Chemistry and Materials Research
ISSN 2224- 3224 (Print) ISSN 2225- 0956 (Online)
Vol.8 No.6, 2016

Isolation of Sesquiterpene from the Leaves Extract of Vernonia Amygdalina and Its Acute Toxicity to the Albino Mice

Alemu Lelago[1*] Desta Ekero [1] Degu Lere Keshebo[2]
1.Wolaita Sodo University, department of chemistry
2. Dilla College of Teachers Education p.box 334

Abstract
Vernonia amygdalina is a shrub or small tree which is widely used traditional medicinal plant for the treatment of schistomiasis, amoebic dysentery, malaria, veneral diseases, wounds, hepatitis and diabetes and gastrointestinal problems. The objective of this study was to extract, isolate and determine the structure of the constituents of biologically active crude extracts of the leaves of *Vernonia amygdalina* and its toxicity investigation on albino mice. Extraction was carried out by maceration; isolation and characterization were carried out by using chromatographic and spectroscopic technique respectively. The screening of secondary metabolites showed the presence of alkaloid, flavonoids, phenols, saponin, steroids and glycosides in methanol extract. Fractionation of crude extract was carried by using column chromatography. The isolation result afforded sesquiterpene which was elucidated by using spectroscopic methods (IR, ^{1}H NMR, ^{13} C NMR and DEPT 135). The acetone extract showed toxicity at higher dosage with LD_{50}=824.6 mg/Kg but methanol extract did not show any toxicity up to 2000 mg/Kg to the albino mice. Due to high applicability of the plant a number of isolated compounds and chronic toxicity should be carried out for further information.
Keywords: Vernonia amygdalina; sesquiterpene; toxicity; albino mice

INTRODUCTION

The World Health Organization stated that about 80% of the people in developing countries still rely on traditional plant derived medicines, mainly due to their lower price. Medicinal plants represent a rich source of secondary metabolites. Due to their secondary metabolites plants are used medicinally in different countries and are a source of many potent and powerful drugs (Srivastava *et al*., 1996). A wide range of medicinal plant parts is used for extract as raw drugs and they possess varied medicinal properties. The different parts used include root, stem, flower, fruit, twigs exudates and modified plant organs. While some of these raw drugs are collected in smaller quantities by the local communities and folk healers for local used, many other raw drugs are collected in larger quantities and traded in the market as the raw material for many herbal industries (Uniyal *et al*., 2006).

Plants have been a valuable source of natural products for maintaining human health, especially in the last decade, with more intensive studies for natural therapies (Nascimento *et al*., 2000). The herbal products today symbolize safety in contrast to the synthetics that are regarded as unsafe to human and the environment (Joy *et al.,* 2001). The characteristics of the plants that inhibit microorganisms are important for human health which have been researched in laboratories since 1926 (Ates and Erdogrul, 2003; Mythili and Ravindhran, 2012).

Medicinal plant extracts may contain many bioactive phytochemicals which present a potential source of natural antioxidants (Zakaria *et al*., 2006). Their ability to act as potential antioxidants has been extensively investigated (Nitta *et al*., 2002). *Vernonia amygdalina,* is traditionally used in the study area for the treatment of different ailments. The plant, with up to 20 cm long elliptical leaves and rough bark, is a member of the Asteraceae family that grows as a small shrub to a height of 2-5 m. It is reputed to have several health benefits. The organic fraction extracts of the plant was shown to possess antimicrobial and antiparasitic activities (Hladik et al., 2005; Akinpelu *et al*., 1999), antioxidant and cytoprotective (Iwalokun *et al*., 2006) activities. It is also effective against amoebic dysentery (Moundipa *et al*., 2000), gastrointestinal disorders (Akah *et al*., 1995) and has cytotoxic effects towards human carcinoma cells of the nasopharynx (Kupchan et al 1969). Its antioxidant activity has been attributed to the presence of flavonoids (Igile *et al*., 1994).

Despite the successful story on the use of plants in the treatments of different ailments the general acceptability has been limited by lack of scientific data to support the indigenous knowledge (Saad *et al*., 2006). It is therefore appropriate to conduct scientific scrutiny on the selected herb in order to determine their effective doses and possible toxicities. Such measure could possibly lead to new findings to treat diseases in lieu of depending solely on drug developments which may be time-consuming aside from causing side effects to human health after prolonged treatments. Keeping in view the importance of medicinal plant, the objective of this study was to screen out different secondary metabolites of *Vernonia amygdalina* and investigate acute toxicity of the plant.

2. Materials and Methods

The leaves of *vernonia amygdalina* were collected on February 2013 from Wolaita Zone, SNNPRS which is located 380 Km from Addis Ababa the capital of Ethiopia. A Voucher specimen is identified at the National

Herbarium (Ethiopia), Department of Biology, Addis Ababa University, Faculty of Natural Sciences.
^{1}H, ^{13}C, and DEPT spectra were recorded on a Bruker advance 400 MHZ spectrometer with trimethyl saline as internal standard. Infrared (IR) spectra were obtained on perkins-Elmer Bx Infrared spectrometer using KBr disc in the range 4000-400cm^{-1}, at Addis Ababa University chemistry laboratory.
TLC analysis was carried out on 0.2mm thickness TLC plates of Merck silica gel 60 F_{254} coated on aluminium plate. Compounds on TLC were detected using UV light: while column chromatography was carried using silicagel, 60 (mesh).

Coding system
In the coding system used for compounds, V stands for the Genus name Vernonia, A stands for the species name Amygdalina, M stands for Methanol extract, the number behind VAM indicates the fraction number of fractionation in which it is obtained in the increasing polarity of the solvent system.

Extraction and isolation
Extraction of plant material
500 gm powder of air-dried leaf of *vernonia amygdalina* was first soaked with n-hexane for 3 days. After filtration, the extract was concentrated under reduced pressure and temperature of $40^{0}C$ using rotary evaporator to recover the solvent and the concentrated marc exhaustively extracted with methanol after soaking for 72 hours at room temperature. The extract was concentrated in rota-vapor and afforded 16 gm yellow residue. The same step was applied for acetone extraction.

Screening of secondary metabolites on methanol extract.
The extract was tested for the presence of bioactive compounds by using standard methods (Sofowra , 1993; Trease *et al*.,1989 and Harborne, 1973)

Isolation of crude extract
When crude extract developed on TLC it showed different number of identifiable colored spots, which was subjected to column chromatography on silica gel from which 33 fractions were collected with increasing polarity of solvent system. The column elution started with 30 ml of pure diethyl ether, followed by increasing polarity of solvent system, diethyl ether, ethyl acetate, ethanol and methanol.

From the fractions, first five fractions were colorless and showed no spot on TLC of different solvent system. The remaining fractions were colorful. Fr-17 obtained with a solvent system EtOAC: C_2H_5OH in the ratio (12:18), Fr-22 C_2H_5OH: CH_3OH (27:3), Fr-26 C_2H_5OH: CH_3OH (15:15), Fr-29 C_2H_5OH: CH_3OH (6:24), Fr-31 CH_3OH (100%), Fr-32 (CH_3OH: H_2O) (9:1) were colored. From these fractions, Fr-17 was taken to spectroscopic analysis due to its amount and level of purity.

Toxicity
A total number of 90 mice were used for the experiment. The extract (soluble in water) was dissolved in distilled water and different concentrations (dosages) as shown in the table 2 and 3. All the mice (age 5 weeks, weighing 24-30 g) were acclimatized for 7 days before the experiment started. The animals were fasted for three hours prior to oral administration of the extract. The last group in each batch received only distilled water (as control) and was provided with normal food after 30 minutes the experiment started. The acute toxicity of the extract of ***Vernonia amygdalina*** was carried out by oral administration of the above dosages by gavages. The food was provided to all mice after 30 minutes of oral administration of the extract. After administrating the extract each cage strictly observed whether there was any physiological, physical or any death for the first one hour continuously then at the interval of 2 hours for one day. Each batch was kept for further seven days to check any delayed effect (Keshebo et al., 2014).
Table 1 Dosage of the methanol extract *Vernonia amygdalina* to the albino mice

Batch	Number of mice per Cage (5 mice)	Dosage in mg/Kg	Observation
1	1	250	Experimental
	2	500	Experimental
	3	750	Experimental
	4	1000	Experimental
	5	distilled water	Controlled
2	1	1250	Experimental
	2	1500	Experimental
	3	1750	Experimental
	4	2000	Experimental
	5	distilled water	Controlled

Table 2 Dosage of the acetone extract *Vernonia amygdalina* to the albino mice

Batch	Number of mice per Cage (5 mice)	Dosage in mg/Kg	Observation
1	1	250	Experimental
	2	500	Experimental
	3	750	Experimental
	4	1000	Experimental
	5	distilled water	Controlled
2	1	850	Experimental
	2	800	Experimental
	3	distilled water	Controlled

3. Results and Discussion

Acute Toxicity result

No death or any physiological change was observed in table 1 this shows that methanol extract of *Vernonia amygdalina* safe to the albino mice up to 2000 mg/Kg for 7 days. But acetone extract of *Vernonia amygdalina* showed toxic effect to the albino mice at the dosage of 1000 mg/Kg table 2 batch 1 cage 4, all of the mice didn`t feed for the first 2 hours and there physiological nature was different from the control and finally they were started dying 15 hours after administration of the extract.

In table 2 batch 2 cage 1, for the first one hour all of the mice did not feed and some of them shivered; one died after three days, the remaining were normal for the next four days. But in cage two there was slight physiological difference for the first one hour and moving to second hour there was no difference from the control up to 7 days. From the toxicity result of acetone LD_{50}= 824.6 mg/Kg. That means acetone extract of *Vernonia amygdalina* is toxic at higher dosage to the albino mice.

Table 3: **Results of preliminary Phytochemical screening of plant secondary metabolites**

Plants	Alkaloids	Flavonoides	Phenols	Steroid	Saponin	Glycoside	Terpene
Vernonia Amygdalina	+	+	+	+	+	+	+

+ = positive result (present - = negative result (absent)

Characterization of compound VAM-17

The compound VAM-17 is a yellow crystalline solid obtained from methanol extract. From TLC plate it shows a yellow color, which is a characteristic of terpenes. Its structure elucidation was determined using spectroscopic techniques. The pure compound has R.f value 0.71 using EtOAC: C_2H_5OH (9:1) as a solvent system.

Spectral data

Compound VAM-17 Yellowish solid, IR Vmax (KBr) cm^{-1},3410, 2926, ,1711,1273,1076,631. ^{13}CNMR14.14,14.30,20.55,22.70,24.89,25.52,25.61,27.23,29.19,29.27,29.70,31.93,127.10,127. 75,128.21,128.31, 130.19,131.95

^{1}H NMR (400 MHZ, $CDCl_3$) δ in ppm, 0.9, 1.0, 1.3, 1.6, 2.1, 2.3, 2.8, 5.4.

From the IR Vmax (KBr) Cm^{-1} spectrum showed absorption band peaks assigned to different functional group stretching. The absorption band at 3410cm^{-1} shows stretching of OH group. The absorption band peak at 2926 shows C-H stretch. The absorption band at 1711 is for carbon-carbon double bond stretch. The peak at 1273 is for C-H bending. The absorption band at 1076 is for =C-H bending. The absorption band at 631 is for C-H bending. Therefore, IR spectrum indicates the presence of hydroxyl group and carbon-carbon double bond in the compound isolated.

From ^{1}H NMR (400MHZ: $CDCl_3$) spectrum showed a multiplate peak at δ 5.4 is for proton of CH group bonded with hydroxyl group and methine group . A singlet peak at δ 2.8 indicates the OH hydrogen. The peak at 2.3 indicates hydrogen bonded with C=C. The multiplet peak at 2.1 indicates the CH group that is bonded with C-O bond. The triplet peak at 1.6 indicates hydrogen bonded with CH_2=CH_2. The triplet peak at 1.3 indicates the CH_2=CH_2 group. The multiplet peak at 1.0 indicates the CH_2 group that is bonded with CH_3 and CH_2. The triplet peak at 0.9 indicates the CH_3 group that is bonded with CH_2.

Table 4: ^{1}H NMR data of compound VAM-17

δ (ppm)	Multiplicity	Remark
0.9	T	
1.0	M	
1.3	T	
1.6	T	
2.1	M	
2.3	S	
2.8	S	
5.4	M	

From ^{13}C NMR spectrum of the compound showed well resolved resonance of 18 carbon atoms. The peaks at δ 14.14 ppm and 14.30 ppm are for methyl group and the peaks at 20.55,22.70,24.89,25.52,25.61,27.23,29.19,29.27,29.70, are for methylene groups. The peaks at δ 31.93, 127.10, 127.75,128.21,128.31,130.19,131.95 are for methine groups. Therefore the compound VAM-17 has a total of 18 carbons.

Table 5: ^{13}C NMR data of compound VAM-17

Carbon No	^{13}C NMR data, δ ppm	DEPT-135 spectra	Type of carbon
1	131.95	CH	Methine
2	127.10	CH	Methine
3	20.55	CH_2	Methylene
4	22.70	CH_2	Methylene
5	24.89	CH_2	Methylene
6	31.93	CH	Methine
7	27.23	CH_2	Methylene
8	128.31	CH	Methine
9	128.21	CH	Methine
10	29.19	CH_2	Methylene
11	25.61	CH_2	Methylene
12	25.52	CH_2	Methylene
13	14.30	CH_3	Methyl
14	29.27	CH_2	Methylene
15	127.75	CH	Methine
16	127.10	CH	Methine
17	29.70	CH_2	Methylene
18	14.14	CH_3	Methyl

From DEPT 135 spectrum showed 18 peaks corresponding to 18 carbons. Compound VAM-17 has two methyl groups at δ 14.14 and 14.30. There are nine methylene groups at δ 20.55,22.70,24.89,25.52,25.61,27.23,29.19,29.27,29.70, and seven methine groups at δ 31.93 127.10,127.75,128.21,128.31,130.19,131.95. The number of carbon atom indicates that it is a sesquiterpene. Based on the above evidences the expected structure of the compound VAM-17 is shown below.

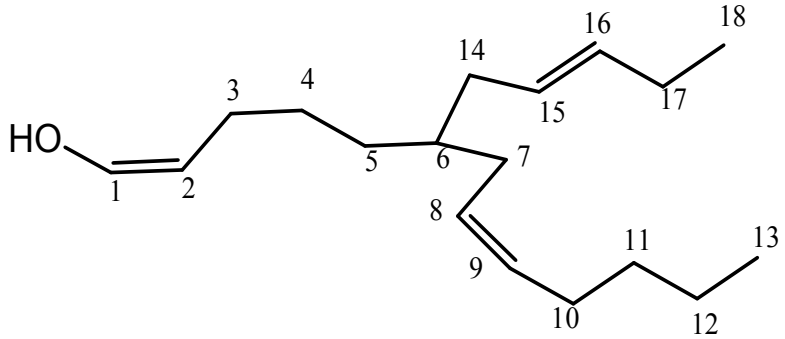

Fig. 1 The Proposed structure of VAM-17

Conclusion

In this work the leaves extract of *vernonia amygdalina* has been analyzed. The plant contains important secondary metabolites alkaloids, flavonoides, phenols, saponin and glycosides which might be the cause for its medicinal values. In addition to screening of different types of secondary metabolites, isolation and characterization of

sesquiterpene was done by using chromatographic and spectroscopic technique. Furthermore, the plant acetone extract is toxic to albino mice but methanol extract is safe to albino mice up to the dosage of 2000mg/Kg. The LD_{50} of toxicity for the plant is 824.6 mg/Kg. Further work is recommended to isolate the active compound (s) due to high applicability of the plant.

Acknowledgements
We thank Wolita Sodo University for financially supporting. The Department of Chemistry and Department of Biology, Addis Ababa University is also acknowledged for providing laboratory facility. We are also grateful to the traditional healers and elders who participated in this work as informants.

References

Akah P.A., Ekekwe R.K. Fitoterapia (1995). Ethnopharmacology of some of the asteraceae family used in the Nigerian traditional medicine. Fitoterapia.66:352–355.
Akinpelu D. A (1999). Antimicrobial activity of Vernonia amygdalina leaves. Fitoterapia. 70:232–234.
Ates D.A., Erdogrul O.A. (2003). Antimicrobial activities of various medicinal and commercial plant extracts. Biol. (27). Pp.157-162
Harborne, J.B. (1973). Phytochemicals Methods. Chapman and Hall Ltd., London, pp. 49-188
Hladik C., Krief S., Haxaire C. J.(2005). Ethnopharmacol. 101:1–5.
Igile, G.O., Oleszek, W., Jurzysta, M., Burda, S., Fafunso, M., Fasanmade, A.A. J. Agric. (1994). Food Chem. 42, 2445-2448
Iwalokun, B.A., Efedede B.U., Alabi-Sofunde J.A., Oduala T., Magbagbeola, O.A., Akinwande (2006). A.I. J. Med. Food. 9, 524-530
Joy PP, Thomas J, Mathew S, Skaria BP (2001). Medicinal Plants: Tropical Horticulture. Vol 2. (Ed. Bose TK, Kabir J, Das P, & Joy PP) India: Naya Prokash Publishers, pp. 449-632.
Keshebo D.L., Choudhury M.K and Dekebo A.H. (2014). *Annals of Biological Research, 5 (6):15-19*
Kupchan S.M., Hemmnigway R.J., Karim A., Werner D. J. Org. (1969). Chem. 34:3908–3911.
Moundipa F.P., Kamini G., Melanie F, Bilong F.C., Bruchhaus I. (2000). Afr. J. Tad. Cam. 62:113–121.
Mythili T. and Ravindhran R. (2012). *Asian J Pharm Clin* Res, Vol 5, Issue 4, 179-182
Nascimento G.G.F., Locatelli J., Freitas P.C., Silva G.L. (2000). Braz. J. Microbiol. (31).pp.247- 256.
Nitta T., Arai T., Takamatsu H., Inatomi Y., Murata H., Iinuma M., Tanaka T., Ito T., Asai F., Ibrahim I., Nakanishi T., and Watabe K.(2002). Journal of Health Science 48:273-276.
Saad B., Azaizeh H., Abu-Hijleh G. and Said S. Evid. (2006). Based Complement. Alternat. Med. 3: 433-439.
Sofowra A. (1993). Medicinal Plants And traditional Medicine in Africa. Spectrum Books Ltd., Ibadan, Nigeria, pp. 191-289.
Srivastava, J., J. Lambert and N. Vietmeyer, (1996). Medicinal plants: An expanding role in development. World Bank Technical Paper. No. 320.
Trease, G.E., Evans, W.C. (1989). Pharmacognosy, 11th edn. Bailliere Tindall, London, pp. 45- 50.
Uniyal S.K., K.N. Singh P. Jamwal and B. Lal, (2006). J. Ethnobiol. Ethnomed., 2: 1-14.
Zakaria Z.A., Fatimah C.A., Jais A.M.M., Zaiton H., Henie E.F.P., Sulaiman M.R., Somchit M.N., Thenamutha M., and Kasthuri D.(2006). International Journal of Pharmacology 2:439-442.