

Alpha Amylase, Alpha Glucosidase Inhibition and Profiling of Volatile Compounds of Biologically Active Extracts from *Momordica cymbalaria* (Hook, Fenzl) Skin and Seeds

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Abstract - *Momordica cymbalaria* (Hook, Fenzl) has been used in traditional medicine for diabetes in India. This study was designed to examine, total phenolic, total flavonoid contents and *in vitro* anti-oxidant activity by using seven different types of solvent extracts. The biologically active extracts, ethanol extract of fruit skin (EESK) and methanol extract of seed (MESE) were evaluated for *in vitro* anti-diabetic activity followed by detection of bioactive metabolites by GC-MS analysis. EESK and MESE exhibited IC₅₀ values of 185.1 µg/ml against α – amylase and 224.4 µg/ml against α – glucosidase, respectively. GC-MS profiling of skin and seed portion of the vegetable revealed the presence of 40 compounds from EESK and 29 compounds from MESE. Thus, this study reveals that EESK and MESE exhibited *in vitro* anti-diabetic activity and GC-MC profiling confirms the presence of anti-diabetic phytocompounds such as L-Cysteine and cis- Vaccenic acid.

Keywords: *Momordica cymbalaria*, Anti-Oxidant Activity, In Vitro Anti-Diabetic Activity, GC-MS

I. INTRODUCTION

Natural products have drawn the attention of the research community due to their enormous health benefits like anti-oxidant, anti-cancer, anti-inflammatory, anti-diabetic activities, very less side effects and economic feasibility compared to synthetic medications. Moreover, consumer demands on functional foods is on rise in the developed and developing nations driven by an insatiable desire to obtain nutritional support from antioxidants, functional fats and dietary fibers. This assistance can improve the health quality of cardiovascular, nervous, immune and reproductive systems of the human body. Fruits and vegetables are the unbeatable sources of bio-actives such as phenolic compounds, flavonoids, terpenoids, carotenoids, natural catalysts and phytosterols with outstanding medicinal values. Additionally, these compounds play a vital role in catalyzing the metabolic processes besides being environmentally safer. Changing life style patterns, unhealthy eating and harmful environmental sources could increase the free radicals, resulting in the disruption of lipid, protein, carbohydrates and nucleic acids [1]. Scavenging ability of the electron hungry molecules by antioxidants is believed to be a significant step in preventing the damage to biologically important macromolecules. Free radicals include mainly Reactive Oxygen Species (Superoxide

radicals, hydroxyl radicals, hydrogen peroxides etc.) and Reactive Nitrogen Species (nitric oxide and peroxy nitrite), which play a critical part in the initiation and upsurge of various diseases such as diabetes, atherosclerosis, cardiovascular diseases, neurodegenerative diseases, cancer and premature aging [2].

To overcome the disease induction by the free radicals and to maintain the oxidative balance, living organisms have enzymatic and non-enzymatic antioxidants which help to scavenge the indigenously formed free radicals [3]. Meanwhile, plant based antioxidants have been reported to play important role in the inhibition of tumor growth, aging in brain and neuronal cells, retardation of neurodegenerative diseases like Alzheimer's and Parkinson's disease [4]. In addition to this, antioxidants possess a wide variety of applications such as food preservatives [5] polymeric membranes [6]. The use of synthetic antioxidants in food preservation results in adverse effects. Those can be replaced by antioxidants from plant origin which are more compatible with mankind and not only considered as safe but also could be more effective than synthetic ones. In recent years, a lot of research works have been carried out in the context of identifying innocuous and potent natural anti-antioxidants.

Momordica cymbalaria (Hook, Fenzl) (MC) is a more prominent nutritious vegetable of South India. It is a perennial climber of Cucurbitaceae family which is available only during the monsoon season, found in the Indian states of Andhra Pradesh, Karnataka, Madhya Pradesh, Maharashtra and Tamil Nadu. MC has been used as traditional medicine for diabetes mellitus and rheumatic disorders [7]. It is a close relative of bitter melon (*Momordica charantia*) which is better known for its anti-diabetic activity and bitter in taste similar to bitter melon. Decreasing postprandial hyperglycemia by inhibition of carbohydrate hydrolyzing enzymes such as, α -amylase and α - glucosidase is one of the therapeutic approaches [8]. Until now, acarbose and voglibose are used either alone or in combination with insulin [9] as an inhibitor of carbohydrate digestive enzymes. However, harmful effects of these compounds, such as liver disorders, flatulence,

abdominal fullness and diarrhea, have been reported [10]. In such a context, the intention of this work are (i) the evaluation and comparison of the quality and quantity of phytochemicals, as well as the anti-oxidant properties, (ii) the investigation of the *in vitro* anti-diabetic property and metabolite profiles of the antioxidant rich extracts by GC-MS and (iii) detection of the appropriate solvent which aids extraction of active ingredients from the skin and seeds of MC.

II. MATERIALS AND METHODS

A. Reagents and *M. cymbalaria* Extracts Preparation

All chemical products used in this study were of analytical grade. α -amylase, α -glucosidase, starch, acarbose, dinitrosalicylic acid (DNS), *p*-nitrophenyl- β -D-glucopyranoside (pNPG), 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid (ABTS) and butylated hydroxyl toluene (BHT), were purchased from Sigma-Aldrich Co. (St. Louis, USA). All the other chemicals used were of analytical grade. Fruits of *M. cymbalaria* were obtained from the local market Sivakasi, Tamil Nadu, India and taxonomically confirmed by the Department of Botany, St. Joseph's College Tiruchirappalli, Tamil Nadu, India (Specimen Number-SJCBOT2561). The skin and seed portions of the vegetable MC were separated lyophilized and ground to powder using electronic grinder. Powdered skin and seed samples of MC, weighing ten grams were extracted with polar and non-polar solvents such as hexane (HX), chloroform (CL), ethyl acetate (EA), ethanol (ET), hydro alcohol (HA), methanol (MT) and aqueous(AQ) respectively, with the help of soxhlet apparatus. After the extraction, solvent was evaporated under reduced pressure at 45 °C by the rotary evaporator (Buchi R-210, Flawil, Switzerland) to obtain the crude extract powder and stored at -20 °C until use.

B. Quantification of Total Phenolic and Flavonoid Contents, *In vitro* antioxidant Assays

The amount of total phenolics content was determined using Foline-Ciocalteu method [11]. Total flavonoids were estimated using the method of Ordonez et al. [12]. The antioxidant capacity of *M. cymbalaria* skin and seed extracts to scavenge the free radicals was studied by the following *in vitro* antioxidants assays, 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity [13], 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid (ABTS) free radical scavenging activity [14], Nitric oxide radical scavenging activity [15] and reducing power activity [16].

C. α - Glucosidase and α - Amylase Inhibitory Activities

α - Glucosidase inhibitory activity was assayed according to the procedure described by Ademiluyi *et al.*, [17] and Chipiti *et al.*, [18] with a slight modification. The inhibition of α -amylase is a modification of the method previously described by Adedayo *et al.*, [17] and Shai *et al.*, [19].

D. Metabolite profiling by GC-MS

A gas-chromatograph (GC 7890 A) coupled with a mass spectrometer (MS 5975 C with Triple-Axis Detector, Agilent Technologies, Santa Clara, CA, USA) was used to analyze the ethanolic skin and methanolic seed extracts derived from MC. Separation was performed on a capillary column, Agilent DB5MS (30 m \times 0.25 mm internal diameter, 0.25 μ m film thickness). The operating conditions of the column were as follows: Initially, GC oven temperature was held at 50 °C for 1.3 min and then the temperature was gradually increased to 250 °C at the rate of 6 °C/min and from 250 °C to 310 °C at the rate of 7 °C / min with hold time of 2 min. Finally the temperature was increased to 325 °C and held for 3 min. Sample injection (1 μ l) was performed in splitless mode at 250 °C. Helium was used as a carrier gas at a flow rate of 1 ml/min. The mass-spectrometer detector was operated under electron multiplier voltage (EMV) mode. The MS temperatures adopted were source 230°C, quadrupole 150°C; the acquisition range 50 –550 m/z. Identification of compounds was obtained by comparing the retention times with those of authentic compounds and the spectral data obtained from NIST (National Institute of Standards and Technology, US) library.

E. Statistical analysis

Statistical analysis was performed using SPSS software (Version 25). All results are expressed as mean \pm standard deviation. Means with different letters are significantly different from each other. Differences with $p < 0.05$ were considered statistically significant. Comparison of mean values was carried out by one-way ANOVA and Duncan's post-hoc test. All experiments were performed with at least performed with three replicates.

III. RESULTS AND DISCUSSION

1. Total Phenolic and Flavonoid Contents

The *M. cymbalaria* ethanol skin extract (EESK) showed the highest phenolics content (37.81 ± 0.36 mg of GAE/g of dry weight of the extract) and flavonoids content (21.13 ± 0.55 mg of RE/g of dry weight of the extract). Among the seed extracts, it was the methanol seed extract (MESE) that showed the highest phenolics content (16.72 ± 0.47 mg of GAE/g of dry weight of the extract) and flavonoids content (17.5 ± 0.04 mg of RE/g of dry weight of the extract). Both in the skin and seed extracts, the lowest TPC (skin = 6.24 ± 0.50 mg of GAE/g of dry weight of the extract; seed = 5.57 ± 0.31 mg of GAE/g of dry weight of the extract) was shown by the chloroform extract and the lowest TFC (skin = 11.46 ± 0.32 mg of RE/g of dry weight of the extract; seed = 11.66 ± 0.47 mg of RE/g of dry weight of the extract) was shown by hexane. The total phenolics content and total flavonoids content of the skin and seed extracts were given in Table I.

TABLE I TOTAL PHENOLIC AND FLAVONOID CONTENTS OF *M. CYMBALARIA* SKIN AND SEED EXTRACTS

Extracts	Total Phenolic Content (mg of GAE/g)		Total Flavonoid Content (mg of RE/g)	
	Skin	Seed	Skin	Seed
Hexane	8.63 ± 0.32 ^e	10.69 ± 0.31 ^d	11.46 ± 0.32 ^f	11.66 ± 0.47 ^e
Chloroform	6.24 ± 0.50 ^f	5.57 ± 0.31 ^d	17.53 ± 0.32 ^b	12.10 ± 0.43 ^{d,e}
Ethyl acetate	17.33 ± 0.29 ^d	6.09 ± 0.27 ^e	14.36 ± 0.41 ^d	13.83 ± 0.47 ^{b,c}
Ethanol	37.81 ± 0.36 ^a	16.66 ± 0.22 ^a	21.13 ± 0.55 ^a	14.53 ± 0.40 ^b
Hydroalcohol	23.90 ± 0.18 ^b	15.15 ± 0.31 ^b	15.9 ± 0.78 ^c	11.90 ± 0.20 ^e
Methanol	17.45 ± 0.63 ^d	16.72 ± 0.47 ^a	17.73 ± 0.40 ^b	17.5 ± 0.04 ^a
Aqueous	20.45 ± 0.18 ^c	12.51 ± 0.54 ^c	12.93 ± 0.45 ^e	13.03 ± 1.25 ^{c,d}

GAE – Gallic Acid Equivalents; RE – Rutin Equivalents. Values are expressed as mean ± standard deviation (n = 3). The mean values followed by different superscripts within the column are significantly different (p < 0.05).

2. In vitro Antioxidant Activity

The kinetic behaviors of scavenging of DPPH radical for various solvent extracts of skin and seed of *M. cymbalaria* are shown in Fig.1 (a) and Fig.1 (b). The ethanol extract showed the inhibition of DPPH radicals with IC₅₀ value of 102.2 3 µg/ml among the skin extracts of MC. On the other hand, MESE showed excellent inhibition with IC₅₀ of 91.11µg/ml. ABTS assay is the widely accepted free radical scavenging method which is based on the hydrogen donating ability of antioxidant compounds. Both ethanol skin extract (IC₅₀ = 81.57 µg/ml) and methanol seed extract (IC₅₀ = 85.01 µg/ml) were equally good at scavenging ABTS radicals than the other solvent extracts [Fig.1(c) and Fig.1 (d)]. Graphs in Fig.1 (e) and Fig.1 (f) shows the nitric oxide scavenging activity of skin extracts of MC. The

ethanol extract of skin once again gave the highest scavenging activity with IC₅₀ of 173 µg/ml than that of the other extracts. Meanwhile, the methanol extract of seeds revealed the scavenging potential of nitric oxide radicals at, IC₅₀ of 194.5 µg/ml. The reducing activity was determined based on the ability of skin and seed extracts to reduce a ferricyanide complex to form ferrous complex Fig.1 (g) and Fig.1 (h). The amount of Fe²⁺ reduced was monitored by measuring the formation of perl'sprussian blue at 700 nm. The reducing power of the *M. cymbalaria* was observed to increase with increasing concentration of extracts. The highest reducing activity was recorded by BHT (0.8213 ± 0.00 at 250 µg/ml) whereas out of 14 skin and seed extracts, ethanol skin extract (0.7573 ± 0.00 at µg/ml) and methanol seed extract (0.7373 ± 0.00 at µg/ml) have exhibited better reducing activity [20].

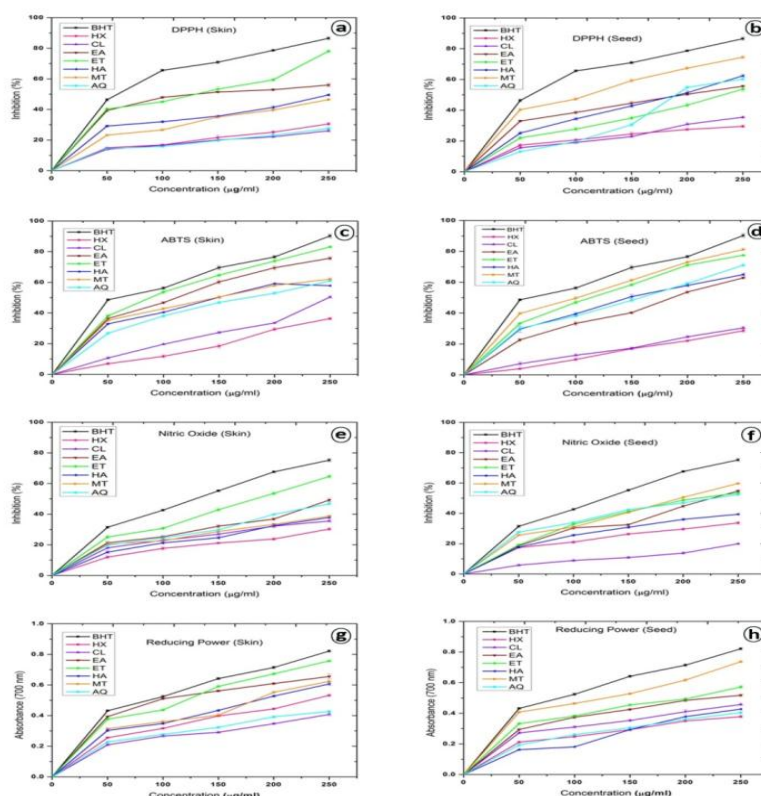


Fig. 1 In vitro anti-oxidant activities of *Momordica cymbalaria* (a) DPPH skin, (b) DPPH seed, (c) ABTS skin, (d) ABTS seed, (e) Nitric oxide skin, (f) Nitric oxide seed, (g) Reducing power skin, (h) Reducing power seed

3. In vitro Anti-Diabetic Activity and GC-MS Profiling

As the ethanolic extract of skin (EESK) and methanolic extract of seed (MESE) revealed higher quantity of total phenolics and flavonoids content, those extracts were chosen to investigate the *in vitro* anti-diabetic activity and metabolite profile by GC-MS. Alpha glucosidase and alpha amylase inhibitors have become a new treatment strategy to combat diabetes mellitus [21]. It has been observed that, the MESE showed 50% of alpha glucosidase inhibition at a concentration of 224.4 µg/ml which was lower than the EESK (245.9 µg/ml) and higher than the standard compound acarbose which showed 50% inhibition at 104.3 µg/ml. The inhibition percentages of alpha amylase enzyme by acarbose, EESK and MESE were presented in Table II. Conversely, in this assay against alpha amylase, EESK showed 50% inhibition at a concentration of 185.1 µg/ml which was much lesser than MESE (231.0 µg/ml). On the other hand, acarbose showed 50% inhibition at a lower concentration of 79.79 µg/ml.

TABLE II IN VITRO ANTI-DIABETIC ACTIVITIES OF *M. CYMBALARIA* EXTRACTS

Samples (µg/ml)	α - Glucosidase		α – Amylase	
Acarbose	Inhibition% ± S.D	IC ₅₀ (µg/ml)	Inhibition% ± S.D	IC ₅₀ (µg/ml)
50	29.38 ± 1.13 ^j	104.3	34.86 ± 1.39 ⁱ	79.79
100	44.24 ± 0.63 ^g		55.78 ± 0.77 ^e	
150	60.53 ± 1.01 ^c		70.97 ± 1.10 ^c	
200	72.96 ± 1.26 ^b		81.00 ± 0.68 ^b	
250	82.61±1.15 ^a		90.07 ± 0.42 ^a	
EESK				
50	14.52 ± 0.76 ⁿ	245.9	16.43 ± 0.76 ^m	185.1
100	26.19 ± 0.50 ^k		28.06 ± 0.34 ^k	
150	31.06 ± 0.63 ⁱ		40.36 ± 0.42 ^h	
200	45.17 ± 0.14 ^g		51.13 ± 0.76 ^f	
250	51.88 ± 0.75 ^e		64.05 ±0.59 ^d	
MESE				
50	19.81 ± 1.13 ^m	224.4	11.79 ± 0.76 ⁿ	231
100	23.92 ± 0.90 ^l		23.35 ± 0.80 ^l	
150	37.53 ± 0.75 ^h		32.76 ± 0.68 ^j	
200	47.85 ± 0.75 ^f		43.02 ± 0.51 ^g	
250	54.49 ± 0.38 ^d		55.55 ± 0.51 ^e	

Values are expressed as mean \pm standard deviation (n = 3). The mean values followed by different superscripts within the column are significantly different (p < 0.05). IC₅₀ – quantity (µg/ml) of MC skin and seed extracts needed to inhibit 50% of the reactive species in the assay.

GC-MS analysis of EESK yielded 40 compounds whereas MESE yielded 29 compounds. The metabolites with their retention time (RT), molecular formula, molecular weight, peak area % are presented in Table III. The skin and seed extracts of MC showed that, it was mainly composed of 8 categories of compound: saturated fatty acids, unsaturated fatty acids, esters, hormones, aromatic compounds, amino acids, heterocyclic compounds and aliphatic compounds. Glycerin, hexadecanoic acid methyl ester, n- hexadecanoic acid, heptadecanoic acid, cis- vaccenic acid and octadecanoic acid were the six co-existing compounds found in the chromatograms of EESK and MESE (Table III). According to the GC-MS analysis of EESK [Fig. 2 (a)], the major peak (27) was observed at the retention time of 21.420 with m/z 73.00 and the compound was identified as n- hexadecanoic acid.

Peak (35) was the second major peak which was identified as octadecanoic acid at the retention time of 23.198 with m/z 73.00. Peak 34 (9, 12, 15 –octadecatrienoic acid), peak 32 (phytol) and peak 21(bicyclo heptanes, 2, 6, 6 – trimethyl 3- octadecyne) were the other major peaks obtained at m/z 79.10, m/z 71.10 and m/z 68.10. In the chromatogram of MESE [Fig.2 (b)], peak 23 was detected as the largest peak (Rt: 23.220) with m/z 73.00 and the compound was identified as octadecanoic acid. Cis –vaccenic acid was detected as the next largest peak with m/z 55.10 at retention time 23.017. Peaks 25 and 15, were the other major peaks, identified as methyl eleostearate and palmitic acid at m/z 79.10 and m/z 73.00 respectively. It is interesting to highlight that, cis-vaccenic acid, an omega – 7 fatty acid which was identified both in the skin and seed extracts, has been reported for graded associations with lower insulin resistance and risk of diabetes, in type 2 diabetic patients [22]. In addition to this, several studies demonstrates the importance of L-cysteine supplementation as an adjuvant therapy for type 2 diabetes mellitus [23] and enhancement of insulin sensitivity [24]. Therefore, these outcomes provide the biochemical exposition to incorporate EESK and MESE as part of dietary assistance for managing diabetes mellitus.

TABLE III TENTATIVE IDENTIFICATION OF VOLATILE COMPOUNDS THROUGH GC-MS ANALYSIS

Identification of phytochemicals in EESK of <i>Momordica cymbalaria</i>					
Peak	Detected Metabolites	*RT(min)	Molecular Formula	Molecular Weight(g/mol)	Area %
1	N,N- Dimethyl- 2- aminoethanol	6.859	C ₄ H ₁₁ NO	89.136	2.12
2	3,4-Dimethyldihydrofuran-2,5-dione	10.290	C ₆ H ₈ O ₃	128.126	6.45
3	Glycerin	11.003	C ₃ H ₈ O ₃	92.094	10.43
4	2,3- Furandione, dihydro-4,4-dimethyl	11.207	C ₆ H ₈ O ₃	128.13	0.80

5	4N-Ethylcytosine	13.528	C ₆ H ₉ N ₃ O	139.158	0.95
6	2-Methoxy -4- vinylphenol	14.592	C ₉ H ₁₀ O ₂	150.177	0.46
7	1,2,3,4 –Butanetetrol	15.000	C ₄ H ₁₀ O ₄	122.12	2.51
8	DL-Proline, 5-oxo-, methyl ester	15.374	C ₆ H ₉ NO ₃	143.141	1.09
9	1-Cyclohexene-1-carboxylic acid	15.509	C ₇ H ₁₀ O ₂	126.155	2.46
10	2-Azetidinone	15.928	C ₃ H ₅ NO	71.078	2.71
11	Cyclopropane, pentyl	16.732	C ₈ H ₁₆	112.213	1.60
12	1,4 – Bis(2-hydroxyethyl) piperazine	17.253	C ₈ H ₁₈ N ₂ O ₂	174.24	0.72
13	Dodecanoic acid	17.332	C ₁₂ H ₂₄ O ₂	200.318	1.01
14	Adipicdihydroxamic acid monohydrate	17.457	C ₆ H ₁₂ N ₂ O ₄	176.171	0.48
15	2- Nonen -1-ol, 2-Methyl-	18.182	C ₁₀ H ₂₀ O	156.265	0.40
16	Butanoic acid, ethyl ester	18.272	C ₆ H ₁₂ O ₂	116.158	0.50
17	Cyclohex-2-enone	18.442	C ₆ H ₈ O	96.1271	0.55
18	Tetradecanoic acid	19.439	C ₁₄ H ₂₈ O ₂	228.371	1.75
19	Cyclohexanone	19.824	C ₆ H ₁₀ O	98.143	1.29
20	1-Aza-7-oxa-bicycloheptan-6-one	19.914	C ₁₀ H ₁₉ N	153.265	0.88
21	Bicycloheptanes, 2,6,6-trimethyl 3- Octadecyne	20.220	C ₁₀ H ₁₈	138.2499	2.32
22	2- Pentadecanone, 6, 10, 14- trimethyl-	20.276	C ₁₈ H ₃₆ O	268.478	1.66
23	Pentadecanoic acid	20.435	C ₁₅ H ₃₀ O ₂	242.398	1.09
24	9 –Octadecyne	20.639	C ₁₈ H ₃₄	250.463	0.97
25	Hexadecanoic acid, methyl ester	21.058	C ₁₇ H ₃₄ O ₂	270.4507	0.75
26	9 – Hexadecenoic acid	21.228	C ₁₆ H ₃₀ O ₂	254.414	0.88
27	n- Hexadecanoic acid	21.420	C ₁₆ H ₃₂ O ₂	256.424	22.72
28	Hexadecanoic acid, ethyl ester	21.681	C ₁₈ H ₃₆ O ₂	284.477	2.02
29	9 – Tricosene, (z)-	22.179	C ₂₃ H ₄₅	322.621	0.50
30	Heptadecanoic acid	22.303	C ₁₇ H ₃₄ O ₂	270.451	0.64
31	cis- Vaccenic acid	22.666	C ₁₈ H ₃₄ O ₂	282.461	0.62
32	Phytol	22.745	C ₂₀ H ₄₀ O	296.531	3.58
33	Methyl 16 –methyl- heptadecanoate	22.858	C ₁₉ H ₃₈ O ₂	298.504	0.42
34	9,12,15- Octadecatrienoic acid	23.017	C ₁₈ H ₃₀ O ₂	278.436	8.86
35	Octadecanoic acid	23.198	C ₁₈ H ₃₆ O ₂	284.477	7.68
36	Octadecanoic acid, ethyl ester	23.424	C ₂₀ H ₄₀ O ₂	312.530	1.05
37	9 – Tricosene (Z)	24.024	C ₂₃ H ₄₆	322.621	1.33
38	N-[Dimethylaminomethyl]aziridine	24.194	C ₅ H ₁₂ N ₂	100.162	1.48
39	1-Methyl-3-phenylindole	24.545	C ₁₅ H ₁₃ N	207.270	0.51
40	Pyridazine	26.108	C ₄ H ₄ N ₂	80.09	1.75
Identification of phytochemicals in MESE of <i>Momordica cymbalaria</i>					
Peak	Detected Metabolite	*RT(min)	Molecular Formula	Molecular Weight(g/mol)	Area %
1	Phenol	10.052	C ₆ H ₆ O	94.111	1.39
2	Formic acid, butyl ester	10.278	C ₅ H ₁₀ O ₂	102.132	1.80
3	Glycerin	10.912	C ₃ H ₈ O ₃	92.094	11.78
4	Phenol, 2-methoxy-	11.637	C ₇ H ₈ O ₂	124.139	0.33
5	Azulene	13.143	C ₁₀ H ₈	128.171	0.77
6	Cysteine	13.437	C ₆ H ₁₂ N ₂ O ₄ S ₂	240.292	0.41
7	N-Benzyl-2-aminocinnamate,methy	13.879	C ₁₇ H ₁₇ NO ₂	267.322	0.32

8	2-Methoxy-4-vinylphenol	14.592	C ₉ H ₁₀ O ₂	150.174	0.22
9	Pyrrolidine, 2-butyl-1-methyl-	15.351	C ₁₈ H ₃₇ N	267.501	0.22
10	1,3-Propanediamine, N-methyl-	15.928	C ₄ H ₁₂ N ₂	88.15	0.12
11	2-Tetradecene, (E)-	16.415	C ₁₄ H ₂₈	196.372	0.30
12	Tetraacetyl-d-xylonic nitrile	19.450	C ₁₄ H ₁₇ NO ₉	343.286	0.32
13	Hexadecanoic acid, methyl ester	21.058	C ₁₇ H ₃₄ O ₂	270.451	0.91
14	Adipamide	21.239	C ₆ H ₁₂ N ₂ O ₂	144.172	0.13
15	n-Hexadecanoic acid	21.409	C ₁₆ H ₃₂ O ₂	256.4241	10.86
16	Carbromal	21.975	C ₇ H ₁₃ BrN ₂ O ₂	237.094	0.14
17	Propanamide	22.065	C ₃ H ₇ NO	73.094	0.16
18	Heptadecanoic acid	22.315	C ₁₇ H ₃₄ O ₂	270.451	0.27
19	9, 12-Octadecadienoic acid (Z,Z)-...	22.609	C ₁₉ H ₃₄ O ₂	294.472	1.02
20	9-Octadecenoic acid (Z)-, methyl...	22.654	C ₁₉ H ₃₆ O ₂	296.488	1.64
21	Octadecanoic acid, methyl ester	22.869	C ₁₉ H ₃₈ O ₂	298.504	1.99
22	cis-Vaccenic acid	23.017	C ₁₈ H ₃₄ O ₂	282.461	17.36
23	Octadecanoic acid	23.220	C ₁₈ H ₃₆ O ₂	284.477	22.15
24	Methyl octadecatrienoate	23.877	C ₁₉ H ₃₄ O ₂	294.472	5.66
25	Methyl 9.cis., 11.trans.t,13.trans - octadecatrienoate	24.206	C ₁₈ H ₃₀ O ₂	278.430	13.00
26	Cyclopentanebutanoic acid methyl ester	24.545	CC ₁₀ H ₁₈ O ₂	170.249	3.55
27	Unknown compound	25.485	-	-	2.07
28	Ethanamine,2-[(4-chlorophenyl)-2-pyridinylmethoxy]-N,N-dimethyl	25.825	C ₁₆ H ₁₉ ClN ₂ O	290.78786	0.43
29	Sarcosine	26.108	C ₃ H ₇ NO ₂	89.094	0.66

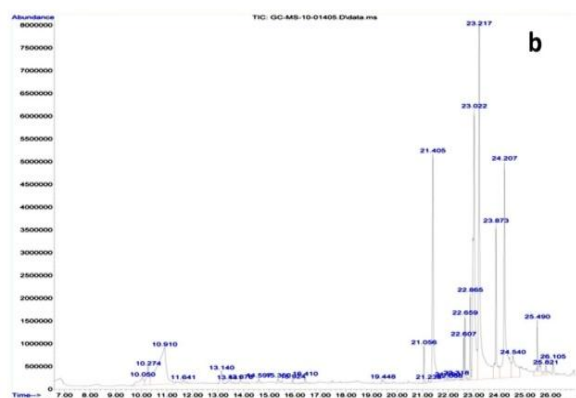
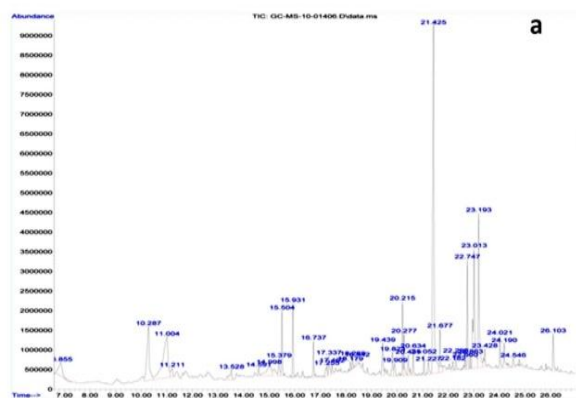


Fig.2 GC-MS chromatogram of *Momordica cymbalaria*
(a) EESK; (b) MESE

IV. CONCLUSION

This study for the first time explored the *in vitro* anti-diabetic effects of skin and seed extracts of *Momordica cymbalaria*. EESK and MESE showed high TPC, TFC, *in vitro* antioxidant activity, inhibition of alpha glucosidase and alpha amylase activity. On the whole, 69 compounds were identified in this vegetable via GC-MS analysis. Hence, both EESK and MESE can be considered as perfect source of natural antioxidants and anti-diabetic agents. Further studies can be directed towards the identification and structural elucidation of the bioactive compounds followed by animal studies and clinical trials to understand the anti-diabetic effects of *M. cymbalaria*.

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