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GC-MS analysis of bioactive components in Jamun, Amla and Kiwi fruits

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Abstract
Fruits are a rich source of a variety of vitamins, minerals, dietary fibre and many other classes of bioactive compounds collectively called phytochemicals. Traditionally, fruits were used for treatment of numerous disorders due to the presence of phytochemicals in addition to micronutrients. In this study, three fruits *viz*., Jamun (*Syzygium cumini*), Amla (*Emblica officinalis*) and Kiwi (*Actinidia deliciosa*) were analysed. Preliminary phytochemical screenings of aqueous extract of fruits were carried out according to standard procedure. Qualitative analysis revealed the presence of phenols, terpenoids, tannins, flavonoids, steroids, alkaloids, carbohydrates, amino acids and carotenoids. Various bioactive compounds were identified from the aqueous extracts of fruits by GC-MS analysis. The identification of these compounds present in aqueous fruit extracts were based on direct comparison of the retention times and mass spectral data as well as by comparison of the mass spectra. This analysis showed the presence of twenty-two chemical compounds and supports the pharmaceutical use of these fruits as medicine.

Keywords: GC-MS analysis, fruit extracts and bioactive compounds

1. Introduction
E. officinalis or Indian gooseberry, commonly known as Amla, Nelli and Amalaki, belongs to the family *Euphorbiaceae*. It is one of the three constituents of 'Tribhala', a rejuvenating medicinal formula, popular for its effectiveness in the treatment of cancer, diabetes, liver, ulcer, anaemia, heart trouble and also is an important constituent in hepato protective formulas available in market (Panda and Kar, 2003) [7]. Amla possess many active phytochemical components like gallic acid, tannin, flavonoids, emblicanin A and B and ellagic acid (Varghese *et al*, 2013) [18].
Jamun (*Syzygium cumini*) commonly known as Indian blackberry, is an underutilized fruit from the Indian subcontinent and it belongs to the *Myrtaceae* family. The jamun fruits were available abundantly during the summer season for a short period. The fruits are also used for the treatment of various diseases as an astringent, antiscorbutic, diuretic, antidiabetic, and in chronic diarrhoea and enlargement of the spleen (Chaturvedi *et al.*, 2009 and Achrekar *et al*., 1991) [4, 1]. Jamun fruits contain high amounts of vitamins, minerals, fibre and are low in calories and fat (Baliga *et al*., 2010) [3].
Kiwifruit (*Actinidia deliciosa*) is a worldwide known fruit among the genus Actinidia (*Actinidiaceae*). Kiwifruit is a very well-known fruit with excellent bioactive properties associated with good health attributes such as healthful skin, better sleep contributed by antioxidants and serotonin content of kiwifruit. Kiwi fruit were a highly nutritious fruit due to its high level of vitamin C and its strong antioxidant including carotenoids, phenolics, flavonoids and chlorophyll. Kiwi fruit was a wealthy source of vitamins E, fructose, galactose and minerals, it contains isoflavones, flavonoids, which were important phytochemical in kiwi extract (Shehata and Soltan, 2013) [12].
Fruits and vegetables have historically been considered rich sources of some essential dietary micronutrients and fibres, and a wide array of phytochemicals that individually, or in combination, may benefit health (Stavric 1994; Rechkemmer 2001) [15, 8]. Experimental dietary studies in animals, cell models and humans demonstrate the capacity of some of these constituents of fruits and vegetables to modify antioxidant pathways, detoxification enzymes, the immune system, cholesterol and steroid hormone concentrations, and blood pressure, and their capacity to act as antioxidant, antiviral and antibacterial agents. Keller and Tukuitonga (2007) [6] stated that 'Low fruit and vegetable intake was identified as an important risk factor for chronic diseases in the WHO World Health Report 2002. Overall, it is estimated that up to 2.7 million lives could potentially be saved each year if fruit and vegetable consumption was

sufficiently increased. Fruit and vegetable consumption has been linked to reduced cardiovascular disease and stroke. In addition, a new scientific base is emerging to support a protective role for fruits and vegetables in prevention of cataract formation, age-related macular degeneration, chronic obstructive pulmonary disease and other digestive disorders, and possibly hypertension.

The emphasis on the micro components of fruits and vegetables in no way implies that the macronutrients lack importance with regard to human health and well-being. It reflects the keen interest that currently exists in possible relationships between the content and profile of the minor constituents in food plants and the prevention of chronic disease. The papers published in recent decades on medicinal plants relate to the function of various plant bioactive composites commonly used in the cure of numerous human diseases. The aim of this work is to analyze and characterize the bioactive compounds of fruits *viz*., jamun, amla and kiwi using GC-MS.

2. Materials and Methods

2.1 Collection of Fruits

Amla and Jamun were acquired from the local market in the season of April and May. Kiwi fruits were procured from Koyambedu market, Chennai.

2.2 Preparation of Fruit Extract

The fruits were thoroughly washed first with tap water and then distilled water separately. The whole fruits were pureed well, using a juicer and then filtered through a sterilized mesh cloth, to separate the aqueous fraction of fruit of particles. The extracts were concentrated under reduced pressure in a rotary evaporator. The aqueous extracts were used for GC-MS analysis.

2.3 Preliminary Phytochemical Screening of Amla, Jamun and Kiwi

Five millilitre of each fruit extract was taken in 250 ml conical flasks and added 50 ml of distilled water, acetone, ethyl acetate, methanol and ethanol each at a proportion of 1:10. The flasks were stoppered tightly and kept in a refrigerator for 48 hrs. The content of the flasks was shaken intermittently during the maceration period. After 48 hrs the contents in the flasks were filtered through Whatmann filter paper No.1. The final filtrates were transferred to the sterilized container and were subjected to series of chemical tests to detect the presence and/or absence of various active principles *viz*. alkaloids, proteins, reducing sugars, tannins, sterols, phenolic compounds, flavonoids, tritrepinoids and saponins (Trease and Evans, 2002; Harborne, 1998 and Sofowora, 1993) [16, 5, 13].

2.4 Gas Chromatography – Mass Spectrometry (GC – MS) Analysis

An Agilent 6890 gas chromatograph was equipped with a straight deactivated 2 mm direct injector liner and a15m Alltech EC-5 column (250µ I.D., 0.25µ film thickness). A split injection was used, for sample introduction and thesplit ratio was set of 10:1. The oven temperature program was programmed to start at 35 C, hold for 2 minutes, then rampat 20 °C per minute, to 300 °C and hold for 5 minutes. The helium carrier gas was set to 2 ml/minute flow rate (constant flow mode).A JEOL GCmate II bench top double-focusing magnetic sector mass spectrometer, operating in electron ionization (EI) mode with TSS-20001 software was used, for all analyses. Low-resolution mass spectra were acquired, at a resolving power of 1000 (20% height definition) and scanning from m/z 25 to m/z 700 at 0.3 seconds per scan, with a 0.2 secondinter-scan delay. High resolution mass spectra were acquired at a resolving power of 5000 (20% height definition) and scanning the magnet from m/z 65 to m/z 750, at 1 second per scan.

Interpretation of mass spectrum GC-MS was conducted, using the database of National Institute Standard and Technology (NIST), having more than 62,000 patterns. The spectrum of the unknown component was compared to theNIST library. The Name, Molecular weight and structure of the components of the test Material, were ascertained. Theentire analysis was done in SAIF facility, available at IIT Madras.

3. Results and Discussions

3.1 Phytochemical analysis of amla, jamun and kiwi

The results of qualitative tests for detection of phytochemicals present in the different solvent extract of the fruits are shown in Table 1. The qualitative tests were carried to find out the presence of active principles *viz.* phenols, alkaloids, terepenoids, saponin, flavonoids, steroids, tannins, carbohydrates, proteins, amino acids and carotenoid compounds with different solvent extracts of selected fruits.

Table 1: Phytochemical analysis of Amla, Jamun and Kiwi [@]

Parameters	Tests	Amla					Jamun					Kiwi				
		A	B	C	D	E	A	B	C	D	E	A	B	C	D	E
Phenols	Ferric chloride Test	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Terpenoids	Salkowski's Test	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Tannins	Gelatin Test	+	+	+	+	+	+	+	+	+	+	-	-	-	-	+
Saponins	Foam test	+	+	+	+	+	-	-	-	-	+	-	-	-	-	-
Flavonoids	Alkaline Reagent Test	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Steroids	Libermann Test	-	-	-	-	-	+	+	+	+	+	+	+	+	+	+
Alkaloids	Mayer's Test	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+
	Wagner's Test	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	Dragendroff's Test	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carbohydrate	Molisch's Test	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	Benedict's Test	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	Fehling's Test	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Proteins	Biruet Test	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Amino acids	Ninhydrin Test	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carotenoids	Test for Carotenoids	-	-	-	-	-	+	+	+	+	+	+	+	+	+	+

@- Average of six trials

A-Aqueous, B-Acetone, C-Ethanol, D-Methanol & E-Ethyl acetate

\+ Present, - Absent

From table 1 shows the active principles *viz*., phenols, alkaloids, terepenoids, flavonoids, carbohydrates, proteins, amino acids are present in amla, jamun and kiwi. Steroids and Carotenoids are absent in amla which correlated with the findings recorded by Badoni *et al* (2016) [2]. Saponin are absent in both jamun and kiwi which were similar to Reginold *et al*., (2013) [9] and Soham *et al*., (2020) [14]. Tannin is absent in kiwi and similar results were reported by Salawu *et al*., (2014) [11] and Salama Zeinab *et al*., (2018) [10].

3.2 Identification of bioactive compounds of Amla -GC-MS Analysis

Figure 1 shows the GC- MS chromatogram of aqueous extract of amla along with their retention time (RT). Table 2 shows the major phyto-components present in amla along with

retention time, peak area percent of peak area, molecular formula, molecular weight and structure. The GC-MS chromatogram of amla showed the presence of various bioactive compounds in aqueous extract of amla.

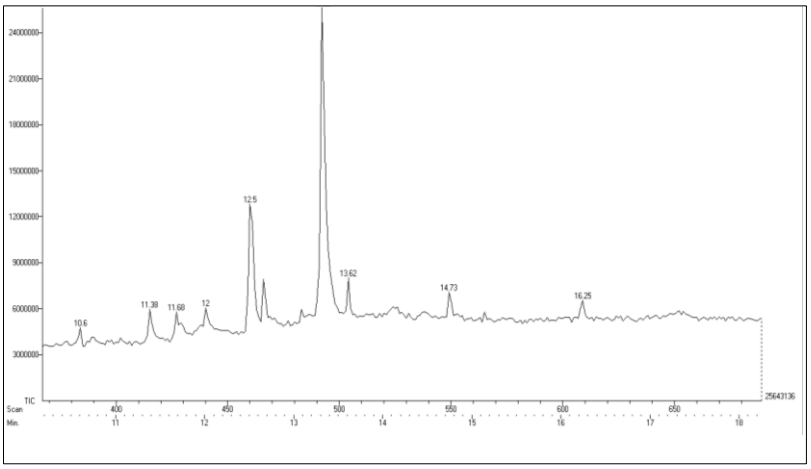
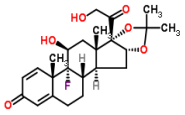
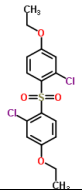
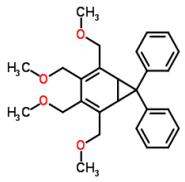
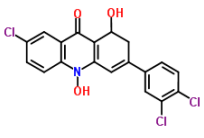
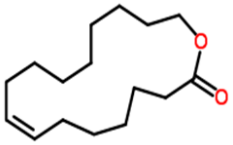
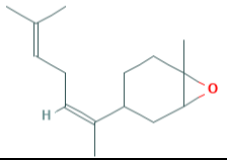
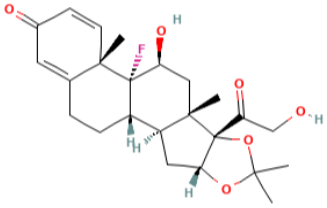
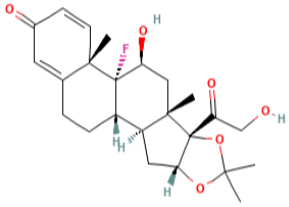
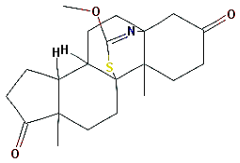


Fig 1: GC-MS Chromatogram of aqueous extract of amla

Table 2 shows the presence of Triamcinolone acetonide, Bis [2-chloro-4-ethoxyphenyl] sulfone, Bicyclo [4.1.0] hepta-2,3,4,5-tetrakis (methoxymethyl) -7,7-diphenyl, 7-chloro-3-[3,4-dichlorophenyl] -1,10-dihydroxy-9[2H]- acridinone, Oxacycloheptadec-8-en-2-one, Trans-Z-a-Bisabolene epoxide, Triamcinolone acetonideand (2-Methoxy-1,3,2-thiozin) [5,10,9] androstan-3,17-dione in aqueous extract of amla.

Table 1: Compounds identified in aqueous extract of amla

RT	Name of the bioactive compounds	Peak area	Peak area %	Molecular formula	Molecular weight	Structure
10.6	Triamcinolone acetonide	4769392	5.767755	$C_{24}H_{31}FO_6$	434.497	
11.38	Bis[2-chloro-4-ethoxyphenyl]sulfone	5943936	7.188163	$C_{16}H_{16}Cl_2O_4S$	375.266	
11.68	Bicyclo[4.1.0] hepta-2,3,4,5-tetrakis (methoxymethyl)-7,7-diphenyl	5800144	7.014272	$C_{27}H_{32}O_4$	420.541	
12.00	7-chloro-3-[3,4-dichlorophenyl]-1,10-dihydroxy-9[2H]-acridinone	6061568	7.330419	$C_{19}H_{12}Cl_3NO_3$	408.663	
12.5	Oxacycloheptadec-8-en-2-one	12822432	15.50652	$C_{16}H_{28}O_2$	252.392	

13.32	Trans-Z-a-Bisabolene epoxide	25643136	31.01094	$C_{15}H_{24}O$	220.35	
13.62	Triamcinolone acetonide	8003024	9.678274	$C_{24}H_{31}FO_6$	434.5	
14.73	Triamcinolone acetonide	7095840	8.581192	$C_{24}H_{31}FO_6$	434.5	
16.25	(2-Methoxy-1,3,2-thiozin) [5,10,9]androstan-3,17-dione	6551136	7.922467	$C_{21}H_{29}NO_3S$	375.52486	
	Total	82690608	100	-	-	-

3.3 Identification of bioactive compounds of Jamun-GC-MS Analysis

Figure 2 shows the GC- MS chromatogram of aqueous extract of jamun along with their retention time (RT). Table 3 shows the major phyto-components present in jamun along with retention time, peak area, molecular formula, molecular weight and structure. The GC-MS chromatogram of jamun showed the presence of various bioactive compounds in aqueous extract of jamun.

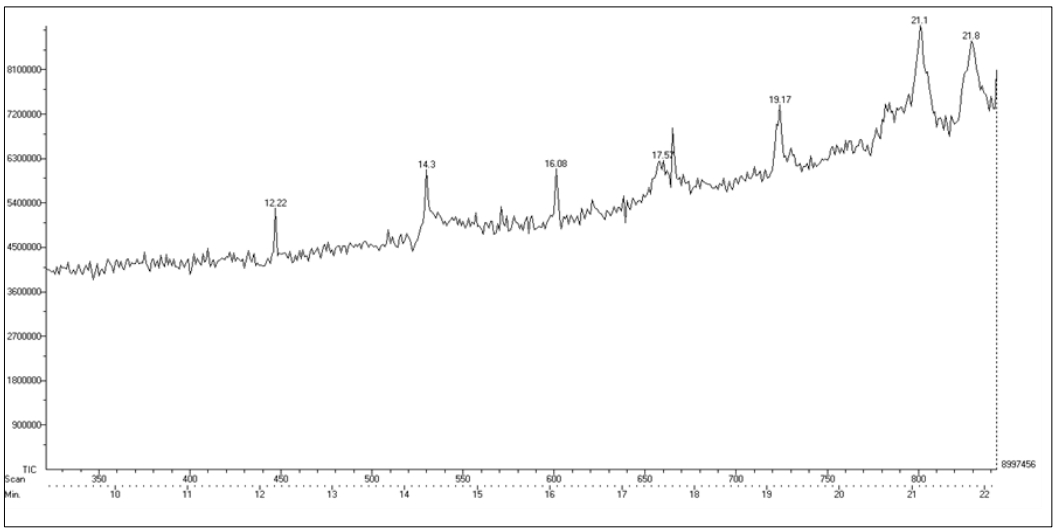
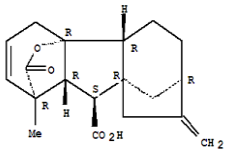
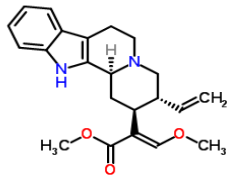
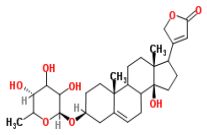
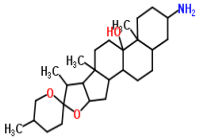
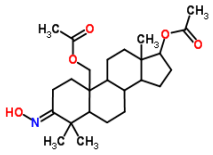
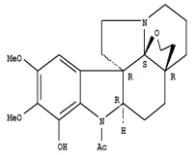
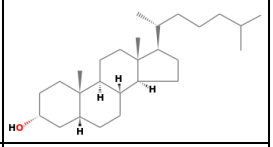


Fig 2: GC-MS Chromatogram of aqueous extract of jamun

Table 3 shows the presence of Gibb-3-ene-1,10-dicarboxylic acid, 2,4a-dihydroxy-1-methyl-8-methylene-,1,4a-lactone,10-methyl ester, (1a, 2a, 4aa, 4b, 10a)-, 18,19-Secoyohimban-19-oi acid,16,17,20,21-tetradehydro-16-(hydroxymethyl)-,methyl ester, (15a,16E)-, Carda-5,20 (22)-dienolide, 3-[(6-deoxy-a-L-mannopyranosyl)oxy]-14-hydroxy-,(3a)-,Spirostan-9-ol,3-amino-,3a,5a,25R)-, Aceticacid,17- acetoxy3 hydroxyimino-4,4,13-trimethyl-hexadecahydrocyclopenta(a)phenanthren-10-ylmethylester, Aspidosermidin-17-ol, 1-acetyl-19,21-epoxy-15,16-dimethoxy- and Cholestan-3-ol,2-methylene-, (3a,5a)-in aqueous extract of jamun.

Table 2: Compounds identified in aqueous extract of jamun

RT	Name of the bioactive compounds	Peak area	Peak area %	Molecular formula	Molecular weight	Structure
12.22	Gibb-3-ene-1,10-dicarboxylic acid, 2,4a-dihydroxy-1-methyl-8-methylene-, 1,4a-lactone,10-methyl ester, (1a,2a,4aa,4b,10a)-	5294912	10.85203	$C_{19}H_{22}O_4$	314.3756	
14.3	18,19-Secoyohimban-19-oi acid,16,17,20,21-tetradehydro-16-(hydroxymethyl)-,methyl ester,(15a,16E)-	6071104	12.44285	$C_{22}H_{26}N_2O_3$	366.453	
16.08	Carda-5,20 (22)-dienolide, 3-[(6-deoxy-a-L-mannopyranosyl)oxy]-14-hydroxy-, (3a)-	6092384	12.48646	$C_{29}H_{42}O_8$	518.63898	
17.57	Spirostan-9-ol,3-amino-, (3a,5a,25R)-	6268912	12.84826	$C_{27}H_{45}NO_3$	431.651	
19.17	Acetic acid,17-acetoxy-3-hydroxyimino-4,4,13-trimethyl-hexadecahydrocyclopenta(a)phenanthren-10-ylmethyl ester	7381248	15.12802	$C_{25}H_{39}NO_5$	433.581	
21.1	Aspidosermidin-17-ol, 1-acetyl-19,21-epoxy-15,16-dimethoxy-	8997456	18.44047	$C_{23}H_{30}N_2O_5$	-	
21.8	Cholestan-3-ol,2-methylene-, (3a,5a)-	8685888	17.8019	$C_{27}H_{48}O$	388.66	
	Total	48791904	100	-	-	-

3.4 Identification of bioactive compounds of Kiwi-GC-MS Analysis

Figure 3 shows the GC- MS chromatogram of aqueous extract of kiwi along with their retention time (RT). Table 4 shows the major phyto-components present in kiwi along with retention time, peak area, molecular formula, molecular weight and structure. The GC-MS chromatogram of kiwi showed the presence of various bioactive compounds in aqueous extract of kiwi.

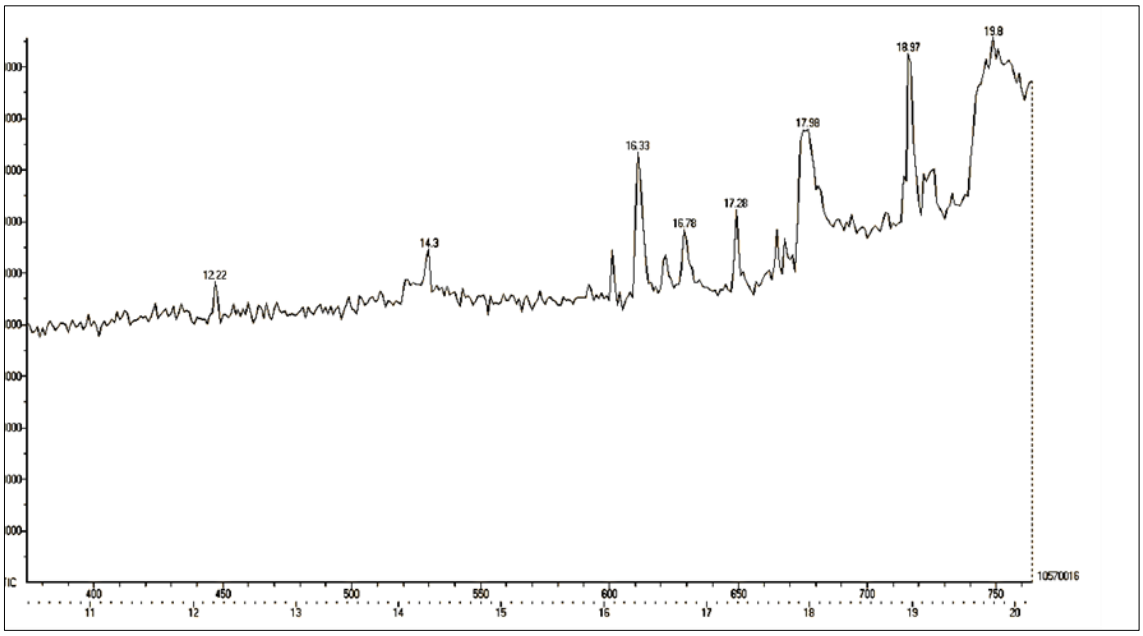
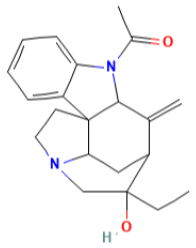
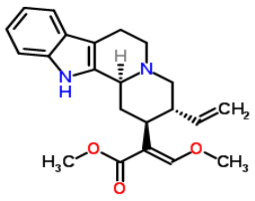
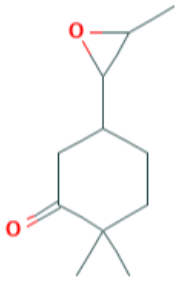
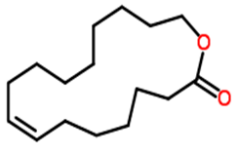
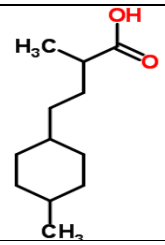
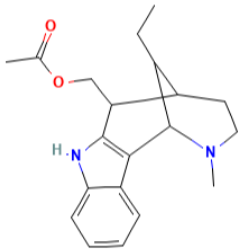
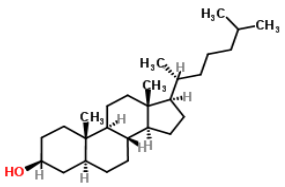
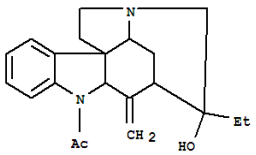


Fig 3: GC-MS Chromatogram of aqueous extract of kiwi

Table 4 shows the presence of Strychane,1-acetyl-20a-hydroxy-16-methylenre -, 18, 19-Secoyohimban-19- oic, 16, 17, 20, 21- tetradehydro-16- (hydroxymethyl)-,methylester,(15a,16E), Cyclohexanone,2,2-dimethyl-5- [3-methyloxianyl], [2a (R*),3a], Oxacycloheptadec-8-en-2-one, Cyclohexanebutanoic acid, a,4-dimethyl, Dasycarpidan-1-methanol, acetate (ester), Oxacyclotetradecane-2,11-dione,13-methyl and Dasycarpidan-1-methanol, acetate (ester) in aqueous extract of kiwi.

Table 4: Compounds identified in aqueous extract of kiwi

RT	Name of the bioactive compounds	Peak area	Peak area %	Molecular formula	Molecular weight	Structure
12.22	Strychane,1-acetyl-20a-hydroxy-16-methylenre-	5828736	9.061889	$C_{21}H_{26}N_2O_2$	338.4	
14.3	18,19-Secoyohimban-19-oic,16,17,20,21-tetradehydro-16-(hydroxymethyl)-,methyl ester,(15a,16E)	6457168	10.03891	$C_{22}H_{26}N_2O_3$	366.453	
16.33	Cyclohexanone,2,2-dimethyl-5- [3-methyloxianyl], [2a(R*),3a]	8350016	12.9817	$C_{11}H_{18}O_2$	182.26	

16.78	Oxacycloheptadec-8-en-2-one	6840864	10.63544	$C_{16}H_{28}O_2$	252.392	
17.28	Cyclohexanebutanoic acid, a,4-dimethyl	7225632	11.23363	$C_{12}H_{22}O_2$	198.302	
17.98	Dasycarpidan-1-methanol, acetate (ester)	8785424	13.65863	$C_{20}H_{26}N_2O_2$	326.4	
18.97	Oxacyclotetradecane-2,11-dione,13-methyl	10263568	15.95669	$C_{27}H_{48}O$	388.669	
19.8	Dasycarpidan-1-methanol, acetate (ester)	10570016	16.43312	$C_{21}H_{26}N_2O_2$	-	
	Total	64321424	100	-	-	-

4. Conclusion

The bioactive compounds were extracted through different solvents. The phytochemical analysis showed that the fruits are rich in alkaloids, flavonoids phenols, terepenoids, flavonoids, carbohydrates, proteins, amino acids, carotenoids and steroids. GC-MS study showed various bioactive compounds present in the selected fruits. Isolation of individual compounds from the fruits and subjecting it to the pharmacological activity will definitely give fruitful results.

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