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Evaluation of the Hypoglycemic Potential of Endophytic Fungi from Leaves Extract of Moringa Oliefera (Pakistan), its Identification and GC-MS Analysis

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Abstract: *Crude ethyl acetate extract of endophytic fungi MOL1 and MOL3 of Moringa oliefera was taken to evaluate the hypoglycemic activity in normal and alloxan-induced diabetic rabbits.100mg/kg of MOL1 the values of body weight from 1.42±0.09 decreased to 1.18±0.075 while 200mg/kg of extract reduced body weight from 1.36±0.15 to 1.11±0.132. 100 mg/kg of MOL1 treatment on 15th day showed that the BGL count was decreased from 390.2±10.9 to 200.5±7.65. At a concentration of 200 mg/kg reduced BGL count was 195.5±7.69 from 382.7±15.3. 100mg/kg MOL3 treatment decreased weight to 1.21±0.09 from 1.49±0.08 and 200mg/kg reduced body weight from 1.39±0.18 to 1.11±0.075. In group#4, which received 100 mg/kg body weight of MOL3, the BGL count on the 15th day was 387.4±6.80 and after 1 hour of post-treatment it decreased to 184.4±44.0. MOL3 concentration of 200 mg/kg reduced BGL from 410.4±45.67 to 211.6±33.33 after 3 hours of post-treatment it decreased to 180.3±34.1.*

Key Words: Ethyl Acetate Extract, Endophytic Fungi, Moringa Oliefera, Hypoglycemic Activity

Introduction

Diabetes mellitus is a dysmetabolic syndrome. categorized by an increase in blood sugar reason can be faults in producing insulin or insulin sensitivity, and sometimes both. Major complications of diabetes include abnormal BGL and BVD (blood vessel diseases) which lead to long-standing damage to vital organs: nerves, eyes, heart, and kidneys. ("Diagnosis and Classification of Diabetes Mellitus," 2010) Worldwide, it is predicted by the researchers that the number of diabetic patients in 2045 will increase to 693m from 451m in 2017 (Ogurtsova et al., 2017).

Pakistan is of developing country and facing difficulties to prevail diabetes. Relatively also many researches have been conducted to investigate for incidence of diabetes. Every next project gives different estimations of the frequency of diabetes. The aim of this study was to find out the antidiabetic activities of fungal extract of *the M. olifera* plant. leaves which are already used for the purpose of curing diabetes. Plants which are studied in detail for having potency of anti-diabetic activities enlisted; A. sativum, Aloe vera, M. oliefera, A. cepa, C. cajan, C. bonducella, C. indica, F. bengalenesis, M. charantia, P. marsupium, O. sanctum, S. cumini, S. chirayita, T. foenum graecum and T. cordifolia (Eldeen et al.,2013). *N. oryzae* endophytic fungi reveal excellent antidiabetic effects. It was proven that endophytic fungi could be a substitute source for diabetes-controlling novel compounds (Uzor et al., 2017).

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Moringa oliefera commonly known as Drumstick tree, Ben tree, Magic tree or Suhanjna (Urdu) is a tree of immense medicinal importance. It is native species of Pakistan and India extending up to Afghanistan and Bangladesh. *M. oliefera* was designated as ethno-medicine about 2000 BC ago (Rosa, 1993).

To investigate MOL1 and MOL2 fungal metabolites against the hypoglycemic effects of alloxan-treated rabbits was the goal because it has not been documented yet. Basically, the alloxan drug induces diabetes mellitus resulting in damage to B-pancreatic cells which produce insulin but do not harm other cells (Malaisse-Lagae et al., 1981).

Methodology

Sample preparation

For the production of secondary metabolites, the fresh fungal strains were cultured. For fungal endophytes PDB was used after autoclave (121°C) (Kjer et al., 2010). In each PDB flask, the culture of five days was introduced. Which was then kept in an incubator (shaking) at "25°C" 150 revolts/min (rpm). After the incubation period forty per cent hypocaloric acid, 200 to 500 µL was supplemented to all flasks respectively to allow the components in the media to detach.

Filtration of culture takes place by using filter paper after vigorous mixing and grinding. To each flask containing media equal volumes of ethyl acetate (EtOAc) were added and then mixed through a shaking process for 20 min and left at a standing position for 2 hours. The three-time procedure was repeated. To separate the funnel mixture.

To dehydrate the organics layer Anhydrous sodium sulfate was added and then filtered. By using Whatman filter paper EtOAc was filtered. To concentrate the extracts a rotary evaporator was used at 45°C. In a fume hood the concentrated EtOAc extract was further dried to obtain solid residues (Khattak et al., 2014).

Materials and animal management

Experimental animals: Young and fit male rabbits were bought from Board Bazar, Peshawar weighing '1 to 2 kg' body. Before the experiment for 15 days, test animals were kept in the Pharmacy animal house, for observation. Animals were provided with well-adjusted nourishment containing pulses, greeneries, water and feedstuff. During the whole experiment, all rabbits were kept in wooden cages.

Other requirements were: Disposable syringes, mouth gag, needles, oral feeding (blunt curved) needle, strips, Glucometer Normal saline, distilled water

Drugs: 1. Alloxan monohydrate purchased from Sigma Chemical Company, USA 2. Tolbutamide

Induction of diabetes mellitus

For induction of diabetes mellitus in normal glycemic male rabbits Alloxan Monohydrate was used. Rabbits were endorsed for 12 hrs. at the fast state and were injected "120mg/kg" alloxan monohydrate injection in the vein of the marginal ear. "0.9%" normal saline was used for the preparation of the injection. The dose was enough to abolish the β cells of the pancreas. Diabetes rabbits were those rabbits who have blood serum glucose levels≥250mg/dl. After 3 days of induction of alloxan in healthy and fit rabbits' the spectrophotometric method was used to check BGL (9).

Experimental design

The weights of the rabbits were 1 to 1.5 kg. Before the experimental study Rabbits were weighed, numbered, and casually separated into six sets, 6 animals in each set. The present study was conducted in the "Department of Pharmacy", at the University of Peshawar. Rabbits were divided into five experimental groups.

1. Group -1: Non-diabetic rabbits treated with normal saline10 ml/kg,
2. Group -2: rabbits that are diabetic were treated with 10 ml/kg normal saline,
3. Group- 3: rabbits that are non-diabetic treated with 300mg/kg Tolbutamide,
4. Group -4: rabbits that are diabetic treated with 100mg/kg MOL1 and MOL3extract,
5. Group- 4: rabbits that are diabetic treated with 200mg/kg MOL1 and MOL3 extract.

Treatment of rabbits

Rabbits that are diabetic were treated with two different concentrations of MOL1 and MOL3 extracts for weight analysis for 15 days. The same concentrations of extracts were selected for BGL determination after 15 days of drug induction.

Statistical Analysis

Results are expressed as mean- ± standard deviation (SD). 'e In vivo data were analyzed using one-way

ANOVA, followed by Dunnett's post hoc test to compare blood glucose levels between control and test groups. A p-value less than 0.05 was considered statistically significant.

Identification of isolated endophytic fungi

Morphological identification

To accelerate the growth rate and the production of enough conidia PDA was used as reported by (Diba et al., 2007). With mycological identification keys and taxonomic description, the characteristics of fresh cultures were compared (Fawole, & Oso, 2007) in order to determine the isolated fungi to the genus level.

Macroscopic identification

Identification depended on colony characteristics as well as microscopic features, among the colonial features such as surface appearance, surface texture, and colony colour both from the upper as well as from the lower side. The identified fungi were kept up on the Potato Dextrose Agar (PDA) slant at (+4oC) for more investigation.

Microscopic identification

In order to identify spores and mycelia of pure fungal isolates slide culture was prepared. Thus, the morphology of spores as well as mycelia of fungal isolates was analysed and recognized through lacto phenol cotton blue staining by using a microscope and identified after developing them on slide according to Stevens (Stevens, 1974).

Molecular identification

DNA extraction

DNA of selected endophytic fungi will be extracted using a fungal DNA isolation kit (NORGEN BITEC CORP E5038).

DNA amplification

By using PCR isolated DNA is then amplified. By use of the "HotStarTaq Master Mix Kit (QIAgen) kit" the PCR was processed.

Blast and Phylogeny

The sequence (ITS region) was checked for homology using BLAST using MEGA 7.0 software. The aligned sequences were then used to construct a phylogenetic tree, after conducting their bootstrap test.

Gas Chromatography-Mass Spectrometry (GC-MS) Analysis

GC/MS conditions

HPLC Fractions of MOL1 and MOL3 fungal metabolites and crude extracts of both codes in methanol will be determined by the use of "Gas chromatography-mass spectrometry (GC-MS)". GC-MS analysis will show the presence of various metabolic compounds. By the use of "The Thermo Scientific (DSQII) GC instrument, the sample was analyzed. with a TR-5MS capillary column of length '30M', Film Thickness 'of 0.25µm' and Internal Diameter of '0.25mm' the GC instrument was equipped. Helium (He) was used as a carrier gas with a flow rate of 1ml/min. With a temperature of 250C, the injector was operated in split mode. The voltage of ionization was 70eV. In split mode the samples were injected as 10:1. Mass spectral scan range was fixed at '45-450 (m/z)'. The spectrum obtained through GC-MS-MS compounds present in the extracts was identified by comparing and using computer searches on a NIST Ver.2.1 MS data library. (Gopalakrishnan, & Vadivel 2011)

Identification of bioactive compounds

By using the database of the National Institute Standard and Technology (NIST) having more "than 62,000" patterns interpretation on mass-spectrum GC-MS-MS was conducted. With the spectrum of known components stored in the NIST library, the spectrum of the unknown components was compared. The name, molecular weight, Retention time, and molecular formula of the extract's samples were identified.

Results

Hypoglycemic effects of M. olifera fungal endophytes on normal and alloxan-diabetic rabbits

Change in weight levels of diabatic animals after treatment with MOL1

Tables: 1-6 and **Figure: 1-6** show the effect of oral administration of different concentrations of MOL1 extract on the weight change of treated rabbits. The initial weight of group#1 negative control and group#3 standard were (1.3±40.12) and (1.42±0.09), body weight gradually changed on 15 days

(1.49±0.08) and (1.16±0.12) respectively. After treatment with 100mg/kg of MOL1, the values decreased to 1.18±0.075 from 1.42±0.09. 200mg/kg of extract reduced body weight from 1.36±0.15 to 1.11±0.132. The differences were statistically significant because body weight gradually decreased.

Weight level of test Rabbits

Table 1

Group # 1 (-control)

S. NO	Day 0	Day 5	Day 10	Day 15
1	1.5 kg	1.4	1.5	1.6
2	1.3 kg	1.4	1.4	1.5
3	1.3	1.3	1.4	1.4
4	1.4	1.4	1.5	1.5
5	1.5	1.5	1.6	1.6
6	1.2	1.3	1.3	1.4

**Normal saline used as control 10 ml/kg*

Table 2

Group # 2 (disease + control)

S. NO	Day 0	Day 5	Day 10	Day 15
1	1.4 kg	1.3	1.2	1.1
2	1.4 kg	1.3	1.3	1.2
3	1.5	1.4	1.3	1.3
4	1.3	1.3	1.2	1.2
5	1.4	1.3	1.2	1.1
6	1.5	1.4	1.3	1.2

**Alloxan used 80mg/kg*

Table 3

Group # 3 (+control)

S. NO	Day 0	Day 5	Day 10	Day 15
1	1.3 kg	1.3	1.2	1.1
2	1.3 kg	1.2	1.1	1.0
3	1.5	1.4	1.4	1.3
4	1.3	1.2	1.2	1.1
5	1.5	1.4	1.3	1.3
6	1.4	1.3	1.3	1.2

**Tolbutamide used as a standard drug 300mg/kg*

Table 4

Group # 4 (MOL1 100 mg/kg)

S. NO	Day 0	Day 5	Day 10	Day 15
1	1.5 kg	1.4	1.3	1.2
2	1.4 kg	1.4	1.3	1.2
3	1.5	1.4	1.3	1.2
4	1.3	1.2	1.2	1.1
5	1.4	1.3	1.2	1.1
6	1.5	1.4	1.4	1.3

Table 5

Group # 5 (MOL1 200 mg/kg)

S. NO	Day 0	Day 5	Day 10	Day 15
1	1.3 kg	1.2	1.1	1.0
2	1.4 kg	1.3	1.3	1.2
3	1.3	1.2	1.1	1.0
4	1.5	1.4	1.4	1.3
5	1.5	1.4	1.3	1.2
6	1.2	1.1	1.1	1.0

Table 6

Effect of MOL1 isolated from Moringa oliefera on body weight of alloxan-induced diabetic and normal male rabbits (Mean ± SD).

Experimental groups	Treatment dose	Day 0	Day 5	Day 10	Day 15
Group#1 (-control)	Saline	1.3±40.12	1.38±0.07	1.44±0.10	1.49±0.08
Group#2 (disease+control)	Alloxan+saline	1.42±0.09	1.33±0.05	1.24±0.05	1.18±0.07
Group#3 (+control)	Tolbutamide	1.42±0.09	1.2±0.08	1.24±0.10	1.16±0.12
Group#4	MOL1	1.42±0.09	1.34±0.083	1.28±0.075	1.18±0.075
Group#5	MOL1	1.36±0.15	1.26±0.12	1.21±0.132	1.11±0.132

Figure 1

Weight of rabbits after introducing normal saline.

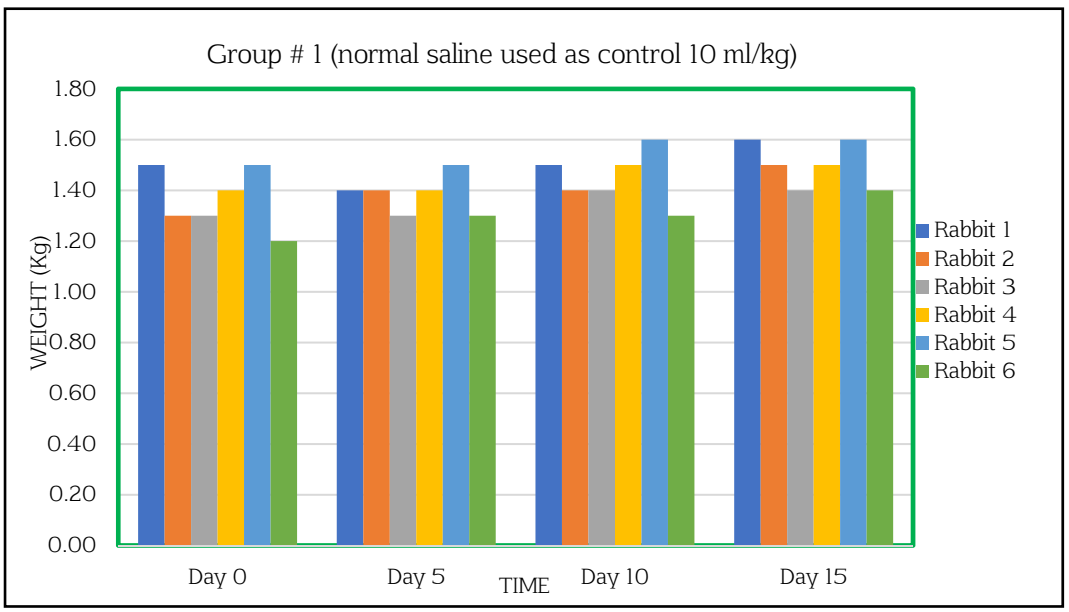


Figure 2

Weight after introducing alloxan to normal rabbits.

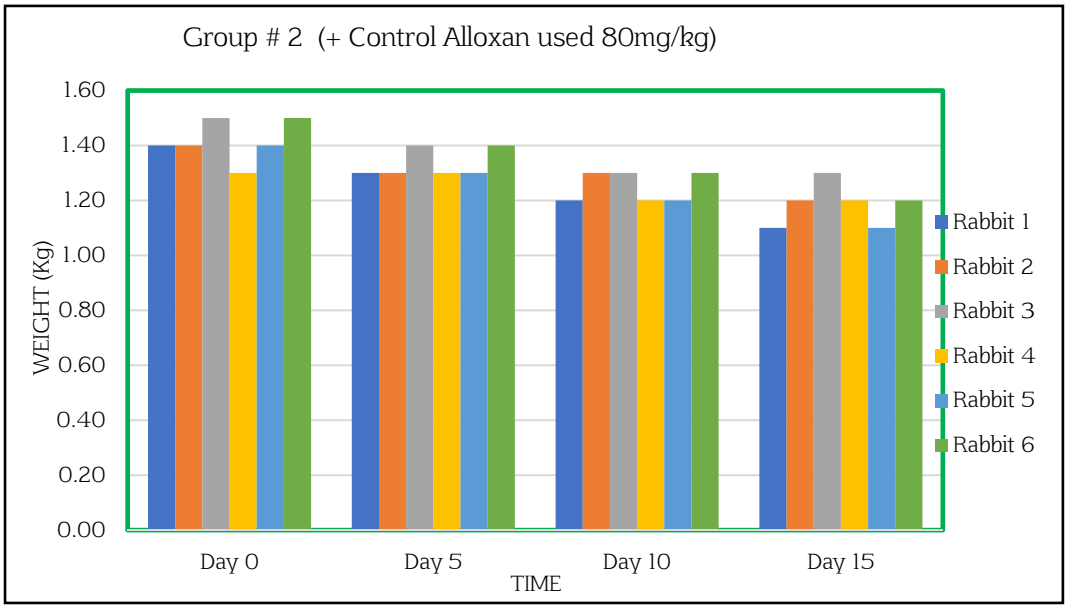


Figure 3

Weight after introducing tolbutamide to diabatic rabbits.

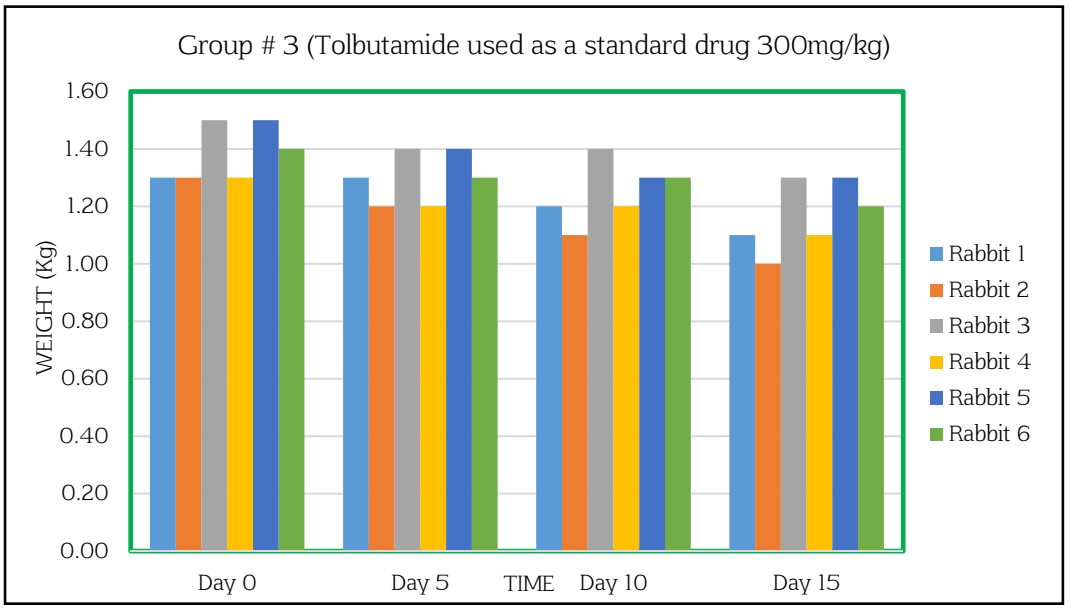


Figure 4

Diabetic rabbits treated with 100mg/kg MOL1 extract

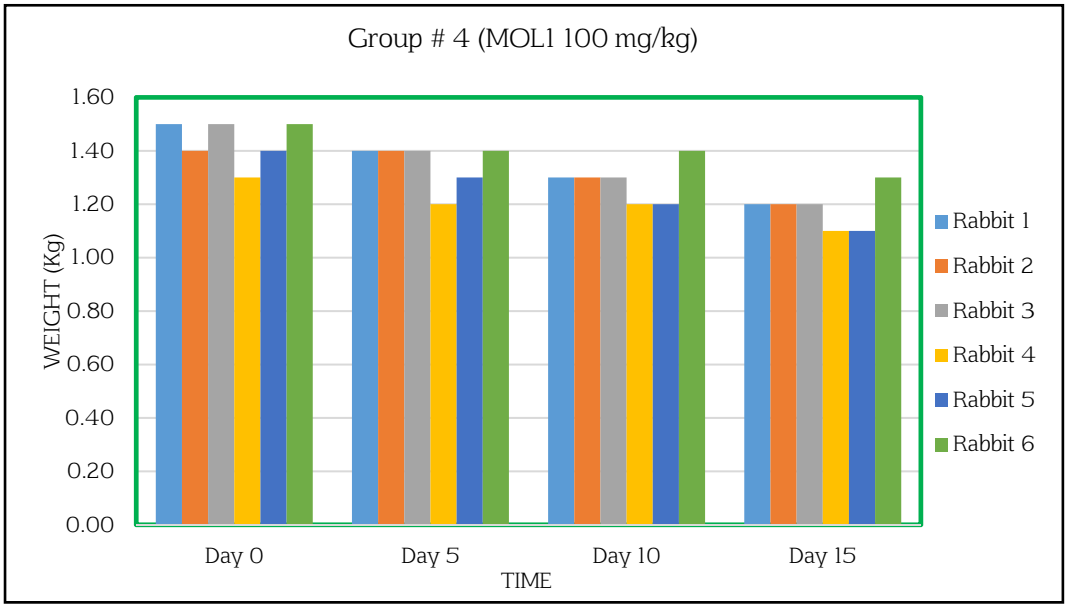
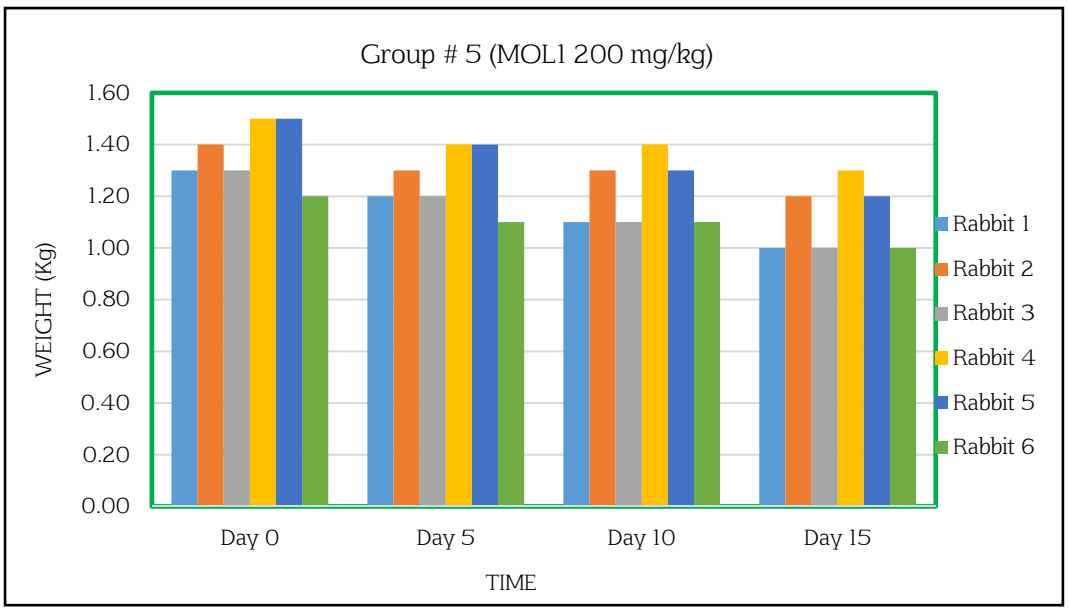


Figure 5

Diabetic rabbits treated with 200mg/kg MOL1 extract



Change in Blood glucose level of diabetic animals after treatment with MOL1

The drugs were administered orally to animals once daily for 15 days. Non-glycemic along with alloxan-induced diabetic rabbits were tested for several treatments which are displayed in **Tables:3.7-12** and **Figure:6-10.** Mean and SD values of the glycemic level of all groups of animals showed variation in blood glucose levels from day 0 to day 15. Mean BGL of non-diabetic negative control group#1 was varied between 91.2 to 98.7 and diabetic animals with negative control group#2 were between 367.9 to 417.5 mg/dl during the study of both samples MOL1 and MOL3. In group #3 there was a significant reduction of BGL. In group#4, which received 100 mg/kg of MOL1, the BGL count on the 15th day was 390.2±10.9 and after 1 hour of post-treatment it decreased to 200.5±7.65, while after 3 hours we noticed that it increased to 203.11±5.15. MOL1 concentration of 200 mg/kg reduced BGL to 195.5±7.69 from 382.7±15.3, after 3 hours of post-treatment it increased to 198.3±3.6.

So, both concentrations of MOL1 showed activity but at 200 mg/kg, it was higher than 100mg/kg.

BGL of test animals:

Table 7

Group #1 (-Control)

S. NO	Day 0	Day 5	Day 10	Day 15	1hour post-treatment	3hour
1	98	101	105	99	85	95
2	89	86	93	91	98	87
3	102	94	89	86	103	98
4	83	87	86	89	105	100
5	84	98	82	98	99	97
6	97	92	95	100	104	99

Table 8

Group # 2 (disease + control)

S. NO	Day 0	Day 5	Day 10	Day 15	1hour post-treatment	3hour
1	88	386	327	395	398	391
2	85	375	386	412	398	402
3	89	390	412	450	407	412
4	90	405	389	431	487	436
5	102	342	415	398	416	401
6	101	342	403	367	406	399

Table 9

Group # 3 (standard)

S. NO	Day 0	Day 5	Day 10	Day 15	1hour post-treatment	3hour
1	90	386	363	394	187	186
2	95	405	329	382	124	136
3	99	390	389	399	130	128
4	97	413	397	386	127	133
5	93	386	374	378	186	172
6	92	412	398	379	191	201

Table 10

Group # 4 (MOL1 extract 100mg/kg)

S. NO	Day 0	Day 5	Day 10	Day 15	1hour post-treatment	3hour
1	98	401	391	400	212	207
2	98	312	387	398	201	198
3	85	390	388	381	198	206
4	88	389	405	392	205	210
5	88	412	399	373	189	200
6	97	403	415	398	199	198

Table 11

Group # 5 (MOL1 extract 200mg/kg)

S. NO	Day 0	Day 5	Day 10	Day 15	1hour post-treatment	3hour
1	98	329	336	355	196	203
2	89	363	391	397	189	200
3	88	390	383	380	201	197
4	82	405	395	388	195	193
5	88	388	367	396	186	201
6	95	339	359	382	207	196

Table 12

Hypoglycemic effect of graded doses of secondary metabolites of fungal endophytes from M. oliefera in diabetic rabbits

Mean blood glucose Concentration ± SD							
EG	TD (mg/kg)	Day 0	Day 5	Day 10	Day 15	1hour post-treatment	3hour
Group#1 (-control)	Saline	91.8±7.93	92.8±5.93	91.3±8.04	93.6±5.91	98.7±7.40	95.8±4.73
Group#2	Alloxan+Saline	92.27±7.1	372.5±26.1	387.4±32.41	407.9±29.1	417.5±34.1	406.5±15.7
Group#3 (+control)	Tolbutamide	94.2±3.32	398.4±12.8	374.1±26.3	386.2±8.5	154.4±33.5	156.8±31.0
Group#4	MOL1	92.1±5.95	382.9±36.5	397.3±11.0	390.2±10.9	200.5±7.65	203.11±5.15
Group#5	MOL1	89.8±5.69	367.9±30.4	371.2±22.3	382.7±15.3	195.5±7.69	198.3±3.6

Figure 6

BGL of rabbits after introducing normal saline

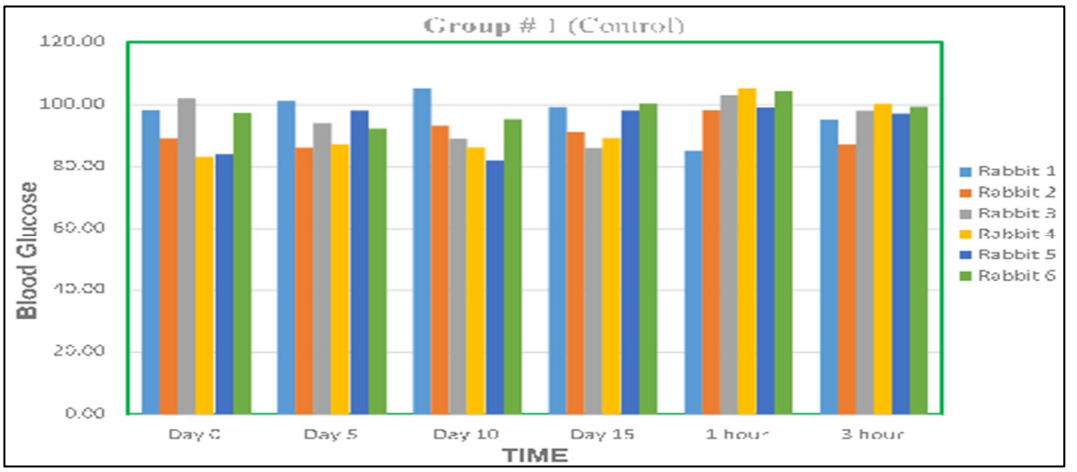


Figure 7

BGL after introducing alloxan to normal rabbits.

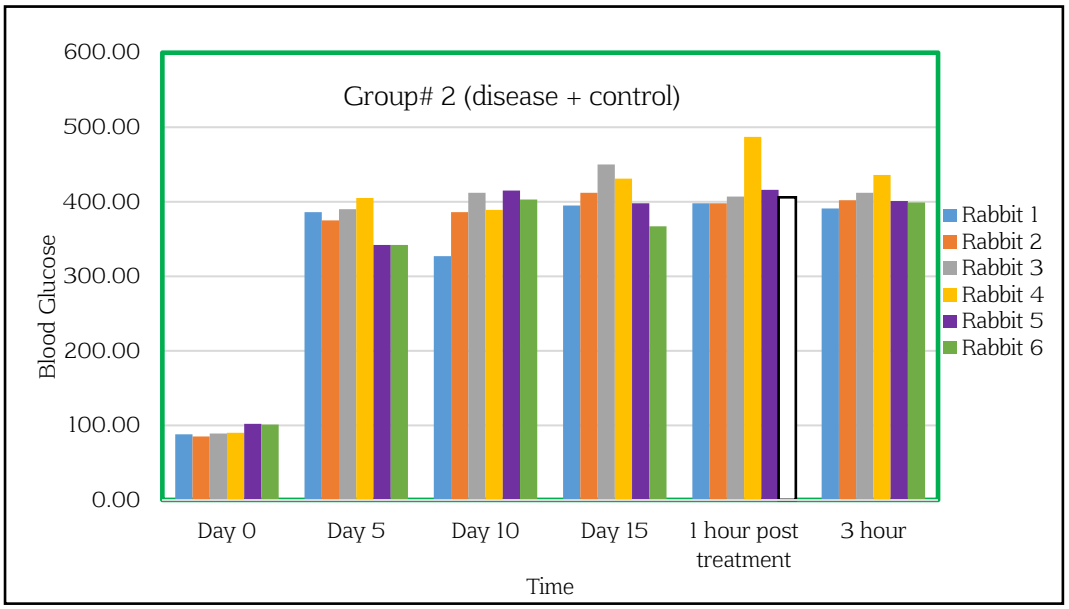


Figure 8

BGL after introducing SD to diabatic rabbits.

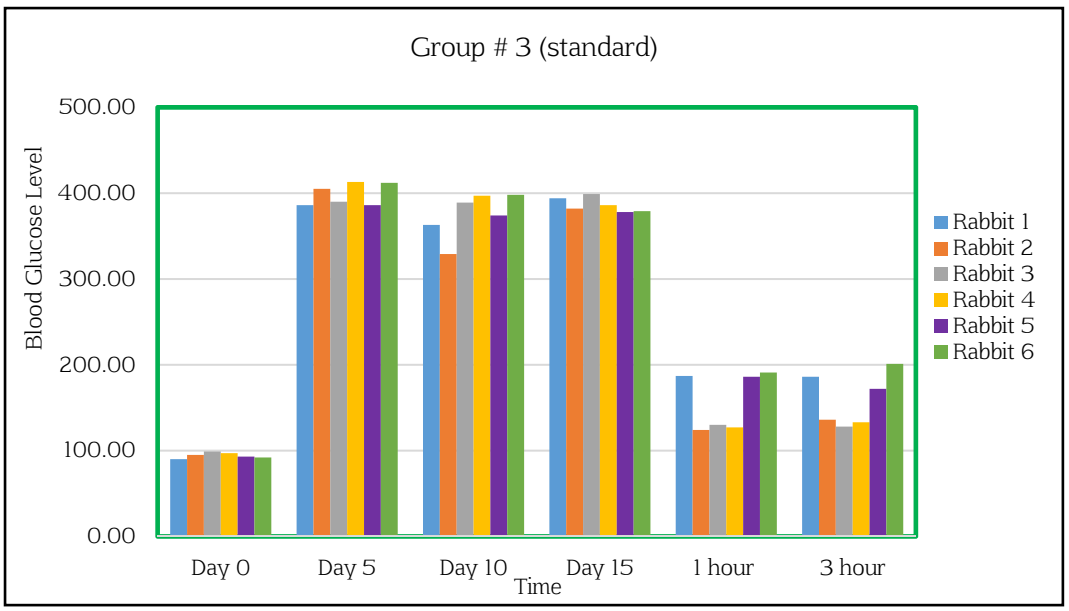


Figure 9

BGL after introducing 200mg/kg MOL1 extract to diabetic rabbits.

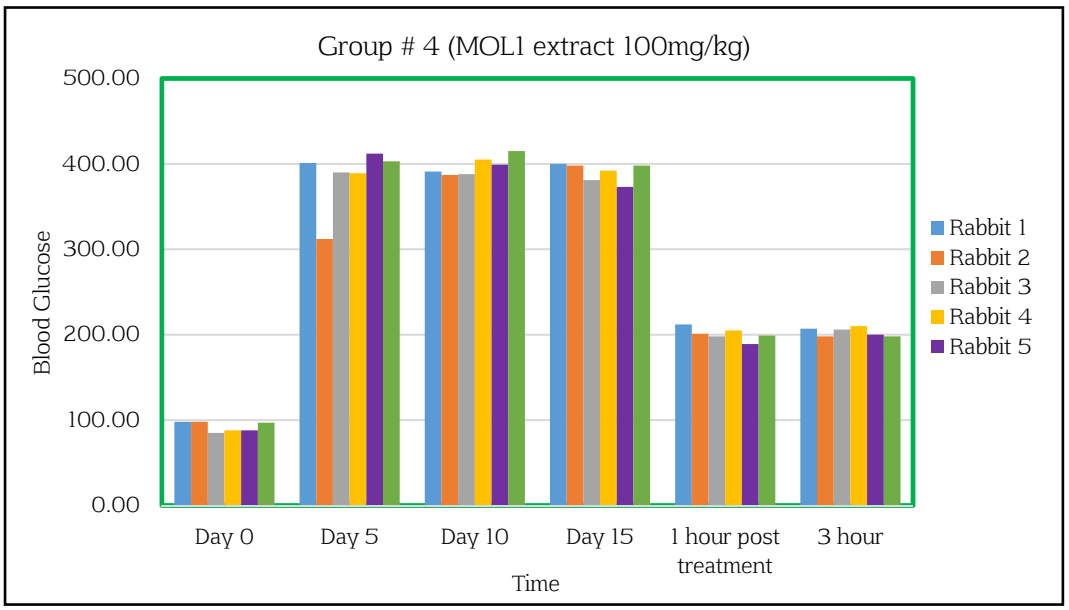
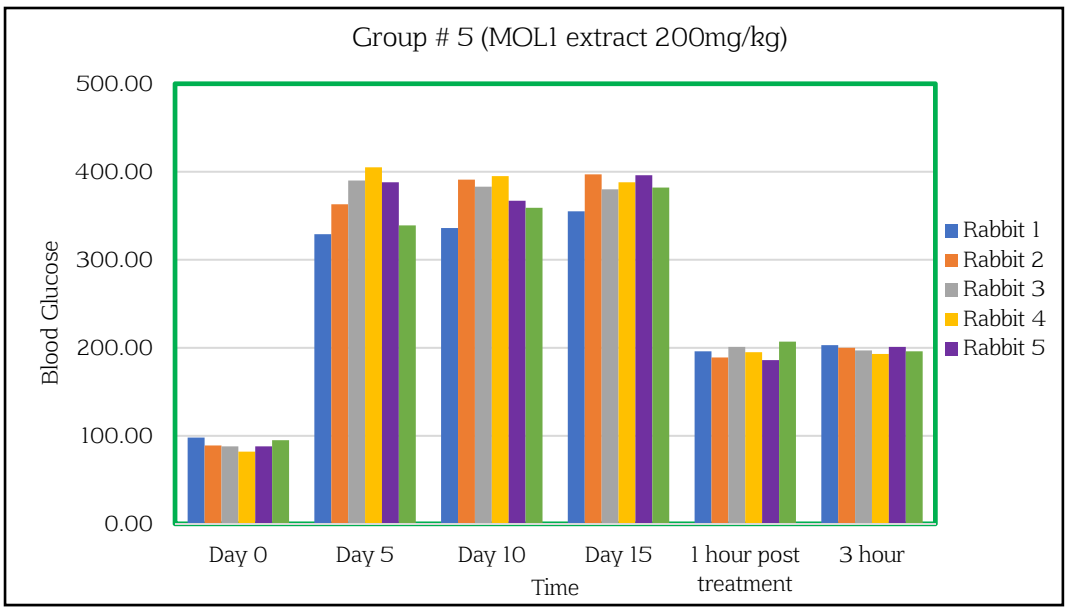


Figure 10

BGL after introducing 200mg/kg MOL1 extract to diabetic rabbits.



Change in weight levels of diabatic animals after treatment with MOL3

The various aforesaid measures shown in the procedure resulted in variation which is represented in **Table**: 13-18 and **Figure**: 11-15. After treatment with 100mg/kg of MOL3 weight decreased to 1.21±0.09 from 1.49±0.08. 200mg/kg of extract reduced body weight from 1.39±0.18 to 1.11±0.075. Both preparations formed hypoglycemic effects but high concentration showed more control. The experiment was dose-dependent.

Weight level of test animals:

Table 13

Group # 1 (- control)

S. NO	Day 0	Day 5	Day 10	Day 15
1	1.2 kg	1.3	1.3	1.4
2	1.3 kg	1.4	1.5	1.5
3	1.2	1.3	1.4	1.4
4	1.4	1.4	1.5	1.5
5	1.5	1.5	1.5	1.6
6	1.2	1.3	1.4	1.4

**Normal saline used as control 10 ml/kg*

Table 14

Group # 2 (disease + control)

S. NO	Day 0	Day 5	Day 10	Day 15
1	1.5 kg	1.4	1.2	1.1
2	1.4 kg	1.3	1.3	1.2
3	1.5	1.4	1.3	1.3
4	1.3	1.3	1.2	1.1
5	1.4	1.3	1.3	1.1
6	1.5	1.4	1.3	1.2

*Alloxan used 80mg/kg

Table 15

Group # 3 (+ control)

S. NO	Day 0	Day 5	Day 10	Day 15
1	1.3 kg	1.3	1.2	1.2
2	1.3 kg	1.2	1.1	1.0
3	1.6	1.4	1.4	1.3
4	1.4	1.3	1.2	1.1
5	1.5	1.5	1.4	1.3
6	1.4	1.3	1.2	1.2

*Tolbutamide used as a standard drug 300mg/kg

Table 16

Group # 4 (MOL3 extract 100 mg/kg)

S. NO	Day 0	Day 5	Day 10	Day 15
1	1.6 kg	1.5	1.4	1.3
2	1.4 kg	1.4	1.3	1.2
3	1.6	1.6	1.5	1.3
4	1.4	1.3	1.2	1.1
5	1.5	1.3	1.2	1.1
6	1.5	1.5	1.4	1.3

***** MOL3 test sample used as 100 mg/kg.

Table 17

Group # 5 (MOL3 extract 200 mg/kg)

S. NO	Day 0	Day 5	Day 10	Day 15
1	1.4 kg	1.4	1.3	1.2
2	1.4 kg	1.3	1.2	1.1
3	1.6	1.5	1.4	1.2
4	1.5	1.4	1.3	1.1
5	1.2	1.1	1.1	1.0
6	1.3	1.2	1.1	1.1

*MOL3 test sample used as 200 mg/kg.

Table 18

Effect of secondary metabolites MOL3 isolated from Moringa oliefera plant on body weight of alloxan-induced diabetic and normal male rabbits.

Experimental groups	Treatment dose(mg/kg)	Day 0	Day 5	Day 10	Day 15
Group#1 (-control)	Saline	1.31±0.15	1.36±0.081	1.43±0.081	1.46±0.08
Group#2 (disease+control)	Alloxan+saline	1.42±0.09	1.34±0.05	1.26±0.05	1.16±0.08
Group#3 (+control)	Tolbutamide	1.47±0.09	1.33±0.10	1.24±0.12	1.17±0.11
Group#4	MOL3	1.49±0.08	1.42±0.12	1.32±0.12	1.21±0.098
Group#5	MOL3	1.39±0.18	1.30±0.14	1.22±0.12	1.11±0.075

Weight of rabbits after the introduction of saline introduction.

Figure 13

Weight after introducing tolbutamide to diabatic rabbits.

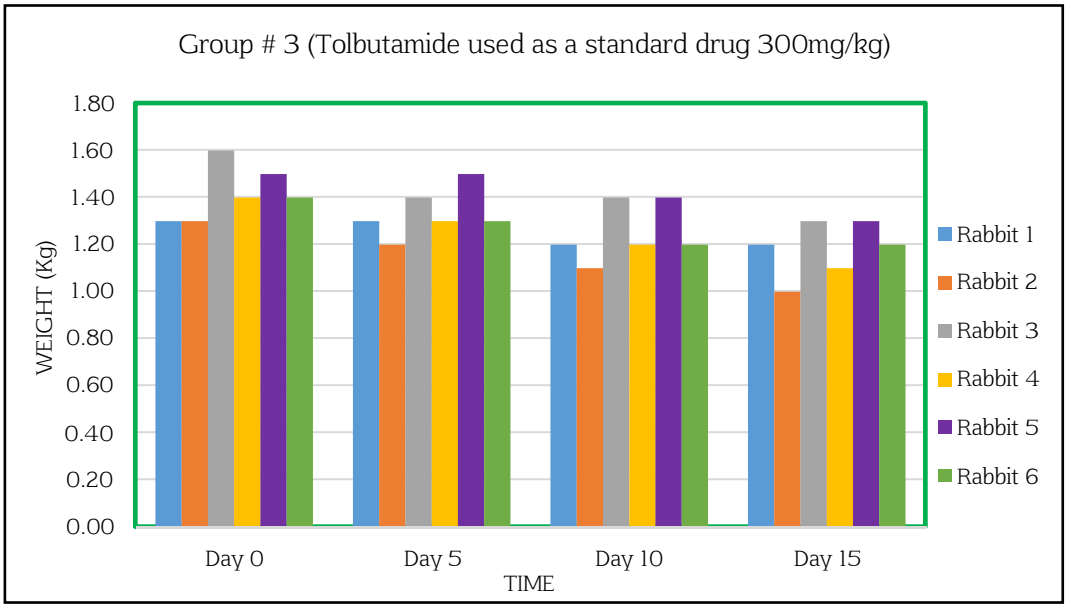


Figure 14

Weight after introducing MOL3 100mg/kg to diabatic rabbits.

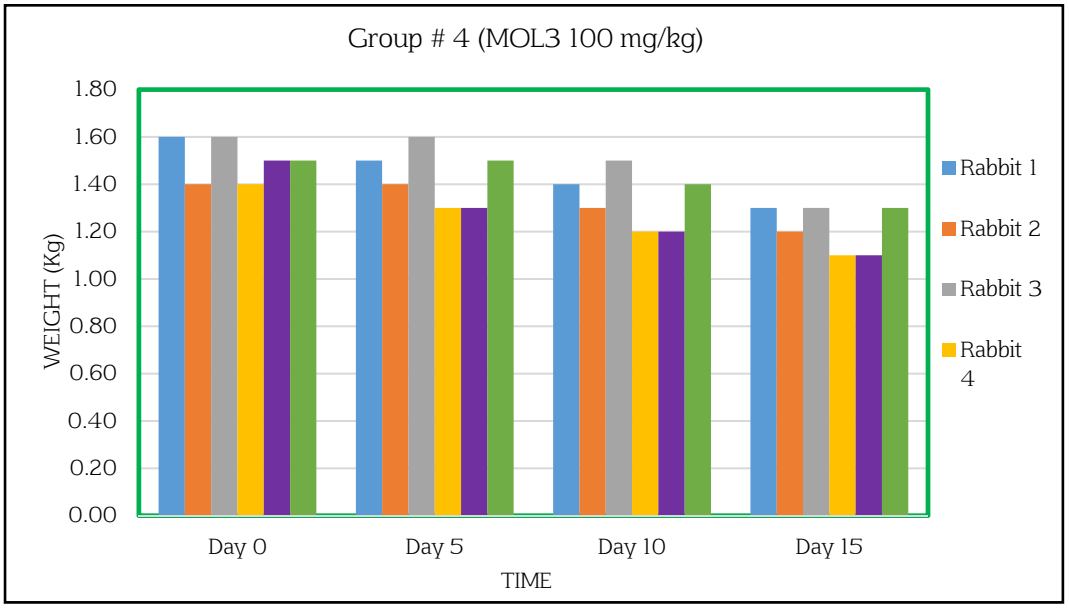
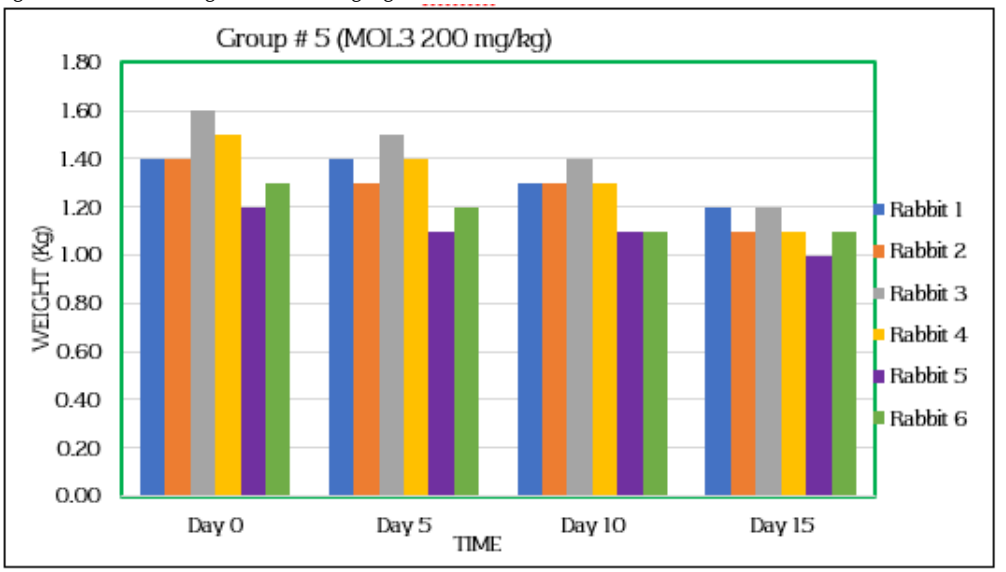


Figure 15

Weight after introducing MOL3 200mg/kg to diabatic rabbits.



Change in BGL levels of diabatic animals after treatment with MOL3

The drugs were administered orally to animals once daily for 15 days. Non-glycemic along with alloxan-induced diabetic rabbits were tested for several treatments which are displayed in Tables: 19-24 and Figure 16- 20. Mean BGL of non-diabetic negative control group#1 was varied between 93.3 to 94 and diabetic animals with negative control group#2 were between 367.9 to 405 mg/dl during the study of MOL3. In group #3 there was a significant reduction of BGL. In group#4, which received 100 mg/kg body weight of MOL3, the BGL count on the 15th day was 387.4±6.80 and after 1 hour of post-treatment it decreased to 184.4±44.06, MOL3 concentration of 200 mg/kg reduced BGL to 211±33.33 from 410.4±45.67 after 3 hours of post-treatment it further decreased to 180.3±34.1.

Blood glucose level

Table 19

Group # 1 (Control)

S. NO	Day 0	Day 5	Day 10	Day 15	1hour post-treatment	3hour
1	99	101	101	99	86	92
2	98	91	93	91	93	86
3	101	94	91	86	101	98
4	86	87	86	102	89	100
5	82	98	81	95	99	80
6	96	95	94	93	100	98

Table 20

Group # 2 (disease + control)

S. NO	Day 0	Day 5	Day 10	Day 15	1hour post-treatment	3hour
1	89	384	337	394	395	392
2	85	365	376	412	398	402
3	89	390	412	450	407	412
4	90	400	360	430	447	436

5	102	344	413	388	411	401
6	100	330	400	366	401	350

Table 21

Group # 3 (standard)

S. NO	Day 0	Day 5	Day 10	Day 15	1hour post-treatment	3hour
1	91	376	373	398	197	186
2	94	400	349	387	114	130
3	96	399	384	399	133	138
4	95	411	399	376	117	123
5	91	388	379	398	188	162
6	92	450	328	479	132	191

Table 22

Group # 4 (MOL3 100mg/kg)

S. NO	Day 0	Day 5	Day 10	Day 15	1hour post-treatment	3hour
1	89	331	354	380	242	230
2	99	312	356	388	200	198
3	85	380	388	386	108	207
4	90	401	390	392	199	210
5	81	312	386	381	200	211
6	87	333	395	398	189	199

Table 23

Group # 5 (MOL3 200mg/kg)

S. NO	Day 0	Day 5	Day 10	Day 15	1hour post-treatment	3hour
1	99	332	433	455	198	211
2	81	333	365	401	280	202
3	98	390	323	390	200	199
4	83	305	309	381	201	101
5	84	381	370	366	190	201
6	91	309	341	482	212	200

Table 24

Effect of secondary metabolites of MOL3 isolated from Moringa oliefera plant on body BGL of alloxan-induced diabetic and normal male rabbit.

Experimental groups	Treatment dose(mg/kg)	Day 0	Day 5	Day 10	Day 15	1hour post-treatment	3hour
Group#1 (-control)	Saline	93.3±87.76	94.2±4.96	90.7±6.8	94.1±5.7	94.4±6.2	92.03±7.94
Group#2	Alloxan+saline	92.2±6.83	367.9±27.52	381.9±28.0	405.7±30.37	409.4±19.12	397.9±28.2
Group#3 (+control)	Tolbutamide	93.14±2.13	403.3±25.4	367.8±25.7	404.8±36.7	143.3±36.3	152.7±29.1
Group#4	MOL3 100mg/kg	88.33±6.05	367.93±30.4	377.7±18.2	387.4±6.80	184.4±44.06	208.9±11.58
Group#5	MOL3 200mg/kg	89.04±7.86	340.1±35.95	354.6±44.1	410.4±45.67	211.6±33.33	180.3±34.1

Figure 16

BGL after introducing with normal saline

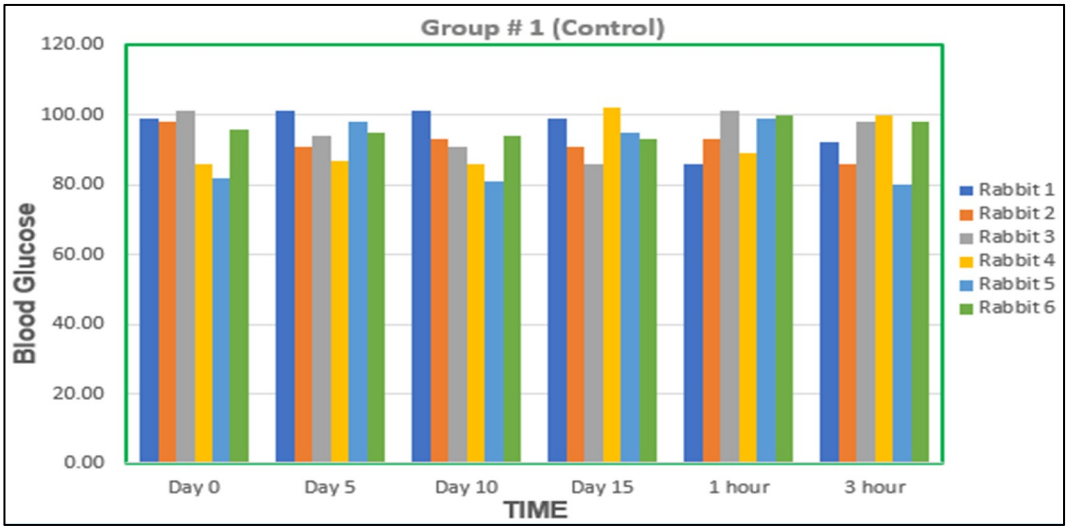


Figure 17

BGL after introducing alloxan.

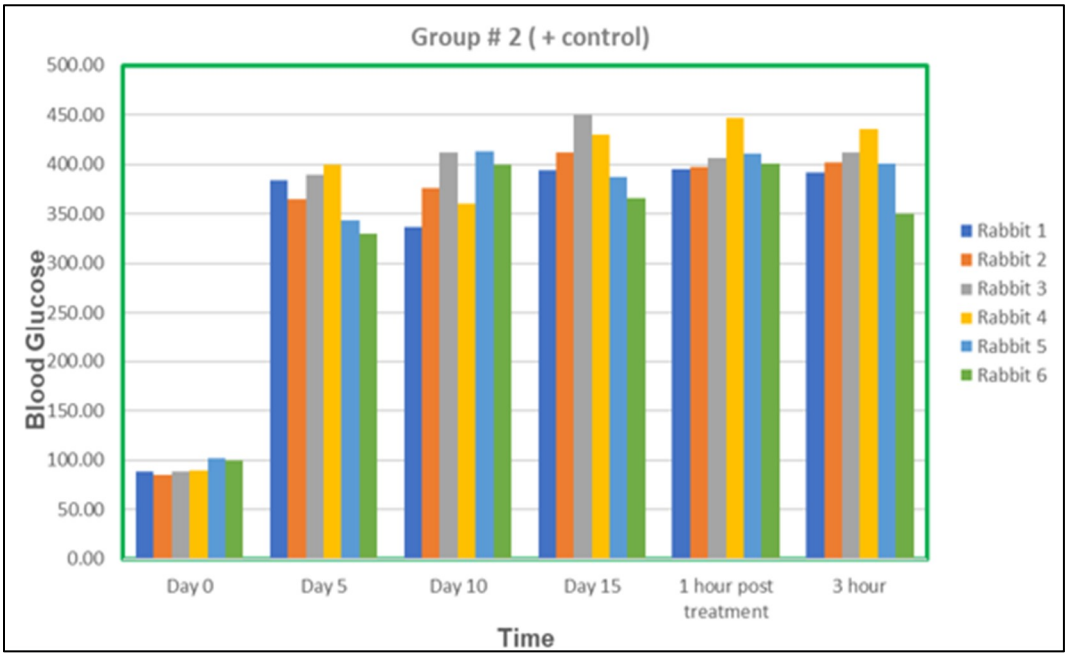


Figure 18

BGL after the introduction of Tolbutamide in 15 day diabatic rabbits.

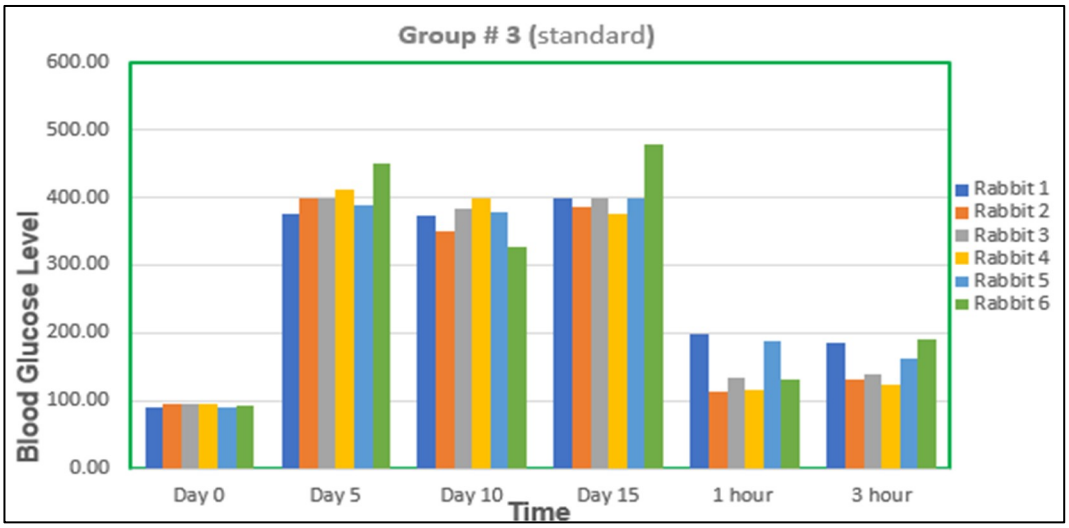


Figure 19

BGL after treatment with 100mg/kg of MOL3 extract in diabetic rabbits.

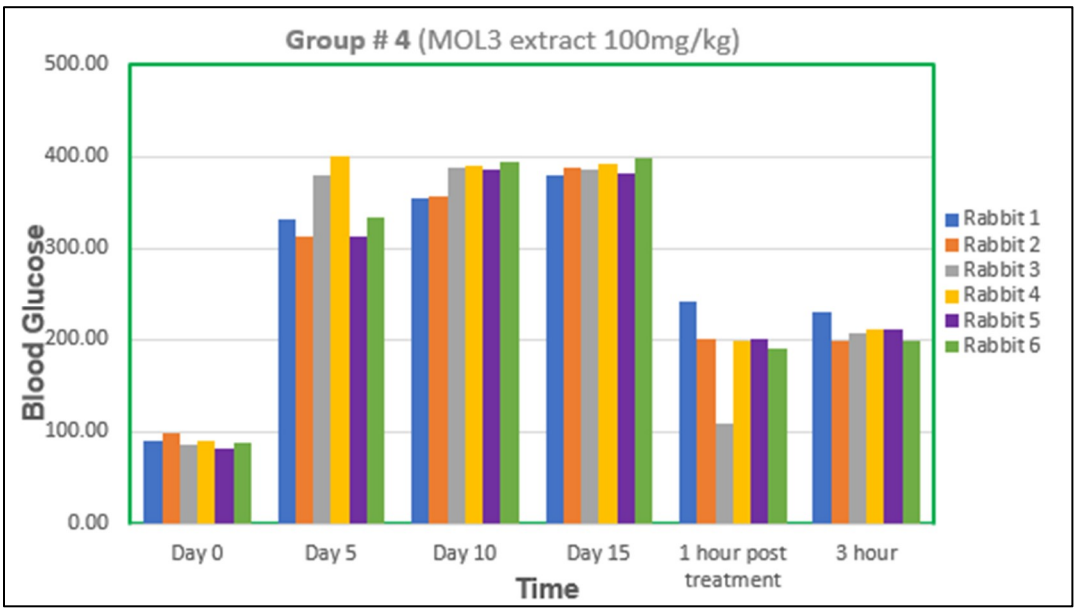
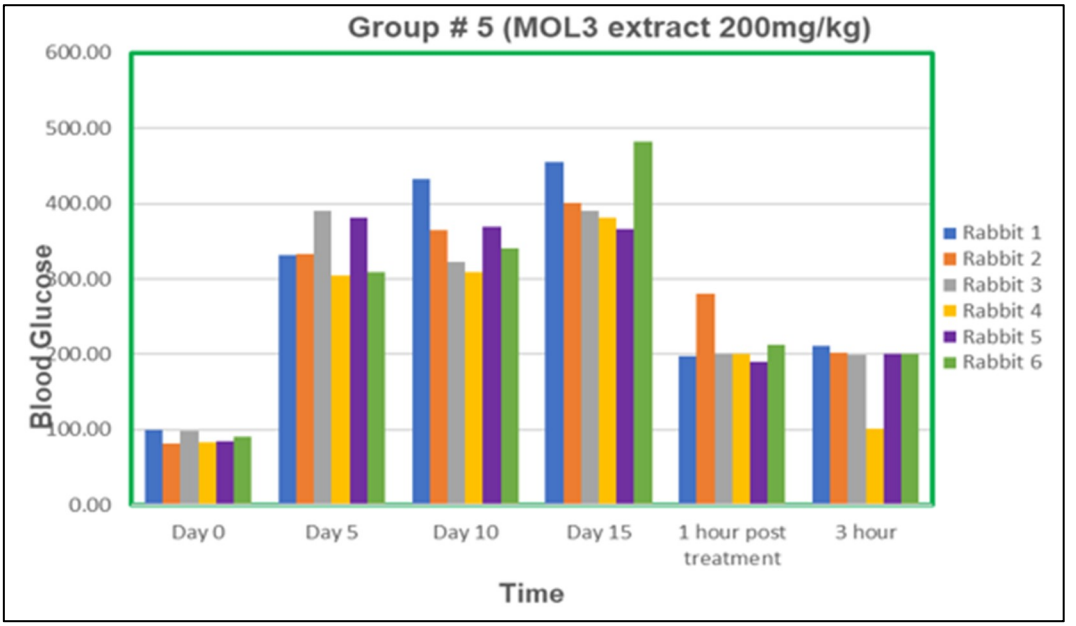


Figure 20

BGL after treatment with 200mg/kg of MOL3 extract in diabetic rabbits.



Macro and Microscopic Characterization of the fungal endophytes

Isolated endophytic fungi from the Leaves and roots of M. oliefera (located in PFI) were identified via morphological (microscopic and macroscopic) characters. The morphology of identified endophytic fungi is displayed in Table: 3.25 and Figure: 21. They were identified on the basis of colony surface, reverse side color, growth rate, margin of colony and microscopic features such as Hyphae, conidiophore, conidia, fruiting body.

MOL1 appeared as Green, velvety surfaced while the reverse side of the plate showed a yellowish to white colour appearance. Growth was rapid and margins were irregular. MOL3 colonies appeared white at the start and then they quickly became black. The reverse side of the plate was pale yellow growth was fast with the entire margin.

MOL1 under the microscope showed Septate hyphae, Phialides grouped brush-like clusters and unicellular. Conidiophores were oval and conidia were arranged in smooth chains. MOL3 hyphae were septate, conidia appeared black, rough and globose. Smooth, hyaline, darker apex conidiophores.

Micro and Macroscopic features of MOL1 & MOL3 isolated from M. oliefera leaves.

CAACGGGTAACGGGGAATTAGGGTTCGATTCCGGAGAGGGAGCCTGAGAA
ACGGCTACCACATCCAAGGAAGGCAGCAGGCGCGCAAATTACCCAATCCC
GACACGGGGAGGTAGTGACAATAAATACTGATACGGGGCTCTTTTGGGTC
TCGTAATTGGAATGAGTACAATCTAAATCCCTTAACGAGGAACAATTGGA
GGGCAAGTCTGGTGCCAGCAGCCGCGGTAATTCCAGCTCCAATAGCGTAT
ATTAAAGTTGTTGCAGTTAAAAAGCTCGTAGTTGAACCTTGGGTCTGGCT
GGCCGGTCCGCCTCACCGCGAGTACTGGTCCGGCTGGACCTTTCCTTCTG
GGGAATCTCATGGCCTTCACTGGCTGTGGGGGGAACCAGGACTTTTACTG
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GGAATAATAGAATAGGACGTGCGGTTCTATTTTGTTGGTTTCTAGGACCG
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GGTGAAATTCTTGGATTTGCTGAAGACTAACTACTGCGAAAGCATTCGCC
AAGGATGTTTTCATTAATCAGGGAACGAAAGTTAGGGGATCGAAGACGAT
CAGATACCGTCGTAGTCTTAACCATAAACTATGCCGACTAGGGATCGGAC
GGTGTTTCTATTATGACCCGTTCGGCACCTTACAAGAAATCAAAGTTTTT
GGGTTCTGGGGGGGAATATGGTCCGCAAGGCGAAAACTTAAAGAAATTGA
CGGAGGGGCCCACCCCGGGGTGGAACCCGCGGCTTAATTTTGTCCCCCCCGG

Table 27

Sequence of MOL3 (Aspergillus niger)

ACATGACGGGGTTGACCACTTTCCGGCTCTGGGGGGTCGTTGCCAACCCT
CCTGAGCCAGTCCGAAGGCCTCACCGAGCCATTCAATCGGTAGTAGCGAC
GGGCGGTGTGTACAAAGGGCAGGGACGTAATCGGCACGAGCTGATGACTC
GTGCCTACTAGGCATTCCTCGTTGAAGAGCAATAATTGCAATGCTCTATC
CCCAGCACGACAGGGTTTAACAAGATTACCCAGACCTCTCGGCCAAGGTG
ATGTACTCGCTGGCCCTGTCAGTGTAGCGCGCGTGCGGCCCAGAACATCT
AAGGGCATCACAGACCTGTTATTGCCGCGCACTTCCATCGGCTTGAGCCG
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AGGGCCGAGGTCTCGTTCGTTATCGCAATTAAGCAGACAAATCACTCCAC
CAACTAAGAACGGCCATGCACCACCATCCAAAAGATCAAGAAAGAGCTCT
CAATCTGTCAATCCTTATTTTGTCTGGACCTGGTGAGTTTCCCCGTGTTG
AGTCAAATTAAGCCGCAGGCTCCACGCCTGGTGGTGCCCTTCCGTCAATT
TCTTTAAGTTTCAGCCTTGCGACCATACTCCCCCCAGAACCCAAAAACTT
TGATTTCTCGTAAGGTGCCGAACGGGTCATAATAGAAACACCGTCCGATC
CCTAGTCGGCATAGTTTATGGTTAAGACTACGACGGTATCTGATCGTCTT
CGATCCCCTAACTTTCGTTCCCTGATTAATGAAAACATCCTTGGCGAATG
CTTTCGCAGTAGTTAGTCTTCAGCAAATCCAAGAATTTCACCTCTGACAG
CTGAATACTGACGCCCCCGACTATCCCTATTAATCATTACGGCGGTCCTA
GAAACCAACAAAATAGAACCGCACGTCCTATTCTATTATTCCATGCTAAT
GTATTCGAGCAAAGGCCTGCTTTGAACACTCTAATTTTTTCACAGTAAAA
GTCCTGGTTCCCCCACAGCCAGTGAAGGCCATGAGATTCCCCAGAAGGAA
AGGTCCAGCCGGACCGTACTCGCGGTGAGGCGGACCGGCCAGCCGAACCC
AAGGTTAACTACAAGCTTTTTACTGCACAACTTTAAAAACGCCTTTGGAG
CGGGAATACCCCGGCTGCGGGGACCAGAATGCCCTCCAATGTTCCCGCTT
AAGGGGTTAAATGGGCCCCTTCCCTTTCAGAACCCAAAGAACCCGCGCCC
CAATTAATTTTCTCCCTCCCCCCCCCGGGAGGGGAATTCCGCCCCCCCCC
CCCCCCCCAGAGG

Figure 22

The phylogenetic tree of MOL1 was identified as Penicillium sp.

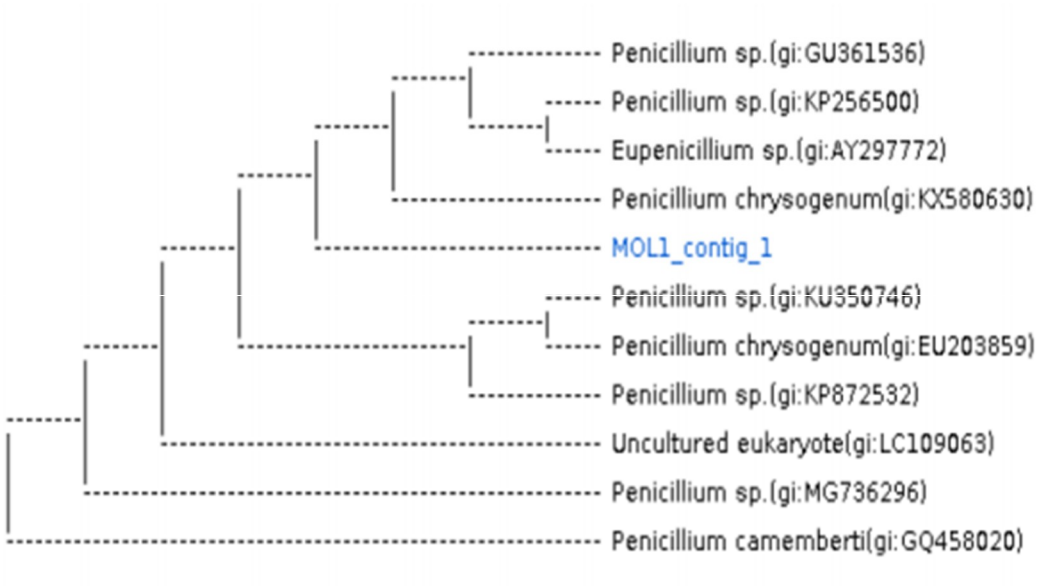
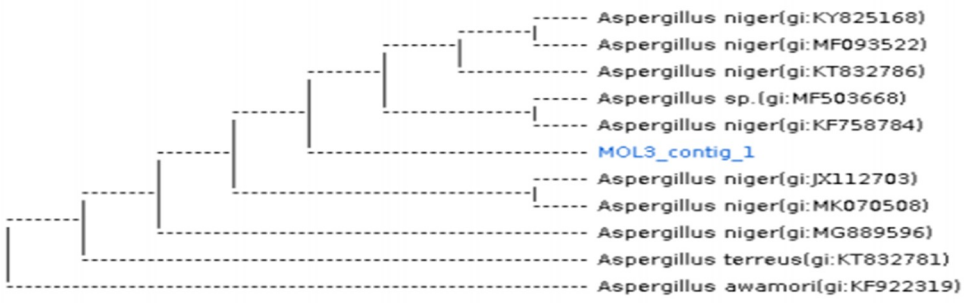


Figure 23

The phylogenetic tree of MOL3 was identified as Aspergillus niger.



GC-MS analysis

Plant endophytes are a significant source of compounds. Fungi contain secondary metabolites for example flavonoids, alkaloids, terpenes, steroids and phenolics. The chemical composition of two selected endophytic fungal extracts was screened through GC-MS analysis.

GC-MS analysis of Crude metabolic extract from MOL1 fungi

Eighteen bioactive compounds were acknowledged from the ethyl acetate extract of MOL1 fungi. The identification of fungal compounds was based on the molecular weight, peak area, molecular formula, retention time, MS Fragment- ions and Pharmacological activities mentioned below in Figure 24, Tables 28, 29 &30. GC-MS investigation of the extract discovered the presence of Fatty acids

Macrocyclic lactones, Lactam, Steroid, Linoleic acid ester, lipid, Sterols, Carotenoid, Amino acid, Polyphenols, Straight chain fatty acids and Ester such as; (1) Butane, 1,1-diethoxy-3-methyl- (2)Strychane, 1-acetyl-20à-hydroxy-16-methylene, (3) 9-Octadecenoic acid, (2-phenyl-1,3-dioxolan-4-yl)methyl ester, cis, (4) Milbemycin b, 13-chloro-5-demethoxy-28-deoxy-6,28-e poxy-5-(hydroxyimino)-25-(1-methyleth yl)-, (6R,13R,25R) (4)2,7-Diphenyl-1,6-dioxopyridazino[4,5:2 ',3']pyrrolo[4',5'-d]pyridazine, (5)Ethyl iso-allocholate, (6) Cholestan-3-one, cyclic 1,2-ethanediyl aetal, (5à), (7) 9,12,15-Octadecatrienoic

acid, 2,3-bis[(trimethylsilyl)oxy]propyl ester, (Z,Z,Z, (8) Stearic acid, 3-(octadecyloxy)propyl ester, (9) Ergosta-5,22-dien-3-ol, acetate, (3á,22E), (10) Lycopene, (11) Olean-12-ene-3,15,16,21,22,28-hexol, (3á,15à,16à,21á,22à), (12) Glycine, N-[(3à,5á,7à,12à)-24-oxo-3,7,12-tris[(tr imethylsilyl)oxy]cholan-24-yl]-, methyl ester, (13)Quercetin, (14) Butanoic acid, (15) Bis(2-ethylhexyl) phthalate, (16)1-Monolinoleoylglycerol trimethylsilyl ether, (17)Hexasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11-dodecamethyl.

Figure 24

GC-MS Chromatogram of MOL1 compounds

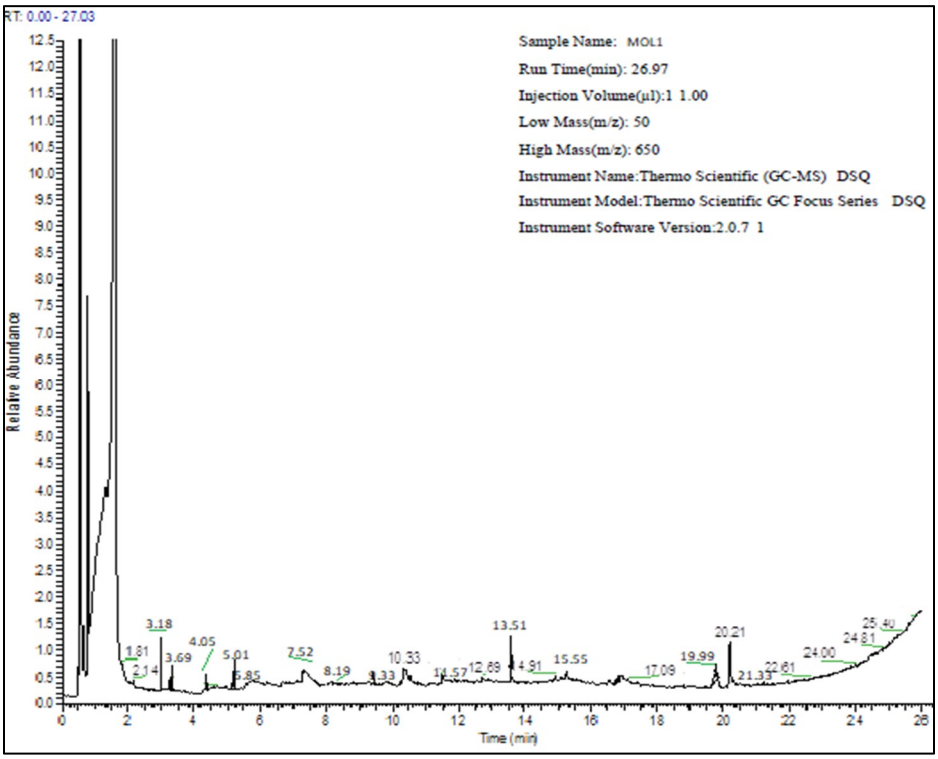


Table 28

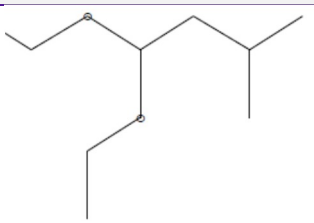
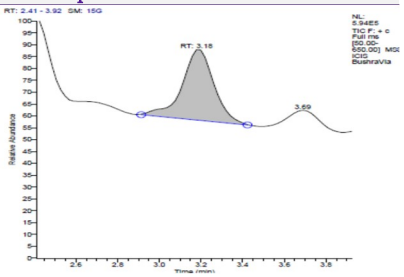
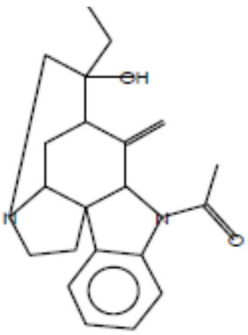
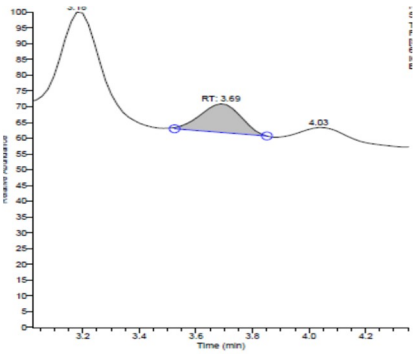
Compounds detected in the secondary metabolites of MOL1

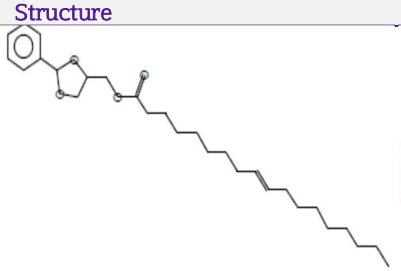
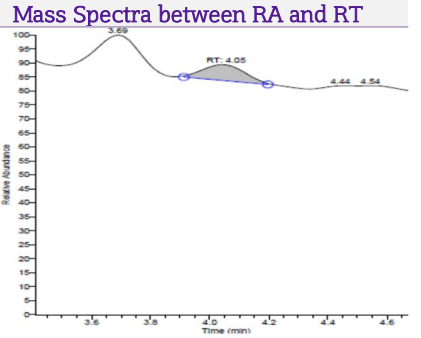
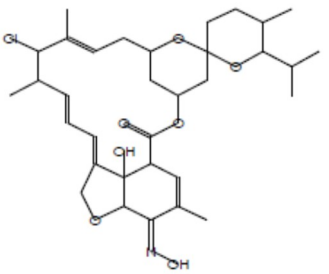
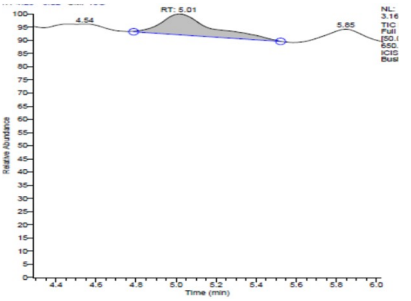
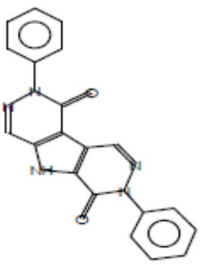
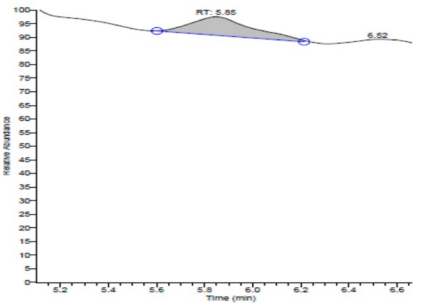
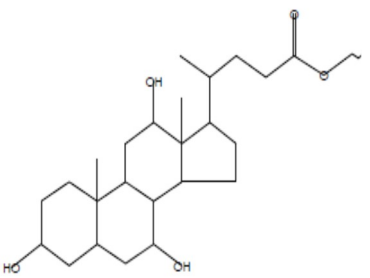
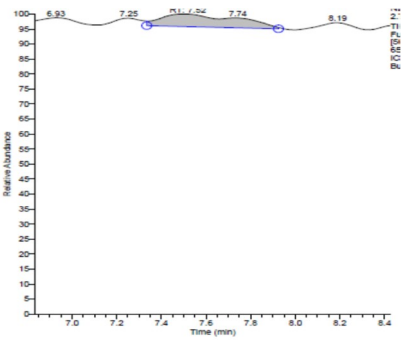
S.No	RT	Name of the Compound	Formula	M.W	Peak height
1	3.18	Butane, 1,1-diethoxy-3-methyl-	$C_9H_{20}O_2$	160	176890.17
2	3.69	Strychane, 1-acetyl-20à-hydroxy-16-methylene	$C_{21}H_{26}N_2O_2$	338	47481.91
3	4.05	9-Octadecenoic acid, (2-phenyl-1,3-dioxolan-4-yl)methyl ester, cis	$C_{28}H_{44}O_4$	444	21048.14
4	5.01	Milbemycin b, 13-chloro-5-demethoxy-28-deoxy-6,28-e poxy-5-(hydroxyimino)-25-(1-methyleth yl)-, (6R,13R,25R)	$C_6H_5NO_2$	123	24963.22
5	5.85	2,7-Diphenyl-1,6-dioxopyridazino[4,5:2',3']pyrrolo[4',5'-d]pyridazine	$C_{20}H_{13}N_5O_2$	353	20887.22
6	7.52	Ethyl iso-allocholate	$C_{26}H_{44}O_5$	436	11410.82

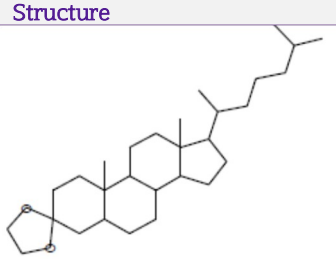
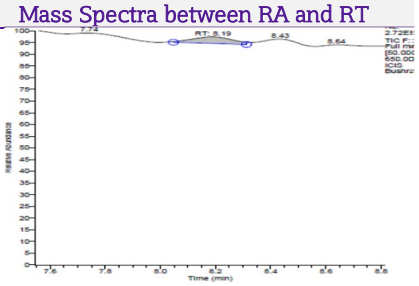
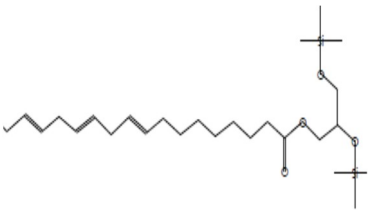
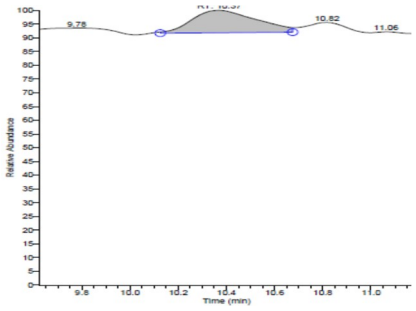
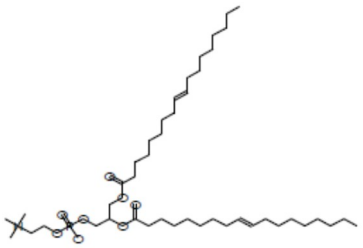
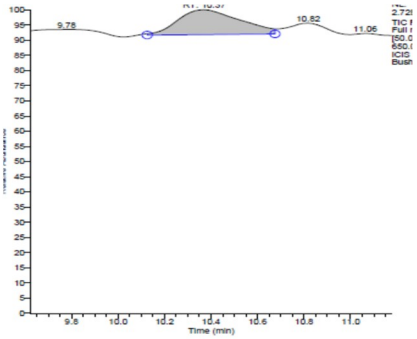
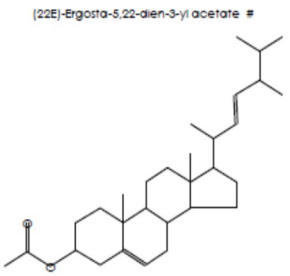
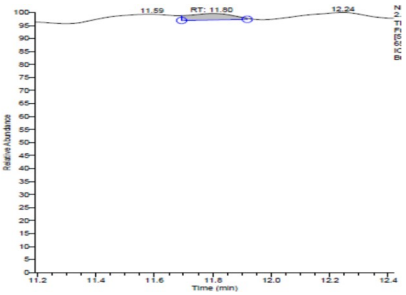
7	8.19	Cholestan-3-one, cyclic 1,2-ethanediyl aetal, (5á)	$C_{29}H_{50}O_2$	430	7847.80
8	9.33	9,12,15-Octadecatrienoic acid, 2,3-bis[(trimethylsilyl)oxy]propyl ester, (Z, Z, Z	$C_{33}H_{46}ClNO_7$	603	5238.72
9	10.33	Stearic acid, 3-(octadecyloxy)propyl ester	$C_{44}H_{84}NO_8P$	785	22277.26
10	11.57	Ergosta-5,22-dien-3-ol, acetate, (3á,22E)	$C_{25}H_{43}BO_4$	418	6804.70
11	12.63	Lycopene	$C_{40}H_{56}$	536	15545.39
12	13.51	Olean-12-ene-3,15,16,21,22,28-hexol, (3á,15à,16à,21á,22à)	$C_{30}H_{50}O_6$	506	8602.84
13	14.55	Glycine, N-[(3à,5á,7à,12à)-24-oxo-3,7,12-tris[(tr imethylsilyl)oxy]cholan-24-yl]-, methyl ester	$C_{36}H_{69}NO_6Si_3$	695	11123.58
14	15.55	Quercetin	$C_{15}H_{10}O_7$	302	15464.80
15	19.99	Butanoic acid	$C_{27}H_{54}O_4Si_2$	498	436253.70
16	20.21	Bis(2-ethylhexyl) phthalate	$C_{24}H_{38}O_4$	390	529279.46
17	21.33	1-Monolinoleoylglycerol trimethylsilyl ether	$C_{27}H_{54}O_4Si_2$	498	18092.78
18	22.61	Hexasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11-dodecamethyl	$C_{16}H_{50}O_7Si_8$	578	15099.11

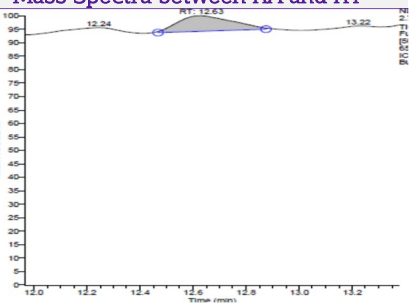
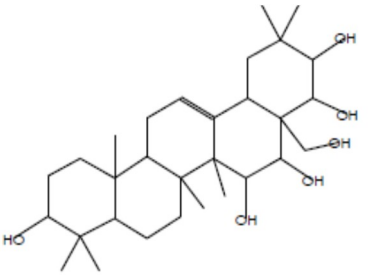
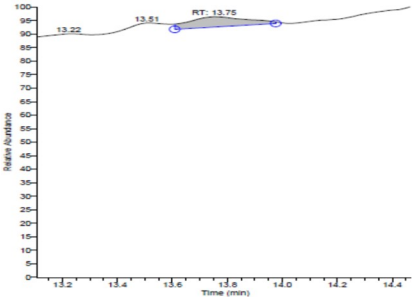
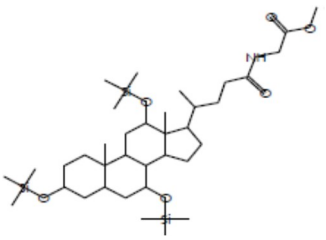
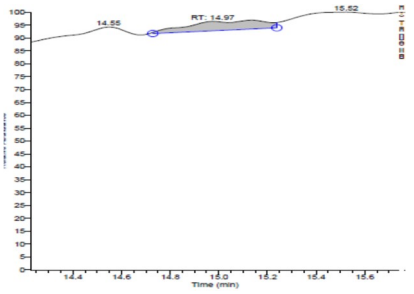
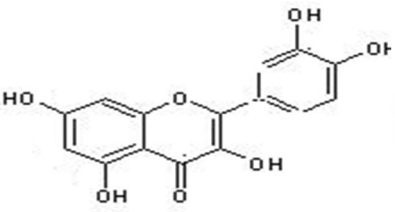
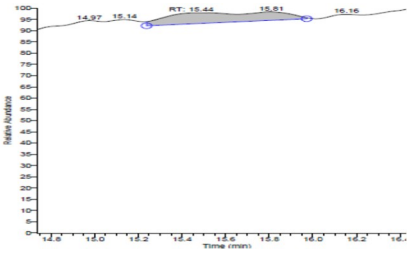
Table 29

Structure and Mass spectrum of major bioactive compounds of MOL1

Compound Name	Structure	Mass Spectra between RA and RT
Butane, 1,1-diethoxy-3-methyl-		
Strychane, 1-acetyl-20à-hydroxy-16-methylene		

Compound Name	Structure	Mass Spectra between RA and RT
9-Octadecenoic acid		
Milbemycin b, 13-chloro-5-demethoxy-28-deoxy-6,28-e poxy-5-(hydroxyimino)-25-(1-methyleth yl)-, (6R,13R,25R)		
2,7-Diphenyl-1,6-dioxopyridazino[4,5:2',3']pyrrolo[4',5'-d]pyridazine		
Ethyl iso-allocholate		

Compound Name	Structure	Mass Spectra between RA and RT
Cholestan-3-one, cyclic 1,2-ethanediyl aetal, (5á)		
9,12,15-Octadecatrienoic acid, 2,3-bis[(trimethylsilyl)oxy]propyl ester, (Z, Z, Z		
Stearic acid, 3-(octadecyloxy)propyl ester		
Ergosta-5,22-dien-3-ol, acetate, (3á,22E)		

Compound Name	Structure	Mass Spectra between RA and RT
Lycopene		
Olean-12-ene-3,15,16,21,22,28-hexol, (3á,15à,16à,21á,22à)		
Glycine, N-[(3à,5á,7à,12à)-24-oxo-3,7,12-tris[(trimethylsilyl)oxy]cholan-24-yl]-, methyl ester		
Quercetin, 3,5,7,3',4'-pentahydroxyflavone		

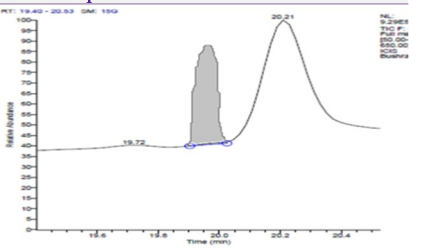
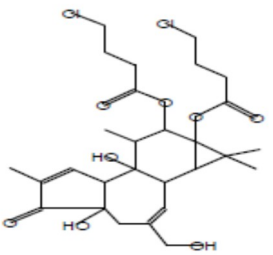
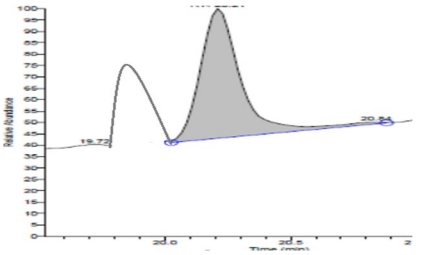
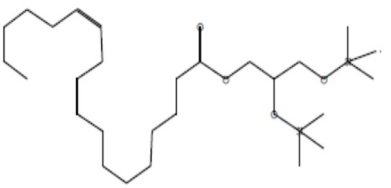
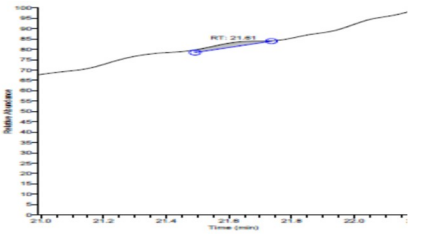
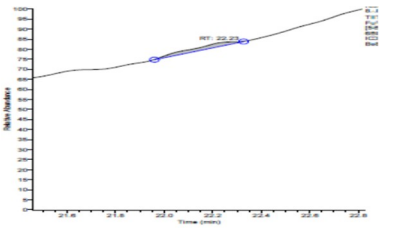
Compound Name	Structure	Mass Spectra between RA and RT
Butanoic acid		
Bis(2-ethylhexyl) phthalate		
1-Monolinoleoylglycerol trimethylsilyl ether		
Hexasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11-dodecamethyl		

Table 30

Activity of major compounds identified by GC – MS analysis of secondary metabolites from MOL1 endophytic fungi.

Name of the Compound	Nature of Compound	Pharmacological activities
Butane, 1,1-diethoxy-3-methyl-	Aroma compounds	Food additives and flavouring agent (15)
Strychane, 1-acetyl-20à-hydroxy-16-methylene	-	New chemical compound (16)

Name of the Compound	Nature of Compound	Pharmacological activities
9-Octadecenoic acid	fatty acids	Anti-inflammatory, Hypocholesterolemic Cancer preventive, Hepatoprotective, Nematicide Insectifuge, Antihistaminic Antieczemic, Antiacne, 5-Alpha reductase inhibitor Antiandrogenic, Antiarthritic, Anticoronary, Insectifuge (16)
Milbemycin b, 13-chloro-5-demethoxy-28-deoxy-6,28-e poxy-5-(hydroxyimino)-25-(1-methyleth yl)-, (6R,13R,25R)	macrocyclic lactones	Anti-infective, Antiparasatic (17)
2,7-Diphenyl-1,6-dioxopyridazino[4,5:2 ',3']pyrrolo[4',5'-d]pyridazine	Lactam	anti-angiogenic effects and anti-tumor efficacy (18)
Ethyl iso-allocholate	Steroid	anti-inflammatory, anticancer antimicrobial, antiasthma, diuretic (19)
Cholestan-3-one, cyclic 1,2-ethanediyl aetal, (5á)	Steroid	analgesic, antiulcer, anticancer (20)
9,12,15-Octadecatrienoic acid, 2,3-bis[(trimethylsilyl)oxy]propyl ester, (Z, Z, Z	Linoleic acid ester	cancer preventive, Anti-inflammatory, hepatoprotective (21)
Stearic acid, 3-(octadecyloxy)propyl ester	lipid	Unknown (22)
Ergosta-5,22-dien-3-ol, acetate, (3á,22E)	sterols	Anticancer activities (23)
Lycopene	carotenoid	antioxidant properties (24)
Olean-12-ene-3,15,16,21,22,28-hexol, (3á,15à,16à,21á,22à)		Unknown
Glycine, N-[(3à,5á,7à,12à)-24-oxo-3,7,12-tris[(tr imethylsilyl)oxy]cholan-24-yl]-, methyl ester	Amino acid	immune-modulating substance, cytoprotective (25)
Quercetin	polyphenols	Antidiabetic (26)
Butanoic acid	straight chain fatty acids	Antiviral, antitumor, anti-HIV and antinociceptive activities (27)
Bis(2-ethylhexyl) phthalate	ester	Plasticizers (28)
1-Monolinoleoylglycerol trimethylsilyl ether	Steroid	Antimicrobial, Antioxidant, Antiinflammatory Antiarthritic Antiasthma, Diuretic (29)
Hexasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11-dodecamethyl	fatty acids	Anti-microbial, anti-septic, emollient, antibacterial, hair conditioning agent (30)

GC-MS analysis of Crude metabolic extract from MOL3 fungi

The library statistics of main peaks present in chromatograms were extracted via the database NIST shown in Figure 25. Several peaks with different retention times (RT) were detected in the chromatograph Table 31, 32. The molecular formula and molecular weight were presumed from the spectra. Biological activities of all compounds were reviewed in Table 3.34.

Eleven myco-compounds were identified in analysis (1)Cyclotetrasiloxane, octamethyl, (2) Butanedioic acid, dimethyl ester, (3) 8,14-Seco-3,19-epoxyandrostane-8,14dione, (4) 4-Amino-1,5-pentandioic acid (5) 9-Octadecenoic acid (Z)-, methyl ester, (6)16-Nitrobicyclo[10.4.0]hexadecan-1-ol13-one, (7) Ricinoleic acid, (8) Dasycarpidan-1-methanol,

acetate (ester), (9) Benzeneacetonitrile, 4-hydroxy-, (10) Phenol, 3,5-dimethoxy, (11)Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro-3-(2-methylpropyl).

Figure 25

GC-MS Chromatogram of MOL3 compounds

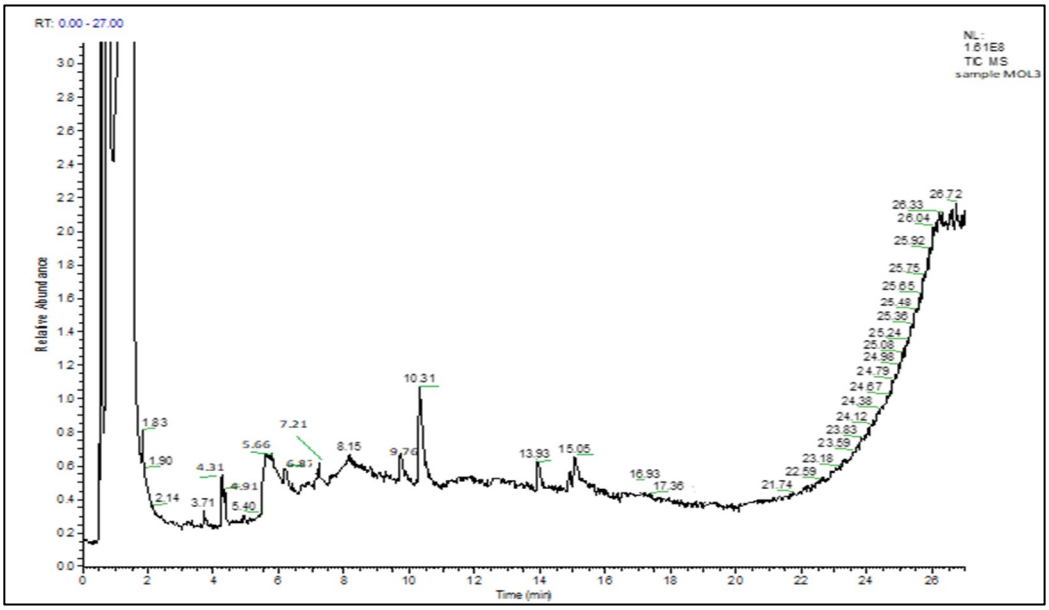


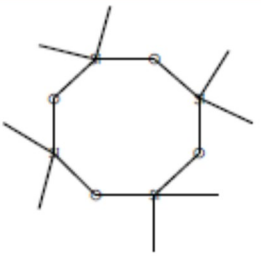
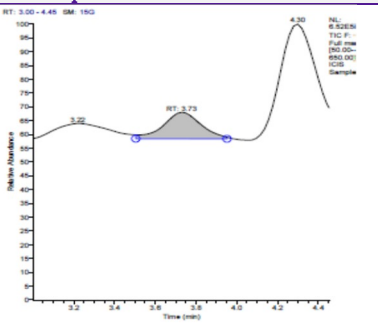
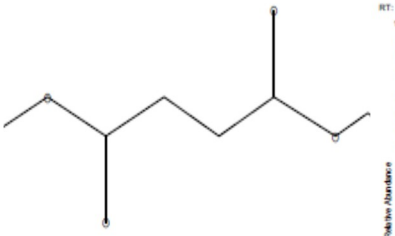
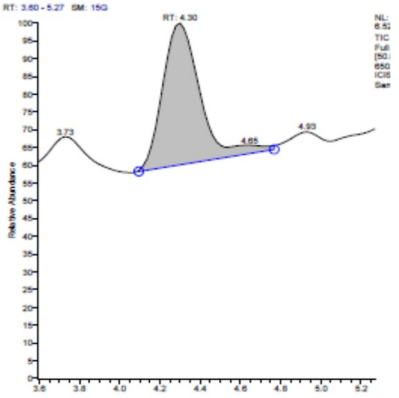
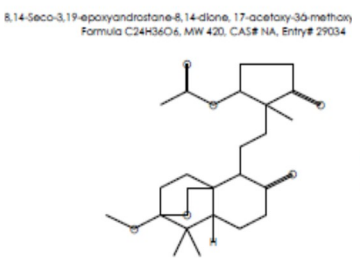
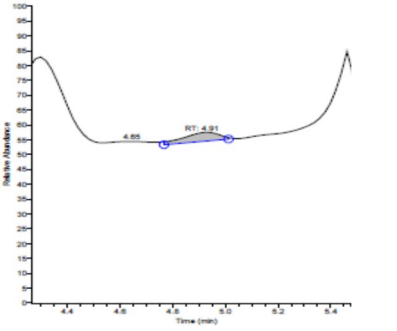
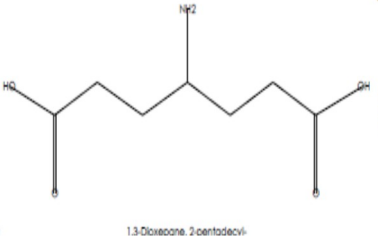
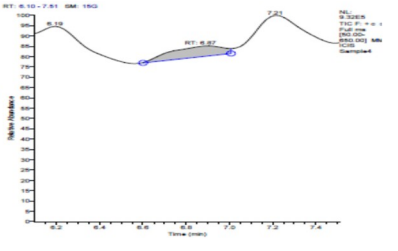
Table 31

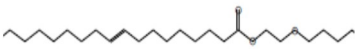
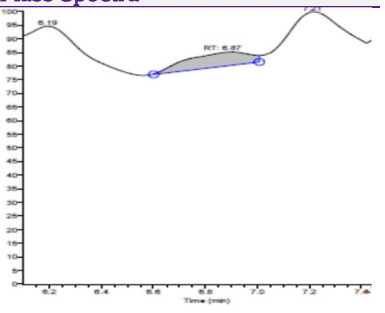
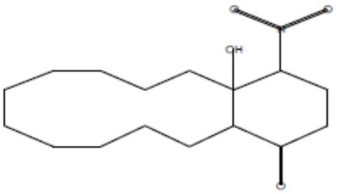
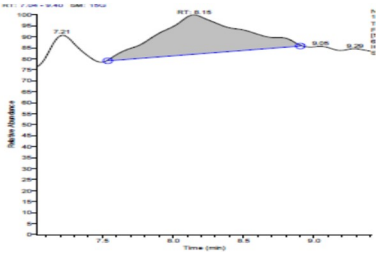
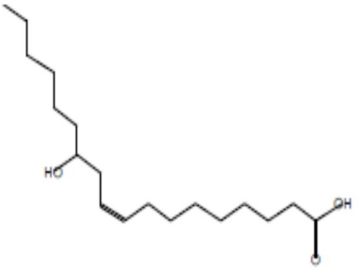
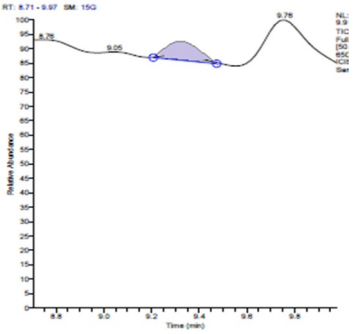
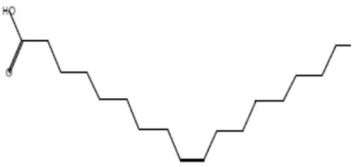
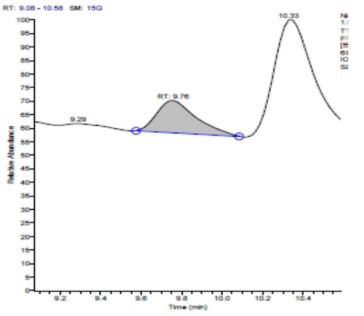
Compounds detected in the secondary metabolites of MOL3

S.No	RT	Name of the Compound	Formula	M.W	Peak Area%
1	3.73	Cyclotetrasiloxane, octamethyl	C8H24O4Si4	296	62747.35
2	4.31	Butanedioic acid, dimethyl ester	C6H10O4	146	259786.56
3	4.91	8,14-Seco-3,19-epoxyandrostane-8,14dione,	C24H36O6	420	23357.86
4	5.66	4-Amino-1,5-pentandioic acid	C7H13NO4	175	496874.24
5	6.87	9-Octadecenoic acid (Z)-, methyl ester	C24H46O3	38	46973.30
6	7.21	16-Nitrobicyclo[10.4.0]hexadecan-1-ol13-one	C16H27NO4	297	153120.55
7	8.15	Ricinoleic acid	C18H34O3	298	184111.79
8	9.76	Dasycarpidan-1-methanol, acetate (ester)	C20H26N2O2	326	169077.03
9	10.31	Benzeneacetonitrile, 4-hydroxy-	C8H7NO	133	616265.76
10	13.93	Phenol, 3,5-dimethoxy	C8H10O3	154	20388.68
11	15.05	Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro-3-(2-methylpropyl)	C11H18N2O2	210	228502.75

Table 32

Structure and Mass spectrum of major bioactive compounds of MOL3

Compound Name	Structure	Mass Spectra
Cyclotetrasiloxane, octamethyl		
Butanedioic acid, dimethyl ester		
8,14-Seco-3,19-epoxyandrostane-8,14dione		
4-Amino-1,5-pentandioic acid		

Compound Name	Structure	Mass Spectra
9-Octadecenoic acid (Z)-, methyl ester		
16-Nitrobicyclo[10.4.0]hexadecan-1-ol13-one		
Ricinoleic acid		
Dasycarpidan-1-methanol, acetate		

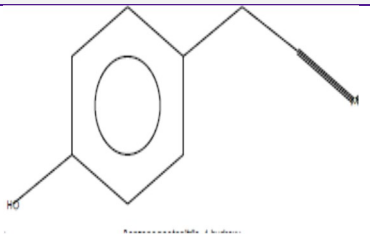
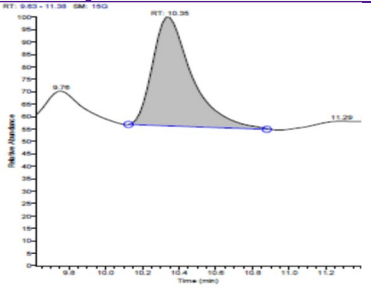
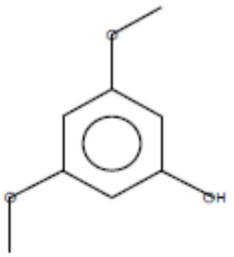
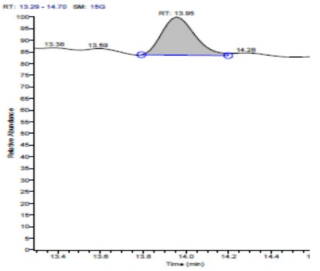
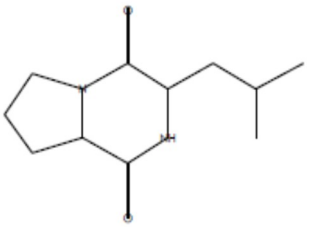
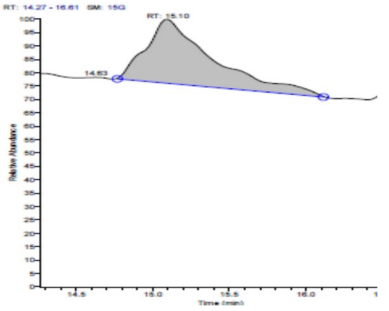
Compound Name	Structure	Mass Spectra
Benzeneacetonitrile, 4-hydroxy-		
Phenol, 3,5-dimethoxy		
Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro-3-(2-methylpropyl)		

Table 33

Nature and biological activities of bioactive compounds of MOL3

Name of the Compound	Nature of Compound	Pharmacological activities
Cyclotetrasiloxane, octamethyl	Organosilicon	Antibacterial (31)
Butanedioic acid, dimethyl ester	fatty acid methyl esters	Industrial use, Flavoring agent, Membrane stabilizer (32)
8,14-Seco-3,19-epoxyandrostane-8,14dione	Unknown	Antimicrobial (33)
4-Amino-1,5-pentandioic acid	vitamins	Anticancer agent (34)
9-Octadecenoic acid (Z)-, methyl ester	Linoleic acid ester	Anti-cancer activity (35)
16-Nitrobicyclo[10.4.0]hexadecan-1-ol13-one		Antibacterial activity (36)
Ricinoleic acid	Fatty acid	Anticancer, antimicrobial, activities, anti-inflammatory, analgesic properties (37, 38)

Name of the Compound	Nature of Compound	Pharmacological activities
Dasycarpidan-1-methanol, acetate	Fatty acid ester	Unknown (39)
Benzeneacetonitrile, 4-hydroxy-	benzene	Antibacterial (40)
Phenol, 3,5-dimethoxy	Phenolic compounds	antioxidant and antibacterial activities (41)
Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro-3-(2-methylpropyl)	Carboxylic acid	Antioxidative (42)

Conclusion

Our study revealed that the MOL1 and MOL3 possess anti-hypyerglycemic effects and they reduced the weight in diabetic rabbits by reducing BGL. Notably, the more concentrated extracts showed good effects. The results demonstrate that consumption of MOL1 and MOL3 extracts of leave endophytes from *M. oliefera* is linked to stopping the increase in blood glucose levels. These discoveries elevate that both identified endophytic fungal strains *Aspergillus niger* and Penicillium sp. need more study for their possible applications. further experimental investigation is needed to establish the mode of action during the immune response of both extracts on diabetic rabbits. This is of great importance to developing countries such as Pakistan. The existence of phytochemicals diversities in *M. olifera* endophytes showed a broad spectrum of diverse biological activities cancer preventive, Anti-inflammatory, hepatoprotective, Anticancer activities, Immune modulating substance, Cytoprotective, Antidiabetic Antiviral, antitumor, Plasticizers and Antimicrobial. These results of the GCMS profile can be used as a pharmacogenetic tool for the identification of novel drugs from *Moringa oliefera* fungal endophytes.

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