Department of Pharmaceutical Chemistry and Pharmacognosy

Antiproliferative Activity of the Leaf Latex of *Aloe secundiflora* Engl. and its Major Constituents

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This is to certify that the thesis prepared by Yabibal Berie, entitled: **"Antiproliferative Activity of the Leaf Latex of *Aloe secundiflora* Engl. and its Major Constituents"** and submitted in partial fulfillment of the requirements for the Degree of Master of Science in Medicinal Chemistry complies with the regulations of the university and meets the accepted standards with respect to originality and quality.

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List of abbreviations

ABTS	2,20-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid
^{13}C-NMR	Carbon-13 nuclear magnetic resonance
^{1}H-NMR	Proton nuclear magnetic resonance
AGEs	Advanced glycation end products
DCM	Dichloromethane
DEPT	Distortionless enhancement by polarization transfer
DMSO	Dimethyl sulfoxide
DPPH	2,2-diphenyl-1-picrylhydrazyl
ESI	Electron spray ionizer
GLOBOCAN	Global cancer incidence, mortality and prevalence
MGIR	Microbial growth inhibition rate
MTT	3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazoliumbromide
PBS	Phosphate-buffered saline
PTLC	Preparative thin layer chromatography
RPMI	Roswell Park media institute
TLC	Thin layer chromatography
TOF-HRMS	Time of flight-high resolution mass spectrometry
UV	Ultraviolet light

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Abstract

Antiproliferative Activity of the Leaf Latex of *Aloe secundiflora* Engl. and its Major Constituents

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Addis Ababa University, 2023

Globally, an estimated 19.3 million new cancer cases and almost 10.0 million cancer deaths occurred in 2020. The various methods of treatment of cancer that can be employed alone or in combination, depending upon different factors, are chemotherapy, radiotherapy and surgical interventions. The currently available chemotherapeutic agents have severe toxicities and face multiple drug resistance. This calls for researchers to look for safe and effective chemotherapeutic agents from natural products. Thus, the antiproliferative efficacy of *Aloe secundiflora*, a plant traditionally used to treat cancer, was evaluated. The antiproliferative activity of *A. secundiflora* leaf latex was investigated against four cancer cell lines, breast (MCF-7), lung (A427), urinary bladder (RT-4), and cervical (SiSo) using MTT assay. The latex possesses better antiproliferative activity with an IC_{50} value of 15.3 μg/mL against the A427 cell line over the other three cell lines. Phytochemical analysis of the latex using preparative thin layer chromatography resulted in the isolation of two glycosides and the structure of these two glycosides were characterized as Aloenin B and Aloeresin D using spectroscopic methods, and by comparison with reported spectroscopic data. Aloenin B and Aloeresin D displayed antiproliferative activity against A-427 cell lines with IC_{50} value of 3.2 and 5.5 μg/mL, respectively. The activity observed for the latex as well as isolated compounds of *A. secundiflora* support the traditional use of the plant against cancer.

Keywords: Cancer, *Aloe secundiflora,* aloenin B, aloeresin D, cancer cell line, MTT assay, antiproliferative

1. Introduction

1.1. Overview of cancer

Cancer is a group of diseases in which the cells that produce it lose control over cell division (Ramdasi, 2016). One of the defining characteristics of cancer cells is abnormal cell signaling pathways that cause them to divide rapidly. The initiation and progression of cancer depends on both external and internal factors. External stimuli can be different chemicals, viruses, radiation, and physical agents like asbestos, etc., while internal factors include hormone issues, inherited traits, and mutation (Hanahan and Weinberg, 2000, Anand *et al*., 2008). At molecular level, normal cells undergo a chain of events to develop in to cancer including epigenetic modifications, abnormal DNA repair machinery behavior, loss of cell cycle regulation, genetic mutations, and modifications to signal transduction pathways (Bertram, 2000). Accumulation of multiple mutations is required for the progression of cancer from *in situ* dysplasia to malignant tumor (Ma *et al*., 2007).

Metastasis is the most common cause of death from cancer accounting for more than 90% of cancer-associated mortality and it impairs the function of key organs in the later stages of cancer growth (Poste and Fidler, 1980). Metastasis occurs when genetically unstable cancer cells adapt to a tissue microenvironment that is distant from the primary tumor. This process involves both the selection of traits that are advantageous to cancer cells and the concomitant recruitment of traits in the tumor stroma that accommodate invasion by metastatic cells (Gupta and Massagué, 2006). Cancer cells can acquire both endocrine and paracrine signaling pathways in the advance stages, which aids in their ability to first attack nearby tissues before spreading to distant places and ultimately metastasizing

the entire cancer. However, autocrine signaling is the peculiar mechanism acquired by cancer cells, giving them the ability to divide autonomously. Autocrine signaling in cancer cells not only allows them to synthesize their own growth factors but also cells get stimulated by bearing cell surface receptors for these growth factors (Ramdasi, 2016).

The toll of cancer is increasing worldwide. An estimated 19.3 million new cancer cases and almost 10.0 million cancer deaths occurred in 2020 in the world. Female breast cancer has surpassed lung cancer as the most commonly diagnosed cancer, with an estimated 2.3 million new cases (11.7%), followed by lung (11.4%), colorectal (10.0 %), prostate (7.3%), and stomach cancers(5.6%) (Sung *et al*., 2021). Due to a number of complex reasons, such as an aging and burgeoning population, accelerated socioeconomic development, and changes in the prevalence of related risk factors, the burden of cancer has increased over time in both developed and developing nations (Bray *et al*., 2018).

Cancer is a public health issue in Africa with 1.1 million new cancer cases and 711,429 deaths in 2020 (Sharma *et al*., 2020).

In Ethiopia, cancer is becoming a frequently diagnosed disease with a considerably high mortality rate. The Global Cancer Incidence, Mortality and Prevalence (GLOBOCAN) 2020 report estimates that there were 77,352 new cases and over 51,865 fatalities in the nation, with the majority of cases (50 598; 65.4%) affecting women (Wondimagegnehu *et al*., 2022).

Treatment modalities of cancer: there are various methods of treatment that can be employed alone or in combination, depending upon different factors such as: specific pathological and molecular characteristics of the cancer, its location, the severity of the disease, and the patient's health status. Treatment usually includes surgery, radiotherapy, and/chemotherapy (Sudhakar, 2009). There are

also other systemic therapy alternatives including biological molecules, targeted medicines, and immune-mediated therapies (Antonia et al., 2006, Wu *et al*., 2006). Chemotherapy is the major treatment modality for advanced stages of cancer. The currently available class of anticancer drugs are alkylating agents, antimetabolites, antitumor antibiotics, topoisomerase inhibitors, and tubulin-binding drugs. In general, this class of compounds face two major problems; drug resistance and severe toxicities on normal cells (Fernando and Jones, 2015).

1.2. The role of medicinal plants in cancer treatment

Natural remedies have been used for generations to cure a variety of illnesses, including cancer (Song *et al*., 2014). They are highly sought after for primary healthcare in underdeveloped countries due to their low cost, higher cultural acceptance, better compatibility with human bodies, and minimal side effects (Nema *et al*., 2013).

Over 60% of anticancer drugs that are clinically utilized come in some form from natural sources (Cragg *et al*., 2005). The major source has, by far, been the kingdom of plants (Raina *et al*., 2014). From 1981 to 2006, around 75% of anticancer drugs that were marketed were either derived from medical plants or were chemicals that originated from plants. These medicines includes paclitaxel(*Taxus chinensis* (Pilger) Rehd (bark)), (Vinca alkaloids-vindesine, vincristine, vinorelbine, and vinblastine- *Vinca rosea* L. (*Catharanthus roseus* (L.) G. Don) (leaf)), etoposide (rhizome of the wild mandrake (Podophyllum peltatum), and irinotecan **(**Camptotheca acuminata (bark and stem)) (Siddiqui *et al*., 2022).

Ethiopians and other inhabitants of emerging nations in Asia and Africa continue to place a high value on medicinal plants (Agidew, 2022). Medicinal plants not only supplement or replace the insufficiently available modern medical treatments, but also improve the local population's health

(Russell-Smith *et al*., 2006). Plants like *Ricinus communis L*., *Jatropha curcas* L., *Kniphofia foliosa Hochst**., *Gloriosa superba* and *Prunus africana* (Hook.f.) show notorious anticancer activities and the class of secondary metabolites responsible for these plants notorious anticancer effects are terpenoids, phenolic compounds, alkaloids, and Steroids (Tesfaye *et al*., 2020). These plants' ability to produce secondary metabolites, which include flavonoids, alkaloids, and other complex and diverse sorts of molecules, makes them therapeutic (Twaij, B.M. and Hasan, M.N., 2022).

Different ethnobotanical studies have reported the traditional use of Ethiopian medicinal plants such as *Aloe spp* for prevention and treatment of various ailments including cancer. Those medicinal plants that are used for the treatment of cancer are summarized in table Table 1.

Table 1: Ethiopian medicinal plants that are reported for treatment of cancer

No.	Scientific name of plant	Vernacular name	Family	Part used	Method of preparation	Type of cancer treated	References
1	*Acacia seyal* Delile	Girar Wacho (A)	Fabaceae	L	Chewing, and spitting	Not specified	(Regassa, 2013, Reta, 2013)
2	*Aerva javanica* Burm.f.	Tobiaw (A)	Amaranthaceae	R	The root is powdered and combined with the bat's blood and given orally before breakfast in the morning	Not specified	(Teklehaymanot, 2009)
3	*Aloe pirottae* A. Berger.	Daar (S), Iret (A), Argeesa (O)	Asphodelaceae	L	A spoonful of the plant's pulp or leaf is mixed with honey and consumed twice a day	Not specified	(Tuasha *et al*., 2018)
4	*Aloe rivae* Baker	Argeesa (O)	Asphodelaceae	E	The exudate is taken orally	Not specified	(Demissew and Brandham, 1992)
5	*Aloe secundiflora* Engl.	Iret (A), Argeesa (O)	Asphodelaceae	R	Fresh roots are crashed, and the sap is applied on the affected area	Not specified	(Esubalew *et al*., 2017, Anywar *et al*., 2022)
6	*Bersama abyssinica* Fresen.	Afageshign (A)	Melianthaceae	B	The bark is pounded, boiled, and a small amount of the preparation is drunk	Breast	(Ayele, 2018, Tesfaye *et al*., 2020)
7	*Brucea antidysenterica* J.F.Mill.	Abalo (A)	Simaroubaceae	B	Dry bark is ground, macerated and drunk before meal.	Not specified	(Wabe *et al*., 2011)
8	*Clematis simensis* Fresen.	Yeazo hareg (A)	Ranunculaceae	L	Leaf of the plant is macerated and drunk	Breast	(Chekole *at al*., 2015, Kidane *et al*., 2018)
9	*Clerodendrum myricoides* (Hochst.) Vatke	Misirch (A)	Lamiaceae	L	Fresh leaf is pounded, decocted, and drunk with tea or coffee	Blood	(Nigatu *et al*., 2019)
10	*Dovyalis abyssinica* (A. Rich.) Warb.	Koshm (A)	Flacourtiaceae	B	The raw bark is chewed and swallowed	Not specified	(Enyew *et al*., 2014, Tesfaye *et al*., 2021)
11	*Euphorbia schimperiana* Scheele	Abidamo (A)	Euphorbiaceae	R	Fresh roots are pounded, and the sap is applied on the affected area	Skin	(Tesfaye *et al*., 2020)
12	*Foeniculum vulgare* Mill.	Ensilal (A)	Apiaceae	R	The roots of the plant are mixed with other herbs and taken orally	Lung	(Tesfaye *et al*., 2020)
13	*Jatropha curcas* L.	-	Euphorbiaceae	Se	Tumours are treated with a paste made from the plant seed powder mixed with honey	Not specified	(Agize *et al*., 2022)
14	*Millettia ferruginea* (Hochst.) Bak.	Birbira (A)	Fabaceae	B	The bark is washed, pounded, filtered, and given orally	Not specified	(Tesfaye *et al*., 2021)
15	*Prunus Africana* (Hook.f.) Kalkman	Tiqur Inchet (A)	Rosaceae	B	Powdered bark	Breast & skin	(Kefalew *et al*., 2015)
16	*Ricinus communis* L.	Gulo (A)	Euphorbiaceae	R	Fresh root is chewed and swallowed	Breast	(Regassa, 2013, Tuasha *et al*., 2018)
17	*Sideroxylon oxyacanthum* Baill.	-	Sapotaceae	L	The leaf part, often mixed with leaf of *Zanthoxylum chalybeum* and honey, is macerated and given orally	Not specifed	(Tuasha *et al*., 2018)
18	*Vernonia auriculifera* Hiern.	Gujo (A)	Asteraceae	L	The leaves of the plant in a fresh state are grounded, and the sap is applied to it	Skin	(Ayele, 2018, Tesfaye *et al*., 2020)
19	*Zanthoxylum chalybeum* Engl.	-	Rutaceae	L	The leaf/shoot (often with the shoot of *Olea capensis* and *Clerodendrum myricoides*) is boiled, mixed with honey, and drunk	Not specifed	(Kidane *et al*., 2014, Tekle, 2015)

*A-Amharic, O-Afaan Oromo, S-Somali, L-leaf, R-root, Se-seed, E-exudate, B-bark

1.3. The genus *Aloe*

1.3.1. Distribution and botanical description

The genus *Aloe* is from the family Asphodelaceae and it is a monoecious, perennial species with shallow roots and species of the genus are distinguished by having fleshy and cuticularized leaves usually with spiny margins. *Aloe* species are mostly inhabitants of arid climates, and are widely distributed in Africa, India, and other arid areas. The genus *Aloe* comprises of more than 400 species with the majority being found in South Africa (Diriba and Deresa, 2022). However, they could also be grown in subtropical summer rainfall and winter rainfall regions. In Ethiopia, there are about 46 species of *Aloe* of which about 66% of these *Aloe* species are endemic to the country (Belayneh *et al*., 2020). They are distributed in all floristic regions. The main factors limiting genus distribution are temperature, soil moisture, fire tolerance, and rainfall (Van Jaarsveld, 1989).

1.3.2. Ethnopharmacological use

There are a number of ethnobotanical uses for various *Aloe* species around the globe. The fleshy leaves and roots of most species within the *Aloe* family are the most frequently used parts in many traditional treatments. The majority of known uses of *Aloe* plants are from Asia (India and Nepal), but there are also a few records from other regions of the world including Africa. The most frequently reported uses of *Aloe* species include gastrointestinal problems, liver disease, and for skin problems (Salehi *et al*., 2018).

In Ethiopia different *Aloe* species are used to prevent and treat various illnesses and *Aloe* species ethnobotanical uses are summarized in Table 2.

Table 2: Ethnobotanical uses of *Aloe* species in Ethiopian traditional medicine.

S.Nº	Scientific Name	Use	Part used	Use description	References
1	*Aloe calidophila* Reynolds	Sexually transmitted infections	Leaf	Smoke-bathe the genitals	(Belayneh Desta, 2020)
2	*Aloe citrina* Carter & Brandham	Malaria	Exudate	Taken orally	(Diriba and Deresa, 2022)
3	*Aloe lateritia* Engler	Skin infection	Gel & exudate	Apply on skin	(Amir *et al*., 2019)
4	*Aloe macrocarpa* Todaro	Sexual impotency	Root	Pulverized, mix with fresh butter and use as ointment & smoke-bathe the penis	(Singh *et al*., 2021)
5	*Aloe megalacantha* Baker subs. megalacantha	Eye infection	Exudate	Drop in infected ear	(Nawrot *et al*., 2021)
6	*Aloe pirottae* Berger	Antiparasite	Exudate	Taken orally	(Anywar *et al*., 2021)
7	*Aloe pubescens* Reynolds	Colon cleaner	Exudate	Powder of exudate locally called SIBRI in water solution taken orally	(Feyisa *et al*., 2021)
8	*Aloe rivae* Baker	Snakebite	Exudate	Taken orally	(Kumar *et al*., 2020)
9	*Aloe secundiflora* Engler	Ectoparasite	Leaf & Exudate	Concocted for external use on skin	(Abihudi *et al*., 2019)
10	*Aloe tewoldei* Gilbert & Sebsebe	Crop pest control	Exudate	Powder used in crop storage	(Belayneh Desta, 2020)
11	*Aloe yavellana* Reynolds	Mosquito repellent	Leaf	Smoking around to stifle mosquito	(Oda and Erena, 2017)
12	*Aloe ruspoliana* Baker	Itching skin on goat locally called CHITO	Leaf	Warm & rub the skin while warmer	(Newton, 2004)
13	*Aloe rugosifolia* Gilbert & Sebsebe	Wound healing	Exudate	Apply externally	(Demissew, 1996)
14	*Aloe megalacantha* Baker subs alticola	Diabetics	Exudate	Powder in water solution taken orally	(Belayneh *et al*., 2021)
15	*Aloe mcloughlinii* Chris.	Laxative	Exudate	Collect & drink	(Belayneh *et al*., 2020)

1.3.3. Phytochemistry

The most widely used part of the *Aloe* plant is its leaves, which can be divided into three main sections: (i) the outer, green epidermis, which is primarily made up of structural elements; (ii) the outer pulp region below the epidermis, which is made up of vascular bundles where the bitter latex or sap is derived; and (iii) the inner leaf pulp, which is made up of *Aloe* gel and contains parenchyma cells. It is expected that the various biological activities of leaves are a result of the diverse composition of these leaf parts, which is also likely to have different classes of bioactive constituents (Cock, 2015). These parts are likely to have distinct classes of bioactive compounds; alkaloids, anthraquinones, pre-anthraquinones found in outer green epidermis, while phenolic compounds, including anthraquinones, pre-anthraquinones, anthrones, chromones, coumarins, and flavonoids found in the outer pulp region below the epidermis (Salehi et al., 2018). Besides leaves and roots are also the site of storage for many interesting secondary metabolites such as anthraquinones. Compounds reported from different *Aloes* species are listed in appendix I.

1.3.4. Biological Activities

According to a number of studies, *Aloe* species have shown a wide range of biological activities, including anticancer, antioxidant, antiinflammatory, immunomodulatory, hepatoprotective, anti ulcer, and antidiabetic (Table 3). *Aloe* is also used to treat skin conditions caused by radiation and infection. This plant's widespread use is due to the presence of pharmacologically active chemical constituents that are reported from different *Aloe* species (Hamman, 2008).

Table 3: Summary of biological activities of *Aloe* species

***Aloe* species**	**Activities**	**Model**	**Test dose**	**Result**	**References**
Aloe vera	Anticancer	*In-vitro*	10-200µmol/L	10.45 ± 0.31µg/ml (IC_{50})	(Shalabi *et al*., 2015)
Aloe castellorum	Anticancer	*In-vitro*	5-80 µg/ml	89.09 ± 5.12683	(Ahamed *et al*., 2020)
Aloe pseudorubroviolacea	Anticancer	*In-vitro*	50-80µg/ml	98.26% ± 5.669111	(Ahamed *et al*., 2020)
Aloe barbadensis	Antidiabetic	*In-vitro*	0.031–0.500 mg/ml	Decreased formation of AGEs	(Muñiz-Ramirez *et al*., 2020)
Aloe barbadensis	Anthelmintic	*In-vitro*	50-150 mg/ml	Took less time to cause paralysis and death	(Shelke *et al*., 2020)
Aloe ferox	Anthelmintic	*In-vivo*	200 mg/kg	Had the highest WCR (85%)	(Mwale and Masika, 2015)
Aloe viridiflora	Antimalarial	*In-vitro*	32 to 77µg ml^{-1}	50% of the parasite growth was inhibited	(Van Zyl *et al*., 2002)
Aloe wickensii	Antimalarial	*In-vitro*	0.5-50 µg ml^{-1}	IC_{50} value of 13.46 ± 1.36 µg ml^{-1}	(Van Zyl *et al*., 2002)
Aloe arborescens	Antimicrobial	*In-vitro*	80-600 µg ml^{-1}	31% MGIR	(Kupnik *et al*., 2021)
Aloe barbadensis	Antimicrobial	*In-vitro*	80-600 µg ml^{-1}	52% MGIR	(Kupnik *et al*., 2021)
Aloe schelpei	Antioxidant	DPPH assay	100, 50, 25, 12.5 and 6.25 µg/mL	strong free radical scavenging activity reaching a maximum of 84.3%	(Teka and Kassahun, 2020)
Aloe ferox	Antioxidant	DPPH assay	0.025–0.5 mg/ml	IC_{50} value of methanol extract was 0.086 mg/ml	(Wintola and Afolayan, 2011)
Aloe vera	Hepatoprotevtive	*In-vivo*	60 mg/kg	Improving the liver enzyme activities	(Davis *et al*., 1994, Hajhashemi *et al*., 2012, Salama et al., 2016)
Aloe littoralis	AntiInflammatory	*In-vivo*	2.5 and 5 ml/kg	Had significant (P<0.05) anti-inflammatory activity	(Al-Sobarry *et al*., 2013, Hajhashemi et al., 2012)
Aloe perryi	Analgesic	*In-vivo*	100 and 250 mg/ kg	Showed Significant peripheral analgesic action	(Al-Sobarry *et al*., 2013)
Aloe vera	Peptic ulcer	*In-vivo*	200mg/kg	Showed Significant anti-ulcer activity	(Borra et al., 2011)
Aloe vera	Wound healing	*In-vivo*	25 and 50 mg daily	A significant improvement healing	(Lakhanpal *et al*., 2015)
Aloe secundiflora	Antimicrobial	*In-vivo*	1000 -1 mg/ml	MIC (5 mg/ml - 9 mg/ml)	(Rachuonyo *et al*., 2016)

1.4. *Aloe secundiflora* Engl.

1.4.1. Distribution and botanical description

Aloe secundiflora is an evergreen, succulent, perennial plant that forms a thick rosette of approximately 20 spear-shaped leaves that can be 30 to 75 cm long and 8 to 30 cm broad at the base (Kimani *et al*., 2018) (Figure 1). The plant is usually solitary, although occasionally suckers develop to form small groups. It can be stemless or have a short stem up to 30 cm long (Ngumbau *et al*., 2020). The plant can be found in the tropics and subtropics of east Africa in Sudan, Ethiopia, Kenya, Rwanda, and Tanzania. It is well adapted to dry semi-arid conditions of grassland and open woodland on sandy soil at elevations between 600 and 2,000 meters (Kimani *et al*., 2018).

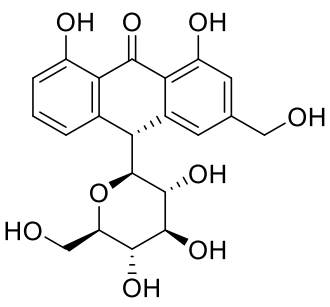
Figure 1: Picture of *Aloe secundiflora*. Available at: https://www.bing.com/images/search. Accessed date Jan 21/01/2023.

1.4.2. Ethnobotanical uses

In Ethiopia the exudate of *A. secundiflora* is used traditionally for the treatment of skin infections, wound healing, inflammation, ectoparasite, malaria, and diarrhea (Belayneh *et al*., 2020). The exudate of *A. secundiflora* is also used traditionally for the treatment of cancer in east Africa (Anywar *et al*., 2022).

1.4.3. Phytochemistry

The phytochemical profile of the leaf exudate of *A. secundiflora* from Kenya and Tanzania was analyzed by HPLC-MS and identified eight compounds that are mainly phenolic, anthrones (isobarbaloin **(1)**, barbaloin **(2)**, pyrones (aloenin **(3),** aloenin B **(4)** and other aloin derivatives), chromones (aloesin) **(5)**, and phenyl pyrones (10-O-β-d-glucopyranosyl aloenin) **(6)**, aloinoside **(7)**, and aloinoside B **(8)** with only a small amount of polysaccharides and aliphatic compounds (Rebecca *et al*., 2003). Induli *et al* (2012) reported two naphthoquinones, 5-hydroxy-3,6-dimethoxy-2-methylnaphthalene-1,4-dione **(9)** and 5,8 dihydroxy-3-methoxy-2-methylnaphthalene-1,6-dione **(10)** from the roots of *A. secundiflora*. From the stem of *A. secundiflora*, a total of seven compounds were isolated and characterized as the monomeric anthraquinones chrysophanol **(11)**, aloesaponarin II **(12)**, aloesaponarin I **(13)**, laccaic acid D-methyl ester **(14)**, emodin **(15)**; the pr-eanthraquinone aloesaponol I **(16)** and the naphthoquinone derivative 8-hydroxy-2,7- dimethoxy-3-methylnaphthalene-l,4-dione **(17)** (Cheloti, 2010).



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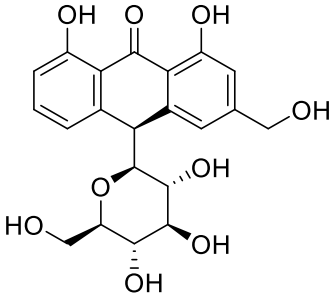

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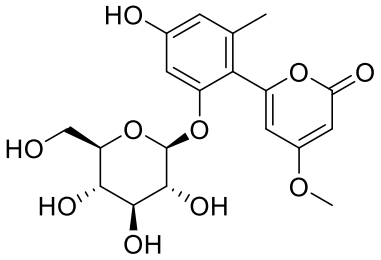

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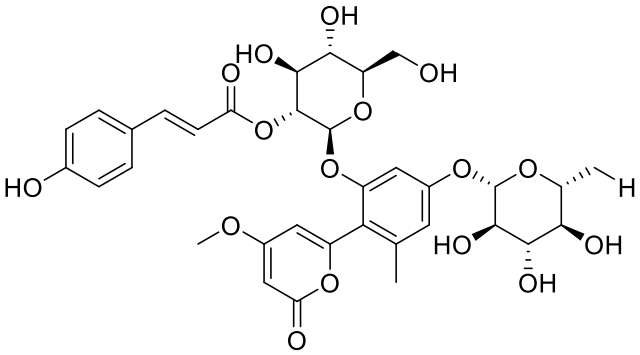

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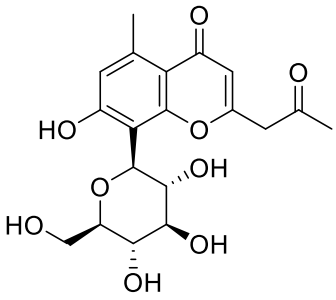

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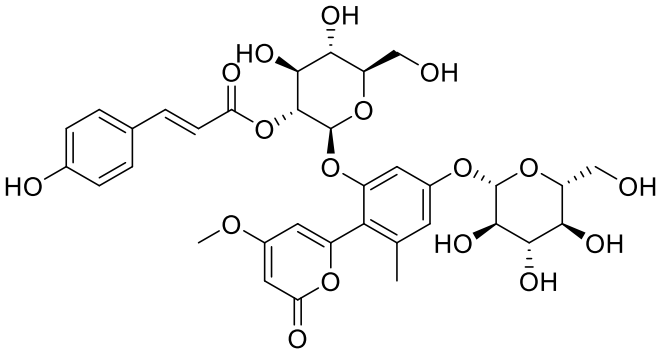

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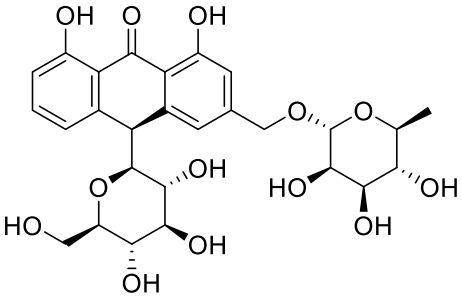

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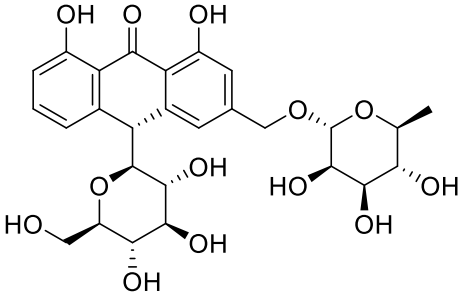

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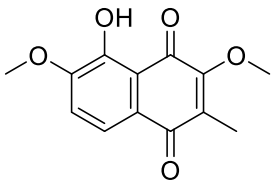

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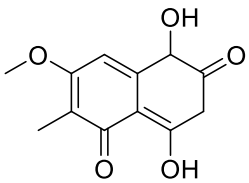

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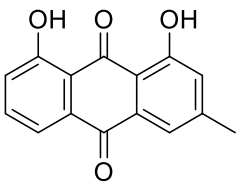

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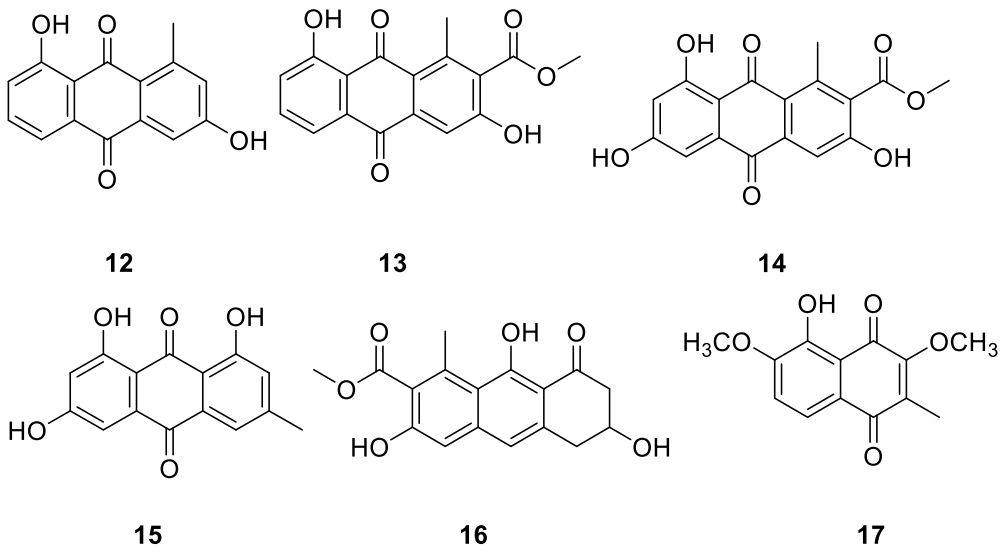

Figure 2: Compounds isolated from *Aloe secundiflora* Engl.

1.5. Statement of the problem

Cancer continues to be a major public health issue and the world's leading cause of death despite tremendous efforts to combat it (Zargar *et al*., 2019). The only major treatment modality for treating advanced stages of cancer is chemotherapy, but it is highly detrimental to healthy tissues (van den Boogaard *et al*., 2022). In addition to severe toxicity on normal tissues, a key cause of failed cancer therapy remains drug resistance (Alfarouk *et al*., 2015). Moreover, it is challenging to synthesize innovative compounds that are safe and effective against a variety of ailments since physiologically active substances have complex structures. As a result, there has been an upsurge in interest in the research and discovery of natural products including anticancer agents from plants, particularly those from developing nations with a variety of floral resources *(N Nwodo et al*., 2016). Along with the aforementioned reasons, the prevalence and incidence of cancer are constantly rising, necessitating the development of new anticancer agents from natural products with improved safety profiles and new mode of action (Siddiqui *et al*., 2022).

The anticancer potential of the genus *Aloe* has interested researchers owing to the potent and cell line selective antiproliferative activities exhibited by different compounds such as aloin, aloe-emodin, acemannan, barbaloin, physcion, chrysophanol, aloesin, diethylhexyl phthalate, and an N-terminal octapeptide (Salehi *et al*., 2018). Thus, the main goal of this study is to assess the possible antiproliferative activity of *A. secundiflora* and isolated compounds from the latex.

2. Objectives

2.1. General objective

- To evaluate the possible antiproliferative activity and major constituents isolated from *Aloe secundiflora* Engl.

2.2. Specific objectives

- To determine the antiproliferative activity of the leaf latex of *A. secundiflora*
- To isolate major constituents from *A. secundiflora*
- To elucidate the structures of the isolated constituents; and
- To evaluate the antiproliferative activity of the isolated constituents

3. Materials and Methods

3.1. Materials

3.1.1. Plant material

The fresh leaves of *A. secundoflora* were collected from Oromia region of Ethiopia, Borena zone, Yabelo wereda in a specific location known as "Dida Chena" (GPS location: 04º 24' 58.2"North & 038º 16' 24.0" East) and identified by Professor Sebsebe Demisew, National Herbarium, Department of Biology, College of Natural and Computational Sciences, Addis Ababa University (AAU), where a botanical specimen was deposited (collection number AAU106) for future reference.

3.1.2. Chemicals and instruments

The chemicals utilized in this study were distilled water (AAU laboratory), chloroform, methanol, acetone, and ethyl acetate (LOBA-Chemie, India), which were used for extraction and isolation. Pre-coated silica gel 60 F_{254} plates (aluminum backed, 200 µm, Merck KGaA, Darmstadt, Germany) were used for the analytical TLC and silica gel HF_{254} were used for preparative TLC (LOBA-Chemie, India). 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), and dimethylsulfoxide (DMSO) were utilized in the biological experiment (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, PTLC coating machine, and oven.

3.2. Methods

3.2.1. Preparation of plant material

The leaf of *A. secundiflora* was collected in April, 2022 by cutting the leaves transversely near the base allowing the yellow sap to come down into a plastic plate. The water was evaporated by leaving at room temperature for three days to yield a golden coarse powder. The dried latex was kept in a closed sample bottle and stored at 4 °C until used.

3.2.1.1. Isolation

Analytical thin layer chromatography (TLC) was used to choose a solvent system that would allow a better resolution of the components of the latex after many trials and errors and then chloroform: methanol (4:1) was selected as the mobile phase for isolation by preparative thin layer chromatography (PTLC). The latex was initially dissolved in methanol and directly applied to preparative thin layer chromatography (PTLC) plates over silica gel of 0.5 mm thickness (Merck, G 6; 20 cm × 20 cm). The chromatograms were then developed in a solvent system of chloroform: methanol (4:1) and visualized under UV light of 254 and 366 nm. Two major bands were observed (AS-2 and AS-5). AS-2 and AS-5 were further analysed using ethyl acetate: methanol: water at a ratio of 40:8:5 and 40:5:5, respectively as a solvent system using 0.25 mm-thick silica gel chromatographic plates. After separation AS-2 gave compound-1 and compound-2 while AS-5 gave compound-3 and compound-4. The compounds were scraped off, washed with ethyl acetate: methanol (50: 50) filtered and were kept for further analysis.

3.2.2. Structural elucidations

An ABSCIEX Triple TOF 5600 mass spectrometer (Concord, ON, Canada) was used to record mass spectra, and ESI was used to ionize all of the samples. Using a Bruker Avance III HD

spectrometer (Faellanden, Switzerland), ^{1}H and ^{13}C NMR spectra were acquired at 500 and 125 MHz, respectively. DMSO was used to dissolve the sample. Chemical shifts are reported in units of ppm and coupling constants (J) are expressed in Hz. Tetramethylsilane (TMS) was used as an internal standard during spectral determination. The number of ^{1}H NMR signals is given as s = singlet, d = doublet, t = triplet, q = quartet, dd = doublet of doublets and m = multiplet.

3.2.3. Antiproliferative activity test

3.2.3.1. Cell culture

The cancer cell lines in this study, breast adenocarcinoma (MCF-7), lung cancer(A427), urinary bladder cancer (RT-4), and cervical cancer (SiSo) were obtained from the German Collection of Microorganisms and Cell Culture (DMSZ Braunschweig, Germany). These cell lines were routinely maintained in 75 cm^2 culture flasks (Sarstedt, Nümbrecht, Germany), in a humid atmos phere of 5% CO_2 at 37 ˚C. Cells were grown in 90% RPMI-1640 media containing, 10% (*v*/*v*) heat-inactivated fetal bovine serum (Sigma-Aldrich, Munich, Germany) and supplemented with 30 mg/L penicillin and 40 mg/L streptomycin. Cells were incubated in a 5% CO_2 humidified incubator (Heracell, Thermo Fisher Scientific,Waltham, MA, USA), at 37 ◦C, and passaged weekly (Tesfaye *et al.*, 2021).

3.2.3.2. MTT cell viability assay

The 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2*H*-tetrazolium bromide (MTT) assay
was used in this experiment to determine the inhibition of the viability of suspension cells by the extracts (Yao *et al*., 2017). Briefly, 20,000 cells were seeded out in 50 µL medium per well and immediately exposed to seven serial dilutions test solutions which was prepared in two

fold serial dilutions of the crude latex first and then the isolated compounds ranging from 50 to 0.78 µg/mL.

After 24 h of incubation, 20 µL of freshly prepared solution of MTT in PBS (2.5 mg/mL) was added and the plates were returned to the incubator for an additional 4 h. Following the incubation, 100 µL of a 0.04 N HCl solution in isopropanol was added into each well, followed by sonication of the plates to dissolve the formed formazan crystals and the optical density was measured at λ = 570 nm with a Spectramax 384 Plus plate reader or a Sunrise plate reader (Tecan; Männedorf, Switzerland). Control experiments without cells were performed under the same assay conditions to rule out that the latex or the compounds themselves were reducing MTT to the blue formazan. No evidence for such a chemical reduction observed in the OD readings, even at the highest concentrations if the cells aren't viable.

3.2.3.3. Statistical Analysis

Antiproliferative tests were independently performed in triplicate and each triplicated test doubled. IC_{50} values were calculated with the software GraphPad Prism 9 ((Graph Pad Software, CA, USA)). The results are presented as means ± standard error of mean, where appropriate.

4. Results and Discussion

4.1. Compound characterization

TLC analysis of the latex using chloroform: methanol (4:1) as a solvent system showed 5 bands designated as AS-1 to AS-5 with R_f values of 0.36, 0.42, 0.56, 0.62, and 0.68, respectively (Figure 3). Among the bands, two major bands, AS-2 and AS-5 were taken for further purification using a solvent systems (ethyl acetate: methanol: water) with a ratio of 40:8:5 and 40:5:5, respectively. The separated compounds from AS-2 are designated as compound-1 and compound-2 with R_f value of 0.48 and 0.52, respectively (Figure 4) whereas AS-5 gave compound-3 and compound-4 with R_f values of 0.67 and 0.69, respectively (Figure 5). Despite the isolation of four compounds, the low yield only allowed the structure of two compounds to be determined.

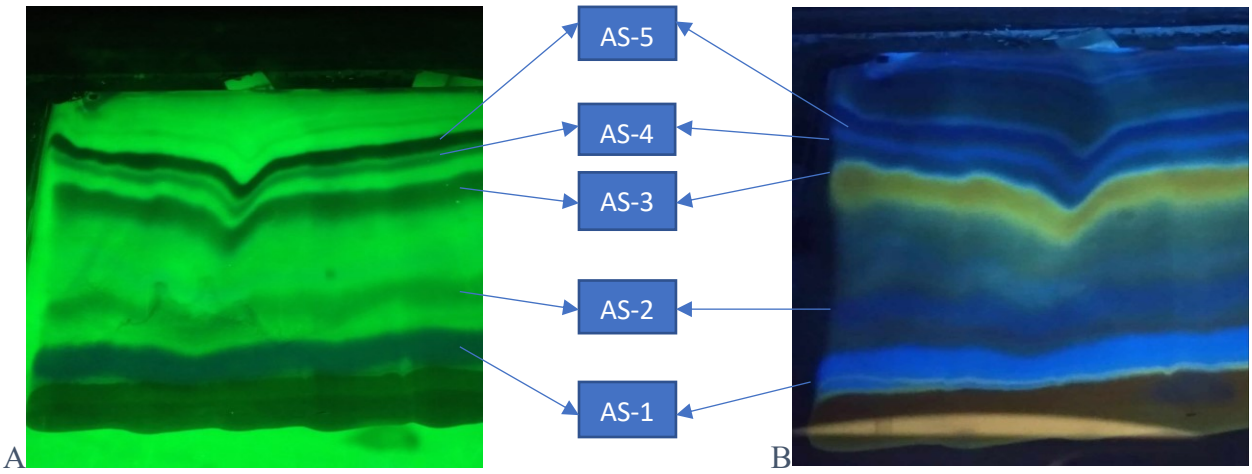

Figure 3: TLC chromatogram of the leave latex of *A. secundiflora* viewed under UV 254 (A) and 366 nm (B)

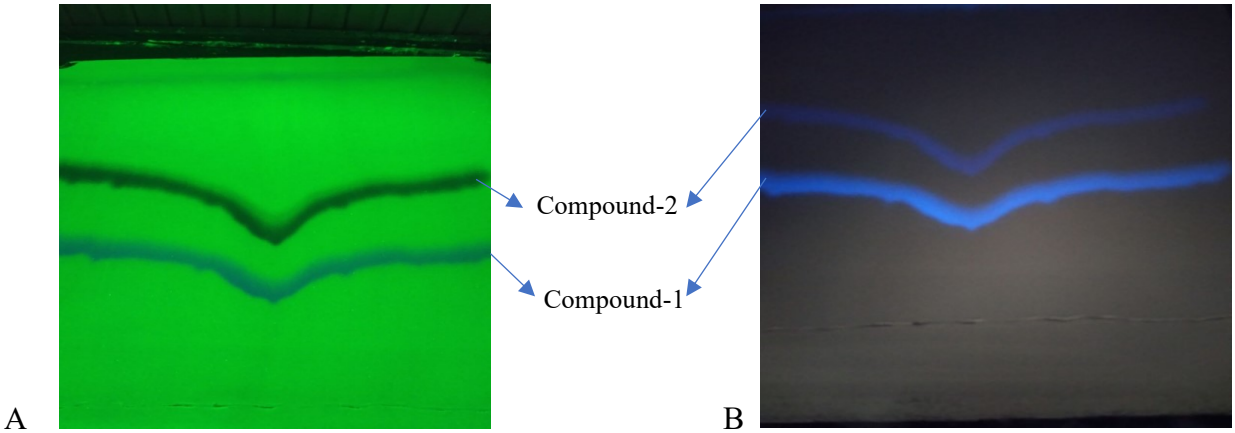

Figure 4: TLC chromatogram of compound-1 and compound-2 viewed under UV 254 (A) and 366 nm (B)

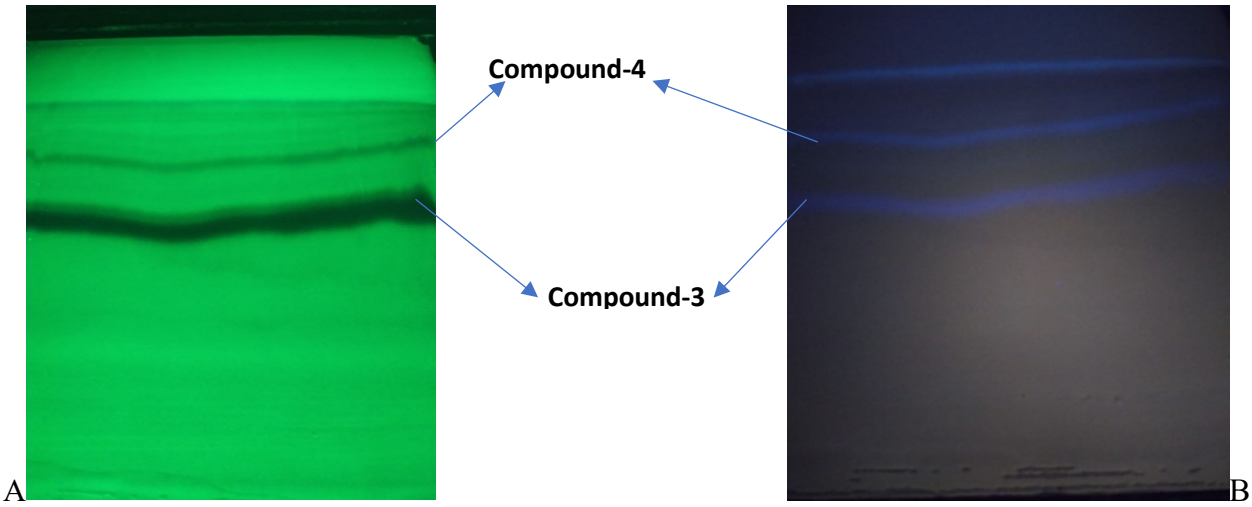

Figure 5: TLC chromatogram of compound-3 and compound-4 viewed under UV 254 (A) and 366 nm (B) wavelength

4.1.1. Characterization of compound-2

Compound-2 (Figure 4) was isolated as a white amorphous compound with an R_f value of 0.42 in ethyl acetate: methanol: water (40:8:5) solvent system. The negative TOF-HRMS/ESI

(Appendice III) spectra showed a pseudomolecular ion at m/z $[M-H]^-$ = 717.2029 corresponding to a molecular formula $C_{34}H_{38}O_{17}$ ((exact calculated masses = m/z 718.203075 $[M-H]^-$.

^{1}H-NMR spectral (Appendice II) data of compound-2 revealed the presence of eight aromatic proton signals assigned to δ = 7.45 (*d*, J=7.45Hz, H-5”/9’’), 6.86 (*d*, J=6.83Hz, H-11), 6.66 (*d*, J=6.69Hz, H-9), 6.84 (*d*, J=6.83, H-6’’/8’’), 6.82 (d, *J*=6.83Hz, H-5), 5.57 (*s*, H-3). Two olefinic protons were also observed and assigned as δ=7.46 (*d*, J=7.45Hz, H-3”) and 5.99 (*d*, J=5.98Hz, H-2’’). The typical anomeric proton signals at 5.57 *d* and 5.03 *d* indicated the presence of a sugar unit in compound-2.

The presence of a sugar moiety is further corroborated by the proton peaks from δ 5.05 to 3.32. The ^{13}C NMR spectral data of compound-2 revealed the presence of 34 carbon atoms. From these, nine are quaternary carbons; δ=173.48 C-4, 168.01 C-1”, 167.76 C-2, 161.36 C-6, 160.72 C-7”, 157.52 C-8, 159.00 C-10, 141.11 C-12, 127.25 C-4’’. Two carbonyl carbons were also observed and assigned as δ=167.76 C-2 and 168.01 C-1” and 17 of the carbons were identified as aromatic carbons (δ between 89.17 and 167.76), two olefinic carbons δ=117.18 C-2’’ and 146.95 C-3’’, and the remaining were assumed to be sugar carbons and other sp^3 hybridized carbons. Based on ^{1}H and ^{13}C NMR spectral data of compound-2 and also by comparing its spectral data with those reported in the literature (Abd-Alla *et al*., 2009), compound-2 was identified as Aloenin B (Figure 6). This compound was also isolated from different *Aloe spp*. such as from the leaves and roots of *A. hijazensis* and from the leaves of *A. arborescens* (Park *et al*., 1998, Abd-Alla *et al*., 2009). The full ^{1}H and ^{13}C NMR spectral data is presented in Table 4.

Table 4: ^{1}H NMR and ^{13}C NMR spectral data of compound-2 in comparison with literature reported by Abd-Alla *et al*., 2009.

Carbon and hydrogen numbering	^{1}H NMR chemical shift (δ, ppm)		^{13}C NMR chemical shift (δ, ppm)	
	Compound-2	Literature	Compound-2	Literature
2			167.76	163.7
3	5.57 *d*	5.52 *d*	89.17	88.0
4			173.48	170.5
5	6.82 *d*	5.79 *d*	106.57	104.0
6			161.36	158.7
7			113.5	115.3
8			157.52	156.9
9	6.66 d	6.63 *d*	100.20	99.9
10			159.00	155.7
11	6.86 *d*	6.48 *d*	113.5	111.4
12			141.11	138.7
1’	5.03 *d*	5.06 *d*	101.28	98.0
2’	5.05 *dd*	4.77 *dd*	74.79	73.9
3’	4.05 *dd*	4.29	77.96	72.6
4’	3.52 *dd*	3.58	71.85	70.0
5’	3.32 *m*		78.40	77.0
6’	3.48 *m*	3.69 *m*	62.76	60.8
1’’			168.01	165.0
2’’	5.99 *d*	6.13 *d*	117.18	114.0
3’’	7.46 *d*	7.38 *d*	146.95	144.6
4’’			127.25	125.3
5’’	7.45 *d*	7.44 *d*	140.79	130.1
6’’	6.84 *d*	6.74 *d*	116.83	115.7
7’’			160.72	159.7
8’’	6.84 *d*	6.74 *d*	116.83	115.7
9’’	7.45 *d*	7.44	140.79	130.1
1’’’	5.57 *d*	4.87 *d*	101.58	99.5
2’’’	3.95 *m*		74.31	73.1
3’’’	3.45 *m*		76.03	76.7
4’’’	3.40 *m*	4.77~3.12 (m, glc protons H-2’/2’’’, 3’/3’’’, 4’/4’’’, 5’/5’’’, 6’/6’’’)	71.79	70.1
5’’’	3.30 *m*		78.31	77.1
6’’’			62.94	60.7
OCH_3-4		3.69 *s*	56.89	56.0
CH_3-12		2.09 *s*	20.00	19.3

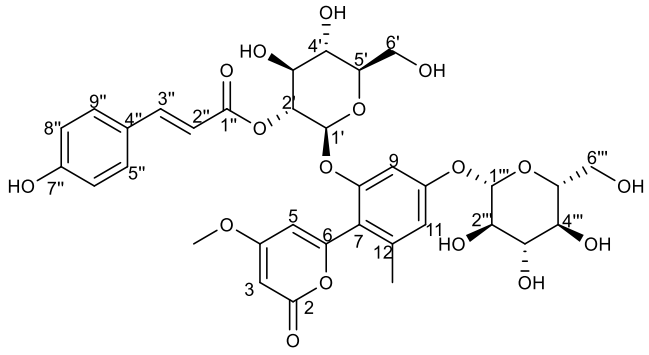

Figure 6: Chemical structure of compound-2 (Aloenin B).

4.1.2. Characterization of compound-3

Compound-3 (Figure 6) was isolated as a white amorphous compound with an R_f value of 0.63 in (ethyl acetate: methanol: water; 40: 5: 5) solvent system. The negative TOF-HRMS/ESI (Appendice IV) spectra showed a pseudomolecular ion $[M-H]^-$ = 555.1893, corresponding to a molecular formula $C_{29}H_{32}O_{11}$ ((exact calculated masses = m/z 556.1866 $[M-H]^-$.

^{1}H-NMR spectral data of compound-3 (Figure 7, Table 5) revealed the presence of five aromatic proton signals assigned to δ=6.67 (1H, *s*, J= 6.84Hz, H-6), 7.32 (1H, *d*, J=7.29Hz, 5" & 9"-H), and 6.61 (1H, *d,* J=6.74Hz, 6" & 8"-H). Three olefinic protons were also observed and assigned as δ=5.98 (1H, s, J=5.98Hz, H-3), 6.74 (1H, *d*, J=6.74Hz, 2"-H), and 7.27 (1H, *d*, J=7.29Hz, 3"-H). The typical anomeric proton signal at 5.47 *d* indicated the presence of a sugar unit in compound-3. The presence of a sugar moiety is further corroborated by the proton peaks from δ 5.35 to 3.29. The ^{13}C and DEPT-135 NMR spectral data of compound-3 revealed the presence of 29 carbon atoms, in which 12 of the carbons were identified as aromatic carbons (δ between 113.13 and 159.98), four olefinic carbons (δ between 111.23 and 167.90) and the rest 17 were assumed to be sugar carbons and other sp^3 hybridized carbons. The 29 carbons in compound-3 are composed of three methyl, two methylene, six sp^3 methine, eight sp^2 CH, and ten sp^2 quaternary carbons, according to the DEPT-135 plots. Signals suggesting the structural

characteristics listed below were seen in the ^{1}H and ^{13}C NMR spectra (and were further supported by DEPT experiment): A hydroxypropyl substitution is present in the 2-position of the chromone nucleus, a methoxy group is present at C-7 of the chromone moiety, a p-coumaroyl ester residue is present at C-2' of the carbohydrate moiety, and a methyl group is attached to C-5. Based on the spectroscopic data and comparison with those given in the literature (Meng *et al*., 2004), compound-3 was identified as Aloeresin D (Figure 7). The compound was also isolated from the leaf exudate of *A. vera, A. rabaiensis, A. ngongensis, A. leachii, A. dawei, A. classenii, A. cheranganiensis, A. canarina,* and *A. cameronii* (Viljoen *et al*., 2001).

Table 5: ^{1}H NMR and ^{13}C NMR spectral data of compound-3 in comparison with literature reported by Meng *et al*., 2004.

Carbon and hydrogen numbering	^{1}H NMR chemical shift (δ, ppm)		^{13}C NMR chemical shift (δ, ppm)	
	Compound-3	Literature	Compound-3	Literature
2			167.90	164.93
3	5.98 *s*	6.04 *s*	111.23	111.24
4			179.17	178.71
4a			116.24	115.79
5			142.10	141.67
6	6.77 *s*	6.84 *s*	111.73	111.27
7			159.98	159.75
8			111.13	110.65
1a			157.92	157.47
9	2.81	2.67	43.81	43.24
10	4.26 *m*	4.25 *m*	64.29	63.85
11	1.28 *d*	1.21 *d*	23.33	23.66
12	2.69	2.67	-	22.86
13	3.90 *s*	3.85 *s*	56.93	56.46
1’	5.47 *d*	4.99 *d*	71.01	70.55
2’	5.98 *t*	5.60 *t*	76.3	72.21
3’	3.54 *m*	3.54 *m*	72.64	75.86
4’	3.29 *m*	3.3 *m*	71.26	70.82
5’	3.29 *m*	3.3 *m*	82.42	81.92
6’a	3.64	3.48 *m*	62.19	61.54
6’b	3.71	3.75 *m*	62.19	61.54
1”			165.87	165.36
2”	6.74 *d* (J=6.74)	6.11 *d*	114.08	113.93
3”	7.27 *d* (J=7.29)	7.26 *d*	144.90	144.33
4”			125.01	124.95
5”	7.32 *d* (J=7.29)	7.45 *d*	130.66	130.17
6”	6.61 *d* (J=6.74)	6.75 *d*	116.32	115.73
7”			159.98	159.53
8”	6.63 *d* (J=6.74)	6.75 *d*	116.32	115.66
9”	7.32 *d* (J=7.29)	7.45 *d*	130.66	130.06
A		4.80 *d*		
B		4.56 *t*		
C		5.25 *d*		
D		5.34 *d*		
E		10.0		

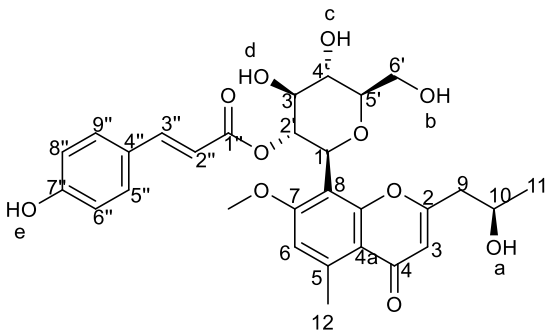

Figure 7: Chemical structure of compound-3 (Aloeresin D).

4.2. Antiproliferative activity

In the present study, the antiproliferative activity of the leaf latex of *A. secundiflora* and its isolated constituents (Aloenin B and Aloeresin D) were evaluated against four cancer cell lines namely, breast adenocarcinoma (MCF-7), lung cancer(A427), urinary bladder cancer (RT-4), and cervical cancer (SiSo). The latex displayed antiproliferative effect against the cancer cell lines (Table 6) with better effect against A427 than the other cell lines. In a previous report by (Tesfaye *et al*., 2021) *A. debrana* was found to exert similar activity against cervical cancer (SiSo), lung cancer(A427), and urinary bladder cancer (RT-4). In *A. debrana* chromones and anthrones are isolated.

Table 6: Antiproliferative T/C corrected (%) of *A. secundiflora* latex

T/C corrected (%) at (50µg/mL)			
MCF-7	A427	RT-4	Siso
49.4 ± 11.5	15.3 ±15.3	53.4 ± 22.2	88.7 ± 0.2

The two isolated compounds were also tested against the four cell lines (Table 7) and they exhibited antiproliferative effect. Both compounds showed better activity against A427 than the other cancer cell lines and compound-2 displayed better activity than compound-3. The results found in this study are comparable to other studies on the anti-proliferative effects of *Aloe* latexes and compounds isolated from them (Abdissa *et al*., 2014, Jose *et al*., 2014).

Table 7: IC_{50} values(µg/ml) for the activities of isolated compounds from *Aloe secundiflora* against A427, MCF-7, RT-4, and SiSo

	$IC_{50} \pm$ SEM (µg/mL)			
Compounds	A427	MCF-7	RT-4	SiSo
Aloenin B	3.2 ± 0.9	18 ± 8	21.9 ± 0.6	32 ± 4.1
Aloeresin D	5.5 ± 1.9	26.5 ± 9.9	29.8 ± 10	56.1 ± 26.4

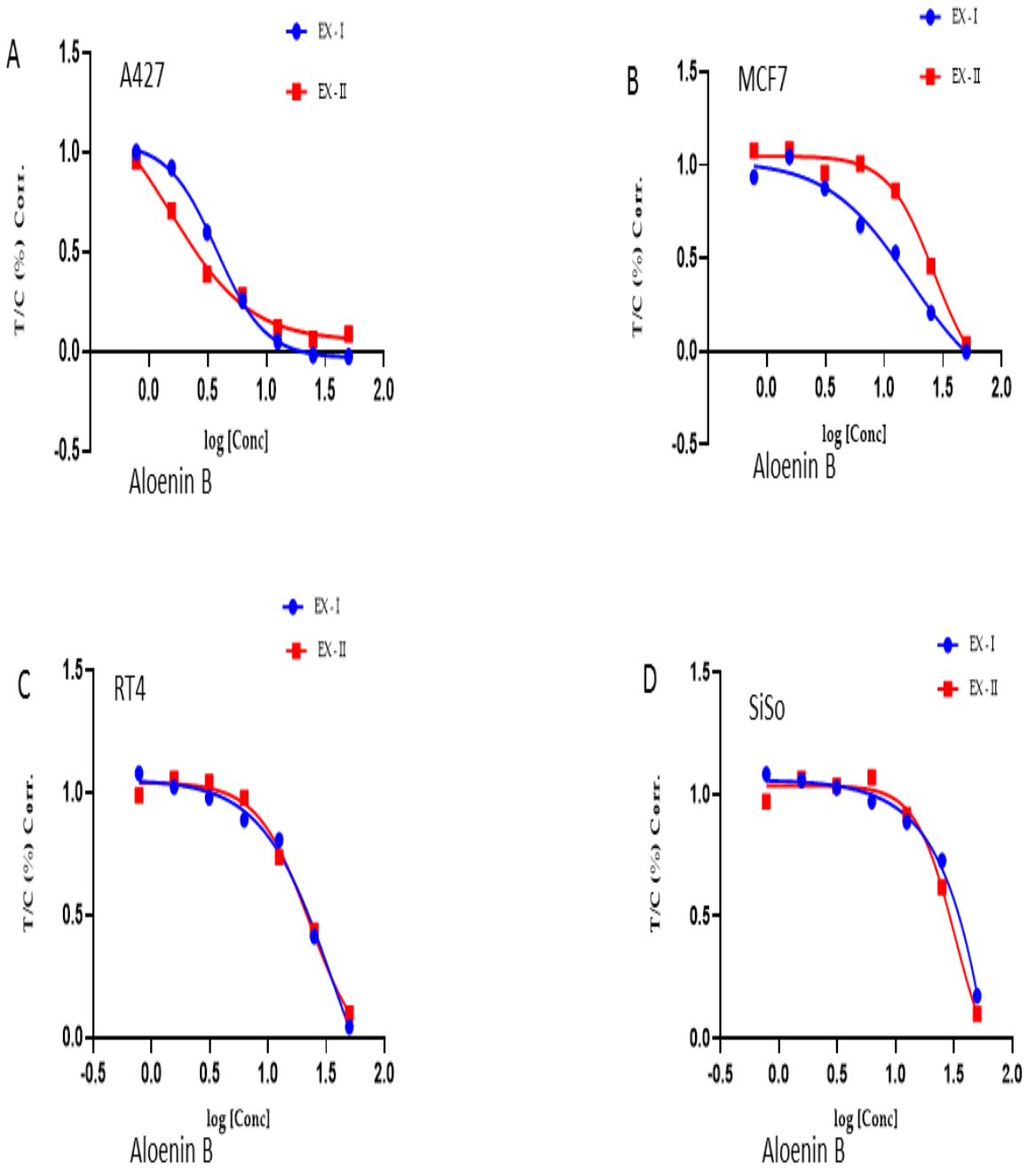

Figure 8: Antiproliferative activity of Aloenin B against A(A427), B(MCF-7), C(RT-4), and ,(D)SiSo respectively

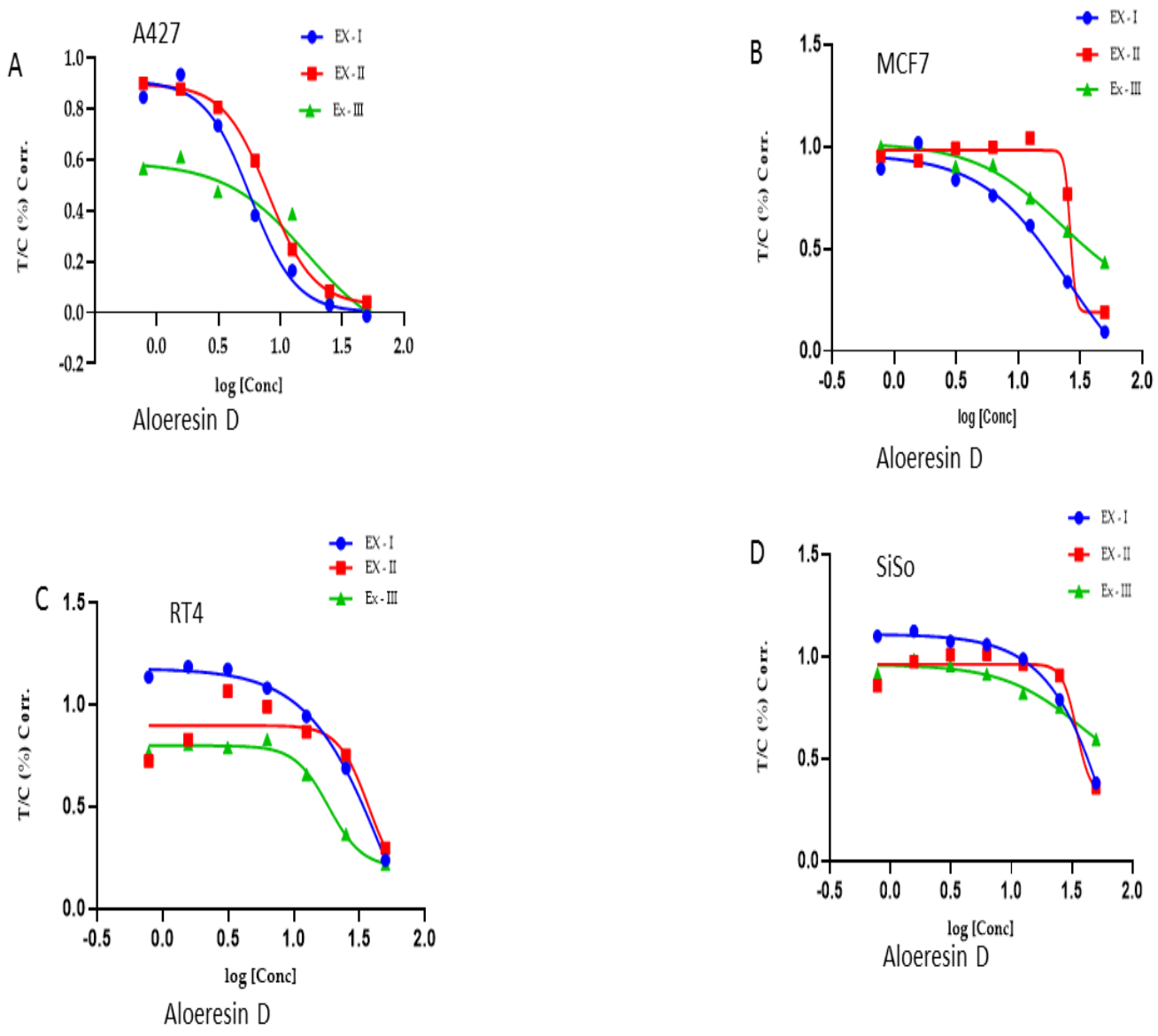

Figure 9: Antiproliferative activity of Aloeresin D against A(A427), B(MCF-7), C(RT-4), and D(SiSo) respectively

5. Conclusion

The antiproliferative effect of the leaf latex of *A. secundiflora* and two major compounds isolated from the latex, namely Aloenin B and Aloeresin D was determined against four cancer cell lines namely, breast adenocarcinoma (MCF-7), lung cancer (A427), urinary bladder cancer (RT-4), and cervical cancer (SiSo) using MTT assay. The latex, Aloenin B, and Aloeresin D exhibited antiproliferative activity against the tested cell lines, and their effect was found to be more pronounced against the lung cancer cell line (A427).

6. Recommendations

The following future works are recommended based on the present study:

- Minor compounds that are found in this plant should be isolated and their activities should be investigated.
- The mechanism of action of the isolated major compounds should be explored.

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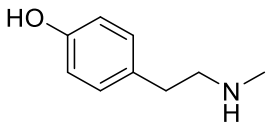
Appendices

Appendice I: Structure of compounds isolated from the genus *Aloe*

Class of compounds	Isolated compounds	Source	No	References
	N-methyltyramine	*A. secundiflora* Engl.	**1**	(Nash *et al*., 1992)
	O, N-dimethyltyramine	*A. pirottae* A.Berger	**2**	(Nash *et al.*, 1992)
	γ-coniceine	*A.gillilandii*	**3**	(Dring *et al*., 1984)
	Coniine	*A. viguieri* Perrier	**4**	(Cock, 2015)
	Prechrysophanol	*A. graminicola*	**5**	(Yenesew *et al*., 1993)
	Desoxyerythrolaccin	*A. saponaria* Haw.	**6**	(YAGI et al., 1974)
	1,5- dihydroxy-3-hydroxy methylanthraquinone	*A. excelsa*	**7**	(Mebe, 1987)
	Helminthosporin	*A. graminicola*	**8**	(Yenesew *et al*., 1993)
	Isoxanthorin	*A. secundiflora* Engl.	**9**	(Induli *et al*., 2012)
	Laccaic acid-D-methyl ester	*A. graminicola*	**10**	(Yenesew *et al*., 1993)
	Nataloe emodin	*A. nyeriensis* var. kedongensis	**11**	(Conner *et al*., 1987)
	O-methoxy-nataloe-emodin-8- methyl ether	*A. vera*	**12**	(Chinchilla *et al*., 2013)
Alkaloids	Aloechrysone	*A. berhana*	**13**	(Dagne *et al*., 1992)
	Aloesaponol II	*A. graminicola*	**14**	(Yenesew *et al*., 1993)
	Aloe emodin-11-O-rhamnoside	*A. rabaiensis*	**15**	(Conner *et al*., 1989)
	Nataloe emodin-2-O-glucoside	*A. nyeriensis* var. kedongensis	**16**	(Conner *et al*., 1987)
	Aloesaponol I-6-O-β-D-glucopyranoside		**17**	(YAGI *et al*., 1977)
	Aloesaponol II-6-O-β-D-glucopyranoside	*A. Saponaria* Haw.	**18**	
	Aloesaponol III-8-O-β-D-glucopyranoside		**19**	
	Aloesaponol-O-methyl-4-O-glucoside	*Aloe spp.*	**20**	(Cock, 2015)
	Asphodelin	*A. Saponaria* Haw., *A. elgonica*. Bullock	**21**	(Salehi *et al*., 2018)
	Bianthracene	*A. Saponaria* Haw., *A. elgonica*. Bullock	**22**	
	Barbaloin	*A. vera* and *A. ferox*	**23**	(Dagne *et al*., 2000)
	Aloin A	*A. trichosantha* A.Beger.	**24**	(Oumer *et al*., 2014)
	Aloin B	*A. tweediae* Christian, *A. trichosantha* A.Beger.	**25**	(Viljoen and van Wyk, 2001), (Oumer *et al*., 2014)
	Homonataloin	*A. marlothii* Berger	**26**	(McCarthy and Price, 1966)
	Nataloin	*A. mutabilis*	**27**	(Chauser-Volfson and Gutterman, 1998)
	Aloe barbendol	*A. barbadensis*	**28**	(Saleem *et al*., 1997)
	Aloe emodin-10-C-rhamnoside	*A. rabaiensis*	**29**	(Conner *et al*., 1989)
	8-*O*-methyl-7-hydroxyaloin B	*A. vera* (L.) Burm. f.	**30**	(Xiao *et al*., 2000)
Anthrones	6*'*-*O*-cinnamoyl-8-*O*-methyl-7-hydroxyaloin	*A. barbadensis*	**31**	(Dagne et al., 2000)
	6*'*-*O*-*p*-coumaroyl-7-hydroxyaloin A/B	*A. barbadensis*	**32**	
	7-hydroxyaloin-4*',*6*'*-*O*-diacetate	*A. succotrina*	**33**	(Sigler and Rauwald, 1994)
	6*'*-*O*-cinnamoyl-5-hydroxyaloin A	*A. broomii*	**34**	(Holzapfel *et al*., 1997)
	Microstigmin A	*A. microstigma* Salm-Dyck.	**35**	(Dagne *et al*., 1997)
	Deacetyllittoraloin	*A. littoralis*	**36**	(Dagne et al., 1998)
	Littoraloin	*A. littoralis*	**37**	
	Littoraloside	*A. littoralis*	**38**	
	Microdontin A/B	*A. microdonta*	**39**	(Farah *et al*., 1992)
	Homonataloside	*A. citrina*	**40**	(Girma *et al*., 2015)
	Pluridone	*A. pluridens*	**41**	(Confalone *et a*l., 1983)
	Protocatechuic acid	*A.greatheadii* var. davyana	**42**	(Botes *et al*., 2008)
	Methyl -*p*-coumarate	*A. vera*	**43**	(Yang *et al*., 2004)
Benzene and Naphtalene Derivatives	Isoeleutherol	*A. graminicola*	**44**	(Dagne *et al*., 1994)
	Isoeleutherol-5-*O*-glucoside	*A. saponaria*	**45**	(Dagne *et al*., 2000)
	Feroxidin	*A. barbadensis*	**46**	(Chiodelli *et al*., 2017)
	Plicataloside	*A. plicatalis*	**47**	(Wessels *et al*., 1996)
	5,8-dihydroxy-3-methoxy-2-methylnaphthalene-1,6-dione	*A. secundiflora*	**48**	(Induli *et al*., 2012)
Coumarins	Feralolide	*A. ferox Mill.*	**49**	(Speranza *et al*., 1993)

	Vacillanoside	*A. vera*	**50**	(Rahman *et al*., 2017)
	Aloeribide	*A. vera*	**51**	
	Naringenin	*A. pretoriensis*, *A. thorncroftii*, *A. lineata*	**52**	(Viljoen *et al*., 1998)
	Apigenin	*A. vera*	**53**	(Bhalla and Chauhan, 2015)
Flavonoids	Isovitexin	*A. greenii*	**54**	(Grace *et al*., 2010)
	Dihydro-isorhamnetin	*A. vera*	**55**	(Kumar *et al*., 2022)
	Quercetin		**56**	(Abd-Alla *et al*., 2009)
	Kaempferol	*A. hijazensis*	**57**	
	Cosmosiin		**58**	
	β-Sitosterol	*A. berhana*, *A. rivae*	**59**	(Dagne and Alemu, 1991)
Sterols	Cholesterol	*A.barbadensis* Miller.	**60**	(Waller, 1978)
	Campesterol	*A. barbadensis* Miller.	**61**	
	Lupeol	*A. vera*	**62**	(Gangadharan *et al*., 2019)
Pyrans and pyrones	Bisbenzopyran	*A. barbadensis*	**63**	(Saleem *et al*., 1997)
Chromones	Aloesin	*A. secundiflora*	**64**	(Rebecca *et al*., 2003)



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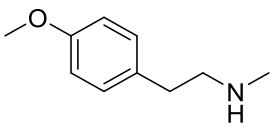

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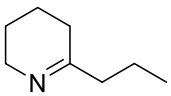

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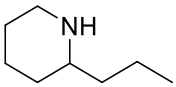

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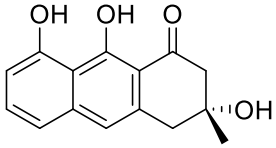

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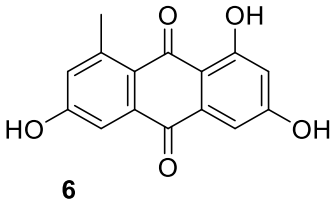

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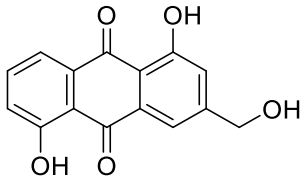

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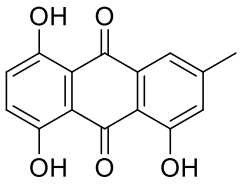

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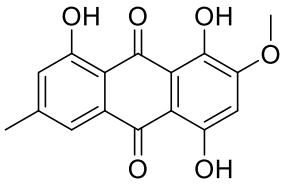

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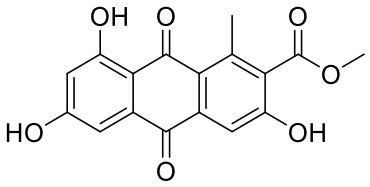

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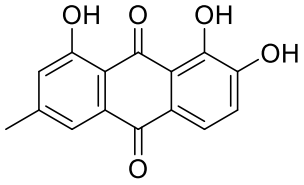

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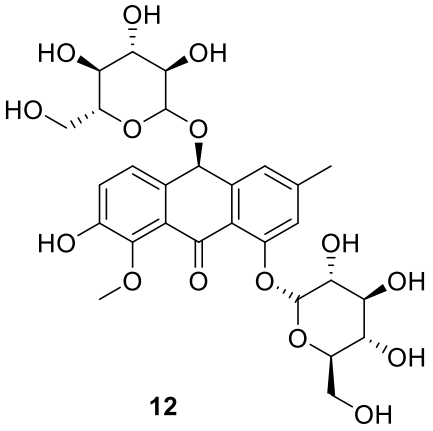

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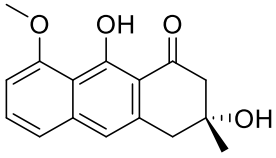

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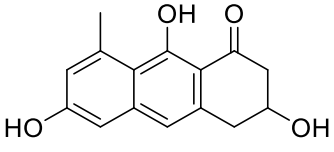

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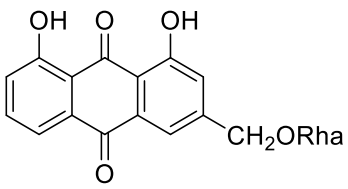

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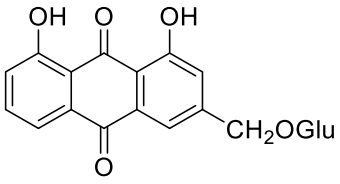

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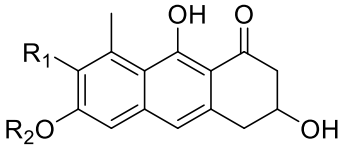
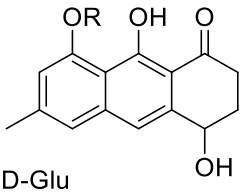


17: R1=COOCH3, R2=D-Gluc

18; R1=H, R2=D-Gluc



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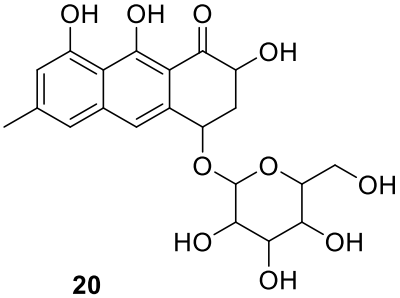

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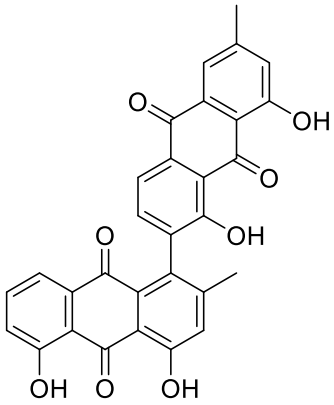

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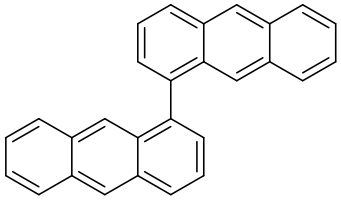

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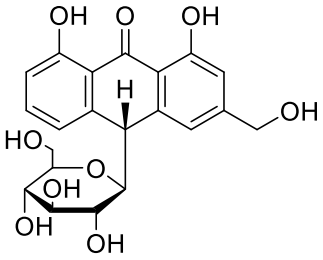

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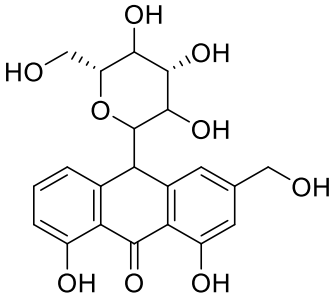

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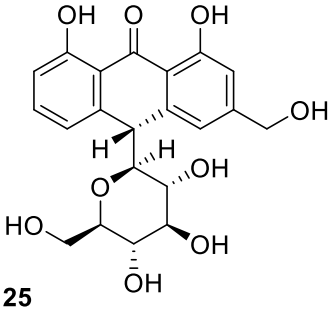

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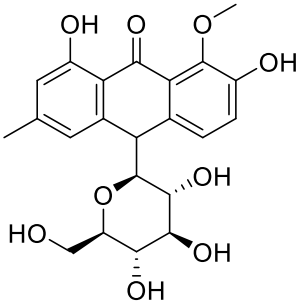

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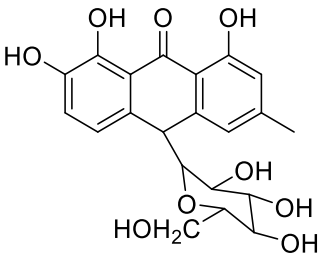

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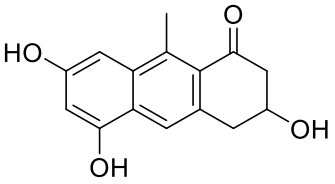

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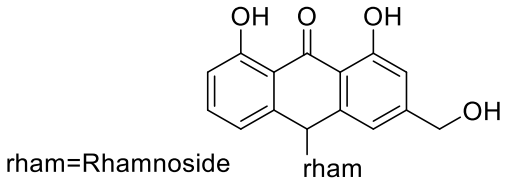

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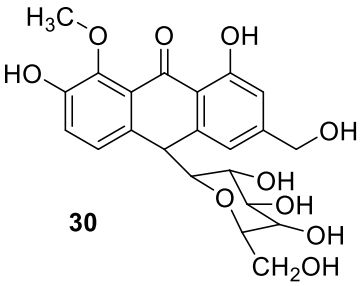

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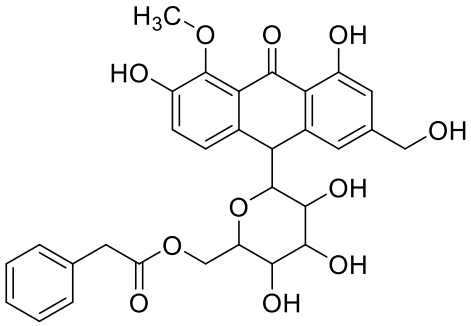

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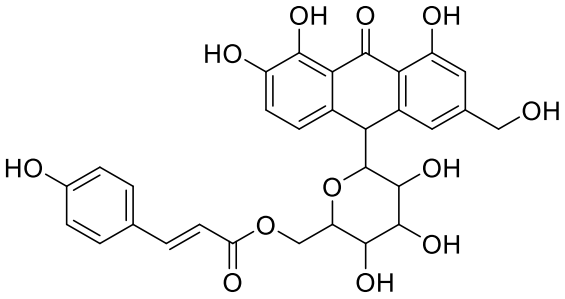

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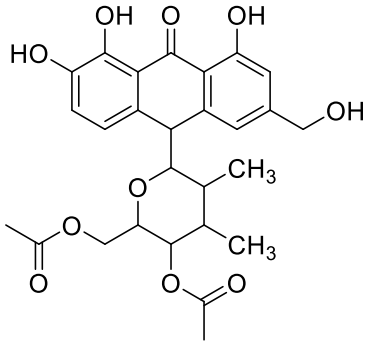

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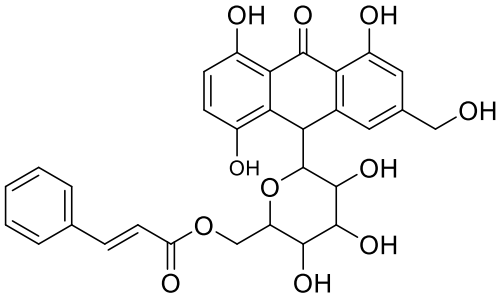

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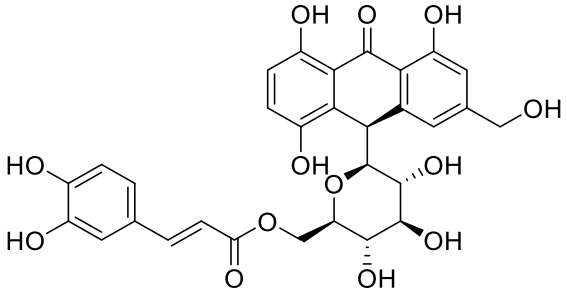

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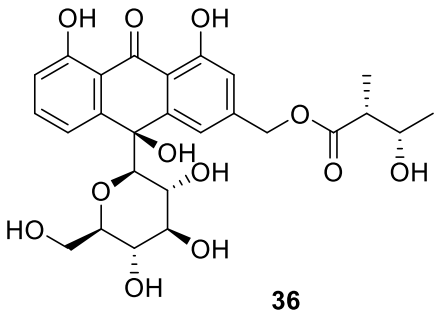

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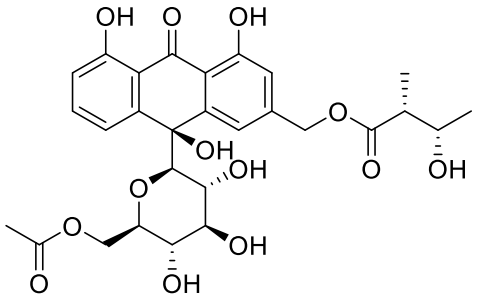

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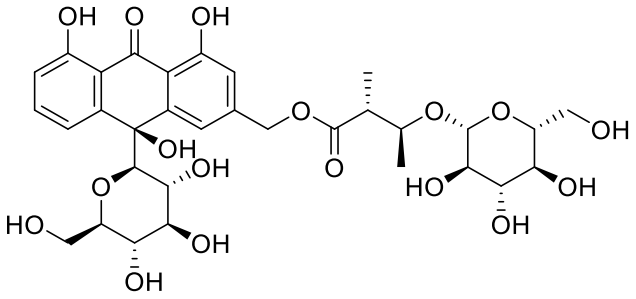

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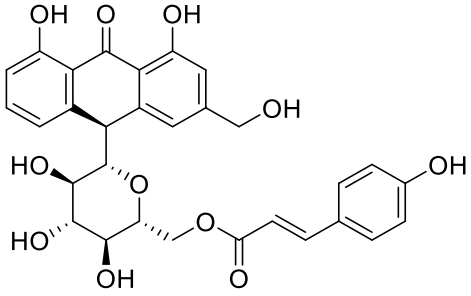

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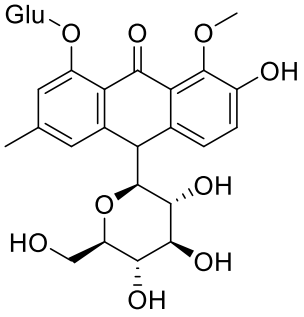

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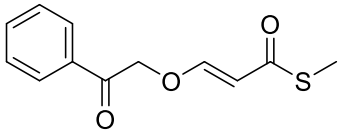

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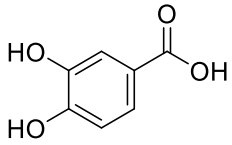

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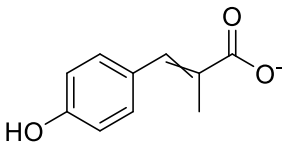

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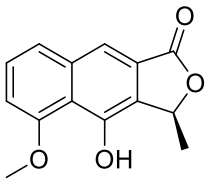

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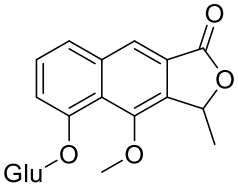

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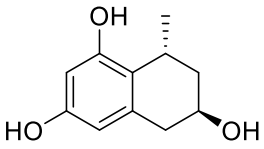

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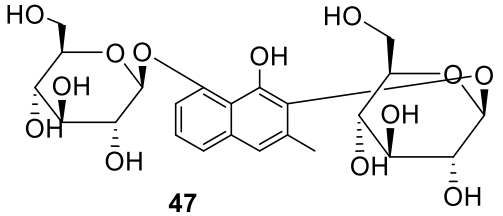

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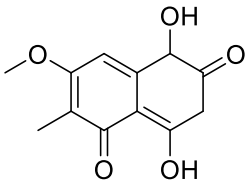

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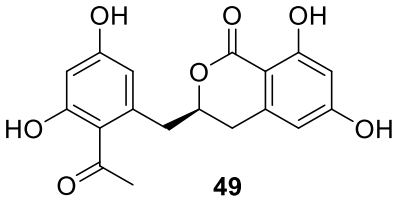

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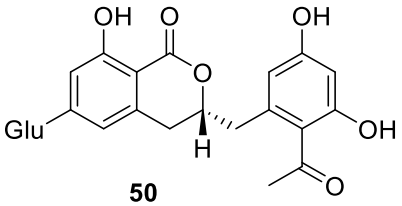

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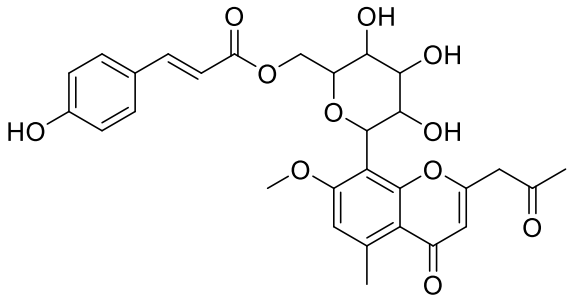

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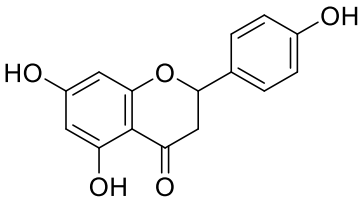

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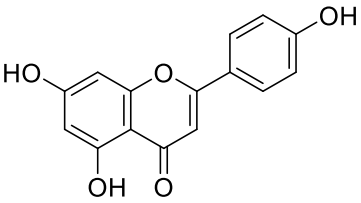

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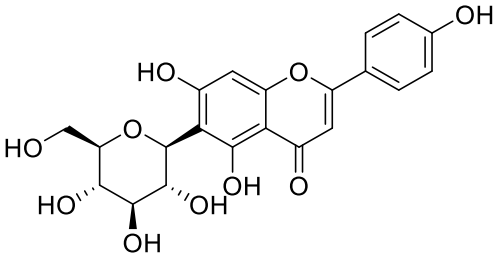

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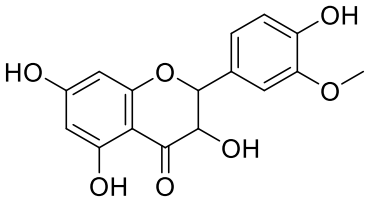

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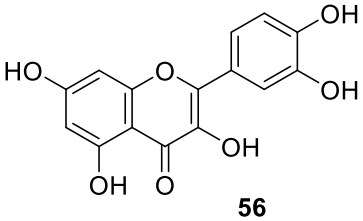

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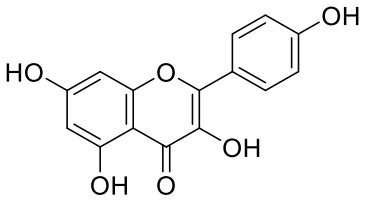

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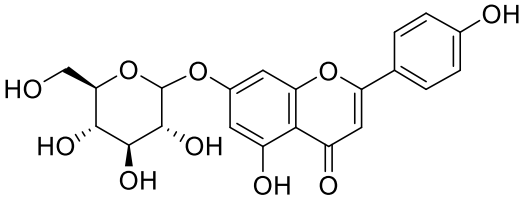

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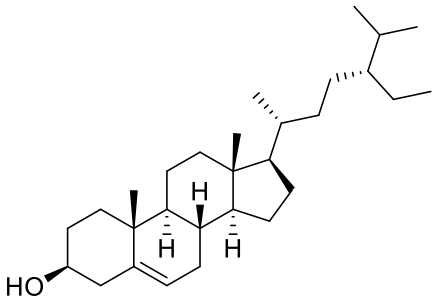

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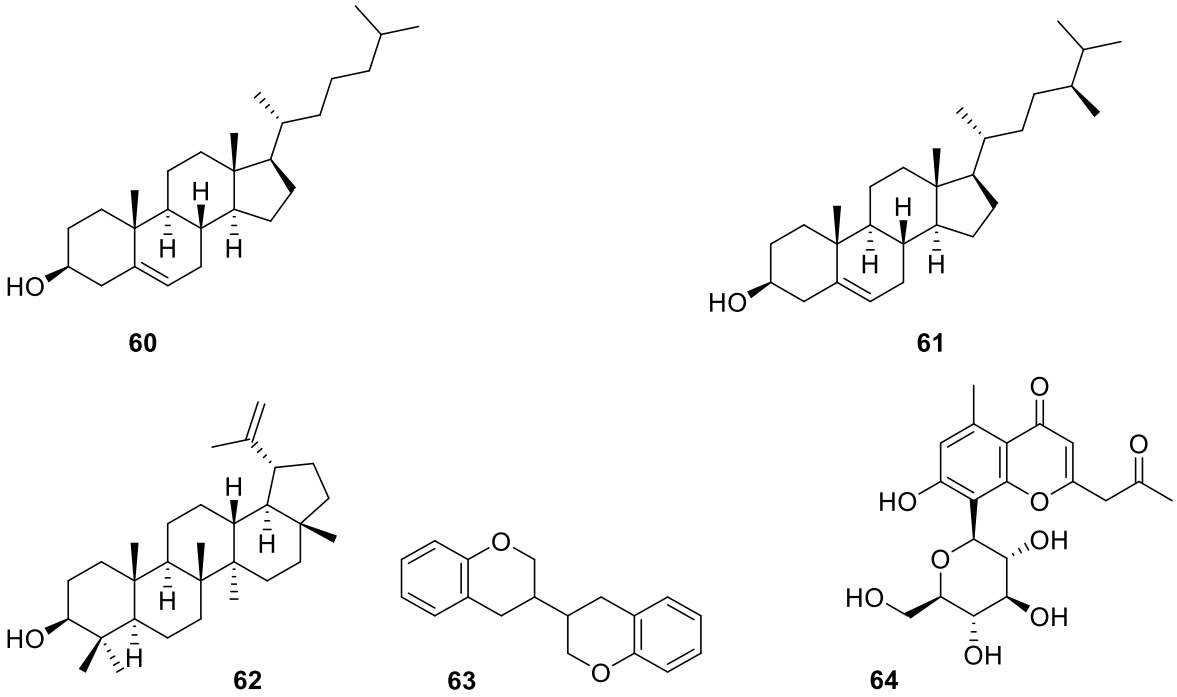
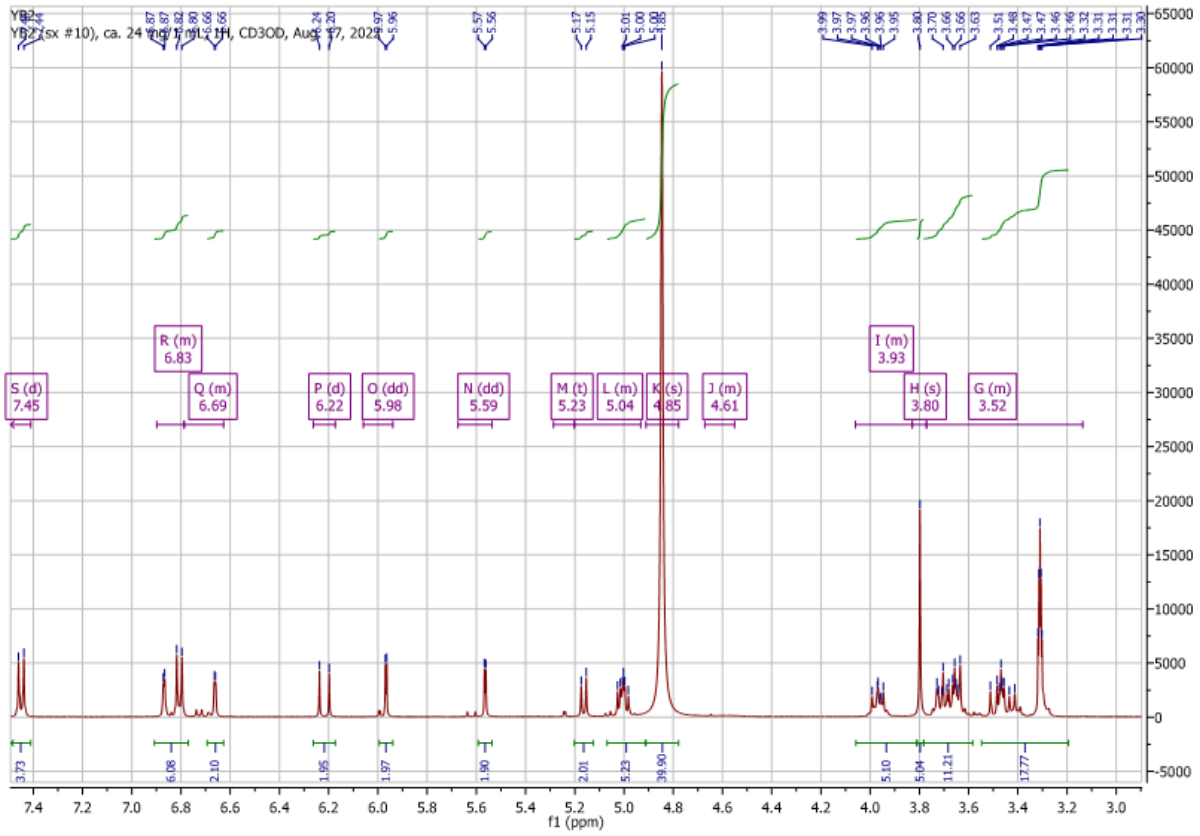
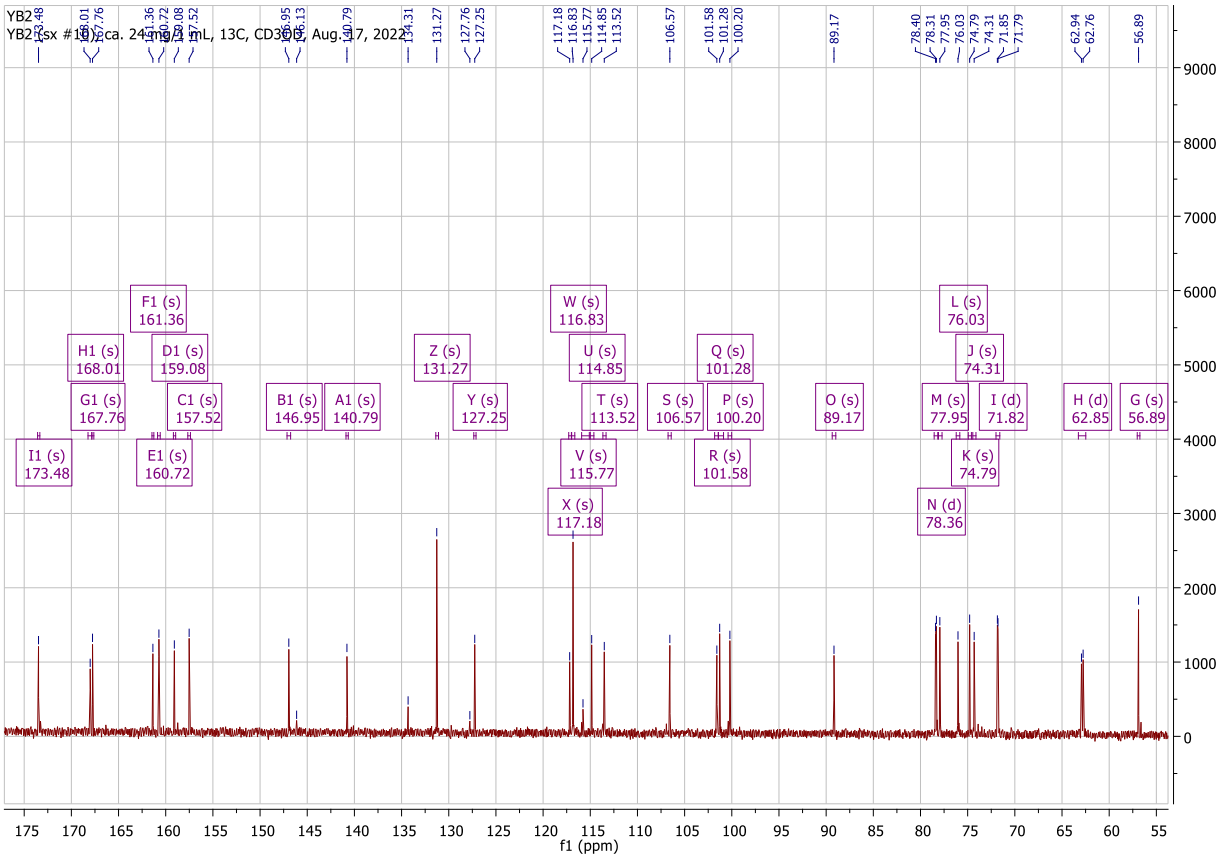


Figure 10: Structure of compounds isolated from the genus *Aloe*

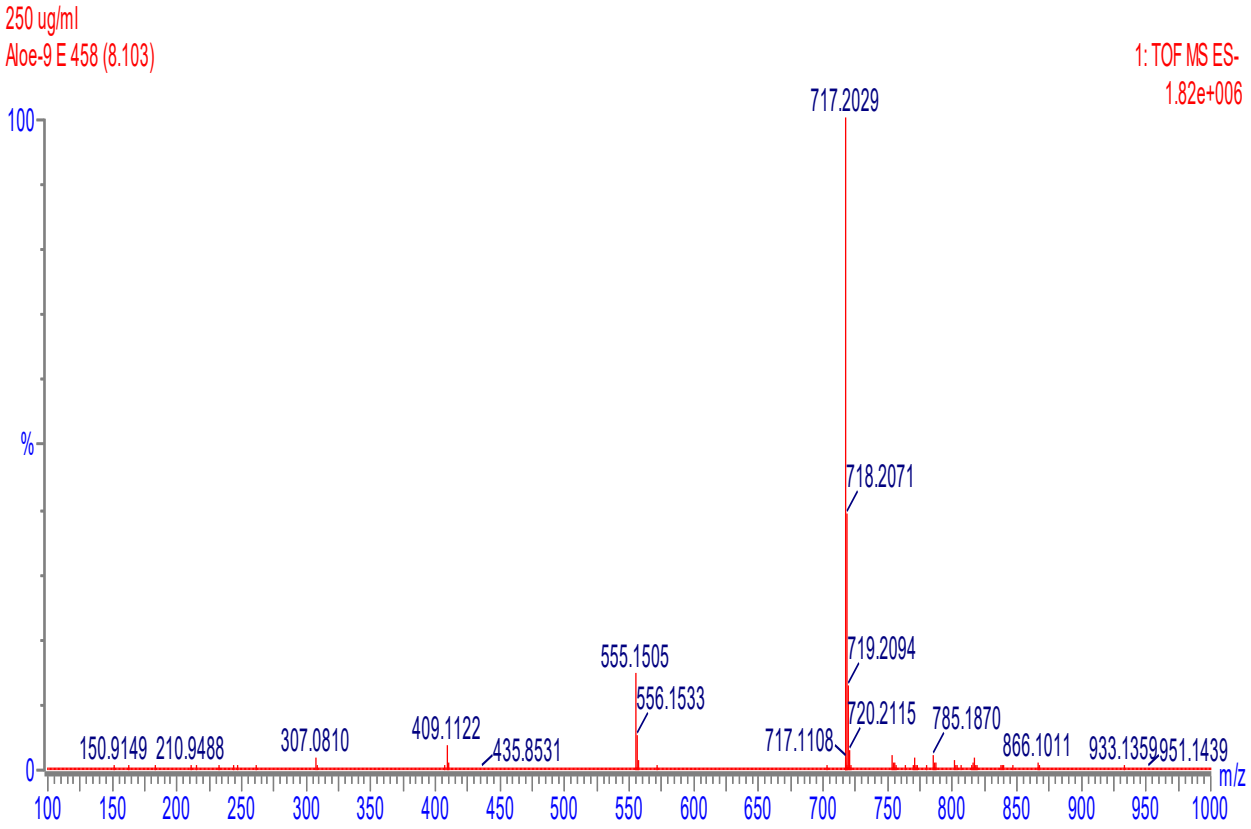
Appendice II: ^{1}H NMR of compound-2 (Aloenin B)



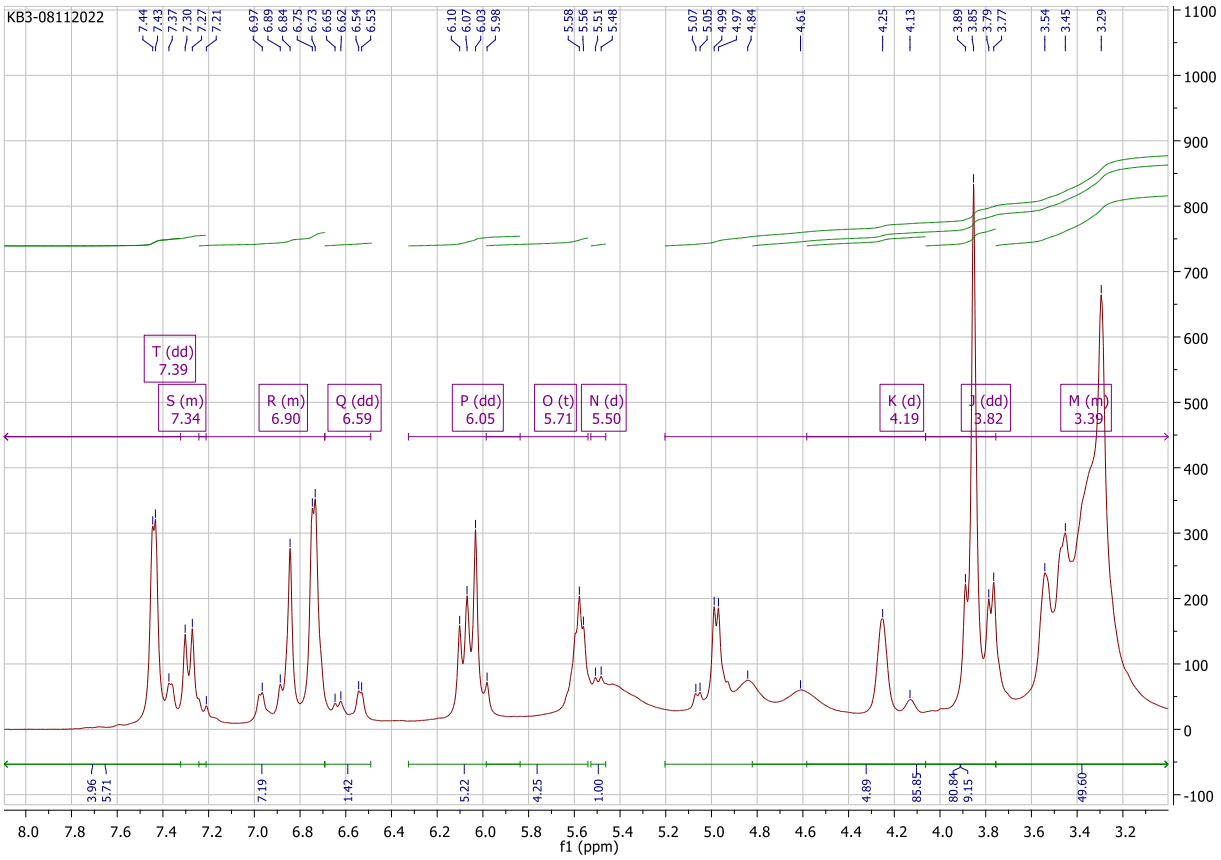
Appendice III: ^{13}C NMR of compound-2 (Aloenin B)



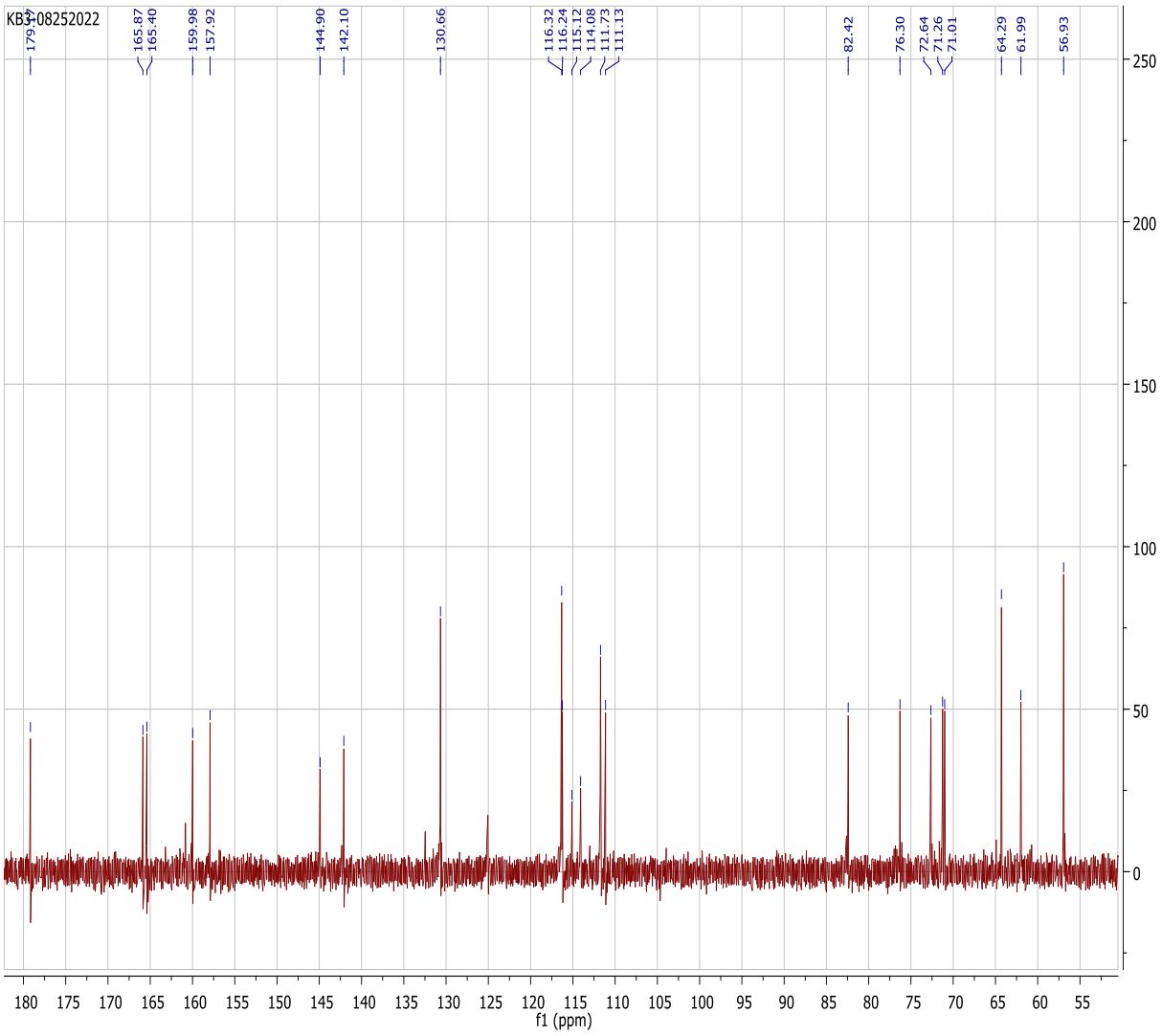
Appendice IV: Mass Spectrum of Compound-2 (Aloenin B)



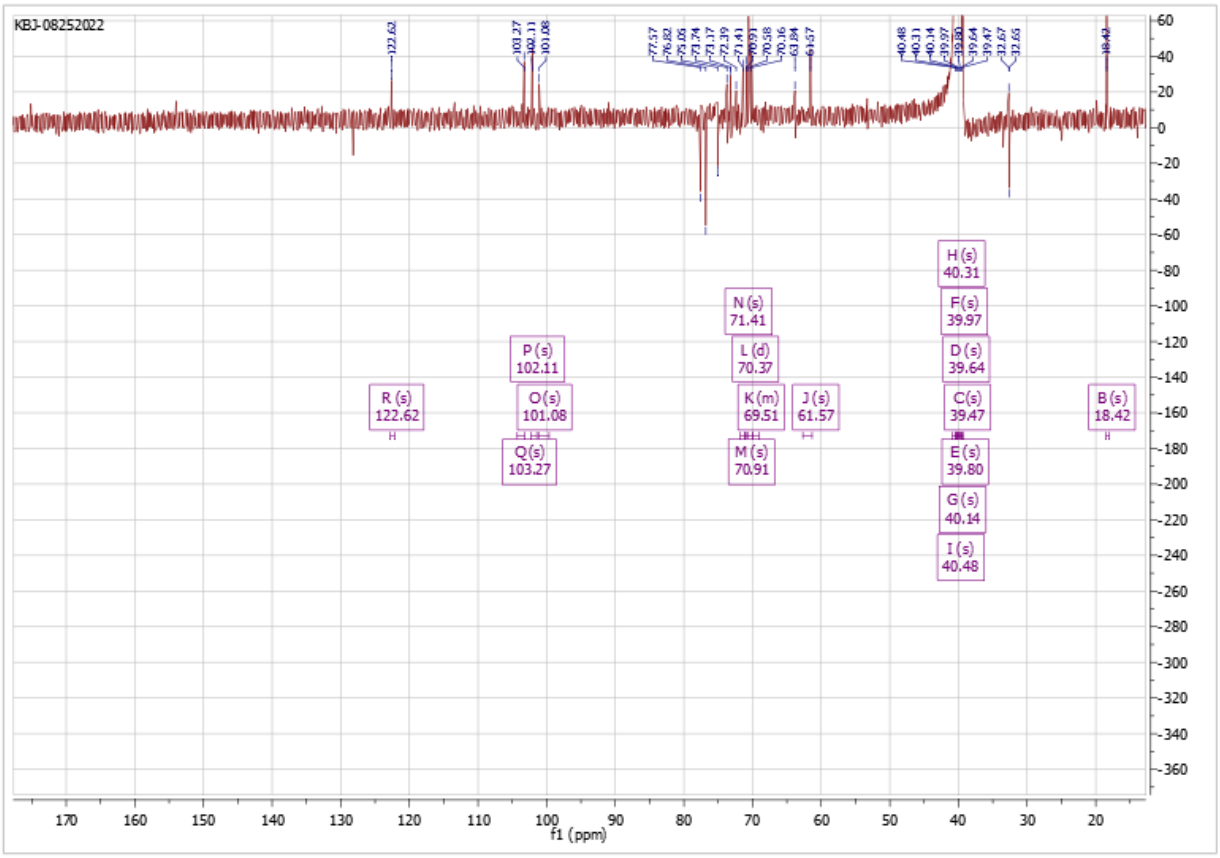
Appendice V: 1H NMR of compound-3 (Aloeresin D)



Appendice VI: ^{13}C NMR of compound-3 (Aloeresin D)



Appendice VII: DEPT-135 of compound-3 (Aloeresin D)



Appendice VIII: Mass Spectrum of Compound-3 (Aloeresin D)