboxylic acid Hydrazide 3- furancarboxylic acid Methyl ester	0.48	In cocoa and cocoa products. Anticancer. Used to produce detergents and biodiesel.
5.884	3- heptane 3- ethyl- aminocaproic acid Aminocaproic acid	0.40	Used as a flavouring agent. In the treatment of acute bleeding. Stops bleeding affecting the blood clotting system.
6.105	4H- Pyran-4 –one 2,3- dihydro- 3,5- dihydroxy-6 –methyl-4H- pyran- 4- one 2,3- dihydro- 3,5- dihydroxy -6 –methyl-3,4 –difluoroanisole	8.88	Antifungal activity. To study thermal degradative pathway. Reduce harm from tobacco use.
6.423	L-arabinitol Xylitol	8.99	Indicate over growth of intestinal microbes and fungus species. Used as sugar substitute.
6.597	Propanoic acid 2- mercapto- methyl ester benzofuran 2,3- dihydro- benzenemethanamine	0.45	Preservative and flavouring agent. Drugs for the disorder of metabolism Insecticides.
6.788	2(3H)- furanone Dihydro- 4- hydroxyl-2 –butanamine 3- methyl- pentanal	0.64	Antifungal activity. Used in lotion and cream. Indicator of fungal activity.
6.926	1,2,3,4- cyclopentanetetrol Alpha- d- xylopyranose D- lyxose	0.49	Strong inhibitor activity. Used in enzyme analysis. Pentose and glucoronate interconversion.
6.926	Ethyl 2- ethylbutyl carbonate 1H-(1, 2, 3)triazole- 4- carboxylic acid. 1- (4- aminofurazan -3 –yl) -5 – pyrrolidin- 1- ylmethyl- thiophene.	0.39	Antibacterial activity. Kinase inhibitor compounds. Used as protein kinase inhibitors.
6.968	2(3H) –furanone, 5- butyldihydro	1.45	Used in identifying ligands. Also used as flavouring agent.
7.070	5-hydroxymethylfurfural 4- mercaptophenol	12.02	Used in food industry as a food additive as a flavouring agent. Leukotriene antagonist.
7.237	Acetic acid, (acetyloxy)-, ethyl ester Heptanoic acid, 1- methylethyl este pantanoic acid 3- hydroxyl-4 methyl-, methyl ester	0.40	Used in industrial synthesis of esters and salts. Used as a solvents and to make flavouring agents. Anti - asthmatics and in drugs for disorder of urinary system.
7.351	4H- pyran- 4- one, 2,3- dihydro- 3,5- dihydroxy- 6- methyl Acetamide, N- (2- acetyl- 3- oxo- 4- isoxazolidinyl) 2,4- dihydroxy- 2,5- dimethyl- 3(2H)- furan- 3- one	2.72	Matrix metalloproteinase inhibitors. Anti–asthmatics and drugs for dermatological studies. Used in flavour and perfume industry.
7.495	1- di(tert –butyl) silyloxycyclopentane 2- butanone, 4- hydroxyl- 3- methyl -2- azabutane N- amidini-4, 4- dimethoxy	1.42	Tertiary ether used as a solvent. Used in the treatment of bronchial asthma. Modulate intracellular calcium.
7.669	1,3- benzenediol 2- choloro- barbituric acid 2- thio- 2,4(1H, 3H)- pyrimidinedithione	0.54	Used as antiseptic and disinfectants. Used extensively for anesthesia. Biodegradable and thermosensitive.
7.771	1- isoleucine, N-methoxycarbonyl- methylester 4,5- dihydro- 2- methylimidazole- 4- on	1.29	Used for chemical synthesis. Present as natural additives in general food.

	methanamine N- bis(5- methyl- 1,3,4- oxadiazol- 2-yl)		Used as antimicrobial in animal models.
7.860	Naphthalene 1- methyl- 2,5- cyclohexadiene-1,4 – dione, 2- chloro 5,6- epoxy- 6- methyl- 2- heptanone	2.23	Used as insecticide and pest repellent. Used for dyeing keratinous fibres. Inhibitors and Conjugates cell binding and cytotoxic agents.
7.986	Butanedioic acid, 2- hydroxyl- 2- methyl (S)- 2- propanol 1- (1,3- dimethylbutoxy) 8- chlorooctyl 2-methylbutanoate	0.62	Anti microbial property. Widely used as universal cleaning agent. Used as a flavouring agent.
8.058	1,2,3- benzenetriol	0.46	Drug or therapeutic agent.
8.274	Pyrrolidine, 1,2-dedihydro-5-[3-acetoxy-1-butyl]-2-methylthio-L-Lysine, N2-acetyl-2-Propen-1-amine, N,N-bis(1-methyl ethyl)-	0.49	Antifungal property. Used as fungicides in metallo enzyme inhibitor.
8.441	6,8-Doixatetradecane, Acetoxyacetic acid, 3-pentadecyl ester 6,8-Dioxahexadecane	0.36	Used for photo damaged skin and acne treatments. Used in pharmaceuticals. Used in extraction of dye.
8.561	3-Deoxy-d-mannoic lactone 1,3-Methylene-d-arabitol 3-Piperidinol	0.25	Used as food supplement and muscle strengtheners. Acts as an herbal drug. They are modulators of ATP binding cassette transporters.
8.687	2,4-Methylene-D-epirhamnitol 1,3,4-Thiadiazole-2(3H)-thione, 5-Methyl- Propanedioic acid, oxo-, diethyl ester	1.51	Used as herbicide and defoliant. Therapeutic uses and in bronic acid derivatives. C- Met kinase inhibitors and hepatitis c virus inhibitors.
8.795	1,6-Anhydro-2,4-dideoxy-.beta.-D-ribo-hexopyranose 3-Butenoic acid Chloroacetic acid, 2,2-dimethylpropyl ester.	24.06	Used as a chemical tracer for biomass burning in atmospheric chemistry studies. Intermediate compound in metabolism. In Chemically amplified positive resist composition and patterning process.
9.142	Dodecanoic acid Methyl-.alpha.-d-ribofuranoside	0.54	Used in many soaps and shampoos. For treating viral infection such as hepatitis b virus infection
9.651	1,5-Anhydro-1-rhamnitol Octane, 1-(ethenylthio) Methyl-.alpha.-d-ribofuranoside	0.44	Blood Glucose Regulator in Diabetes and Metabolic Syndrome Phosphite ester stabilizers. Tricyclic-Nucleoside Compounds for Treating Viral Infections.
9.687	Glycine, N-(3-fluorobenzoyl)-, methyl ester Glycine, N-(4-fluorobenzoyl)-, methyl ester Pyridine, 1,2,3,6-tetrahydro-1-(1-oxobutyl)-	0.80	Used in treatment of diabetic neuropathy. Drugs that are used for their effects on dopamine receptors.
9.837	Rhamnitol, 1- O - decyl- Lactose 2-Hexenal diethyl acetal, trans	0.69	Used as primary immunization. Used as filler or filler binders in pharmaceutical tablets or capsules. Used for fragrance.
9.951	N-Methoxymethyl-N-methylacetamide 2R,3S-9-[1,3,4-Trihydroxy-2-butoxy methyl] guanine N-Aminomorpholine	1.07	Involved in cell cycle kinase mediated cellular proliferation. Antifungal activity. In industries that use wood fuel. Kinase inhibitors.
10.119	3-Deoxy-d-mannoic lactone dl-Allo-cystathionine	0.54	Have cellulolytic enhancing activity. Used to treat different pathologies.

			Used as a substrate to differentiate and analyse cystathionine.
10.178	Hexadecanoic acid, methyl ester	0.43	Used as a natural additive in organic product. The aluminium salt is used as a thickening agent of napalm used in military actions.
10.256	Estra-1,3,5(10)-trien-17.beta.-ol 4-Oxopentyl formate Z-(13,14-Epoxy) tetradec-ll-en-l-ol Acetate	0.37	It has an anticoagulant property. Used as feed/food additive and for pharmaceutical applications. Used in traditional and folklore medicine.
10.460	n-Hexadecanoic acid	2.27	Sodium palmitate is permitted as a natural additive in organic products. The aluminium salt is used as a thickening agent of napalm used in military actions.
11.251	9,12-Octadecadienoic acid (Z,Z)-, methyl ester 8,11-Octadecadienoic acid, methyl ester 10,13-Octadecadienoic acid, methyl ester	0.64	Shows the antioxidant effect of polyphenols and natural phenols. It is used in cosmetic industries.
11.568	cis-13-Octadecenoic acid	1.36	Stearic acid is used along with simple sugar or corn syrup as a hardener in candies. Stearic acid is used to produce dietary supplements
11.634	9,12- Octadecadienoic acid (Z,Z)-	6.93	Shows the antioxidant effect of polyphenols and natural phenols. It is used in cosmetic industries.
13.185	1H-Inden-1-one, octahydro-7a-hydroXy- Phenol, 3-[N,N-diethylamine1-O-[4-[1-cycloazapropyl]-n-butyl]- Bicyclohexyl-2,3'-dione	0.27	Used for fragrance in perfume industry. Used industrially as UV stabilizers and antioxidants. Antibiotic activity and act as antimalarials
14.521	Butane-1,4-dioic acid, monocyclododecyl monophenyl ester Phenyl isobutyrate 1,7-Dimethyl-4-oxa-tricyclo[5.2.1. O(2,6)]decane-3,5,8- trione	0.32	Used as a food ingredient as a flavorant and gelling aid. Used as food additives and flavouring agent. Has antibacterial and antifungal activity.
14.857	Phthalic acid, di(2-propylpentyl) ester Bis(2-ethylhexyl) phthalate Phthalic acid, hept-3-yl isohexyl Ester	0.73	They are used as plasticizers. And used to soften poly vinyl chloride. It is also used as a hydraulic fluid and as a dielectric fluid in capacitors. DEHP also finds use as a solvent in glow sticks.
16.558	Fumaric acid, cyclohex-3-enylmeth l isobutyl ester N-Vinylimidazole Pyrrole, 4-Aethyl-2-methyl-	0.33	Used as food preservatives and in therapeutic uses. Used in manufacturing of perfumes and in hair treatment. Used as flavouring agent and in manufacturing of perfumes.

Table – 1: GC- MS chromatogram of seeds of *Vitis Venifera*

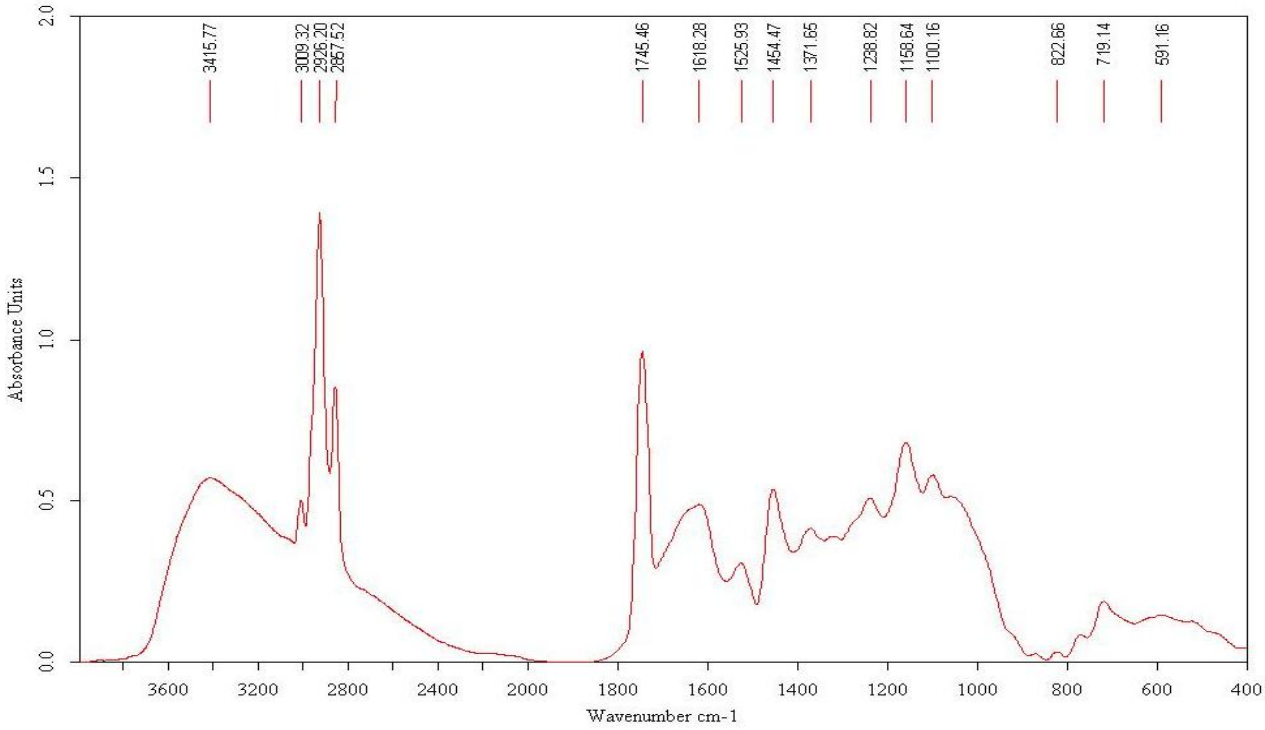

Figure – 2: Infrared spectroscopy spectrum for *Vitis Vinifera* seeds powder extract.

S.No	Frequency	Group	Intensity
1	3415.77	Alcohol (O-H)	Medium, broad
2	3009.32	Alkene (C-H)	Medium to strong
3	2926.20	Alkane (C-H)	Medium
4	2857.52	Alkane (C-H)	Medium
5	1745.46	Ester (C=O)	Strong
6	1618.28	C=C	Weak to medium
7	1525.93	Arene (C=C)	Medium
8	1454.47	Arene (C=C)	Medium
9	1371.65	Alkyl	Strong
10	1238.82	Alcohol (C-O)	Strong
11	1158.64	Alcohol (C-O)	Strong
12	1100.16	Alcohol (C-O)	Strong
13	822.66	Alkyl halide (C-Cl)	Strong
14	719.14	Alkyl halide (C-Cl)	Strong
15	591.16	Alkyl halide (C-Br)	Strong

Table – 2: Infrared spectroscopy table for *Vitis Vinifera* seeds dry powder extract

DISCUSSION

Gas Chromatography Mass Spectrometry (GCMS) Analysis: From the Figure – 1 and Table – 1 the GC-MS chromatogram of methanolic extract of seed showed nearly 130 compounds. 2-Tetrazaboroline compounds having the retention time of 5.500 and measuring the peak area of 3.63 are used to study spectroscopic properties [9]. 5-ethyl- 1,4- dimethyl- thymine having the retention time of 5.500 and measuring the peak area of 3.63 is used as antidepressants [10]. 4H- pyran-4- one having the retention time 6.105 and measuring the peak area of 8.88 shows antifungal activity [11]. 2,3-dihydro- 3,5- dihydroxy-6 –methyl- 4H- pyran- 4-one having the retention time 6.105 and measuring the peak area of 8.88 are used to study thermal degradative pathway. 2,3- dihydro- 3,5- dihydroxy -6 –methyl- 3,4 -difluoroanisole having the retention time of 6.105 and measuring the peak area of 8.88 reduces harm from tobacco use [12]. 5-hydroxymethyl furfural having the retention time of 7.070 and measuring the peak area of 12.02 are involved in formation of ether and halides and also used in food industry as a food additive as a flavouring agent. 4- mercaptophenol having the

retention time of 7.070 and measuring the peak area of 12.02 are used for leukotriene antagonist [13]. 9, 12-Octadecadienoic acid (Z, Z) - having the retention time 11.634 and measuring the peak area of 6.93 shows the antioxidant effect of polyphenols and natural phenols. It is used in cosmetic industries. It has antimicrobial and antifungal activity [14].

Fourier Transform Infrared Spectrophotometer (FTIR) Analysis: From the Figure – 2 and Table – 2, the medium broad absorption bands is observed at 3415.77 cm^{-1} which is representative for O-H stretching vibrations, characteristic of the presence of alcohol. The bands at 3009.32 cm^{-1} is due to the stretching vibration of C-H groups indicative of the alkene. The 2926.20 cm^{-1} and 2857.52 cm^{-1} are due to stretching vibration of alkanes. The strong bands at 1745.46 cm^{-1} represent the bending vibrations of C=O indicative of the esters. The 1618.28 cm^{-1} band in all samples, predict the presence of C=C. The C=C groups exhibit strong bands at 1525.93$cm^{-1}$and 1454.47$cm^{-1.}$ The band at 1371.65 cm^{-1} represents alkyl group. The band at 1238.82 cm^{-1}, 1158.64 cm^{-1} and 1100.16 cm^{-1} represents alcohol C-O. The band at 822.66 cm^{-1} and 719.14 cm^{-1} represents alkyl halide C-Cl. The band at 591.16 cm^{-1} represents alkyl halide C-Br [15].

CONCLUSION

The GC-MS chromatogram of the methanolic extract of seeds showed nearly 130 compounds. Most of the compounds which were reported from seeds were found to be rich in 2- tetrazaboroline, 5- ethyl- 1,4- dimethyl- thymine, 4H- Pyran-4 – one, 2,3- dihydro- 3,5- dihydroxy-6 –methyl- 4H-pyran- 4- one, 2,3- dihydro- 3,5- dihydroxy -6 – methyl- 3,4 –difluoroanisole, 5-hydroxymethylfurfural, 4- mercaptophenol, 9,12-Octadecadienoic acid (Z,Z)- at retention time 5.500, 6.105, 7.070, 11.634 with peak area 3.63, 8.88,12.02, 6.93 respectively were proved to possess various medicinal activities. As per the data analysis of FTIR from using infrared spectroscopy correlation table, it was found that alkane groups at the frequency of 2926.20 and esters at a frequency of 1745.46 gave maximum peaks. Hence, we can conclude that the *Vitis Vinifera* extract is rich in alkane and ester groups.

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