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Sesquiterpene and Ecdysteroid from Achyranthes aspera L.

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Abstract
Background:- *Achyranthes aspera* L. (Amaranthaceae) locally known as "Telenge or ambulale" (Amharic) and Muchele (Tigrigna)" is one of the traditional medicinal plant used as contraceptive, for relieving asthma and cough, and anti snake bite in the indigenous health care delivery system of Ethiopia **Objective:-** Identifying and characterization of the chemical constituents of elucidations of the n-buthanol fractions of the methanolic leaves extracts of *Achyranthes aspera***Method:-** Structural elucidations of the compounds was based on IR, UV, ^{1}D NMR (^{1}H, ^{13}C &DEPT) and ^{2}D NMR (COSY, HMQC &HMBC) spectroscopic techniques.**Result:** Phytochemical investigation of the leaves of this plant resulted in the isolation and characterization of two compounds **AA-1**and **AA-2**. Compound **AA-1** is sesquiterpene named as 4,4a,7,8-tetrahydro-4-hydroxy-4a,7,8-trimethyl-8-(tetrahydro-3,4,5-trihydroxy-6-(hydroxymethyl)-2H-pyran-2-yloxy) naphthalen-2(3H)-one. Compound AA-2 is an insect molting ecdysteroid hormone named as, 20-hydroxyecdysone. **Conclusion:** From this study, two compounds were isolated and structurally identified. Compound **AA-1** is the first report from *A. aspera* and plants belonging to the same species and family. While compound **AA-2** was reported previously from the same plant.
Keywords: Achyrannthes aspera, sesquiterpene, ecdysteroid, structural elucidation
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Introduction

Plants are used medicinally in different countries and are a source of many potent and powerful drugs (Periyasamy and Mahalingam, 2010). Medicinal plants have multipurpose uses such as for pharmaceutical purpose, preparing foodstuffs, insecticides, antioxidants, coloring matters, flavors and fragrances, extraction of enzymes, pheromones, etc (Theoneste, 2002). The genus Achyranthes belongs to the family Amaranthaceae. It is perennial stiff erect herb, growing up to 1 m height distributed as weed up to an altitude of 2000m in many regions of Ethiopia. Some of the species of belonging to the genus *Achyranthes viz. A. fauriei, A. bidentata, A. japonica, A. ferruginea* have been investigated for their constituents and screened for pharmacological activity (Shendkar, 2011). *Achyranthes aspera* L., locally known as "Telenge or ambulale" is one of the traditionally used anti-fertility plants in the indigenous health care delivery system of Ethiopia (Workineh, *et al*., 2006).

The plant is highly esteemed by traditional healers and used for the treatment of asthma, bleeding, boils, bronchitis, cold, cough, colic, debility, dropsy, dog bite, dysentery, ear complications, headache, leucoderma, pneumonia, renal complications, scorpion bite, snake bite, skin diseases and in facilitating delivery (Abdul and Athar, 2008., Singh, 2010).

Chemical investigations of the different parts of *Achyranthes aspera* resulted in the isolation & identification Alkaloids, flavonoids, saponins, steroids and terpenoids from the leaves of the plant (Saurabh and Khosa, 2011). *A. aspera* is one of the important medicinal plants having many therapeutic uses as Odontalgic, Rheumatism, Bronchitis, skin disease, as an anti-asthmatic and for insect bite (Tullanithi, 2011, Amrutia, 2011). The chloroform and ethanol extracts of roots of the *Achyranthes aspera* are reported to have anti-implantation & abortifacient activity. The ethanol extract of the root posses spermicidal activity. The aqueous and methanolic extracts of the whole plant have hypoglycemic effect (Shendkar, *et al*., 2012 and Pradip *et al*., 2012). Previous investigation of the leaves of *Achyranthes aspera* revealed the presence of steriodal and triterenoidal saponins and flavonoid glycosides (Kunert *et al*., 2000 and Michl *et al* 2000). In the current study we report the isolation and characterization of sesquiterpene and ecdysteroid, from the leaves of *Achyranthes aspera* using different spectroscopic techniques.

2. Materials and Methods

2.1 General

^{1}H, ^{13}C and ^{2}D-NMR spectra were recorded with Bruker advance 400MHz spectrometer with using CD_3OD and TMS as internal started. The ultraviolet and visible (UV-Vis) spectra were taken on GENESY'S 2PC UV-Vis scanning spectrometer in the range 200-1000 Cm^{-1} Infrared (IR) spectra were obtained on Perkin-Elmer BX Infrared spectrometer using KBr and MeOH in the range 4000- 400 Cm^{-1}. Solvents were removed using the Buchi type rota vapor under reduced pressure at 30 ^{0}C. Mixtures of compounds were separated using chromatoron (model 79247), Column Chromatography and Preparatory TLC. TLC analyses were carried out on TLC plates 0.2 mm thick layer of Merck silica gel 60 F_{254} coated on aluminum foil. Compounds on TLC were detected using UV light on the wave length of 254 and365 nm and spraying with 1% vanillin in sulfuric acid.

2.2. Plant material

The fresh leaves and stem containing the fruits of the plant were collected from Mekelle**,** capital City of Tigray regional state of Ethiopia, which is 780 km from Addis Ababa, and identified by the botanist national herbarium of Ethiopia. The leaves and the stems were collected separately and the collected leaves are dried at room temperature.

2.3. Method of extraction and isolation

Air dried and finely powdered leaves of the plant (500 g) were defatted with petroleum ether in a percolator at room temperature for 72h. After the extract was filtered, the solvent free powder(the marc) was exhaustively extracted with methanol in a percolator and the solvent was removed using Rota evaporator to afford a greenish gum (45 g). The crude methanol extract was then taken up in water and re-extracted with diethyl ether until all the chlorophyll pigments were removed. The aqueous phase was then partitioned with n-butanol saturated with water (300 ml) three times. The n-butanol partitioned was concentrated under reduced pressure using vacuum distillation to give crude mixture (3 g). This mixture was dissolved in the solvent system of MeOH/ $CHCl_3$ (8:2) and subjected to Chromatotron and 30 fractions each 20ml were collected using 100 ml Erlenmeyer flask by monitoring the TLC of the eluants using $CHCl_3$/MeOH/EtOAc (6:2:2) as solvent system to identify the components of the fractions collected. The first seventeen fractions (i.e F1-F17) were collected using MeOH/CHCl3 (8:2) as solvent system. TLC was checked fractions that had similar retention factor (RF) were combined together. Based on this F4, F5 and F6 were mixed and the solvent was removed using rotavapor. The combined fractions are also subjected to Column chromatography (CC) on silica gel (20g) to afford 12 fractions collecting in a test tube. Fraction 6 was subjected to Preparatory Thin Layer Chromatography (PTLC) to give compounds AA-1.

Compound AA-1 is yellowish semisolid with RF value 0.38. It is UV active and showed a purple color seeing in UV lamp at 254 nm. After spraying with 1% vanillin in H_2SO_4 and heated in hotplate a purple color was observed. Repeated column chromatography on silica gel (solvent system, CHCl3/MeOH (8:2)) of fourteenth fraction collected above afforded 11mg of compound **AA-2**. It gives a yellow color spot on TLC after spraying with 1% vanillin in H_2SO_4 with RF value of 0.44. It is less polar compared to compound **AA-1.**

3. Result

Phytochemical investigation of the leaves of this plant resulted in the isolation and characterization of two compounds **AA-1**and **AA-2**. Compound **AA-1** is sesquiterpene named as 4,4a,7,8-tetrahydro-4-hydroxy-4a,7,8-trimethyl-8-(tetrahydro-3,4,5-trihydroxy-6-(hydroxymethyl)-2H-pyran-2-yloxy) naphthalen-2(3H)-one. Compound AA-2 is an insect molting ecdysteroid hormone named as, 20-hydroxyecdysone. Compound **AA-1** is the first report from *A. aspera* and plants belonging to the same species and family. While compound **AA-2** was reported previously from the same plant.

4. Discussion

Compound **AA-1** was isolated as a yellowish semi solid crystal/ amorphous powder, with RF value 0.38 in $CHCl_3$/MeOH/ EtOAc (6:2:2). In the IR spectrum strong and sharp absorption band at 3411 cm^{-1} indicated the presence of OH group. An absorption band at 1654 cm^{-1} indicated the C=O stretch of α, β-unsaturated carbonyl group. The UV spectrum absorption band at λ max 236 nm (in MeOH) shows the presence of α, β-unsaturated carbonyl chromophore.

^{1}H NMR spectrum analysis of the compound **AA-1** showed 1H doublet at δ 6.01 indicating a methine attached to carbon 9 and 1H singlet at δ 5.89 indicating a methine attached to the olefenic carbon 4. On the other hand, 1H a doublet of doublet at δ 5.75 indicating a methine attached to the olefenic carbon 8. A 1H triplet at δ 4.55 indicating the presence of methine attached to oxygen and sharp singlet peak at δ 3.32 indicating the presence of OH group. The spectrum also indicated two 3H singlet at δ 1.96 and 1.03 indicating the presence of two methyl group attached to quaternary carbons. A 3H doublet at δ 1.29 showed the presence of methyl group attached to methine (Table 1).

Comparison of the ^{13}C with DEPT-135 NMR spectra revealed a quaternary carbon atom at δ 199.85, which indicated the presence of conjugated carbonyl group. The quaternary carbon peak at δ 165.70 showed an olefenic carbon β to the carbonyl group. The peaks at δ 132.36, 132.30 and 125.72 indicated the existence of three olefinic carbon atoms. In addition, two quaternary carbon atoms in the aliphatic regions were also observed at δ 78.61 and 41.03. DEPT 135 NMR displayed two peaks at δ 61.43 and 49.35 that showed the presence of two methylene groups, in which the down field shifted peak at δ 61.43 is oxygenated carbon The nine peaks left were assigned to six methine at δ 76.96, 76.80, 73.55, 73.23, 70.28 and 22.0~~)~~which are attached to oxygen and three methyl groups at δ 23.30, 20.8 and 18.16~~)~~. The ^{1}H, ^{13}C and DEPT NMR data of the compound is summarized in **Table 1.**

The ^{2}D NMR (COSY, HMQC and HMBC) spectra of the compound **AA-1** also further supported in predicting the structure. From COSY spectrum (**Table 2**) the proton peak at δ 6.01(H-9) is correlated with the proton peak at δ 5.75 (H-8). The spectrum also shows that the proton peak at δ 1.96 (H-12) is correlated with the proton peak at δ 1.29 (H-13), 1.05 (H-7) and 5.89 (H-4).

From HMQC spectrum (**Table 3**), the protons at δ 6.01 (1H, d) and 5.75 (1H, dd) correlated with the carbon peaks at δ 132.36 and 132.30 respectively. And the proton peak at δ 4.55 (1H, t) correlated with the carbon peak at δ 73.2. The HMBC spectrum (**Table 4**) also shows the proton on C-2 has long range correlation with C-3, C-4, C-6 and C-10 as shown in **Figure 1**. On the other hand, the proton on C-9 correlates with C-1, C-5, C-6 and the proton on C-8 also correlates with C-13, C-6 and C-1 as shown in **Figure 2**.

Based on the spectroscopic data, the proposed structure of compound **AA-1** is indicated in **Figure 3.** (4,4a, 7, 8-tetrahydro-4-hydroxy-4a, 7, 8-trimethyl-8-(tetrahydro-3, 4, and 5-trihydroxy-6 (hydroxymethyl)-2H-pyran-2 yloxy) naphthalen-2(3H)-one). The isolation and characterization of this compound is the first report from *A. aspera* and plants belonging to the same species and family.

Compound AA-2 is previously reported in the same plant was also isolated as a yellowish amorphous solid with RF value of 0.44 in $CHCl_3$/ MeOH/ EtOAc (6: 2: 2). In the IR spectrum absorption band at 3350 cm^{-1} indicated the presence of hydroxyl group. An absorption band at 1450 cm^{-1} indicated the C=O stretch of α, β-unsaturated carbonyl group. The ^{1}H NMR spectrum of compound **AA-2** shows that 1H doublet at δ 5.83 indicates the methine attached to the C-7 and 1H triplet at δ 3.17 indicates methine attached to C-9. A sharp singlet peak at δ 4.6 indicates the presence of OH group. The ^{1}H NMR spectrum also shows that there are five 3H singlet peak at δ 1.30, 1.22, 1.21, 0.95 and 0.90 which indicated that the presence of five methyl groups attached to the quaternary carbons**.** ^{13}C NMR spectrum showed that compound **AA-2** has 27 carbon atoms. The peak at δ 206.5 indicates that the presence of ketone carbonyl group. The ^{13}C NMR also revealed the presence of two olefenic carbons at δ 168 and 122.1. DEPT-135 spectra indicates that there are seven quaternary carbon, seven methine, eight down ward methtylene groups at (δ 42.4, 37.3, 35.1, 32.5, 31.8, 30.8, 27.3, 23.7) and five quaternary methyl carbons, from these carbon groups six are resonated in the region corresponding to oxygenated carbons (δ 85.2, 78.4, 77.9, 71.3, 68.7, and 68.5) **Table 5**.

The above spectral data were further supported by ^{2}D NMR data (COSY, HMQC and HMBC). From COSY spectrum of compound **AA-2 (Table 6)**, the proton peak at δ 5.83 correlated with the proton peak at δ (3.17 and 1.79). The spectrum also shows that the proton peak at δ 2.41 correlated with the proton peak at δ 2.08. From HMQC spectrum (**Table 7)**, the proton peak at δ 5.83 (1H, s) is correlated with carbon peak at δ 122.1 and the proton peak at δ 3.17 (1H, t) correlated with the carbon peaks at δ 35.1. The proton peak at δ 0.95(3H, t) and 0.90 (3H, t) correlated with the carbon peak at δ 24.4 and 18.0 respectively. HMBC spectrum of compound **AA-2 (Table 8)** also shows correlation of some proton with the carbons. The carbon -proton correlation is shown in **Figure 4**.

Based on the spectroscopic data obtained and in comparison with literature (Kunert *et al*., 2000), the structure of compound **AA-2** is proposed to be as 20-hydroxyecdysone (**Figure 5).**

5. Conclusion

In present study, two polar compounds were isolated and characterized from the leaves of A. aspera. Compound AA-1 is the first report from *A. aspera* and plants belonging to the same species and family. While compound AA-2 was reported previously from the same plant. It is highly recommended that further studies were required to assess the pharmacological effect of the new compounds reported from this plant.

6. Acknowledgement

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7. Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper

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9. Abbreviation and Acronyms

AA…… Achyranthes aspera
^{13}C NMR…… Carbon 13 Nuclear Magnetic Resonance
CC……Column chromatography
COSY……Correlation Spectroscopy
^{1}D NMR…… One dimensional Nuclear Magnetic Resonance
^{2}D NMR……Two dimensional Nuclear Magnetic Resonance
DEPT……Distortionless Enhancement by Polarization Transfer
^{1}H NMR……...Proton Nuclear Magnetic Resonance
HMQC……..Hetronuclear Multi Quantum Correlation
HMBC……..Hetronuclear Multiple Bond Correlation
IR……..Infrared Spectroscopy
PTLC……Preparatory Thin Layer Chromatography
TLC……..Thin Layer Chromatography
UV……Ultra Violent
δ……delta (symbol for chemical shift)
d……doublet
dd……..doublet of doublet
m……....multiplet
s……singlet
t…… triplet

10. Tables

Table 1: ^{1}H, ^{13}C and DEPT NMR shifts of compound AA-1 (TMS, CD_3OD, 30^{0}C)

Position	δ_H	δ_C	δ_C (DEPT-135)
1	4.55(1H, t)	73.2	73.2
2	2.62 (1H, d) & 2.19 (1H, d)	49	49
3	-	199.8	-
4	5.89 (1H, s)	125.7	125.7
5	-	165	-
6	-	78	-
7	1.05 (1H, m)	22.0	22.0
8	5.75 (1H,	132.30	132.30
9	6.01 (1H, d)	132.36	132.36
10	-	41	-
11	1.03 (3H, s)	23.3	23.3
12	1.96 (3H, s)	18.0	18.0
13	1.29 (3H, d)	20.6	20.6
1’	4.29 (1H, d)	99.8	99.8
2’	3.29(1H, m)	76.8	76.8
3’	3.20(1H, m)	73.5	73.5
4’	3.28(1H,m)	70.2	70.2
5’	3.18(1H,m)	76.9	76.9
6’	3.87 (1H, d) &3.65 (1H, d)	61.4	61.4

Table 2: COSY (1H↔1H) correlation of **AA-1**

Carbon No	COSY(^{1}H↔^{1}H)
C-9 (δ 132.36)	H-9↔H-8
C-8 (δ 132.30)	H-8↔H-9
C-4 (δ125.7)	H-4↔H-12
C-1' (δ 99.8)	H-1'↔H-3'
C-5' (δ 76.9)	H-5'↔H-6'
C-2' (δ 76.8)	H-2'↔H-4'
C-3' (δ 73.5)	H-3'↔H-1'
C-1 (δ 73.2)	H-1↔H-13,H-9
C-4' (δ 70.2)	H-4'↔H-2'
C-6' (δ 61.4)	H-6'↔H-5',H-13
C-2 (δ 49.0)	H-2↔H-7,H-11
C-11 (δ 23.3)	H-11↔H-2,H-7
C-7 (δ 22.0)	H-7↔H-11
C-13 (δ 20.6)	H-13↔H-1,H-6',H-12
C-12 (δ 18.0)	H-12↔H-4,H-13

Table 3: HMQC correlation of **AA-1**

Carbon No	Hydrogen No	Remark
C-9 (δ 132.36)	δ 6.01(1H,d)	CH
C-8 (δ 132.30)	δ 5.75(1H,dd)	CH
C-4 (δ125.7)	δ 5.89(1H,s)	CH
C-1' (δ 99.8)	δ 4.29(1H,d)	CH
C-5' (δ 76.9)	δ 3.18(1H,m)	CH
C-2' (δ 76.8)	δ 3.29(1H,m)	CH
C-3' (δ 73.5)	δ 3.20(1H,m)	CH
C-1 (δ 73.2)	δ 4.55(1H,t)	CH
C-4' (δ 70.2)	δ 3.28(1H,m)	CH
C-6' (δ 61.4)	δ 3.87(1H,d) & 3.65(1H,d)	CH_2
C-2 (δ 49.0)	δ 2.62(2H,d) & 2.19(2H,d)	CH_2
C-11 (δ 23.3)	δ 1.03(3H,s)	CH_3
C-7 (δ 22.0)	δ 1.05(1H,m)	CH
C-13 (δ 20.6)	δ 1.29(3H,d)	CH_3
C-12 (δ 18.0)	δ 1.96(3H,s)	CH_3

Table 4: HMBC Correlation of **AA-1.**

Proton No	Carbon's correlated
H-9	H-9↔C-1,C-5,C-6
H-8	H-8↔C-1,C-6,C-13
H-4	H-4↔C-2,C-6,C-12
H-1'	H-1'↔C-2',C-5',C-6
H-5'	H-5'↔C-6'
H-2'	H-2'↔C-3'
H-3'	H-3'↔C-2',C-4'
H-1	H-1↔C-9,C-8,C-13
H-4'	H-4'↔C-3',C-5'
H-6'	H-6'↔C-4',C-5'
H-2	H-2↔C-3,C-6,C-4,C-10
H-11	H-11↔C-2,C-3,C-7,C-10
H-7	H-7↔C-6,C-10,C-11
H-13	H-13↔C-1,C-8
H-12	H-12↔C-4,C-5,C-6

Table 5: ^{1}H, ^{13}C and DEPT NMR shifts of compound AA-2 (TMS, CD_3OD, 30^0C)

Position	δ_H	δ_C	δ_C (DEPT-135)
1	1.83(1H,d),1.78(1H,m)	37.4	37.4
2	3.87(1H,m)	68.7	68.7
3	3.97(1H,m)	68.5	68.5
4	1.73(2H,m)	30.8	30.8
5	2.38(1H,m)	51.8	51.8
6	-	206.5	-
7	5.83(1H,s)	122.1	122.1
8	-	168.0	-
9	3.17(1H,t)	35	35
10	-	39.3	-
11	1.79(1H,m),1.64(1H,m)	21.5	21.5
12	2.08(1H,m),1.82(1H,m)	32.5	32.5
13	-	49*	-
14	-	85.2	-
15	1.95(1H,m),1.62(1H,m)	31.8	31.8
16	1.99(2H,m)	23.7	23.7
17	2.41(1H t)	50.5	50.5
18	0.90(3H,s)	18.0	18.0
19	0.95(3H,s)	24.4	24.4
20	-	77.9	-
21	1.30(3H,s)	21.0	21.0
22	3.32(1H,t)	78.4	78.4
23	1.59(2H,m)	27.3	27.3
24	1.72(1H,m),1.45(1H,m)	42.4	42.4
25	-	71.3	-
26	1.22(3H,s)	29.7	29.7
27	1.21(3H,s)	28.9	28.9

The symbol * indicates the peak that observed at δ 49 is overlapped by the solvent

Table 6: COSY Correlation of AA-2

Carbon No	COSY($^{1}H\leftrightarrow^{1}H$) correlation
C-7(δ122.1)	H-7↔H-9
C-22(δ78.4)	H-22↔H-21
C-2(δ68.7)	H-2↔H-4,H-3,H-1
C-3(δ68.5)	H-3↔H-2,H-1
C-17(δ50.5)	H-17↔H-15,H-12
C-1(δ37.4)	H-1↔-3,H-2
C-9(δ35.1)	H-9↔H-7,H-1
C-12(δ32.5)	H-12↔H-17,H-11
C-15(δ31.8)	H-15↔H-17,H-16
C-4(δ30.8)	H-4↔H-2
C-16(δ27.3)	H-16↔H-15
C-11(δ21.5)	H-11↔H-12
C-21(δ21)	H-21↔H-22,H-18
C-18(δ18)	H-18↔H-21

Table 7: HMQC Correlation of **AA-2**

Carbon No	Hydrogen No	Remark
C-7(δ122.1)	5.83(1H,s)	CH
C-22(δ 78.4)	3.32(1H,t)	CH
C-2(δ68.7)	3.87(1H,m)	CH
C-3(δ68.5)	3.97(1H,m)	CH
C-5(δ51.8)	2.38(1H,m)	CH
C-17(δ50.1)	2.41(1H,t)	CH
C-24(δ42.4)	1.72(1H,m,1.45(1H,m)	CH_2
C-1(δ37.4)	1.83(1d),1.78(1H,m)	CH_2
C-9(δ35.1)	3.17(1H,t)	CH
C-12(δ32.5)	2.08,1.82	CH_2
C-15(δ31.8)	1.95,1.62	CH_2
C-4(δ30.8)	1.73(2H,m)	CH_2
C-26(δ29.7)	1.22(3H,s)	CH_3
C-27(δ28.9)	1.21(3H,s)	CH_3
C-16(δ27.3)	1.99(2H,m)	CH_2
C-19(δ24.4)	0.95(3H,s)	CH_3
C-23(δ23.7)	1.59(2H,m)	CH_2
C-11(21.5)	1.79(1H,m),1.64(1H,m)	CH_2
C-21(δ21)	1.30(3H,s)	CH_3
C-18(δ18)	0.90(3H,s)	CH_3

Table 8: HMBC Correlation of **AA-2**.

Proton No	Carbons correlated
H-27	C-24,C-25,C-22
H-19	C-5,C-1
H-18	C-14,C-13,C-12

10. Figures

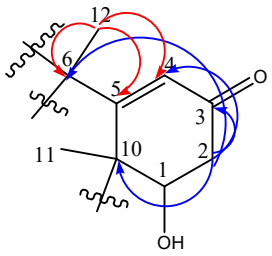

Figure 1: Partial structure I: HMBC Correlation of compound AA-1

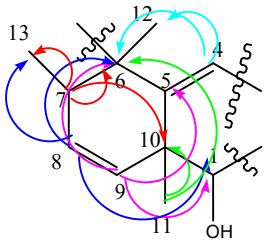

Figure 2: Partial structure II: HMBC correlation of AA-1

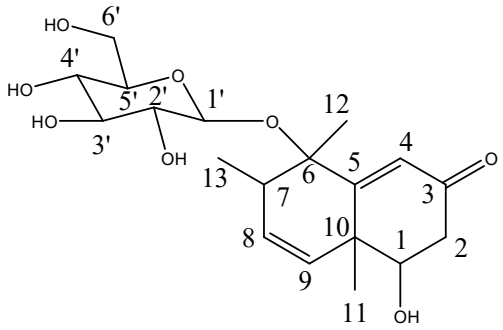

Figure 3: **Structure of Compound AA-1**

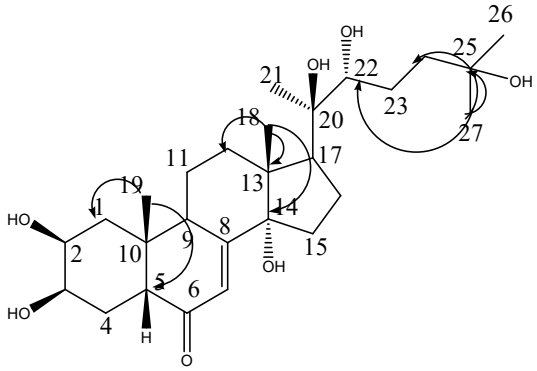

Figure 4: HMBC correlation of compound AA-2.

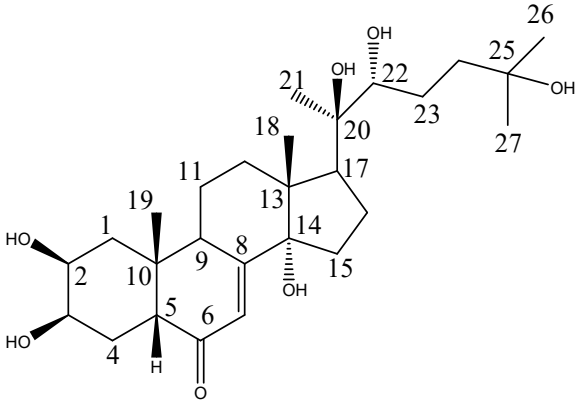

Figure 5: Structure of compound AA-2.