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Research Article

Phytochemical analysis of Momordica dioica, a selected Indian medicinal plant by HR-LCMS spectra method

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Abstract

Plants have been used for various medical applications since the beginning of human history and are considered as the basis for modern medicines. Phytochemicals present in plants have already been reported as potential candidates in this regard. Due to the tremendous applications of medicinal plant products in the pharmaceuticals and biotechnology field, phytochemical analysis of medicinal plants has become an important and challenging task. An analytical technique like high resolution liquid chromatography mass spectrometry (HR-LCMS) is found to be an important technique in the analysis of complex bioactive phytoconstituents. The present study was aimed at bioactive constituent analysis from *Momordica dioica* fruits by using HR-LCMS analysis. The study confirms presence of compounds having potential of being therapeutic agents, which includes alkaloid, flavonoid, phenol, saponins, cardiac glycosides, tannin, carbohydrates, terpenoids and steroids.

Keywords: HR-LCMS, Momordica dioica, Phytoconstituents

INTRODUCTION

The role of phytoconstitutes extracted from medicinal plants in maintaining sustainable human health documented worldwide. The traditional medicinal practices including Ayurveda, Rig-Veda (3700 B.C.), Unani and Homeopathy mentioned the use of medicinal plant products for the cure of various human diseases (Balkrishna et al., 2017; Pandey, 2013). In the last few years, many drugs were explored with low side effects from medicinal plants. There is an increasing demand for the identification of novel, potent drug molecules from medicinal plant products that are safe with low side effects to treat various diseases (Lahlou, 2013; Patra, 2012). In the phytochemical analysis of plant, the first step is the identification and isolation of bioactive phytoconstitute from the medicinal plants.

It is well known that some plant products and vegetables, which were used as dietary supplements, might reduce the effects of cancer proliferation. Hence, ethno-medicinal plants had tremendous contribution in the development drugs to prevent or treat various diseases, including the cancer also (Fatma et al., 2019). Their preventive effects might induce a decrease in cell proliferation as well as reduce cancer invasion and spread. It has been proposed that the whole-plant effects might be much better than its active components (Aggarwal et al., 2013).

In the present study, *Momordica dioica* plant was selected from the native places of Nanded district and their extracts were analyzed using HR-LCMS for the identification of bioactive molecules. The plant was traditionally used as an astringent, febrifuge, antiseptic, antihelmintic, antibacterial, anti-inflammatory, hepatoprotective, hypoglycemic and analgesic properties (Bawara et al., 2010). The fruits of *Momordica dioica* shows various medicinal properties like analgesics, anti-tumorogenic, anti-inflammatory, anti-diabetic activity and anti-cancer activity (Ahirrao et al., 2019).

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MATERIALS AND METHODS

Collection of sample

The fruits of the *Momordica dioica* plant were collected from the rural areas of Nanded district. The fruits were cleaned by washing thoroughly 2-3 times with running tap water and once sterile distilled water. It was followed by cutting into small pieces, shade drying, grinding and storing in well closed containers for further use (Revathy et al., 2015).

Extraction of Bioactive Compounds

The fruits of *Momordica dioica* were finely powdered and bioactive compounds were extracted with petroleum ether and acetone using a Soxhlet extractor (Redfern et al., 2014). The extracts were then collected and stored at 4°C for further analysis.

High Resolution-Liquid Chromatography Mass Spectrometry (HR-LCMS) Methodology

The fruit extracts of *Momordica dioica* prepared in petroleum ether and acetone were subjected to HR-LCMS analysis individually and chemical fingerprints were prepared using high-resolution liquid chromatography and mass spectrometry (model-G6550A of Agilent technologies) with 0.01% mass resolution (Pitt, 2009) with following parameters:

a. MS- minimum range 150 (M/Z) and maximum 1000 daltons with scanning rate each per second.

b. The source parameter for gas chromatography was maintained at 250°C with a gas flow of 13 psi/minute.

c. The auxiliary draw speed was 100 µl/minute, eject speed at 100.0 µL/min, draw position offset 0.0 mm wait time after drawing 2.0 s, Sample flush out factor was 5.0 (Tables 1 and 2).

RESULTS AND DISCUSSION

The High Resolution-Liquid Chromatography-Mass spectrometry analysis (HR)-LCMS of petroleum ether extract of *Momordica dioica* fruit was found to contain 38 compounds which were confirmed based on their mass and molecular formula as shown in Table 3, chromatogram Figure 1. The chromatogram gives information on the

Table 1. Solvent Composition.

Sl. no	Channel	Ch. 1 Solv.	Name 1	Ch2 Solv.	Selected	Used	Percent
1	A	100.0% Water V.02	0.1% FA in water	100.0% Water V.02	Ch. 1	Yes	95.00%
2	B	100.0% Acetonitrile V.02	90% ACN +10% H2O+ 0.1% FA	100.0% Acetonitrile V.02	Ch. 1	Yes	5.00%

Table 2. Timetable.

Sl. no	Time	A	B	Flow	Pressure
1	1.00 min	95.00%	5.00%	0.300 mL/min	1200.00 bar
2	20.00 min	0.00%	100.00%	0.300 mL/min	1200.00 bar
3	25.00 min	0.00%	100.00%	0.300 mL/min	1200.00 bar
4	26.00 min	95.00%	5.00%	0.300 mL/min	1200.00 bar
5	30.00 min	95.00%	5.00%	0.300 mL/min	1200.0 bar

Table 3. Bioactive Compounds in petroleum ether extract of *Momordica dioica* fruit.

Sl. No.	Name of compound	Compound formula	Mass
1	Clenbuterol	$C_{12}H_{18}Cl_2N_2O$	276.0816
2	Lycoperdic acid	$C_8H_{11}NO_6$	217.0619
3	Thiabendazole	$C_{10}H_7N_3S$	201.0325
4	3-tert-Butyl-5-methylcatechol	$C_{11}H_{16}O_2$	180.1146
5	19-Noretiocholanolone	$C_{18}H_{28}O_2$	276.2081
6	Beta-Cortol	$C_{21}H_{36}O_5$	368.2556
7	1-Naphthylacetylspermine	$C_{22}H_{34}N_4O$	370.2713
8	Triphenyl phosphate	$C_{18}H_{15}O_4P$	326.07
9	9Z-Octadecen-12-ynoic acid	$C_{18}H_{30}O_2$	278.2241
10	Linoleoyl Ethanolamide	$C_{20}H_{37}NO_2$	323.2818
11	3-Methylcyclopentadecanone	$C_{16}H_{30}O$	238.2319
12	Camelledionol	$C_{29}H_{44}O_3$	440.3296

13	Palmitic amide	$C_{16} H_{33} N O$	255.256
14	Oleamide	$C_{18} H_{35} N O$	281.2716
15	Monoolein	$C_{21} H_{40} O_4$	356.2918
16	4'-Apo-beta,psi-caroten-4'-carotenal	$C_{35} H_{46} O$	482.3593
17	Corchorifatty acid F	$C_{18} H_{32} O_5$	328.2297
18	9Z-Octadecenedioic acid	$C_{18} H_{32} O_4$	312.2352
19	Dibutyl decanedioate	$C_{18} H_{34} O_4$	314.2508
20	Estradiol-17-phenylpropionate	$C_{27} H_{32} O_3$	404.2379
21	Sorbitan laurate	$C_{18} H_{34} O_6$	346.2328
22	Nandrolone phenpropionate	$C_{27} H_{34} O_3$	406.2535
23	12S,13R-EpOME	$C_{18} H_{32} O_3$	296.2405
24	Milbemectin	$C_{31} H_{44} O_7$	528.315
25	Practolol	$C_{14} H_{22} N_2 O_3$	266.1596
26	Lauryl hydrogen sulfate	$C_{12} H_{26} O_4 S$	266.16
27	α-Linolenic Acid	$C_{18} H_{30} O_2$	278.2296
28	Linalyl caprylate	$C_{18} H_{32} O_2$	280.2457
29	Docosanedioic acid	$C_{22} H_{42} O_4$	370.3161
30	Carpaine	$C_{28} H_{50} N_2 O_4$	478.3768
31	Isopalmitic acid	$C_{16} H_{32} O_2$	256.2453
32	Praziquantel	$C_{19} H_{24} N_2 O_2$	312.1819
33	Pachymic acid	$C_{33} H_{52} O_5$	528.3936
34	Petroselinic acid	$C_{18} H_{34} O_2$	282.2618
35	Rhodoxanthin	$C_{40} H_{50} O_2$	562.3744
36	N-Nitrosotomatidine	$C_{27} H_{44} N_2 O_3$	444.3351
37	Stearic acid	$C_{18} H_{36} O_2$	284.2775
38	Calpeptin	$C_{20} H_{30} N_2 O_4$	362.2211

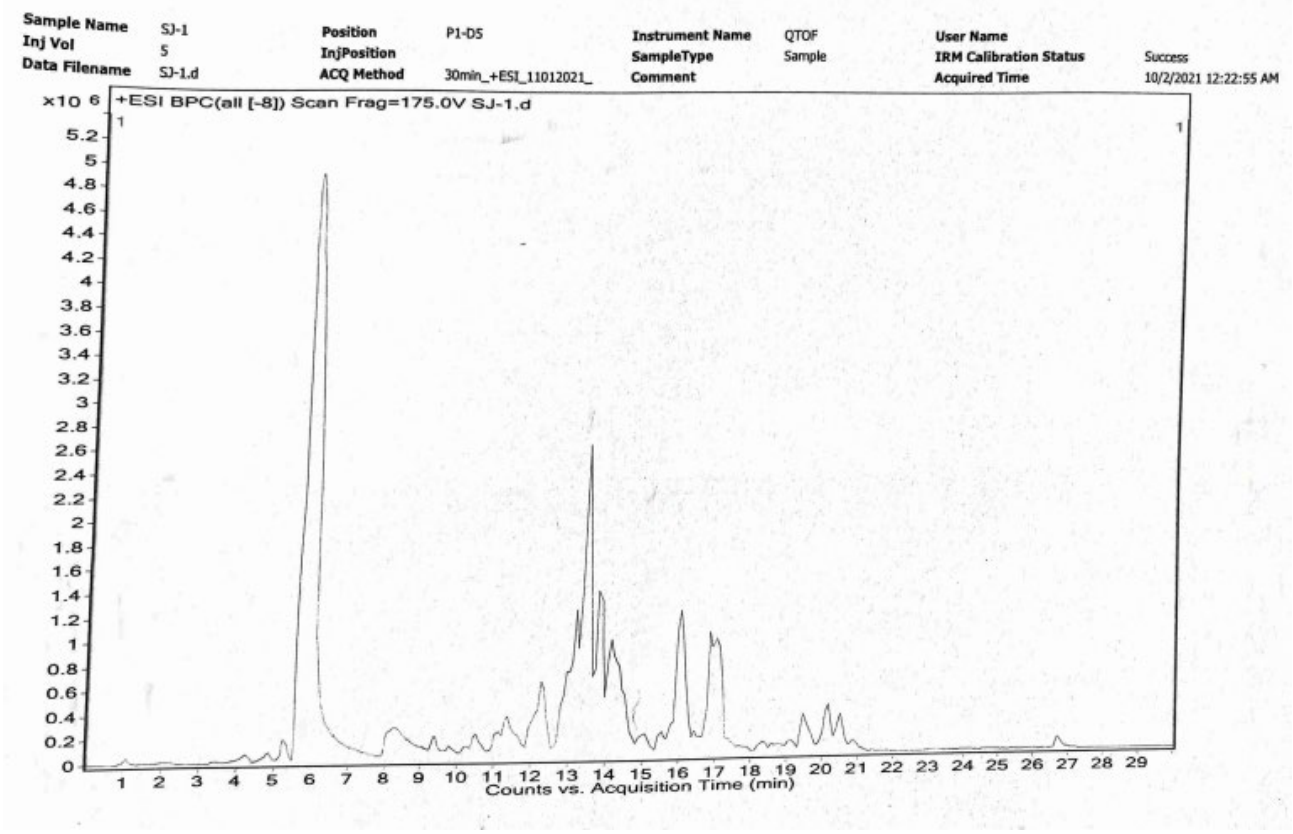

Figure 1. HR-LCMS Spectrogram of petroleum ether extracts of Momordica dioica fruit.

relative concentrations of various compounds eluted as a function of retention time.

The height of the peak indicates the relative concentrations of bioactive compounds. Mass Spectrometer analyses the structure of unknown compounds which are eluted at different times. The important phytoconstituents confirmed by HR-LCMS Analysis were Clenbuterol, Lycoperdic acid, Palmitic amide, Oleamide, Milbemectin, etc. The compounds have reported various activities like antioxidant, antineoplastic, antiviral, anticarcinogenic, antiviral. Most of them were prominently reported anticancer activity. Lycoperdic acid shows the anticancer activity in the form of dietary phenolics compound which is used in cancer treatment (Anantharaju et al., 2016). The Palmitic amides were reported in the treatment for bladder cancer in the form of heterocyclic derivative of fatty acids (Jozwiak et al., 2020). Oleamide has shown the anticancer activity against the MDA-MB-231 Cell Line in In vitro Bioassay (Wisitpongpun et al., 2020). Milbemectin has shown the anticancer activity against leukemia (El-Saber et al., 2020) (Figure 1).

The Phytochemicals found in the extract including Adenosine, Cucurbic acid, Leukotriene E3, Methanophenazine, Momordicoside I, Vulgarone A, Pyropheophorbide a, Camelledionol, Azelaic acid, Retamine, Petroselinic acid were shown in Table 4. It was also reported that these compounds found in the different species of plants exhibit different pharmacological activities (Tsuchiya & Nishizaki, 2015). Among these: Leukotrienes are lipid mediators which play impotant roles in acute and chronic inflammation and allergic diseases. They also play roles in various allergic diseases, including asthma, atopic dermatitis, allergic rhinitis, allergic conjunctivitis and anaphylaxis (Jo-Watanabe et al., 2019). Pyropheophorbide a isolated from *G. elliptica* is a potential glioblastoma-specific anticancer agent without side effects on normal cells. In addition, specifically it had cytostatic activity on glioblastoma cells rather than human umbilical vein endothelial cells (Cho et al., 2014). The in vitro cytotoxic activity of azelaic acid was studied with 25 human melanoma primary cultures and with 5 established cell lines characterized by different contents of melanotic pigment (Zaffaroni et al., 1990).

The HR-LCMS High analysis of acetone extract of *Momordica dioica* fruit spectrum profile (Figure 2) shows 45 compounds which were confirmed based on their retention time, mass and molecular formula.

Table 4. Bioactive Compounds in acetone extract of *Momordica dioica* fruit.

Sl. No.	Name of compound	Compound formula	Mass
1	Adenosine	$C_{10}H_{13}N_5O_4$	267.0967
2	Butopyronoxyl	$C_{12}H_{18}O_4$	226.1201
4	Isocarbostyril	C_9H_7NO	145.0523
5	Cucurbic acid	$C_{12}H_{20}O_3$	212.1403
6	Leukotriene E3	$C_{23}H_{39}NO_5S$	441.2493
7	Dasytrichone	$C_{18}H_{16}O_4$	296.1045
8	Dihydrodeoxystreptomycin	$C_{21}H_{41}N_7O_{11}$	567.2884
9	3-tert-Butyl-5-methylcatechol	$C_{11}H_{16}O_2$	180.1147
10	Aegle marmelos Alkaloid C	$C_{23}H_{27}NO_3$	365.1969
11	9Z-Octadecen-12-ynoic acid	$C_{18}H_{30}O_2$	278.2241
12	Methanophenazine	$C_{37}H_{50}N_2O$	538.3879
13	Momordicoside I	$C_{36}H_{58}O_8$	618.4123
14	Vulgarone A	$C_{15}H_{22}O$	218.1667
15	LysoPE(24:0/0:0)	$C_{29}H_{60}NO_7P$	565.4196
16	Islanditoxin	$C_{24}H_{31}C_{l2}N_5O_7$	571.1714
17	Linoleoyl Ethanolamide	$C_{20}H_{37}NO_2$	323.2819
18	3-Ketosphinganine	$C_{18}H_{37}NO_2$	299.2843
19	Epoxyganoderiol C	$C_{30}H_{48}O_3$	456.3593
20	Oleoyl Ethanolamide	$C_{20}H_{39}NO_2$	325.2974
21	4,4'-Methylenebis(2,6-di-tert-butylphenol)	$C_{29}H_{44}O_2$	424.3342
22	Pheophorbide a	$C_{35}H_{36}N_4O_5$	592.2677
23	Pyropheophorbide a	$C_{33}H_{34}N_4O_3$	534.2621
24	Camelledionol	$C_{29}H_{44}O_3$	440.3314
25	4'-Apo-beta,psi-caroten-4'-al	$C_{35}H_{46}O$	482.3597
26	Azelaic acid	$C_9H_{16}O_4$	188.1071
27	Hericenone B	$C_{27}H_{31}NO_4$	433.2289
28	Cilazapril	$C_{22}H_{31}N_3O_5$	417.2344
29	Muricatacin	$C_{17}H_{32}O_3$	284.2412

30	2alpha-Fluoro-17beta-hydroxyandrost-4-en-3-one	$C_{19} H_{27} F O_2$	306.2009
31	Corchorifatty acid F	$C_{18} H_{32} O_5$	328.231
32	Phygrine	$C_{16} H_{28} N_2 O_2$	280.2096
33	Pimozide	$C_{28} H_{29} F_2 N_3 O$	461.2252
34	Retamine	$C_{15} H_{26} N_2 O$	250.1993
35	Momordin Ia	$C_{42} H_{66} O_{13}$	778.4667
36	Sorbitan laurate	$C_{18} H_{34} O_6$	346.2333
37	Phlegmarine	$C_{16} H_{30} N_2$	250.2357
38	Formimidoyl-fortimicin A	$C_{18} H_{36} N_6 O_6$	432.2736
39	(-)-Ormosanine	$C_{20} H_{35} N_3$	317.2794
40	Practolol	$C_{14} H_{22} N_2 O_3$	266.1602
41	Ricinoleic acid	$C_{18} H_{34} O_3$	298.2572
42	Ethyl 2E,4Z-hexadecadienoate	$C_{18} H_{32} O_2$	280.2465
43	Petroselinic acid	$C_{18} H_{34} O_2$	282.2627
44	Homodolicholide	$C_{29} H_{48} O_6$	492.3574
45	2-Dodecylbenzenesulfonic acid	$C_{18} H_{30} O_3 S$	326.1956

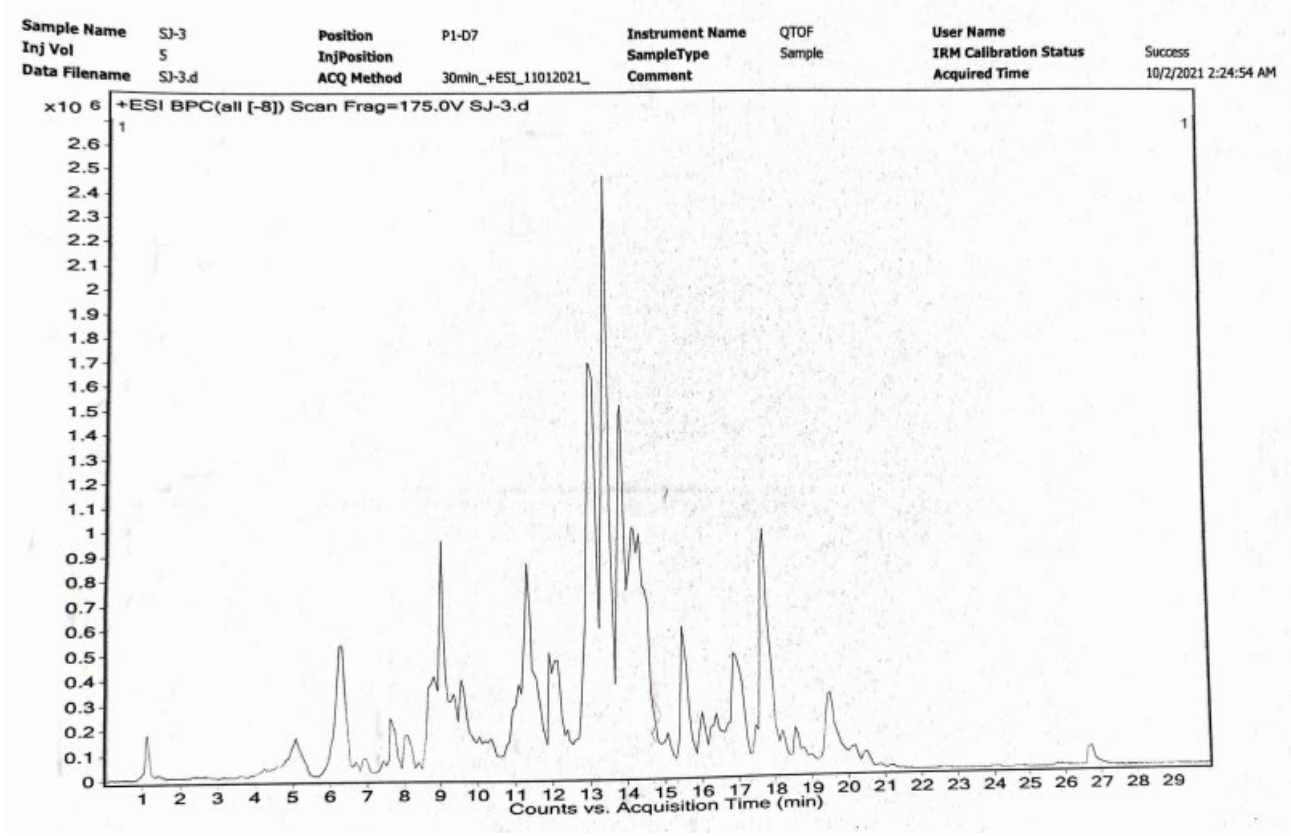

Figure 2. HR-LCMS Spectrogram of acetone extracts of Momordica dioica fruit.

CONCLUSION

The petroleum ether and acetone extract of *Momordica dioica* fruits revealed the presence of therapeutically important bioactive phytocompounds like alkaloids, Flavonoids, Phenols, Saponins, Cardiac glycosides, Tannins, Carbohydrates, Terpenoids and Steroids using (HR)-LCMS high-resolution liquid chromatography-mass spectrometer analysis. These bioactive phytoconstitutes possess important pharmacological activities and could be useful for treating various human ailments.

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