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GCMS AND FTIR ANALYSIS ON THE METHANOLIC EXTRACT OF RED VITIS VINIFERA PULP

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ABSTRACT

Red *Vitis Vinifera* pulp has major components such as Organic acids, Malate, Tartrate and Sugars and contains higher moisture content. Our work is designed to identify the possible phytochemicals compounds present in the methanolic extract of red grape pulp by using GCMS along with functional group analysis through FTIR. From the GCMS analysis of red Vitis Vinifera pulp nearly 61 compounds were identified. By FTIR analysis, strong absorption band was found at 3400 cm–1, which represents the amine groups and ketones at a frequency of 1066.02 cm^{-1} gave maximum peaks. Hence, we can conclude that the red *Vitis Vinifera* pulp extract is rich in amines, ether and ester groups.

KEYWORDS: GCMS, FTIR, spectral analysis, NIST.

1. INTRODUCTION

Red Grapes or *Vitis vinifera* is a Berry fruit and belongs to the group of versatile fruits which are used in a wide range as popular foods - from raisins to jelly to wine. Over 72 million tons of grapes are grown every year and 7.2 trillion gallons of wine is produced. Grapes are rich source of many vital nutrients and anti-oxidants.[1] Many berry fruit together form a bunch of grapes. The essential parts of fruit are Peel, Pulp, and Seeds. Inside the skin layer is the Pulp or flesh. Cells in the pulp have cell sap with large vacuoles. When berries are crushed, the cells break to release the juice in the pulp. The pulp majorly contains water (65%-85% of grape weight), sugars (15%-25%), it directs the sweetness. It is attributed to different mix of acids such as malic acid, tartaric acid, succinic acid, ascorbic acid, citric acid, phenols, and flavonoids.[2] These are considered to have biological properties, not only limited to anti-oxidant, anti-inflammatory, anti-cancer, antimicrobial, antiviral, cardioprotective, neuroprotective, hepatoprotective activities but also as a nutraceuticals.[3]

GC-MS is a method that couples two different analytical techniques, gas-liquid chromatography and mass spectrometry to identify different phytochemical compounds present in a test sample. GC can separate volatile as well as semi-volatile compounds with higher resolution, but it cannot identify them. MS can be used to obtain structural information of the compound, but it cannot separate readily. It is used to analyse complex biochemical and organic mixtures and it is also highly compatible.[4] Infrared spectroscopy is most powerful technique for materials analysis and used in the laboratories. Infrared absorption spectroscopy is the study of interaction of infrared radiation with matter as a function of photon frequency. Fourier Transform Infrared Spectroscopy (FTIR) ensures accurate information about the rotation, vibration, of the particular chemical bonding and molecular structures, thereby helping to analyze inorganic as well as organic compounds. An infrared spectrum relates sample fingerprint with absorption peaks which corresponds the frequencies of vibrating mode among the bonds of the atoms that it is made up of. Each different material is a combinational mix of atoms; no two compounds produce the exact same infrared spectrum. Therefore, infrared spectroscopy can be used for qualitative analysis identification of test samples. In addition, the size of the peaks determined by the spectrum is a directly proportional to the amount of material present in the test sample. With modern software algorithms, infrared is an excellent tool for quantitative analysis. It is most important technique for identification of the functional groups present in the sample. The characteristic of chemical bond can be analysed by different wavelength of light absorbed.[5]

Our present work was aimed to identify the possible phytochemical compounds using GCMS along with its

functional groups using FTIR, present in the methanolic extract of red grape pulp.

2. MATERIALS AND METHODOLOGY

2.1. Preparation of plant materials and extract for In Vitro studies

The Red *Vitis Vinifera* is collected from The Horticultural Department, University of Agricultural sciences, Gandhi Krishi Vignan Kendra, Bangalore. 10 grams of the dried pulp 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 was subjected for GCMS analysis [6].

2.2. Gas Chromatography-Mass Spectrometry

The methanolic extract of the Red *Vitis Vinifera* pulp was subjected to analysis on a GC- MS Clarus 500 Perkin Elmer system comprising a AOC-20i autosampler 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.

2.3. 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.

2.4. 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 methanolic solvent extract of *vitis vinifera* pulp was used for FTIR analysis. 10mg of the sample was encapsulated in 100mg of KBr pellet, to prepare translucent sample disc. The powdered sample of methanolic extract was loaded in FTIR spectroscope (Burker make Tensor 27 model FT-IR, 64 scans at a spectral resolution of 4 cm^{-1}).

3. RESULTS

3.1. Gas Chromatography Mass Spectrometry (GCMS) Analysis

The GCMS chromatogram for the methanolic extract of red grapes pulp is shown in the Figure – 1 and the interpretation is given in Table – 1.

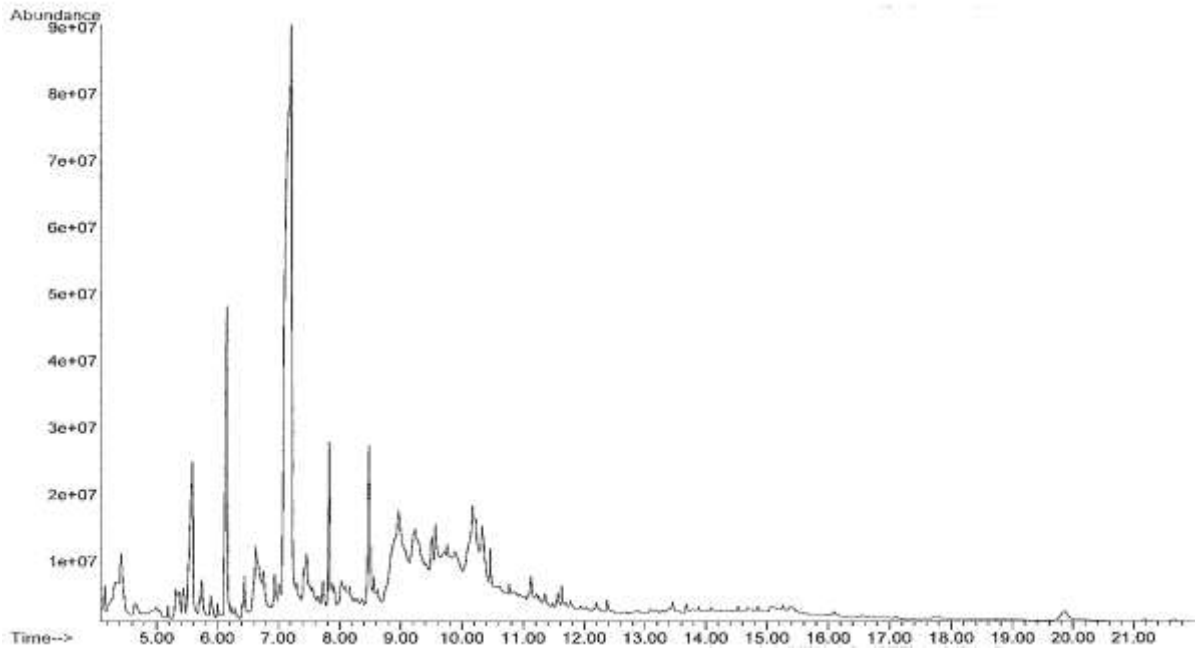

Figure 1: GC- MS chromatogram of red grapes pulp.

Table 1: GC- MS chromatogram of red grape pulp.

Retention time	Name of the compound	Peak area	Activity
4.338	Formaldehyde, dimethylhydrazone	2.40	Lipid formulation. Treating neurodegenerative diseases. modified iRNA agents
4.338	Thietane, 2,4-dimethyl-	2.40	Sweetener, Pesticide.
4.338	Ethanimidic acid, ethyl ester	2.40	Antitumor agent. Antagonist.
4.416	4H-Pyran-4—one, 2,3-dihydro-3,5-dihydroxy-6-methyl.	2.30	Flavour improvement
4.416	Isopropyl isothiocyanate.	2.30	Used as medicament
4.416	Propane, 1-isothiocyanato.	2.30	Used as medicament
4.662	2-Furancarboxaldehyde, 5-methyl-	0.52	HIV inhibiting pyrimidines derivatives
5.309	1,2,4 – Cyclopentanetrione, 3-methyl	0.80	Used in ink.
5.309	4,5-Diamino-2-hydroxypyrimidine	0.80	Antiviral, antitumor.
5.309	Pyrazole-5-carboxamide, 4-amino-	0.80	Novel kinase inhibitors.
5.441	2-(tert-Butylamino)ethanol	0.63	Antiviral compounds.
5.441	Methylazoxymethanol acetate	0.63	Methylating agent act on cell proliferation, cell survival, DNA synthesis
5.590	1,3,5-Triazine-2,4,6-triamine	6.29	Antibacterial, Antifungal
5.590	Thymine	6.29	Antibacterial, Antifungal
5.590	4,5-Diamino-2-hydroxypyrimidine	6.29	Anti-viral, antitumor, antimetastatic immune system
5.734	2-Furancarboxylic acid, hydrazide	1.02	Treatment of cancer.
5.734	Furyl hydroxymethyl ketone	1.02	Prebiotic preparation.
5.734	Methyl 2-furoate	1.02	Treatment of inflammation and pain.
5.884	Malonic acid,ethyl 2-methylpent-3 -yl ester	0.65	No activity reported
5.884	Silane, ethenylethoxydimethyl-	0.65	Antimicrobial, Antibacterial agent, Biofilm development controller.
6.159	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	9.02	Affect autonomic neurotransmission, blood pressure, body activity,Anti proliferative and pro-apoptotic effects.
6.159	2,4-dihydroxy-2,5-dimethyl-3(2H)-Furan-3- one.	9.02	Flavourant analysis. Anti microbial activity, Anti fungal.
6.441	Ethanamine, N- ethyl-N-nitroso-	0.79	Antitumoral agents.
6.441	2-Imidazolidinethione	0.79	Serine protease inhibitors.
6.626	Propanal, 2,3-dihydroxy-, (S)-	4.21	CDK9 Kinase Inhibitors.
6.626	Propylamine, N,N,2,2-tetramethyl- n-oxide	4.21	No activity reported
6.938	2(3H) -Furanone, dihydro-4-hydroxy-	0.82	HIV Integrase inhibitors.
6.938	Urea, trimethyl-	0.82	Angiotensin, Antagonists.
6.938	2-Heptanamine, 5-methyl-	0.82	Cardiovascular agents.
7.004	5—Acetoxymethyl-2-furaldehyde	0.48	Antibacterial agents.
7.213	Benzenemethanol,3-fluoro-	43.87	Tyrosine kinase enzyme inhibitors, Anti-inflammatory agents.
7.213	4-Fluorobenzyl alcohol	43.87	Effect on Lipid peroxidation, anti-microbial activity, more scavenging activity.
7.411	2,5-Dimethyl-4-hydroxy-3(2H)-furanone	0.45	Antimicrobial and cell cycle arrest activity.
7.411	Cyclohexanone, 4-ethyl-	0.45	Hepatitis b antiviral agent, Inhibitors of viral replication, inhibitor of beta secretase.
7.411	Glycine, N- (3- methyl-1- oxobutyl)-, methyl ester	0.45	Antimicrobial, Anticancer and Antioxidant.
7.729	2,8,4,6-(Epoxyethanediylidenoxy) [1,3]dioxino[5,4-]-1,3-dioxin,tetrahydro	0.63	No activity reported
7.729	2(3H) -Thiazolimine, 3-hydroxy-4,5-dimethyl-	0.63	HMG CoA Reductase Inhibitor, anticancer agent, sweetener and flavouring agent.
7.729	5-Nonanone, O-methyloxime	0.63	Microsomal triglyceride transfer protein, in treatment of benign prostatic hypertrophy.

7.830	1,3-benzenediol, 2-chloro	3.29	Apoptosis-inducing agents for the treatment of cancer and immune and autoimmune diseases.
7.830	2-Naphthalenol	3.29	Protease inhibitors.
7.830	2,6-piperazinedione, dioxime	3.29	Useful in treatment of ocular disorder and as herbicide.
8.022	Butanedioic acid, 2-hydroxy-2-methyl-,(S)-	0.73	Bone cancer therapy, Antiaging, skin renovation.
8.022	2,2-dimethylpropanoic acid, nonyl ester	0.73	Electrolyte salt, Used in battery industry.
8.022	2-Dimethylsilyloxytetradecane	0.73	Industrial Applications
8.483	10-Chlorodecyl(E)-2-methylbut-2-enoate.	4.44	Use of calendula glycosides for the treatment of psoriasis
8.483	Butanoic acid 3-butenyl ester	4.44	Used in Triazine derivatives as herbicides
8.483	Succinic acid, hex-4-yn-3-yl propyl ester	4.44	Used for arthritis and joint pain. In cycloaddition of azides and alkynes.
8.963	Thiophene, 3- (1,1-dimethylethyl)-	12.97	Agrochemicals, anti-microbial, analgesic, anti-inflammatory, antiallergic, anti-tumor activities.
8.963	Pyrimidine, 2,4,5-triamino-	12.97	Colouring agents.
8.963	2-Fluoro-5-methylaniline	12.97	Immunosuppressive agents.
9.508	1-isobutyl-7, 7-dimethyl-octahydro-isobenzofuran-3a-ol.	0.76	No activity reported
9.508	Fumaric acid, pent-4-en-2-yl pentyl ester	0.76	No activity reported
9.508	4-Pyridinol, 2,6-dimethyl-3- [methylthio)-	0.76	No activity reported
9.573	Pyrimidine-2,4(1H,3H)-dione, 6-hydroxy-5-methyliminomethyl	0.96	No activity reported
9.573	8-Chloro-1,2,4-triazolo(4,3-b)pyridazin-7-amine	0.96	No activity reported
10.466	n-Hexadecanoic acid	0.58	Enzyme inhibition.
11.119	4-Chlorophenyl isothiocyanate	0.59	Anti microbial activity.
11.119	2-Chlorophenyl isothiocyanate	0.59	Agonists of APJ receptor.
11.119	(1,1'-Biphenyl]-3-amine	0.59	No activity reported
19.852	alpha-Santalol	0.80	Fragrance delivery system for liquid detergent compositions.
19.852	8-Azabicyclo(3.2.1]octane-3,6-diol, 8-methyl-, 3-acetate	0.80	No activity reported
19.852	3-Phenoxylactamide	0.80	No activity reported

3.2. Fourier Transform Infrared Spectrophotometer (FTIR) Analysis

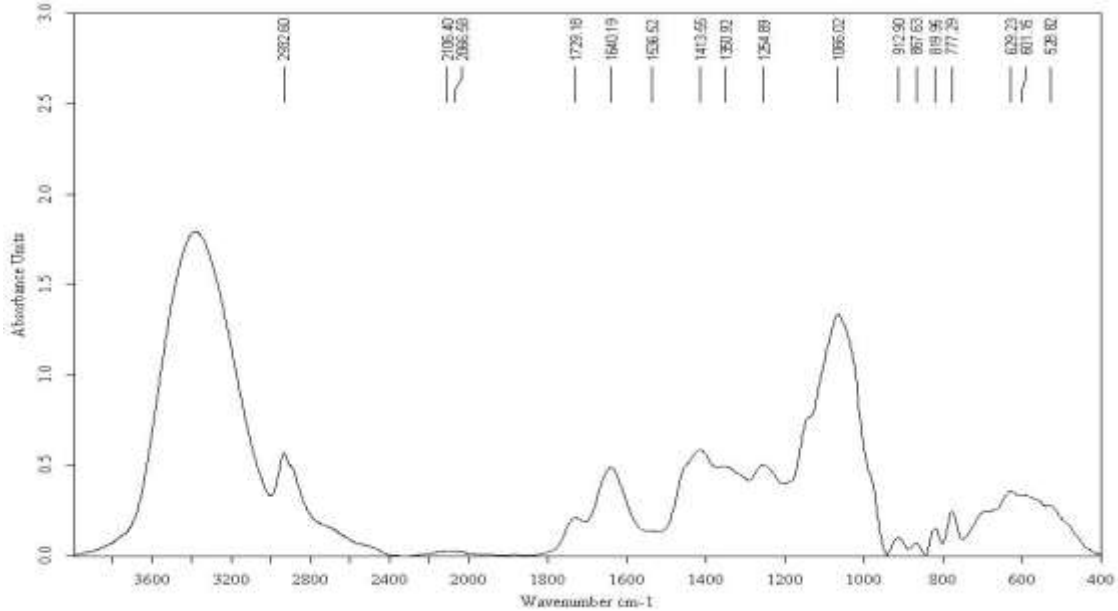

Figure 2: Infrared spectroscopy spectrum for red grape pulp methanolic extract.

Table 2: Infrared spectroscopy table for red grape pulp methanolic extract.

S. No.	Frequency	Group	Intensity
1	3400.00	Amines (N-H [stretching])	Medium
2	2932.00	Alkane (C-H [stretching])	Medium-Small
3	1729.18	Aldehyde (C=O [stretch])	strong
4	1640.19	Amide (N-H [bending])	strong
5	1536.52	Nitro(N-O[stretch]	strong
6	1413.55	Amine	Medium
7	1350.92	Amine(C-N[stretch])	Medium-weak
8	1254.89	Amine(C-N-[stretching])	Medium-weak
9	1066.02	Ether and ester (C- O- [stretching])	Strong
10	819.96	Alkene (=C-H[bending])	Small
11	777.29	Alkyl Halide (C-Cl [Stretch])	Small
12	629.23	Alkyl Halide (C-Cl [stretch])	Medium-weak

The spectrum obtained from FTIR analysis for methanolic extract of pulp are given in the Figure – 2 and their group identification are given in Table – 2.

4. DISCUSSION

4.1. Gas Chromatography Mass Spectrometry (GCMS) Analysis

From the Figure – 1 and Table – 1 the GC-MS chromatogram of a methanolic extract of pulp showed nearly 63 compounds. Most of the compounds which were reported from pulp were found to be rich in 1,3,5-Triazine-2,4,6-triamine, Thymine, 4,5-Diamino-2-hydroxypyrimidine, 4H-Pyran-4-one,2,3-dihydro-3,5-dihydroxy-6-methyl-, 2,4-dihydroxy-2, 5-dimethyl-3(2H)-Furan-3-one, Benzenemethanol,3-fluoro-, 4-Fluorobenzyl alcohol, Butanedioic acid, 2-hydroxy-2-methyl-,(S)-, 2,2-dimethylpropanoic acid, nonyl ester, 2-Dimethylsilyoxytetradecane, 10-Chlorodecyl (E) -2-methylbut-2-enoate, butanoic acid 3-butenyl ester, Succinic acid, hex-4-yn-3-yl propyl ester, at retention time 5.590, 5.590, 5.590, 6.159, 6.159, 7.213, 7.213, 8.022, 8.022, 8.022, 8.483, 8.483, 8.483, with peak area, 6.29, 6.29, 6.29, 9.02 , 9.02 , 43.87 , 43.87 , 0.73 , 0.73 , 0.73, 4.44, 4.44 , and 4.44 respectively. As per literature chloroform was reported as a better solvent system for compound extraction from pulp of red grapes. Our present study shows methanol can also be used as a compound for extraction from pulp of red grapes. Methanol solvent could be used along with chloroform as solvent system for various bioactive compound extractions. 1,3,5-triazine-2,4,6- triamine compounds having the retention time of 5.590 and measuring the peak area of 6.29 are used in Anti-microbial Activity, anti-fungal.[7] Thymine having the retention time of 5.590 and measuring the peak area of 6.29 is used as Anti-microbial activity and anti-fungal.[8]

4,5-Diamino-2-hydroxypyrimidine having the retention time 5.590 and measuring the peak area of 6.29 is used as anti-viral, antitumor, antimetastatic immune system.[9] 4H-Pyran-4-one,2,3-dihydro-3,5-dihydroxy-6-methyl- having the retention time 6.159 and measuring the peak area of 9.02 is used affect autonomic neuro transmission, blood pressure and body activity.[10,11] 2,4-dihydroxy-2,5-dimethyl-3(2H)-Furan-3-one having the retention time 6.159 and measuring the peak are of 9.02 are used as flavourant analysis, anti-microbial and antifungal activity [12]. Benezenemethanol,3-fluoro-having th retention time 7.213 and measuring the peak area 43.87 is used as anti-inflammatory agents and as tyrosine kinase enzyme inhibitor.[13,14]

4-Fluorobenzyl alcohol having the retention time of 7.213 and measuring the peak are of 43.87 may be employed as the Effect of lipid peroxidation, scavenging activity, antimicribial activity.[15] Butanedioic acid, 2-hydroxy-2-methyl-, (S) - having the retention time of 8.022 and measuring the peak area of 0.73 is used in bone cancer therapy and Antiaging, Skin renovation.[16,17] 2, 2-dimethylpropanoic acid, nonyl ester having the retention time 8.022 and measuring the peak area of 0.73 is used in batteries.[18] 2- Dimethylsilyloxytetradecane having the retention time of 8.022 and measuring the peak area of 0.73 is used in industries.[19] 10-Chloro (E)-2-methylbut-2-enoate having the retention 8.483 and measuring the peak area of 4.44 is used in treatment of psoriasis.[20] Butanoic acid 3-butenyl ester having the retention time of 8.483 and measuring the peak area of 4.44 is used as herbicide.[21] Succinic acid, hex-4-yn-3-yl propyl ester having the retention time of 8.483 and measuring the peak area of 4.44 is used for arthritis and joint pain.[22]

4.2. Fourier Transform Infrared Spectrophotometer (FTIR) Analysis

FT-IR spectroscopy is used to identify some qualitative aspects regarding the organic compounds in a fruit pulp of red grapes. Several indicator bands that are pertained to functional groups represent chemical components or metabolic products. From the Figure – 2 and Table – 2, the very strong absorption bands at 3400.00 cm^{-1} which is representative for N-H stretching vibrations, characteristic of the presence of amino acids. The bands at 2932.00 cm^{-1} is due to the stretching vibration of Alkanes (–C-H) groups. The 1729.18 cm^{-1} band is due to stretching vibration of Aldehyde group. The strong band at 1640.19 cm^{-1} represents the bending vibrations of Amines. The strong bands at 1536.52 cm^{-1} represent the

stretching vibrations of Nitro groups. The 1413.55 cm^{-1} band in the samples, predict the presence of Amine. The Medium-weak bands at 1350.92 cm^{-1} and 1254.89 cm^{-1} represent the stretching vibrations of Amines respectively. The (C-O-) Ether and Ester groups exhibit strong bands at 1066.02cm^{-1}. The band at 819.96 cm^{-1} represents Alkene (-C=H) bending vibration. The bands at 777.29 cm^{-1} and 629.23 cm^{-1} represent the alkyl halide (C – Cl) stretching vibrations of small and medium-weak intensity respectively.[23]

5. CONCLUSION

The GC-MS chromatogram of a methanolic extract of red grapes pulp showed nearly 61 compounds. Most of the compounds which were reported from Pulp were found to be rich in 1,3,5-Triazine-2,4,6-triamine, Thymine,4,5-Diamino-2-hydroxypyrimidine, 4H-Pyran- 4-one,2,3-dihydro-3,5-dihydroxy-6-methyl-,2,4-dihydroxy-2,5-dimethyl- 3(2H) Furan-3- one, Benzenemethanol, 3-fluoro-, 4-Fluorobenzyl alcohol, Butanedioic acid, 2-hydroxy- 2-methyl-,(S)-, 2,2-dimethylpropanoic acid, nonyl ester, 2-Dimethylsilyoxytetradecane, 10-Chlorodecyl (E) -2-methylbut-2-enoate, butanoic acid 3-butenyl ester, Succinic acid, hex-4-yn-3-yl propyl ester, at retention time 5.590, 5.590, 5.590, 6.159, 6.159, 7.213, 7.213, 8.022, 8.022, 8.022, 8.483, 8.483, 8.483, with peak area, 6.29 , 6.29 , 6.29, 9.02 , 9.02 , 43.87 , 43.87 , 0.73 , 0.73 , 0.73, 4.44, 4.44 , and 4.44 respectively. Our present study shows methanol can also be used as a compound for extraction from red grapes pulp. Methanol solvent could be used along with chloroform as solvent system for various bioactive compound extractions. As per the data analysis of FTIR from using infrared spectroscopy correlation table for red grapes pulp methanol extract, it was found that amines groups at the frequency of 3400.00 cm^{-1} and ketones at a frequency of 1066.02 cm^{-1} gave maximum peaks. Hence, we can conclude that the pulp methanolic extract is rich in amines, ether and ester groups.

6. ACKNOWLEDGEMENT

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