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Bio activity study of *Momordica dioica* Rox.ex willd. Rispa johns, Arun O.S, Dr. Sarala Samuel. Kerala Ayurveda Ltd.

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Abstract
The work aims to evaluate the bioactive study of *Momordica dioica* rox.ex willd by analysing antioxidant, anti-bacterial activity and cytotoxic effect. Separation of components using GC-MS analysis. *Momordica dioica* Fruit have good medicinal value and rich with number of phytoactives but not well explored. For its medicinal activity *Momordica dioica* Fruit is taken for this study and extracted its constituents sequentially using solvents of petroleum ether, chloroform, ethyl acetate, ethanol and distilled water. Phytochemical screening shows the presence of different phytoactives in different solvent extract. The GC MS study also proves the presence of therapeutically active component. DPPH activity and IC50 value prove the anti oxidant activity of the study material. Anti microbial activity revels the anti microbial activity against *E.aerogenes, K.pneumoniae, S.aureus, E.coil, P.aeruginosa, proteus species* and cytotoxicity effect proved by the *In vitro* cytotoxic study respectively.

Keywords: *Momordica Dioica* Rox. ex willd, antioxidant activity, anti-bacterial activity, cytotoxic effect and separation of components using GC-MS analysis.

Introduction
Medicinal plants have been subjects of man's curiosity since time immemorial. Almost every civilisation has a history of medicinal plant use [3]. The ethno botany provides a rich resource of natural drug research development [4]. But it was only in period of the Ayurveda samhitas that there were serious attempts in studying plants scientifically [1].
Ayurveda system has described a large number of such medicines based on plants or plant product and the determination of their morphological, pharmacological, pharmacogonstical characters can provide a better understanding of their active principles and mode of action [8].
Momordica dioica belongs to the family Cucurbitaceae and under the genus Momordica, a genus of annual or perennial, dioceous climbers that contains about 80 species. Fruits are green and generally used as vegetable. It possesses many medicinal properties. Fruits are diuretic, alexiteric stomachic laxative, hepatoprotective, and have antivenum property. It is also used to cure asthma, leprosy, excessive salivation, prevent the inflammation caused by lizard, snake bite, elephantiasis, fever, mental disorders, digestive disorders and troubles of heart and to treat discharge from mucous membrane [7].
Fresh fruit juice is prescribed for hypertension. The fruit is cooked in a small amount of oil and consumed for treating diabetes. Tender fruits are rubbed on skin for pimples and acne. Seeds are roasted and taken for eczema and other skin problems. The powder or infusion of the dried fruits, when introduced into the nostrils produces a powerful errhine effect.

Plant Profile
Classification
Class - Dicotyledonae
Subclass - Polypetalae
Order - Cucurbitales
Family - Cucurbitaceae
Genus - *Momordica*
Species - *dioica*

Common names
English - Small bittergourd,spine gourd
Hindi - Kakora, Parora, Golbandra
Malyalam - Venpaval, Erima pasel
Sanskrit -vahisi

Fig 1: Habit of *Momordica dioica*

Materials and methods

Collection of plant materials

The fruit of *Momordica dioica* were collected from peechi forest the month of January and were identified in the Department of Botany, St. Mary's College, Thrissur Kerala. The plant was further authenticated with the help of Flora of presidency of madras by J.S Gamble., and 1995.The plant name was picked with international plant names index (IPNI). Vocher specimen (Vr. No. 003) was dried and preserved on herbarium and was deposited to the herbarium collection of Department of Botany St. Mary's college Thrissur.

Preparation of Extracts

The shade dried plant material was ground to a coarse powder and 50gm of the powdered material was soaked in solvents of increasing polarity starting petroleum ether, chloroform, ethyl acetate, ethanol and distilled water (1:5) for 75hours. The solvent was then removed by rotary evaporation. Each residue was weighed and the yield percentage was determined. Percentage yield was calculated as below.

Extract yield %=$(W1/W2) \times 100$

Where W1 is net weight of powder in grams after extraction and W2 is total weight of powder in grams taken before extraction.

Qualitative phytochemical screening of plant extracts

Qualitative analysis of the petroleum ether, chloroform, ethyl acetate, ethanol and distilled water of the fruit of *M.dioica* were further carried out to test the presence of phytochemicals such as alkaloids, flavonoids, terpenoids, sterols, tannins, glycosides, phenols etc. using standard protocol given by (Harborne 1998) [5]. The following tests were done for the preliminary phytochemical screening

Test for primary and secondary metabolites

Test for carbohydrates

Molisch's test

A few drops of molisch's solution was added to 2ml of the extract, thereafter a small volume of concentrated sulphuric acid was allowed to run down the side of the test tube to form a layer without shaking. The interface was observed for a purple cum violet colour ring as indicative of positive for carbohydrates.

Test for Sugar

Benedict's test

To 1ml of extract, add 5ml of Benedict's reagent and boil for 5 minutes. Depending on the concentration, the amount and colour of the precipitate produced varied. A positive Benedict's test appears green, yellow, orange or red.

Test for Reducing Sugar

Two millimetres (2ml) of the extract in a test tube was added into 5ml mixture of equal volumes of Fehling's solution s 1 and 2 and boiled in a water bath for about 2 min. The brick red precipitate was indicative of the presence of reducing sugars.

Seliwanoff's test

To 3 ml of Seliwanoff reagent, 1ml of plant extract was added and heated on water bath for 2 min. the change in colour to red indicates the presence of hexose sugar.

Test for Amino Acids

Ninhydrin's test

To 1ml of extract, few drops of 0.2% Ninhydrin was added and heated for 5 minutes. Formation of blue colour indicates the presence of amino acid.

Millon's test

To 3ml extract 5ml of million's reagent was added and heated. The appearance of white precipitate which changed to brick red on heating indicates the presence of proteins.

Test for Protein

Biuret test

Test solution was treated with 2ml biuret reagent and boiled for 5 minutes and observed the formation of violet/pink colour.

Xanthoprotein test:

To the 5ml of test solution, added 1ml of concentrated nitric acid and boiled, yellow precipitate is formed. After cooling it, added 40% sodium hydroxide solution, orange colour is formed.

Test for Starch

KI test

5ml extract was treated with the reagent of the starch (iodine). Any shift to blue violet indicates the presence of starch.

Test for Quinone
H_2SO_4 test
To the test solution added 5 drops of $H_2SO_4$and shaken vigorously; a red colour formation indicates the presence of quinone.

Test for Cardiac Glycoside
Keller kiliani test
To the extract added chloroform and evaporate it to dryness. Add o.4ml of glacial acetic acid containing trace amount of ferric chloride. Transfer to a small test tube, added 0.5ml of concentrated sulphuric acid by the side of the test tube. Brown ring observed at the interface, indicates the presence of cardiac glycoside.

Picric acid test
Treat the test solution with picric acid, and orange colour formation indicates the presence of protein.

Test for Steroid
Salkowski test
The extracts (1mg) was taken in a test tube and dissolved with chloroform (10ml), then add equal volume of concentrated sulphuric acid to the test tube by sides. The upper layer in test tube was turns in to red and sulphuric acid layer showed yellow with green fluorescence. It showed the presence of steroids.

Test for Triterpenoids
Salkowski test
5ml of the extract was added to chloroform along with a few drops of conc. Sulphuric acid. The mixture was shaken well and kept aside for some time. Appearance of yellow colour in the lower layer indicates the presence of triterpenoids.

Test for Flavonoids
Alkaline reagent test
The extract (1ml) was taken in a test tube and added few drop of dilute NaOH solution. An intense yellow colour was appeared in the test tube. It became colourless when an addition of a few drops of dilute acid that indicates the presence of flavonoids.

Test for Phenol
Phenol reagent test
To 1ml of extract add 5ml of folin'sciocalteau reagent and 20% sodium carbonate mixed and placed in a water bath. Appearance of blue colour shows the presence of phenol.

Test for Saponins
Foam test
The extract (5ml) was mixed with distilled water (2.5ml) and shaken vigorously until a stable persistent froth. The frothing was mixed with olive oil (2drops) and shaken vigorously. The formation of emulsion indicated the presence of saponins.

Test for Alkaloids
Dragendorff's test
To the 2ml extract, 15ml o 1% Hcl was added. After heating the solution in water bath, dragendroff's reagent was added. Formation of yellow precipitate indicates the presence of alkaloid.

Test for Tannins
$FeCl_3$ test: Each sample (0.25g) was boiled in distilled water (10ml) and then filtered. Few drops of 0.1% ferric chloride solution were added to the filtrate and the change in colour was observed. The presence of brownish green or a blue-black colour confirmed the presence of tannins.

Test for Coumarin
NaOH test
10% NaOH was added to the extract, and chloroform was added. Formation of yellow colour shows the presence of coumarin.

Pharmacological activity screening
1antioxidant property screening
The antioxidant activity of petroleum ether, chloroform, ethyl acetate, ethanol and distilled water extracts of fruit were determined on the basis of their scavenging effects on the stable DPPH free radical. The dried fruit extracts were re-dissolved in dimethyl sulfoxide and to get the solution of 10 mg/10 ml for each extract which was subjected to analysis of *invitro* antioxidant activities.

DPPH Radical scavenging assay
Free radical scavenging activity of the plant extract was assessed on the basis of the radical scavenging effect of the stable 2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH), by a modified method (Braca et al., 2002) [2]. Methanol (900µl) with DPPH solution (6.34µM, 100µl) was taken as control and methanol as blank. The optical density was recorded and % of inhibition was calculated using the formula given below:
Percentage (%) inhibition of DPPH activity =A-B/A×100
Where A is optical density of the control and B is optical density of the sample. Using the DPPPH scavenging activity IC_{50} value was calculated.

Anti Bacterial Activity Screening
The pathogenic strains of bacteria were collected from Polyclinic laboratory, Thrissur. Organisms used were *Enterobacter aerogenes*, *Klebsiella pneumoniae*, *Staphylococcu aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Proteus* sp. The cultures were maintained on agar medium at 4c. Medium selected as nutrient agar and Screening of bacteriotoxicity using Disc diffusion assay method was used to determine the bacteriotoxicity of plant extracts (Heately., 1944).. Gentamycin was used as standard.

***In vitro* cytotoxicity study**
The chloroform extract was studied for short term in vitro cytotoxicity using Dalton's ascites cells (DLA). Cells were aspirated from the peritoneal cavity of tumor bearing mice and it was washed three times using PBS. The viability of cells were checked using trypan blue (cell viability should be above 98%). The cell suspension (1×10^6cells in 0.1m) was added to tubes containing various concentrations of the test compounds and the volume was made up to 1ml using phosphate buffered saline (PBS). Control tubes containing only cell suspension. These assay mixtures was incubated for 3h at 37^0C and then 1ml of trypan blue was added after incubation and the number of dead cell was counted using a haemocytometer (Shrivatava and Ganesh, 2010) [9]. Dead cells take up the blue colour of trypan blue while live cells do not take up the dye. The numbers of the stained and unstained cells were counted separately.
The percentage cytotoxicity was calculated using the formula given below:

$$\% \text{ cytotoxicity} = \left[\frac{\text{No.of dead cells}}{\text{No.of viable cells+no.of dead cells}}\right] \times 100$$

GC-MS (gas chromatography- Mass spectometry) analysis

GC-MS analysis of the chloroform extract of fruit of *M. dioica* was performed GC-MS analysis were conducted using shimadzu GC-MS. using this study Identification of phytocomponents were done.

Results

Yield of Extract

The extraction yields from fruit of *M. dioica* using different solvents are presented in Fig 2.Comparatively, ethanol extract of fruit exhibited higher extraction yield (1.79%) (Table.1).

Table 1: Percentage yield in the sequential extraction of fruit of *M. dioica*

Solvents	Yield Percentage (gm)
Petroleum ether	1.50
Chloroform	0.16
Ethylacetate	0.04
Ethanol	1.79
Distilledwater	1.70

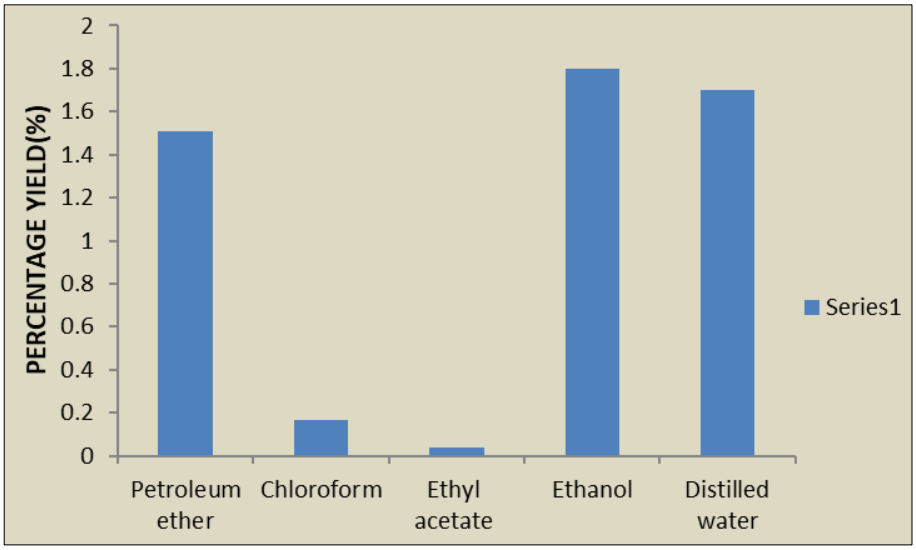

Fig 2: Different solvent extracts

Phytochemical Screening

Petroleum ether, chloroform, ethyl acetate, ethanol, aqueous extracts of *M.dioica* fruit were subjected to preliminary phytochemical screening

Table 2: Phytochemical screening of different fruit extracts of *M.dioica*+ indicates the presence of metabolite, - indicates the absence of metabolite.

Primary /Secondarymetabolites	Petroleumether	Chloroform	Ethyl acetate	Ethanol	Distilledwater
Quinone	+	-	-	+	-
Cardiacglycoside	+	+	-	+	+
Steroid	-	+	+	-	-
Flavanoid	+	+	+	-	-
Alkaloid	+	+	+	+	+
Phenol	-	-	-	-	+
Saponin	+	+	-	+	+
Tannin	-	-	-	-	-
Coumarin	_	+	-	+	+
Terpenoid	-	+	-	-	-
Sugar	+	+	-	+	+
Protein	-	-	-_	-	-
Picric acid	-	-	-	+	+
Starch	-	-	-	-	-
Carbohydrate	-	-	+	-	_
Aminoacid	-	-	-	-	+

Table 3: Percentage inhibition of DPPH free radical by different fruit extracts of *M.dioica* at 517nm.

Sl. No.	Concentration (µg/ml)	Percentage inhibition of DPPH free radical				
		Petroleum ether	chloroform	Ethyl acetate	Ethanol	Distilled water
1	50	14.31±0.63	8.37±0.25	9.11±0.22	5.33±1.04	16.66±0.71
2	100	25.71±0.60	17.34±1.39	22.26±1.40	8.34±1.07	22.83±1.57
3	150	33.85±0.39	22.92±2.39	29.03 ± 0.59	15.67±1.06	30.83±0.59
4	200	36.71±0.75	33.58±0.63	37.62±1.63	22.02±0.35	35.36±1.33
5	250	42.58±2.08	53.29±0.67	48.82±0.39	41.10±0.80	42.33±0.36
6	300	51.5 ± 1.07	58.53±1.64	57.87±0.64	68.55±0.53	44.59±0.62
7	350	59.12±0.40	85.12±1.58	63.27±1.91	74.56±0.65	50.63±0.88

In vitro* Anti-oxidant property screening of fruit of *M.dioica

DPPH free radical scavenging assay

The free radical scavenging activity of different extracts of fruit of *M. dioica* was show in the table 3. Chloroform showed maximum activity of 85.12% at 350µg/ml.

Table 4: Comparison of IC_{50}Values of different fruit extracts of *M.dioica.*

Sl. No.	Solvents	IC_{50} (µ g /ml)
1	Petroleum ether	292.38±4.42
2	Chloroform	242.85±2.34
3	Ethyl acetate	256.91±1.17
4	Ethanol	268.58±1.17
5	Distilled water	345.01±0.69

Values are presented as mean± standard deviation (n=3)

Using this result IC_{50} value were calculated and lower IC_{50} value was observed for Chloroform extract (242.85±2.34) and highest IC_{50} for distilled water extract (345.01±0.69) (Table.4).

Antibacterial activity screening of fruit of *M.dioica*

Using disc diffusion method, the result of antibacterial screening is given in the table (5, 6, 7, 8 and 9) among the tested organisms *Enterobacter aerogenes* shows comparatively higher zone of inhibition (0.99±0.05) as illustrated in Fig: 9, 10, 11, 12 and 13.

Table 5: Antibacterial activity of Petroleum ether extract of *M.dioica,*

Sl. No:	Organism	Zone of inhibition(mm)(Mean ±SD)			
		Gentamycin(25µg)	100µg	250µg	500µg
1	*E. aerogenes*	1.83±0.28	0.73±0.05	0.77±0.06	0.83±0.05
2	*K.pneumoniae*	2.83±0.28	0.57±0.06	0.64±0.05	0.69±0.06
3	*S.aureus*	3.83±0.28	0.67±0.05	0.73 ±0.05	0.87±0.05
4	*E.coli*	3.83±0.28	0.66±0.05	0.66±0.05	0.73±0.05
5	*P.aeruginosa*	1.83±0.28	0.53±0.05	0.63±0.05	0.73±0.05
6	*Proteus species*	1.66±0.28	R	R	R

Table 6: Antibacterial property of Chloroform extract *M.dioica.*

Sl. No:	Organism	Zone of inhibition(mm)(Mean ±SD)			
		Gentamycin(25µg)	100µg	250µg	500µg
1	*E. aerogenes*	1.83±0.28	0.73±0.05	0.77±0.06	0.83±0.05
2	*K.pneumoniae*	2.83±0.28	0.57±0.06	0.64±0.05	0.69±0.06
3	*S.aureus*	3.83±0.28	0.67±0.05	0.73 ±0.05	0.87±0.05
4	*E.coli*	3.83±0.28	0.66±0.05	0.66±0.05	0.73±0.05
5	*P.aeruginosa*	1.83±0.28	0.53±0.05	0.63±0.05	0.73±0.05
6	*Proteus species*	1.66±0.28	R	R	R

Table 7: Antibacterial property of Ethyl acetate extract of *M.dioica.*

Sl No:	Organism	Zone of inhibition(mm)(Mean ±SD)			
		Gentamycin(25µg)	100µg	250µg	500µg
1	*E. aerogenes*	1.83±0.28	0.77±0.05	0.83±0.05	0.93±0.05
2	*K.pneumoniae*	1.66±0.28	0.67±0.05	0.76±0.05	0.77±0.05
3	*S.aureus*	1.83±0.28	0.63±0.05	0.73±0.05	0.73±0.05
4	*E.coli*	1.66±0.28	0.63±0.05	0.67±0.05	0.77±0.05
5	*P.aeruginosa*	1.83±0.28	R	R	R
6	*Proteus species*	1.66±0.28	R	R	R

Table 8: Antibacterial property of Ethanol extract of *M.dioica.*

Sl No:	Organism	Zone of inhibition(mm)(Mean ±SD)			
		Gentamycin(25µg)	100µg	250µg	500µg
1	*E. aerogenes*	1.66±0.28	0.63±0.05	0.67±0.06	0.76±0.11
2	*K.pneumoniae*	1.83±0.28	0.83±.05	0.86±0.05	0.87±0.11
3	*S.aureus*	3.7±0.26	0.64±0.05	0.67±0.04	0.73±0.05
4	*E.coli*	1.83±0.28	R	R	R
5	*P.aeruginosa*	2.5±0.5	0.63±0.05	0.63±0.05	0.67±0.06
6	*Proteus species*	1.66±0.28	R	R	R

Table 9: Antibacterial property of Distilled water extract of *M.dioica.;* R: resistant. Values are presented as mean± standard deviation (n=3).

Sl NO:	Organism	Zone of inhibition(mm)(Mean ±SD)			
		Gentamycin(25µg)	100µg	250µg	500µg
1	*E. aerogenes*	1.66±0.28	0.83±0.057	0.86±0.06	0.99±0.05
2	*K.pneumoniae*	2.5±0.5	0.64±0.051	0.70±0.07	0.80±0.06
3	*S.aureus*	1.83±0.28	0.66±0.057	0.66±0.06	0.67±0.06
4	*E.coli*	1.66±0.28	0.67±0.057	0.63±0.06	0.73±0.05
5	*P.aeruginosa*	1.66±0.28	R	R	R
6	*Proteus species*	1.66±0.28	R	R	R

In vitro Cytotoxicity Study

In a short term cytotoxicity study using Dalton's ascites (DLA) cells, chloroform fruit extracts of *M.dioica* displayed cytotoxicity activity. Result noted in the table 10

Table 10: *In vitro* cytotoxicity study of Chloroform extract of *M.dioca*

Sl. No.	Drug concentration (µg/ml)	Percent cell death (DLA)
1	200	100%
2	100	95%
3	50	80%
4	20	60%
5	10	42%

GC-MS Analysis of Chloroform extract from *M.dioica*

The GC-MS chromatogram of chloroform fruit extract from *M.dioica* is shown in the Fig.14. The compounds with their respective molecular weight and the peak area covered them are given in the Table.11.

Table 11: Identified Phytoconstituents from Chloroform extract of *M.dioica* by GC-MS analysis

Sl. No.	Reten-tionTime	Name of the compounds	Molecular Formulae	Molecular Weight	Peak Area %
1	27.592	TRIDECANOIC ACID	$C13H_{26}O_2$	214.349	2.49
2	27.725	BUTANOIC ACID, ANHYDRIDE	C8H14O3	158.197	4.54
3	27.800	2,2'-OXYBIS(ETHANE-2,1-DIYL) DIPENTANOATE	C22H30O9	502.598	5.13
4	27.867	BUTANOIC ACID, 3-OXO-, PROPYL ESTER	C7H12O3	144.168	2.93
5	27.908	3-PENTANOL, 2,2,4,4-TETRAMETHYL	C9H20O	144.255	4.09
6	27.969	HEXADECANOIC ACID	C16H32O2	256.43	7.29
7	31.746	(+/-)-CITRONELLOL	C10H20O	156.269	9.48
8	42.315	1,2-BENZENEDICARBOXYLIC ACID	$C8H6O_4$	166.132	59.58
9	43.570	DOCOSANE	C22H46	310.619	1.82
10	45.372	PENTADECANE	C15H32	212.421	2.64

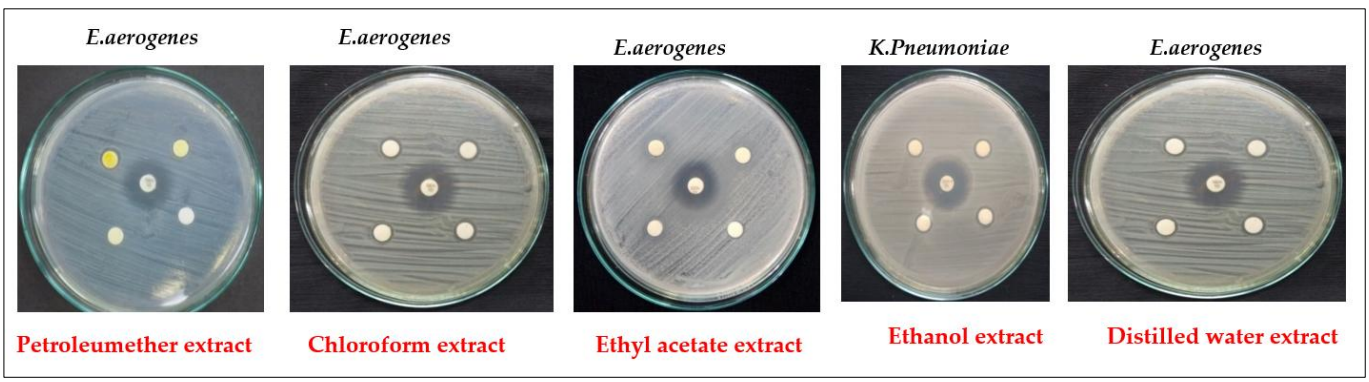

Fig 3, 4, 5, 6 and 7: Antibacterial Activity

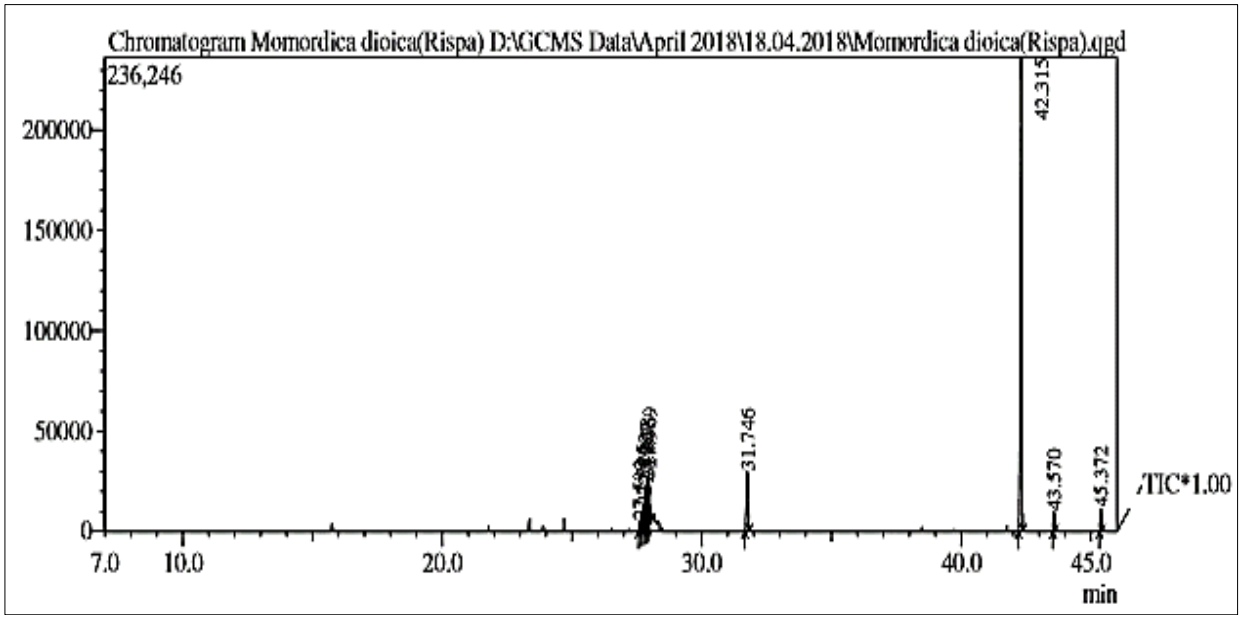

Fig 8: GC-MS Chromatogramofchloroform fruit extract of *M.dioica*

Discussion and conclusion

This study has explored the various phytochemicals in the fruits of *M.dioica*. The antioxidant efficacy of chloroform extract in DPPH is high with IC_{50} value of 242.85µg/ml, indicates that this fruit has great scope for isolation and identification of important antioxidant molecules which can be formulated to make antioxidant dosage forms. On top of that, these natural antioxidants can have potential advantages among various diseases with oxidative stress. So, further study is necessary to get maximum benefit from this fruit. These *in vitro* results should be confirmed *in vivo*. It is concluded that the plant extract possess antibacterial activity against tested organisms. The zone of inhibition varied suggesting the varying degree of efficacy and different phytoconstituents on the target organism. The antibacterial activity of the plants may be due to the presence of various active principles in their fruits. Further studies are needed to isolate and characterize the bioactive principles to develop new antibacterial drugs. In DLA cell line cytotoxicity study chloroform extract was conducted and the cell death was 100% respectively at 200µg extract. GC-MS profiling of the chloroform extracts confirmed the presence of 10 compounds. The compounds in chloroform exhibit biological activities as well as chemicals responsible could be isolated and as anticancer, antioxidant, antiinfective, antibacterial, and antifungal, immunomodulators, and antihelminthic, etc. The mechanism by which it exerts its effects remains unknown,so the mechanism of action need to be studied in future. The local ethnomedical preparations and prescriptions of plant sources should be scientifically evaluated and then disseminated properly and the knowledge about the botanical preparation of traditional sources of medicinal plants can be extended for future investigation into the field of pharmacology, phytochemistry, ethanobotany and other biological actions for drug discovery.

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