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Determination of Bioactive Components of Ethyl Acetate Fraction of *Punica granatum* Rind Extract

J. Sangeetha, K. Vijayalakshmi*

Department of Biochemistry, Bharathi Women's College, North Chennai -600108, Tamil Nadu, India

ABSTRACT
Punica granatum belongs to a Punicaceae family. The *Punica granatum* is valued as a powerful medicinal plant and used in folk medicines. Hence the present investigation was carried out to determine the possible chemical components from ethyl acetate fraction of *Punica granatum* rind extract by GC-MS Technique. This analysis revealed that ethyl acetate fraction of *Punica granatum* rind extract contain Pyrogallol (41.88%), 5-Hydroxymethylfurfural (14.10%), D-Allose (9.17%), 2-Methoxy-1, 4-Benzenediol (8.34%) and 2, 3 Dimethylfumaric acid (3.96%) justifying the use of this plant to treat many disease in folk and herbal medicine.

Keywords: *Punica granatum,* GC-MS Technique, Pyrogallol, Herbal medicine.

INTRODUCTION

Plants constitute an important source of active natural products which differ widely in terms of structures, biological properties and mechanisms of actions. Various phytochemical components, especially polyphenols (such as flavonoids, phenyl propanoids, phenolic acids, tannins, etc) are known to be responsible for the free radical scavenging and antioxidant activities of plants.

A Pomegranate (*Punica granatum*) is a fruit-bearing deciduous shrub or small tree growing between five and eight meters tall. The pomegranate is mostly native to the Iranian Plateau and the Himalayas in Northern India. Powder prepared from rind is used as a tooth powder and also as medicine a cosmetic industries. Rind powder is excellent source of beta-carotene, potassium, phosphorous and calcium. The pomegranate rind exploited in folk medicine is their strong astringency, making them a popular remedy throughout the world, in the form of an aqueous decoction (i.e., boiling the hulls in water for 10-40 minutes), for dysentary and diarrhea, and also for stomatitis. [1-3] The decoction can be drunk, used as a mouthwash, douche or enema.

Pure drugs that are technologically produced or isolated from plants may be chosen for their high activity against a human disease, but they have disadvantages. They rarely have the same level of activity as the crude extract at parallel dose or concentrations of the active component. [4] This phenomenon is attributed to the absence of interacting substances present in the extract. Furthermore, many plants contain substances that inhibit multi-drug resistance (MDR). A further disadvantage is that pure drugs are often more expensive to produce and distribute, and so are often unavailable and/or unaffordable to the poorest populations in remote areas who need them most. In contrast, herbal medicines can sometimes be grown and produced locally, at lower cost, by or close to those who need them. [5] Since there is no report on the phytoconstituents of ethyl acetate fraction of *Punica granatum* rind extract it was chosen as the subject of this study. The aim of this paper is to determine the organic compounds present in the active fraction of *Punica granatum* rind extract with the aid of GC-MS Technique, which may provide an insight in its use in folklore medicine.

***Corresponding author: Mrs. K. Vijayalakshmi,** Department of Biochemistry, Bharathi Women's College, North Chennai -600108, Tamil Nadu, India;
E-mail: sangeetha_joe@rediffmail.com

MATERIALS AND METHODS

Plant material

Ganesh variety of plant specimen for the proposed study was collected from local fruit market. Care was taken to select healthy fruits. It was identified as *P. granatum Linn* having yellowish red rind (Ganesh variety) belongs to Punicaceae family. The required fruit rind were cut and removed from the fruit and fixed and cast in to paraffin blocks. It was authenticated by Dr. P. Jayaraman, Director of National Institute of Herbal Science, Plant Anatomy Research Centre, Chennai. A voucher specimen is maintained in plant anatomy research centre, Chennai (PARC/2009/459). The fresh rind of the fruit was used for the microscopic studies.

Preparation of plant extract

Punica granatum rind was collected from local market. They were dried in shade and powdered mechanically. 30 g of

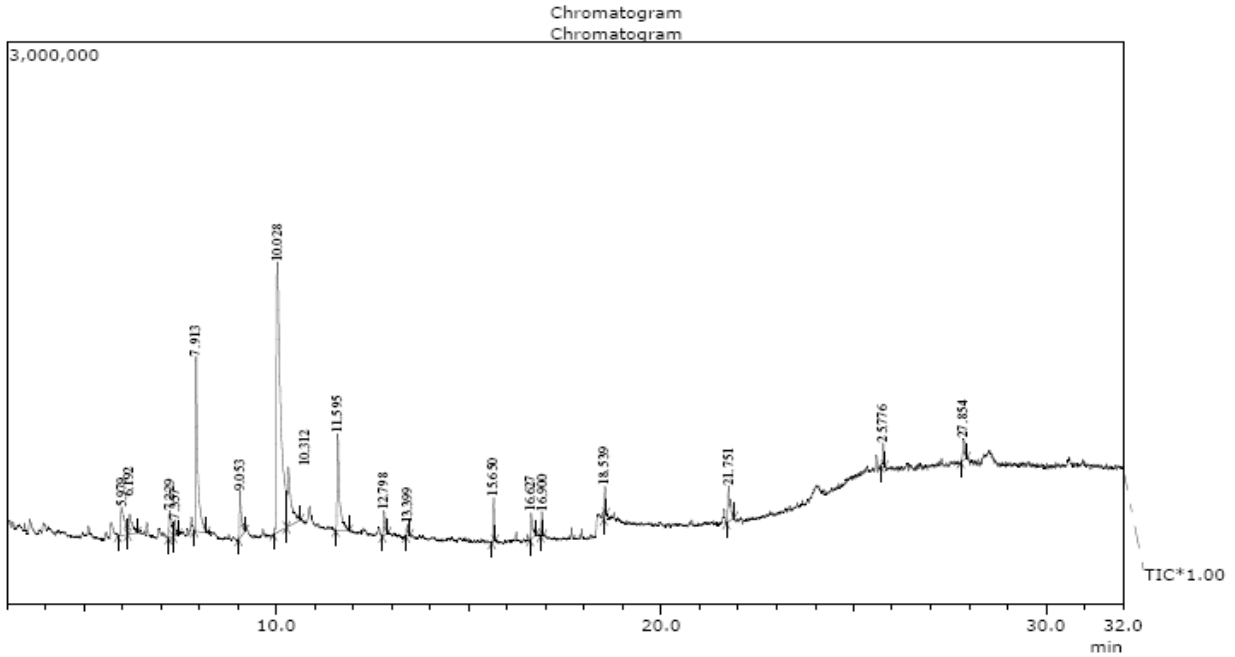


Fig 1: Chromatogram obtained for ethyl acetate fraction of *Punica granatum* rind extract

Table 1: Total ionic chromatogram showing the compounds of ethyl acetate fraction of *Punica granatum* rind, Retention time and concentration of the compound

Peak	RT	Area	Area%	Name of the compound
1	5.979	1101017	3.96	2,3- Dimethylfumaric acid
2	6.192	690003	2.48	Levoglucosenone
3	7.229	672822	2.42	2-furanone, 3,4-dihydroxytetrahydro
4	7.357	334926	1.21	Heptanal
5	7.913	3917687	14.10	5-Hydroxymethylfurfural
6	9.053	998359	3.59	3-methyldecanoic acid
7	10.028	11632804	41.88	Pyrogallol
8	10.312	2316920	8.34	2-Methoxy-1,4-Benzenediol
9	11.595	2547135	9.17	D-Allose
10	12.798	465169	1.67	1,6-Anhydro-beta,-d-glucofuranose
11	13.399	153233	0.55	Methyl-(2-hydroxy-3-ethoxy-benzyl)ether
12	15.650	417908	1.50	1,2-Benzenedicarboxylic acid,bis(2-ethylpropyl)est
13	16.627	479727	1.73	Palmitic acid
14	16.900	203911	0.73	Ethyl Palmitate
15	18.539	297587	1.07	Ethyl oleate
16	21.751	789104	2.84	Triphenylphosphine oxide
17	25.776	275222	0.99	Stigmast-5-en-3-ol, oleat
18	27.854	483247	1.74	Gamma,-Sitosterol

powdered *Punica granatum* rind was macerated with methanol and stored for 72 hours in ice cold condition for the extraction of phytochemicals. At the end of the third day extract was filtered using whatmann No. 1 filter paper and the organic layer was allowed to evaporate. The residue settled was used for further analysis.

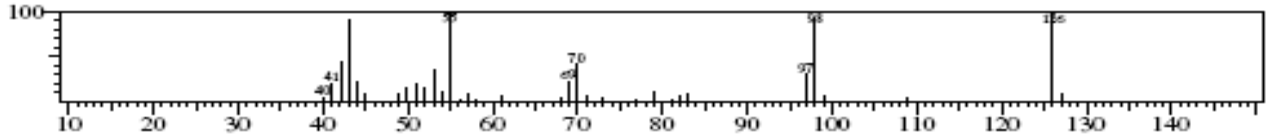
Column chromatography

10 g of the crude extract was subjected to column chromatography over silica gel (100-200 mesh) and eluted with n-hexane, chloroform, ethyl acetate and alcohol respectively. n-Hexane and Chloroform did not elute much of the compounds. The ethyl acetate fraction of the *Punica granatum* rind was taken for GC-MS analysis.

Gas Chromatography- Mass Spectrum Analysis (GC-MS)

GC-MS technique was used in this study to identify the phytocomponents present in the extract. GC-MS technique was carried out at Sargam laboratory, Chennai, Tamil Nadu. GC-MS analysis of this extract was performed using GC SHIMADZU QP2010 system and gas chromatograph interfaced to a Mass Spectrometer (GC-MS) equipped with Elite-1 fused silica capillary column (Length : 30.0 m, Diameter : 0.25 mm, Film thickness : 0.25 µm Composed of 100% Dimethyl poly siloxane). For GC-MS detection, an electron ionization energy system with ionization energy of 70eV was used .Helium gas (99.999%) was used as the carrier gas at a constant flow rate of 1.51ml/min and an injection volume of 2µl was employed (split ratio: 20). Injector temperature 200°C; Ion-source temperature 200°C. The oven temperature was programmed from 70°C (isothermal for 2 min.), with an increase of 300°C for 10 min. Mass spectra were taken at 70eV; a scan interval of 0.5 seconds with scan range of 40 – 1000 m/z. Total GC running time was 35 min. The relative percentage amount of each component was calculated by comparing its average peak area to the total areas. Software adopted to handle mass spectra and chromatograms was a GC MS solution ver .2.53.

Line # 1 R.Time:5.975(Scan#:358) MassPeaks:34
Raw Mode:Averaged 5.967-5.983(357-359) BasePeak:126.05(20001)
BG Mode:Calc. from Peak



Hit#:1 Entry:12662 Library:NIST08.LIB
SI:84 Formula:C6H8O4 CAS:0-00-0 MolWeight:144 RetIndex:1293
CompName:2,3-Dimethylfumaric acid

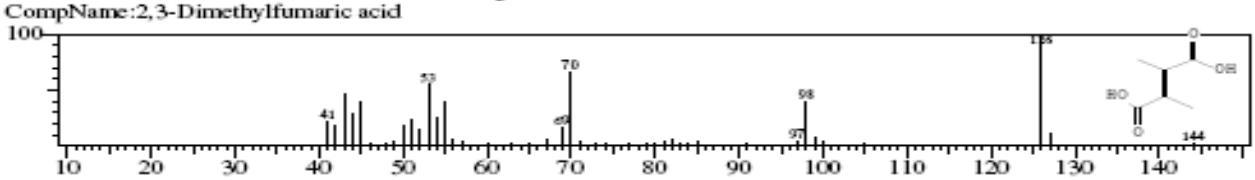
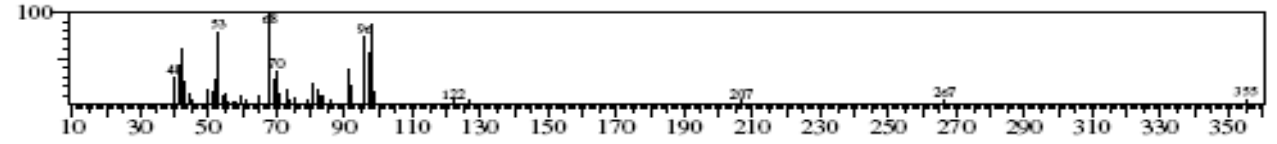


Fig 2: Molecular formula, M.W and Molecular structure of the first compound

Line # 2 R.Time:6.192(Scan#:384) MassPeaks:45
Raw Mode:Averaged 6.183-6.200(383-385) BasePeak:68.00(9874)
BG Mode:Calc. from Peak



Hit#:1 Entry:6350 Library:NIST08.LIB
SI:84 Formula:C6H6O3 CAS:37112-31-5 MolWeight:126 RetIndex:993
CompName:Levoglucosenone $$ 6,8-Dioxabicyclo[3.2.1]oct-2-en-4-one # $$

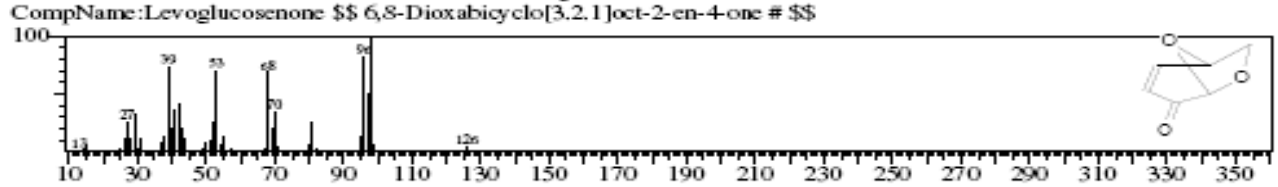
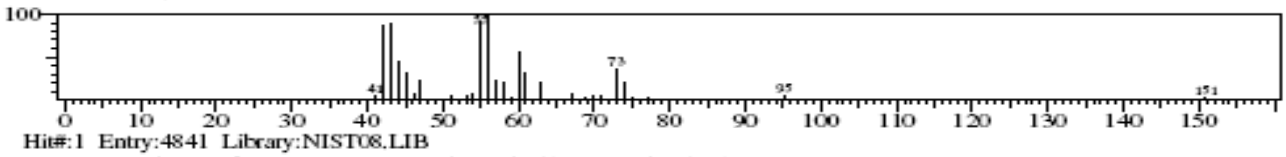


Fig 3: Molecular formula, M.W and Molecular structure of the second compound

Line # 3 R.Time:7.225(Scan#:508) MassPeaks:30
Raw Mode:Averaged 7.217-7.233(507-509) BasePeak:56.00(19761)
BG Mode:Calc. from Peak



Hit#:1 Entry:4841 Library:NIST08.LIB
SI:89 Formula:C4H6O4 CAS:0-00-0 MolWeight:118 RetIndex:1201
CompName:2-Furanone, 3,4-dihydroxytetrahydro

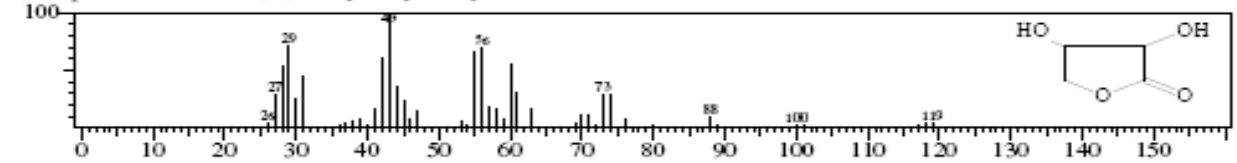
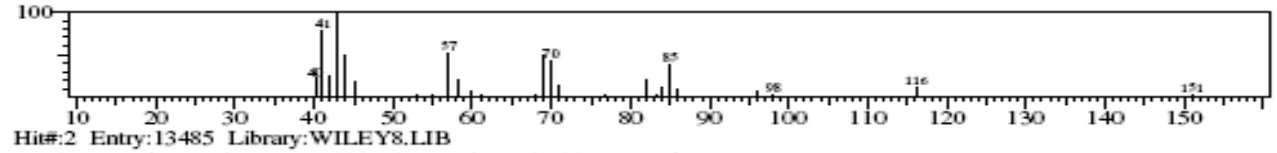


Fig 4: Molecular formula, M.W and Molecular structure of the third compound

Line # 4 R.Time:7.358(Scan#:524) MassPeaks:31
Raw Mode:Averaged 7.350-7.367(523-525) BasePeak:43.00(11331)
BG Mode:Calc. from Peak



Hit#:2 Entry:13485 Library:WILEY8.LIB
SI:81 Formula:C7H14O CAS:111-71-7 MolWeight:114 RetIndex:0
CompName:HEPTANAL $$ ENANTHALDEHYDE $$ 1-HEPTALDEHYDE $$ 1-HEPTANAL $$ 2077204001 HEPT

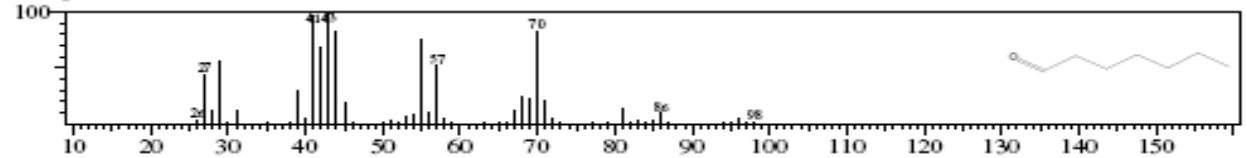
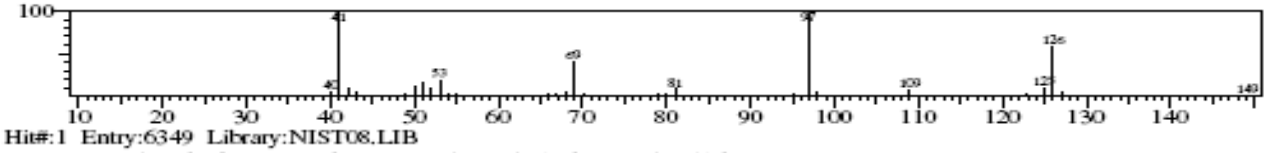


Fig. 5: Molecular formula, M.W and Molecular structure of the fourth compound

Line # 5 R.Time:7.917(Scan#:591) MassPeaks:50
Raw Mode:Averaged 7.908-7.925(590-592) BasePeak:41.05(214774)
BG Mode:Calc. from Peak



Hit#:1 Entry:6349 Library:NIST08.LIB
SI:93 Formula:C6H6O3 CAS:67-47-0 MolWeight:126 RetIndex:1163
CompName:2-Furancarboxaldehyde, 5-(hydroxymethyl)- $$ 2-Furaldehyde, 5-(hydroxymethyl)- $$ 5-Hydrxoymethylfurf

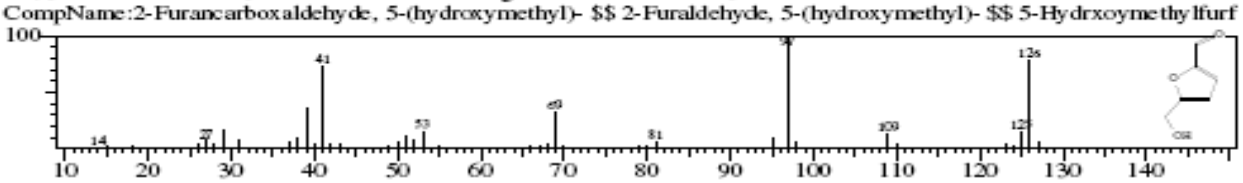
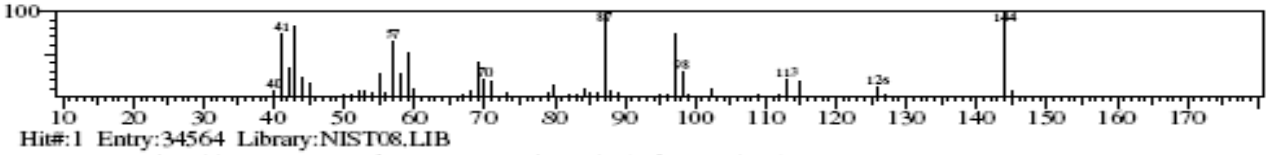


Fig 6: Molecular formula, M.W and Molecular structure of the fifth compound

Line # 6 R.Time:9.050(Scan#:727) MassPeaks:49
Raw Mode:Averaged 9.042-9.058(726-728) BasePeak:144.00(25589)
BG Mode:Calc. from Peak



Hit#:1 Entry:34564 Library:NIST08.LIB
SI:77 Formula:C11H22O2 CAS:60308-82-9 MolWeight:186 RetIndex:1407
CompName:Decanoic acid, 3-methyl- $$ 3-Methyldecanoic acid $$

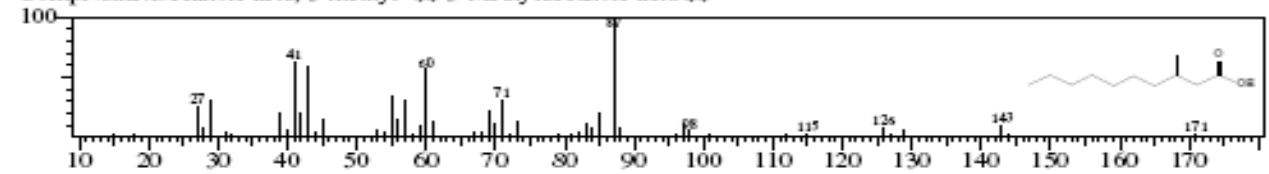
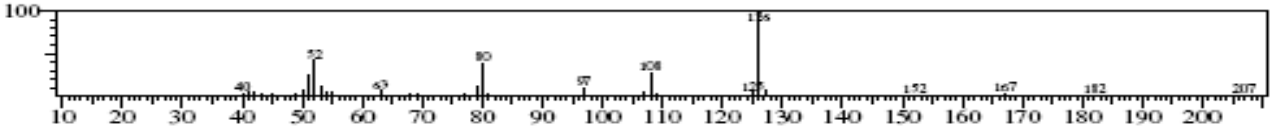


Fig 7: Molecular formula, M.W and Molecular structure of the sixth compound

Line # 7 R.Time:10.025(Scan#:844) MassPeaks:86
Raw Mode:Averaged 10.017-10.033(843-845) BasePeak:126.00(41877O)
BG Mode:Calc. from Peak



Hit#:1 Entry:6352 Library:NIST08.LIB
SI:97 Formula:C6H6O3 CAS:87-66-1 MolWeight:126 RetIndex:1342
CompName:1,2,3-Benzenetriol $$ Pyrogallol $$ C.I. Oxidation Base 32 $$ C.I. 76515 $$ Fouramine Brown AP $$ Fourri

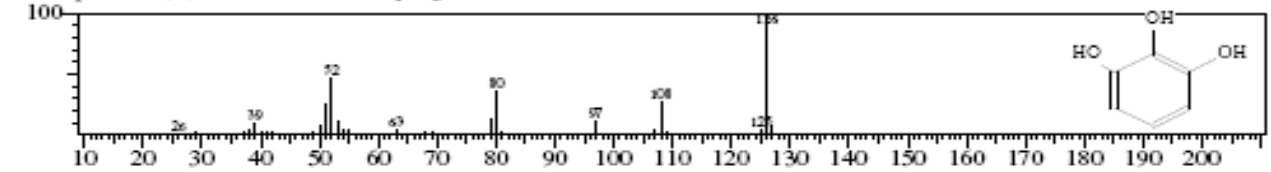
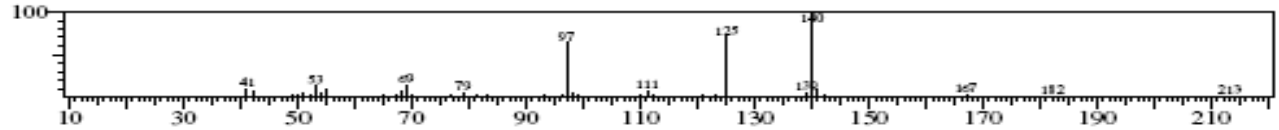


Fig 8: Molecular formula, M.W and Molecular structure of the seventh compound

Line # 8 R.Time:10.308(Scan#:878) MassPeaks:57
Raw Mode:Averaged 10.300-10.317(877-879) BasePeak:140.00(38974)
BG Mode:Calc. from Peak



Hit#:1 Entry:31262 Library:WILEY8.LIB
SI:91 Formula:C7H8O3 CAS:824-46-4 MolWeight:140 RetIndex:0
CompName:1,4-BENZENEDIOL, 2-METHOXY- $$ 2-METHOXY-1,4-BENZENEDIOL # $$ 2-METHOXY-1,4-BEN

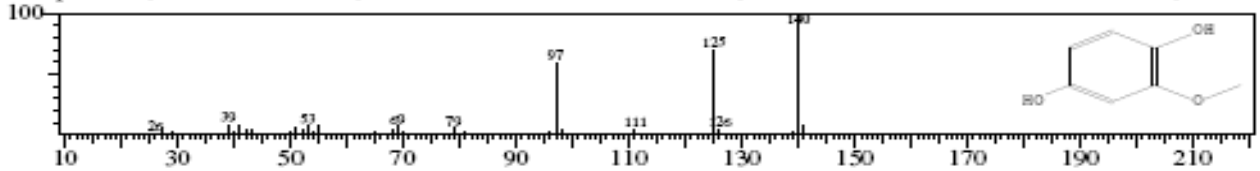
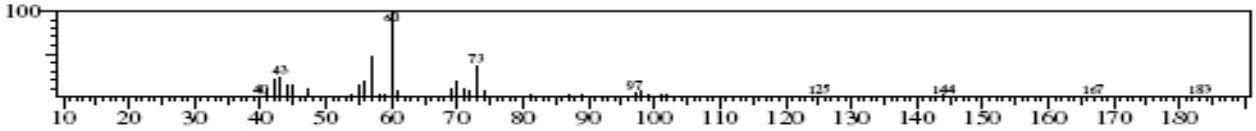


Fig 9: Molecular formula, M.W and Molecular structure of the eighth compound

Line # 9 R.Time:11.592(Scan#:1032) MassPeaks:61

Raw Mode:Averaged 11.583-11.600(1031-1033) BasePeak:60.00(126306)

BG Mode:Calc. from Peak



Hit#:2 Entry:30472 Library:NIST08.LIB

SI:95 Formula:C6H12O6 CAS:2595-97-3 MolWeight:180 RetIndex:1698

CompName:D-Allose $$ .beta.-D-Allose $$ Hexose # $$

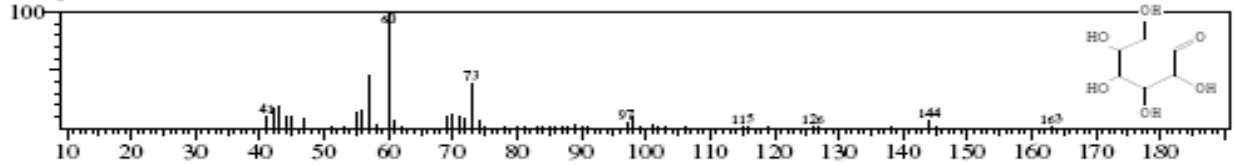
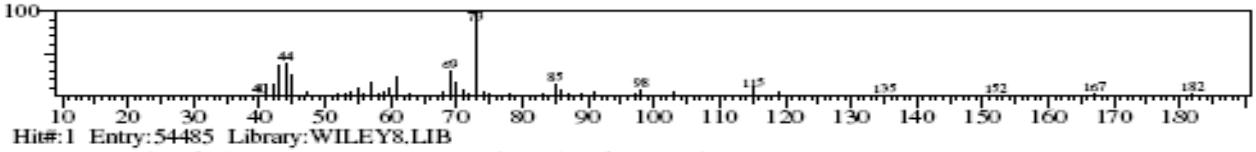


Fig 10: Molecular formula, M.W and Molecular structure of the ninth compound

Line # 10 R.Time:12.800(Scan#:1177) MassPeaks:52

Raw Mode:Averaged 12.792-12.808(1176-1178) BasePeak:73.00(31334)

BG Mode:Calc. from Peak



Hit#:1 Entry:54485 Library:WILEY8.LIB

SI:90 Formula:C6H10O5 CAS:7425-74-3 MolWeight:162 RetIndex:0

CompName:1,6-ANHYDRO-.BETA.-D-GLUCOFURANOSE $$ 1,6-ANHYDRO-BETA-D-GLUCOFURANOSE

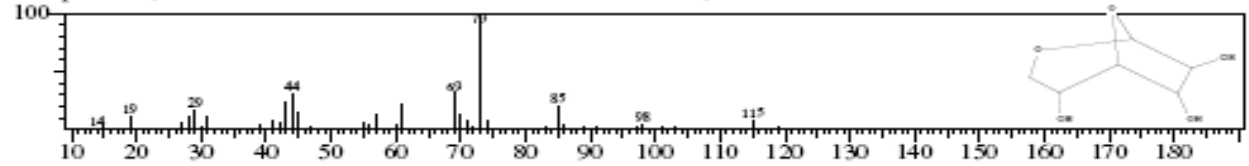
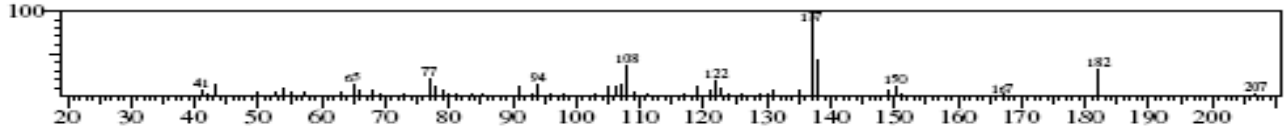


Fig 11: Molecular formula, M.W and Molecular structure of the tenth compound

Line # 11 R.Time:13.400(Scan#:1249) MassPeaks:56

Raw Mode:Averaged 13.392-13.408(1248-1250) BasePeak:137.05(12600)

BG Mode:Calc. from Peak



Hit#:1 Entry:32145 Library:NIST08.LIB

SI:74 Formula:C10H14O3 CAS:0-00-0 MolWeight:182 RetIndex:1478

CompName:Methyl-(2-hydoxy-3-ethoxy-benzyl)ether

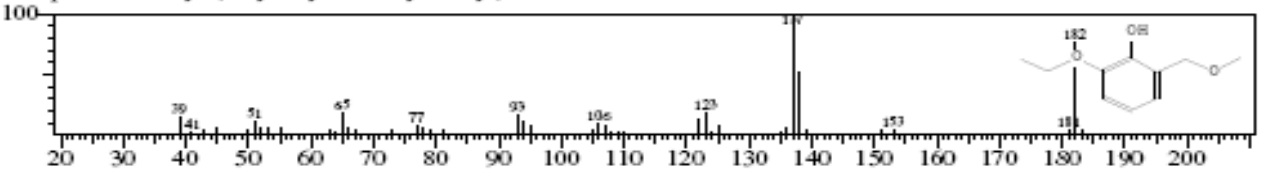
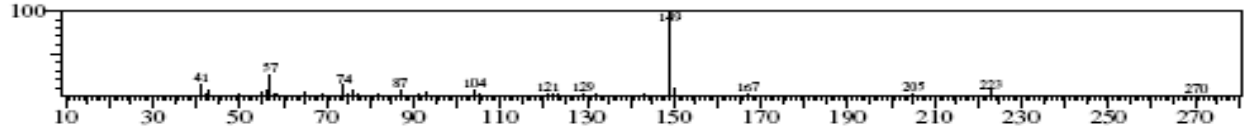


Fig 12: Molecular formula, M.W and Molecular structure of the eleventh compound

Line # 12 R.Time:15.650(Scan#:1519) MassPeaks:60

Raw Mode:Averaged 15.642-15.658(1518-1520) BasePeak:149.00(78032)

BG Mode:Calc. from Peak



Hit#:1 Entry:211760 Library:WILEY8.LIB

SI:89 Formula:C16H22O4 CAS:84-69-5 MolWeight:278 RetIndex:0

CompName:1,2-BENZENEDICARBOXYLIC ACID, BIS(2-METHYLPROPYL) ESTER $$ 1,2-BENZENEDICARBO

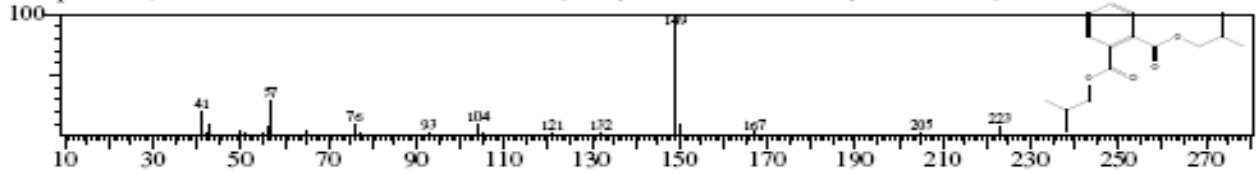
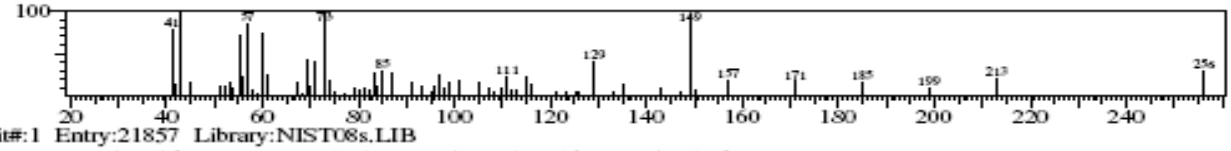


Fig 13: Molecular formula, M.W and Molecular structure of the twelfth compound

Line # 13 R.Time:16.625(Scan#:1636) MassPeaks:66
Raw Mode:Averaged 16.617-16.633(1635-1637) BasePeak:73.05(9161)
BG Mode:Calc. from Peak



Hit#:1 Entry:21857 Library:NIST08s.LIB
SI:84 Formula:C16H32O2 CAS:57-10-3 MolWeight:256 RetIndex:1968
CompName:n-Hexadecanoic acid $$ Hexadecanoic acid $$ n-Hexadecoic acid $$ Palmitic acid $$ Pentadecanecarboxylic

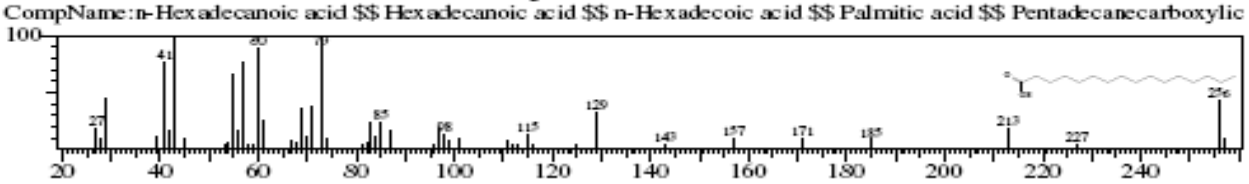
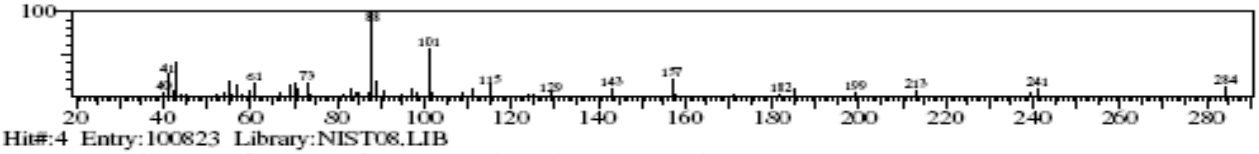


Fig 14: Molecular formula, M.W and Molecular structure of the thirteenth compound

Line # 14 R.Time:16.900(Scan#:1669) MassPeaks:59
Raw Mode:Averaged 16.892-16.908(1668-1670) BasePeak:88.10(20824)
BG Mode:Calc. from Peak



Hit#:4 Entry:100823 Library:NIST08.LIB
SI:92 Formula:C18H36O2 CAS:628-97-7 MolWeight:284 RetIndex:1978
CompName:Hexadecanoic acid, ethyl ester $$ Palmitic acid, ethyl ester $$ Ethyl hexadecanoate $$ Ethyl palmitate $$

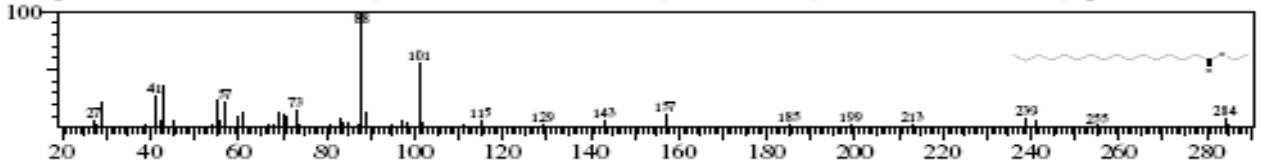
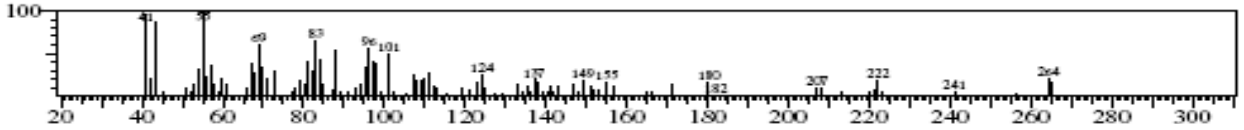


Fig 15: Molecular formula, M.W and Molecular structure of the fourteenth compound

Line # 15 R.Time:18.542(Scan#:1866) MassPeaks:98
Raw Mode:Averaged 18.533-18.550(1865-1867) BasePeak:55.05(8568)
BG Mode:Calc. from Peak



Hit#:1 Entry:24809 Library:NIST08s.LIB
SI:87 Formula:C20H38O2 CAS:111-62-6 MolWeight:310 RetIndex:2185
CompName:Ethyl Oleate $$ 9-Octadecenoic acid (Z)-, ethyl ester $$ Oleic acid, ethyl ester $$ (Z)-9-Octadecenoic acid etl

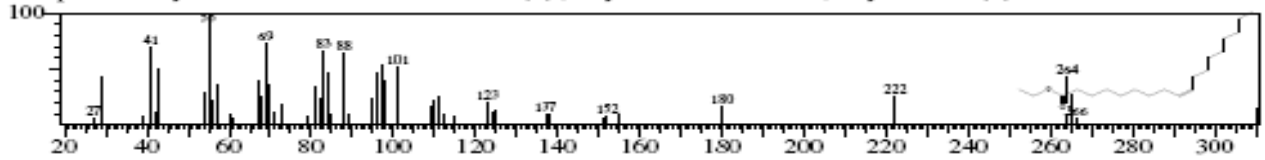
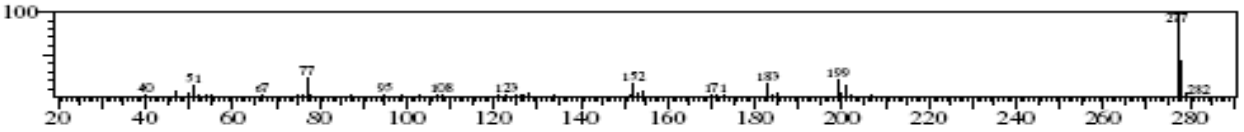


Fig 16: Molecular formula, M.W and Molecular structure of the fifteenth compound

Line# 16 R.Time:21.750(Scan#:2251) MassPeaks:80
Raw Mode:Averaged 21.742-21.758(2250-2252) BasePeak:277.05(38354)
BG Mode:Calc. from Peak



Hit#:1 Entry:212198 Library:WILEY8.LIB
SI:89 Formula:C18H15OP CAS:0-00-0 MolWeight:278 RetIndex:0
CompName:TRIPHENYLPHOSPHINE OXIDE

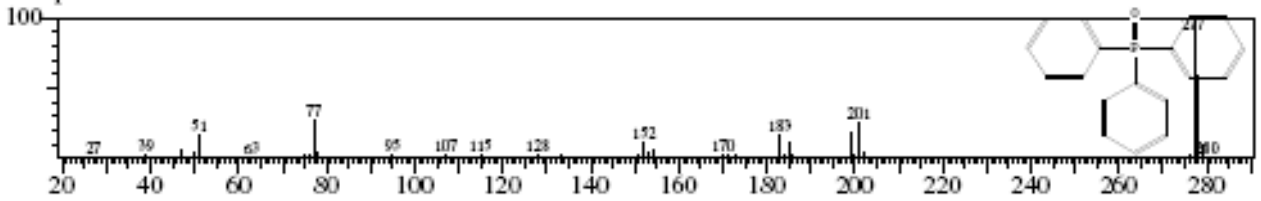


Fig 17: Molecular formula, M.W and Molecular structure of the sixteenth compound

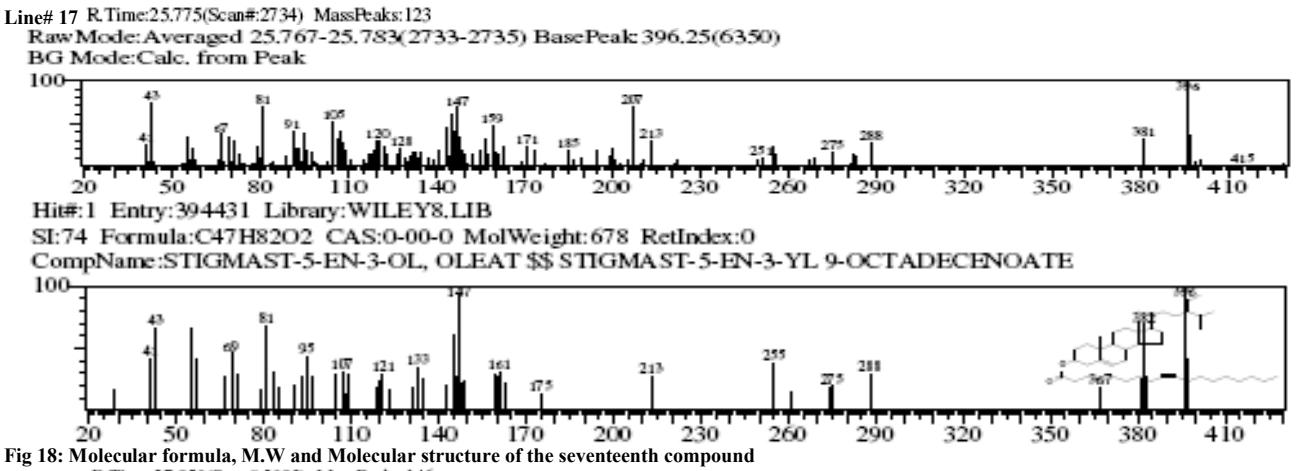


Fig 18: Molecular formula, M.W and Molecular structure of the seventeenth compound

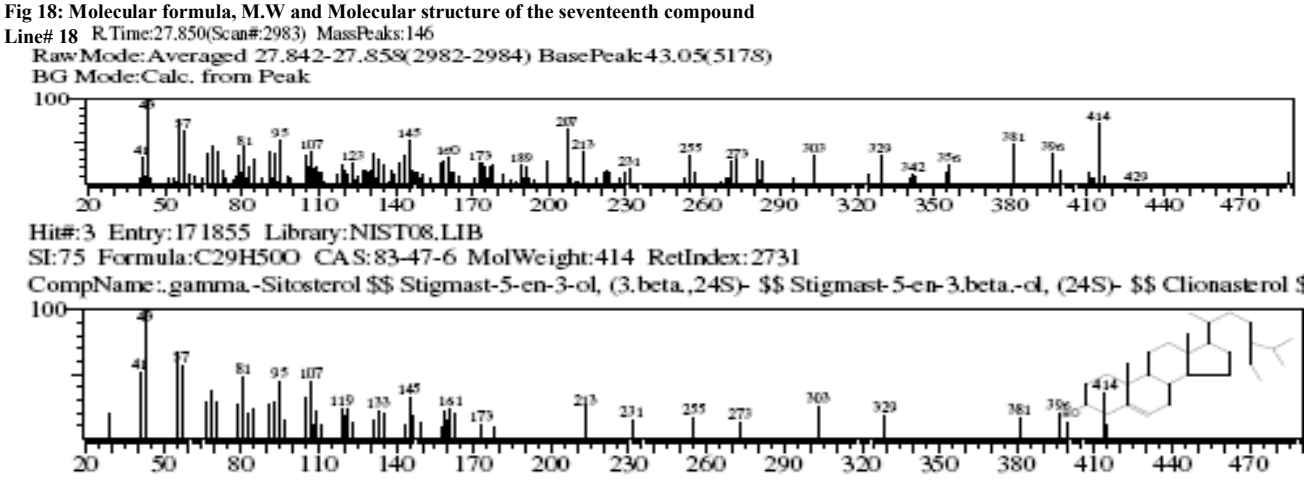


Fig 19: Molecular formula, M.W and Molecular structure of the eighteenth compound

Identification of components

Interpretation of mass spectrum GC-MS was conducted using the database of National Institute Standard and Technique (NIST08s), WILEY8 and FAME having more patterns. The spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST08s, WILEY8 and FAME library. The Name, Molecular weight, Molecular formula and Structure of the component of the test material was ascertained.

RESULT AND DISCUSSION

Eighteen compounds were identified in ethyl acetate fraction of *Punica granatum* rind extract by GC-MS analysis. The chromatogram obtained by ethyl acetate fraction of *Punica granatum* rind was shown in Fig. 1.The active principle, area of the peak, Concentration (%) and Retention Time (RT) are presented in Table 1. The Molecular Weight, Molecular Formula and its structure of the compounds are presented in Fig. 2-19. The prevailing compounds were Pyrogallol (41.88%), 5-Hydroxymethylfurfural (14.10%), D-Allose (9.17%), 2-Methoxy-1, 4-Benzenediol (8.34%) and 2, 3 Dimethylfumaric acid (3.96%).

The present investigation clearly indicates the highest percentage of Pyrogallol compound in ethyl acetate fraction of *Punica granatum* rind extract. The Pyrogallol as various biological activity like Allelochemic; Antibacterial; Abortifacient; Anticlastogen; Antidermatitic; Antilupus; Antimutagenic; Antioxidant; Antipsoriac; Antiseptic; CNS-Active; Candidicide; Cardiovascular; Dye; Ecbolic; Fungicide; Insulin-Sparing; Irritant; Nephrotoxic; Nigrifacient; Pesticide; Prostaglandin-Synthesis-Inhibitor from Dr. Duke's Phytochemical and Ethnobotanical Database. So the compounds present in the active fraction are responsible for the activity of the rind extract.

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