SUPPLEMENTARY MATERIAL

Bioguided Fractionation of Hypoglycaemic Component in Methanol Extract of *Vernonia amygdalina:* An *in vivo* study

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Abstract

Nine components (C1-C9) were isolated from chloroform fraction of fractionated methanol extracts of *Vernonia amygdalina* leaves (FMEVA) by column chromatography. All the components C1 to C9 were purified and screened for hypoglycaemic activities in type-2 diabetic rats. The most potent hypoglycaemic component was elucidated on the basis of extensive spectroscopic (1D-, 2D-NMR, GC-MS, FTIR) data analysis. The Component C5 was found to be the most potent hypoglycaemic in reducing blood glucose by 12.55 ± 3.55% at 4 h post-oral administration, when compared to the positive (18.07 ± 1.20%) and negative (-1.99 ± 0.43%) controls. The spectroscopic data analysis reveals that the isolated compound has a structure consistent with 11β,13-dihydrovernolide. The isolated compound is part of the hypoglycaemic components present in *V. amygdalina* leaves that is responsible for the anti-diabetic activities. Further research is needed in the development of this compound or its derivatives for pharmaceutical use.

Keywords: Anti-diabetic; hyperglycaemia; hypoglycaemic; *Vernonia amygdalina*; Type-2 diabetes

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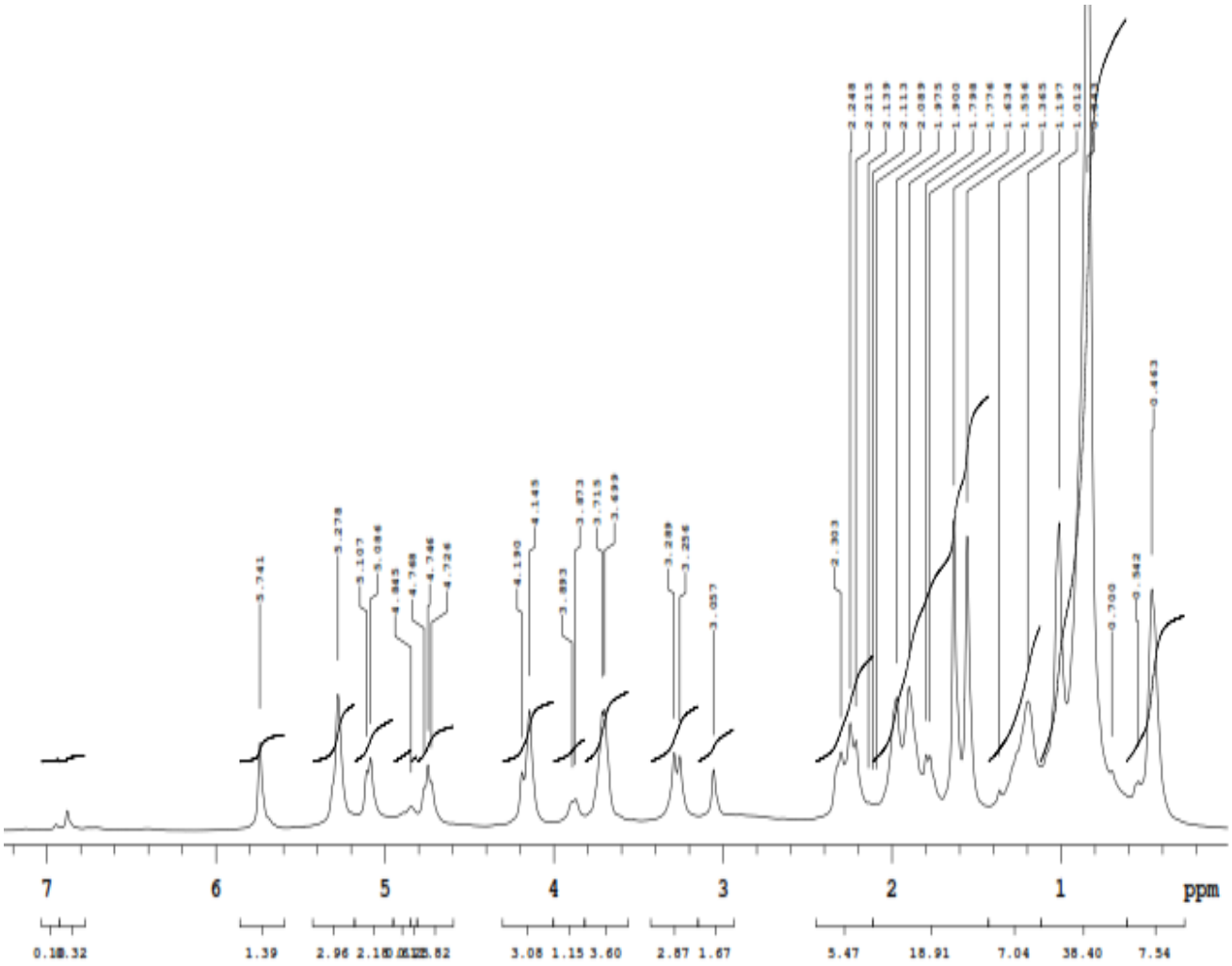

Figure S1: [1]H-NMR Spectra for Purified Component C-5 from Column Fractionated Chloroform Fraction of *V. amygdalina* Methanol Leaf Extract

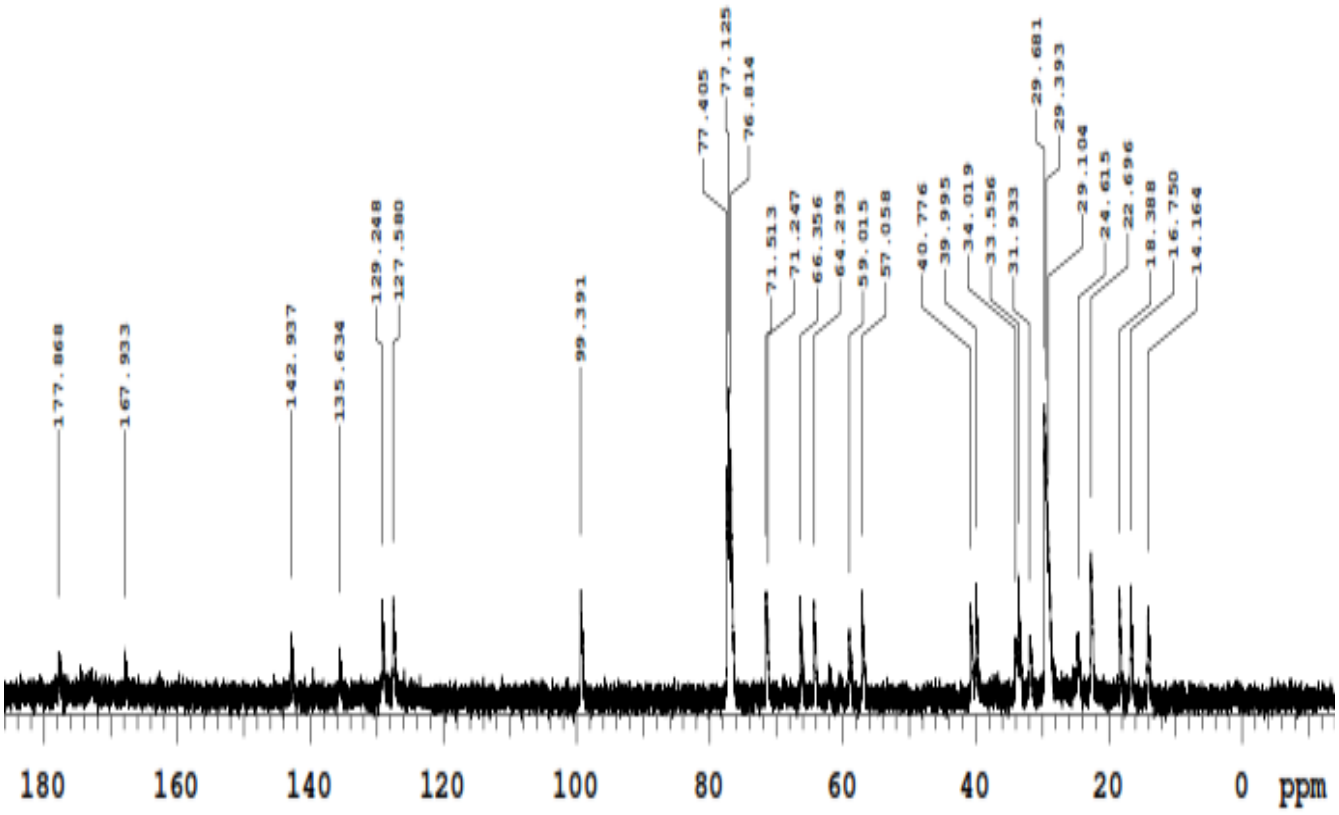

Figure S2: ^{13}C-NMR Spectra for Purified Component C-5 from Column Fractionated Chloroform Fraction of *V. amygdalina* Methanol Leaf Extract

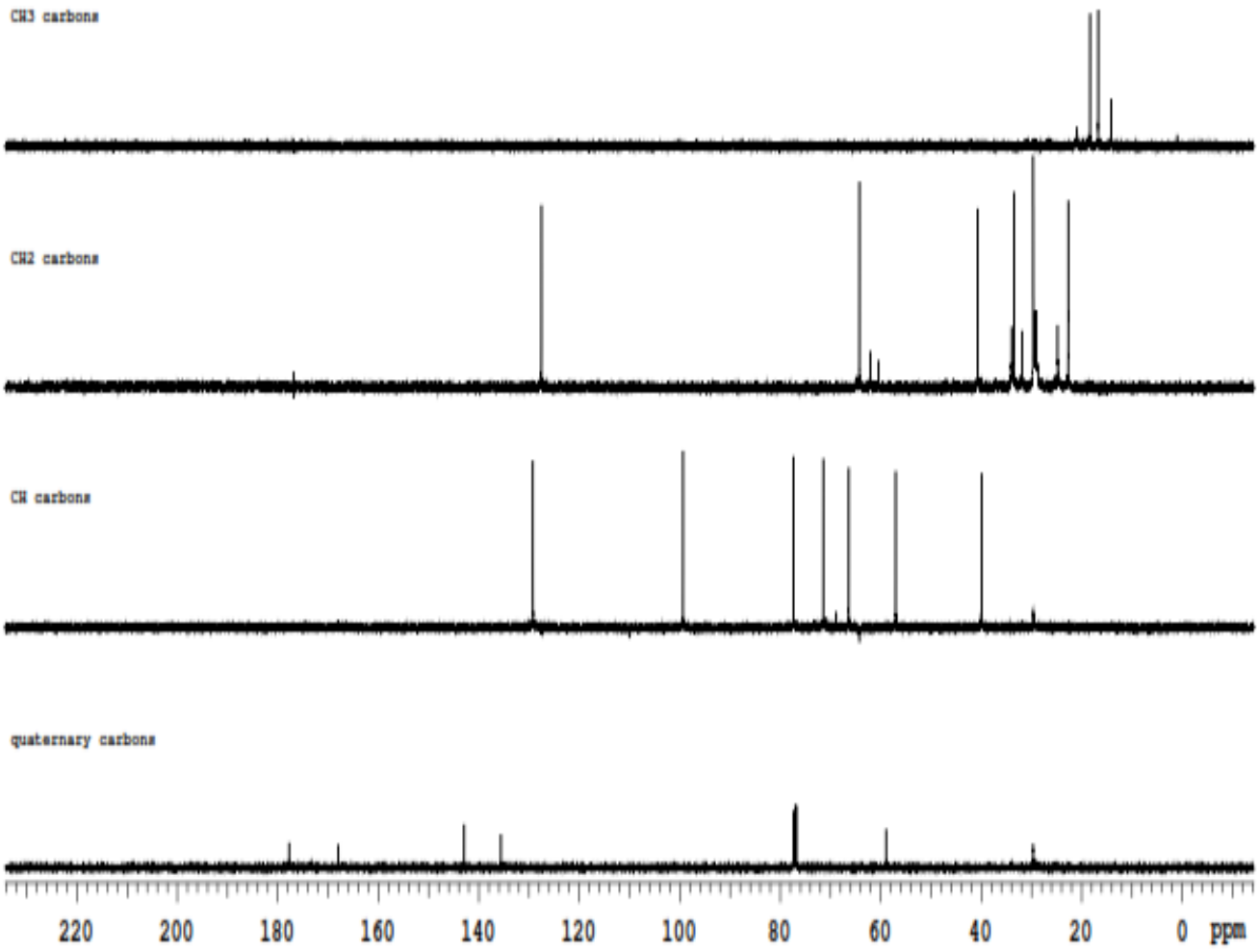

Figure S3: DEPT-NMR Spectra for Purified Component C5 from Column Fractionated Chloroform Fraction of *Vernonia amygdalina* Methanol Leaf Extract

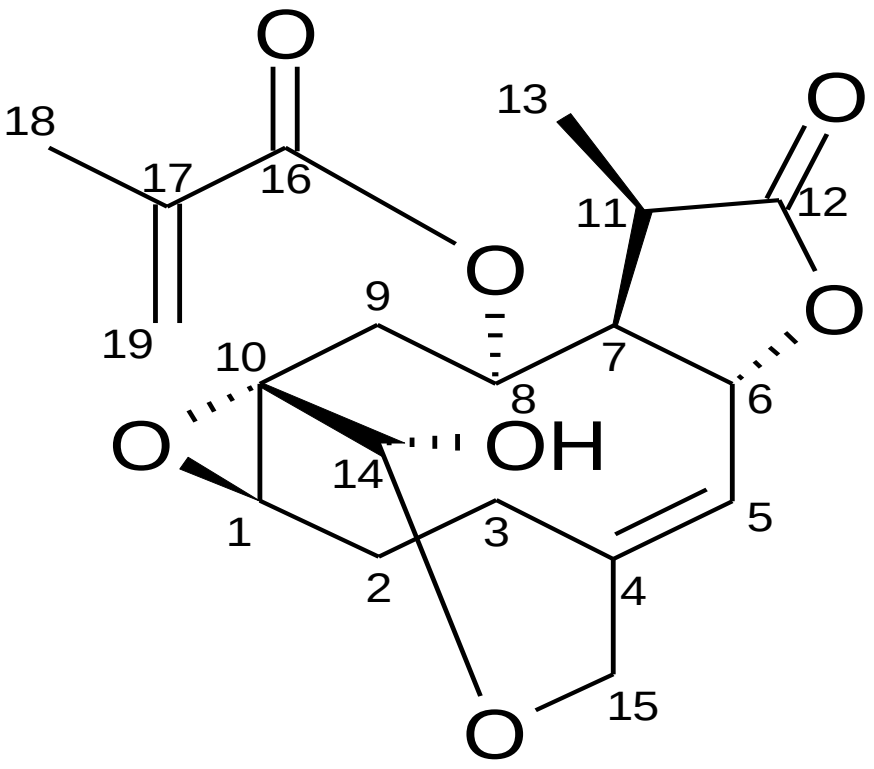

Figure S4: Carbons numbering and chemical structure of 11β,13-dihydrovernolide.

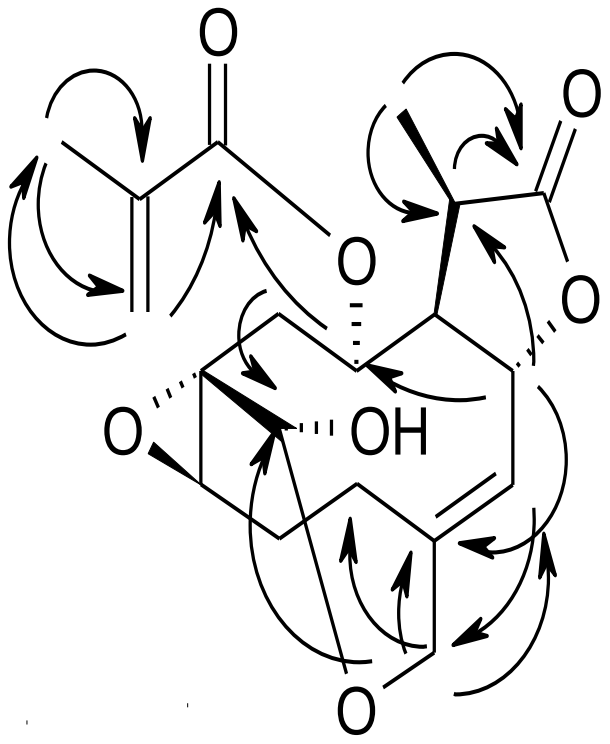

Figure S5: Selected HMBC correlations for 11β,13-dihydrovernolide.

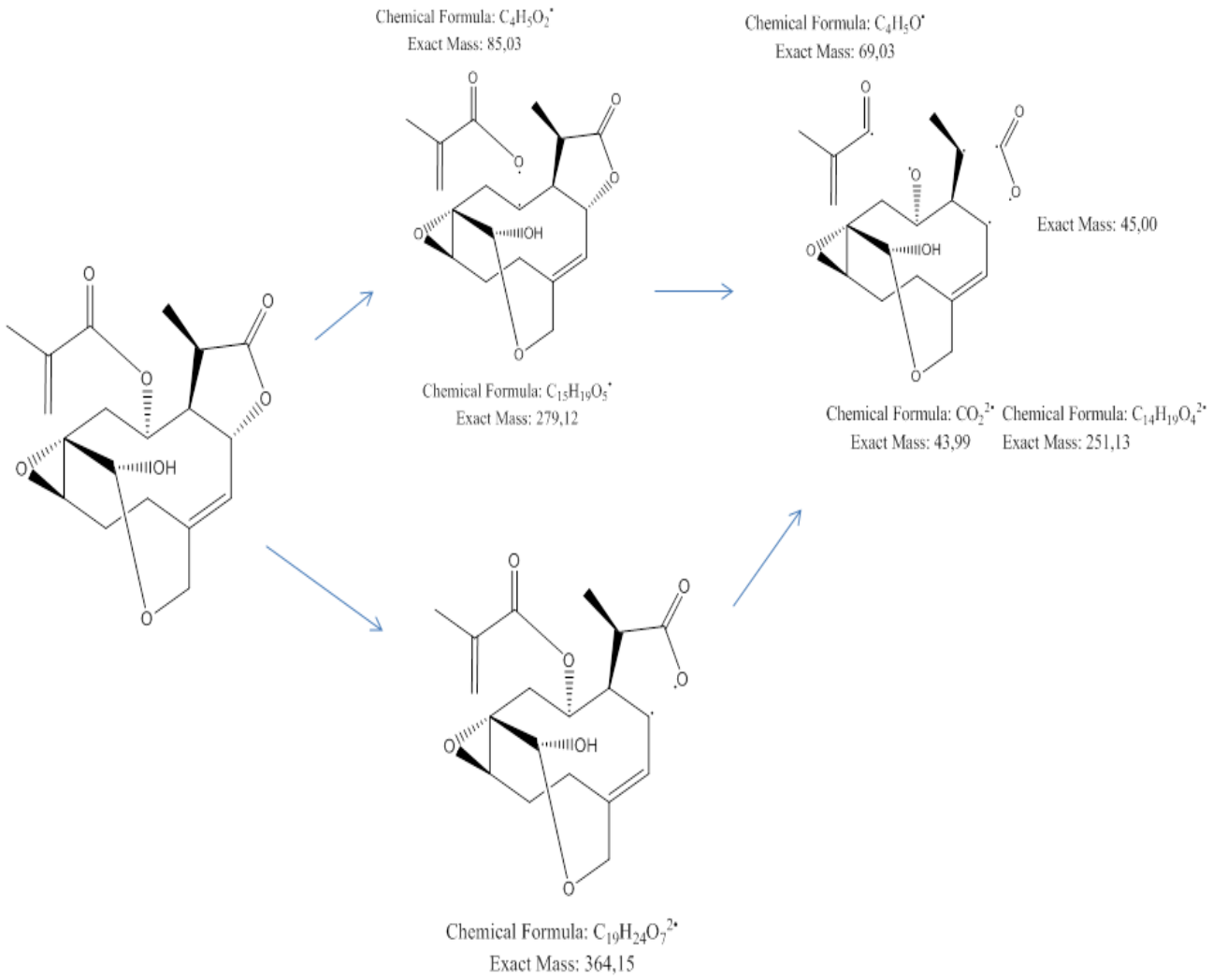

Figure S6: Observed fragmentation pathway for 11β,13-dihydrovernolide.

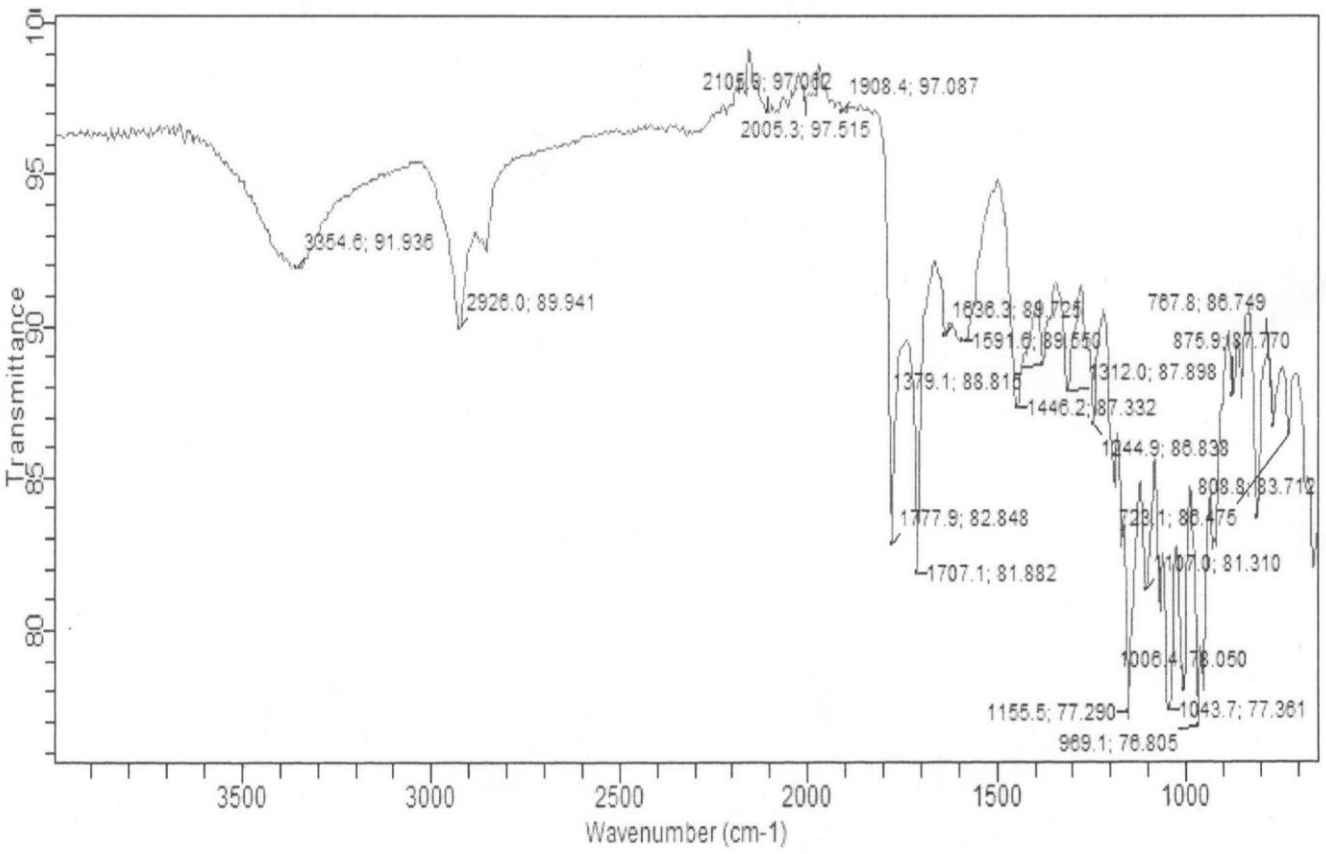

Figure S7: FTIR Spectra for Purified Component C-5 from Column Fractionated Chloroform Fraction of *V. amygdalina* Methanol Leaf Extract.

Tables

Table S1: Change (%) in Fasting Blood Glucose in Type-2 Diabetic Rats After Treatment with Purified Components from Column Fractionated Chloroform Fraction of *V. amygdalina* Methanol Leaf Extract (10 mg/kg bw)

VACF purified components	0.5 h	1 h	2 h	3 h	4 h
C1	0.07 ± 0.42^{cd}	0.84 ± 0.48^{a}	1.30 ± 0.37^{a}	2.10 ± 0.64^{a}	3.75 ± 1.13^{ab}
C2	0.59 ± 0.23^{ab}	2.40 ± 1.97^{a}	6.79 ± 1.72^{bcd}	8.96 ± 1.96^{cd}	9.50 ± 1.75^{cd}
C3	0.22 ± 0.27^{bcd}	2.20 ± 1.78^{a}	3.77 ± 0.86^{ab}	6.15 ± 3.48^{bc}	5.79 ± 3.42^{abc}
C4	0.91 ± 0.15^{a}	1.50 ± 0.12^{a}	2.42 ± 0.38^{a}	4.63 ± 0.87^{ab}	4.74 ± 0.58^{ab}
C5	0.20 ± 0.21^{de}	5.76 ± 3.10^{b}	8.63 ± 2.67^{cd}	11.52 ± 2.98^{d}	12.55 ± 3.55^{d}
C6	0.17 ± 0.21^{bcd}	0.55 ± 0.17^{a}	1.87 ± 2.01^{a}	2.94 ± 1.71^{ab}	3.10 ± 2.55^{ab}
C7	1.79 ± 0.25^{f}	5.41 ± 2.79^{b}	6.24 ± 3.95^{bc}	6.77 ± 3.94^{bc}	6.96 ± 4.41^{bc}
C8	0.49 ± 0.30^{e}	1.42 ± 0.28^{a}	2.62 ± 1.04^{a}	3.40 ± 1.21^{ab}	3.58 ± 1.68^{ab}
C9	0.32 ± 0.18^{bc}	1.32 ± 0.55^{a}	2.48 ± 0.74^{a}	3.09 ± 0.41^{ab}	3.33 ± 0.64^{ab}
DC	0.31 ± 0.10^{bc}	1.00 ± 0.70^{a}	1.15 ± 0.73^{a}	1.90 ± 0.25^{a}	2.11 ± 0.35^{a}
PC	3.09 ± 0.32^{g}	7.41 ± 1.04^{b}	9.50 ± 0.08^{d}	17.48 ± 0.92^{e}	17.80 ± 0.96^{e}

Data are presented as mean ± SD of 3 animals per group. Values with different superscript down the column indicate significant difference ($p<0.05$). VACF: Chloroform fraction of *V. amygdalina* methanol Leaf extract; PC: Positive control; DC: Diabetic control; C1, C2, … and C9: Purified components from column fractionated VACF.

Table S2: Decrease (%) in Fasting Blood Glucose in Type-2 Diabetic Rats after Treatment with Different Doses of Purified Component C5 from Column Fractionated Chloroform Fraction of *V. amygdalina* Methanol Leaf Extract

Group	0.5 h	1 h	2 h	3 h	4 h
G_5	0.51 ± 0.21 [a]	1.06 ± 1.21[a]	2.35 ± 1.29[a]	3.96 ± 0.24[b]	4.10 ± 1.57 [b]
G_{10}	0.70 ± 0.27 [a,b]	4.92 ± 2.23[b]	7.84 ± 1.58 [b]	10.55 ± 1.19[c]	11.97 ± 1.64 [c]
G_{20}	1.23 ± 0.24 [b]	5.32 ± 2.27[b]	9.38 ± 1.60 [b,c]	13.41 ± 2.75[d]	13.62 ± 1.44 [c]
DC	0.28 ± 0.23 [a]	0.84 ± 0.29[a]	1.02 ± 0.13 [a]	1.27 ± 0.16 [a]	1.98 ± 0.12[a]
PC	2.81 ± 0.82 [c]	6.72 ± 2.53[b]	10.40 ± 1.20 [c]	15.21 ± 2.48[d]	18.33 ± 1.53[d]

Data are presented as mean ± SD of 5 animals per group. Values with different superscript down the column indicate significant difference (p<0.05). PC: Positive control; DC: Diabetic control; G_5, G_{10}, … and G_{20} are diabetic rat groups treated with three different doses (5, 10 and 20 mg/kg b.w.) respectively of Purified Components C5 from Chloroform fraction of *V. amygdalina* methanol Leaf extract.

Table S3: ^{13}C- NMR data for Purified Component C5 from *V. amygdalina* Methanol Leaf Extract

Position	* ^{13}C-NMR, 125 MHz, δ	Experimental Data		
		^{13}C-NMR, HSQC, 100 MHz, δ	DEPT	HMBC
1	66.32	66.32	CH	
2	22.74	22.70	CH_2	
3	33.56	33.56	CH_2	
4	142.85	142.94	C	
5	129.23	129.25	CH	C15
6	77.31	78.51	CH	C4, C8, C11
7	57.11	57.06	CH	
8	71.50	71.50	CH	C16
9	40.79	40.78	CH_2	C14
10	58.89	59.02	C	
11	39.93	40.78	CH	C12
12	177.54	177.87	C	
13	16.72	16.75	CH_3	C7, C11, C12
14	99.47	99.39	CH	
15	64.30	64.29	CH_2	C3, C4, C5,C14
16	167.96	167.93	C	
17	135.62	135.63	C	
18	18.28	18.39	CH_3	C16, C17, C19,
19	127.44	127.58	CH_2	C16, C17, C18,

*Rabe *et al*, 2002

Table S4: Functional Groups Identified Using FTIR Spectra Analysis for Purified Component C-5 from Column Fractionated Chloroform Fraction of *V. amygdalina* Methanol Leaf Extract. Wavenumbers (cm^{-1})

	Absorbance	Absorption Ranges	Functional Group
1	3354.60	3100-3500	O-H
2	2926.00	2500-3300	C-H
3	1777.90	1670-1820	C=O
4	1707.10	1700-1725	C=O
5	1446.2	1440-1480	$=C=H_2$
6	969.1	960-990	$=C=H_2$
7	808.8	790-840	-C=CH-

Functional Group region: 4000 cm^{-1} - 1400 cm^{-1} ; Finger print region: 1400 cm^{-1} - 400 cm^{-1}

References

Rabe, T., Mullholland, D., Van-Staden, J., 2002. Isolation and identification of antibacterial compounds from *Vernonia colorata* leaves. Journal of Ethnopharmacology, 80(1): 91-94.