ADDIS ABABA UNIVERSITY

COLLEGE OF HEALTH SCIENCES

SCHOOL OF PHARMACY

Antiproliferative Activities of Alkaloids from *Crinum abyscinicum* Hochst. ExA. Rich Bulb Extract

By: Besufekad Abebe

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A thesis submitted as partial fulfillment for the requirements for the degree of Master of Science in Pharmacognosy

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Antiproliferative activities of alkaloids from *Crinum abyscinicum* Hochst. exA.Rich bulb extract

Besufekad Abebe

Addis Ababa University, 2019

Cancers are group of diseases that are distinguished by increased division of cells caused by genetic changes. Globally, it accounted for 9.6 million deaths in 2015 and is increasing to be a public health burden. Yet the treatment of cancer is mainly through chemotherapy that possesses severe toxicities and facing multiple drug resistance. These problems have urged researchers to look for safe and effective chemotherapeutic agents from natural products, especially from plants. Thus, *Crinum abyscinicum* as one of such plants in Ethiopia that has been used traditionally for treatment of cancer was evaluated for its antiproliferative activity.

In vitro antiproliferative activity of the bulb extract of *C. abyscinicum* was investigated using MTT and resazurine assays on ovarian carcinoma cell line (A2780) and leukemia cell line (MV4-11) respectively**.** It is noted that the bulb extract possesses antiproliferative activity with GI_{50} of 8.289 ± 0.33µg/ml and 20.77 ± 0.354µg/ml against leukemia cell line (MV4-11) and ovarian carcinoma cell line (A2780) respectively.

Further analysis of the bulb extract using preparative thin layer chromatography (PTLC) resulted in the isolation of two alkaloids. The structure of the alkaloids were characterized as 6-hydroxycrinamine (**BCA-1**) and lycorine (**BCA-2**) using spectroscopic methods including HR-TOF-MS, 1D-NMR (^{1}H, ^{13}C-NMR and DEPT) and 2D-NMR (HMBC) spectral data, and by comparison with reported spectroscopic data for the same compounds.

Among the isolated compounds, lycorine displayed antiproliferative activity in a dose-dependent manner against MV4-11 and A2780 cell lines with GI_{50} of 3.372 ± 0.26733µg/ml and 2.849 ± 0.139µg/ml respectively. Likewise, 6-hydroxycrinamine exhibited activity with growth inhibition of 5.323 ± 0.455µg/ml and 2.925 ± 0.769µg/ml against MV4-11 and A2780 respectively. The activity observed for the bulb extract as well as isolated compounds of *C. abyscinicum* support the traditional use of the plant against cancer.

Key words: Cancers, *Crinum abyscinicum*, hydroxycrinamine, lycorine, leukemia cell line, ovarian carcinoma cell line MTT, assay, resazurine assays

List of Abbreviations

^{1}HNMR	Proton nuclear magnetic resonance
^{13}C-NMR	Carbon (13) nuclear magnetic resonance
ATP	Adenosine triphosphate
DEPT	Distortionless enhancement by polarization transfer
DNA	Deoxyribonucleic acid
DU145	Human prostate cancer cell line
ED50	50% effective dose
ESI	Electrospray ionization
HMBC	Heteronuclear multiple bond correlation
HIF-1	Hypoxia-inducible factors 1
GI50	Growth inhibition of 50% of cells
MTT	3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide
PANC-1	Human pancreatic cancer cell line
Ppm	Parts per million
PTLC	Preparative thin layer chromatography
ROS	Reactive oxygen species
TLC	Thin layer chromatography
TOF-MS	Time of flight-mass spectrophotometer
UV	Ultraviolet
WHO	World health organization

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1. Introduction

1.1. Medicinal plants

Plant utilization has been a long history of mankind (Gelaw *et al.,* 2012). Particularly plants were used for the treatment of variety of diseases for centuries. These plants are medicinal due to production of complex and diverse types of compounds called secondary metabolites which comprise of phenols, flavonoids, alkaloids etc. (Yuan *et al.,* 2016).

Because biologically active compounds have complex structures, it is difficult to synthesis novel compounds that are safe and effective against a number of diseases (Seca and Pinto, 2018). Thus, there has been increased interest in the isolation and development of natural products including anticancer agents from the plants, especially from those of the developing countries that have diverse floral capacity (Das *et al.,* 2012; Yuan *et al.,* 2016). This have resulted in the isolation of different drugs which include quinine, vincristine, digoxin digitoxin, emetine and artemisinine (Geyid *et al.,* 2005). In addition, plant constituents have served as a lead compounds and were modified through various methods to produce more selective and potent drugs (Seca and Pinto, 2018).

Plants have also contributed significantly as a source of anticancer agents. In effect, approximately 75% of anticancer agents marketed from 1981 to 2006 are derived from medicinal plants or are plant originated compounds (Newman and Cragg, 2007). Some of these drugs include vinblastine, vincristine, etoposide, topotecan and irinotecan, paclitaxel and docetaxel (Newman and Cragg, 2007; Gelaw *et al.,* 2012; Gordon *et al.,* 2016).

1.2. Ethiopian plants used for the treatment of cancer

In Ethiopia, traditional medicine has played a significant role in treating different public health problems. In fact 80% of the people in Ethiopia rely on traditional medicine due to poor access and unaffordability of modern medicine as well as cultural acceptability of traditional medicines. In addition the country is estimated to have 6,500 to 7,000 species of higher plants, of which about 12% are endemic. However, only small portion has been investigated for their therapeutic values (Suleman and Alemu, 2012; Birhan *et al.,* 2017).

Several Ethiopian plants have been reported in literature to have been used in traditional medical practice for the treatment of cancer which includes *Crinum abyscinicum* Hoechst Ex A. Rich which is mixed with hyena feces and latex and applied topically (Tekilehaymanot, 2009). Plants reported with such activity are listed in Table 1.

Table 1: Plants used for treatment of cancer ("nekersa"or tumor) in Ethiopian traditional medicine

No.	Name of plant	Family	Part used	Method of preparation	References
1	*Aerva javanica* (Burm. f.) Schultes	Amaranthaceae	Root	Powder mixed with bat's blood is taken orally early in m the morning before breakfast	Tekilehaymanot, 2009
2	*Bersama abyssinica* Fresen.	Melianthaceae	Bark	pounded, boiled in water, and a small amount of the preparation is drunk	Tuasha *et al.*, 2018
3	*Buddleja polystachya* Fresen.	Buddlejaceae	Leave	Pounded and macerated with water then given orally	Tuasha *et al.*, 2018
4	*Calpurnia aurea* (Alt.) Benth.	Fabaceae	Leave	Powder mixed with water and taken orally	Teklehaymanot *et al.*, 2007
5	*Carissa spinarum* L	Apocynaceae	Twig & leave	pounded to make paste, and mixed with honey then given orally	Ragunathan and Solomon, 2009
6	*Crinum abyscinicum* Hochst. Ex A. Rich	Amaryllidaceae	Bulb	Powdered and mixed with hyena feces and latex then applied topically	Teklehaymanot, 2009
7	*Croton macrostachyus* Hochst. ex Delile,	Euphorbiaceae	Leave	The leaf juice and its paste used to treat cancer	Ayele, 2018
8	*Dorstenia barnimiana* Schwienf.	Moraceae	Root	Making small opening at affected part and inserting fresh or dry root in the opening	Tekilehaymanot, 2009
			Aerial parts, Root/tube	powder is made in to paste with butter and applied topically,	Ayele, 2018

			r	making small opening at affected part and inserted into the opening	
9	*Ekebergia capensis* Sparrm.	Meliaceae	Fruit	Pounded mixed with water, filtered, and drunk. Few are chewed and swallowed	Tuasha *et al.*, 2018
10	*Gladious candidus* (Rendle)	Iridaceae	Root	Powdered and applied on the wound, or the powder is mixed with water and drunk.	Limeneh*et al.*, 2015
11	*Girardinia bullosa* (Hochst. ex Steud.) Wedd	Urticaceae	Root	Pounded then the solid part is tied on the tumorous body and the liquid part is drunk with salt	Kassa *et al.*, 2016
12	*Myrsine melanophloeos* (L.) R. Br.	Myrsinaceae	Leave	often mixed with *Olea capensis* is pounded, cold macerated with water and drunk	Tuasha *et al.*, 2018
13	*Podocarpus falcatus* (Thunb.)	Podocarpaceae	Root	Powdered and mixed with water is taken orally and applied topically at site of illness	Tekilehayman ot, 2009
14	*Ranunculus multifidus* Forssk	Ranunculaceae	Leave	External dressing of the powder	Tekilehayman ot *et al.*, 2007
15	*Ricinus communis* L.	Euphorbiaceae	Root	Chewed and swallowed	Tuasha *et al.*, 2018
16	*Rubia cordifolia* L.	Rubiaceae	Root	Crushed and smashed in water for 3 days then drunk	Chekole, 2017
17	*Rumex bequaertii De Wild*	Polygonaceae	Leave	chopped and applied on the head	Mekonnen and Abebe, 2017
18	*Senna septemtrionalis* (Viv.) Irwin &Barneby	Fabaceae	Leave	Crushed, smash in water and filter then drink	Chekole, 2017

1.3. The family Amaryllidaceae

The family Amaryllidaceae is comprised of herbs with bulbous root stock of succulent scale leaves; rarely rhizomatous (Zupko *et al.,* 2009). It contains about 90 genera and 1310 species (Aziz *et al.,* 2014). Its members are found worldwide, mainly in tropical and warm temperate regions (Kwembeya *et al.,* 2007). From these regions, South America and sub-Saharan Africa are richest in species, with South America having approximately 300 species in 28 genera and subSaharan Africa having approximately 285 species in 19 genera (Kwembeya *et al.,* 2007). Four genera are indigenous to Ethiopia and Eritrea: *Scadoxus*, *Crinum*, *Ammocharis* and *Pancratium* (Demisew and Nordal, 2010). Few of the species are of agricultural importance (Elgorashi *et al.,* 2003). However, the family includes many desirable ornamentals and those used in traditionalmedicine (Ghosal *et al.,* 1985).

1.4. Genus *Crinum*

1.4.1. Distribution and botanical description

The genus *Crinum* is the only pantropical genus of the family Amaryllidaceae (Jagtap *et al.,* 2014), represented by 111 taxa with 107 species, one subspecies and three varieties (Lekhak *et al.,* 2015). In mainland Africa, the genus includes approximately 50 species (Nordal and Kwembeya, 2004).

Crinum plants are bulbous herbs consisting of 2 to 40 sessile and regular to more less irregular flowers. The flowers are with free tepal segments that are whitish, with or without a red to pink dorsal line. The members produce spirally arranged clumps of strap or linear shaped leaves (Yakandawala and Samarakoon, 2006; Demisew and Nordal, 2010).

1.4.2. Ethnopharmacological use

Members of the genus *Crinum* are used in Asian traditional medicine as rubefacient, tonic and for treatment of allergic disorders and cancer (Tram *et al.*, 2002b).

In India the leaves of *C. defixum* Ker-Gawl were used to treat pimples, body swelling, dropsy, carbuncles, paronychia, leprosy, fever, diarrhea and leucorrhea. The juice prepared from the

leaves of this plant is instilled into the ear to treat otitis whereas the bulb was used for its nauseant, emetic, emollient and diaphoretic properties. It is also used in the treatment of burns, whitlow and carbuncle (Shilpa *et al.*, 2012).

In Bangladesh clan healers use the juice obtained from bulb of *C. latifolium* for chest pain (Udegbunam *et al.*, 2015). They also use the bulb of the plant along with *Allium sativum* against bloating in cattle (Udegbunam *et al.*, 2015).

In Ethiopia the bulb of *C. abyscinicum* is used to treat internal parasites, mastitis, rabies, colic diseases of animals (Tamiru *et al.*, 2013) and cancer (“nekersa”) (Tekilehaymanot, 2009) The ethnopharmacological use of the *Crinum* species in different areas of the world has been reviewed in detail by Fennell and Staden (2001).

1.4.3. Phytochemical constituents

The genus *Crinum* is a representative of the family that possesses most chemical features of the Amaryllidaceae (Nino *et al.*, 2007; Zhang *et al.*, 2009). The isolated compounds include alkaloids, flavonoids, fatty acids, terpenes and other types of compounds. The alkaloids are the major compounds isolated from this genus. They include lycorine, crinine as well as galanthamine type alkaloids. There are also other minor alkaloids reported from this genus (Rahman *et al.*, 2012; Jagtap *et al.*, 2014). Compounds reported from this genus are listed in appendix I.

1.4.4. Pharmacological Activity

Extracts as well as compounds from the *Crinum* plants are used for treatment of different diseases (Zupko *et al.*, 2009).

1.4.4.1. Central nervous system activities

Kaempferol, a flavonol isolated from *C. jagus* administered orally exhibited a dose dependent protection against tonic-clonic convulsions with an effective dose (ED_{50}) value at 95% (confidence interval) CI of 23.46 (18.95- 58.72) mg/kg (Taiwe *et al.*, 2016), whereas alkaloidal fractions obtained from dried bulb of *C. jagus* reversed pre and post treatment glutamate

excitotoxicity at a concentration of 2.9 µg/mL evidenced by a viable nuclei (>70% of cells) and preserved integrity of F-actin in the actin cytoskeleton (Cortes *et al.,* 2015).

The alkaloidal fractions also showed inhibitory activity against acetyl cholinesterase with half maximal inhibitory concentration (IC_{50}) of 18.28±0.29 µg/ml while galanthamine (**59**) used as positive control exhibited a higher inhibition with IC_{50} value of 1.55 µg/ml (Cortes *et al.,* 2015). Oral administration of hydroalcholic crude extract of *C. macowanii* enhanced the memory impaired by scopolamine in the spontaneous alternation performance in the Y-maze at a dose of 40mg/kg but fail to do so in the short-term novel object recognition task (Mugwagwa *et al.,* 2015). In contrast the extract exhibited long term memory enhancement in the long-term novel recognition test which was found to be more than the standard Alzheimer's disease drug donepezil at a dose of 40 mg/kg (Mugwagwa *et al.,* 2015). Treatment of rat with 5 mg/kg and 10 mg/kg aqueous extract of *C. giganteum* resulted in decrease and prevention of ketamine induced schizophrenia and amygdala neurotoxicity (Finbarrs *et al.,* 2016). The result indicated that the plant extract showed higher effect than chlorpromazine by preventing of neurotoxicity at concentration of 25 mg/kg (Finbarrs-Bello *et al.,* 2016).

1.4.4.2. Antibacterial and wound healing activity

The alkaloids crinamine (**40**) was found to be active against *Bacillus subtilis* and *Staphylococcus aureus* with a minimum inhibitory concentration of 10 µg/ml *(*Adesanya *et al.,* 1992). Another species of this genus, *C. asiaticum* was found to be active against Gram-positive *bacteria Bacillus subtilis, B. megaterium and Staphylococcus aureus* and four Gram-negative bacteria *Escherichia coli, Salmonela typhi, Salmnela paratyphi* and *Pseudomonous sp* (I) with a concentration (250mg/disc) of plant methanol extract (Rahman *et al.,* 2012). Accelerated wound healing process was achieved by bulb extract of *C. zeylanicum* (Yahaya *et al.,* 2012).

1.4.4.3. Anticancer activity

Crinamine (**40**) exhibited significant inhibitory activity against the Hypoxia inducible factor-1 (HIF-1) induced transcriptional activation (IC_{50}: 2.7 mM) in a concentration dependent manner without inhibition in the U251-pGL3 control cell line. HIF-1 activates the transcription of genes involved in crucial aspects of cancer biology including angiogenesis, cell survival, and glucose

metabolism (Kim *et al.,* 2006). The alkaloids crinine (**28**) and 6-methoxybuphandrine were also found to exert concentration-dependent cytotoxicity in the panel of human tumor cell lines. As compared to 6-ethoxybuphandrine and crinine was found to be the more active with IC_{50} 14.04 μM against HL-60/Dox (i.e 6-methoxybuphandrine = IC_{50}: 95.22 ± 11.08 μM) (Berkov *et al.,* 2011).

Luo *et al* (2015) demonstrated that lycorine (**1**) is effective against multiple myeloma cell line ARH-77 *via* inducing apoptosis. The same study also found that the mechanisms of lycorine on the 32 multiple myeloma cell line ARH-77 are associated with G1 phase cell cycle arrest, mitochondrial dysfunction, reactive oxygen species (ROS) generation, ATP (adenosine triphosphate) depletion and DNA damage.

The cytotoxic effect of three *Crinum* alkaloids namely 6-hydroxycrinamine, lycorine (**1**), and crinamine (**40**) was tested against human pancreatic (PANC1) and prostate (DU145) cancer cells and the result indicated that the alkaloids, 6-hydroxycrinamine, and crinamine were active with an IC_{50} value of: 22.7, 19.9 and 10.0 μM, respectively, against PANC1 and with IC_{50} value of: 9.3, 21.7 and 18.5 μM, respectively, against DU145 cells (Arai *et al.,* 2015).

The anticancer structural activity relation of lycorine type alkaloids reveals that basic nitrogen, planarity as well as the presence of alkoxy functions are needed. However, substitution of lycorine (**1**) with bulky molecules at ring C, quaternary nitrogen and opening of the dixol ring results in loss of activity (Cheng *et al.,* 1978; Lamoral-Theys *et al.,* 2010; Roy *et al.,* 2018)

1.4.4.4. Antimalarial activity

The antimalarial activity of lycorine was proved against *Plasmodium falciparum* strain with IC_{50} value of 1.026 μM (T9.96) and 0.379 μM (K1) alongside crinine (**28**) which also displayed antimalarial activity with IC_{50} value of 2.110μg/ml and 1.650 μg/ml against the T9.96 and K1 *P. falciparum* strains respectively (Sener *et al.,* 2003). Other alkaloids, cripowellin A, B, C and D which are obtained from *C. erubescens* exhibited antiplasmodial activity, with IC_{50} values of 30 ± 2, 180 ± 20, 26 ±2 and 260 ± 20 nM respectively against the chloroquine/mefloquine-resistant Dd2 strain of *P. falciparum* (Presley *et al.,* 2016).

1.5. *Crinum abyscinicum* Hochst. ExA. Rich

1.5.1. Distribution and botanical description

C. abyscinicum Hochst. ExA. Rich is the most common *Crinum* species growing in most floristic regions in Ethiopia and also in Eritrea. The plant is not known outside the Horn of Africa. The flowering period is from April to July (August). *C. abyscinicum* is distributed in waterlogged valley grasslands and swampy depressions or along stream banks, sometimes in fallow fields, on black clayish and loamy soils, from 1650 to 3100 m altitude (Demissew and Nordal, 2010).
C. abyscinicum has glaucous leaves which are greyish green that are linear to narrowly lanceolate with the size of 40 × 1–3.5 (–5) cm. While flowers are sessile that are pure white or sometimes tinged pink, only rarely with a pink dorsal line. They also produce fruits that are greenish, sometimes tinged red, with a thick fleshy pericarp, without an apical beak yet the seeds are not visible. The morphological appearance of the plant is indicated on figure 7 (Demissew and Nordal, 2010).

In Ethiopian traditional medicine bulb of *C. abyscinicum* is used to treat internal parasites, mastitis and rabies and colic diseases of animals (Tamiru *et al.,* 2013) and the powder mixed with hyena feces and is applied topically for "neqersa" or cancer in Dek island in Lake Tana, Ethiopia (Teklehaymanot, 2009).

A B Figure 1: Picture of *Crinum abyscinicum* Hochst. ExA. Rich (A) in its natural habitat and (B): bulbs

1.6. Overview of cancers

Cancers are group of diseases that are distinguished by uncontrolled division of cells due to changes in the genetic makeup of the cells (Sudhakar *et al.,* 2009, Anitha *et al.,* 2014). The changes in the genomic deoxyribonucleic acid (DNA) are produced by the activity of different mutational processes which include abnormal DNA editing, the incomplete conformity of DNA polymerases, and failure of DNA repair mechanisms (Alexandrov, 2015).

Gene mutations in cancer exert their effect by targeting regulators of G phase progression during which cells respond to extracellular signals by either advancing toward another division or withdrawing from the cycle into a resting state (Sherr *et al.,* 1996). Because cell cycle exit can facilitate maturation and terminal differentiation, these processes are destabilized in cancer cells (Sherr *et al.,* 1996). Thus cancer cells become refractory to extracellular growth regulatory signals and instead obligate to the autonomous program that carries them through to division (Sherr *et al.,* 1996) hence abnormal cells can survive and expand against the cell competitive regulation, leading to the formation of a tumor mass (Fouad *et al.,* 2017).

Cancers are caused by both environmental factors and genetic changes. Some of the external factors include ionizing radiation, smoking, alcohol consumption, asbestos, viruses like human papilloma viruses (HPV) and hepatic virus B and C, heavy metals like arsenics and cadmium (Danaei *et al.,* 2005; Parsa, 2012)

Metastasis of tumor cells is the spread of cancer cells to tissues and organs beyond where the tumor originated (Fouad *et al.,* 2017). It involves invasion, intravasation and extravasation. Invasion involves dissociation of tumor cells from primary tumor mass and changes in cell-matrix interaction enable the cells to invade the surrounding stroma through the secretion of substances to degrade the basement membrane and extracellular matrix (Fouad *et al.,* 2017). Then in the process of intravasation the cells will initialize angiogenesis to facilitate a route for the detached cells to enter the circulatory system and spread to distant sites. At the distant sites, tumor cells will interact with the endothelial cells by undergoing biochemical changes, develop adhesion to the endothelial cells and thus penetrates the endothelium and the basement membrane; the process of extravasation (Gupta and Massagué, 2006).

The distinguished characteristics of cancers cells include evading apoptosis, limitless replicative potential, self-sufficiency in growth signals, insensitivity to antigrowth signals; sustained angiogenesis, tissue invasion and metastasis, plus four recently identified additional traits of deregulation of cellular energetics, avoidance of immune destruction, genome instability and tumor promoting inflammations (Hanahan and Weinberg, 2011; Floor *et al.*, 2012, Vega *et al.*, 2018).

The main modalities of cancer treatment are surgery, systemic therapies (chemotherapy, hormonal therapy and biological agents) and radiotherapy, which can be delivered alone or in combination and either with curative or palliative intent (Camacho *et al.,* 2014) of which conventional chemotherapy which kills rapidly dividing cells through induced apoptosis being the basis (Cao, 2016).

In early stage disease, low-risk patients are often cured with surgery alone, but in many other cases a combination of treatments is required (Fernando and Jones, 2015). While in metastatic disease, systemic therapy is the principal therapeutic modality, as delivery through the blood stream facilitates access to disseminated cancer sites. Systemic therapies include hormonal therapy, targeted therapy, immune therapy and chemotherapy (Dienstmann, 2012; Fernando and Jones, 2015).

1.6.1. Epidemiology of cancers

Cancer is the second leading cause of death globally (Siegel *et al.,* 2017). It accounted for 9.6 million deaths in 2018 of which lung, prostate, colorectal, stomach and liver cancer are the most common types of cancer in men, while breast, colorectal, lung, cervix and stomach cancer are the most common among women (WHO, 2018). Cancer burden is increasing owing to a growing and aging global population as well as risk factors like smoking, obesity, and dietary patterns (Belpomme *et al.*, 2015).

Cancer is a public health issue in Africa with estimates of 715 000 new cases and 542 000 cancer deaths in the continent in 2008. Cancer burden projections in Africa suggest that cancer incidence and mortality will double to 1·28 million new cases and 970 000 deaths per year by 2030 (Woldeamanuel *et al.,* 2013).

Cancer is an increasing public health burden for Ethiopia and Sub-Saharan Africa at large (WHO, 2018). Indeed, by the year 2030, cancer and other non-communicable diseases may overtake some infectious diseases as leading causes of death in the African Region. In 2017, four percent of all deaths were due to cancer (WHO, 2017)

In Ethiopia, annual incidence and mortality of all cancer types reported by GLOBOCAN in 2008 were 51 700 and 41 600, respectively. For people under the age of 75 years, the risk of being diagnosed with cancer is 11·3% and the risk of dying from the disease is 9·4% (Woldeamanuel *et al.,* 2013).

The greatest increases are anticipated in the low income countries and longer-term planning is needed to reduce the future cancer burden through resource-appropriate interventions (Ferlay *et al.,* 2015).

1.7. Statement of the problem

Chemotherapy is the only major treatment modality used for the control of advanced stages of malignancies yet it exhibits severe toxicity on normal tissues (Priya *et al.,* 2015). Some of the common side effects which include fatigue, nausea and vomiting affect the daily life of the patient (Aslam *et al.,* 2014). Other side effect is a life-threatening complication, febrile neutropenia (FN) which arise after myelo suppressive chemotherapy (Wang *et al.,* 2015). Together with these side effects and emergence of multidrug resistance has diminished the therapeutic values of chemotherapeutic drugs (Li *et al.,* 2017; Aslam *et al.,* 2014).

Therefore, there is a need for new anticancer drug~~s~~, which specifically reduces the tumor with minimum or no side effects (Aslam *et al.,* 2014).

The anticancer potential of the Amaryllidaceae has interested researchers owing to the potent and cell line selective antiproliferative activities exhibited by representatives of its different alkaloid groups namely pancratistatin, narciclasine and narciprimine which have shown chemotherapeutic activity relative success in clinical trials (Kornienko and Evidente 2008). These discoveries and above mentioned shortcomings of chemotherapeutic drugs have increased the demand for the discovery of new anticancer agents from natural products with minimal side effect and increased potency (Raina *et al.,* 2014). Accordingly, this study aims to investigating

and isolating the antiproliferative compounds from the bulb of *C. abyscinicum,* a member of the Amaryllidaceae family which is used in Ethiopian traditional medicine for the treatment of cancer (Tekilehaymanot, 2009).

2. Objectives

2.1. General objective

- To evaluate the antiproliferative activities of the bulb extract and compounds isolated from *Crinum abyscinicum* Hochst. ex A.Rich

2.2. Specific objectives

- To determine the antiproliferative activities of the bulb extract of *C. abyscinicum*
- To isolate compounds from the bulb extract of *C. abyscinicum*
- To elucidate the structures of the isolated compounds; and
- To evaluate the antiproliferative activities of the isolated compounds

3. Materials and Methods

3.1. Materials

3.1.1. Plant material

The bulb of *C. abyscinicum was* collected from its natural habitat from the town of Alelitu, 44 km North-East of Addis Ababa in April, 2015. Identity of the plant was confirmed by Mr. Melaku Wondafrash, Senior Botanist at the National Herbarium, Addis Ababa University (AAU), where specimens were deposited with a collection number of BA0001.

3.1.2. Chemicals and instruments

The chemicals used in this study include; distilled water (AAU laboratory), chloroform, methanol, butanol, acetic acid (all from Sigma-Aldrich Co., MO, USA), were used for extraction and chromatography. Analytical TLC was performed using pre-coated silica gel 60 F_{254} plates (aluminium backed, 200 µm, Merck KGaA, Darmstadt, Germany) and ethyl acetate. Whereas dimethylsulfoxide (DMSO) (Sigma-Aldrich Co., MO, USA), 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) (Life Technologies, Mulgrave, VIC, Australia) and resazurin were used in the biological assay (Sigma–Aldrich, Castle Hill, NSW, Australia).

Instruments used comprised of UV cabinet, UV spectrophotometer (both from Cammag, switherland), ABSCIEX Triple TOF 5600 mass spectrometer with electron spray ionizer (ESI) (Concord, ON, Canada), and Bruker Avance III HD spectrometer (Faellanden, Switzerland) at 500 MHz for ^{1}H-NMR and 125 MHz for ^{13}C –NMR analyzed using a Bruker Topspin 3.2 program.

3.2. Methods

Extraction of the plant material

The bulbs of *C. abyscinicum* were allowed to dry in open air for two weeks. Then they were pulverized using mortar and pestle. After that the grounded 200g bulbs were macerated for three days using 2 liter of 80% methanol. The maceration was performed in 3x. Then the extract was filtered under vacuum and dried using rotavapor.

3.2.1. Isolation of compounds

3.2.1.1. Analytical and preparative thin layer chromatographic technique

Analytical thin layer chromatography (TLC) was used to select a solvent system with a better resolution of the constituents of the crude drug as well as to monitor the purity of the isolated compounds. Isolation and purification of compounds was performed by preparative thin layer chromatography using silica gel plates (20 cm x 20 cm; 0.5 mm thickness) and butanol: acetic acid: water (4:1:5) as a mobile phase. The solvent was prepared by dissolving butanol, water and then adding acetic acid. Because the solvent forms a two phases, the upper layer was taken and used as s solvent system for the chromatographic separation.

3.2.1.2. Visualization

Chromatographic zones were visualized using ultraviolet light of wave lengths 254 nm and 366 nm. After visualization, bands were coded based on descending order of R_f values. Then, each band was carefully scrapped off separately from the plate and washed using a combination of ethyl acetate and methanol (1:1). The washing was filtered and evaporated *in vacuo*. The residue obtained was weighed and kept in closed umber colored glass vials in cold place until used for biological activity tests and spectroscopic characterization.

3.2.1.3. Structural elucidations

3.2.1.3.1. 1D and 2D-NMR and MS

High-resolution mass spectra were recorded using an ABSCIEX Triple TOF 5600 mass spectrometer (Concord, ON, Canada), and ionization of all samples was carried out using ESI. ^{1}H and ^{13}C NMR spectra were obtained using a Bruker Avance III HD spectrometer (Faellanden, Switzerland) at 500 and 125 MHz, respectively. The 2D experiments carried out included distortionless enhancement bypolarisation transfer (DEPT), and heteronuclear multiple bond correlation (HMBC). Chemical shifts are reported in units of ***d*** (ppm) and coupling constants (*J*) are expressed in Hz. Multiplicity of ^{1}H NMR signals is reported as s = singlet, d = doublet, t = triplet, q = quartet, dd = doublet of doublets and m = multiplet.

3.2.2. Antiproliferative activity test

3.2.2.1. Cell Culture

MV4-11 (human acute myeloid leukemia) and A2780 (ovarian cancer) cells were obtained from the cell bank at the Centre for Drug Discovery and Development, University of South Australia. The cell lines were maintained following ATCC recommendations either in RPMI-1640 (Roswell Park Memorial Institute), DMEM (Dlbecco's Modified Eagle's Medium) or MEM (Minimum Essential Media) with 10% fetal bovine serum. All cell lines were cultured at 37°C in a humidified incubator in the presence of 5% CO_2. All cells were mycoplasma tested.

3.2.2.2. Cell viability assays

MTT and resazurin assays were performed on A2780 and MV4-11 cell lines, respectively, as previously reported (Tadesse *et al.,* 2017). Concentration of crude Extract and isolated compound required to inhibit 50% of cell growth (GI_{50}) was calculated using nonlinear regression analysis.

3.2.2.3. Cell viability assays

3-(4, 5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay was performed on A2780 cell lines as reported by (Wang *et al.,* 2004).
In brief, 1×10^5 cells/well were seeded into 96-well plates and incubated overnight at 37 °C. Test samples were dissolved in DMSO (Dimethyl sulfoxide), and a 3-fold dilution series prepared in 100 μL of cell medium, added to cells (in duplicates), and incubated for 72h at 37 °C. MTT was made up as a stock of 5 mg/mL in cell medium, and the solution was filtersterilized. Medium was removed from cells followed by a wash with 200 μL/well phosphate buffered saline. MTT solution was then added at 20 μL/well and incubated in the dark at 37 °C for 4 h. MTT solution was removed and cells were again washed with 200 μL of PBS. MTT dye was solubilized with 200 μL/well of DMSO with agitation. Absorbance was read at 540 using an EnVision multi-label plate reader (PerkinElmer, Beaconsfield, Buckinghamshire, UK) On the other hand, resazurin assay was done on MV4-11 cell lines as described by (Diab *et al*., 2014).

In short, Cells were seeded at 5×10^3 cells/well into 96-well plates and incubated 19 overnight at 37 ^{0}C, 5% CO_2. Tested samples were diluted from a 10 mM stock solution to prepare a threefold dilution series in 100 mL of cell medium, added to cells (in duplicates), and incubated at the corresponding time point at 37 ^{0}C, 5% CO_2. Resazurin was made up as a stock of 0.1 mg/mL in cell medium, and the solution was filter-sterilized. The resazurin solution was then added at 20 µL/well and incubated in the dark at 37 ^{0}C, 5% CO_2 for 4 h. The plate was left at room temperature for 10–15 min, and absorbance was measured at 585 nm using an EnVision multi-label plate reader (PerkinElmer, Beaconsfield, Buckinghamshire, UK). The concentration of crude extract of *C. abyscinicum* and isolated compound/s required to inhibit 50% of cell growth (GI50) was calculated using nonlinear regression analysis.

3.2.2.4. Cell Cycle Analysis

Cell cycle analysis was performed as described previously (Tadesse *et al.*, 2017). Cells were seeded at 8×10^4 cells per well using 6-well plate and incubated overnight at 37 °C, 5% CO_2. After treatment with each compound, the cells were incubated for 24 h. Cells were transferred to Fluorescence-activated cell sorting (FACS) tubes and centrifuged at 300g for 5 min. Cell pellets were collected and resuspended in 1 mL of phosphate buffered saline (PBS) and centrifuged at 300g for 5 min. The supernatant PBS was removed, and cell pellets were fixed by adding 500 µL ice-cold 70% Ethanol drop wise on ice for 15 min and collected again after being centrifuged at 300g for 5 min. The supernatant ethanol was removed, and collected pellets were incubated with propidium iodide cell cycle solution in PBS (50 µg/mL propidium iodide, 0.1 mg/mL RNase A, 0.05% Triton X-100) at room temperature for 1.5 h and analyzed with a Gallios flow cytometer (Beckman Coulter, Brea, CA, USA). Data were analyzed using Kaluza v1.2 (Beckman Coulter, Brea, CA, USA).

3.2.2.5. Detection of Apoptosis

Cell cycle analysis was performed as described previously (Tadesse *et al.*, 2017). Cells were seeded at 8 × 104 cells per well using a 6-well plate and incubated overnight at 37 °C, 5% CO2. After treatment with each compound, the cells were incubated for 24 h. Cells

were transferred to FACS tubes and centrifuged at 300g for 5 min. Cell pellets were collected and resuspended in 1 mL of warm PBS and centrifuged at 300g for 5 min. The supernatant PBS was removed and cell pellets were diluted to 1 × 105 cells/mL with warm PBS and centrifuged at 300g for 5 min. The supernatant PBS was removed, and cell pellets were resuspended with 1 mL of ice-cold PBS and centrifuged at 300g for 5 min. The supernatant PBS was removed, and cell pellets were resuspended with 100 μL of 1× binding buffer. Then 3 μL of Annexin V and 3 μL of propidium iodide were added to each sample with slight vortexing and cells were incubated in the dark for 15 min. After incubation 200 μL of 1× binding buffer was added to each sample and analyzed by the Gallios flow cytometer (Beckman Coulter, Brea, CA, USA). Data were analyzed using Kaluza v1.2 (Beckman Coulter, Brea, CA, USA).

4. Results and Discussion

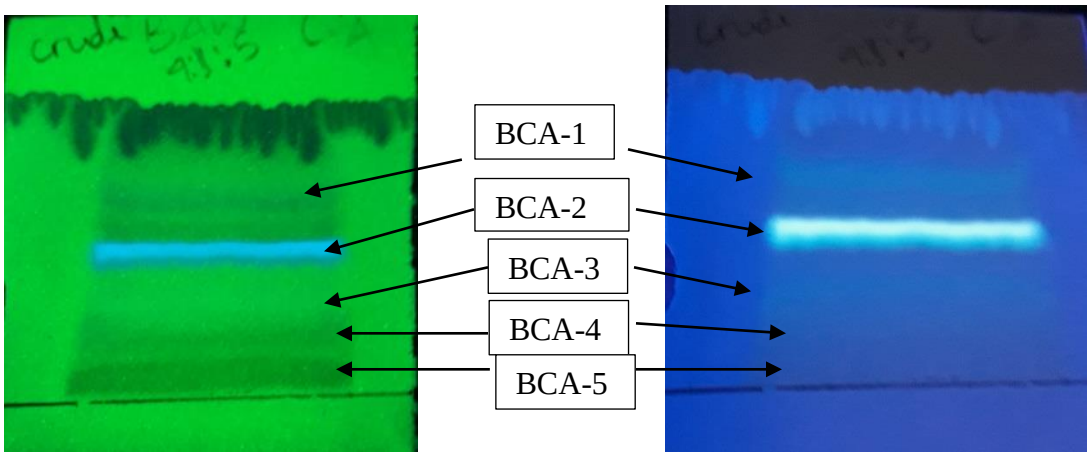
4.1. Extraction Yield

The bulb of *C. abyscinicum* was subjected to extraction with 80% methanol using maceration technique, yielded a brown colored amorphous material. The percentage yield calculated from the dried matter was found to be 8% (w/w).

4.2. Compounds isolated from *C. abyscinicum*

PTLC analysis of the crude extract over silica gel using butanol: acetic acid: water (4:1:5) as a solvent system showed the presence of at least 5 compounds. Of these compounds, two major compounds were isolated with R_f values of 0.62 and 0.44. These compounds were coded as BCA-1 and BCA-2 respectively (Figure 3). The purity of the compounds was confirmed using TLC ethyl acetate: methanol: water (60:25:25) and butanol: acetic acid: water (4:1:3).

BCA 1 quenched and appeared as a dark spot under UV light at 254 nm and green at 366 nm while BCA-2 appeared as blue spot at 254nm and as yellow spot under UV light of 366 nm as indicated in the figure.



A B

Figure 2: TLC profile of the bulb extract of *Crinum abyscinicum* Hochst. ExA. Rich viewed at A: 254nm B: 366nm using butanol: acetic acid: water (4:1:5)

4.2.1. Characterization of BCA-1 and BCA-2

4.2.1.1. BCA-1

BCA-1 was isolated as a pale green colored amorphous substance. The R_f value of the compound was calculated to be 0.66 using the solvent system butanol: acetic acid: water (4:1:5). It gave brown colored precipitate upon treatment with Dragendorff's reagent which indicated that the compound is an alkaloid. The positive TOF MS data of BCA-1 showed a pseudomolecular ion peak at *m/z* 318.1619 $[M-H]^+$ (exact calculated molecular mass = 318.134149 u), indicating a molecular formula of $C_{17}H_{19}NO_5$.

Structural elucidations of the isolated compounds were based on their spectroscopic data and comparison of their spectroscopic characteristics with those reported in the literature. The ^1H NMR spectral data of BCA-1 revealed the presence of 2- aromatic proton signals assigned to H-7 (δ 6.78, 1H, *s*), and H-10 (δ 6.84, 1H, *s*). Two olefinic protons were also observed and assigned as H-1 (δ6.11, 1H, *d*) and H-2 (δ6.31, 1H, *d*). A singlet at δ5.92 was detected due to the presence of a methylene group flanked between two oxygens. The full ^1H NMR spectral data is presented in Table 4.

Table 2: ^1H NMR and ^{13}C NMR chemical shifts for BCA 1

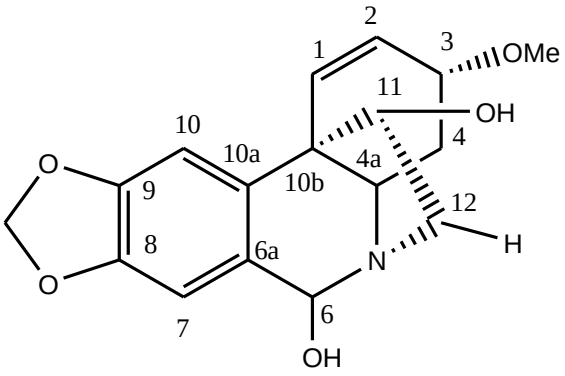
Carbon and hydrogen numbering	^1H NMR chemical shift (δ, ppm)		^{13}C NMR chemical shift (δ, ppm)	
	BCA1	Elgorashi, 2001	BCA 1	Elgorashi, 2001
1	6.11 d	6.26 d	132.94	136.3
2	6.31d	6.25 dd	124.38	123.0
3	3.88t	3.95 t	76.48	75.6
4	1.99m	2.04-2.25m	29.35	29.4
4a	3.53t	3.41 t	59.96	64.8
6	5.50s	5.62 s	87.57	85.5
6a		-	127.89	128.6
7	6.78s	6.97 s	108.89	108.3

8		-	146.3	146.7
9		-	147.88	147.5
10	6.84s	6.74s	102.36	102.7
10a		-	132.9	134.5
10b		-	50.41	50.8
11	4.09m	3.85m	78.23	78.9
12	3.15m	3.01m	57.56	57.6
O-Ch_2-O	5.92s	5.90 d	101.03	101.1
O-Me	3.54s	3.39 s	54.4	55.9

Note: *s*-singlet, *brs*-broad singlet, *d*-doublet, *t*-triplet, *m*-multiplet

The ^{13}C-NMR and DEPT-135 spectral data (Table 1 and Appendix II) indicated the presence of 17 carbon atoms, which were identified as two aromatics (108.89 and 102.36), two olefinics (136.3, 123.0), five quaternary of which four are aromatics (δ 134.5, 147.5, 128.6) and one alkyl (50.8), two attached to nitrogen (57.6, 64.8), one flanked between nitrogen and oxygen (85.5), one attached to a methoxy group (75.6), one methoxy (55.9) and one methylene carbon flanked between two oxygens (101.1). The ^{13}C-NMR spectral data is presented in Table 4.

From the above spectroscopic data and comparison with those reported in the literature the structure of the compound BCA 1 was suggested to be 6-hydroxycrinamine as shown in figure 9. The compound was also isolated from the leaves of *C. latifolium L* (Nguyen *et al.,* 2013).



6-Hydroxycrinamine

BCA-1

Figure 3: Structural formulae of BCA-1 (6-hydroxycrinamine)

The structure of the compound was then further confirmed by 2D NMR.

Some of the important long range couplings between C and H were observed in HMBC as listed below:

(a) ^{3}J coupling between aromatic carbon attached to methoxy group (C-3, 76.48δ) with aromatic proton (H-1, δ 6.11 d).

(b) ^{3}J couplings between hydroxylated carbon (C-6, δ 87.57) with aromatic proton (H-7, δ 6.78)

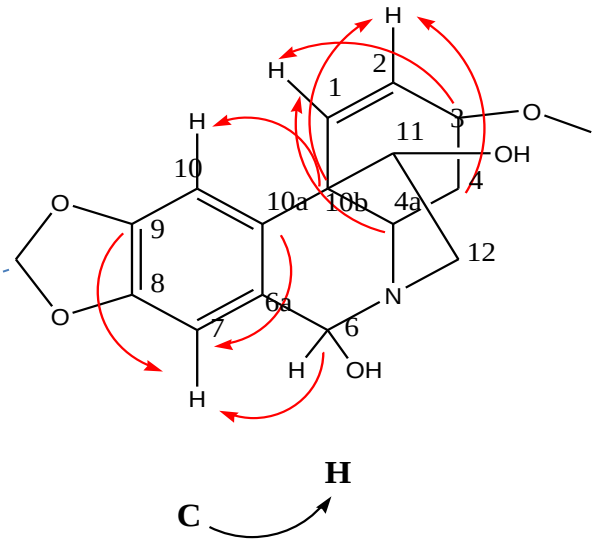


Figure 4: Important HMBC correlations of BCA-1 (6-Hydroxycrinamine)

4.2.1.2. BCA-2

BCA-2 was isolated as a yellow colored amorphous substance. The R_f value of the compound was calculated to be 0.46 using the solvent system butanol: acetic acid: water (4:1:5). The purity of the compounds was confirmed using TLC ethyl acetate: methanol: water (60:25:25) and butanol: acetic acid: water (4:1:3).

BCA-2 gave brown colored precipitate upon treatment with Dragendorff's reagent which indicated that the compound is an alkaloid. The positive TOF MS data of BCA-2 showed a pseudomolecular ion peak at *m/z* 288.1176 $[M-H]^+$ (exact calculated molecular mass = 288.123584 u), indicating a molecular formula of $C_{16}H_{17}NO_4$.

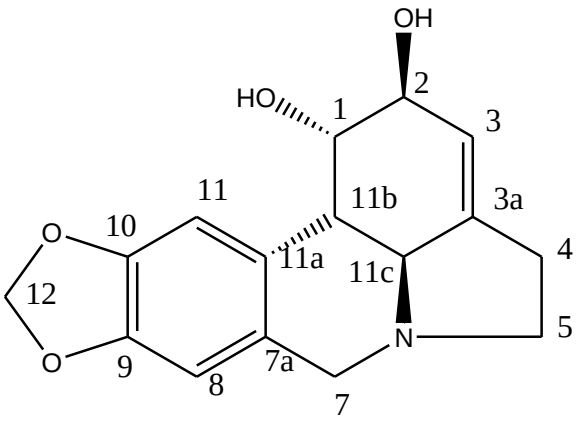
The ^1H NMR spectral data of BCA-2 revealed the presence of 2- aromatic proton signals assigned to H-8 (δ 6.66, 1H, *s*), and H-11(δ6.90, 1H, *s*).Two protons attached to hydroxylated carbon were also observed and assigned as H-1 (δ4.48, 1H, *s*) and H-2 (δ4.15, 1H, *t*). A singlet at δ5.93 was detected due to the presence of a methylene group flanked between two oxygens. The chemical shifts are presented in table 5.

Table 3: ^1H NMR and ^{13}C NMR chemical shifts for compound BCA-2

NO.	**^1H NMR chemical shift (δ, ppm)**		**^{13}C NMR chemical shift (δ, ppm)**	
	BCA-2	Fahim *et al.*, 2009	BCA-2	Fahim *et al.*, 2009
1	4.48s	4.24s	70.56	70.65
2	4.15t	3.94s	70.74	72.16
3	5.56s	5.33 s	117.76	118.93
3a			142.31	142.14
4	2.34m	2.38m	29.36	28.57
5	2.01s	2.15ddd, H-5α 3.15t-like, H-5β	56.42	53.76
7	2.90d H-7α 3.56d H-7β	3.26d (H-7α) 3.96d (H-7β)	53.30	57.18
7a			128.99	130.2
8	6.66s	6.64s	108.6	105.52

9			146.77	146.10
10			146.29	145.66
11	6.90s	6.77s		107.49
11a			128.36	130.02
11b	2.46d	2.46d	39.96	40.61
11c	2.72d	2.55d	61.03	61.29
-OCH2O-	5.93s	5.91-5.92 (-2s)	100.89	101.03

The ^{13}C-NMR and DEPT-135 spectral data indicated the presence of 16 carbon atoms, which were identified as two aromatics (107.3 and105.1), three attached to nitrogen (δ56.42, 53.30 and 61.03), two hydroxylated (70.74 and 70.56) and one methylene carbon flanked between two oxygen (100.89) atoms. From the above spectroscopic data and comparison with those reported in the literature the structure of the compound BCA 2 was suggested to be lycorine as shown in the figure 11.



lycorine

Figure 5: Structural formulae of lycorine

Lycorine (BCA-2) is the first member of the Amaryllidaceae alkaloids isolated in 1877 (Ghosal *et al.*, 1988). It is a widely distributed alkaloid isolated from the genus *Crinum* and the family Amaryllidaceae (Nair *et al.*, 1998).

4.3. Antiproliferative activity

C. abyscinicum is used for the treatment of tumor in Ethiopian traditional medicine therefore it was evaluated against two cancer cell lines namely, human ovarian carcinoma cell line (A2780) and human peripheral lymphoblast (MV4-11) in bioassay guided fractionation approach.

As indicated in Table 4, the crude extract was found to be active against the two cancer cells lines. The extract exerted its effect in a dose dependent manner as shown in Figure 12.The crude extract displayed better effect against MV4-11than A2780. Then two compounds were isolated and tested against the two cell lines.

The isolated compounds were also found to be active against the two cell lines. As stated in the Table 4 both BCA-1 and BCA-2 (lycorine) exhibited better activity than the crude extract. This could be due to increased concentration at the cancer cells. BCA-1 showed activity against MV4-11 with GI_{50} value of 5.323 ± 0.455 while the crude showed with GI_{50} value of 8.289 ± 0.331µg/ml. In addition to the crude extract, both compounds showed better activity against A2780 than MV4-11. However, the extract and the compound isolated showed lower activity than the positive control as indicated in Table 4.

Table 4: GI_{50} of the crude extract and compounds isolated against MV4-11 and A2780 cell lines

Sample Name	GI_{50}* (µg/ml ± SD)	
	MV4-11	A2780
BCA-Crude	8.289 ± 0.331	20.77 ± 0.354
BCA-1 (6-hydroxycrinamine)	5.323 ± 0.455	2.925 ± 0.769
BCA-2 (lycorine)	3.372 ± 0.267	2.849 ± 0.139
Palbociclib	0.057 ± 0.002	0.032 ± 0.008
	*72h Rasazurin Assay	*72h MTT Assay

In vitro anticancer activities of lycorine from previous studies against ovarian carcinoma cell lines (SK-OV-3) revealed significant activity with IC_{50} value of 3.0 ± 0.3µM (Wang *et al.,* 2014). Although the cell lines are different, the result found in this study is comparable to the above study (2.849 ± 0.139 µM).

As reported in other researches lycorine has also displayed multiple inhibitory properties towards various cancer cell lines, including lymphoma, carcinoma, multiple myeloma, melanoma, leukemia, lung cancer, esophageal cancer and human Hs683 anaplastic oligodendroglioma cell lines (Wang *et al.*, 2014). The antiproliferative effect of lycorine was proposed to be due to induction of apoptosis (Li *et al.*, 2004). This is achieved through down regulation of antiapoptotic Bcl-2 family proteins and up regulation of Bax proteins (Li *et al.*, 2004).

The cytotoxic effect of 6-hydroxycrinamine has also been reported against human pancreatic (PANC1) and prostate (DU145) cancer cells and the result indicated that, 6-hydroxycrinamine, was active with an IC_{50} value of 22.7 µM against PANC1 and with IC_{50}: 9.3µM against DU145 cells (Arai *et al.*, 2015).

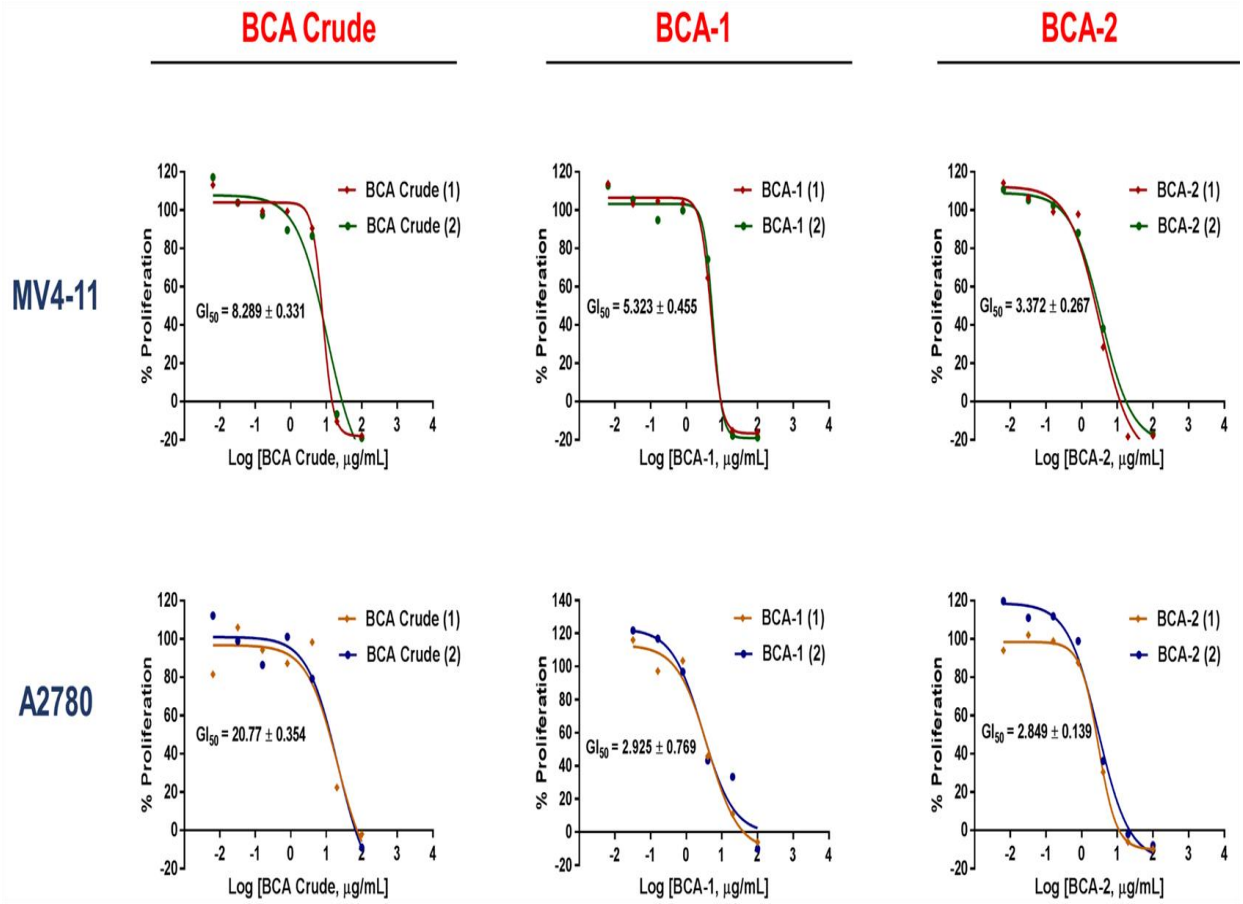


Figure 6: Antiproliferative activity of crude extract of *C. abyscinicum*, 6-hydroxycrinamine (BCA-1) and lycorine (BCA-2)

4.3.2. Cell Cycle Arrest

As previously mentioned in this study 6-hydroxycrinamine and lycorine showed higher antiproliferative effect against A2780 cells as compared to MV4-11 cells. Thus the compounds were evaluated for their effect on the phases of cell cycle.

Cell cycle arrest in a given phase is indicated when cell numbers in that phase increase above control levels. In effect, 6-hydroxycrinamine and lycorine resulted in accumulation of A2780 cells in the G2/M phase of cell cycle in a concentration dependent manner (Figure 13). By comparison 6-hydroxycrinamine showed better effect than lycorine. 6-hydroxycrinamine resulted in increase of cell count from 12.46% to 16.62% (3µmg/ml) and 17.78% (30µmg/ml) while lycorine slightly increased the cell count to 12.46% to 12.56% (3µmg/ml) and 13.52% (30µmg/ml). Cell cycle arrest at the G2/M transition was also reported in other study against ovarian cancer Hey1B particularly by lycorine (He *et al.,* 2011).

The cell cycle arrest on G2/M was observed at lower concentration for both compounds while increasing the concentration of the compounds resulted in S-phase arrest. Both 6-hydroxycrinamine and lycorine caused increment in cell count at S-phase from 14.54% to 16.37% and from 14.54% to 20.42%, respectively, at 30µg/ml. This is consistent with results stated by He *et al.,* (2011) on cisplatin cell cycle effect against same cells, A2780 cells. The study reported that A2780 cells exposed to cisplatin accumulated in G2/M at 1.0 μM at 2.0 μM drug concentration. Concomitant decreases in S- and G1-phase populations were observed, but higher concentrations increased relative distribution of cells in S-phase. These events in S- and G2/Mphase were associated with checkpoint kinase (Chk1) and Checkpoint kinase (Chk2) activation and resultant phosphorylation and proteosomal degradation of Cell division cycle 25 A (Cdc25A).

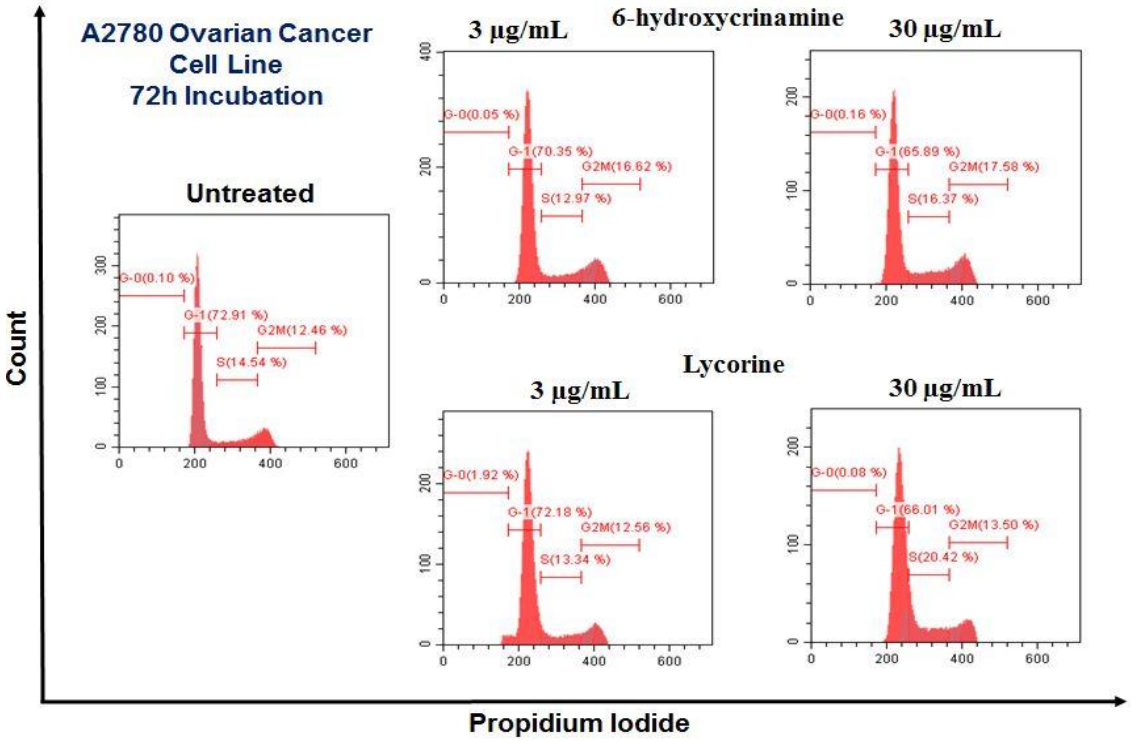


Figure 13: Cell cycle analysis of lycorine and 6-hydroxycrinamine**.**

4.3.3. Apoptosis

In this study, the effect of BCA-1 and BCA-2 on the proliferation of A2780 cells was evaluated. The result showed (Figure 14), BCA-1 and BCA-2 do not induce apoptosis. It was reported that that apoptosis induction is not the primary mechanism responsible for antiproliferative activity of lycorine in some solid cancers (Dasari *et al.*, 2014). This suggests that other mechanisms could be involved in the antiproliferative effect of the compounds. Inhibition of both cell proliferation and cell migration of apoptosis-resistant cancer cell line including Hey1B ovarian cancer cells are proposed to be due to induction of cytostatic effects through an increasing rigidity of actin cytoskeleton (Theys *et al.*, 2009; Dasari *et al.*, 2014).

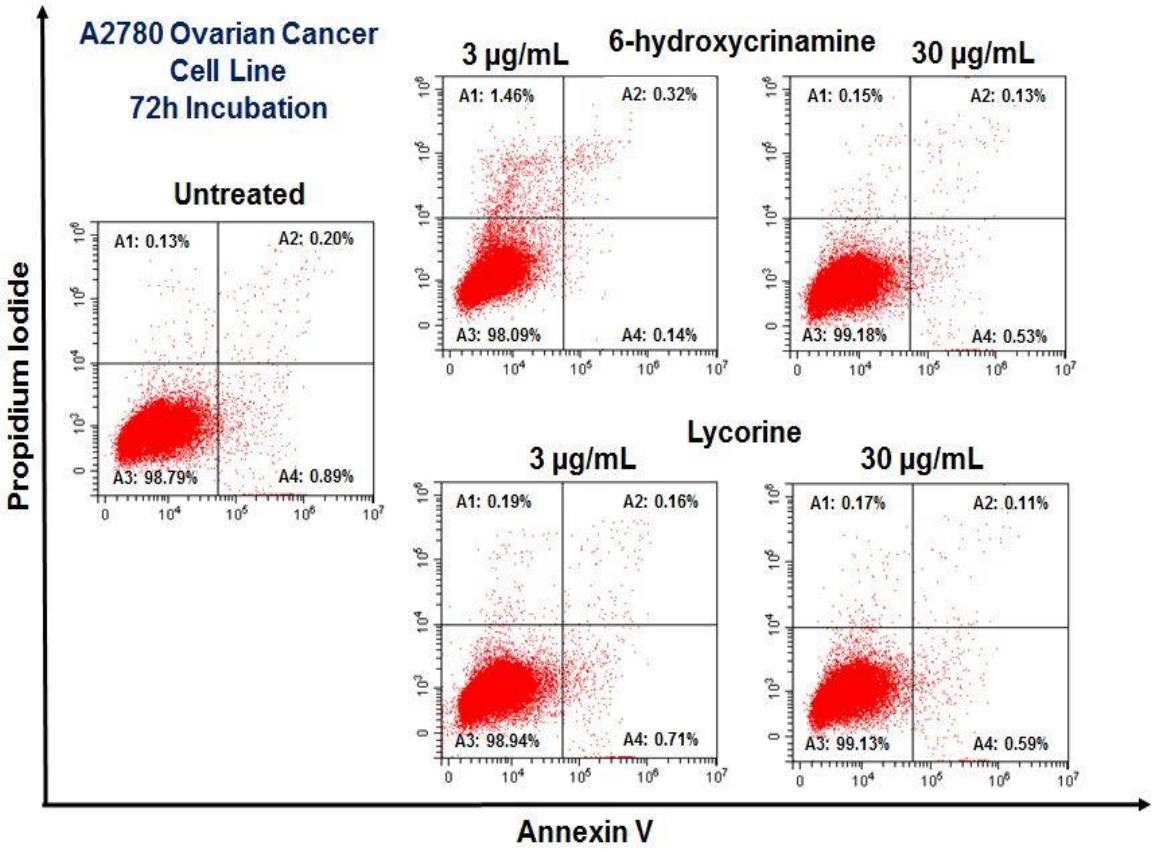


Figure 14: Effects of 6-hydroxycrinamine and lycorine on the induction of apoptosis. A1: Necrotic cells, A2: cell in late apoptosis, A3: Viable cells and A4: cells at early apoptosis. The data are representative of two independent experiments**.**

5. Conclusion

In summary, The antiproliferative effect of the crude extract of *C. abyscinicum* was determined against cancer cell lines, human ovarian carcinoma cell line (A2780) and human peripheral lymphoblast (MV4-11) using MTT and resazurin assays. The crude extract was found to be active. Then two bioactive alkaloids were isolated from the crude extract namely, 6-hydroxycrinamine and lycorine. Thus, the traditional use of the plant may be justified by the presence antiproliferative alkaloids.

6. Recommendations

The following possible recommendation forwarded based on the current findings;

- Other minor compounds that are found in this plant has to be isolated and
- The activities of these minor compounds should be investigated for their antiproliferative activities as well as other biological activities

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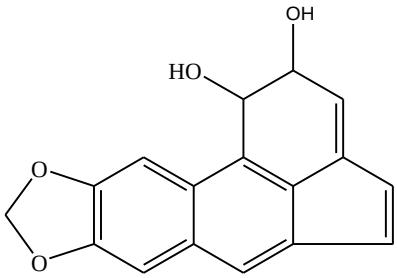
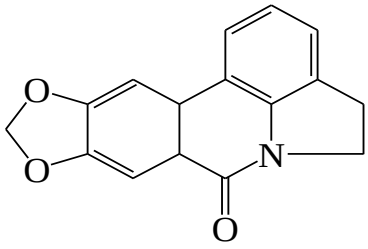
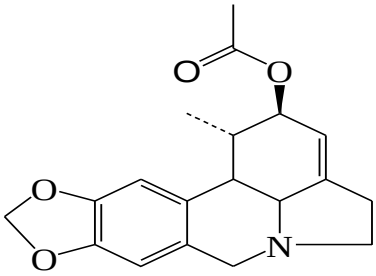
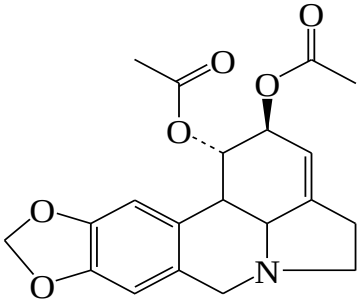
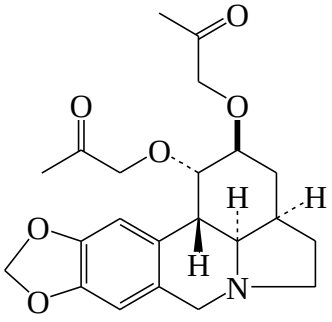
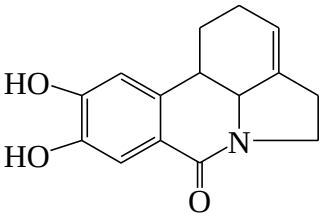
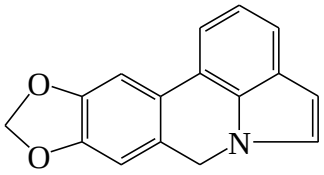
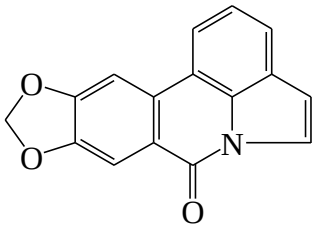
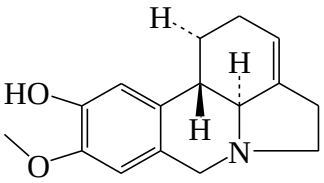
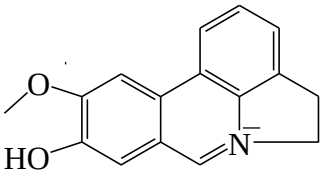
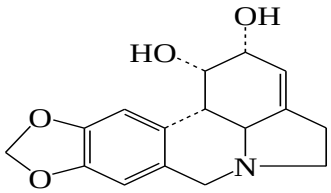
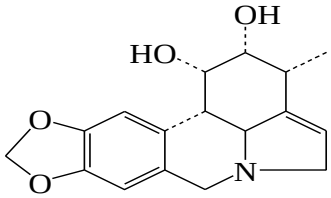
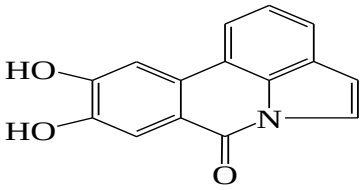
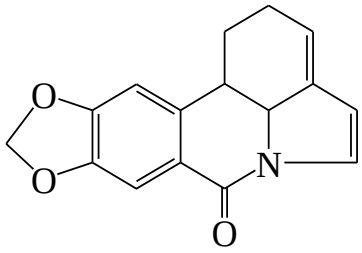
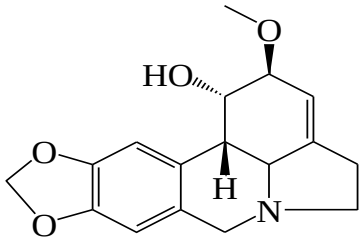
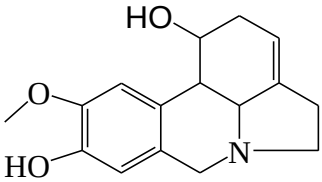
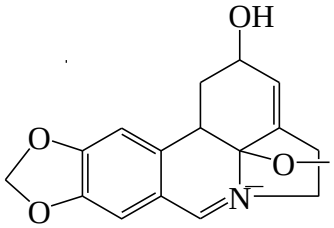
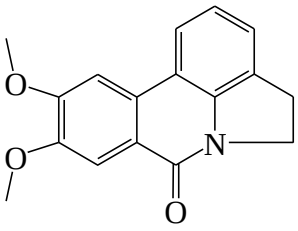
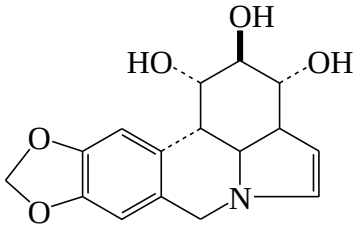
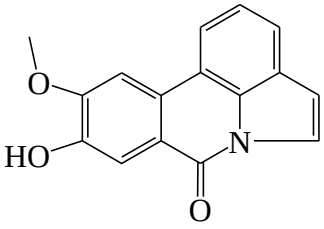
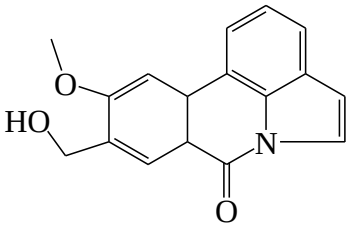
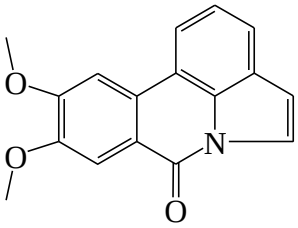
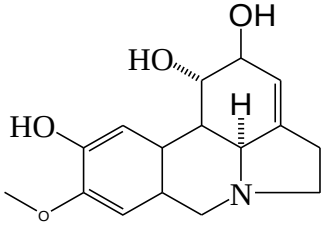
Appendices

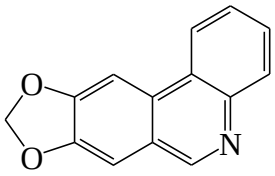
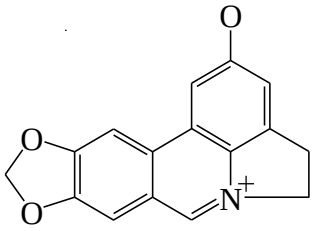
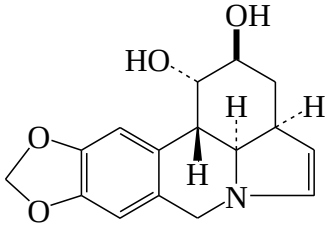
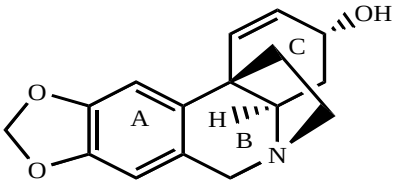
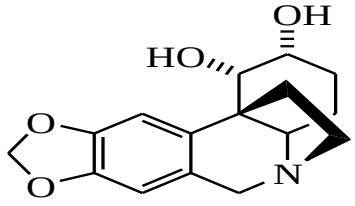
Table 5: Compounds reported from genus *Crinum*

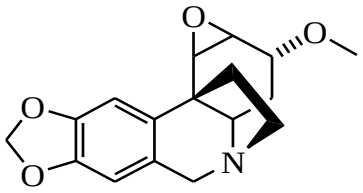
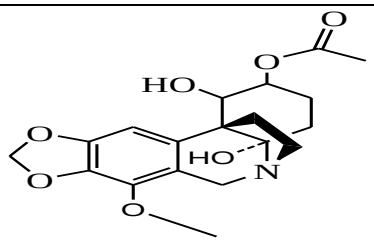
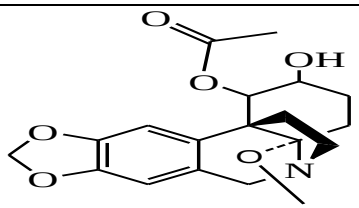
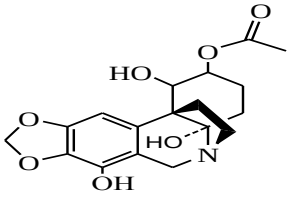
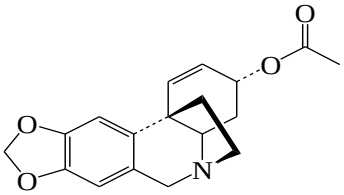
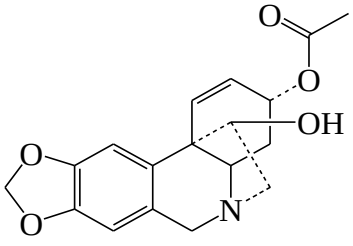
Alkaloids					
Lycorine type					
C. No.	Name	Structural formulae	Plant source	Plant material	References
1	Lycorine		*C. augustum* Rox	Bulb	Ali *et al.*, 1981
					Endo *et al.*, 2019
2	Anhydrolycorin-7-one		*C. pratense* Herb	Bulb	Ghosal *et al.*, 1981
3	2-O-acetyllycorine		*C. powellii* album	Bulb	Nino *et al.*, 2007
			C. amabile	Bulb	Tallini *et al.*, 2019
4	Diacetyllycorine		*C. latifolium* L	Bulb	Kobayashi *et al.*, 1984

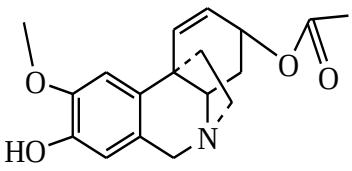
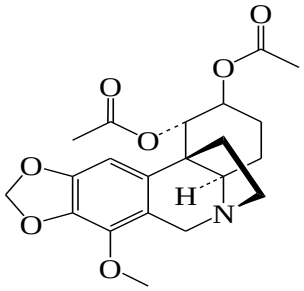
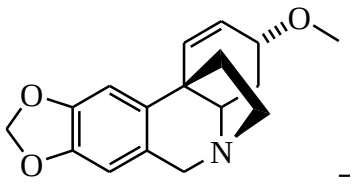
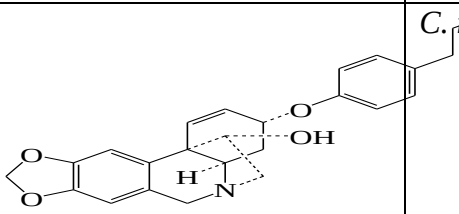
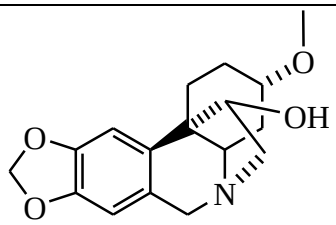
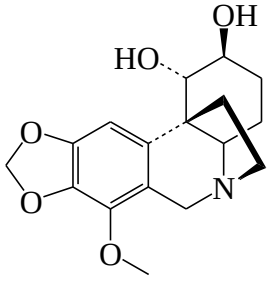
5	1,2-*O*-Acetylzephyranthine		*C. kirkii* Baker	Bulbs	Machocho *et al.*, 2004
6	Criasiaticidine		*C. asiaticum* L	Bulbs	Min *et al.*, 2001
7	4,5-Dehydroanhydrolycorine		*C. latifolium* L	Fruit and stem	Ghosal *et al.*, 1989
8	11,12-dehydroanhydrolycorine		*C. erubescens* Kunth	Fresh Leaves	Guerrieri *et al.*, 2016
9	9-O-demethylpluviine		*C. stuhlmannii* Baker	Bulb	Machocho *et al.*, 1998
10	8-O-demethylvasconine		*C. kirkii* Baker	Bulbs	Bastida *et al.*, 1995

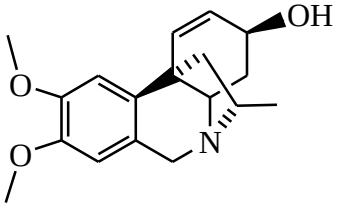
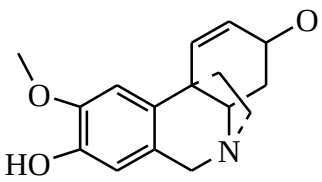
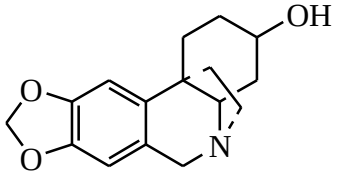
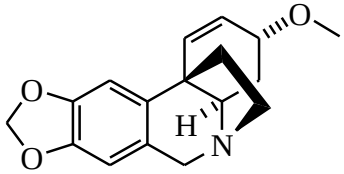
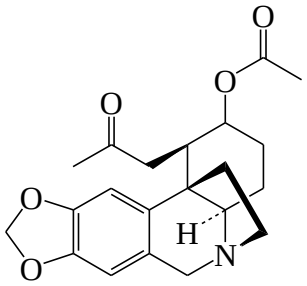
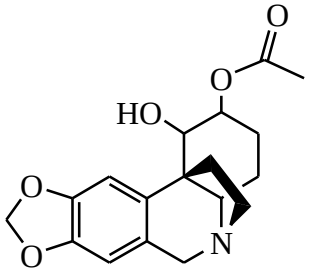
11	2-Epilycorine		*C. latifolium* L	Fruit and stem	Ghosal *et al.*, 1989
12	2-Epipancrassidine				
13	Hippacine		*C. bulbispermum* (Burm.f.) Milne-Redh. & Schweick	Bulbs	Ramadan *et al.*, 2000
14	Hippadine		*Crinum erubescence*	Areal Parts	Presley *et al.*, 2016
15	Hippamine		*C. bulbispermum* (Burm.f.) Milne-Redh. &Schweick	Ornamental part	Aboul-ela*et al.*, 2004
17	Kirkine		*C. kirkii* Baker	Bulb	(Bastida *et al.*, 1995)
18	Mooreine		*C. moorei* Hook.f.	Whole plant	Elgorashi *et al.*, 2001

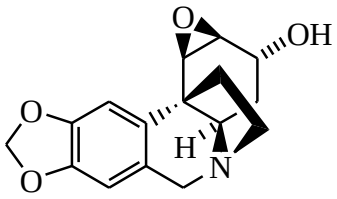
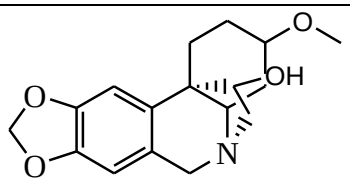
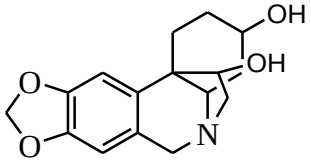
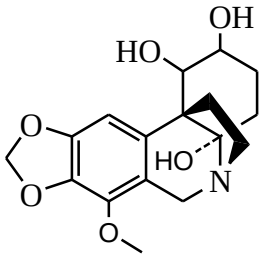
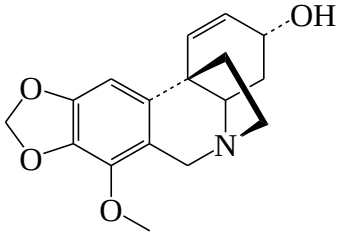
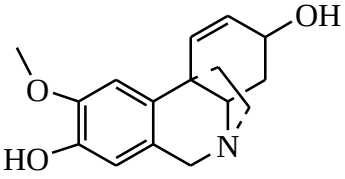
19	Oxoassoanine		*C. latifolium* L	Leaves	Tram *et al.*, 2002
20	Pancrassidine		*C. latifolium* L	Fruit and stem	Ghosal *et al.*, 1989
21	Pratorimine		*C. asiaticum* L	Fruits	Ghosal *et al.*, 1983
22	Pratorinine		*C. pretense* Herb	Bulbs	Ghosal *et al.*, 1981
			C. asiaticum L*var. japonicum* Baker		Min *et al.*, 2001
23	Pratosine		*C. americunum* L	Bulbs	Ali *et al.*, 1986
24	Pseudolycorine		*C. jagus* (J. Thomps.) Dandy	Bulb	Onyiriuka and Jackson, 1978

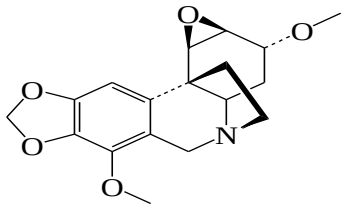
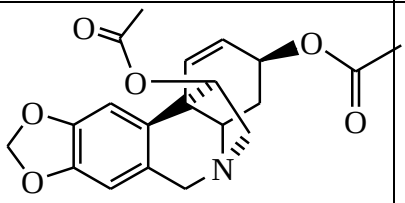
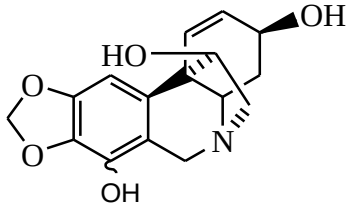
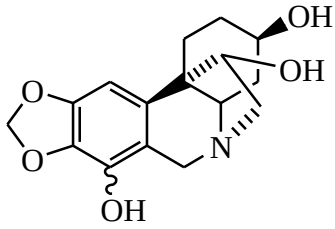
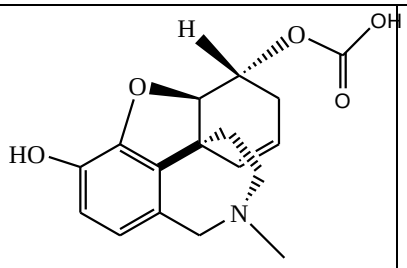
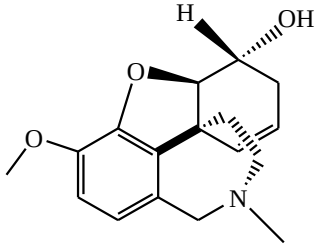
25	Trisphaeridine		*C. erubescens* Kunth	Fresh leaves	Guerrieri *et al.*, 2016
			C. americunum L		Ali *et al.*, 1986
26	Ungeremine		*C. augustum* Rex	Bulbs	Endo *et al.*, 2019
27	Zephyranthine		*C. kirkii* Baker	Bulbs	Machocho *et al.*, 2004
Crinine type					
C. No.	Alkaloid's names	Structural formulae	Plant part	Plant material	References
28	Crinine	 **28**	*C. amabile*, *C. asiaticum* L *var. sinicum* Baker	Bulb	Likhitwitayawuid *et al.*, 1993; Chen *et al.*, 2011
29	Ambelline		*C. amabile* Donn	Bulb	Tallini *et al.*, 2019

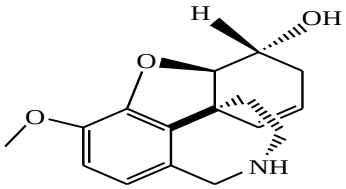
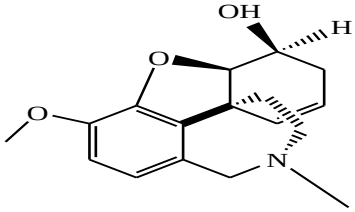
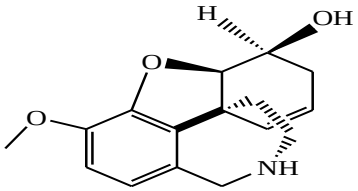
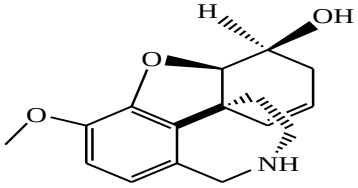
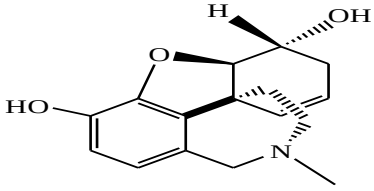
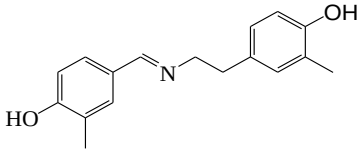
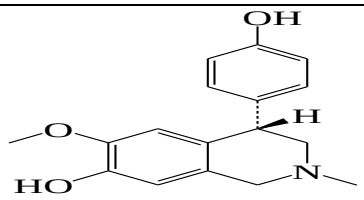
30	Augustine		*C. amabile* Donn	Bulb	Likhitwitayawuid *et al.,* 1993; Pham *et al.,* 1998
			C. augustum Rox.	Whole plant	Ali *et al.,* 1981
31	1-O-Acetylbulbisine		*C. asiaticum* L*var. Sinicum* Baker	Leaves	Chen *et al.,* 2011
32	2-O-Acetylbulbisine		*C. asiaticum* L*var. Sinicum* Baker	Leaves	Chen *et al.,* 2011
33	2-O-Acetylcrinamabine		*C. asiaticum* L *var. Sinicum* Baker	Leaves	Chen *et al.,* 2011
34	O-acetylcrinine		*C. macowanii* Baker	Fresh bulb	Kobayashi *et al.,* 1984)
35	3-O-acetylhymane		*C. latifolium* L	Fresh bulb	Kobayashi *et al.,* 1984

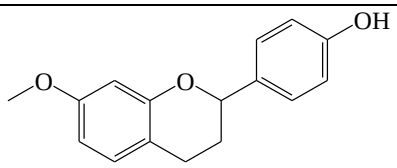
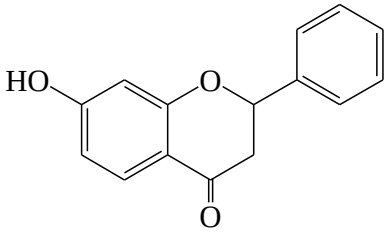
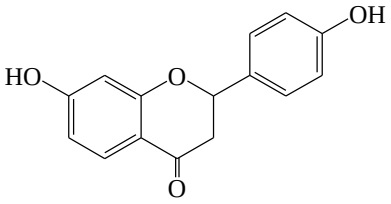
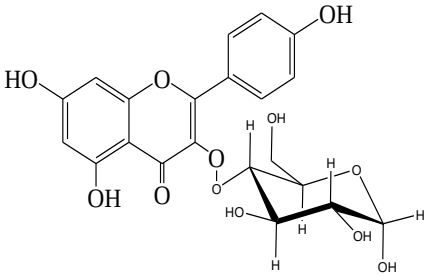
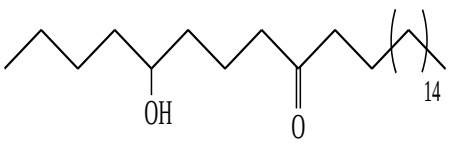
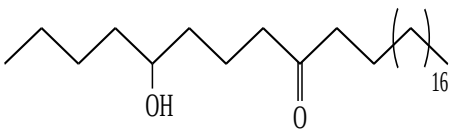
36	3-O-Acetyl-8-O-demethylmaritidine		*C. asiaticum* L*var. Sinicum* Baker	Leaves	Chen *et al.,* 2011
37	Bowdensine		*C.erubescens*Kunth	Fresh leaves	Guerrieri *et al.,* 2016
38	Buphanisine		*C. amabile* Donn	Bulb	Tallini *et al.,* 2019
39	3-[4'-(8'-aminoethyl)phenoxy] bulbispermine		*C. moorei*	Whole plant (non-flowering)	Elgorashi *et al.,* 2001
40	Crinamine			Bulb	Endo *et al.,* 2019
41	Deacetylbuwendisine		*C. macowanii* Baker	Fresh bulb	Kobayashi *et al.,* 1984

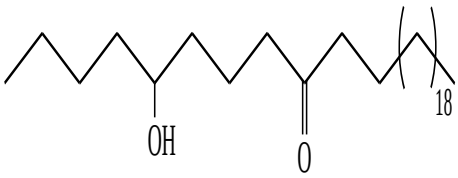
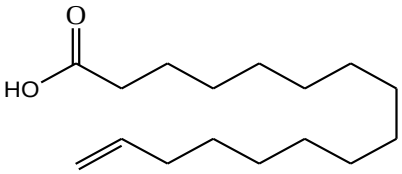
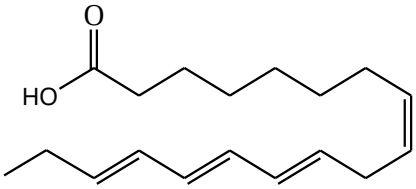
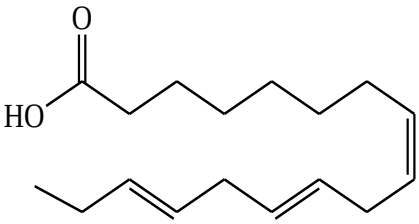
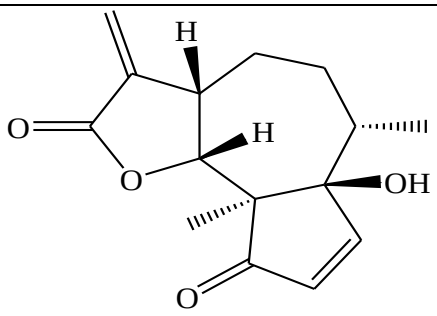
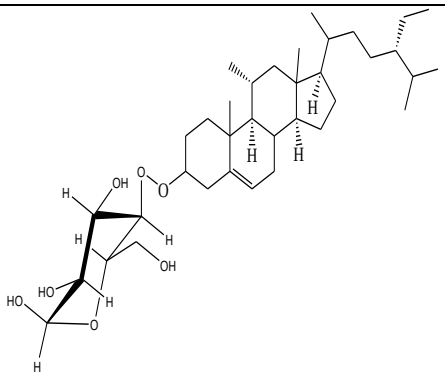
42	Delagoenine		*C. delagoense*I. Verd.	Bulb	Nair *et al.,* 1998
43	8-O-Demethyloxomaritidine		*C. asiaticum* L*var. Sinicum* Baker	Leave	Chen *et al.,* 2011
44	Dihydrovittatine		*C. asiaticum* L*var. Sinicum* Baker	Leave	
45	Epibuphanisine		*Crinum asiaticum var. japonicum*	Rhizome and fruits	Endo *et al.,* 2019
46	1-epidemethoxybowdensine		*C. erubescensb* Kunth	Leave	(Guerrieri *et al.,* 2016)
47	1-Epijosephinine		*C. asiaticum* L*var. Sinicum* Baker	Leave	Chen *et al.,* 2011

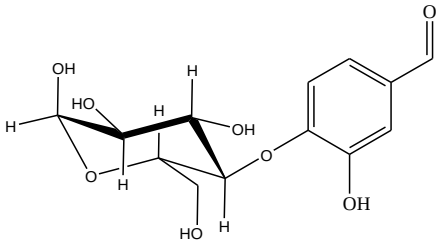
48	Flexinine		*C. amabile* Donn	Bulb	Pham *et al.*, 1998
49	Heamanthamine		*C. ornatum* (Aiton) Herb.	Bulb	Olyede *et al.*, 2010;
50	Hymane		*C. ornatum* (Aiton) Herb.	Bulb	Olyede *et al.*, 2010
			Crinum asiaticum var. japonicum	Rhizome and fruits	Endo *et al.*, 2019
51	7-Methoxycrinamabine		*C. asiaticum* L*var. Sinicum* Baker	Leave	Chen *et al.*, 2011
52	Powelline		*C. latifolium and C. bulbispermum*	Fresh bulb	Kobayashi *et al.*, 1984
53	Siculine		*C. asiaticum* L *var. Sinicum* Baker	Leave	Chen *et al.*, 2011

54	Undulatine		*C. macowanii* Baker	Whole plant	Kobayashi *et al.*, 1984
55	Yemenines A		*C. yemense* Dammann	Bulb	Abdel-Halim *et al.*, 2004
56	Yemenines B		*Crinum asiaticum var. japonicum*	Rhizome and fruits	Endo *et al.*, 2019
57	Yemenines C		*C. yemense* Dammann	Bulb	Abdel-Halim *et al.*, 2004
Galanthamine type					
56	3-*O*-Acetylsanguinine (acetyl-O demethylgalantham ine)		*C. kirkii.*	Bulb	Machocho *et al.*, 2004
57	Epigalanthamine		*C. asiaticum* L	Bulb	Kobashi *et al* 1976

58	Epinorgalanthamine		*C. asiaticum* L	Bulb	Kim *et al.,* 2006
59	Galanthamine		*C. asiaticum* L	Bulb	Kobashi *et al* 1976
60	N-demethylgalanthamine		*C. asiaticum* L	Bulb	Kobashi *et al* 1976
61	Norgalanthamine		*C. asiaticum* L		Kim *et al.,* 2006
62	Sanguinine (O-demethylgalanthamine)		*C. kirkii.*	Bulb	Machocho *et al.,* 2004
Minor alkaloids					
63	Isocraugsodine		*C. asiaticum*	Fruits	Ghosal *et al.,* 1988
64	Cherylline		*C. latifolium* L	Bulb	Kobayashi *et al.,* 1984

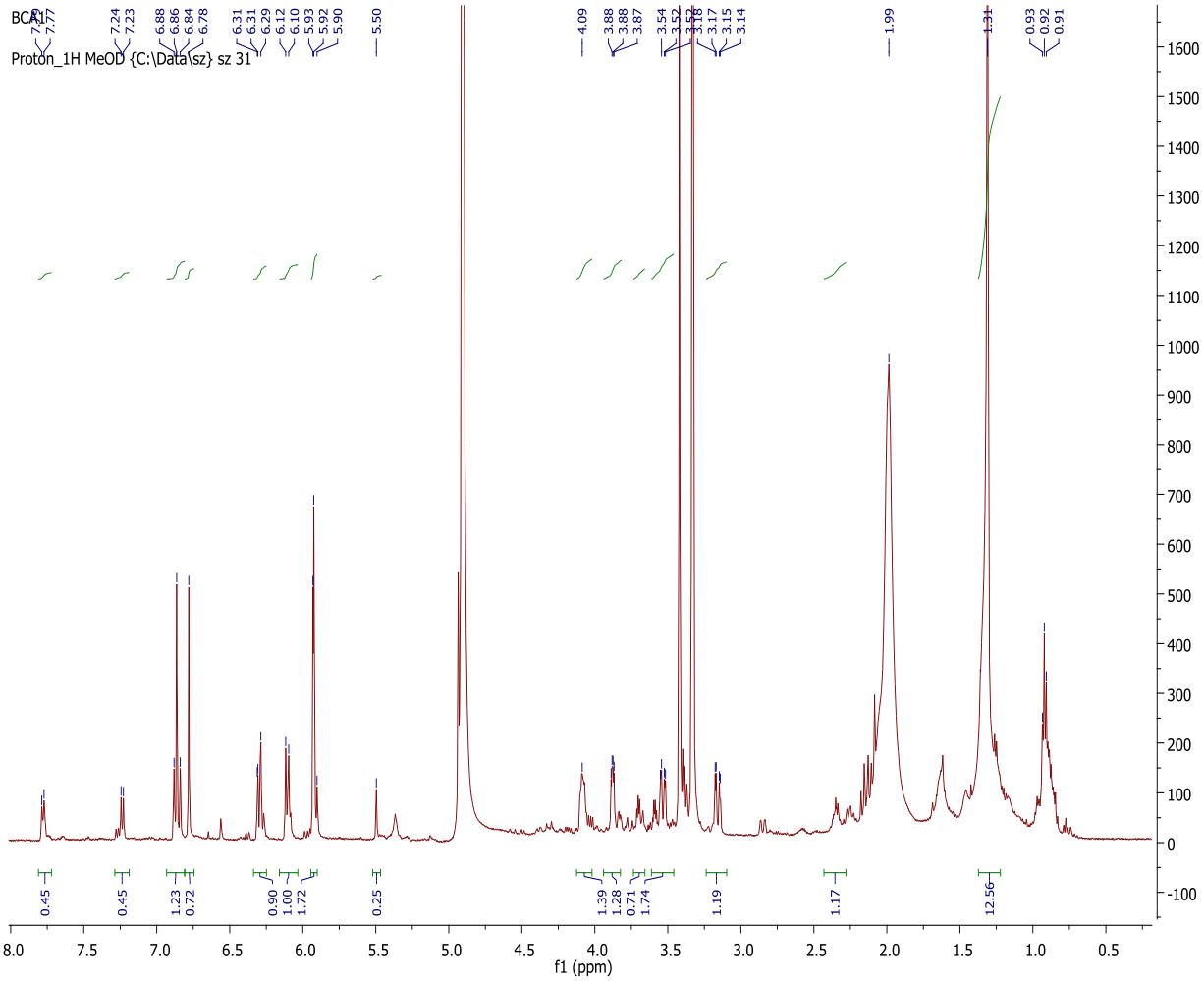
Flavonoids and isoflavonoids					
65	4-hydroxy-7-methoxyflavan		*C. asiaticum* var. *japonicum*	Bulb	Min *et al.*, 2001
66	7-hydroxyflavanone		*C. asiaticum* var. *sinicum*	Bulb	Sun *et al.*, 2009
67	Apigenine		*C. asiaticum*	Flowers	Kale *et al.*, 2012
68	Astrangalin		*C. woodrowii* Baker	Bulb	Jagtap, 2015
Fatty acids and polyketides					
69	S-hydroxyhexacosan-9-one		*C. auugus rum*	Bulb	El Hafiz *et al.*,1991
70	5-hydroxyoctacosan-9-one		*C. auugus rum*	Bulb	

71	S-hydroxytriacontan-9-one		*C. auugus rum*	Bulb	
72	n-hexadecanoic acid		*C. asiaticu m*	Leave	Indradevi *et al.*, 2012
73	9, 12, 15-octadecatrienoic acid		*C. asiaticu m*	Leave	
74	9,12-octadecadienoic acid		*C. asiaticu m*	Leave	
Terpenes					
	Asparthenin		*C. ensifoliu m*	Bulb	Khoi *et al.*, 2011
	Sitosterol-α D-glucopyranoside		*C. purpura scens*	Leaves	Nkanwen *et al.*, 2009

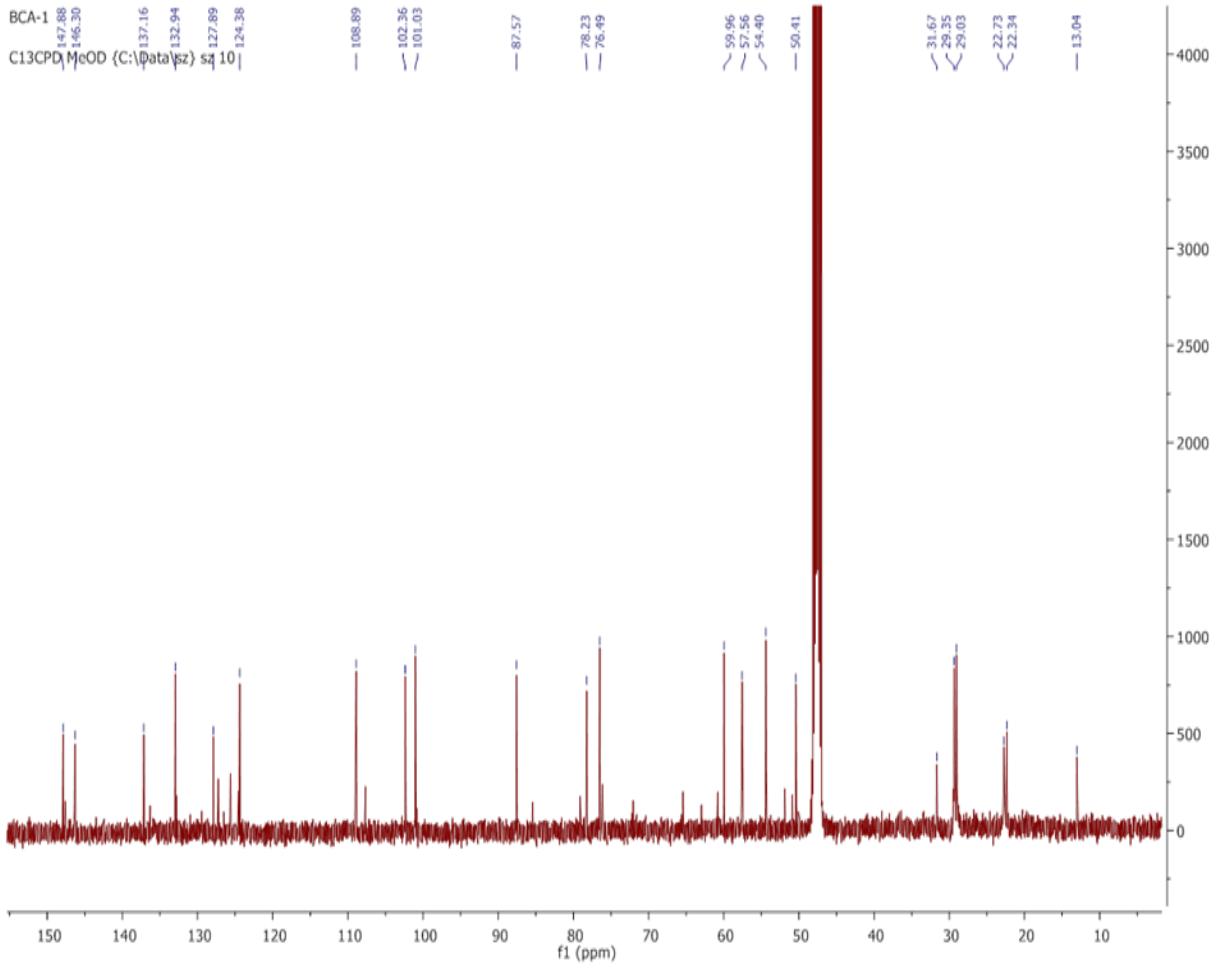
Glycoside					
	Amabiloside (3-hydroxy-4-0-β-D-glucopyranosylbenzaldehyde)		*C. amabile*	Bulbs	Likhitwitayawuid *et al.,* 1993

Appendix I: ^{1}HNMR, ^{13}C NMR, and DEPT NMR of BCA-1

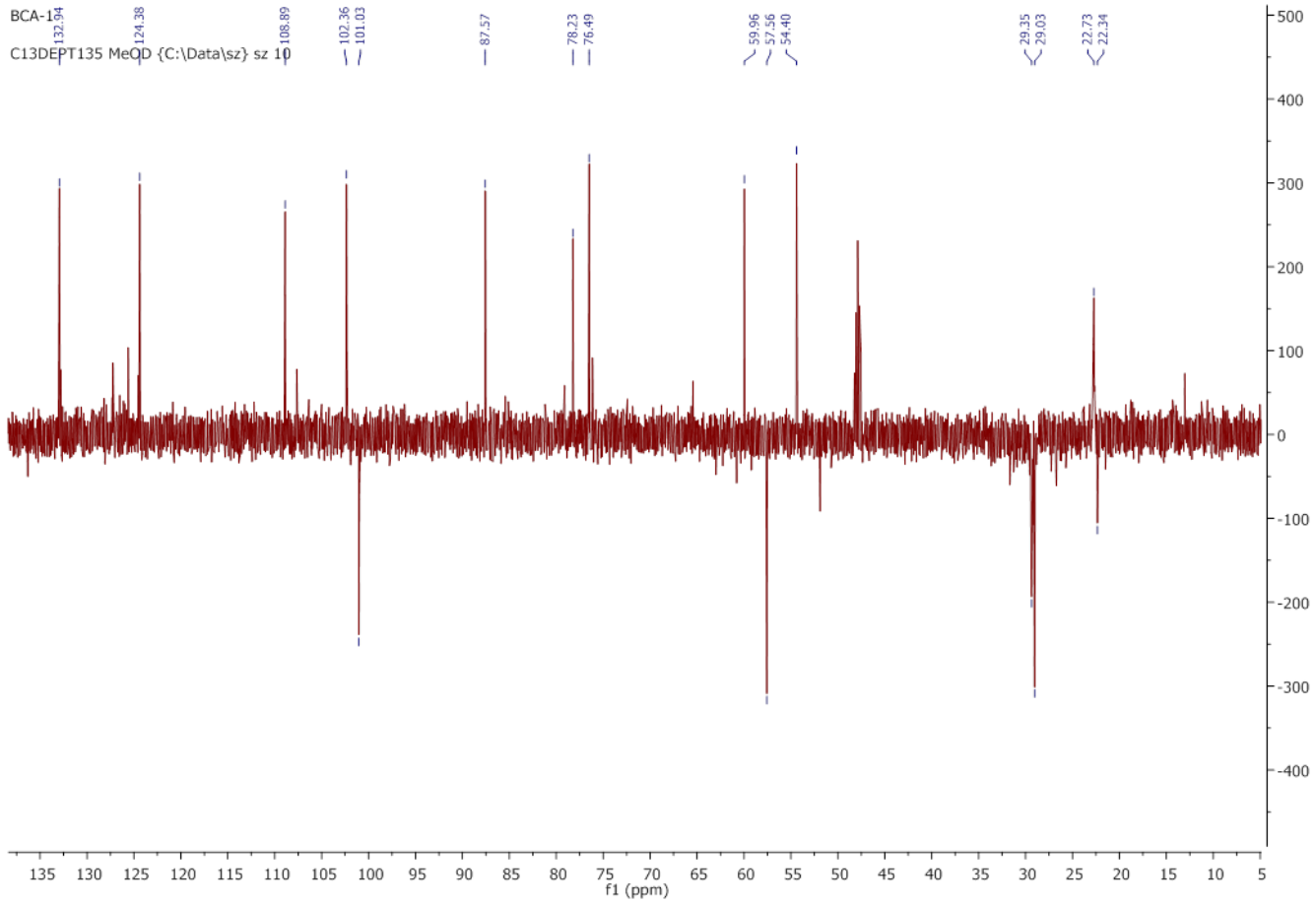
^{1}HNMR of BCA-1



^{13}C NMR of BCA-1

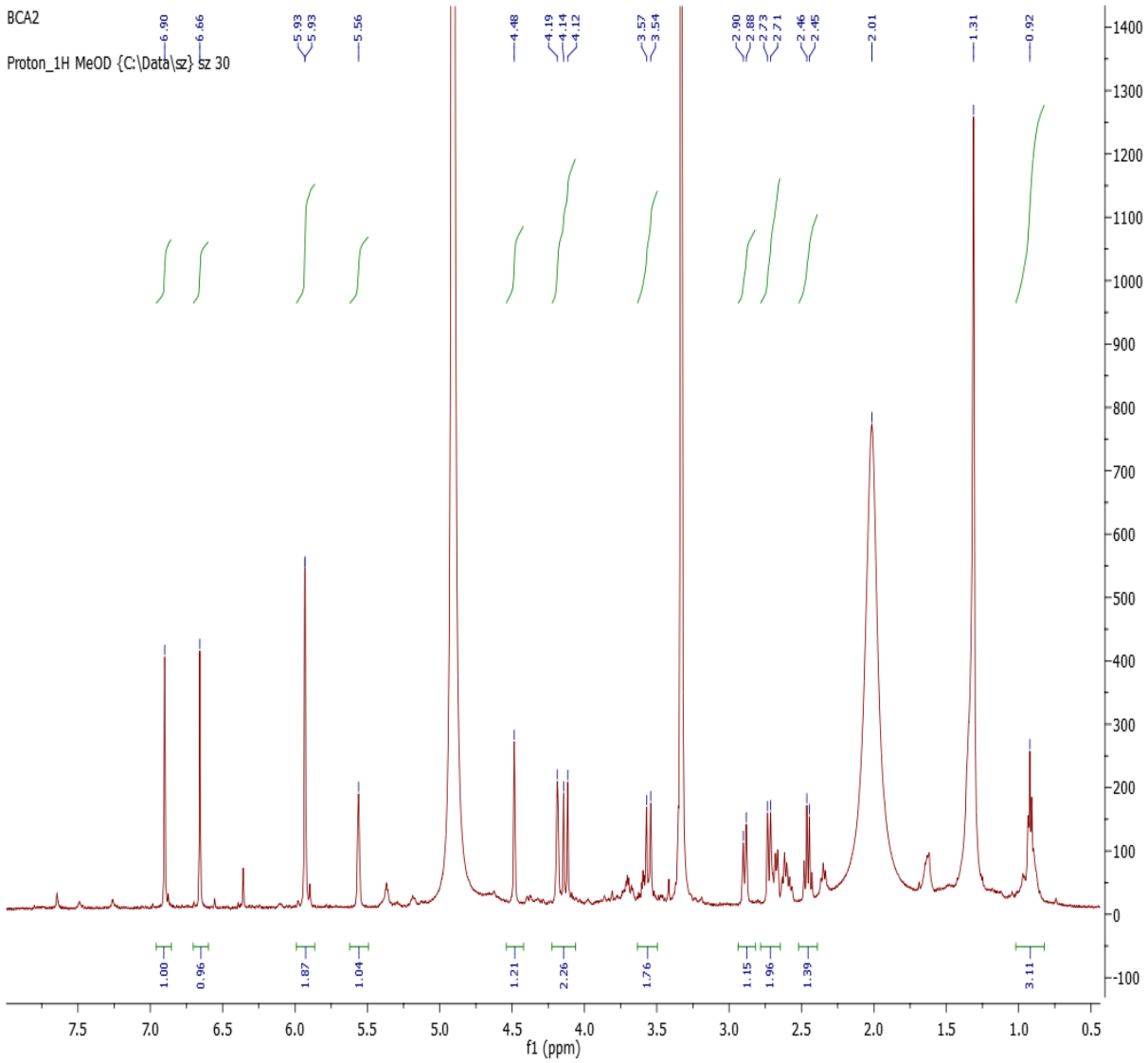


DEPT NMR of BCA-1

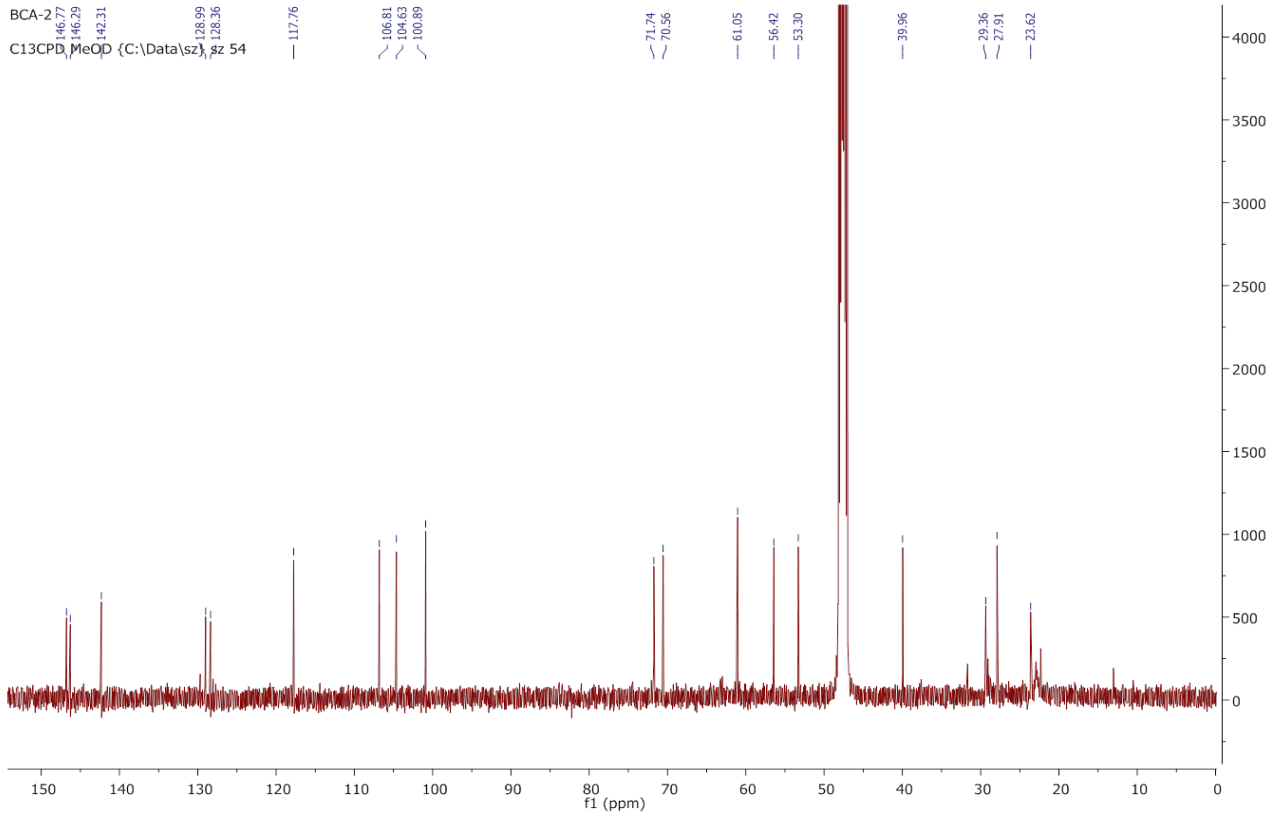


Appendice II: ^{1}HNMR, ^{13}CNMR and DEPT spectrum of BCA-2

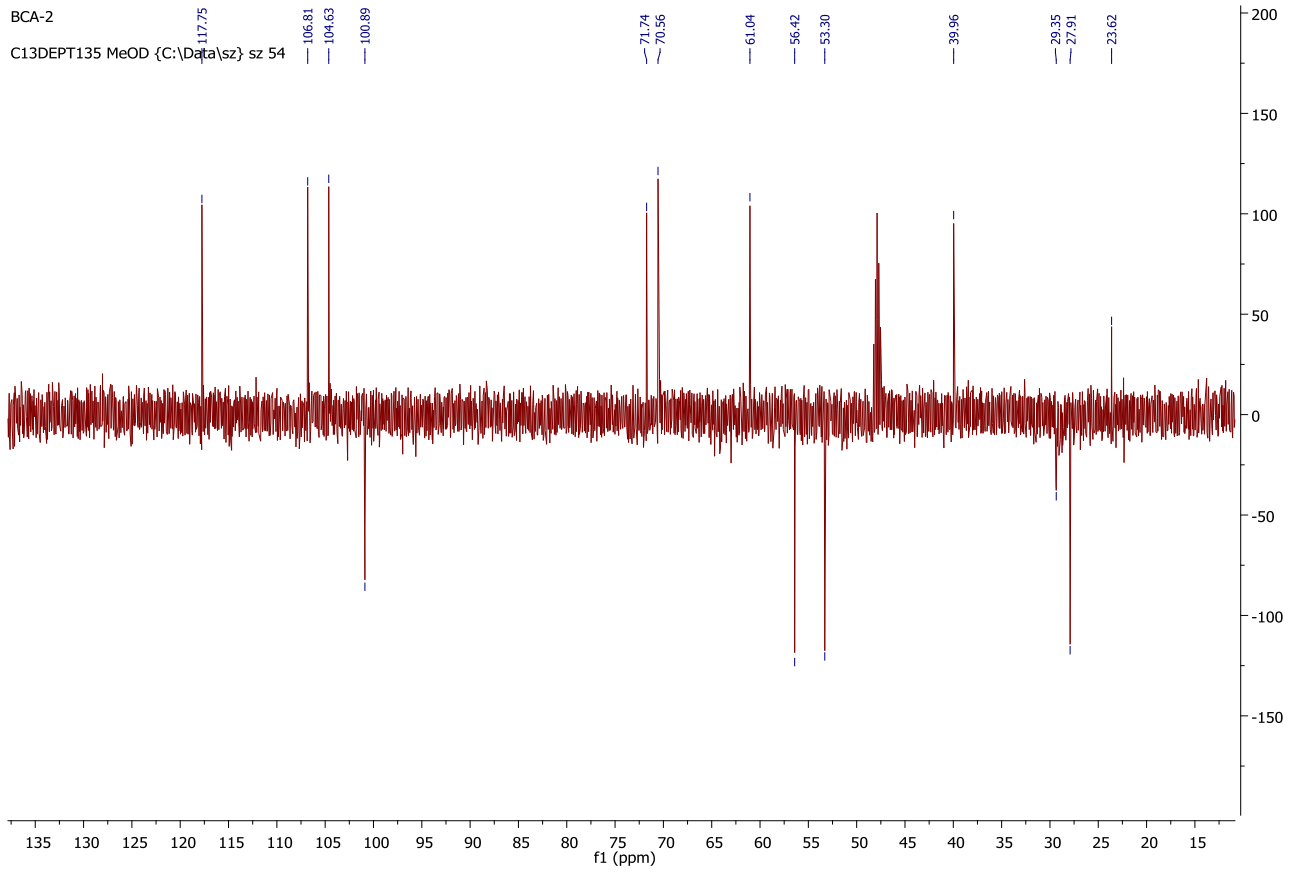
^{1}HNMR of BCA-2



^{13}C NMR of BCA-2

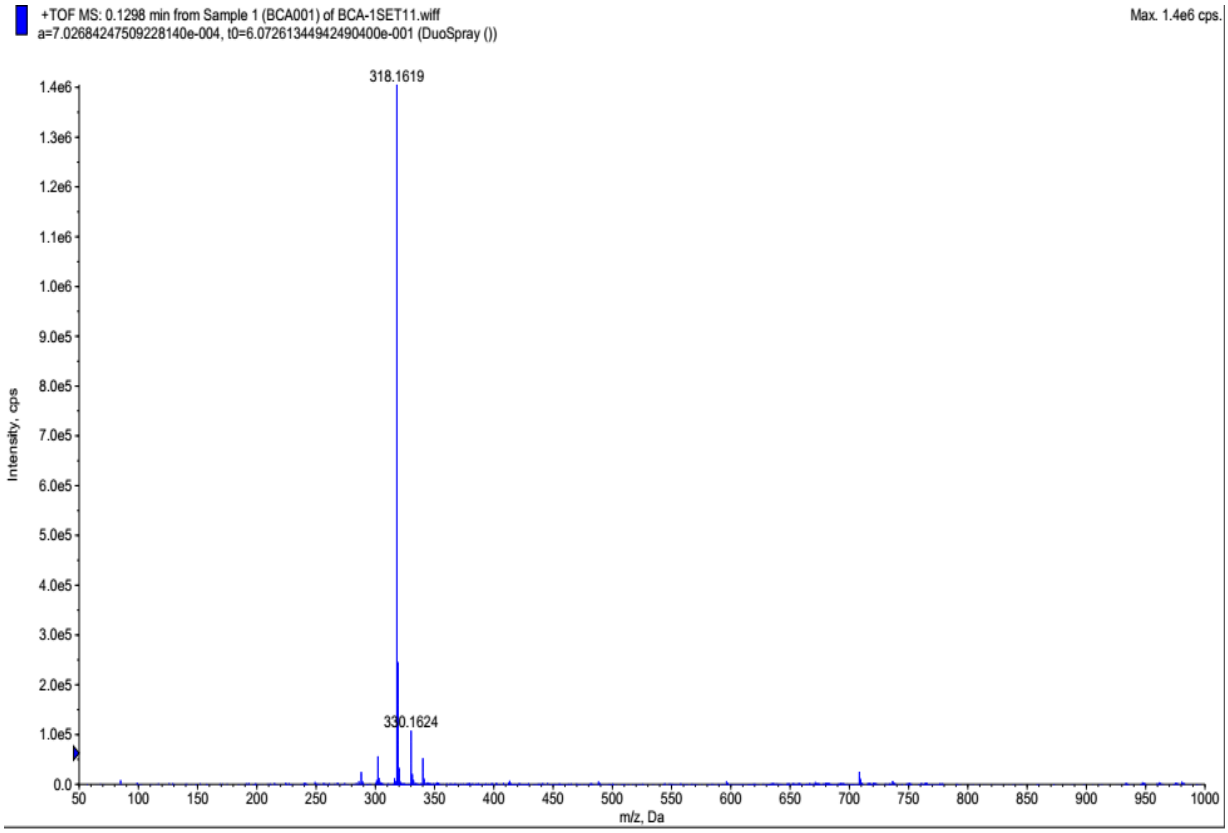


DEPT NMR of BCA-2

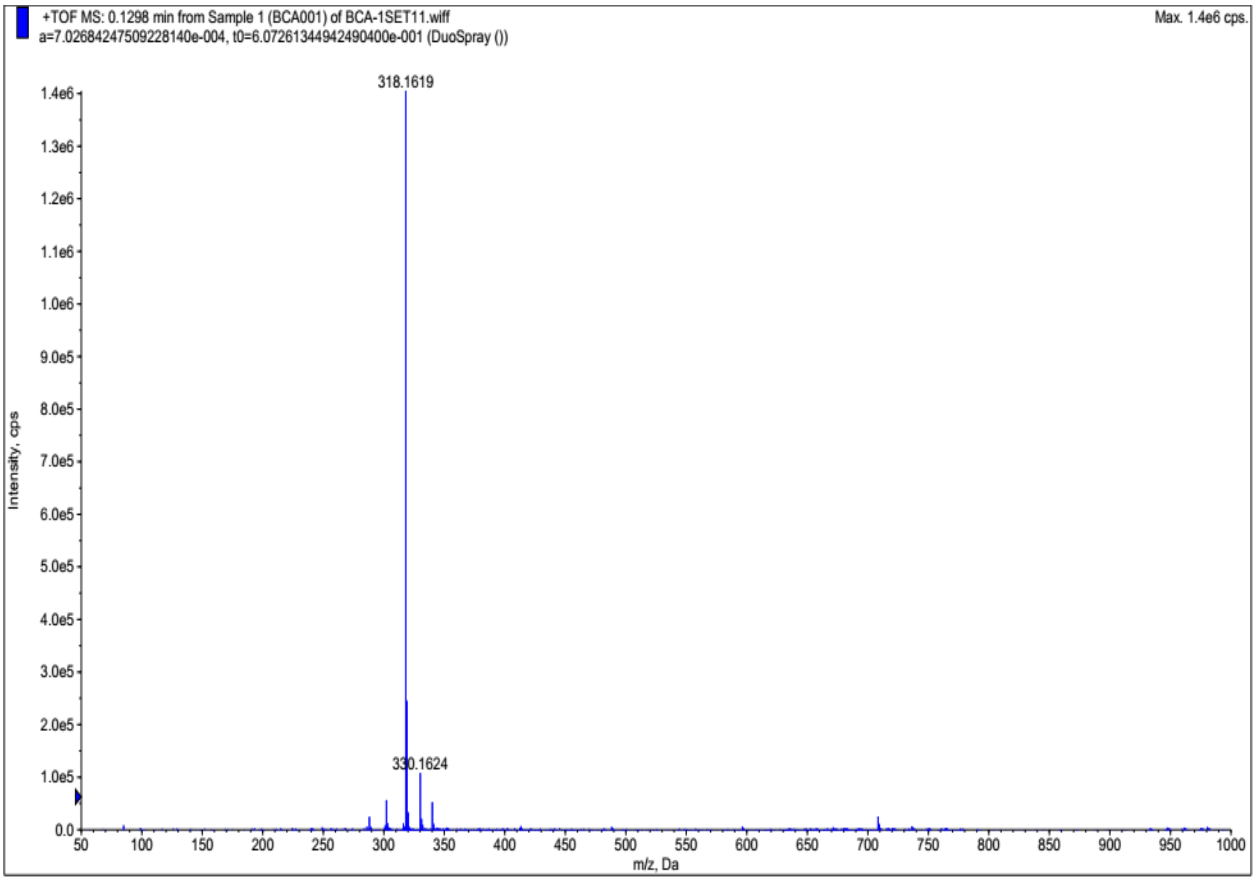


Appendices III: TOF-MS of BCA-1 and BCA-2

TOF-MS BCA 1



TOF-MS BCA 2


+TOF MS: 0.1298 min from Sample 1 (BCA001) of BCA-1SET11.wiff
a=7.02684247509228140e-004, t0=6.07261344942490400e-001 (DuoSpray ())
Max. 1.4e6 cps.
318.1619
330.1624
Intensity, cps
m/z, Da