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Studies on the Chemical Constituents of *Thymus serpyllum*

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Phytochemical investigations on the methanolic extract of *Thymus serpyllum* resulted in the isolation of 2 new compounds, 3-ketopentatriacontanoic acid (**1**) and 27-ketotriacontanol (**2**). The extract also afforded 3 known compounds, 3 β-hydroxyolean-12-en-28-oic acid (**3**), 3-O-β-D-glucopyranosyl-sitosterol (**4**), and dihydroursonic acid (**5**), detected for the first time from *Thymus serpyllum*. The structures of the new compounds were elucidated by spectroscopic methods, while the known compounds were identified on the basis of their spectral data and literature evidence. The hexane and ethyl acetate soluble portions of the methanolic extract showed significant antifungal activity, whereas the chloroform soluble portion and the remaining methanol extract showed moderate activity.

Key Words: *Thymus serpyllum,* chemical constituents, isolation, structures, antifungal

Introduction

Thymus serpyllum (Lamiaceae) has been extensively used in folk medicine for many years. The plant is commonly known as wild thyme and is widely found in Jammu and Kashmir at an elevation of 8000 through 12,000 feet above sea level.[1] It is the most versatile herb for use in home remedies. The plant is aromatic, antiseptic, diaphoretic, analgesic, and diuretic. It acts as an emmanagogue, carminative, and stimulant. Its essential oil contains certain compounds that are powerful and proven antibiotic and disinfectant that enhances the immune system and fight infections.[2] It can also relieve rheumatism, gout, and sciatica. The herb decoction may help prevent hair loss. The plant is a proven remedy for ailments of the respiratory, digestive, and genito-urinary system. A strong decoction, sweetened with honey, is good for easing the spasms of whooping cough. The extract of the plant also showed antioxidant, antimicrobial, antiseptic, antibacterial, and antifungal activities.[3,4]

The medicinal importance and bioactivity of *Thymus serpyllum* prompted us to carry out phytochemical investigations on this species.

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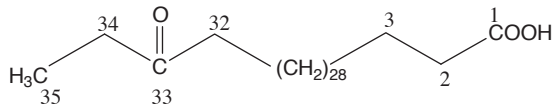
The methanolic extract of the plant showed strong activity in an antifungal assay. It was subsequently divided into hexane, chloroform, ethyl acetate, and methanol fractions. The hexane and ethyl acetate fractions showed good antifungal activity, whereas chloroform and methanol extract showed moderate antifungal activity. The methanol extract of the plant was subjected to a series of column and flash chromatographic techniques as described in the experimental section to obtain 2 new compounds, ketopentatriacontanoic acid **(1)** and ketotriacontanol **(2)**, and 3 known compounds, 3-β-hydroxyolean-12-en-28-oic acid **(3)**, 3-O-β-D-glucopyranosyl-sitosterol (**4**), and dihydroursonic acid **(5)**. The known compounds were detected for the first time in this plant. Compound **3**, a principle constituent of the plant, has been found to be a potential anti-HIV agent and it also inhibits HIV-1 replication in acutely infected H_9 cells with an EC50 value of 1.7 μg/mL.[5]

Results and Discussion

3-Ketopentatriacontanoic acid (1): The mass spectrum of compound **1** showed the molecular ion peak at m/z 536.5167, corresponding to the molecular formula $C_{35}H_{68}O_3$, indicating 2 degrees of unsaturation in the molecule. The other prominent peaks were found to occur at m/z 522, 508, 396, 368, 340, and 57. The peak at m/z 522 showed the loss of a methyl group from the molecular ion. The peak at m/z 508 indicated the loss of CH_2-CH_2 group from the molecule. The base peak at 396 corresponds to the formula $C_{27}H_{56}O$ whereas the peaks at 368 indicated the loss of ethylene moiety from the M^+ 396 fragment. Another major peak at m/z 57 indicated the presence of a $COCH_2CH_3$ group in the molecule.



1

The UV spectrum (MeOH) of compound **1** showed λ_{max} absorptions at 179, 205, and 280 nm, suggesting the aliphatic keto-acid type chromophore. The IR spectrum ($CDCl_3$) showed ν_{max} absorptions at 2849, 1700, 1680, and 1027 cm^{-1}, indicating C-H, acidic C=O, ketonic C=O, and C-C functionalities in the molecule, respectively.

The ^{1}H-NMR spectrum of compound **1** showed 68 proton resonances in the molecule. A 3H triplet at δ 0.79 was assigned to the 35-H methyl protons while a 2H multiplet at δ 2.20 was assigned to the 34-H methylenic protons. Its downfield shift is due to the α ketonic group**.** A 2H triplet at δ 2.20 (7.1 Hz) was assigned to the 2-H methylenic protons. A 2H doublet at δ 1.51 (J = 7.1 Hz) was assigned to the 3-H methylenic protons. An upfield broad singlet at δ 1.17 was assigned to the 56 methylenic protons, 4-H through 31-H. A 2H doublet that appeared at δ 3.04 (J = 7.5 Hz) was assigned to 32-H. Its downfield chemical shift is also due to ketonic function at α disposition. The acidic proton appeared at δ 10.5. The ^{1}H-NMR chemical shifts assigned are given in Table 1.

The ^{13}C-NMR spectrum ($CDCl_3$, 100 MHz) of compound **1** showed the presence of 35 carbon resonances in the molecule. The multiplicities of carbon signals were determined by DEPT experiments. The signals at δ 33.81 and 40.24 were assigned to 2-C and 34-C adjacent to the acidic and kenotic functions. The signal at δ 176.35 was assigned to the 1-C acidic carbonyl carbon while the signal at δ 203.00 was assigned to the 33-C ketonic carbonyl carbon. The signal at δ 13.58 was assigned to the 35-C methyl carbon. The

Table 1. ^{1}H-NMR chemical shift assignments for compounds **1**, **2**, **3**, **4**, and **5**.

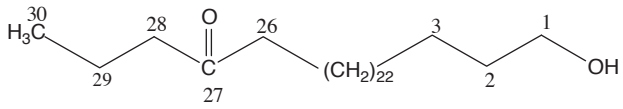
Proton No.	Chemical Shift (δ)	Integration	Multiplicity	Coupling Constant (J)
Compound 1				
2-H	2.20	2H	t	7.1 Hz
3-H	1.51	2H	d	7.1 Hz
4-H through 31-H	1.17	56H	s	-
32-H	3.04	2H	d	7.5 Hz
34-H	2.20	2H	m	-
35-H	0.79	3H	t	7.9 Hz
Compound 2				
1-H	3.56	2H	m	9.79 Hz
2-H	1.95	2H	m	-
3-H	2.50	2H	m	-
4-H through 25-H	1.17	44H	s	-
26-H	3.20	2H	d	-
28-H	2.12	2H	d	7.65 Hz
29-H	1.44	2H	M	
30-H	0.71	3H	t	-
Compound 3				
3-OH	3.21	1H	m	**-**
12-H	5.27	1H	t	7.5 Hz
18-H	2.82	1H	d	9.4 Hz
23-H	0.98	3H	s	-
24-H	0.74	3H	s	-
25-H	0.96	3H	s	-
26-H	0.91	3H	s	-
27-H	1.12	3H	s	-
29-H	0.75	3H	s	-
30-H	0.89	3H	s	-
Compound 4				
1′-H	5.24	1H	d	6.8 Hz
3-H	3.64	1H	m	-
6-H	5.37	2H	s	-
18-H	0.71	3H	s	-
19-H	0.88	3H	s	-
21-H	1.02	3H	s	-
26-H	0.83	3H	s	-
27-H	0.81	3H	s	-
28-H	0.86	3H	s	-
Compound 5				
3-Hα	2.76	1H	dd	10.0 Hz, 4.5 Hz
23-H	1.04	3H	s	-
24-H	0.82	3H	s	-
25-H	0.99	3H	s	-
26-H	0.85	3H	s	-
27-H	1.21	3H	s	-
29-H	0.80	3H	d	6.8 Hz
30-H	0.95	3H	d	6.6 Hz

signals of 1C each at δ 29.14, δ 29.00, δ 28.96, and δ 28.84 and an intense signal at δ 29.33 were assigned to the other methylenic carbons. The signals at δ 31.58 and δ 24.64 were assigned to 32-C and 3-C, respectively. The ^{13}C-NMR chemical shifts are presented in Table 2.

Table 2. ^{13}C-NMR shift assignments for compounds **1** and **2**.

Compound 1		**Compound 2**	
Carbon No.	Chemical Shift (δ)	Carbon No.	Chemical Shift (δ)
1-C	176.35	1-C	64.71
2-C	33.81	2-C	31.74
3-C	31.58	3-C	29.06
4-C	29.14	4-C	22.48
5-C	29.00	5-C through 25-C	29.50
6-C	29.14	27-C	174.34
7-C	28.84	28-C	36.24
8-C	24.64	30-C	13.85
9-C through 31-C	29.33	-	-
33-C	203.00	-	-
34-C	40.24	-	-
35-C	13.58		

27-Ketotriacontanol (2): The mass spectrum of compound **2** showed the molecular ion peak at m/z 452.3236, corresponding to the molecular formula $C_{30}H_{60}O_2$, indicating one degree of unsaturation in the molecule. Other prominent peaks were found to occur at m/z 424, 382, 368, 129, and 97. The peak at m/z 424 indicated the loss of $-CH_2$-CH_2- group from the molecule.



2

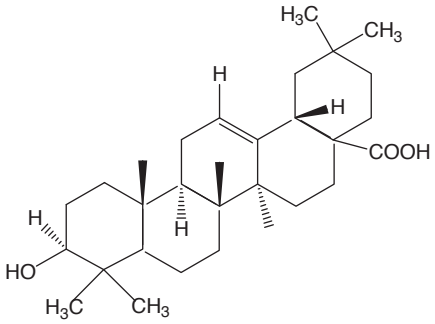
The UV spectrum (MeOH) of compound **2** showed λ_{max} absorptions at 192, 204, and 389 nm in the molecule. The IR spectrum ($CDCl_3$) showed intense νmax absorptions at 3411, 2849, 1708, 1467, and 1027 cm^{-1}, indicating O-H, C-H, C=O, C-O, and C-C functionalities in the molecule, respectively.

The ^{1}H-NMR spectrum ($CDCl_3$, 300 MHz) of compound **2** showed 60 proton resonances in the molecule. The 2H multiplet at δ 1.44 was assigned to 29-H. Another 2H multiplet at δ 3.56 was assigned to 1-H adjacent to the OH group. A 2H doublet and a 2H triplet at δ 3.20 (J = 9.79 Hz) and 2.12 (J = 7.65 Hz) were assigned to 26-H and 28-H, respectively. A 3H triplet at δ 0.71 was assigned to the 30-H methyl protons. An upfield broad singlet at δ 1.17 was assigned to the 44 methylenic protons, 4-H through 25-H. Two multiplets each of 2H at δ 1.95 and at δ 2.50 were assigned to 2-H and 3-H methylenic protons, respectively. The ^{1}H-NMR chemical shifts assignments are given in Table 1.

The ^{13}C-NMR spectrum ($CDCl_3$, 75 MHz) of compound **2** showed the presence of 30 carbon resonances in the molecule. The multiplicities of carbon signals were determined by DEPT experiments. The downfield

signal at δ 174.34 was assigned to keto carbonyl carbon. The methylenic carbon signals at δ 31.74 and δ 29.06 were assigned to 2-C and 3-C, respectively. A rather downfield signal at δ 36.24 was assigned to 28-C, adjacent to the ketonic carbon. An upfield signal at δ 13.85 was assigned to the 30-C methyl carbon. The signal at δ 64.71 was assigned to 1-C. The intense signal at δ 29.50 was assigned to other methylenic carbons in the molecule. The ^{13}C-NMR chemical shift assignments of compound **2** are presented in Table 2.

3-β-Hydroxyolean-12-en-28-oic acid (**3**): The mass spectrum of compound **3** showed the molecular ion peak at m/z 456.3603, corresponding to the formula $C_{30}H_{48}O_3$, and indicating 7 degrees of unsaturation in the molecule. The other major fragmented peaks were found to occur at m/z 248, 203, 133, and 55. The base peak at 248 corresponding to the formula $C_{16}H_{24}O_2$ is a characteristic fragment of triterpenes. The peak at m/z 203 indicated the loss of 45 a.m.u. (COOH) from the fragmented ion at m/z 248.



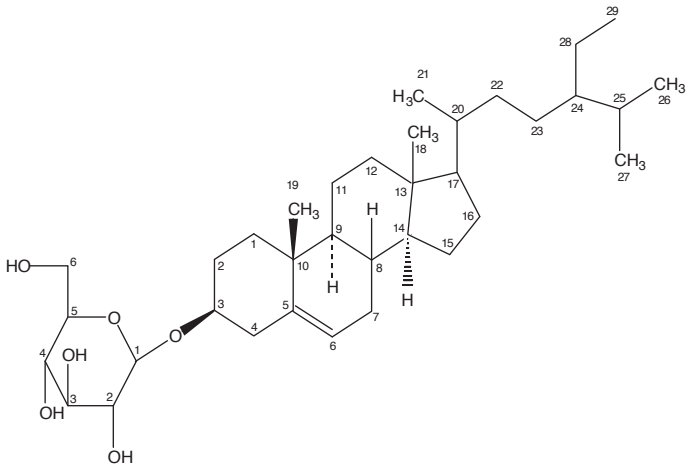
3

The UV spectrum (MeOH) of compound **3** showed λ_{max} absorptions at 204 and 198 nm. The IR spectrum ($CDCl_3$) showed an absorption band at 1694 cm^{-1}, together with a strong absorption at 2940 cm^{-1}, indicating the presence of carboxylic acid.

The ^{1}H-NMR spectrum ($CDCl_3$300 MHz) of compound **3** showed 7 singlets for methyl protons resonating at 0.74, 0.75, 0.89, 0.91, 0.96, 0.98, and 1.12, which were assigned to 24-H, 29-H, 30-H, 26-H, 25-H, 23-H, and 27-H, respectively. A broad doublet at δ 2.82 (J = 9.4 Hz) was assigned to 18-H, indicating an olean type skeleton. A downfield triplet at δ 5.27 (J = 7.5 Hz) was assigned to the 12-H olefinic proton. A multiplet at δ 3.21 indicated a hydroxyl group at C-3.

The EIMS spectrum indicated the position of the double bond between C-12 and C-13. The loss of 45 a.m.u. (COOH) from the fragment m/z 248 indicated the presence of an acid function at the 17-C position. The comparison of the spectral data with literature values indicated that the compound in hand was known but detected for the first time in this plant.[10]

3-0-β-D-Glucopyranosyl-Sitosterol (**4**)**:** The mass spectrum of compound **4** showed the molecular ion peak at m/z 414 (M^+-glycosidic unit). The other major fragmentation peaks were found to occur at m/z 396, 381, 329, 303, 275, and 57 (100%). The loss of 18 a.m.u. indicated the loss of a water molecule. The peak at m/z 396 is characteristic for β-sitosterol. The negative ion FABMS exhibited the M^+ at m/z 575 [M^+-H], corresponding to the formula $C_{35}H_{60}O_6$ with 6 degrees of unsaturation.

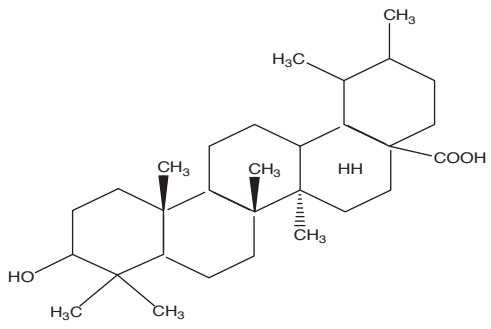


4

The UV spectrum (MeOH) of compound **4** showed λ_{max} absorption at 204 and 198 nm. The IR spectrum ($CDCl_3$) showed ν_{max} absorptions at 3396-3389, 1654, and 2896 cm^{-1}, indicating the presence of O-H, C=C, and C-H functionalities in the molecule.

The ^{1}H-NMR spectrum ($CDCl_3$, 300 MHz) of compound **4** showed a broad singlet at δ 5.37, assigned to H-6. H-3 appeared as a multiplet at δ 3.64. The methyl signals appeared at δ 0.71, 0.81, 0.83, 0.86, 0.88, and 1.02, corresponding to the 18-H, 27-H, 26-H, 29-H, 19-H, and 21-H methyl protons, respectively. The C-1' anomeric proton appeared at δ 5.24 as a doublet, indicating the presence of a β-glycosidic unit. On the basis of spectral data and literature studies, compound **4** was identified as 3-β-D-glucopyranosyl-β-sitosterol.[9]

Dihydroursonic acid (5): The mass spectrum of compound **5** exhibited the molecular ion peak at m/z 456, corresponding to the molecular formula $C_{30}H_{48}O_3$, indicating 6 degrees of unsaturation in the molecule. The other prominent peaks were found to occur at m/z 248 (100%), 207, 203, 189, 175, 133, 105, and 69. The peak at 203 indicated the loss of COOH from the molecular ion peak. The peak at m/z 189 corresponded to the loss of H_2O from the m/z 207.



5

The UV spectrum (MeOH) of compound **5** showed λ_{max} absorptions at 204 and 198 nm. The IR spectrum ($CDCl_3$) showed ν_{max} absorptions at 3423, 2941, and 1692 $cm^{-1}$and indicated the presence of O-H, C-H, and C=O functionalities in the molecule.

The ^{1}H-NMR spectrum ($CDCl_3$, 300 MHz) spectrum of compound **5** showed 7 methyl proton singlets at δ 1.21 (3H, s, 27-Me), 1.04 (3H, s, 23-Me), 0.99 (3H, s, 25-Me), 0.95 (3H, d, J = 6.6 Hz, 30-Me), 0.85 (3H, s, 26-Me), 0.82 (3H, s, 24-Me), and 0.80 (3H, d, J = 6.8 Hz, 29-Me). A double doublet at δ 2.76 (J a, a = 10.0 Hz, J a, e = 4.5 Hz) was assigned to

3-H in its α-stereochemistry.[16] The spectral data and literature evidence led us to identify the compound **5** as dihydroursonic acid.[6,7]

Antifungal activities: The antifungal activities of the different fractions of methanolic extract were studied through applying the agar tube dilution protocol, which is one of the most precise and reliable methods. The hexane fraction showed significant activity against *Fusarium solani* while the ethyl acetate fraction also exhibited significant activity against *Aspergillus flavus*. The chloroform and methanolic fractions showed moderate activity against *Fusarium solani* and *Microsporum canis* respectively at 400 μg/mL of DMSO.

Experimental

Instrumentation: UV spectra were recorded on a Shimadzu double beam UV spectrophotometer; IR spectra were recorded on a model FTIR spectrophotometer. Mass spectra were recorded on a Varian MAT 312 mass spectrometer connected to a DDP 11/34 DEC computer system. The ^{1}H-NMR spectra were recorded at 300 MHz on a Bruker AM 300 NMR spectrometer while the ^{13}C-NMR spectra were recorded at 75 MHz on the same instrument. The optical rotations were recorded on an Australian K-157 digital polarimeter. TLC experiments were performed on silica gel (GF-254, 0.2 mm) from E. Merck.

Plant Material: The plant material (40 kg) was collected in July 2005 from Pir Chanassi, District Muzaffarabad, Azad Jammu and Kashmir, Pakistan. The plant was identified by the plant taxonomist at the Department of Botany of the University of Azad Jammu and Kashmir, Muzaffarabad, where a voucher specimen is deposited in the herbarium of the department.

Extraction Procedure: Dried powder (5 kg) of *Thymus serpyllum* was extracted with methanol. The MeOH extract was concentrated under reduced pressure on a rotary evaporator to afford a viscous material (80 g). This crude extract was subjected to silica-gel column chromatography. The column was eluted with increasing polarities of petroleum ether, chloroform, methanol, and water. The elution with 20% chloroform in pet. ether afforded a main fraction **D** (2.0 g). This fraction **D** was rechromatographed by subjecting it to the silica gel through a column with chloroform/hexane (15:85) as the solvent system to afford 10 fractions, D_1 through D_{10}. The elution of the column with 80% chloroform in pet. ether afforded a main fraction **E** (2.5 g). The main fraction **E** was further chromatographed on a silica gel (3.0 g, 240-300 mesh) column with chloroform pet. ether (9.0:1.0) as the solvent system to afford fractions E_1 through E_{20}.

Isolation of 1: Fraction $\mathbf{D}_1$ (98.0 mg) was subjected to the flash column (silica gel, 150.0 mg). The column was eluted with pet. ether/chloroform (9.0:1.0) to afford compound **1** as a white amorphous solid.

Physical Data: Melting point: 85-87 °C; $[\alpha]_D$: 56° (C = 0.06, $CDCl_3$); UV (MeOH): 179, 205, and 280 nm, IR ($CDCl_3$) νmax cm^{-1}: 2849 (C-H), 1700 (C=O), 1680 (C=O ketonic) and 1027 (C-C); EIMS m/z (rel. int): 536, 522 (20), 508 (12), 396 (100), 368 (99), 340 (38), and 57 (90); ^{1}H-NMR ($CDCl_3$, 300 MHz) δ: Table 1; ^{13}C-NMR ($CDCl_3$, 75 MHz) δ: Table 2.

Isolation of 2: Fraction **D5** (70.1 mg) was subjected to the flash column (silica gel 150.0 mg). The column was eluted with pet. ether/chloroform (2.5:7.5) to afford compound **2** as a white crystalline compound.

Physical Data: Melting point: 87 °C; $[\alpha]_{D:}$ 30° (C = 0.02, CDCl3); UV (MeOH): λmax nm: 192, 204, and 389; IR ($CDCl_3$) νmax cm^{-1}: 3411 (O-H), 2849 (C-H), 1708 (C=O), 1467 (C-O), and 1027 (C-C); EIMS m/z (rel. int %): M^+452, 424 (14), 382 (18), 368 (47), 129 (40), and 97 (28); HREIMS m/z: 452.4567 (calcd 452.4593 for $C_{30}H_{60}O_2$); ^{1}H-NMR ($CDCl_3$, 300 MHz) δ: Table 1; ^{13}C-NMR ($CDCl_3$, 75 MHz) δ: Table 2.

Isolation of 3: Fraction $\mathbf{E}_1$ was subjected to the flash (silica gel) column. The column was eluted with chloroform/ethyl acetate (6.5:3.5) as the solvent system. This afforded the white powdered compound **3,** identified as oleanolic acid.

Physical Data: $[\alpha]_D$: 57° (C = 0.5, $CDCl_3$); UV (MeOH): λmax, nm: 204 and 198; IR ($CDCl_3$) νmax, cm^{-1}1694 (O-H) and 2940 (carboxylic); EIMS m/z (rel. int): M^+456, 482, 203 (62), 133 (28), and 55 (39); HREIMS m/z:(calcd. 456.3604 $C_{30}H_{48}O_3$); ^{1}H-NMR ($CDCl_3$, 300 MHz) δ: Table 1.

Isolation of 4: Fraction $\mathbf{E}_5$ was subjected to the flash (silica gel 150.0 mg) column with chloroform/ethyl acetate (5.0:5.0) as the solvent system. This resulted in the isolation of compound **4** (25.0 mg), identified as 3-β-D-glucopyranosyl-β-sitosterol.[14,15]

Physical Data: Melting point: 272-274 °C [Reported 270-272 ° C]; $[\alpha]_D$: -37.4° (C = 0.0025, $CDCl_3$); UV (MeOH) λmax, nm: 204 and 198; IR ($CDCl_3$) νmax cm^{-1}: 3455 (O-H), 3042 (C-H and 1645 (C=C); EIMS m/z (rel. int %): 414 (M^+-glycosidic unit) 396 (100), 381 (11), 329, 303 (11), 275, and 57 (90); ^{1}H-NMR ($CDCl_3$, 300 MHz) δ: Table 1.

Isolation of 5: Fraction $\mathbf{E}_8$ was subjected to the flash (silica gel 150.0 mg) column. The column was eluted with chloroform/ethyl acetate (4.5:5.5), as the solvent system. This afforded compound **5** as a white amorphous powder (30.0 mg), which was identified as dihydroursonic acid **5**.[6,7]

Physical Data: Melting point: 280-282 °C; $[\alpha]_{D:}$ +70.5 (C = 0.006, CDCl3); UV (MeOH) λmax, nm: 204 and 198; IR ($CDCl_3$)ν max, cm^{-1}: 3423 (O-H), 2941 (C-H), and 1692 (C=O); EIMS m/z (rel. int %): M^+456, 248 (100), 207 (19), 203 (69), 189 (10), 175, 133 (17), and 105 (14); ^{1}H-NMR ($CDCl_3$, 300 MHz) δ: Table 1.

***In Vitro* Antifungal Bioassay of Hexane Fraction**

Name of the Fungus	Linear growth (mm)		% Inhibition	Std. Drugs MIC μg/mL	% Inhibition of Std. Drug
	Sample	Control			
Trichophyton longifusus	50	100	50	Miconazole	70
Candida albicans	100	100	0	Miconazole	110.8
Aspergillus flavus	100	100	0	Amphotericin B	20
Microsporum canis	70	100	30	Miconazole	98.4
Fusarium solani	30	100	70	Miconazole	73.25
Candida glaberata	100	100	0	Miconazole	110.8

Key: Concentration of sample 400 μg/mL of DMSO Incubation temp. 27 (28 ± 1 °C)

***In Vitro* Antifungal Bioassay of Ethyl Acetate Fraction**

Name of the Fungus	Linear growth (mm)		% Inhibition	Std. Drugs MIC μg/mL	% Inhibition of Std. Drug
	Sample	Control			
Trichophyton longifusus	50	100	50	Miconazole	70
Candida albicans	100	100	0	Miconazole	110.8
Aspergillus flavus	30	100	70	Amphotericin B	20
Microsporum canis	70	100	30	Miconazole	98.4
Fusarium solani	40	100	60	Miconazole	73.25
Candida glaberata	100	100	0	Miconazole	110.8

Key: Concentration of sample 400 μg/mL of DMSO Incubation temp. 27 (28± 1 °C)

***In Vitro* Antifungal Bioassay of Chloroform Fraction**

Name of the Fungus	Linear growth (mm)		% Inhibition	Std. Drugs MIC μg/mL	% Inhibition of Std. Drug
	Sample	Control			
Trichophyton longifusus	80	100	20	Miconazole	70
Candida albicans	100	100	0	Miconazole	110.8
Aspergillus flavus	100	100	0	Amphotericin B	20
Microsporum canis	60	100	40	Miconazole	98.4
Fusarium solani	50	100	50	Miconazole	73.25
Candida glaberata	100	100	0	Miconazole	110.8

Key: Concentration of sample 400 μg/mL of DMSO Incubation temp. 27 (28 ± 1 °C)

***In Vitro* Antifungal Bioassay of Methanol Fraction**

Name of the Fungus	Linear growth (mm)		% Inhibition	Std. Drugs MIC μg/mL	% Inhibition of Std. Drug
	Sample	Control			
Trichophyton longifusus	50	100	50	Miconazole	70
Candida albicans	100	100	0	Miconazole	110.8
Aspergillus flavus	85	100	15	Amphotericin B	20
Microsporum canis	40	100	60	Miconazole	98.4
Fusarium solani	90	100	10	Miconazole	73.25
Candida glaberata	100	100	0	Miconazole	110.8

Key: Concentration of sample 400 μg/mL of DMSO Incubation temp. 27 (28 ± 1 °C)

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