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Cucurbitacins isolated from the fruits of *Momordica cymbalaria* Hook f.

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ABSTRACT

Fruits of *Momordica cymbalaria* were collected from the southern regions of Tamil Nadu, India and botanically identified. The fruit powder was subjected to successive solvent extraction in Soxhlet apparatus and the individual extracts were processed through solvent fractionation followed by column chromatography. Six compounds were isolated from fruits of *Momordica cymbalaria*. The compounds were characterized by using IR, mass, ^{1}H NMR and ^{13}C NMR spectral data. Four compounds, which had been isolated previously from *Momordica charantia* and a novel compound, 21, 22-didehydroxy momordicoside were isolated. Quercetin was also isolated and identified from the plant.

KEY WORDS: *Momordica cymbalaria*; Cucurbitacin; Chromatography; Isolation; NMR

INTRODUCTION

Momordica cymbalaria Hook f is a wild crop, well known as "Athalakkai" in Tamil. The synonyms of *Momordica cymbalaria* are *Momordica tuberosa* Roxb. Cogn., *Luffa tuberosa* Roxb. It is available in various *part* of India, and it is a highly acceptable wild vegetable across South India. The nutritional study of the fruits of *Momordica cymbalaria* has been reported to possess a high level of calcium, potassium and vitamin C in addition to its high crude fiber content (1). The fruits of *Momordica cymbalaria* have been reported to possess hypoglycemic activity in rats (2, 3). The extracts of dried fruits of *Momordica cymbalaria* were shown to have antidiabetic and hypolipidemic properties (4, 5). Roots are used by the natives of north interior Karnataka and Andhra Pradesh to treat gynecological ailments and to induce abortions (6).

The decoction of *Momordica cymbalaria* fruits have been used in traditional medicine as a treatment for gastric ulcer. Further there is no report about any phytoconstituents isolated from this plant. Our main focus of this study is to isolate the possible phytoconstituents from *Momordica cymbalaria* through systematic phytochemical approach.

MATERIALS AND METHODS

Plant material and chemicals - **Momordica** *cymbalaria* was collected from Aruppukottai, near Madurai, Tamil Nadu. The fruits and aerial portions were botanically identified and authenticated by botanist Dr. R. Kannan. A voucher specimen of the herb (TUH No.266) was deposited in the department of Environmental and Herbal Sciences, Tamil University, Thanjavur. All the other chemicals and solvent used were of laboratory grade unless otherwise mentioned and purchased from S.D. Fine-chem Ltd, Mumbai, India.

Apparatus and general experimental procedure

Thin layer chromatography was performed using pre-coated plates of silica gel $60F_{254}$ (E. Merck, Germany) for each extracts obtained from fruits and aerial portions of *Momordica cymbalaria*. The solvent systems for each extracts were standardized after much trial and error. The developed spots were visualized by various means UV 254nm, 366nm, and Iodine chamber or sprayed with reagents such as anisaldehyde-sulphuric acid, vanillin- sulphuric acid, sulphuric acid or Ninhydrin reagent to visualize the spots. The maximum numbers of resolvable spots were identified on TLC plates. Alliance HPLC (Waters Inc., Milford, MA) equipped with quaternary pump, an auto sampler and a variable wavelength detector was used in the study.

Symmetry C_{18} reverse phase (RP) column, (4.6mmx 250mm, 5 µm particle, Waters Inc.) was used for the analysis of the purity of all the compounds. The column was maintained at room temperature 25°C. The mobile phase used consists of 0.01% trifluoroacetic acid (solvent A) and Acetonitrile (solvent B) in gradient flow. (Gradient used was initially 80:20 (A:B) to 60:40 (A:B) at 22min to 20:80 (A:B) at 40 min, the flow rate was 1.0 ml/min, injection volume: 10µL. The compounds were detected in UV detector between 205 to 210nm. The area % was determined for each compound as its peak purity.

Melting points were checked with Lab India melting point apparatus (Mumbai, India). UV spectra were recorded on Agilent-8543 UV-Visible spectrophotometer at a sample concentration of 100µg/ml in methanol. Infra red (IR) spectra were recorded on Perkin-Elmer Model Paragon 1000 FT-IR spectrometer (CT, USA) in KBr disc with 1 mg of each sample for all the six compounds. Mass spectra were obtained from Quattro LC-MS (Micromass UK Limited, Manchester, UK) equipped with ES-MS. Symmetry C_{18} reverse phase (RP) column, (4.6mmx 250mm, 5 µm particle, Waters Inc.) was used for the LC separation. The column was maintained at room temperature 25°C. The NMR data was obtained on a Bruker AVANCE DPX-400 spectrometer (PBI in Saskatoon, SK) operating at 400 MHz and 125 MHz for proton and carbon respectively. Deuterated chloroform ($CDCl_3$) was used as solvent for dissolving compounds 1 to 3 and deuterated dimethylsulfoxide (DMSO) was used as solvent for dissolving compounds 4 to 5. 10mg of each compound was employed for the ^{1}H-NMR analysis and 50 mg of each compound was employed for the ^{13}C-NMR analysis.

Extraction and isolation

5000g of the fruit powder was extracted through successive solvent extraction in Soxhlet apparatus using the solvents Pet-ether (60-80), chloroform, ethyl acetate, methanol and water. The extracts were concentrated in rotary vacuum evaporator at temperature not exceeding 45°C and dried under high vacuum. The dried extracts from ethyl acetate and methanol were combined and fractionated between butanol and water to get Fraction A (98g) and Fraction B (130g). The aqueous extract was processed separately to isolate the phenolic compound present in it.

Fraction-A (96g), was subjected to column chromatography using silica gel (60-120 mesh) and eluted with hexane: ethyl acetate followed by crystallization using acetone to yield compounds 1, 2 and 3 respectively. Fraction-B (130g) was subjected to column chromatography using silica gel (60-120 mesh), eluted with chloroform: methanol and repeatedly crystallized using methanol to yield compounds 4, 5 respectively. The aqueous extract residue was subjected to column chromatography followed by crystallization in using neutral alumina, eluted with acetone: methanol and crystallized using aqueous methanol 95% to yield compound 6.

RESULTS

Compound **1.** (201 mg), 3,7,23-trihydroxy-cucurbita-5,24-diene-19-al, HRMS: *m/z* at 472.69 ($C_{30}H_{48}O_4$), mp 123-126°C, IR $\nu^{KBr}cm^{-1}$: 3462, 2944, 1698, 1463, 1388, 1268, 1193, 1143, 1089, 1017, 991, 654, 571, UV λ_{max}: 224.0, 251.1.

Compound **2.** (208 mg), 3,7,25-trihydroxy-cucurbita-5,23-diene-19-al, HRMS: *m/z* at 472.69 ($C_{30}H_{48}O_4$), mp 188-191°C, IR $\nu^{KBr}cm^{-1}$: 3462, 2944, 1698, 1463, 1388, 1268, 1193, 1143, 1089, 1017, 991, 654, 571, UV λ_{max}: 224.0, 251.1.

Compound **3.** (190 mg), 3,7-dihydroxy-25-methoxy-cucurbita-5,23-diene-19-al, HRMS: *m/z* at 486.72 ($C_{31}H_{50}O_4$), mp 182-185°C, IR $\nu^{KBr}cm^{-1}$: 3424, 2948, 1698, 1468, 1305, 1288, 1178, 1029, 998, 944, 813, 800, 637, 599, UV λ_{max}: 255.5.

Compound **4.** (120 mg), 21,22,23,24-tetrahydroxy-cucurbita-5-ene-3-O-biglucoside, HRMS: *m/z* at 802.98 ($C_{41}H_{70}O_{15}$), mp 180-186°C, IR $\nu^{KBr}cm^{-1}$: 2976, 2966, 1674, 1456, 1398, 1258, 1219, 1180, 1103, 1072, 1026, 983, 779, 550, 494 , UV λ_{max}:255.8.

Compound **5.** (98 mg), 23,24-dihydroxy-cucurbita-5,21-diene-3-O-biglucoside, HRMS: *m/z* at 768.97 ($C_{41}H_{68}O_{13}$), mp 200-204°C, IR $\nu^{KBr}cm^{-1}$: 3448, 3109, 3062, 2966, 1676, 1479, 1391, 1114, 1076, 1011, 994, 791, 693, 618, UV λ_{max}: 255.0.

Compound **6.** (140 mg), 2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxy-4Hchromen-4-one, HRMS: *m/z* at 302.23 ($C_{15}H_{10}O_7$), mp 316°C, IR $\nu^{KBr}cm^{-1}$: 3407, 1660, 1520, 1460, 1406, 1362, 1513, 1200, 1132, 1095, 942, 879, 785, 687, 604, UV λ_{max}: 245.7.

The HPLC purity of the isolated compounds was determined. The results of analysis indicated the purity of the isolated compounds were not less than 98%. The HPLC method showed single peak with reproducible retention time.

Compounds 1, 2, and 3 (Fig. 1, 2 and 3) were found to be 3,7,23-trihydroxy-cucurbita-5,24-diene-19-al, 3,7,25-trihydroxy-cucurbita-5,23-diene-19-al and 3,7-dihydroxy-25-methoxy-cucurbita-5,23-diene-19-al respectively, which have been isolated previously from

Table 1. : ^{1}H and ^{13}C NMR (400 MHz) data of compound 1, 2 and 3 in $CDCl_3$

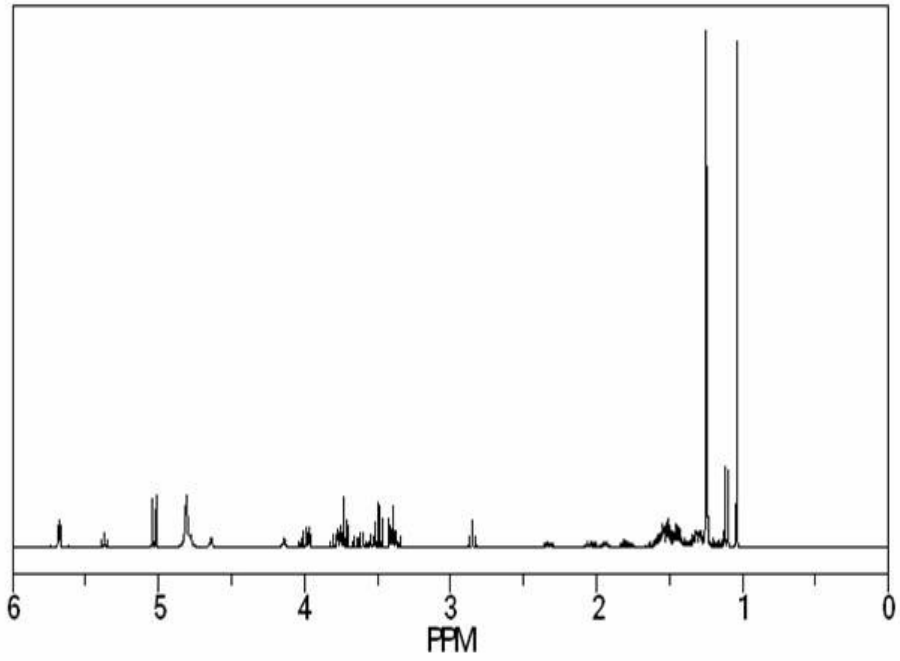
Position	^{1}H NMR (δ value)			^{13}C NMR (δ value)		
	1	2	3	1	2	3
1	1.42*s*,1.17*d*	1.42*s*,1.17*d*	1.42*s*,1.17*d*	23.0	23.0	23.0
2	1.56*m*, 1.31*s*	1.56*m*, 1.31*s*	1.56*m*, 1.31*s*	27.6	27.6	27.6
3	3.23 (OH – 4.81)*t*	3.23 (OH – 4.81)*t*	3.23 (OH – 4.81)*t*	76.4	76.4	76.4
4	-	-	-	41.0	41.0	41.0
5	-	-	-	145.7	145.7	145.7
6	5.37*t*	5.37*t*	5.37*t*	124.3	124.3	124.3
7	3.68 (OH – 4.14)*t*	3.68 (OH – 4.14)*t*	3.68 (OH – 4.14)*t*	65.7	65.7	65.7
8	-	-	-	50.6	50.6	50.6
9	-	-	-	50.6	50.6	50.6
10	2.16*m*	2.16*m*	2.16*m*	36.2	36.2	36.2
11	2.08*m*, 1.83*m*	2.08*m*, 1.83*m*	2.08*m*, 1.83*m*	22.7	22.7	22.7
12	1.56, 1.31*m*	1.56, 1.31*m*	1.56, 1.31*m*	30.1	30.1	30.1
13	1.04	1.04	1.04	49.2	49.2	49.2
14	1.04	1.04	1.04	48.3	48.3	48.3
15	1.55, 1.30*m*	1.55, 1.30*m*	1.55, 1.30*m*	35.0	35.0	35.0
16	1.60, 1.35	1.60, 1.35	1.60, 1.35	28.1	28.2	28.2
17	1.47	1.47	1.47	52.6	52.0	52.0
18	1.04	1.04	1.04	18.6	18.6	18.6
19	9.52*s*	9.52*s*	9.52*s*	206.4	206.4	206.4
20	1.64	1.64	1.68	33.2	36.5	36.5
21	0.96	0.96	0.96	19.8	19.5	19.5
22	1.44	2.04, 1.79	2.04, 1.79	44.8	39.2	39.2
23	3.90 (OH – 4.14)*t*	5.69*s*	5.69*s*	66.7	125.5	128.5
24	5.39*t*	5.67*s*	5.67*s*	130.5	139.5	139.5
25	-	(OH – 2.0)	-	135.4	70.9	74.9
26	1.82	1.28	1.28	24.9	29.9	26.2
27	1.70*m*	1.28*m*	1.28	18.9	29.9	26.2
28	1.04	1.04	2.98*t*	14.7	14.7	50.3
29	1.25	1.25	1.04	25.4	25.4	14.7
30	1.25*m*	1.25*m*	1.25*m*	25.4	25.4	25.4
31	-	-	1.25	-	-	25.4

Table 2 : ^{1}H and ^{13}C NMR (400 MHz) data of compound 4 and 5 in $CDCl_3$

Position	^{1}H NMR (δ value)		^{13}C NMR (δ value)	
	4	5	4	5
1	1.42*s*, 1.17*d*	1.42*s*, 1.17*d*	26.0	26.0
2	1.54*m*, 1.29*s*	1.54*m*, 1.29*s*	27.3	27.3
3	2.85*t*	2.85*t*	85.9	85.9
4	-	-	41.9	41.9
5	-	-	144.1	144.1
6	5.37*t*	5.37*t*	123.0	123.0
7	2.04, 1.79*m*	2.04, 1.79*m*	32.5	32.5
8	1.44	1.44	51.2	51.2
9	1.45	1.45	45.2	45.2
10	1.94	1.94	39.1	39.1
11	1.53*m*, 1.28	1.53*m*, 1.28	25.5	25.5
12	1.56*m*, 1.31	1.56*m*, 1.31	34.4	34.4
13	-	-	49.2	49.2
14	-	-	52.5	52.5
15	1.55, 1.30	1.55, 1.30	31.4	31.4
16	1.60*m*, 1.35	1.60*m*, 1.35	28.4	28.4
17	1.47	1.51	40.3	51.6
18	1.04	1.04	14.7	18.3

19	1.72*m*	2.33	36.5	40.4
20	0.96	1.11	13.5	20.2
21	3.28 (OH-4.81)	5.69*t*	71.2	141.2
22	3.37 (OH-4.81)*t*	5.67*t*	69.8	129.8
23	3.36 (OH-4.81)*t*	3.97 (OH-4.14)*t*	71.5	84.3
24	(OH-4.81)*t*	(OH-4.47)*t*	74.0	76.0
25	1.24	1.24	26.0	25.3
26	1.24	1.24	26.0	25.3
27	1.04	1.04	30.5	30.5
28	1.25	1.25	25.7	25.7
29	1.25	1.25	25.7	25.7
30	5.03*s*	5.03*s*	106.3	106.3
31	3.73 (OH-4.81)*t*	3.73 (OH-4.81)*t*	74.1	74.1
32	3.49 (OH-4.81)*t*	3.49 (OH-4.81)*t*	76.8	76.8
33	3.40 (OH-4.81)*t*	3.40 (OH-4.81)*t*	104.9	104.9
34	4.0*m*	4.0*m*	77.0	77.0
35	3.63*m*, 3.38	3.63*m*, 3.38	68.6	68.6
36	5.03*dd*	5.03*dd*	71.8	71.8
37	3.73 (OH-4.81)*t*	3.73 (OH-4.81)*t*	73.8	73.8
38	3.49 (OH-4.81)*t*	3.49 (OH-4.81)*t*	76.8	76.8
39	3.40 (OH-4.81)*t*	3.40 (OH-4.81)*t*	71.5	71.5
40	3.76*m*	3.76*m*	77.8	77.8
41	3.79, 3.54 (OH-4.78)*t*	3.79, 3.54 (OH-4.78)*t*	62.2	62.2

P.B.Dasan; Expt.No.: PBD-MC-29; COMPOUND-V ^{1}H NMR SPECTRUM: 29, Nov, 2006



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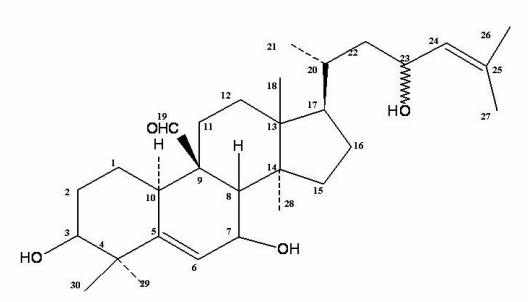
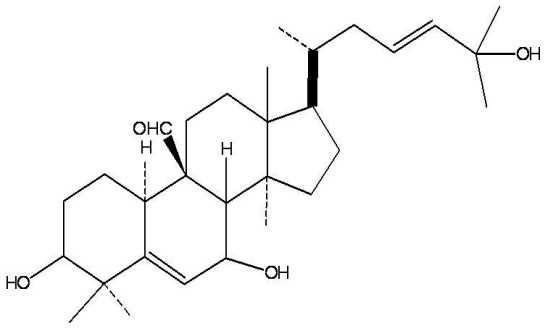


Fig. 1



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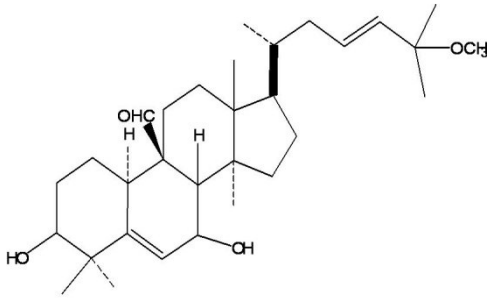

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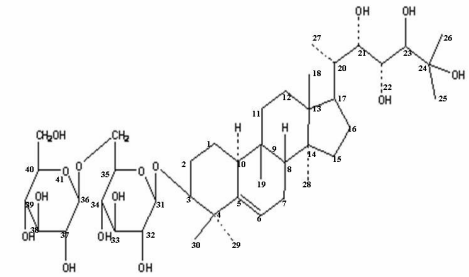

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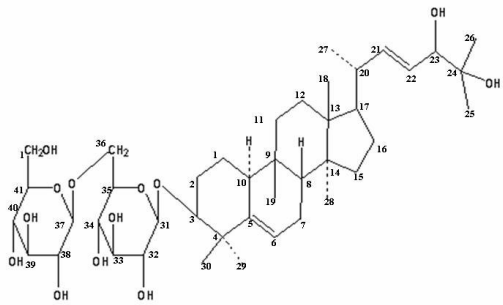

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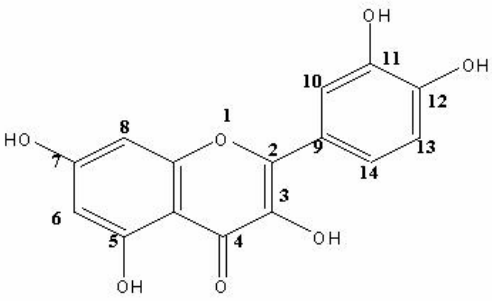

Fig. 6

Momordica foetida and *Momordica charantia* (7, 8). Structures were assigned from mass, ^{1}H-NMR and ^{13}C-NMR (Table 1) and IR spectroscopic data.

Compound 4 (Fig. 4) was found to be 21,22,23,24-tetrahydroxy-cucurbita-5-ene-3-O-biglucoside (momordicoside-A), which had been isolated previously from *Momordica charantia* (8). Compound 5 has not been reported previously and was identified as 23,24-dihydroxy-cucurbita-5,21-diene-3-O-biglucoside (21,22-didehydroxy momordicoside-A). High resolution mass spectroscopy of compound 5 (Fig. 5) indicated a molar mass of 768.97 g mol^{-1} corresponding to a molecular formula of $C_{41}H_{68}O_{13}$. The mass spectrum exhibited peak at *m/z* 769 [M], 770 [M]$^{+}$, 753 [M-CH_3]$^{+}$, 722 [M-HCOOH]$^{+}$, 704 [M-HCOOH.H_2O]$^{+}$, 690 [M-CH_3OH]$^{+}$. The spectral data of compound 5 was very close to that of compound 4, but having minor difference. The IR spectra of compound 5 differs from that of compound 4 by lacking peaks at 1258, 1219 and 1180 but having additional peaks at 3448, 3109 and 3062 which indicated that there is a change in number of hydroxyl groups attached with the side chain of compound 5. ^{1}H-NMR spectra clearly indicate that OH peak at δ 4.81 for C-21 and C-22 were missing in compound 5 and this change causes a positive shift of protons at C-19, C-20, C-21 and C-22 (Table 2). The ^{13}C-NMR spectra showed a major shifting for carbons at C-21, C22 from δ 71.2, 69.8 to δ 141.2 and 129.8 respectively. On comparison of the complete spectral data of compounds 4 and 5 it was concluded that compound 5 differs from that of compound 4 by two hydroxyl groups were absent at C-21 and C-22 in compound 5. Therefore the structure of the compound is proposed as 21,22-di-dehydroxy momordicoside-A. Compound 6 (Fig. 6) was identified as quercetin by comparing the spectral data and melting point with the reported data (10).

DISCUSSION

Six compounds have been isolated from fruits of *Momordica cymbalaria*. The first three compounds were identified as 3,7,23-trihydroxy-cucurbita-5,24-diene-19-al, 3,7,25-trihydroxy-cucurbita-5,23-diene-19-al, 3,7-dihydroxy-25-methoxy-cucurbita-5,23-diene-19-al and 21,22,23,24-tetrahydroxy-cucurbita-5-ene-3-O-biglucoside (momordicoside-A), respectively which were reported earlier from *Momordica foetida* (7). The same compounds had been previously isolated from *Momordica charantia* (11). Compounds 1, 2, 3 and 4 belong to a special group of cucurbitacins called momordicosides after their occurrence in *Momordica charantia* (12). The common feature of momordicosides is that C_{19} has been oxidised to an aldehyde group. All the aglycones had the common feature of lacking the C_{11} ketone-group present in ordinary cucurbitacins, instead having an aldehyde group at the C_{19} position. This is the first report of momordicosides from this species.

Compound 5 was found to be a novel compound found to be a derivative of momordicoside-A. The compound was characterized as 21, 22-didehydroxy momordicoside-A.

CONCLUSION

Four known cucurbitacin triterpenoids (Momordicosides) and a novel momordicoside derivative were isolated and characterized from the fruits of *Momordica cymbalaria*. Further pharmacological investigations are underway to study the biological activity of the plant.

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