**ADDIS ABABA UNIVERSITY**

**COLLEGE OF HEALTH SCIENCES**

**SCHOOL OF PHARMACY**

**DEPARTMENT OF PHARMACEUTICAL CHEMISTRY**

**AND PHARMACOGNOSY**

# *In vitro* Antileishmanial and Molecular Docking Studies of a Sesquiterpene Lactone from *Cirsium dender* Friis (Asteraceae)

**BY: Aklilu Samuel (B.Pharm)**

**OCTOBER, 2023**

**ADDIS ABABA, ETHIOPIA**

# *In vitro* Antileishmanial and Molecular Docking Studies of a Sesquiterpene Lactone from *Cirsium dender* Friis (Asteraceae)

**By: Aklilu Samuel (B.Pharm)**

**A Thesis Submitted to the Department of Pharmaceutical Chemistry and Pharmacognosy, School of Pharmacy, College of Health Sciences in Partial Fulfillment for the Requirements of Master of Science in Medicinal Chemistry**

**Advisor: Dr. Solomon Tadesse (PhD)**

**October, 2023**

**Addis Ababa, Ethiopia**

**ADDIS ABABA UNIVERSITY**

**SCHOOL OF GRADUATE STUDIES**

**DEPARTMENT OF PHARMACEUTICAL CHEMISTRY AND PHARMACOGNOSY**

This is to certify that the thesis conducted by Mr. Aklilu Samuel, entitled **"*In vitro* Antileishmanial and Molecular Docking Studies of a Sesquiterpene Lactone from *Cirsium dender* Friis (Asteraceae)"** and submitted to Department of Pharmacognosy and Pharmaceutical Chemistry, School of Pharmacy, College of Health Sciences, Addis Ababa University; in partial fulfillment for the requirement of Master of Science in Medicinal Chemistry compiles with the regulations of the University and meets the accepted standards originality and quality.

Signed by the examining committee:

| **Name** | **Signature** | **Date** |
|---|---|---|
| Advisor: Dr. Solomon Tadesse | _____ | _____ |
| Examiner (External): | _____ | _____ |
| Examiner (Internal): | _____ | _____ |

Addis Ababa, Ethiopia

October, 2023

## DECLARATION

I, the undersigned, declare that this thesis is my original work and has not been presented for a degree in any other university.

Name: Aklilu Samuel

Signature: _____

## Abstract

***In vitro* Antileishmanial and Molecular Docking Studies of a Sesquiterpene Lactone from *Cirsium dender* Friis (Asteraceae)**

By: Aklilu Samuel

Addis Ababa, Ethiopia

Leishmaniasis caused by genus of *Leishmania* has impact on the Africa and Asian countries due to high prevalence, resistance to current therapy and unavailability of standard medicine for the treatment. Asteraceae plants (a family widely used in folk medicine worldwide) are emerging as an interesting source for new leishmanicidal compounds and this study was aims to extract and isolate active metabolite from the root of *Cirsium dender* Friis (Asteraceae) to study molecular docking and to investigate *in vitro* antileishmanial activity against promastigote and amastigote forms of *L. aethopica* and *L. donovani.* Air dried roots of the plant was macerated with 80% methanol which resulted in 9.98% brown methanol extract. Phytochemical analysis was investigated by using thin layer and preparative chromatography which result in isolation of a white crystalline compound named AS01. Characterization of AS01 on the basis of APCI-MS, $^{13}$CNMR and $^{1}$HNMR spectral assignments and by comparing with reported data of the compound the AS01 was identified as dehydrocostus lactone. Antileishmanial activity of the extract and dehydrocostus lactone was studied on clinical isolates of *L. aethopica* and *L. donovani* and the $IC_{50}$ of the dehydrocostus lactone was 24.33μ/mL, 12.35 μ/mL against promastigote and 12.54 μ/mL, 3.29 μ/mL against amastigote of *L. aethopica* and *L. donovani* respectively. The compound dehydrocostus lactone and the crystalline

structure of pteridine reductase1 (PDB ID: 2XOX) extracted from *L.donovani*, which is a potential target for drug development against Leishmania, underwent a molecular docking study using the autodock vina and Pymol visualization tools. The result of the study discloses that the compound dehydrocostus lactone has antileishmanial activity and it has binding affinity to docking site of pteridine reductase1 enzyme.

**Keywords:** Anti-leishmanial, *Cirsium dender* Friis, Dehydrocostus lactone, L*eishmania aethopica* and *Leishmania donovani*

## Acknowledgments

First and foremost, I thank Almighty God. Next, I would like to express my deepest gratitude to my advisor Dr. Solomon Tadesse for his guidance, support, and constructive comments and to Mr. Alemu Tadesse for his support and comments.

I am deeply thankful to Dr. Daniel Bisrat and Mr. Biniam Paulos from the School of Pharmacy, Addis Ababa University for their advice, support during laboratory activities and compound isolation procedures. I also wish to thank Mr. Dawit Araya and Mr. Mulugeta Gichile for their technical support during antileishmanial activity test.

I would also like to acknowledge Wolaita Sodo University for sponsoring the study and Addis Ababa University for supporting my research work and graduate study.

Lastly, I would extend my heartfelt thanks to Mr. Semeyat Koyra for his valuable financial support during the study and to my family; friends; staff members of department of pharmaceutical chemistry and Pharmacognosy for their assistance.

## Table of Content

## List of Figures

## List of Tables

## List of Acronyms and Abbreviations

| | |
|---|---|
| **$^{1}$H NMR** | Proton Nuclear Magnetic Resonance |
| **$^{13}$C NMR** | Carbon thirteen Nuclear Magnetic Resonance |
| **2D NMR** | Two-dimensional Nuclear Magnetic Resonance |
| **AAU** | Addis Ababa University |
| **CHS** | College of Health Sciences |
| **CL** | Cutaneous Leishmaniasis |
| **COSY** | Correlation Spectroscopy |
| **DEPT** | Distortionless Enhancement by Polarization Transfer |
| **DoP** | Department of Pharmacology |
| **$IC_{50}$** | Half maximal inhibitory concentration |
| **LA** | *Leishmania aethopica* |
| **LD** | *Leishmania donovani* |
| **MCL** | Mucocutaneous Leishmaniasis |
| **MS** | Mass Spectrometry |
| **NMR** | Nuclear Magnetic Resonance |
| **NNN** | Novy-MacNeal-Nicolle |
| **PBS** | Phosphate Buffer Saline |

| | |
|---|---|
| **PCV** | Packed Cell Volume |
| **PDB** | Protein Database |
| **PKDL** | Post Kalaazar Dermal Leishmaniasis |
| **PTR1** | Pteridine Reductase 1 |
| **RBCs** | Red Blood Cells |
| **R*f*** | Retention Factor |
| **Rpm** | Revolution per Minute |
| **RPMI-1640** | Roswell Park Memorial Institute-1640 |
| **SNNPR** | Southern nations and nationalities people's region |
| **SoM** | School of Medicine |
| **SoP** | School of Pharmacy |
| **TLC** | Thin Layer Chromatography |
| **TMS** | Tetramethyl silane |
| **UV** | Ultraviolet |
| **VL** | Visceral Leishmaniasis |
| **WHO** | World Health Organization |

# 1. Introduction

## 1.1 Leishmaniasis

Leishmaniasis is a spectral disease, which is spread by the bite of phlebotomine sand flies (Torres-Guerrero, 2017). It is a global health problem, which is widely distributed in Asia, Africa, Mexico, Central America, and South America. Generally leishmaniasis is found in 98 countries with over 350 million people at risk (Farrell, 2002; Torres-Guerrero, 2017). About 95% of CL cases occur in Americas, the Mediterranean basin, the Middle East and central Asia and over 90% mucocutaneous leishmaniasis cases occur in Bolivia, brazil, Ethiopia and Peru (Torres-Guerrero, 2017; WHO, 2023).

According to the 2023 report by WHO, there is a significant global occurrence of leishmaniasis, with an estimated 50,000 to 90,000 new cases of visceral leishmaniasis (VL) and 600,000 to 1 million new cases of cutaneous leishmaniasis (CL) reported annually (WHO, 2023).

Based on the available mortality data, an estimated annual death toll of 20,000 to 40,000 can be derived for leishmaniasis. This estimation is in line with the overarching case-fatality rate of 10%, which is consistent with previous estimations provided by the World Health Organization (Alvar, 2012).

## 1.2 Etiology

Parasites of the genus Leishmania belong to the family Trypanosomatidae and to the order Kinetoplastid. During their life cycle these parasites present in two stages; promastigotes and amastigotes (Gossage, 2003). Promastigotes are elongated flagellated

cells which develop and multiply in the digestive tract of the vector whereas the amastigotes are round or oval shaped cells, with a very short flagellum that does not protrude from the flagella bag, they transform and multiply in the host's macrophages and are responsible for generating the disease (Gluenz, 2010).

Over 90 sand fly species can transmit more than 20 species of Leishmania to humans that result in different clinical symptoms as Cutaneous Leishmaniasis (CL), mucosal leishmaniasis (ML), Mucocutaneous leishmaniasis (MCL) Visceral Leishmaniasis (VL) and Post-kala-azar dermal leishmaniasis (PKDL) which is dermal sequela of visceral leishmaniasis as shown table 1 below (Postigo 2010; Reithinger 2007; Ready, 2014; Ganguly, 2010; David, 2009).

**Table 1 Human Pathogenic Leishmania species** (Reithinger et al., 2007; Boelaert et al., 2000; Ready, 2014)

| **Species** | **Clinical Manifestation** | **Distribution** |
|---|---|---|
| *L. donovani* | VL | Bangladesh, Nepal and East Africa<br>Northeast India Bangladesh, Sri Lanka |
| *L. braziliensis* | CL, ML | Mexico South and Central America |
| *L. panamensis* | CL, ML | Northern, Central and southern America |
| *L. peruviana* | CL | Peru |
| *L. guyanensis* | CL | South America |
| *L. lainsoni* | CL | South America |
| *L. colombiensis* | CL | Northern South America |
| *L amazonensis* | CL | South America |
| *L Mexicana* | CL | Central America, Mexico, USA |
| *L pifanoi* | CL | South America |
| *L venezuelensi* | CL | Northern and South America |
| *L garnhami* | CL | South America |
| *L aethiopica* | CL | Ethiopia, Kenya |
| *L killicki* | CL | North Africa |
| *L major* | CL | Central Asia, north and east Africa |
| *L tropica* | VL, CL | Asia, Africa |
| *L infantum* | VL, CL | North Africa, China , South America, SW Asia |

CL-Cutaneous Leishmaniasis; ML- Mucosal Leishmaniasis VL- Visceral Leishmaniasis

### 1.2.1 Cutaneous Leishmaniasis

Cutaneous leishmaniasis (CL) is most common form of infection and is characterized by the emergence of skin lesions, usually within several weeks or months of initial infection. Lesions develop into ulcer which are susceptible to secondary infection and last for months or years. The etiologic agents of CL include *L. donovani*, *L. tropica, L. infantum, L.aethiopica, L. major in the old world , L. mexicana, L. amazonensis, L. venezuelensis braziliensis, L. (Viannia), L. (Viannia) panamensis, L. (Viannia) guyanensis, L. (Viannia) peruviana and L. infantum* in the New World (Bailey & Lockwood, 2007; Reithinger et al., 2007).

### 1.2.2 Mucosal Leishmaniasis

Mucosal leishmaniasis (ML), which manifests from days to years after CL is the result of dissemination of parasites from the skin to the mucosal tissues in particular naso-oropharyngeal mucosa and it is caused by *L.(Viannia) braziliensis, L.(Viannia) panamensis, L. L.(Viannia) guyanensis and L. (Leishmania) amazonensis* (Strazzulla et al., 2013).

### 1.2.3 Visceral Leishmaniasis

Visceral leishmaniasis (VL) also known as kala-azar, which affects spleen, liver, and bone marrow is caused by the species *L. donovani* and *L. infantum* (Boelaert et al., 2000; Ready, 2014). VL encompasses a broad range of severity and manifestations and patients become gradually ill over a period of a few months, and nearly always die if untreated (Boelaert et al., 2000; Ready, 2014).

### 1.2.4 Post Kala-azar Dermal Leishmaniasis

Post-kala-azar dermal leishmaniasis (PKDL) is a complication of visceral leishmaniasis (VL) caused by *L donovani* and it is a syndrome characterized by skin lesions such as papules, erythematous and nodular rash in a patient who has recovered from VL (Zijlstra, 2016; Ganguly et al., 2010; Zijlstra et al., 2003; Zijlstra, 2016).

## 1.3 Life cycle of leishmaniasis

The life cycle of Leishmania, as shown figure 1 below, alternates between two main morphological forms: intracellular amastigotes in the mammalian host and motile promastigotes in the sand fly vector (Gossage et al., 2003; Killick-Kendrick, 1990; Rougeron et al., 2010).

The life cycle starts when a parasitized female sandfly takes a blood meal from a vertebrate host (e.g., a human) (Rougeron et al., 2010). During the sand fly takes a blood meal the promastigote form is injected and incubated into the skin of a mammalian host. It transforms into amastigote form after phagocytosed by tissue macrophage and survive within the macrophage. The amastigote start to develop and spread to other macrophage of the host cause mucosal and visceral disease and then the life is completed when a female sandfly ingests infected macrophages during a blood meal (Gossage et al., 2003; Killick-Kendrick, 1990).

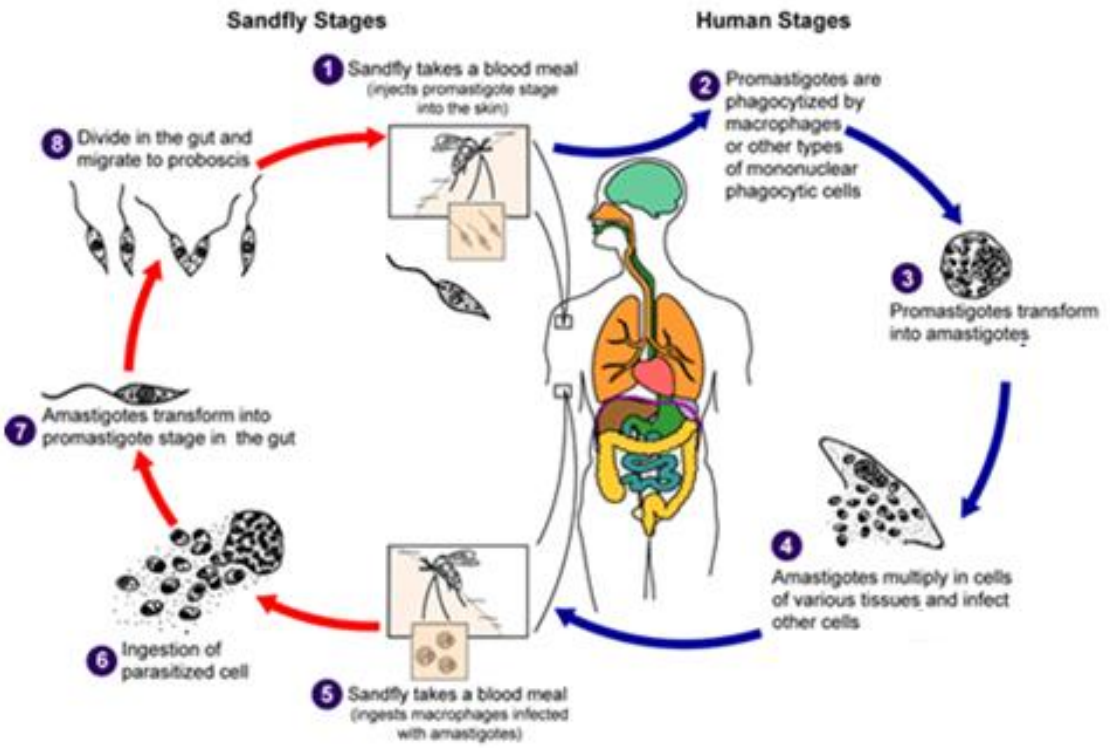



Figure 1: Life cycle of *leishmania* parasite (https://www.cdc.gov/dpdx/leishmaniasis)

### 1.4 Treatment of Leishmania

Leishmaniasis which is classified as a major tropical disease by World Health Organization has no effective vaccine against it and chemotherapy is the only effective way to treat all forms of disease (Monzote, 2009).

The primary treatment against leishmaniasis includes pentavalent antimonials (Wyllie et al., 2004), amphotericin B, pentamidine (Ramos et al., 1996; Yardley & Croft, 1997), miltefosine (Croft et al., 1987) and Paromomycin (Neal, 1968).

The drug of choice as the first-line treatment for VL in Ethiopia is a combination of sodium stibogluconate and paromomycin, which is WHO recommended combination

treatment regimen for VL caused by *L.donovani* in East Africa. In absence of paromomycin, Sodium stibogluconate and pentavalent antimonials are used as monotheraphy. Liposomal Amphotericin B is a recommended as first-line treatment for VL in special situations as patients with pregnancy, severe illness, HIV-co-infection, severe malnutrition, severe anemia and extremes of age (below 2 years or above 45 years). Miltefosine originally developed as an oral anticancer drug that has shown to have anti-leishmanial activity and taken orally at a dose of 2-3mg/kg per day (100mg/day for patients weighting more than 25kg) for 28 days (FMOH, 2013). Summary of Medicines used for the Treatment of Leishmaniasis are shown in table 2.

Table 2 Summary of Medicines used for the Treatment of Leishmaniasis (Moore & Lockwood, 2010; Matos et al., 2020; Haldar et al., 2011 and Al-Natour, 2009)

| **Medicine** | **Regimen** | **Adverse effects** |
|---|---|---|
| **Pentavalent antimoniales** | 20 mg/Kg/day for 30days | Anorexia , Lethargy Abdominal Pain |
| **Liposomal Amphotericin B** | 5mg/kg/day for 6days | High fever, thrombophlebitis, nephrotoxicity, hypokalemia |
| **Miltefosine** | 2-3mg/day for 28days (100mg/day for patient weighing more than 25kg) | anorexia, nausea, vomiting and diarrhea, allergy, teratogenic |
| **Paromomycin** | 15mg/kg for 17days | Reversible oto-toxicity, Renal and hepatotoxicity |
| **Sodium Stibogluconate** | 20mg/kg body for 30days | Muscle Pain, Joint Stiffness, Vomiting, Cardiac and Liver toxicity |
| **Pentamidine** | 2 to 4 mg/kg for 15days | Diabetes Mellitus, Hypotension |

### 1.5 Medicinal Plants used against Leishmaniasis

Many plants are used ethnobotanically to treat various ailments and their isolated chemicals showed promising pharmacological activity as antimicrobials, antiprotozoal, anticancer and this provides the scientific basis for the use of plants in treatment of diseases (Van Wyk & Prinsloo, 2020).

In Brazil medicinal plants such as *Ruta graveolens*, *Aloe vera*, *Chenopodium ambrosioides*, *Pfaffia glomerata* (Spreng.) and *Hyptis pectinata* are used to treat cutaneous leishmaniasis (De Queiroz et al., 2014).

Based on Traditional Persian medicine some plants that are effective against CL are *Vitis vinifera*, *berberis vulgaris*, *Rheum ribes*, *Santalum album*, *Cinnamomum camphora*, *Brassica nigra*, *Crocus sativus* and *Juniperus excelsa* (Parvizi et al., 2020). In the Saudi Arabia, plants used for dermatological disorders among which have antileishmanial activities are *Achillea biebersteinii Afan* of which the Leaves paste is applied; *Asparagus africanus Lam* of which the Leaves paste is applied; *Calotropis procera (Aiton) Dryand* of wich the latex is applied; *Commiphora gileadensis (L*.) C.Chr. of which Oleogum resin application topical in CL (Almoshari, 2022).

In Morocco, plants used for treatment leishmaniasis are *Lavandula dentate, Berberis hispanica, Cistus salviifolius, Crataegus oxyacantha, and Ephedra altissima* (Zeouk et al., 2020). And in Northern Nigeria the plant *Bauhinia reticulata* of which bark and leaves used effective in the treatment of CL (Jumare et al., 2022).

In Ethiopia, Amhara Regional State, “Azohareg” *Clematis hirsuta* Perr. & Guill of which fresh leaf is pounded and applied on the affected area with salt to treat Leishmaniasis (Yimam et al., 2022).

## 1.6 Compound Isolated from Plants with antileishmanial Activity

Two compounds di-2-ethylhexyl phthalate(**1**) and 1,4,5-trihydroxy-7-methoxy-2-methyl-anthraquinone(**2**) isolated from of *Drechslera rostrata* and *Eurotium tonpholium* showed activity against *L. major* with $IC_{50}$ of 3.2 and 10.38 µg/mL, respectively (Awaad et al., 2014).

Triterpenoids ursolic acid (**3**) and oleanolic acid (**4**) from the methanolic extract of *Pourouma guianensis* (Moraceae) showed activity against intracellular amastigotes ($IC_{50}$ of 27µg/ml and 11µg/ml, respectively(Torres-Santos et al., 2004). Triterpenoids ursolic acid (**3**) from methanol extract of the leaves of *Terminalia arjuna* Roxb (Combretaceae) demonstrate activity against promastigotes of *L. donovani* with $IC5_0$ value of 3.51µg /mL (Moulisha et al., 2010).

The *Plumeria bicolor* extract plumericin (**5**) and isoplumericin (**6**) showed activity against both promastigote and amastigote forms of *L. donovani*. Plumericin consistently showed high activity with the $IC_{50}$ of 3.17±0.12 and 1.41±0.03µM whereas isoplumericin showed the $IC_{50}$ of 7.2±0.08µM and 4.1±0.02µM against promastigote and amastigote forms, respectively (Sharma et al., 2011).

Jacaranone (**7**) from Asteraceae *Pentacalia desiderabilis* (Vell.) has activity against promastigotes of *L.chagasi, L.(V) braziliensis, and L. amazonensis* showing an $IC_{50}$ of 17.22, 12.93, and 11.86µg/mL respectively (Morais et al., 2012).

Mikanolide (**8**) and deoxymikanolide(**9**) which isolated from *Mikania variifolia* and *M. micrantha* were active on *L. braziliensis* promastigotes with $IC_{50}$ of 5.1 and 11.5µg/mL respectively (Laurella et al., 2017).

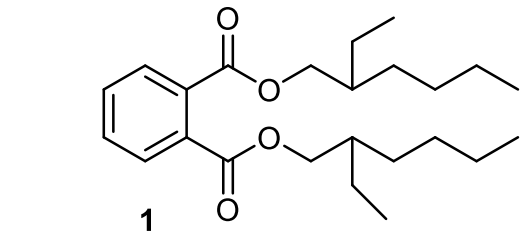
Psilostachyin(**10**) and peruvin(**11**) extract of *Ambrosia tenuifolia Sprengel (Asteraceae)* has $IC_{50}$ values of 0.12µg/ml and 0.39µg/ml against promatigote form of *Leishmania mexicana* respectively and the compounds are considered as lead molecules and may be potential candidates for novel therapeutics for the treatment Leishmaniasis (Sülsen et al., 2008).

Sesquiterpene elatol (**12**), the major constituent of the Brazilian red seaweed *Laurencia dendroidea* (Hudson) has $IC_{50}$ of 4.0µM and 0.45µM for promastigote and intracellular amastigote forms of *L. amazonensis* (Dos Santos et al., 2010). And another sesqueterpene Parthenolide (**13**) is a sesquiterpene lactone purified from aerial parts of *Tanacetum parthenium* showed significant activity against the promastigote form of *L. amazonensis* with $IC_{50}$ of 0.37µg/ml and 0.81µg/ml for the intracellular amastigote form (Tiuman et al., 2005).

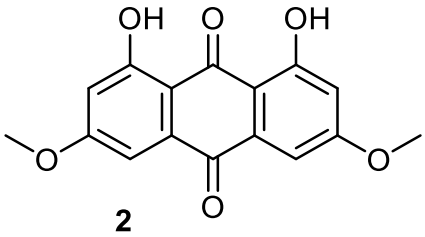
Lasidiol p-methoxibenzoate (**14**), a daucane sesquiterpene from *Eryngium foetidum* L. (Apiaceae) inhibit the growth of both *L. tarentolae* and *L. donovani* with $IC_{50}$ values of 14.33 and 7.84µM, respectively (Rojas-Silva et al., 2014).

Two sesquiterpene lactones calein C (**15**) and calealactone C (**16**) from leaves of *Calea pinnatifida* shows activity against *L. amazonensis* promastigotes with EC50 of 1.7 and 4.6 µg /mL, respectively (Caldas et al., 2019).

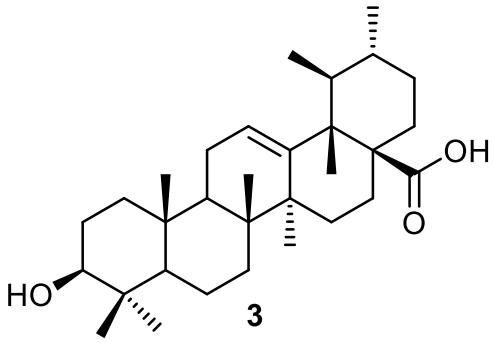
Natural guaianolid dehydrozaluzanin (**17**) petroleum ether extract of the leaves of *Munnozia maronii* was found to inhibit in vitro the growth of promastigote forms of *Leishmania* with an $IC_{50}$ of 25 pg/mL (Fournet et al., 1993).



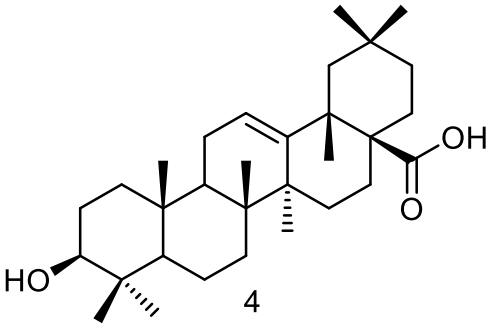

**1**



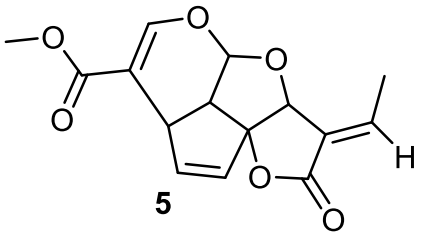

**2**



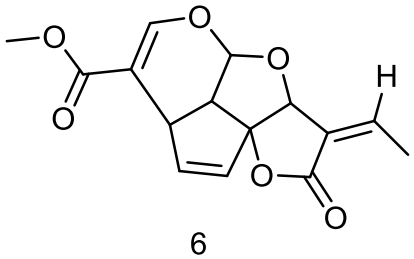

**3**




4




**5**




6

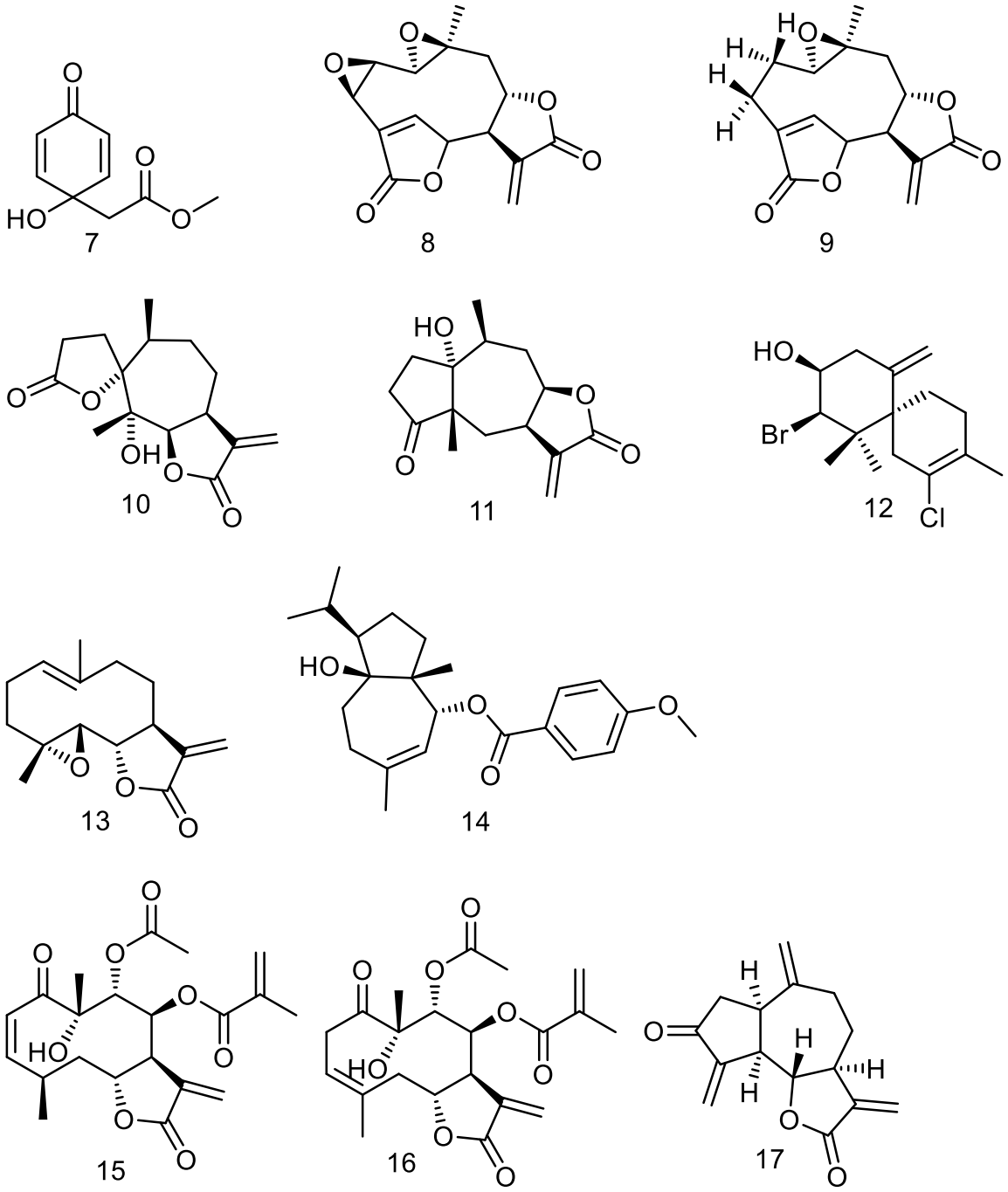



Figure 2: Compound Isolated from Plant with antileishmanial Activity

## 1.7 Ethnobotanical and Pharmacological Activities of the Genus Cirsium

*Cirsium* is a genus of annual, perennial or biennial flowering plants which is monoecious and rarely dioecious in the family of Asteraceae, commonly known as plume thistles (Luo et al., 2021). According to the Plants of the World online collections at the Royal Botanic Gardens of Kew, there are around 450–480 recognized species in this genus (Aggarwal et al., 2022; Powell et al., 2011).

It is distributed across the world in Asia, Central America, Europe, North Africa, and North America, Ethiopia, Kazakhstan, Siberia China, Afghanistan Nepal, Pakistan, Myanmar, Vietnam, West Himalaya Korea Italy and Arabian Peninsul and Japan (Del Guacchio et al., 2022). Distribution and ethinobotanic uses of Cirsium Species for the different diseases are shown below in table 3.

**Table 3: Ethnobotanical and Pharmacological Activities of the Genus Cirsium**

| **Crisium Species** | Part used | Ethinobotanic uses and pharmacological activity | Reference |
|---|---|---|---|
| *C. setidens* | Root | Pyrexia, detoxify and improve blood circulation | Lee et al., 2006 |
| *C. japonicum* | Root | Anti-inflammatory, diuretic, cancer, Hemorrhages, hypertension and hepatitis | Miyazawa et al., 2003<br>Lee et al., 2020 |
| *C. falconeri* Hook.f., | Root | Arthritis | Chakraborty et al., 2017 |
| *C. verutum* D. Don | Leaf<br>Root | Gastrointestinal disorder and Rheumatism | Singh et al., 2009; Singh & Borthakur 2011 |
| *C. tenoreanum* | Root | Treatment of Varicose | Loizzo et al., 2004 |
| *C. arvense (L.) Scop.* | Leaf | Pharyngitis, astringent, tumor, diuretic, toothache | Guarrera, 2005<br>Aggarwal et al., 2022 |
| *C. rivulare* | Root | anxiolytic effects | Aggarwal et al., 2022 |
| *C. vulgare* (Savi) Ten | Flower head | Boils (furuncles), Anxiolytic | Karpavičienė, 2022<br>Arya & Parmar, 2009 |
| *C. wallichii* | Root | Pyrexia, bleeding, inflammation | Aggarwal et al., 2022 |
| *C. englerianum* O. Hoffm. | Root | Dermal infections, snake bite, cough, intestinal parasite | Bibiso et al., 2021<br>Chekole, 2017 |
| *C. dender* Friis | Root | “mich” | Luizza et al., 2013 |

### 1.7.1 *Cirsium dender* Friis

#### 1.7.1.1 Botanical description

*Cirsium dender* Friis (1975 )(figure 3) is a 3m tall species of herb in the family Asteraceae, native to Ethiopia and First published in Norwegian Journal of Botany (Friis & Bidgood, 1998; IPNI, 2023).

**Figure 3: Photograph of *Cirsium dender*** Friis

#### 1.7.1.2 Ethnobotanical Uses

The root of the *Cirsium dender* Friis (in local language Oromiffa called Yehaheya means "donkey"; traditional healer call "borsa" in wolaitigna) being chewed to alleviate symptoms of "mich"; which is unknown diseases with uncertain causes, characterized by fever, headache, sweating, swelling and muscle spasms (Luizza et al., 2013).

## 1.8 Statement of Problem

Current therapies for the leishmaniasis have serious shortcomings mainly due to extensive toxicity, emerging resistance and variation in efficacy based on species and strain of the Leishmania parasite and there is a high unmet medical need to improve diagnosis, control the spread of disease and to develop novel drugs and identify new chemical starting points for drug discovery to tackle the disease ( Chem, 2013; Zulfiqar & Avery, 2022). Because of the drawback of current drug the search for new leishmanial compound is necessary and the use of medicinal plant is alternative to achieve the goal.

## 1.9 Significance of Study

In traditional medicine dried root of *Cirsium dender* Friis are chewed for alleviating "*mich*" (Luizza et al., 2013). But in this study methanol extract , hexane extract and isolated compound was evaluated for antileishmanial activity as Asteraceae plants (a family widely used in folk medicine worldwide) are emerging as an interesting source for new leishmanicidal compounds (Moraes Neto, 2019).

The present study of the isolation of compound and antileishmanial activity of the root extract of *Cirsium dender* Friis and its isolated compound against the promastigote and amastigote of *L. donovani* and *L. aethopica* provide important information on both the chemistry of the plant and its antileishmanial activity and the finding will provide information for those who are interested in antileishmanial activity of chemicals from plant origin.

## 2. Objectives

### 2.1 General objectives

The main objective of this study is to isolate chemical constituents from the root of *Cirsium dender* Friis and screen for antileishmanial potency of methanol extract and isolated compound

### 2.2 Specific objectives

- To investigate the *in vitro* anti-amastigote activity of the methanol extract of *Cirsium dender* Friis
- To determine the *in vitro* anti-promastigote activity of the methanol extract of *Cirsium dender* Friis
- To evaluate the *in vitro* anti amastigote activity of isolated compound
- To investigate the *in vitro* anti promastigote activity of isolated compound
- To isolate major compound(s) from the active extract
- To interpret structure(s) of the isolated compound
- To determine $IC_{50}$ value for methanol extract
- To determine $IC_{50}$ value for isolated compound
- To perform molecular docking study on isolated compound

# 3. Materials and Methods

## 3.1 Materials

### 3.1.1 Plant Materials

The roots of *Cirsium dender* Friis were collected in March 2022 from Matala Walana Kebele Boloso Bombe Woreda, Wolaita zone of SNNP regional state which is located in South West direction and 345km far from the capital city Addis Ababa. The plant was then authenticated and identified by Mr. Melaku Wondafrash a botanist at College of Natural and Computational Sciences, Addis Ababa University (AAU), where a botanical specimen with voucher number AS005/2022 was deposited for further reference.

### 3.1.2 Instruments and Supplies

Instruments and supplies used in the study include analytical balance (Mettler Toledo, Switzerland), analytical TLC plates silica gel coated (60 F254, 0.2 mm thick, Merck KGaA, Darmstadt, Germany), Whatman filter paper No 1(Sigma-Aldrich), Nylon filter cloth, Rotary evaporator (BUCHI Rotavapor™ R-300, Switzerland), Tissue Drying Oven (Meditec-Medizintechnik, Germany), UV Cabinet (designed for inspecting thin-layer chromatograms), UV Lamp (254nm and 366nm), Preparative TLC (Glass-Backed, Silica, 1000µm, 20x20cm, F254), MS, $^{1}$HNMR, $^{13}$CNMR and analytical TLC were used for the extraction, purification, isolation and identification of compound.

Polystyrene sterile tissue culture flasks (50ml) (Corning incorporated, USA), Chamber slides (16 wells) (Nalge Nunc international, USA), 96 well Microtiter plates (Nalge Nunc international, USA), Autoclave machine (Timo, Italy), Biosafety cabinet with UV

(Laboculture ESCO class IIA), Centrifuges, eppendorf 5804R, carbon dioxide incubator (Thermo Electron), Digital water bath (Julabo TW20), Hemocytometer (Improved Double Neubauer type), Olympus inverted type light microscope (Shinjuku, Tokyo, Japan), table-top vacuum filter (micropore, Brazil), Micro pipettes (Pipetman ultra), multichannel pipettes (Hamilton), Microplate reader (VICTOR3 Perkin Elmer), pipette tips and its rack were used for biological assay.

### 3.1.3 Chemicals and Reagents

n-Hexane, ethyl acetate, petroleum ether, methanol, Chloroform and distilled water, trisodium citrate (BDH Chemicals Ltd, England), Giemsa (ESJAY Chemicals, Maharashtra, India), resazurin sodium salt(Sigma-Aldrich, Germany), dimethyl sulfoxide (DMSO), triton X-114 and potato starch powder (Sigma-Aldrich Laborchemikaien GmbH, Germany), phosphate buffer saline (PBS) (Gibco, USA), NaOH (Ranchem Industry, Turkey) and HCl (Loba Chemie, India), $Na_2CO_3$ (Loba Chemie - India), gallic acid (MerCK, Germany), Folin–Ciocalteu's solution, quercetin (Sigma Aldrich - Germany), $AlCl_3$ (Loba Chemie - India), $NaNO_2$, Bromocresol green (Sisco research laboratories, India), calcium chloride, potassium chloride, sodium bicarbonate, sodium chloride(all from sigma Aldrich USA), $Na_2HPO_4$(BDH Chemicals, England), citric acid (Avonchem, UK) were of analytical grade.

### 3.1.4 Culture Media

The culture medium and supplements include: minimum essential medium (MEM), heat inactivated new born calf serum (HINBCS), penicillin streptomycin solution, (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid) (HEPES), medium -199 with Earle's salts

(M199), nutrient agar (all from Sigma-Aldrich), USA) and Roswell Park memorial institute (RPMI-1640) (Sigma-Aldrich, UK) were used to prepare complete medium. D (+) glucose (anhydrous), and defibrinated and heat inactivated sheep blood were used to prepare NNN (Novy-MacNeal-Nicolle) media and Locke's solution.

### 3.1.5 Test Organism

Clinical isolates of *L. aethopica* (CL-068/22) and *L. donovani* (VL-029/22) were acquired from Leishmaniasis Research and Diagnostic Laboratory (LRDL), for the department of Microbiology, Immunology and Parasitology (DMIP), College of Health Science (CHS), School of Medicine (SoM), Addis Ababa University (AAU).

The parasite strain of *L. aethopica* (CL-068/22) was isolated from 33 years old male patient who lives in Arsi Zone, Oromia region, Ethiopia and *L. donovani* strain (VL-139/19) was isolated from 23 years old male patient who lives in Amhara region, Ethiopia. Red Blood Cells were collected from healthy 28 years old male volunteer with no underlying chronic disease.

### 3.1.6 Reference Drug

Amphotericin B (Laborchemikaien GmbH, Germany) was used as reference drug.

### 3.1.7 Experimental Animals

White Swiss Albino mice of either sex weighing 22 - 30g and age 5 - 6 weeks were obtained from Laboratory Animal House, Department of Pharmacology and Clinical Pharmacy, School of Pharmacy, College of health science, Addis Ababa University. The

animals were held in mice cages at room temperature and a 12h light/12h dark cycle. They were provided with water and food pellets ad libitum in the animal house of the School of Pharmacy, College of health science, Addis Ababa University.

All the experiments were conducted in accordance with Guide for the Care and Use of Laboratory Animals (Clark et al., 1997) and were approved by the institutional Review Board of the School of pharmacy, AAU with reference number ERB/SOP/545/15/2023.

## 3.2 Methods

### 3.2.1 Extraction of the Root

The roots of *Cirsium dender* Friis were washed with tap water to remove dust and any other debris present on it. Then roots were air dried under a shaded area at room temperature and crushed using a mortar and pestle to get a coarse powder. A total of 450g coarsely powdered root was macerated with 80 % (v/v) methanol accordance with methods by International Centre for Science (Handa, 2008). The contents were shaken manually and allowed to remain within the solvent. After 72hours, the extract was filtered first using nylon filter cloth and then by Whitman filters paper No 1. The marc was re-macerated twice using the same solvent to exhaustively extract the plant material. The combined filtrate was concentrated under vacuum using Rotary evaporator at a 38°C and dried in Tissue Drying Oven at 38°C to yield 44.67g (9.93%) of methanol extract (Azwanida 2015; Singh 2008).

### 3.2.2 Isolation of a Compound

The resulting dry methanol extract obtained by maceration was fractionated by using different solvent system hexane, ethyl acetate, chloroform, methanol and water. From the hexane fraction, which has antileishmanial activity; the compound was isolated by preparative thin layer chromatography with solvent system of petroleum ether and ethyl acetate in the ratio of 4 to 1 as a mobile phase. The major band was scrapped off from the plate and wash with chloroform filtered using Whitman no. 1 and concentrated to dryness result in white crystalline solid and the purity of isolated compound monitored by TLC which was visualized using UV light of wave lengths 254 nm and 366 nm.

### 3.2.3 Spectroscopic Analysis

NMR spectral data were obtained at room temperature on FT-NMR spectrometer operating at 400 MHz for $^{1}H$ and 100 MHz for $^{13}C$ using deuterated dimethyl sulfoxide. A region from 0 to 12ppm for 1H and 0 to 205ppm for 13C was employed for scanning. Signals were referred to an internal standard tetramethylsilane (TMS). Chemical shifts are reported in δ units and coupling constants (J) in Hz. Multiplicities of 1H NMR signals are indicated as broad singlet(brs), doublet (d), doublet of doublet (dd), triplet (t), triplet of doublet of doublet (tdd) and multiplet (m).

### 3.2.4 Antileishmanial Assay

#### 3.2.4.1 Promastigote Cultures

The parasites which were clinically isolated *L. aethopica* and *L. donovani* were cultured in Lock's treated Novy-MacNeal-Nicolle (NNN) medium containing antibiotic solution

(penicillin 100 IU/ml and streptomycin 100 µg/ml)(Magill et al., 2012). The logarithmic stage parasites were transferred from NNN media into tissue culture flasks containing complete RPMI-1640 medium (RPMI-1640 medium supplemented with 10% HIFCS and 100 IU penicillin/ml-100µg/ml streptomycin solution) at 22℃ for *L. aethopica* and 26℃ for *L. donovani* (Barati et al., 2014). The growth of parasite monitored every day by inverted light microscope to confirm growth of parasites. The logarithmic phase of the parasites was used for antipromastigote assay, while stationary phase (metacyclic phase) was used for macrophage infection (antiamastigote assay).

#### 3.2.4.2 Antipromastigote Assay

Antipromastigote activities of extracts and isolated compound were done as methods previously described by Ketema and Tariku et al (Ketema, 2023; Tariku, 2010) with slight modification. To 96-well plates filled with 100µl of complete culture medium (RPMI-1640), 100µl of test substance (200µg/ml) dissolved in 1% DMSO was added on the first well. Then, 100µl was taken into subsequent wells then the last 100µl was discarded, to achieve serial dilution from 200µg/ml to 1.56µg/ml.

Then 100µl of suspension of parasites ($3.5x10^{-6}$ promastigotes/ml of *L. aethopica* or *L. donovani)* were added to each well and contents of the plates were incubated at 22℃ for *L. aethopica* and 26 ℃ for *L. donovani*. After 68 h of incubation 20µl of fluorochrome resazurin solution (0.125mg/ml, PH=7.2) was added to each well and incubated accordingly for additional 4 hrs. After that fluorescence intensity was measured by a Multilabel Reader at an excitation wavelength of 530 nm and emission wavelength of 590 nm.

Assay with DMSO 1% and AMB (same serial dilution as the test substances) were conducted as negative and positive control respectively. Blank well containing complete RPMI-1640 medium was used to monitor background fluorescence intensity of resazurin and the average value of absorbance was subtracted from every well. The Antipromastigote activity of the extract and isolated compounds were expressed as percent of parasite inhibition and determined by using the following formula and then $IC_{50}$ was calculated (Ketema, 2023; He et al., 2016).

$$\%\text{inhibition} = 100 - \frac{ODt - ODb}{ODn - ODb} * 100$$

Where: ODt-Absorbance of triplicate of test substance; ODb-Average absorbance of blank and ODn -Average absorbance of media

#### 3.2.4.3 Macrophage Collection and Culture

Six to eight weeks old Swiss albino mice were injected into the peritoneal cavity with 2% freshly prepared starch (Sigma-Aldrich, Co., St. Louis, USA) and 10mL of sterile ice-cold phosphate-buffered saline (PBS) (Sigma, Co., St. Louis, USA) supplemented with 3% HINBCS after 2days, and 6–8mL exudates were recovered. The Contents were centrifuged at1500 rpm for 10 min and macrophages were resuspended in an essential medium (MEM) (Sigma-Aldrich, Co., St. Louis, USA) containing 10% HINBCS, 25mM HEPES, 2mM L-glutamine and 100IU/mL penicillin and 100µg/mL streptomycin. The cells were counted and adjusted to $3.5\times10^6$ cells/ml in complete MEM medium by using hemocytometer.

Macrophages were cultivated in 25cm$^3$ cell culture flasks with filter cap containing 10ml of complete RPMI 1640 medium at 37°C under 5%$CO_2$ humidified air and then macrophages were allowed to reach a high density population of about 70% confluence.

### 3.2.4.4 Axenic Amastigote Assay

*In vitro* cell culture method for biological evaluation of antileishmanial activity of methanol extract , hexane extract and isolated compound against intracellular Leishmania spp. amastigotes by using published methods with slight modification (Corral et al., 2013; Koutsoni et al., 2019; Riss et al., 2016).

Mouse peritoneal macrophages suspended in complete MEM medium ($3\times10^5$cells/ml, 200µl) were seeded in white 96-well plates and incubated for 24 hours at 37$^o$C in 5% $CO_2$ to promote cell adhesion. After 24 hours the plates were washed with pre-warmed complete medium to remove non-adherent macrophages. The stationary stages of *L. donovani* and *L. aethopica* promastigotes (leishmania to macrophage ratio; 10:1) were seeded in the plates containing adherent macrophages and maintained at 31$^o$C (*L. aethopica*) or 37$^o$C (*L. donovani*), 5% $CO_2$ and 95% relative humidity for further 24 hours to allow infection and amastigote differentiation.

Then, non-internalized promastigotes were removed by extensive washing three times with prewarmed MEM medium to remove remaining extracellular promastigotes and serially diluted concentrations of methanol extract extracts, hexane extract and isolated compound (1.5 - 200µg/ml), Triplicates of reference drug, negative controls in triplicates were added the wells was filled with complete RPMI-1640 medium until the final volume

of 200µl per well and incubated for further 48 hours at 37 °C under 5% $CO_2$ humidified air.

Finally, culture supernatants was removed with a multichannel pipette and macrophages' membranes was disrupted by adding 50µl of lysis solution, containing ATP and luciferin to each well; which initiates the enzymatic reaction of luciferin to oxyluciferin catalyzed by luciferase expressed by metabolically active amastigotes. After 20 min incubation of the plate in a non-inverted position at room temperature, and cell viability was measured fluorometrically (excitation λ, 550 nm; emission λ, 590 nm) (Shimony & Jaffe, 2008). The results were expressed as the percentage of reduction in the parasite burden and it was computed by using the following formula and then $IC_{50}$ was calculated (He et al., 2016).

$$\%\text{inhibition} = 100 - \frac{ODt - ODb}{ODn - ODb} * 100$$

Where: ODt-Absorbance of triplicate of test substance; ODb-Average absorbance of blank and ODn -Average absorbance of media

#### 3.2.4.5 *In vitro* Haemolysis Test

Haemolytic activity of methanolic methanol extract , hexane extract and the isolated compound was determined by using RBCs which was prepared from 2ml blood collected from $O^+$ individual and added to of 48ml of PBS then centrifuged at 3500 rpm for 10 min at 4℃ and washed with PBS results 1ml of RBC pellets. By adding 49ml of PBS to 1ml RBC pellets 2% RBC suspension was prepared and the concentration was adjusted to $1.9x10^9$ RBC/ml.

Serially titrated (95.24, 47.62, 23.81, 11.905, 5.9525, 2.9585, 1.488 and 0.724/ml) methanol extract , hexane extract and isolated compound were mixed with 2% blood suspension in eppendorf tubes and incubated at 37°C for two hours with Triton X-14 (5µL/mL) (Sigma-Aldrich, Co., St. Louis, USA) and 2.5% DMSO as positive and negative controls. The mixture was then centrifuged at 1000 g for 10 min. 75µL of the resulting supernatant were transferred to 96-well plates In column 1, 2 and 3 for methanol extract; column 4, 5 and 6 for isolated compound; column 7, 8 and 9 for hexane fraction; column 10 for blank (RBC suspension only), column 11 for triton X114 (positive control) and column 12 for DMSO (negative control). The free hemoglobin in the supernatant was measured in UV-Vis spectrophotometer at 540nm. Each experiment was performed in triplicates at each concentration. Percentage of hemolysis by substances calculated by using the following formula:

$$\%\text{Hemolysis} = \frac{ODt - ODnc}{ODpc - ODnc} * 100$$

Where: ODt-Absorbance of triplicate of test substance; ODb-Average absorbance of blank and ODn -Average absorbance of media

### 3.2.5 Molecular Docking Study and *in silico* Prediction

*In silico* prediction of the compound dehydrocostus lactone drug likeness properties by ADMET lab 2.0 online software tools (Armaković & Armaković, 2023) and molecular docking study of the compounds was carried out by using Crystal structure of pteridine reductase 1 (PTR1)(PDB ID: 2XOX) from Leishmania donovani(Barrack et al., 2011) and computer applications AutoDock MGL Tools, AutoDock Vina and Pymol visualization tool.

### 3.2.6 Statistical analysis

Anti-promastigotes and anti-amastigotes activity, which expressed as $IC_{50}$ of the methanol extract, hexane extract and isolated compound was analyzed by using Graphpad prism 9.3.0 and Microsoft Excel 2010 and the result was reported mean ± standard error. All the analyses were carried out at 5% level of significance.

*In silico* prediction of the compound dehydrocostus lactone drug likeness and molecular docking study of the compounds was carried out by AutoDock MGL Tools, AutoDock Vina and Pymol visualization tool and result expressed as affinity energy toward docking site of enzyme.

### 3.2.7 Ethical Clearance

All procedures conducted in this research work, including use of Vero cell lines were reviewed and approved by Ethical Review Committee, School of Pharmacy, College of Health Sciences; Addis Ababa University with letter number ERB/SOP/545/15/2023 dated June 23, 2023. Informed consent was obtained from volunteer man to use the collected red blood for research purpose.

## 4. Result and Discussion

### 4.1 Extraction yield

Four hundred fifty gram of air dried root of *Cirsium dender* Friis was macerated with 80% methanol and which gave 44.65gm (9.93%) brown methanol extract as plant secondary metabolite.

### 4.2 TLC Analysis of Extract

Antileishmanial activity demonstrated by the methanolic extract of *Cirsium dender* Friis and hexane extract was further subjected to phytochemical analysis to identify the compound(s) responsible for bioactivity. Analytical TLC of hexane extract using PE: EA (4:1) solvent system showed the presence of more than 5 compounds in hexane extract (Figure 4).

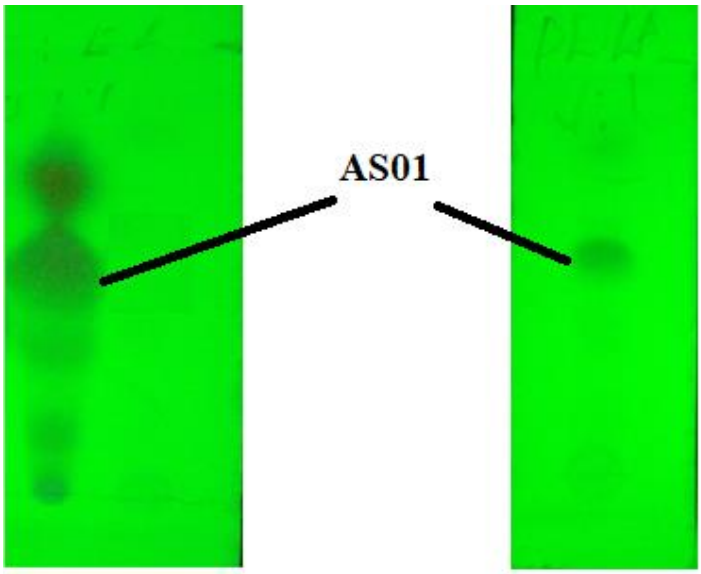



Figure 4: TLC chromatogram of hexane extract of *Cirsium dender* Friis extract and isolated compound AS01 in PE: EA (4:1) viewed under UV light of 254nm

Solid yellow precipitates formed in hexane fraction, which were decanted and isolated by PTLC using PE and EA in the ratio of 4 to 1 as eluent solvent. The compound designated AS01 was isolated and appeared dark when viewed under 254-nm UV radiation, but was not visible at 366 nm. AS01 is white solid in the form of crystals $R_f$ = 0.54 in the PE/EA (4:1) system.

### 4.3 Characterization of compound AS01

AS01 was obtained as a white crystalline powder with an Rf value of 0.54 in petroleum ether: ethyl acetate (4:1) solvent system. Compound was subjected to GC-APCI-MS analysis, resolution (figure 5). The mass spectrum showed MH+ ion, a pseudo molecular ion (M+1) peak at 231.1 m/z.

According to the NIST database and PubChem, 2023 national library of medicine, the weight and pattern of fragmentation correspond to the compound DHCL whose molecular formula is $C_{15}H_{18}O_2$ (figure 10). The exact calculated molecular mass was found to be 230.1306798220 amu which corresponds to a relative molecular formula of $C_{15}H_{18}O_2$ (Lemmon et al., 2011).

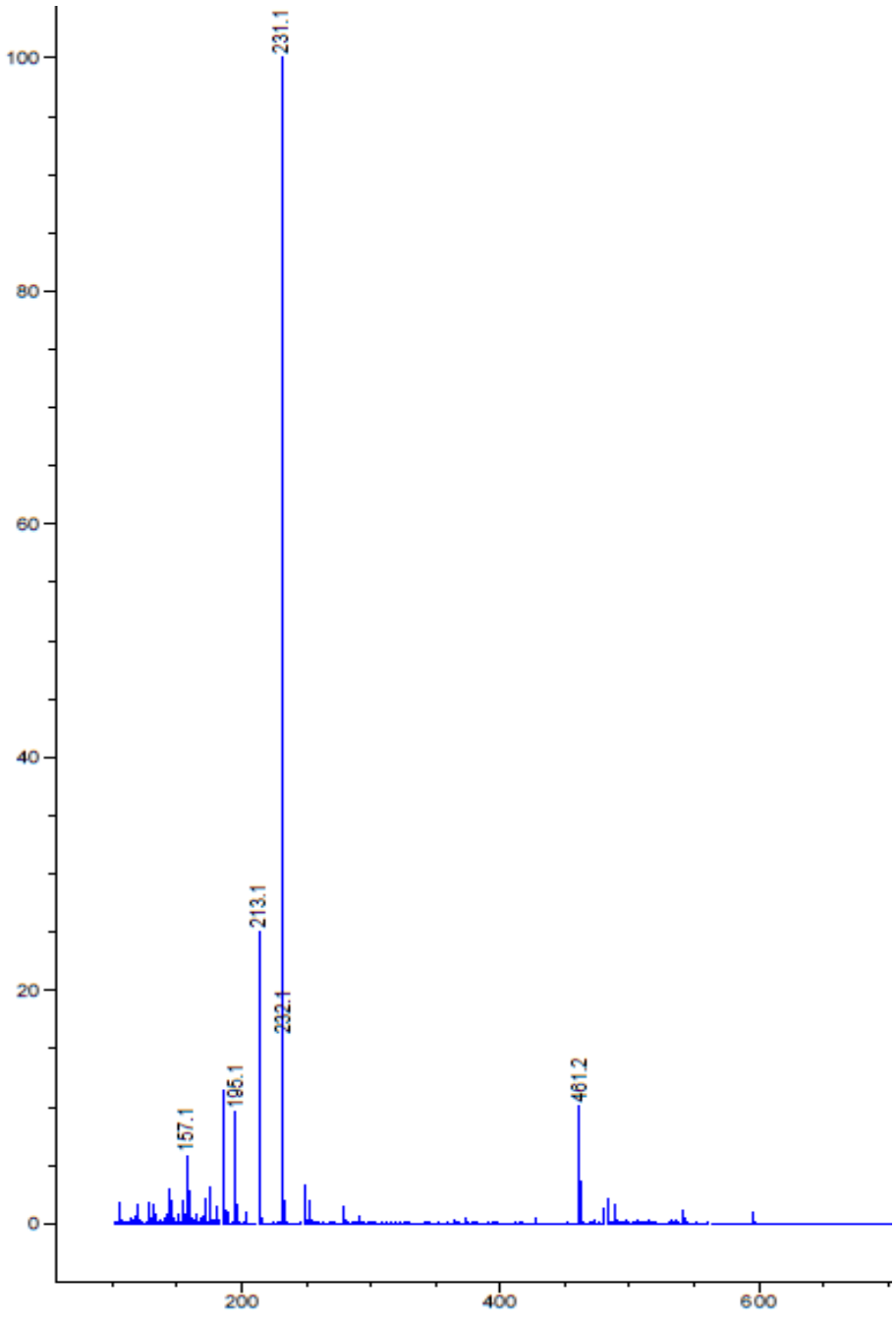



**Figure 5: APCI mass spectrum of AS01**

The $^{1}$H-NMR (500 MHz, DMSO) spectrum (figure 6) of compound AS01 showed signal at δ 3.94(1H, t, J=9.2) assigned for oxygenated methane proton at C-6 and coupled to triplet by neighboring protons at C-5 and C-7 each at δ 2.86(1H, m) and δ 2.99(1H, m)

respectively. Signals at δ 5.08(1H, m) and δ 4.98(1H, m), δ 4.86(1H, s) and δ 4.74(1H, s) and δ 6.04(1H, d) and δ 5.64(1H, d) confirms the presence of three different methylene bonds each attached with different protons. The methylene protons at δ 6.04 and δ 5.64 coupled each other as indicated by doublet.


**Figure 6: $^{1}$H-NMR spectrum of AS-01**

The $^{13}$C-NMR spectrum and DEPT 135 (figure 7 and figure 8) of compound AS01 showed there are four quaternary carbons at δ152.15, 149.77, 139.86 and 169.62 for C-4, C-10, C-11 and C-12 respectively. There are seven methylene carbons at δ 29.57, 32.2, 30.34, 39.91, 119.99, 111.95 and 108.27 for C-2, C-3, C-8, C-9, C-13, C-14 and C-15. The remaining tertiary carbons C-1, C-5, C-6 and C-7 are at δ 43.95, 51.53, 84.81 and 46.58, respectively.

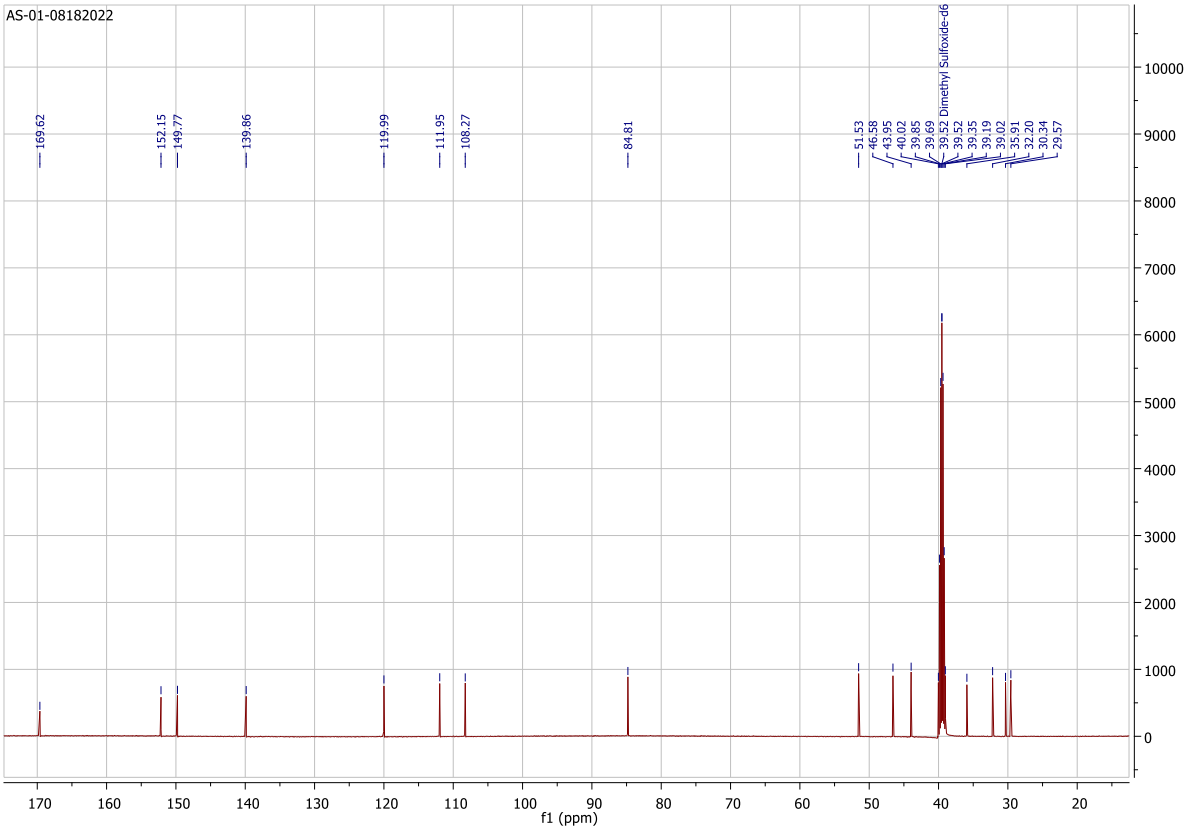



**Figure 7: $^{13}$C-NMR spectrum of AS01**

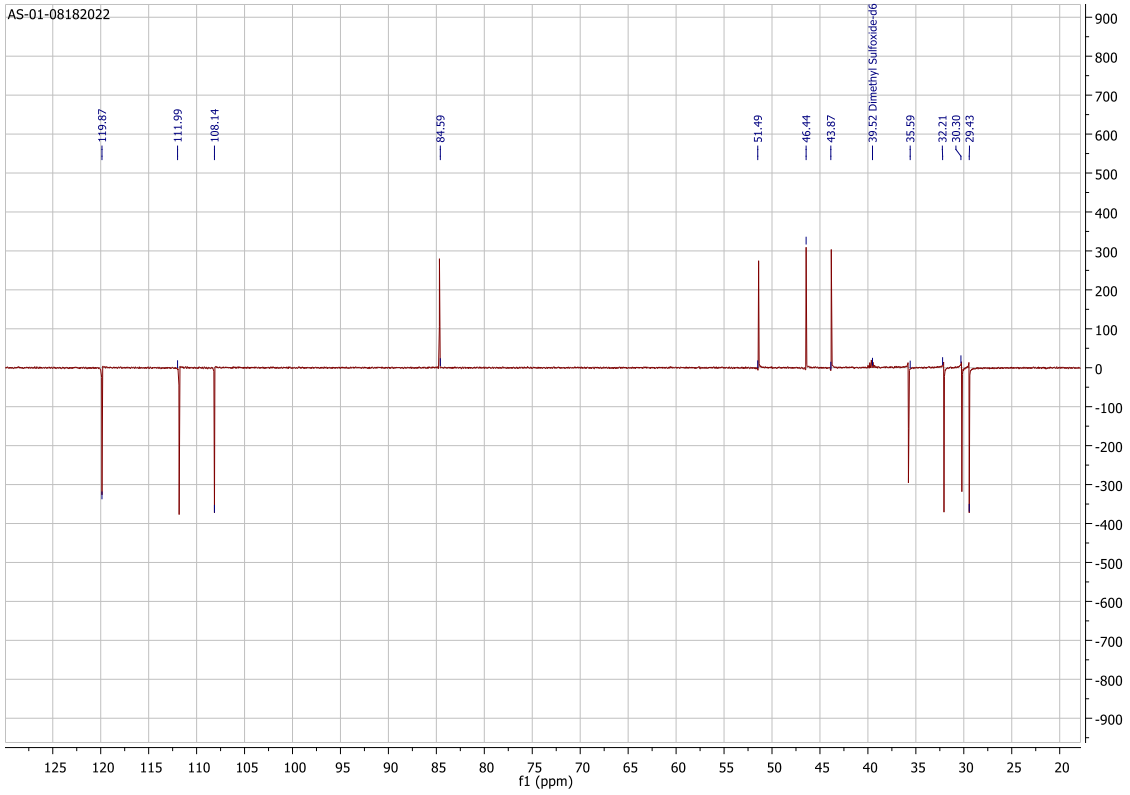



**Figure 8: DEPT-135 spectrum of AS01**

2DNMR of the compound AS01 (figure 9) indicates the connectivity of carbon and proton and it also indicate two coupling protons as shown below in figure 10. 2DHHNMR indicates that H at δ 6.05 (d, J = 3.5 Hz, 1H) and δ 5.65 (d, J = 3.2 Hz, 1H) are on the adjacent carbon as confirmed by carbon proton connectivity. Proton δ 5.09 (m, 1H) and δ 5.00 (m, 1H) are the same carbon. Similarly there are two at δ 4.87 (brs, 1H) and δ 4.76 (brs, 1H) on the same carbon and show broad singlet. The four carbons connected with four different protons are clearly indicated these protons and carbons are H at δ 3.95 connect with C at δ 84.91; H at δ 2.99 connect with C at δ 43.95; H at δ 2.94 connect with C at δ 46.58 and H at δ 2.87 connect with C at δ 51.53.

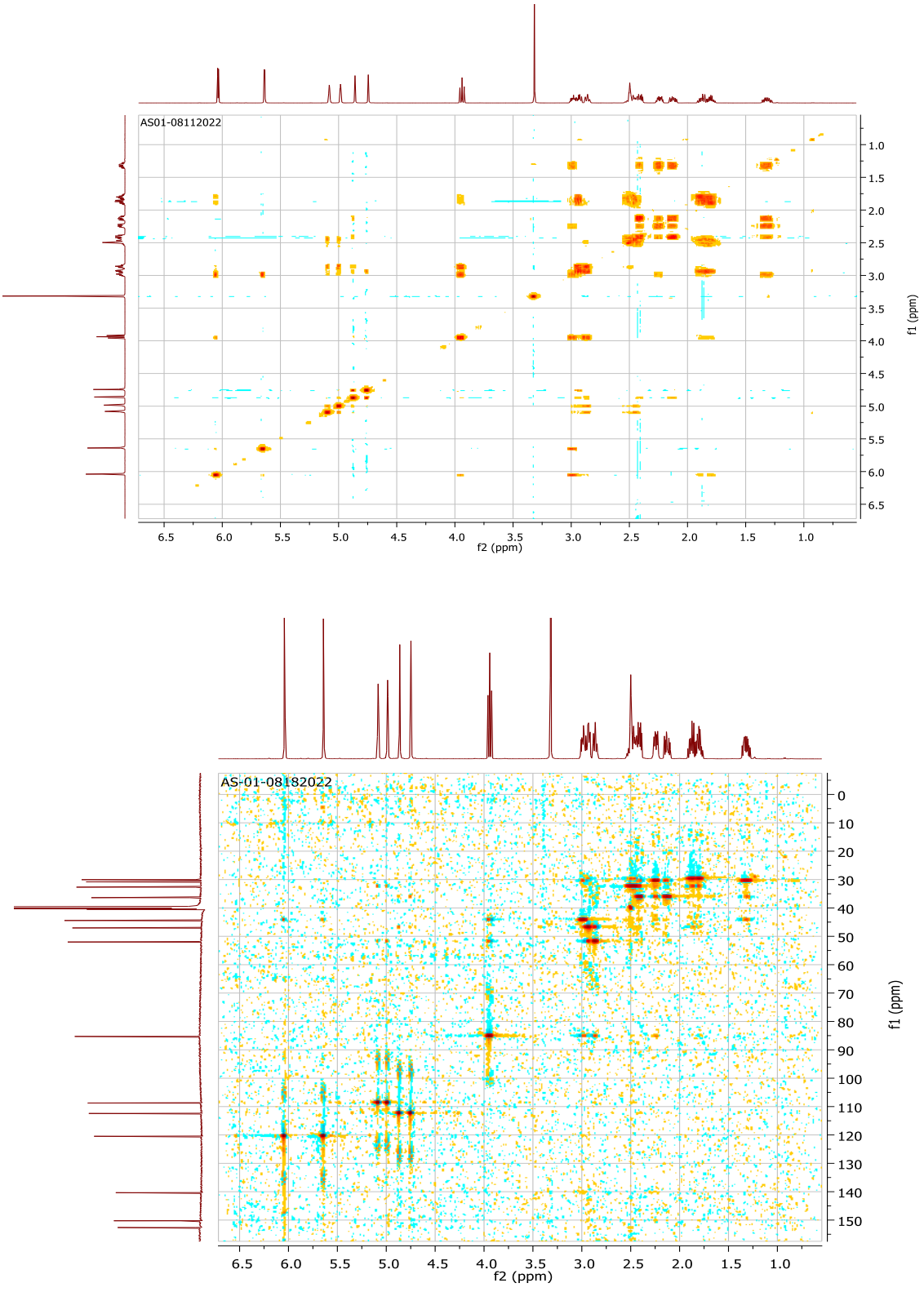



**Figure 9. 2DNMR of the compound AS01**

$^{1}$HNMR, $^{13}$C-NMR and 2DNMR spectral data of compound AS01 was found to be consistent with the one reported for the same compound, dehydrocostus lactone (figure 11)(Cho et al., 2010).

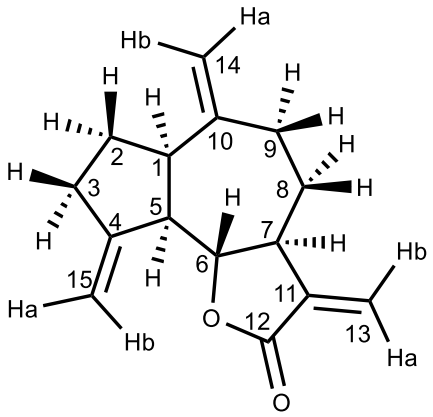



**Figure 10: The structure of dehydrocostus lactone**

**Table 4. Comparison of the $^{1}$HNMR and $^{13}$CNMR spectral data of the AS01 and dehydrocostus lactone reported by Cho et al.**

| | | $^{1}$HNMR chemical shift (δ, ppm) | | | $^{13}$CNMR chemical shift (δ, ppm) | |
|---|---|---|---|---|---|---|
| No. | | Experimental data of AS01 | Literature report of DHC | No. | Experimental data of AS01 | Literature report of DHC |
| 1 | H-1a | 2.94m (qdd) | 2.92(1H, m) | C-1 | 46.58 | 47.55 |
| 2 | H-2a | 1.81m | 1.94 (2H m) | C-2 | 29.57 | 30.24 |
| | H-2b | 1.89dtd | | | | |
| 3 | H-3a | 2.51ddd | 2.54(2H, m) | C-3 | 32.2 | 32.54 |
| | H-3b | 2.46m | | | | |
| 4 | - | - | - | C-4 | 152.15 | 151.21 |
| 5 | H-5 | 2.87m | 2.88(1 H, m) | C-5 | 51.53 | 45.07 |
| 6 | H-6 | 3.95t | 3.93(1H, t) | C-6 | 84.81 | 85.19 |
| 7 | H-7 | 2.99m (qdd) | 2.86(1 H, m) | C-7 | 43.95 | 51.97 |
| 8 | H-8a | 2.25ddd | 2.24(1 H, m) | C-8 | 30.34 | 30.88 |
| | H-8b | 1.33m | 1.42(1 H, m) | | | |
| 9 | H-9a | 2.42m | 2.48 (1 H, m) | C-9 | 39.91 | 36.21 |
| | H-9b | 2.14m | 2.17(1 H, m) | | | |
| 10 | - | - | - | C-10 | 149.77 | 149.18 |
| 11 | - | - | - | C-11 | 139.86 | 139.70 |
| 12 | - | - | - | C-12 | 169.62 | 120.13 |
| 13 | H-13A | 6.05d | 6.22(1H, d, 3.5) | C-13 | 119.99 | 170.21 |
| | H-13a | 5.65d | 5.39(1H, d, 3.2) | | | |

| | | | | | | |
|---|---|---|---|---|---|---|
| 14 | H-14a | 4.87brs | 4.9(1H, brs) | C-14 | 111.95 | 112.56 |
| | H-14b | 4.76brs | 4.82(1H, brs) | | | |
| 15 | H-15a | 5.09m | 5.27(1H, d, 4.3) | C-15 | 108.27 | 109.53 |
| | H-15B | 5.00m | 5.07(1H, d, 4.2) | | | |

Note: d=doublet, t=triplate, s=singlet, m=multiplate , qdd=quartet doublet of doublet, ddd=doublet doublet of doublet

The above table presents the chemical shifts of experimental data of compound AS01. By comparing the $^{1}$H- NMR and $^{13}$C-NMR spectral data of AS01 with the literature reported data of $^{1}$H- NMR and $^{13}$C-NMR spectral data the compound AS01 was identified as dehydrocostus lactone as in figure 10.

## 4.4 Antileishmanial Activity of Extracts

Antileishmanial assay were done on both promastigote and amastigote forms of *L. aethopica* and *L. donovani*, which are the major causes of cutaneous leishmaniasis and Visceral leishmaniasis (kala-azar) in Ethiopia respectively (Ashford et al., 1973; Leta et al., 2014).

The $IC_{50}$ value of both hexane and 80% methanol extract of root of *Cirsium dender* Friis against promastigote of *L. aethopica and L. donovani* were determined**.** The $IC_{50}$ of the hexane and 80% methanol extract of the medicinal plant *Cirsium dender* Friis against promastigote of *L. aethopica* were 10.54±3.04µg/ml and 120.60±8.69µg/ml respectively (Table 5). Likewise, the $IC_{50}$ of the hexane and 80% methanol extract against promastigote of *L. donovani* were 11.86±3.51µg/ml and 8.78±2.21µg/ml respectively (Table 5). The $IC_{50}$ of both hexane and 80% methanol extract were higher than that of the reference drug (AMB).

The $IC_{50}$ of hexane and 80% methanol extract of root of *Cirsium dender* Friis against axenically grown intracellular amastigotes of *L. aethopica and L. donovani* were determined by using axenically grown amastigotes The $IC_{50}$ of hexane and 80% methanol extract against intracellular amastigote of *L. aethopica* were 8.78±2.29µg/ml and 8.07±1.62µg/ml and $IC_{50}$ against intracellular amastigote of *L. donovani* were 6.19±3.98µg/ml and 9.86±2.62µg/ml, respectively (Table 5).

**Table 5. *In vitro* Antileishmanial activity of 80% methanol and hexane root extract of *Cirsium dender* Friis against Promastigote and amastigote form of *L. aethopica* and *L. donovani***

| | **Antileishmanial activity $IC_{50}$ (µg/ml)** | | | |
|---|---|---|---|---|
| | **Promastigotes** | | **Amastigotes** | |
| | **LA** | **LD** | **LA** | **LD** |
| ME-80 | 120.60±8.69 | 11.86±3.51 | 8.78±2.29 | 6.19±3.98 |
| HE | 10.54±3.04 | 8.78±2.21 | 8.07±1.62 | 9.86±2.62 |
| AMB | 0.0286±0 | 0.0283±0 | 0.054±0 | 0.033±0 |
| Medium+1%DMSO | 0.00 | 0.00 | 0.00 | 0.00 |

LA- Leishmania aethopica, LD- Leishmania donovani; The values are expressed as mean±SEM; n=3; ME-80: methanol 80% extract; HE: hexane extract; AMB: amphotericin B; NC: negative control; R2: regression coefficient; IC50: Concentration causing 50% suppression of parasite growth.

The hexane and 80% methanol extract of root of *Cirsium dender* Friis revealed anti-leishmanial activities with varying level of activities against *L. donovani* and *L. aethopica*. But both extract have lower anti-leishmanial activities as compared to reference drug AMB.

The anti-leishmanial activity of the hexane extract of the plant was higher than the activities exhibited by 80% methanol extract against both *L. donovani* and *L. aethopica*. Then, the hexanes extract which, exhibit higher antileishmanial activity against both promastigote and axenically grown amastigotes of *L. aethopica and L. donovani* was

investigated for active metabolite(s) responsible for antileishmanial activity and the compound labeled as AS01 was isolated by using thin layer and preparative chromatography.

### 4.5 Antileishmanial activity of AS01

The $IC_{50}$ of the isolated compound AS01 against promastigote of *L. aethopica* and *L. donovani* were 24.33±5.69µg/ml and 12.35±1.87µg/ml, respectively (Table 6). Whereas the activity against axenically grown amastigotes of *L. aethopica* and *L. donovani* were 12.54±1.63µg/ml and 3.29±1.52µg/ml, respectively as in table 6 below. The standard dug AMB was found to be much more active than dehydrocostus lactone against the promastigote and amastigote forms of both *L. aethopica* and *L. donovani.*

**Table 6.Antileishmanial activity of dehydrocostus lactone and amphotericin B**

| | **Antileishmanial activity $IC_{50}$** | | | |
|---|---|---|---|---|
| | **Promastigotes** | | **Amastigotes** | |
| | **LA µg/ml** | **LD µg/ml** | **LA µg/ml** | **LD µg/ml** |
| **Isolated compound** | 24.33±5.69 | 12.35±1.87 | 12.54±1.63 | 3.29±1.52 |
| **AMB** | 0.0286 | 0.0283 | 0.054 | 0.033 |

LA- Leishmania aethopica, LD- Leishmania donovani; The values are expressed as mean±SEM; n=3; AMB: amphotericin B; IC50: Concentration causing 50% suppression of parasite growth.

Based on the experimental data dehydrocostus lactone has higher antileishmanial activity with $IC_{50}$ value of 12.54µg/mL and 3.29µg/mL for *L. aethopica* and L. *donovani* as compared to Triterpenoids ursolic acid(**3**) 27µg/mL, calein C(**15**) 50µg/mL for amastigote and promastigote of *Leishmania amazonensis.* But the compound dehydrocostus lactone has lower antileishmanial activity $IC_{50}$ 12.35 µg/mL as compared to standard drug AMB against both Leishmania species. Other secondary metabolites Parthenolide(**13**), Psilostachyin(**10**), Peruvin(**11**), calealactone C(**16**) has higher antileishmanial activity with $IC_{50}$ value of 0.12µg/ml, 0.39µg/ml, 0.37µg/ml and 4.6 µg /mL against promastigote form of *L.mexicana* respectively as compared to the isolated compound dehydrocostus lactone.

Many previous studies on Asteraceae plants support the antileishmanial activities of hexane, 80% methanol extract and isolated compound AS01(dehydrocostus lactone) and additional *in vivo* study would be needed to further evaluate and justify potential use of the isolated compound AS01 as lead molecule to develop antileishmanial compound.

### 4.6 Hemolytic effect of extract and isolated compound

Hemolytic effect of hexane extract, methanol extract and the isolated compound against human RBC were determined by Alamar Blue reductive assay. Hemolytic property of the methanol and hexane extract as well as the isolated compound AS01 were assayed by mixing serially titrated concentration of extract and isolated compound with 2%blood suspension and triton X114 as positive control which hemolysis 100% and the effect of DMSO which serve as negative control in assay procedure. The percentage of hemolysis of RBC by hexane, methanol and isolated compound were presented in the table 7. The

hemolytic property expressed by LC50 was 0.00408±0.0021µg/ml, 0.00027 ±0.00145µg/ml and 0.0000827±0.001µg/ml for methanol extract, hexane extract and isolated compound AS01 respectively.

**Table 7 Percentage hemolysis of RBC by 80%methanol, hexane extract and isolated compound AS01 from root of *Cirsium dender* friis**

| C(**µg/ml)** | 95.24 | 47.62 | 23.81 | 11.905 | 5.9525 | 2.9585 | 1.488 |
|---|---|---|---|---|---|---|---|
| Methanol extract (%) | 60.6 | 45.45 | 30.3 | 27.2 | 15.15 | 0 | 0 |
| Hexane extract (%) | 51.5 | 51.5 | 33.3 | 18.2 | 9.1 | 0 | 0 |
| Isolated compound AS01 (%) | 36.4 | 33.3 | 18.2 | 15.1 | 15.1 | 9.1 | 3.0 |

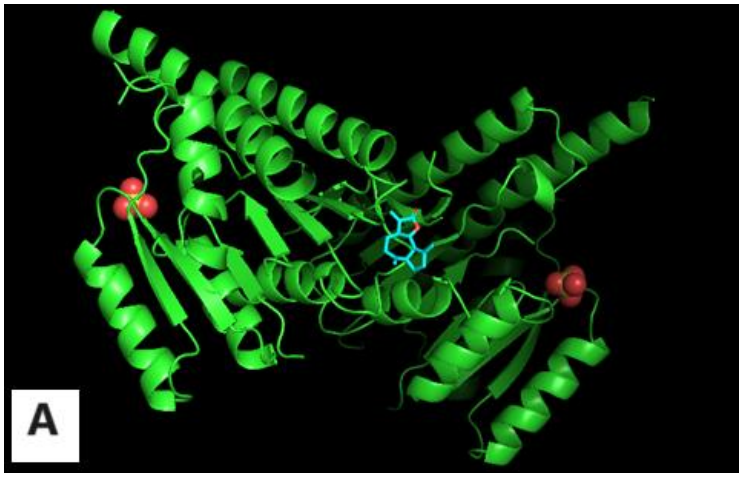
## 4.7 Molecular docking study

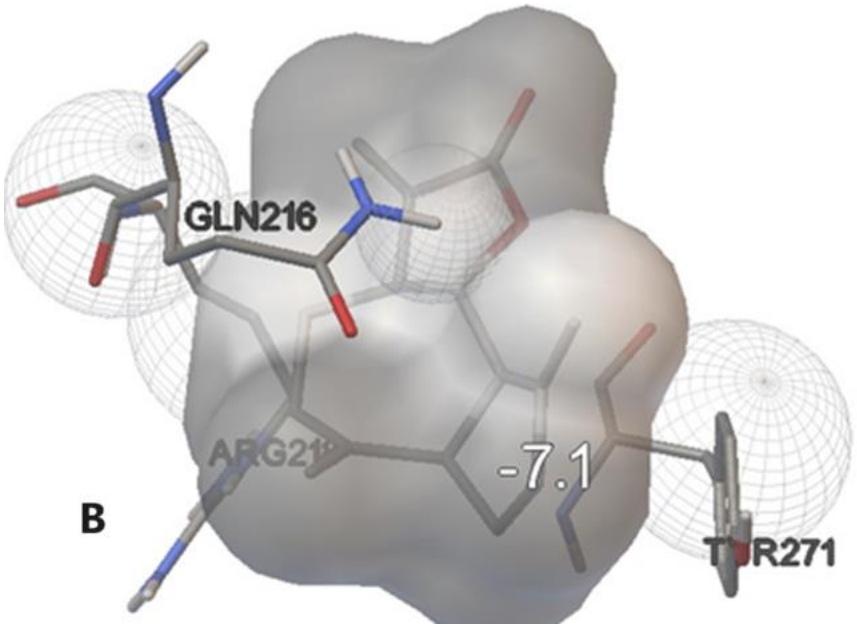
To study the binding interaction and to identify hypothetical binding motif a docking of the compound dehydrocostus lactone with Crystal structure of pteridine reductase 1 (PTR1)(PDB ID: 2XOX) from *Leishmania donovani* (Barrack et al., 2011) was performed by using AutoDock MGL Tools, AutoDock Vina and Pymol visualization tools. The compound has shown high binding affinity of -7.1kcal/mol to ward docking site of the enzyme PTR1. PTR1 is an essential enzyme of pterin and folate metabolism during growth phase of *L. donovani* protozoal parasite (Kumar et al., 2007). The binding mode of compound in active pocket of PTR1 is shown below (figure 13). The compound form non polar interaction with target enzyme PTR1 binding site. On the basis of *in vitro* activity and docking result, the compound had to inhibit PTR1. The result of online ADME calculation in the following table 8 below shows that the compound dehydrocostus lactone has drug likeness characters (Armaković & Armaković, 2023).

**Table 8. The compound dehydrocostus lactone drug likeness (Armaković & Armaković, 2023)**

| Parameter | Value | Reference value |
|---|---|---|
| Number of H bond donors | 0 | <5 |
| Number of H bond acceptors | 2 | <10 |
| Molecular weight | 230.307 | <500 |
| LogP | 3.017 | <5 |
| Mol. Refractivity | 66.238 | 40 – 130 |
| TPSA [Angstrom^2] | 26.3 | <140 |

The compound dehydrocostus lactone has various pharmacological activities as Neuroleptics (Okugawa et al., 1996), Antibacterial (anti-H. pylori) (Lee et al., 2014), anti-inflammatory, ant pain and anticancer (Woo et al., 2019), Antibacterial (Deyno et al., 2021) and molecular docking study of compound with other target protein would be needed to justify the compounds as lead molecule for give pharmacological activity.



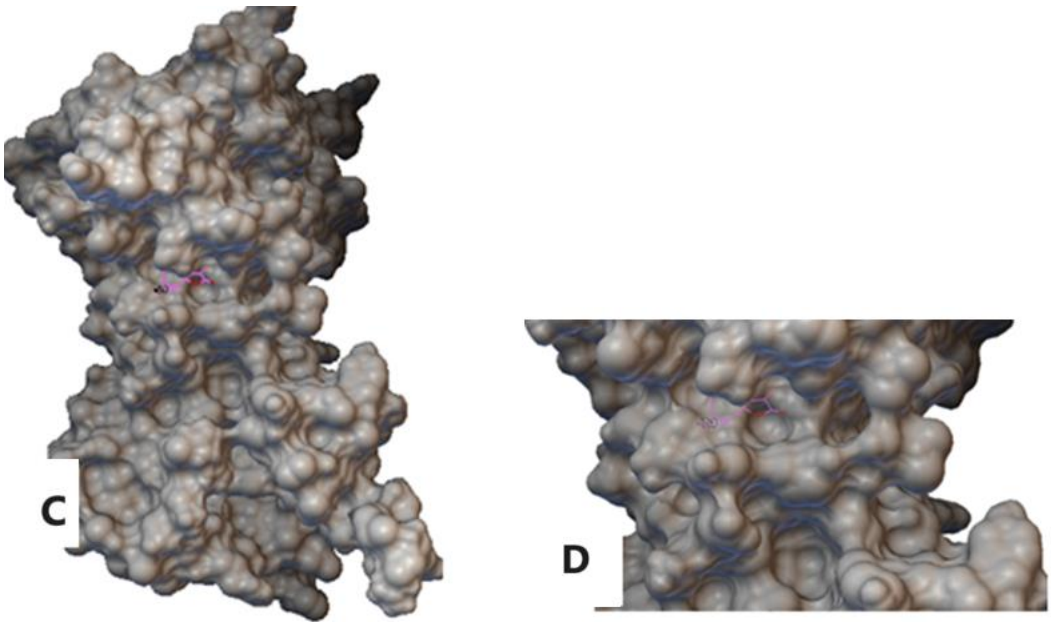



**Figure 11: The binding modes of dehydrocostus lactone with enzyme pteridine reductase 1 (PTR1) (PDB ID: 2XOX).**

(A). Ribbon diagram of docked dehydrocostus lactone- PTR1 complex. (B). Binding interaction of dehydrocostus lactone with amino acid residues of PTR1 (C). Surface representation showing dehydrocostus lactone in the binding site of PTR1. (D). A zoom view of diagram C.

## 5. Conclusion

Antileishmanial activity conducted in this study revealed that the growth of both amastigote and promastigote of leishmania was inhibited by hexane and methanol extract of *Cirsium dender* Friis and the hexane extract which has higher antileishmanial activity was further assessed to isolate compound dehydrocostus lactone. Experimental investigation for antileishmanial activity has demonstrated that the compound dehydrocostus lactone inhibit the growth of promastigote and amastigote form of *L. donovani* and *L. aethopica*. This study has provided scientific evidence for the activity of hexane and methanol extract of *C. dender* frees against leishmania and it is the first to demonstrate antileishmanial activity of the compound dehydrocostus lactone. Molecular docking of isolated compound dehydrocostus lactone showing that pteridine reductase 1 (PTR1) (PDB ID: 2XOX) from *Leishmania donovani* and dehydrocostus lactone interact with amino acid residue in the binding site. the study contribute to further study of the compound, structural modification and additional *in vivo* studies would be needed to justify antileishmanial activity to discover new antileishmanial compound.

# 6. Recommendations

Based on the present study the following recommendation are drawn

- Isolation of other compounds from the hexane and methanol extract of plant material.
- Modify the compound AS01 as lead compound to develop semisynthetic chemicals and evaluate their antileishmanial activity.
- To perform additional antiprotozoal activities of the compound.

## References

Acebey, L., V. Jullian, D. Sereno, S. Chevalley, Y. Estevez, C. Moulis, S. Beck, A. Valentin, A. Gimenez and M. Sauvain (2010). "Anti-leishmanial lindenane sesquiterpenes from Hedyosmum angustifolium." Planta medica **76**(04): 365-368.

Aggarwal, G., G. Kaur, G. Bhardwaj, V. Mutreja, H. S. Sohal, G. A. Nayik, A. Bhardwaj and A. Sharma (2022). "Traditional Uses, Phytochemical Composition, Pharmacological Properties, and the Biodiscovery Potential of the Genus Cirsium." Chemistry **4**(4): 1161-1192.

Al-Natour, S. H. (2009). "Update in the treatment of cutaneous leishmaniasis." Journal of Family Community Med **16**(2): 41-47.

ALL, G. B. (1975). "The allelopathic activity of Californian thistle (Cirsium arvense (L.) Scop.) in Tasmania." Weed Research **15**(2): 77-81.

Almoshari, Y. (2022). "Medicinal plants used for dermatological disorders among the people of the kingdom of Saudi Arabia: A narrative review." Saudi Journal of Biological Sciences: 103303.

Armaković, S. and S. J. Armaković (2023). "Atomistica. online–web application for generating input files for ORCA molecular modelling package made with the Anvil platform." Molecular Simulation **49**(1): 117-123.

Arya, V. and R. K. Parmar (2009). "A Perspective on therapeutic potential of weeds." New Zealand Journal of Ecology **33**: 2.

Ashford, R., M. Bray, M. Hutchinson and R. Bray (1973). "The epidemiology of cutaneous leishmaniasis in Ethiopia." Transactions of the Royal Society of Tropical Medicine and Hygiene **67**(4): 568-601.

Ashmita, P., L. SINGH, D. KUMAR, R. ANTIL and P. DAHIYA (2020). "Cirsium arvense: A Multi-potent Weed." Annals of Biology **36**(3): 442-447.

Awaad, A. S., H. M. Al-Zaylaee, S. I. Alqasoumi, M. E. Zain, E. M. Aloyan, A. M. Alafeefy, E. S. Awad and R. M. El-Meligy (2014). "Anti-leishmanial Activities of Extracts and Isolated Compounds from Drechslera rostrata and Eurotium tonpholium." Phytotherapy Research **28**(5): 774-780.

Azwanida, N. (2015). "A review on the extraction methods use in medicinal plants, principle, strength and limitation." Med Aromat Plants **4**(196): 2167-0412.

Bailey, M. S. and D. N. Lockwood (2007). "Cutaneous leishmaniasis." Clinics in dermatology **25**(2): 203-211.

Banaras, S., A. Javaid, A. Shoaib and E. Ahmed (2017). "Antifungal activity of Cirsium arvense extracts against phytopathogenic fungus Macrophomina phaseolina." Planta Daninha **35**.

Barati, M., I. Sharifi, F. Sharififar, M. Hakimi Parizi and A. Shokri (2014). "Anti-leishmanial activity of Gossypium hirsutum L., Ferula assa-foetida L. and Artemisia aucheri Boiss. Extracts by colorimetric assay." Anti-Infective Agents **12**(2): 159-164.

Barrack, K. L., L. B. Tulloch, L.-A. Burke, P. K. Fyfe and W. N. Hunter (2011). "Structure of recombinant Leishmania donovani pteridine reductase reveals a disordered active site." Acta Crystallographica Section F: Structural Biology and Crystallization Communications **67**(1): 33-37.

Bibiso, M., M. Anza and B. Alemayehu (2021). "Antibacterial and antioxidant activity of Cirsium Englerianum (Asteraceae), an endemic plant to Ethiopia." Res Journal of Pharmacogn **8**(3): 5-12.

Boelaert, M., B. Criel, J. Leeuwenburg, W. Van Damme, D. Le Ray and P. Van Der Stuyft (2000). "Visceral leishmaniasis control: a public health perspective." Transactions of the Royal Society of Tropical Medicine and Hygiene **94**(5): 465-471.

Boğa, M., P. K. Yılmaz, D. B. Cebe, M. Fatima, B. S. Siddiqui and U. Kolak (2014). "Chemical constituents and biological activities of Cirsium leucopsis, C. sipyleum, and C. eriophorum." Zeitschrift für Naturforschung C **69**(9-10): 381-390.

Caldas, L. A., M. L. Yoshinaga, M. J. Ferreira, J. H. Lago, A. B. de Souza, M. D. Laurenti, L. F. D. Passero and P. Sartorelli (2019). "Antileishmanial activity and ultrastructural changes of sesquiterpene lactones isolated from Calea pinnatifida (Asteraceae)." Bioorganic chemistry **83**: 348-353.

Cantrell, C. L., I. S. Nunez, J. Castaneda-Acosta, M. Foroozesh, F. R. Fronczek, N. H. Fischer and S. G. Franzblau (1998). "Antimycobacterial activities of dehydrocostus lactone and its oxidation products." Journal of natural products **61**(10): 1181-1186.

Chakraborty, T., S. Saha and N. S. Bisht (2017). "First report on the ethnopharmacological uses of medicinal plants by Monpa tribe from the Zemithang region of Arunachal Pradesh, Eastern Himalayas, India." Plants **6**(1): 13.

Chekole, G. (2017). "Ethnobotanical study of medicinal plants used against human ailments in Gubalafto District, Northern Ethiopia." Journal of ethnobiology and ethnomedicine **13**(1): 1-29.

Chem, F. M. (2013). "Drug discovery for the treatment of leishmaniasis, African sleeping sickness and Chagas disease." Future Med Chem **5**(15): 1709-1718.

Cho, K.-M., X.-H. An, J.-K. Chon, H.-S. Kim and J.-C. Chun (2010). "Foliage contact herbicidal activity of dehydrocostus lactone derived from Saussurea lappa." Korean Journal of Weed Science **30**(4): 421-428.

Clark, J. D., G. F. Gebhart, J. C. Gonder, M. E. Keeling and D. F. Kohn (1997). "The 1996 guide for the care and use of laboratory animals." ILAR journal **38**(1): 41-48.

Corral, M. J., E. González, M. Cuquerella and J. M. Alunda (2013). "Improvement of 96-well microplate assay for estimation of cell growth and inhibition of Leishmania with Alamar Blue." Journal of microbiological methods **94**(2): 111-116.

Croft, S., R. Neal, W. Pendergast and J. Chan (1987). "The activity of alkyl phosphorylcholines and related derivatives against Leishmania donovani." Biochemical pharmacology **36**(16): 2633-2636.

De Queiroz, A. C., T. d. L. M. F. Dias, C. B. B. Da Matta, L. H. A. C. Silva, J. X. de Araújo-Júnior, G. B. de Araújo, F. d. B. P. Moura and M. S. Alexandre-Moreira "Research Article Antileishmanial Activity of Medicinal Plants Used in Endemic Areas in Northeastern Brazil."

De Toledo, J. S., S. R. Ambrósio, C. H. Borges, V. Manfrim, D. G. Cerri, A. K. Cruz and F. B. Da Costa (2014). "In vitro leishmanicidal activities of sesquiterpene lactones from Tithonia diversifolia against Leishmania braziliensis promastigotes and amastigotes." Molecules **19**(5): 6070-6079.

Del Guacchio, E., P. Bureš, D. Iamonico, F. Carucci, D. De Luca, F. Zedek and P. Caputo (2022). "Towards a monophyletic classification of Cardueae: restoration of the genus Lophiolepis (= Cirsium pp) and new circumscription of Epitrachys." Plant Biosystems-An International Journal Dealing with all Aspects of Plant Biology **156**(5): 1269-1290.

Deyno, S., A. G. Mtewa, D. Hope, J. Bazira, E. Makonnen and P. E. Alele (2021). "Antibacterial activities of Echinops kebericho Mesfin tuber extracts and isolation of the most active compound, dehydrocostus lactone." Frontiers in Pharmacology **11**: 608672.

Dietze, R., S. Carvalho, L. Valli, J. Berman, T. Brewer, W. Milhous, J. Sanchez, B. Schuster and M. Grogl (2001). "Phase 2 trial of WR6026, an orally administered 8-aminoquinoline, in the treatment of visceral leishmaniasis caused by Leishmania chagasi." The American journal of tropical medicine and hygiene **65**(6): 685-689.

Dos Santos, A. O., P. Veiga-Santos, T. Ueda-Nakamura, B. P. D. Filho, D. B. Sudatti, É. M. Bianco, R. C. Pereira and C. V. Nakamura (2010). "Effect of elatol, isolated from red seaweed Laurencia dendroidea, on Leishmania amazonensis." Marine drugs **8**(11): 2733-2743.

Farrell, J. (2002). Leishmania. Boston, Mass., Kluwer Academic Pub.

Flaih, M. H. (2022). "Geographical Distribution of Cutaneous Leishmaniasis and Pathogenesis." Leishmaniasis: General Aspects of a Stigmatized Disease: 99.

Fournet, A., V. Muñoz, F. Roblot, R. Hocquemiller, A. Cavé and J. C. Gantier (1993). "Antiprotozoal activity of dehydrozaluzanin C, a sesquiterpene lactone isolated from Munnozia maronii (Asteraceae)." Phytotherapy Research **7**(2): 111-115.

Friis, I. and S. Bidgood (1998). "Dombeya kefaensis, sp. nov.(Sterculiaceae) from SW. Ethiopia." Nordic Journal of Botany **18**(2): 215-220.

Ganguly, S., N. K. Das, J. N. Barbhuiya and M. Chatterjee (2010). "Post-kala-azar dermal leishmaniasis–an overview." International journal of dermatology **49**(8): 921-931.

Gossage, S. M., M. E. Rogers and P. A. Bates (2003). "Two separate growth phases during the development of Leishmania in sand flies: implications for understanding the life cycle." International journal for parasitology **33**(10): 1027-1034.

Guarrera, P. M. (2005). "Traditional phytotherapy in Central Italy (marche, abruzzo, and latium)." Fitoterapia **76**(1): 1-25.

Haldar, A. K., P. Sen and S. Roy (2011). "Use of antimony in the treatment of leishmaniasis: current status and future directions." Molecular biology international **2011**.

Handa, S. P. S. Khanuja, G. Longo and D. D. Rakesh (2008). Extraction technologies for medicinal and aromatic plants, Earth, Environmental and Marine Sciences and Technologies.

He, Y., Q. Zhu, M. Chen, Q. Huang, W. Wang, Q. Li, Y. Huang and W. Di (2016). "The changing 50% inhibitory concentration (IC50) of cisplatin: A pilot study on the artifacts of the MTT assay and the precise measurement of density-dependent chemoresistance in ovarian cancer." Oncotarget **7**(43): 70803.

IPNI (2023). "International Plant Names Index." Published on the Internet https://www.ipni.org/p/2643-2. The Royal Botanic Gardens, Kew, Harvard University Herbaria & Libraries and Australian National Herbarium. [Retrieved 17 January 2023].

Ishida, H., T. Umino, K. Tsuji and T. KOSUGE (1987). "Studies on antihemorrhagic substances in herbs classified as hemostatics in Chinese medicine. VII. On the antihemorrhagic principle in Cirsium japonicum DC." Chemical and pharmaceutical bulletin **35**(2): 861-864.

Jumare, A. I., A. D. Mahmoud and W. E. Amlabu (2022). "Traditional medicinal plants used in the management of cutaneous Leishmaniasis diseases in Sokoto State, Northern Nigeria." Ethnobotany Research and Applications **23**: 1-21.

Jung, H. A., S. E. Jin, B.-S. Min, B.-W. Kim and J. S. Choi (2012). "Anti-inflammatory activity of Korean thistle Cirsium maackii and its major flavonoid, luteolin 5-O-glucoside." Food and chemical toxicology **50**(6): 2171-2179.

Kafetzis, D. A. (2003). "An overview of paediatric leishmaniasis." Journal of postgraduate medicine **49**(1): 31.

Karpavičienė, B. (2022). "Traditional Uses of Medicinal Plants in South-Western Part of Lithuania." Plants **11**(16): 2093.

Khramova, D. S., V. V. Golovchenko, A. S. Shashkov, D. Otgonbayar, A. Chimidsogzol and Y. S. Ovodov (2011). "Chemical composition and immunomodulatory activity of a pectic polysaccharide from the ground thistle Cirsium esculentum Siev." Food Chemistry **126**(3): 870-877.

Killick-Kendrick, R. (1990). "The life-cycle of Leishmania in the sandfly with special reference to the form infective to the vertebrate host." Annales de Parasitologie humaine et comparée **65**: 37-42.

Koutsoni, O. S., K. Karampetsou and E. Dotsika (2019). "In vitro screening of antileishmanial activity of natural product compounds: Determination of IC50, CC50 and SI values." Bio-protocol **9**(21): e3410-e3410.

Kumar, P., S. Sundar and N. Singh (2007). "Degradation of pteridine reductase 1 (PTR1) enzyme during growth phase in the protozoan parasite Leishmania donovani." Experimental parasitology **116**(2): 182-189.

Laurella, L. C., N. Cerny, A. E. Bivona, A. Sanchez Alberti, G. Giberti, E. L. Malchiodi, V. S. Martino, C. A. Catalan, M. R. Alonso and S. I. Cazorla (2017). "Assessment of sesquiterpene lactones isolated from Mikania plants species for their potential efficacy against Trypanosoma cruzi and Leishmania sp." PLoS Neglected Tropical Diseases **11**(9): e0005929.

Lee, H.-K., H. E. Song, H.-B. Lee, C.-S. Kim, M. Koketsu, L. Thi My Ngan and Y.-J. Ahn (2014). "Growth inhibitory, bactericidal, and morphostructural effects of dehydrocostus lactone from Magnolia sieboldii leaves on antibiotic-susceptible and-resistant strains of Helicobacter pylori." PloS one **9**(4): e95530.

Lee, J.-H., S.-I. Choi, Y.-S. Lee and G.-H. Kim (2008). "Antioxidant and anti-inflammatory activities of ethanol extract from leaves of Cirsium japonicum." Food Science and Biotechnology **17**(1): 38-45.

Lee, J. S., L. A. Paje, J. P. Rodriguez, K. S. Kang, D.-H. Hahm, J. S. Shim, Y.-J. Choi and S. Lee (2020). "Validation of an HPLC/UV analysis method for cirsimaritin in Cirsium japonicum var. maackii." Korean Journal of Pharmacognosy **51**(3): 217-221.

Lee, S.-H., Y.-S. Jin, S.-I. Heo, T.-H. Shim, J.-H. Sa, D.-S. Choi and M.-H. Wang (2006). "Composition analysis and antioxidative activity from different organs of Cirsium setidens Nakai." Korean Journal of Food Science and Technology **38**(4): 571-576.

Lee, S. H., S.-I. Heo, L. Li, M. J. Lee and M.-H. Wang (2008). "Antioxidant and hepatoprotective activities of Cirsium setidens NAKAI against CCl 4-induced liver damage." The American Journal of Chinese Medicine **36**(01): 107-114.

Lee, Y.-J., J.-H. Lee, Y.-H. Kim, J.-H. Kim, S.-Y. Yu, D.-B. Kim, J. S. Lee, M. L. Cho, J.-H. Cho and B. K. Kim (2015). "Assessment of the pectolinarin content and the radical scavenging-linked antiobesity activity of Cirsium setidens Nakai extracts." Food science and biotechnology **24**: 2235-2243.

Lemmon, E., M. McLinden, D. Friend, P. Linstrom and W. Mallard (2011). "NIST chemistry webbook." NIST standard reference database(69): 20899.

Leta, S., T. H. T. Dao, F. Mesele and G. Alemayehu (2014). "Visceral leishmaniasis in Ethiopia: an evolving disease." PLoS neglected tropical diseases **8**(9): e3131.

Lim, H., K. H. Son, H. W. Chang, K. Bae, S. S. Kang and H. P. Kim (2008). "Anti-inflammatory activity of pectolinarigenin and pectolinarin isolated from Cirsium chanroenicum." Biological and Pharmaceutical Bulletin **31**(11): 2063-2067.

Liu, S., X. Luo, D. Li, J. Zhang, D. Qiu, W. Liu, L. She and Z. Yang (2006). "Tumor inhibition and improved immunity in mice treated with flavone from Cirsium japonicum DC." International Immunopharmacology **6**(9): 1387-1393.

Liu, S., J. Zhang, D. Li, W. Liu, X. Luo, R. Zhang, L. Li and J. Zhao (2007). "Anticancer activity and quantitative analysis of flavone of Cirsium japonicum DC." Natural product research **21**(10): 915-922.

Loizzo, M. R., G. A. Statti, R. Tundis, F. Conforti and F. Menichini (2004). "Antimicrobial activity and cytotoxicity of Cirsium tenoreanum." Fitoterapia **75**(6): 577-580.

Luizza, M. W., H. Young, C. Kuroiwa, P. Evangelista, A. Worede, R. Bussmann and A. Weimer (2013). "Local knowledge of plants and their uses among women in the Bale Mountains, Ethiopia." Ethnobotany Research and Applications **11**: 315-339.

Luo, W., B. Wu, L. Tang, G. Li, H. Chen and X. Yin (2021). "Recent research progress of Cirsium medicinal plants in China." Journal of Ethnopharmacology **280**: 114475.

Magill, A. J., E. T. Ryan, D. R. Hill and T. Solomon (2012). Hunter's Tropical Medicine and Emerging Infectious Disease: Expert Consult-Online and Print, Elsevier Health Sciences.

Matos, A., A. Viçosa, M.-I. Ré, E. Ricci-Júnior and C. Holandino (2020). "A review of current treatments strategies based on paromomycin for leishmaniasis." Journal of Drug Delivery Science and Technology **57**: 101664.

Miyazawa, M., C. Yamafuji, K. Kurose and Y. Ishikawa (2003). "Volatile components of the rhizomes of Cirsium japonicum DC." Flavour and fragrance journal **18**(1): 15-17.

Monzote, L. (2009). "Current treatment of leishmaniasis: a review." The Open Antimicrobial Agents Journal **1**(1).

Moore, E. M. and D. N. Lockwood (2010). "Treatment of visceral leishmaniasis." J Glob Infect Dis **2**(2): 151-158.

Morais, T. R., P. Romoff, O. A. Fávero, J. Q. Reimão, W. C. Lourenço, A. G. Tempone, A. D. Hristov, S. M. Di Santi, J. H. G. Lago and P. Sartorelli (2012). "Anti-malarial, anti-

trypanosomal, and anti-leishmanial activities of jacaranone isolated from Pentacalia desiderabilis (Vell.) Cuatrec.(Asteraceae)." Parasitology research **110**(1): 95-101.

Moulisha, B., G. A. Kumar and H. P. Kanti (2010). "Anti-leishmanial and anti-cancer activities of a pentacyclic triterpenoid isolated from the leaves of Terminalia arjuna Combretaceae." Tropical Journal of Pharmaceutical Research **9**(2).

Murray, H. W., E. B. Brooks, J. L. DeVecchio and F. P. Heinzel (2003). "Immunoenhancement combined with amphotericin B as treatment for experimental visceral leishmaniasis." Antimicrobial agents and chemotherapy **47**(8): 2513-2517.

Nazaruk, J. (2008). "Antioxidant activity and total phenolic content in Cirsium five species from north-east region of Poland." Fitoterapia **79**(3): 194-196.

Nazaruk, J., S. K. Czechowska, R. Markiewicz and M. H. Borawska (2008). "Polyphenolic compounds and in vitro antimicrobial and antioxidant activity of aqueous extracts from leaves of some Cirsium species." Natural Product Research **22**(18): 1583-1588.

Nazaruk, J. and P. Jakoniuk (2005). "Flavonoid composition and antimicrobial activity of Cirsium rivulare (Jacq.) All. flowers." Journal of ethnopharmacology **102**(2): 208-212.

Nazaruk, J., E. Karna and D. Kalemba (2012). "The chemical composition of the essential oils of Cirsium palustre and C. rivulare and their antiproliferative effect." Natural product communications **7**(2): 1934578X1200700242.

Neal, R. (1968). "The effect of antibiotics of the neomycin group on experimental cutaneous leishmaniasis." Annals of Tropical Medicine & Parasitology **62**(1): 54-62.

Oja, S. M., J. P. Guerrette, M. R. David and B. Zhang (2014). "Fluorescence-enabled electrochemical microscopy with dihydroresorufin as a fluorogenic indicator." Analytical chemistry **86**(12): 6040-6048.

Okugawa, H., R. Ueda, K. Matsumoto, K. Kawanishi and A. Kato (1996). "Effect of dehydrocostus lactone and costunolide from Saussurea root on the central nervous system in mice." Phytomedicine **3**(2): 147-153.

Parvizi, M. M., F. Zare, F. Handjani, M. Nimrouzi and M. M. Zarshenas (2020). "Overview of herbal and traditional remedies in the treatment of cutaneous leishmaniasis based on Traditional Persian Medicine." Dermatologic Therapy **33**(4): e13566.

Phondani, P. C., R. K. Maikhuri, L. S. Rawat, N. A. Farooquee, C. P. Kala, S. R. Vishvakarma, K. Rao and K. Saxena (2010). "Ethnobotanical uses of plants among the Bhotiya tribal communities of Niti Valley in Central Himalaya, India." Ethnobotany Research and Applications **8**: 233-244.

Postigo, J. A. R. (2010). "Leishmaniasis in the world health organization eastern mediterranean region." International journal of antimicrobial agents **36**: S62-S65.

Powell, K. I., K. N. Krakos and T. M. Knight (2011). "Comparing the reproductive success and pollination biology of an invasive plant to its rare and common native congeners: a case study in the genus Cirsium (Asteraceae)." Biological Invasions **13**(4): 905-917.

Ramos, H., E. Valdivieso, M. Gamargo, F. Dagger and B. Cohen (1996). "Amphotericin B kills unicellular leishmanias by forming aqueous pores permeable to small cations and anions." The Journal of membrane biology **152**(1): 65-75.

Ready, P. D. (2014). "Epidemiology of visceral leishmaniasis." Clinical epidemiology **6**: 147.

Reithinger, R., J.-C. Dujardin, H. Louzir, C. Pirmez, B. Alexander and S. Brooker (2007). "Cutaneous leishmaniasis." The Lancet infectious diseases **7**(9): 581-596.

Riss, T. L., R. A. Moravec, A. L. Niles, S. Duellman, H. A. Benink, T. J. Worzella and L. Minor (2016). "Cell viability assays." Assay Guidance Manual.

Rojas-Silva, P., R. Graziose, B. Vesely, A. Poulev, F. Mbeunkui, M. H. Grace, D. E. Kyle, M. A. Lila and I. Raskin (2014). "Leishmanicidal activity of a daucane sesquiterpene isolated from Eryngium foetidum." Pharmaceutical Biology **52**(3): 398-401.

Rougeron, V., T. De Meeûs, S. Kako Ouraga, M. Hide and A.-L. Bañuls (2010). "“Everything you always wanted to know about sex (but were afraid to ask)” in Leishmania after two decades of laboratory and field analyses." PLoS pathogens **6**(8): e1001004.

Sahli, R., C. Rivière, C. Dufloer, C. Beaufay, C. Neut, J. Bero, T. Hennebelle, V. Roumy, R. Ksouri and J. Quetin-Leclercq (2017). "Antiproliferative and antibacterial activities of Cirsium scabrum from Tunisia." Evidence-Based Complementary and Alternative Medicine **2017**.

Salgado-Almario, J., C. A. Hernández and C. Ovalle-Bracho (2019). "Geographical distribution of Leishmania species in Colombia, 1985-2017." Biomédica **39**(2): 278-290.

Savoia, D., T. Allice and P.-A. Tovo (2005). "Antileishmanial activity of HIV protease inhibitors." International Journal of Antimicrobial Agents **26**(1): 92-94.

Sharma, U., D. Singh, P. Kumar, M. Dobhal and S. Singh (2011). "Antiparasitic activity of plumericin & isoplumericin isolated from Plumeria bicolor against Leishmania donovani." The Indian journal of medical research **134**(5): 709.

Shimony, O. and C. L. Jaffe (2008). "Rapid fluorescent assay for screening drugs on Leishmania amastigotes." Journal of microbiological methods **75**(2): 196-200.

Shin, S., K. Saravanakumar, A. V. A. Mariadoss, X. Hu, A. Sathiyaseelan and M.-H. Wang (2022). "Functionalization of selenium nanoparticles using the methanolic extract of Cirsium setidens and its antibacterial, antioxidant, and cytotoxicity activities." Journal of Nanostructure in Chemistry **12**(1): 23-32.

Singh, A., M. Lal and S. Samant (2009). "Diversity, indigenous uses and conservation prioritization of medicinal plants in Lahaul valley, proposed Cold Desert Biosphere Reserve, India." International Journal of Biodiversity Science & Management **5**(3): 132-154.

Singh, B. and S. Borthakur (2011). "Wild medicinal plants used by tribal communities of Meghalaya." J Econ Taxon Bot **35**(2): 331-339.

Singh, J. (2008). "Maceration, percolation and infusion techniques for the extraction of medicinal and aromatic plants." Extraction technologies for medicinal and aromatic plants **67**: 32-35.

Stachon, W. and R. Zimdahl (1980). "Allelopathic activity of Canada thistle (Cirsium arvense) in Colorado." Weed Science **28**(1): 83-86.

Strazzulla, A., S. Cocuzza, M. R. Pinzone, M. C. Postorino, S. Cosentino, A. Serra, B. Cacopardo and G. Nunnari (2013). "Mucosal leishmaniasis: an underestimated presentation of a neglected disease." BioMed Research International **2013**.

Sülsen, V. P., F. M. Frank, S. I. Cazorla, C. A. Anesini, E. L. Malchiodi, B. Freixa, R. Vila, L. V. Muschietti and V. S. Martino (2008). "Trypanocidal and leishmanicidal activities of sesquiterpene lactones from Ambrosia tenuifolia Sprengel (Asteraceae)." Antimicrobial Agents and Chemotherapy **52**(7): 2415-2419.

Tariku, Y., A. Hymete, A. Hailu and J. Rohloff (2011). "In vitro evaluation of antileishmanial activity and toxicity of essential oils of Artemisia absinthium and Echinops kebericho." Chemistry & biodiversity **8**(4): 614-623.

Tiuman, T. S., T. Ueda-Nakamura, D. g. A. c. Garcia Cortez, B. P. Dias Filho, J. A. Morgado-Díaz, W. de Souza and C. V. Nakamura (2005). "Antileishmanial activity of parthenolide, a sesquiterpene lactone isolated from Tanacetum parthenium." Antimicrobial agents and Chemotherapy **49**(1): 176-182.

Torres-Guerrero, E. (2017). "Leishmaniasis: a review." F1000Research **6**.

Torres-Santos, E., D. Lopes, R. R. Oliveira, J. Carauta, C. B. Falcao, M. Kaplan and B. Rossi-Bergmann (2004). "Antileishmanial activity of isolated triterpenoids from Pourouma guianensis." Phytomedicine **11**(2-3): 114-120.

Van Wyk, A. S. and G. Prinsloo (2020). "Health, safety and quality concerns of plant-based traditional medicines and herbal remedies." South African Journal of Botany **133**: 54-62.

WHO. (2023). Leishmaniasis. Retrieved October 17, 2023, from https://www.who.int/news-room/fact-sheets/detail/leishmaniasis/

Woo, J.-H., J.-H. Ahn, D. S. Jang and J.-H. Choi (2019). "Effect of dehydrocostus lactone isolated from the roots of Aucklandia lappa on the apoptosis of endometriotic cells and the alternative activation of endometriosis-associated macrophages." The American Journal of Chinese Medicine **47**(06): 1289-1305.

Wyllie, S., M. L. Cunningham and A. H. Fairlamb (2004). "Dual action of antimonial drugs on thiol redox metabolism in the human pathogen Leishmania donovani." Journal of Biological Chemistry **279**(38): 39925-39932.

Yardley, V. and S. L. Croft (1997). "Activity of liposomal amphotericin B against experimental cutaneous leishmaniasis." Antimicrobial agents and chemotherapy **41**(4): 752-756.

Yimam, M., S. M. Yimer and T. B. Beressa (2022). "Ethnobotanical study of medicinal plants used in Artuma Fursi district, Amhara Regional State, Ethiopia." Tropical Medicine and Health **50**(1): 85.

Yin, Y., S.-I. Heo and M.-H. Wang (2008). "Antioxidant and anticancer activities of methanol and water extracts from leaves of Cirsium japonicum." Journal of applied biological chemistry **51**(4): 160-164.

Zeouk, I., A. E. O. Lalami, Y. Ezzoubi, K. Derraz, M. Balouiri and K. Bekhti (2020). "Cutaneous Leishmaniasis: Medicinal Plants Used in Sefrou City (Center of Morocco), a Focus of Leishmaniasis." Phytothérapie **18**(3-4): 187-194.

Zhou, Q., W.-x. Zhang, Z.-q. He, B.-s. Wu, Z.-f. Shen, H.-t. Shang, T. Chen, Q. Wang, Y.-g. Chen and S.-t. Han (2020). "The possible anti-inflammatory effect of dehydrocostus lactone on DSS-induced colitis in mice." Evidence-Based Complementary and Alternative Medicine **2020**.

Zijlstra, E., A. Musa, E. Khalil, I. El Hassan and A. El-Hassan (2003). "Post-kala-azar dermal leishmaniasis." The Lancet infectious diseases **3**(2): 87-98.

Zijlstra, E. E. (2016). "The immunology of post-kala-azar dermal leishmaniasis (PKDL)." Parasites & vectors **9**(1): 1-9.

Zulfiqar, B. and V. M. Avery (2022). "Assay development in leishmaniasis drug discovery: a comprehensive review." Expert Opin Drug Discov **17**(2): 151-166.

# *In vitro* Antileishmanial and Molecular Docking Studies of a Sesquiterpene Lactone from *Cirsium dender* Friis (Asteraceae)

**Aklilu Samuel[1], Dr. Solomon Tadesse[2]**

1Department of Pharmacy, College of Health Sciences, Wolaita Sodo University, P.O. Box 138, Wolaita Sodo, Ethiopia. Email-akyeako@gmail.com

2Department of Pharmaceutical Chemistry and Pharmacognosy, School of Pharmacy, College of Health Sciences, Addis Ababa University, P.O. Box 1176, Addis Ababa, Ethiopia. Email-solomon.tadesse@aau.edu.et

Leishmaniasis caused by genus of *Leishmania* has impact on the Africa and Asian countries due to high prevalence, resistance to current therapy and unavailability of standard medicine for the treatment. Asteraceae plants (a family widely used in folk medicine worldwide) are emerging as an interesting source for new leishmanicidal compounds and this study was aims to extract and isolate active metabolite from the root of *Cirsium dender* Friis (Asteraceae) to study molecular docking and to investigate *in vitro* antileishmanial activity against promastigote and amastigote forms of *L. aethopica* and *L. donovani.* Air dried roots of the plant was macerated with 80% methanol which resulted in 9.98% brown methanol extract. Phytochemical analysis was investigated by using thin layer and preparative chromatography which result in isolation of a white crystalline compound named AS01. Characterization of AS01 on the basis of APCI-MS, $^{13}$CNMR and $^{1}$HNMR spectral assignments and by comparing with reported data of the compound the AS01 was identified as dehydrocostus lactone. Antileishmanial activity of the extract and dehydrocostus lactone was studied on clinical isolates of *L. aethopica* and

*L. donovani* and the $IC_{50}$ of the dehydrocostus lactone was 24.33μ/mL, 12.35 μ/mL against promastigote and 12.54 μ/mL, 3.29 μ/mL against amastigote of *L. aethopica* and *L. donovani* respectively. The compound dehydrocostus lactone and the crystalline structure of pteridine reductase1 (PDB ID: 2XOX) extracted from *L.donovani*, which is a potential target for drug development against Leishmania, underwent a molecular docking study using the autodock vina and Pymol visualization tools. The result of the study discloses that the compound dehydrocostus lactone has antileishmanial activity and it has binding affinity to docking site of pteridine reductase1 enzyme.

## Introduction

Leishmaniasis is a spectral disease, which is spread by the bite of phlebotomine sand flies (Torres-Guerrero, 2017). It is a global health problem, which is widely distributed in Asia, Africa, Mexico, Central America, and South America. Generally leishmaniasis is found in 98 countries with over 350 million people at risk (Farrell, 2002; Torres-Guerrero, 2017). About 95% of CL cases occur in Americas, the Mediterranean basin, the Middle East and central Asia and over 90% mucocutaneous leishmaniasis cases occur in Bolivia, brazil, Ethiopia and Peru (Torres-Guerrero, 2017; WHO, 2023).

According to the 2023 report by WHO, there is a significant global occurrence of leishmaniasis, with an estimated 50,000 to 90,000 new cases of visceral leishmaniasis (VL) and 600,000 to 1 million new cases of cutaneous leishmaniasis (CL) reported annually (WHO, 2023).

Based on the available mortality data, an estimated annual death toll of 20,000 to 40,000 can be derived for leishmaniasis. This estimation is in line with the overarching case-

fatality rate of 10%, which is consistent with previous estimations provided by the World Health Organization (Alvar, 2012).

Parasites of the genus Leishmania belong to the family Trypanosomatidae and to the order Kinetoplastid. During their life cycle these parasites present in two stages; promastigotes and amastigotes (Gossage, 2003). Promastigotes are elongated flagellated cells which develop and multiply in the digestive tract of the vector whereas the amastigotes are round or oval shaped cells, with a very short flagellum that does not protrude from the flagella bag, they transform and multiply in the host's macrophages and are responsible for generating the disease (Gluenz, 2010).

## Materials and Methods

### Plant Materials

The roots of *Cirsium dender* Friis were collected in March 2022 from Matala Walana Kebele Boloso Bombe Woreda, Wolaita zone of SNNP regional state which is located in South West direction and 345km far from the capital city Addis Ababa. The plant was then authenticated and identified by Mr. Melaku Wondafrash a botanist at College of Natural and Computational Sciences, Addis Ababa University (AAU), where a botanical specimen with voucher number AS005/2022 was deposited for further reference.

### Test organism

Clinical isolates of *L. aethopica* (CL-068/22) and *L. donovani* (VL-029/22) were acquired from Leishmaniasis Research and Diagnostic Laboratory (LRDL), for the

department of Microbiology, Immunology and Parasitology (DMIP), College of Health Science (CHS), School of Medicine (SoM), Addis Ababa University (AAU).

The parasite strain of *L. aethopica* (CL-068/22) was isolated from 33 years old male patient who lives in Arsi Zone, Oromia region, Ethiopia and *L. donovani* strain (VL-139/19) was isolated from 23 years old male patient who lives in Amhara region, Ethiopia. Red Blood Cells were collected from healthy 28 years old male volunteer with no underlying chronic disease.

**Extraction of the Root**

The roots of Cirsium dender Friis were washed with tap water to remove dust and any other debris present on it. Then roots were air dried under a shaded area at room temperature and crushed using a mortar and pestle to get a coarse powder. A total of 450g coarsely powdered root was macerated with 80 % (v/v) methanol accordance with methods by International Centre for Science (Handa, 2008). The contents were shaken manually and allowed to remain within the solvent. After 72hours, the extract was filtered first using nylon filter cloth and then by Whitman filters paper No 1. The marc was re-macerated twice using the same solvent to exhaustively extract the plant material. The combined filtrate was concentrated under vacuum using Rotary evaporator at a 38°C and dried in Tissue Drying Oven at 38°C to yield 44.67g (9.93%) of methanol extract (Azwanida 2015; Singh 2008).

**Isolation of a compound**

The resulting dry methanol extract obtained by maceration was fractionated by using different solvent system hexane, ethyl acetate, chloroform, methanol and water. From the hexane fraction, which has antileishmanial activity; the compound was isolated by preparative thin layer chromatography with solvent system of petroleum ether and ethyl acetate in the ratio of 4 to 1 as a mobile phase. The major band was scrapped off from the plate and wash with chloroform filtered using Whitman no. 1 and concentrated to dryness result in white crystalline solid and the purity of isolated compound monitored by TLC which was visualized using UV light of wave lengths 254 nm and 366 nm.

**Spectroscopic analysis**

NMR spectral data were obtained at room temperature on FT-NMR spectrometer operating at 400 MHz for $^{1}H$ and 100 MHz for $^{13}C$ using deuterated dimethyl sulfoxide. A region from 0 to 12ppm for 1H and 0 to 205ppm for 13C was employed for scanning. Signals were referred to an internal standard tetramethylsilane (TMS). Chemical shifts are reported in δ units and coupling constants (J) in Hz. Multiplicities of 1H NMR signals are indicated as broad singlet(brs), doublet (d), doublet of doublet (dd), triplet (t), triplet of doublet of doublet (tdd) and multiplet (m).

**Antileishmanial Assay**

**Antipromastigote Assay**

Antipromastigote activities of extracts and isolated compound were done as methods previously described by Ketema and Tariku et al (Ketema, 2023; Tariku, 2010) with

slight modification. To 96-well plates filled with 100µl of complete culture medium (RPMI-1640), 100µl of test substance (200µg/ml) dissolved in 1% DMSO was added on the first well. Then, 100µl was taken into subsequent wells then the last 100µl was discarded, to achieve serial dilution from 200µg/ml to 1.56µg/ml.

Then 100µl of suspension of parasites ($3.5x10^{-6}$ promastigotes/ml of *L. aethopica* or *L. donovani)* were added to each well and contents of the plates were incubated at 22℃ for *L. aethopica* and 26 ℃ for *L. donovani*. After 68 h of incubation 20µl of fluorochrome resazurin solution (0.125mg/ml, PH=7.2) was added to each well and incubated accordingly for additional 4 hrs. After that fluorescence intensity was measured by a Multilabel Reader at an excitation wavelength of 530 nm and emission wavelength of 590 nm.

Assay with DMSO 1% and AMB (same serial dilution as the test substances) were conducted as negative and positive control respectively. Blank well containing complete RPMI-1640 medium was used to monitor background fluorescence intensity of resazurin and the average value of absorbance was subtracted from every well. The Antipromastigote activity of the extract and isolated compounds were expressed as percent of parasite inhibition and determined by using the following formula and then $IC_{50}$ was calculated (Ketema, 2023; He et al., 2016). $\%\text{inhibition} = 100 - \frac{ODt-ODb}{ODn-ODb} * 100$

Where: ODt-Absorbance of triplicate of test substance; ODb-Average absorbance of blank and ODn -Average absorbance of media

**Axenic amastigote assay**

*In vitro* cell culture method for biological evaluation of antileishmanial activity of methanol extract , hexane extract and isolated compound against intracellular Leishmania spp. amastigotes by using published methods with slight modification (Corral et al., 2013; Koutsoni et al., 2019; Riss et al., 2016).

Mouse peritoneal macrophages suspended in complete MEM medium ($3\times10^5$cells/ml, 200µl) were seeded in white 96-well plates and incubated for 24 hours at 37$^o$C in 5% $CO_2$ to promote cell adhesion. After 24 hours the plates were washed with pre-warmed complete medium to remove non-adherent macrophages. The stationary stages of *L. donovani* and *L. aethopica* promastigotes (leishmania to macrophage ratio; 10:1) were seeded in the plates containing adherent macrophages and maintained at 31$^o$C (*L. aethopica*) or 37$^o$C (*L. donovani*), 5% $CO_2$ and 95% relative humidity for further 24 hours to allow infection and amastigote differentiation.

Then, non-internalized promastigotes were removed by extensive washing three times with prewarmed MEM medium to remove remaining extracellular promastigotes and serially diluted concentrations of methanol extract extracts, hexane extract and isolated compound (1.5 - 200µg/ml), Triplicates of reference drug, negative controls in triplicates were added the wells was filled with complete RPMI-1640 medium until the final volume of 200µl per well and incubated for further 48 hours at 37 °C under 5% $CO_2$ humidified air.

Finally culture supernatants was removed with a multichannel pipette and macrophages' membranes was disrupted by adding 50µl of lysis solution, containing ATP and luciferin

to each well; which initiates the enzymatic reaction of luciferin to oxyluciferin catalyzed by luciferase expressed by living L. amastigotes. After 20 min incubation of the plate in a non-inverted position at room temperature, and cell viability was measured fluorometrically (excitation λ, 550 nm; emission λ, 590 nm) (Shimony & Jaffe, 2008). The results were expressed as the percentage of reduction in the parasite burden and it was computed by using the following formula and then $IC_{50}$ was calculated (He et al., 2016). $\%\text{inhibition} = 100 - \frac{ODt-ODb}{ODn-ODb} * 100$

Where: ODt-Absorbance of triplicate of test substance; ODb-Average absorbance of blank and ODn -Average absorbance of media

**In vitro Haemolysis test**

Haemolytic activity of methanolic methanol extract , hexane extract and the isolated compound was determined by using RBCs which was prepared from 2ml blood collected from $O^+$ individual and added to of 48ml of PBS then centrifuged at 3500 rpm for 10 min at 4℃ and washed with PBS results 1ml of RBC pellets. By adding 49ml of PBS to 1ml RBC pellets 2% RBC suspension was prepared and the concentration was adjusted to $1.9x10^9$ RBC/ml.

Serially titrated (95.24, 47.62, 23.81, 11.905, 5.9525, 2.9585, 1.488 and 0.724/ml) methanol extract , hexane extract and isolated compound were mixed with 2% blood suspension in eppendorf tubes and incubated at 37°C for two hours with Triton X-14 (5µL/mL) (Sigma-Aldrich, Co., St. Louis, USA) and 2.5% DMSO as positive and negative controls. The mixture was then centrifuged at 1000 g for 10 min. 75µL of the

resulting supernatant were transferred to 96-well plates In column 1, 2 and 3 for methanol extract ; column 4, 5 and 6 for isolated compound; column 7, 8 and 9 for hexane fraction; column 10 for blank (RBC suspension only), column 11 for triton X114 (positive control) and column 12 for DMSO (negative control). The free hemoglobin in the supernatant was measured in UV-Vis spectrophotometer at 540nm. Each experiment was performed in triplicates at each concentration. Percentage of hemolysis by substances calculated by using the following formula: $\%\text{Hemolysis} = \frac{ODt-ODnc}{ODpc-ODnc} * 100$

Where: ODt-Absorbance of triplicate of test substance; ODb-Average absorbance of blank and ODn -Average absorbance of media

**Molecular Docking Study and in silico Prediction**

*In silico* prediction of the compound dehydrocostus lactone drug likeness properties by ADMET lab 2.0 online software tools (Armaković & Armaković, 2023) and molecular docking study of the compounds was carried out by using Crystal structure of pteridine reductase 1 (PTR1)(PDB ID: 2XOX) from Leishmania donovani(Barrack et al., 2011) and computer applications AutoDock MGL Tools, AutoDock Vina and Pymol visualization tool.

**Statistical analysis**

Anti-promastigotes and anti-amastigotes activity, which expressed as $IC_{50}$ of the methanol extract, hexane extract and isolated compound was analyzed by using Graphpad prism 9.3.0 and Microsoft Excel 2010 and the result was reported mean ± standard error. All the analyses were carried out at 5% level of significance.

In silico prediction of the compound dehydrocostus lactone drug likeness and molecular docking study of the compounds was carried out by AutoDock MGL Tools, AutoDock Vina and Pymol visualization tool and result expressed as affinity energy toward docking site of enzyme.

**Ethical Clearance**

All procedures conducted in this research work, including use of Vero cell lines were reviewed and approved by Ethical Review Committee, School of Pharmacy, College of Health Sciences; Addis Ababa University with letter number ERB/SOP/545/15/2023 dated June 23, 2023. Informed consent was obtained from volunteer man to use the collected red blood for research purpose.

**Result and Discussion**

**Extraction yield**

Four hundred fifty gram of air dried root of *Cirsium dender* Friis was macerated with 80% methanol and which gave 44.65gm (9.93%) brown methanol extract as plant secondary metabolite.

**TLC Analysis of Extract**

Antileishmanial activity demonstrated by the methanolic extract of *Cirsium dender* Friis and hexane extract was further subjected to phytochemical analysis to identify the compound(s) responsible for bioactivity. Analytical TLC of hexane extract using PE: EA (4:1) solvent system showed the presence of more than 5 compounds in hexane extract (S1).

Solid yellow precipitates formed in hexane fraction, which were decanted and isolated by PTLC using PE and EA in the ratio of 4 to 1 as eluent solvent. The compound designated AS01 was isolated and appeared dark when viewed under 254-nm UV radiation, but was not visible at 366 nm. AS01 is white solid in the form of crystals $R_f$ = 0.54 in the PE/EA (4:1) system.

**Characterization of compound AS01**

AS01 was obtained as a white crystalline powder with an Rf value of 0.54 in petroleum ether: ethyl acetate (4:1) solvent system. Compound was subjected to GC-APCI-MS analysis, resolution (S2). The mass spectrum showed MH+ ion, a pseudo molecular ion (M+1) peak at 231.1 m/z.

According to the NIST database and PubChem, 2023 national library of medicine, the weight and pattern of fragmentation correspond to the compound DHCL whose molecular formula is C15H18O2. The exact calculated molecular mass was found to be 230.1306798220 amu which corresponds to a relative molecular formula of $C_{15}H_{18}O_2$ (Lemmon et al., 2011).

The $^1$H-NMR (500 MHz, DMSO) spectrum (figure S3) of compound AS01 showed signal at δ 3.94(1H, t, J=9.2) assigned for oxygenated methane proton at C-6 and coupled to triplet by neighboring protons at C-5 and C-7 each at δ 2.86(1H, m) and δ 2.99(1H, m) respectively. Signals at δ 5.08(1H, m) and δ 4.98(1H, m), δ 4.86(1H, s) and δ 4.74(1H, s) and δ 6.04(1H, d) and δ 5.64(1H, d) confirms the presence of three different methylene bonds each attached with different protons. The methylene protons at δ 6.04 and δ 5.64 coupled each other as indicated by doublet.

The $^{13}$C-NMR spectrum and DEPT 135 (S4 and S5) of compound AS01 showed there are four quaternary carbons at δ152.15, 149.77, 139.86 and 169.62 for C-4, C-10, C-11 and C-12 respectively. There are seven methylene carbons at δ 29.57, 32.2, 30.34, 39.91, 119.99, 111.95 and 108.27 for C-2, C-3, C-8, C-9, C-13, C-14 and C-15. The remaining tertiary carbons C-1, C-5, C-6 and C-7 are at δ 43.95, 51.53, 84.81 and 46.58 respectively.

2DNMR of the compound AS01 (S6) indicates the connectivity of carbon and proton and it also indicate two coupling protons as shown below in figure 10. 2DHHNMR indicates that H at δ 6.05 (d, J = 3.5 Hz, 1H) and δ 5.65 (d, J = 3.2 Hz, 1H) are on the adjacent carbon as confirmed by carbon proton connectivity. Proton δ 5.09 (m, 1H) and δ 5.00 (m, 1H) are the same carbon. Similarly there are two at δ 4.87 (brs, 1H) and δ 4.76 (brs, 1H) on the same carbon and show broad singlet. The four carbons connected with four different protons are clearly indicated these protons and carbons are H at δ 3.95 connect with C at δ 84.91; H at δ 2.99 connect with C at δ 43.95; H at δ 2.94 connect with C at δ 46.58 and H at δ 2.87 connect with C at δ 51.53.

$^{1}$HNMR, $^{13}$C-NMR and 2DNMR spectral data of compound AS01 was found to be consistent with the one reported for the same compound, dehydrocostus lactone (figure 1)(Cho et al., 2010).

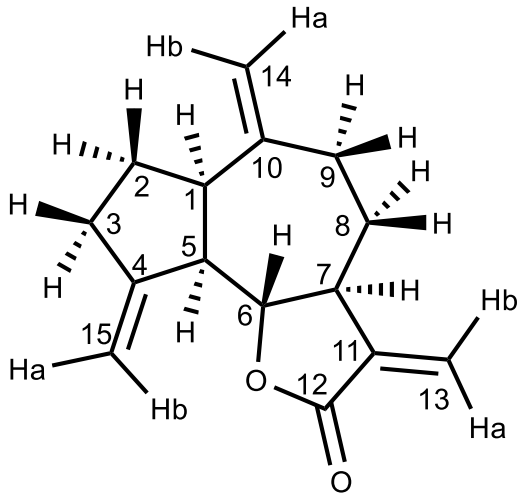



**Figure 1: The structure of dehydrocostus lactone**

**Table 9. Comparison of the $^{1}$HNMR and $^{13}$CNMR spectral data of the AS01 and dehydrocostus lactone reported by Cho et al.**

| | | $^{1}$HNMR chemical shift (δ, ppm) | | | $^{13}$CNMR chemical shift (δ, ppm) | |
|---|---|---|---|---|---|---|
| No. | | Experimental data of AS01 | Literature report of DHC | No. | Experimental data of AS01 | Literature report of DHC |
| 1 | H-1a | 2.94m (qdd) | 2.92(1H, m) | C-1 | 46.58 | 47.55 |
| 2 | H-2a | 1.81m | 1.94 (2H m) | C-2 | 29.57 | 30.24 |
| | H-2b | 1.89dtd | | | | |
| 3 | H-3a | 2.51ddd | 2.54(2H, m) | C-3 | 32.2 | 32.54 |
| | H-3b | 2.46m | | | | |
| 4 | - | - | - | C-4 | 152.15 | 151.21 |
| 5 | H-5 | 2.87m | 2.88(1 H, m) | C-5 | 51.53 | 45.07 |
| 6 | H-6 | 3.95t | 3.93(1H, t) | C-6 | 84.81 | 85.19 |
| 7 | H-7 | 2.99m (qdd) | 2.86(1 H, m) | C-7 | 43.95 | 51.97 |

| | | | | | | |
|---|---|---|---|---|---|---|
| 8 | H-8a | 2.25ddd | 2.24(1 H, m) | C-8 | 30.34 | 30.88 |
| | H-8b | 1.33m | 1.42(1 H, m) | | | |
| 9 | H-9a | 2.42m | 2.48 (1 H, m) | C-9 | 39.91 | 36.21 |
| | H-9b | 2.14m | 2.17(1 H, m) | | | |
| 10 | - | - | - | C-10 | 149.77 | 149.18 |
| 11 | - | - | - | C-11 | 139.86 | 139.70 |
| 12 | - | - | - | C-12 | 169.62 | 120.13 |
| 13 | H-13A | 6.05d | 6.22(1H, d, 3.5) | C-13 | 119.99 | 170.21 |
| | H-13a | 5.65d | 5.39(1H, d, 3.2) | | | |
| 14 | H-14a | 4.87brs | 4.9(1H, brs) | C-14 | 111.95 | 112.56 |
| | H-14b | 4.76brs | 4.82(1H, brs) | | | |
| 15 | H-15a | 5.09m | 5.27(1H, d, 4.3) | C-15 | 108.27 | 109.53 |
| | H-15B | 5.00m | 5.07(1H, d, 4.2) | | | |

Note: d=doublet, t=triplate, s=singlet, m=multiplate , qdd=quartet doublet of doublet, ddd=doublet doublet of doublet

The above table presents the chemical shifts of experimental data of compound AS01. By comparing the $^{1}$H- NMR and $^{13}$C-NMR spectral data of AS01 with the literature reported data of $^{1}$H- NMR and $^{13}$C-NMR spectral data the compound AS01 was identified as dehydrocostus lactone as in figure 1.

**Antileishmanial activity of extracts**

The $IC_{50}$ value of both hexane and 80% methanol extract of root of *Cirsium dender* Friis against promastigote of *L. aethopica and L. donovani* were determined**.** The $IC_{50}$ of the

hexane and 80% methanol extract of the medicinal plant *Cirsium dender* Friis against promastigote of *L. aethopica* were 10.54±3.04µg/ml and 120.60±8.69µg/ml respectively (Table 1). Likewise, the $IC_{50}$ of the hexane and 80% methanol extract against promastigote of *L. donovani* were 11.86±3.51µg/ml and 8.78±2.21µg/ml respectively (Table 1). The $IC_{50}$ of both hexane and 80% methanol extract were higher than that of the reference drug (AMB).

The $IC_{50}$ of hexane and 80% methanol extract of root of *Cirsium dender* Friis against axenically grown intracellular amastigotes of *L. aethopica and L. donovani* were determined by using axenically grown amastigotes The $IC_{50}$ of hexane and 80% methanol extract against intracellular amastigote of *L. aethopica* were 8.78±2.29µg/ml and 8.07±1.62µg/ml and $IC_{50}$ against intracellular amastigote of *L. donovani* were 6.19±3.98µg/ml and 9.86±2.62µg/ml, respectively (Table 1).

**Table 1. *In vitro* Antileishmanial activity of 80% methanol and hexane root extract of *Cirsium dender* Friis against Promastigote and amastigote form of *L. aethopica* and *L. donovani***

| | **Antileishmanial activity $IC_{50}$ (µg/ml)** | | | |
|---|---|---|---|---|
| | **Promastigotes** | | **Amastigotes** | |
| | **LA** | **LD** | **LA** | **LD** |
| ME-80 | 120.60±8.69 | 11.86±3.51 | 8.78±2.29 | 6.19±3.98 |
| HE | 10.54±3.04 | 8.78±2.21 | 8.07±1.62 | 9.86±2.62 |
| AMB | 0.0286±0 | 0.0283±0 | 0.054±0 | 0.033±0 |
| Medium+1%DMSO | 0.00 | 0.00 | 0.00 | 0.00 |

LA- Leishmania aethopica, LD- Leishmania donovani; The values are expressed as mean±SEM; n=3; ME-80: methanol 80% extract; HE: hexane extract; AMB: amphotericin B; NC: negative control; R2: regression coefficient; IC50: Concentration causing 50% suppression of parasite growth.

The hexane and 80% methanol extract of root of *Cirsium dender* Friis revealed anti-leishmanial activities with varying level of activities against *L. donovani* and *L. aethopica*. But both extract have lower anti-leishmanial activities as compared to reference drug AMB.

The anti-leishmanial activity of the hexane extract of the plant was higher than the activities exhibited by 80% methanol extract against both *L. donovani* and *L. aethopica*. Then, the hexanes extract which, exhibit higher antileishmanial activity against both promastigote and axenically grown amastigotes of *L. aethopica and L. donovani* was investigated for active metabolite(s) responsible for antileishmanial activity and the compound labeled as AS01 was isolated by using thin layer and preparative chromatography.

**Antileishmanial activity of AS01**

The $IC_{50}$ of the isolated compound AS01 against promastigote of *L. aethopica* and *L. donovani* were 24.33±5.69µg/ml and 12.35±1.87µg/ml, respectively (Table 2). Whereas the activity against axenically grown amastigotes of *L. aethopica* and *L. donovani* were 12.54±1.63µg/ml and 3.29±1.52µg/ml, respectively as in table 6 below. The standard dug AMB was found to be much more active than dehydrocostus lactone against the promastigote and amastigote forms of both *L. aethopica* and *L. donovani.*

**Table 2.Antileishmanial activity of dehydrocostus lactone and amphotericin B**

| | **Antileishmanial activity $IC_{50}$** | | | |
|---|---|---|---|---|
| | **Promastigotes** | | **Amastigotes** | |
| | **LA µg/ml** | **LD µg/ml** | **LA µg/ml** | **LD µg/ml** |
| **Isolated compound** | 24.33±5.69 | 12.35±1.87 | 12.54±1.63 | 3.29±1.52 |
| **AMB** | 0.0286 | 0.0283 | 0.054 | 0.033 |

LA- Leishmania aethopica, LD- Leishmania donovani; the values are expressed as mean±SEM; n=3; AMB: amphotericin B; IC50: Concentration causing 50% suppression of parasite growth.

Based on the experimental data dehydrocostus lactone has higher antileishmanial activity with $IC_{50}$ value of 12.54µg/mL and 3.29µg/mL for *L. aethopica* and L. *donovani* as compared to Triterpenoids ursolic acid(**3**) 27µg/mL, calein C(**15**) 50µg/mL for amastigote and promastigote of *Leishmania amazonensis.* But the compound dehydrocostus lactone has lower antileishmanial activity $IC_{50}$ 12.35 µg/mL as compared to standard drug AMB against both Leishmania species. Other secondary metabolites Parthenolide(**13**), Psilostachyin(**10**), Peruvin(**11**), calealactone C(**16**) has higher antileishmanial activity with $IC_{50}$ value of 0.12µg/ml, 0.39µg/ml, 0.37µg/ml and 4.6 µg /mL against promastigote form of *L.mexicana* respectively as compared to the isolated compound dehydrocostus lactone.

Many previous studies on Asteraceae plants support the antileishmanial activities of hexane, 80% methanol extract and isolated compound AS01(dehydrocostus lactone) and

additional *in vivo* study would be needed to further evaluate and justify potential use of the isolated compound AS01 as lead molecule to develop antileishmanial compound.

**Hemolytic effect of extract and isolated compound**

Hemolytic effect of hexane extract, methanol extract and the isolated compound against human RBC were determined by Alamar Blue reductive assay. Hemolytic property of the methanol and hexane extract as well as the isolated compound AS01 were assayed by mixing serially titrated concentration of extract and isolated compound with 2%blood suspension and triton X114 as positive control which hemolysis 100% and the effect of DMSO which serve as negative control in assay procedure. The percentage of hemolysis of RBC by hexane, methanol and isolated compound were presented in the table 3. The hemolytic property expressed by LC50 was 0.00408±0.0021µg/ml, 0.00027 ±0.00145µg/ml and 0.0000827±0.001µg/ml for methanol extract, hexane extract and isolated compound AS01 respectively.

**Table 3. Percentage hemolysis of RBC by 80%methanol, hexane extract and isolated compound AS01 from root of *Cirsium dender* friis**

| C(**µg/ml)** | 95.24 | 47.62 | 23.81 | 11.905 | 5.9525 | 2.9585 | 1.488 |
|---|---|---|---|---|---|---|---|
| Methanol extract (%) | 60.6 | 45.45 | 30.3 | 27.2 | 15.15 | 0 | 0 |
| Hexane extract (%) | 51.5 | 51.5 | 33.3 | 18.2 | 9.1 | 0 | 0 |
| Isolated compound AS01 (%) | 36.4 | 33.3 | 18.2 | 15.1 | 15.1 | 9.1 | 3.0 |

**Molecular docking study**

To study the binding interaction and to identify hypothetical binding motif a docking of the compound dehydrocostus lactone with Crystal structure of pteridine reductase 1 (PTR1)(PDB ID: 2XOX) from *Leishmania donovani* (Barrack et al., 2011) was performed by using AutoDock MGL Tools, AutoDock Vina and Pymol visualization tools. The compound has shown high binding affinity of -7.1kcal/mol to ward docking site of the enzyme PTR1. PTR1 is an essential enzyme of pterin and folate metabolism during growth phase of *L. donovani* protozoal parasite (Kumar et al., 2007). The binding mode of compound in active pocket of PTR1 is shown below (figure 13). The compound form non polar interaction with target enzyme PTR1 binding site. On the basis of *in vitro* activity and docking result, the compound had to inhibit PTR1.

The compound dehydrocostus lactone has various pharmacological activities as Neuroleptics (Okugawa et al., 1996), Antibacterial (anti-H. pylori) (Lee et al., 2014), anti-inflammatory, ant pain and anticancer (Woo et al., 2019), Antibacterial (Deyno et al., 2021) and molecular docking study of compound with other target protein would be needed to justify the compounds as lead molecule for give pharmacological activity.

**Conclusion**

Antileishmanial activity conducted in this study revealed that the growth of both amastigote and promastigote of leishmania was inhibited by hexane and methanol extract of *Cirsium dender* Friis and the hexane extract which has higher antileishmanial activity was further assessed to isolate compound dehydrocostus lactone. Experimental investigation for antileishmanial activity has demonstrated that the compound

dehydrocostus lactone inhibit the growth of promastigote and amastigote form of *L. donovani* and *L. aethopica*. This study has provided scientific evidence for the activity of hexane and methanol extract of *C. dender* frees against leishmania and it is the first to demonstrate antileishmanial activity of the compound dehydrocostus lactone. Molecular docking of isolated compound dehydrocostus lactone showing that pteridine reductase 1 (PTR1) (PDB ID: 2XOX) from *Leishmania donovani* and dehydrocostus lactone interact with amino acid residue in the binding site. the study contribute to further study of the compound, structural modification and additional *in vivo* studies would be needed to justify antileishmanial activity to discover new antileishmanial compound.

**Supporting information**

S1 TLC chromatogram of hexane fraction of Cirsium dender *Friis* extract and isolated compound AS01 in PE: EA (4:1) viewed under UV light of 254nm

S2 Atmospheric pressure chemical ionization mass spectrum of AS01

S3 $^{1}$H-NMR spectrum of AS-01

S4 $^{13}$C-NMR spectrum of AS01

S5 DEPT-135 spectrum of AS01

S6 2DNMR of the compound AS01

S7 Binding modes of dehydrocostus lactone with enzyme pteridine reductase 1 (PTR1) (PDB ID: 2XOX).

**Acknowledgment**

First of all, my deepest gratitude belongs to the Almighty God.

To my advisor, Dr Solomon Tadesse for his patience and shared knowledge throughout the entire process of my research work.

To the Addis Ababa University, college of health science and school of pharmacy for the support, use of the facilities and Laboratory equipment of the Pharmacognosy and medicinal chemistry.

To the Woaita Sodo University for sponsoring the study

**References**

Armaković, S., & Armaković, S. J. (2023). Atomistica. online–web application for generating input files for ORCA molecular modelling package made with the Anvil platform. *Molecular Simulation*, *49*(1), 117-123.

Ashford, R., Bray, M., Hutchinson, M., & Bray, R. (1973). The epidemiology of cutaneous leishmaniasis in Ethiopia. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, *67*(4), 568-601.

Barrack, K. L., Tulloch, L. B., Burke, L.-A., Fyfe, P. K., & Hunter, W. N. (2011). Structure of recombinant Leishmania donovani pteridine reductase reveals a disordered active site. *Acta Crystallographica Section F: Structural Biology and Crystallization Communications*, *67*(1), 33-37.

Cho, K.-M., An, X.-H., Chon, J.-K., Kim, H.-S., & Chun, J.-C. (2010). Foliage contact herbicidal activity of dehydrocostus lactone derived from Saussurea lappa. *Korean Journal of Weed Science*, *30*(4), 421-428.

Corral, M. J., González, E., Cuquerella, M., & Alunda, J. M. (2013). Improvement of 96-well microplate assay for estimation of cell growth and inhibition of Leishmania with Alamar Blue. *Journal of microbiological methods*, *94*(2), 111-116.

Deyno, S., Mtewa, A. G., Hope, D., Bazira, J., Makonnen, E., & Alele, P. E. (2021). Antibacterial activities of Echinops kebericho Mesfin tuber extracts and isolation of the most active compound, dehydrocostus lactone. *Frontiers in Pharmacology*, *11*, 608672.

Farrell, J. (2002). *Leishmania*. Kluwer Academic Pub. Publisher description http://www.loc.gov/catdir/enhancements/fy0820/2002025496-d.html

Flaih, M. H. (2022). Geographical Distribution of Cutaneous Leishmaniasis and Pathogenesis. *Leishmaniasis: General Aspects of a Stigmatized Disease*, 99.

He, Y., Zhu, Q., Chen, M., Huang, Q., Wang, W., Li, Q., Huang, Y., & Di, W. (2016). The changing 50% inhibitory concentration (IC50) of cisplatin: A pilot study on the artifacts of the MTT assay and the precise measurement of density-dependent chemoresistance in ovarian cancer. *Oncotarget*, *7*(43), 70803.

Koutsoni, O. S., Karampetsou, K., & Dotsika, E. (2019). In vitro screening of antileishmanial activity of natural product compounds: Determination of IC50, CC50 and SI values. *Bio-protocol*, *9*(21), e3410-e3410.

Kumar, P., Sundar, S., & Singh, N. (2007). Degradation of pteridine reductase 1 (PTR1) enzyme during growth phase in the protozoan parasite Leishmania donovani. *Experimental parasitology*, *116*(2), 182-189.

Lee, H.-K., Song, H. E., Lee, H.-B., Kim, C.-S., Koketsu, M., Thi My Ngan, L., & Ahn, Y.-J. (2014). Growth inhibitory, bactericidal, and morphostructural effects of dehydrocostus lactone from Magnolia sieboldii leaves on antibiotic-susceptible and-resistant strains of Helicobacter pylori. *PloS one*, *9*(4), e95530.

Lemmon, E., McLinden, M., Friend, D., Linstrom, P., & Mallard, W. (2011). NIST chemistry webbook. *NIST standard reference database*(69), 20899.

Leta, S., Dao, T. H. T., Mesele, F., & Alemayehu, G. (2014). Visceral leishmaniasis in Ethiopia: an evolving disease. *PLoS neglected tropical diseases*, *8*(9), e3131.

Okugawa, H., Ueda, R., Matsumoto, K., Kawanishi, K., & Kato, A. (1996). Effect of dehydrocostus lactone and costunolide from Saussurea root on the central nervous system in mice. *Phytomedicine*, *3*(2), 147-153.

Riss, T. L., Moravec, R. A., Niles, A. L., Duellman, S., Benink, H. A., Worzella, T. J., & Minor, L. (2016). Cell viability assays. *Assay Guidance Manual [Internet]*.

Salgado-Almario, J., Hernández, C. A., & Ovalle-Bracho, C. (2019). Geographical distribution of Leishmania species in Colombia, 1985-2017. *Biomédica*, *39*(2), 278-290.

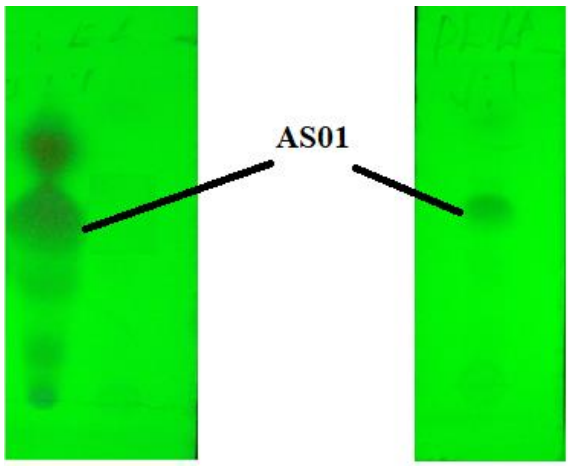
Shimony, O., & Jaffe, C. L. (2008). Rapid fluorescent assay for screening drugs on Leishmania amastigotes. *Journal of microbiological methods*, *75*(2), 196-200.

Torres-Guerrero, E. (2017). Leishmaniasis: a review. *F1000Research*, *6*. https://doi.org/10.12688/f1000research.11120.1

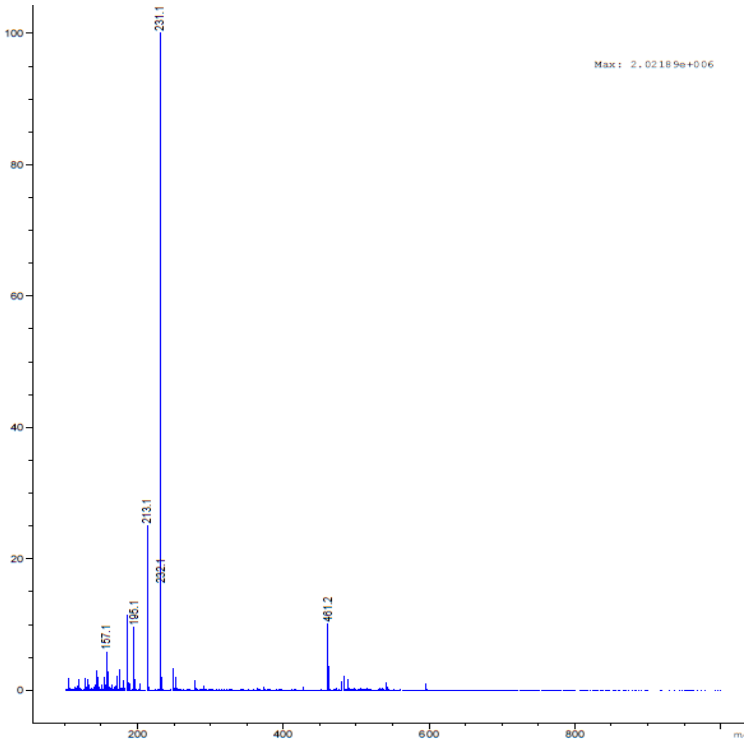
WHO. (2023). *Leishmaniasis*. Retrieved october 17 from https://www.who.int/news-room/fact-sheets/detail/leishmaniasis

Woo, J.-H., Ahn, J.-H., Jang, D. S., & Choi, J.-H. (2019). Effect of dehydrocostus lactone isolated from the roots of Aucklandia lappa on the apoptosis of endometriotic cells and the alternative activation of endometriosis-associated macrophages. *The American Journal of Chinese Medicine*, *47*(06), 1289-1305.

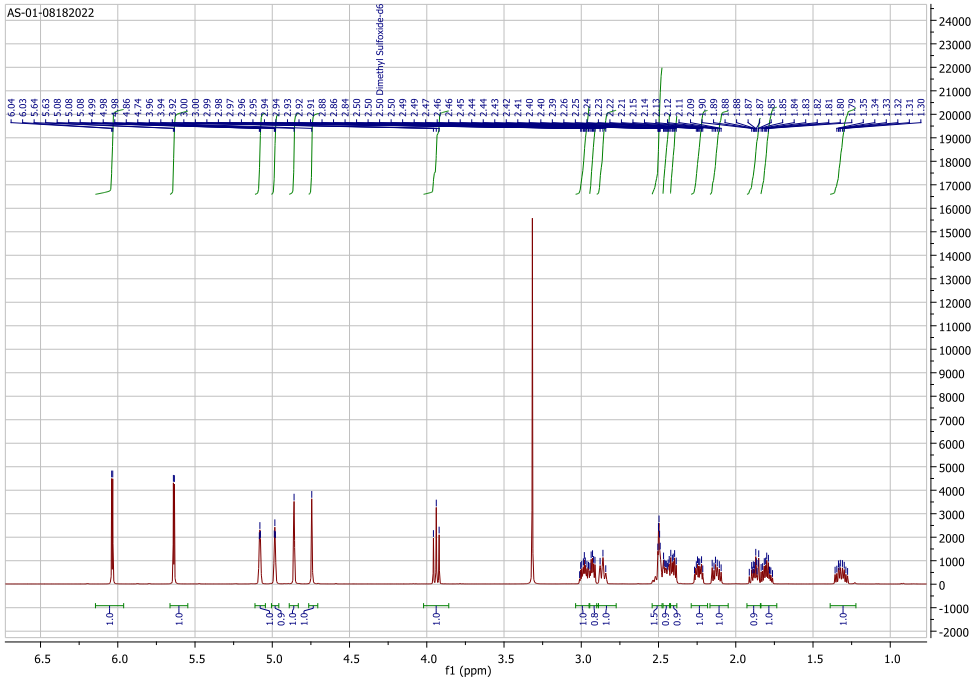
# Supporting figures and tables



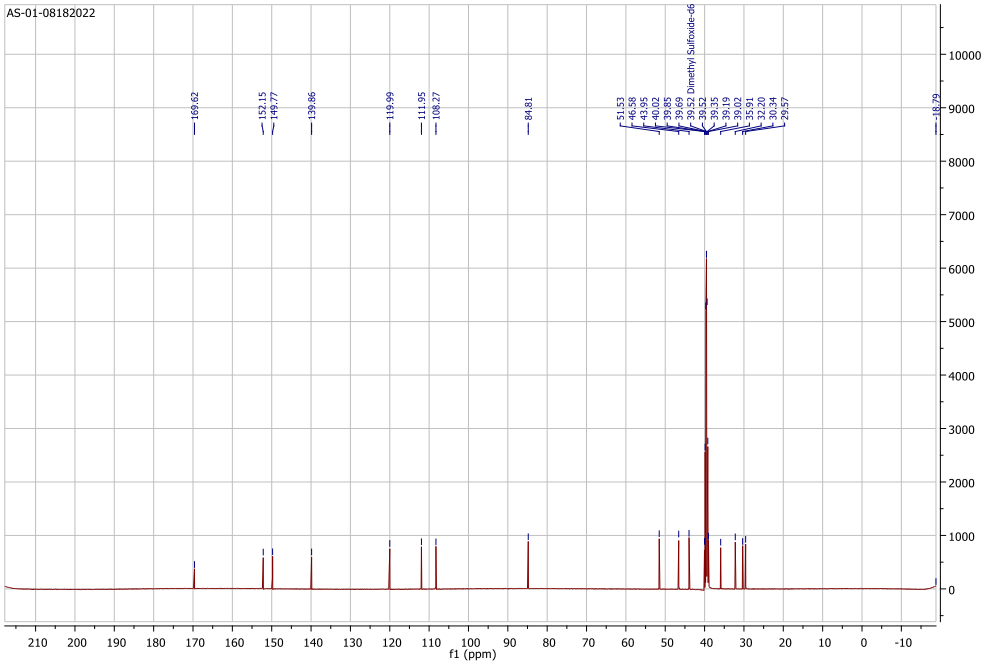

**S1 TLC chromatogram of hexane fraction of Cirsium dender *Friis* extract and isolated compound AS01 in PE: EA (4:1) viewed under UV light of 254nm**



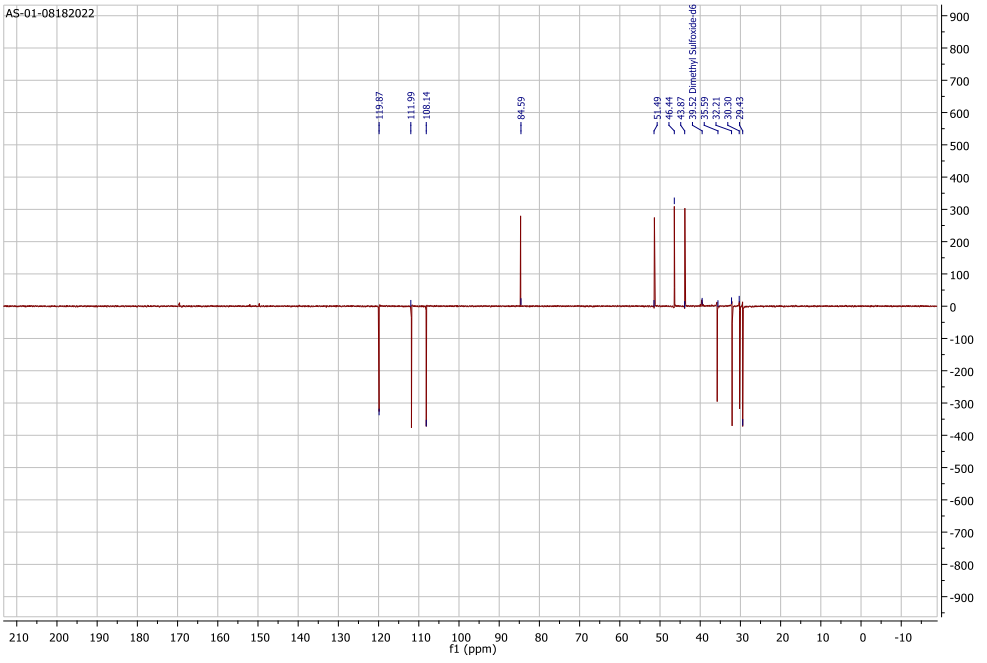

**S2 Atmospheric pressure chemical ionization mass spectrum of AS01**



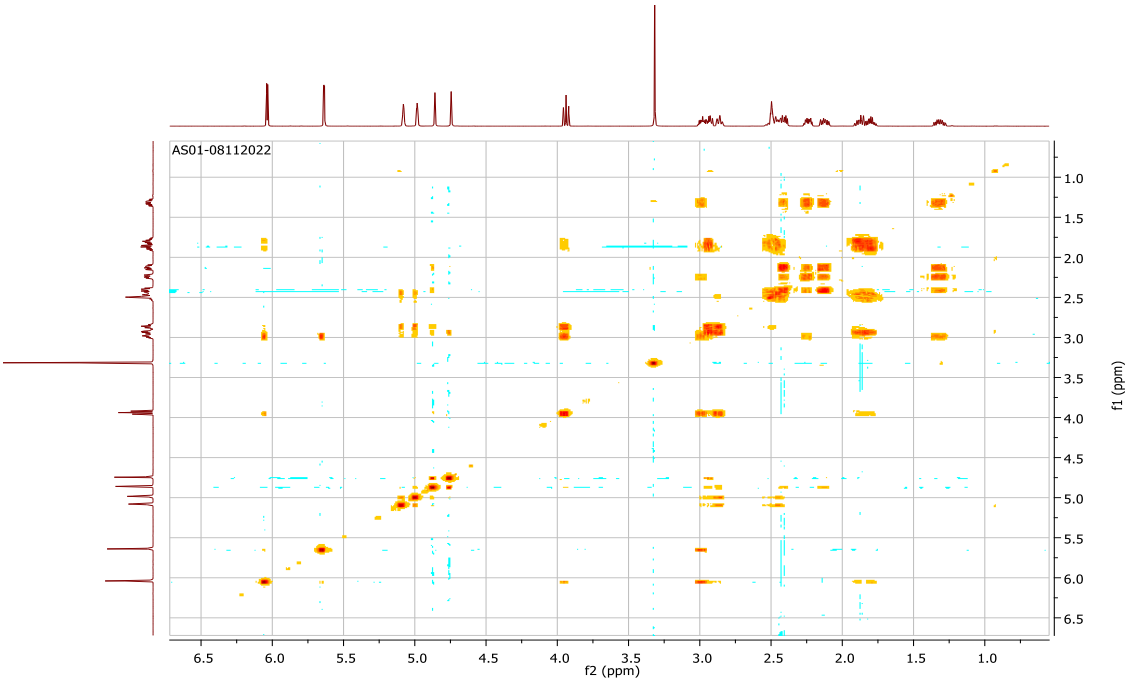

**S3 $^{1}$H-NMR spectrum of AS-01**

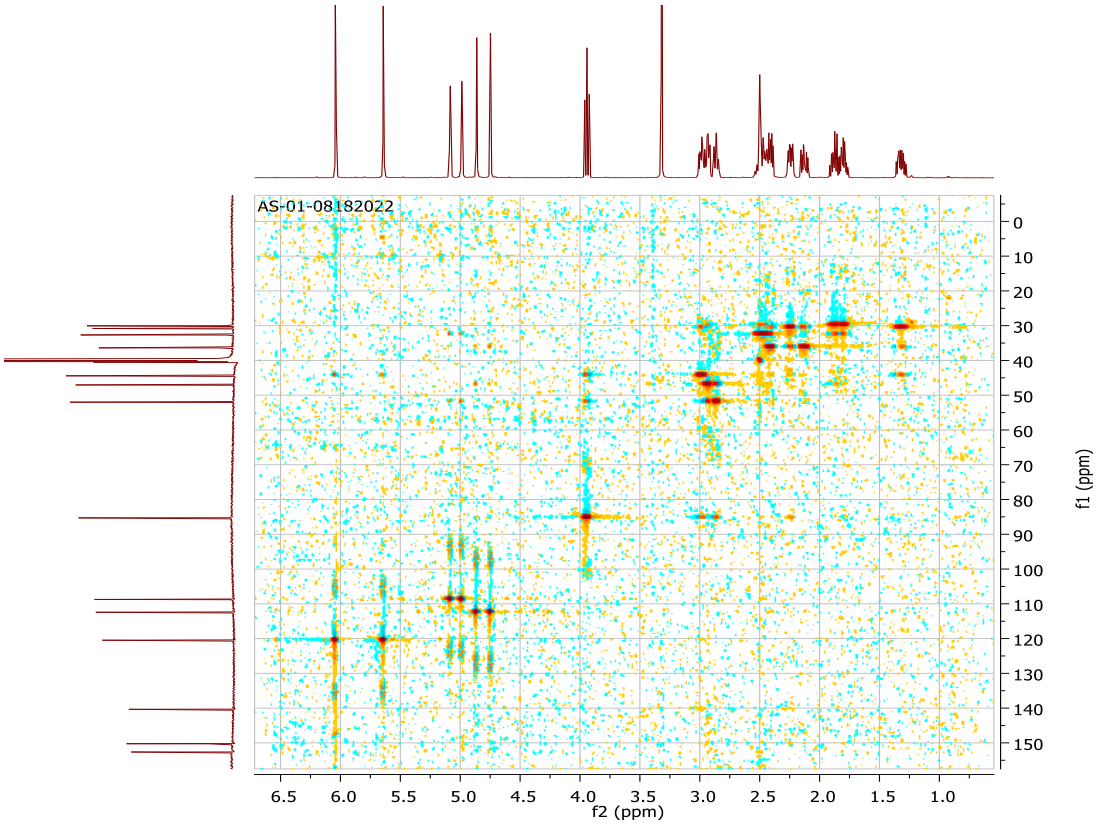



**S4 Figure $^{13}$C-NMR spectrum of AS01**



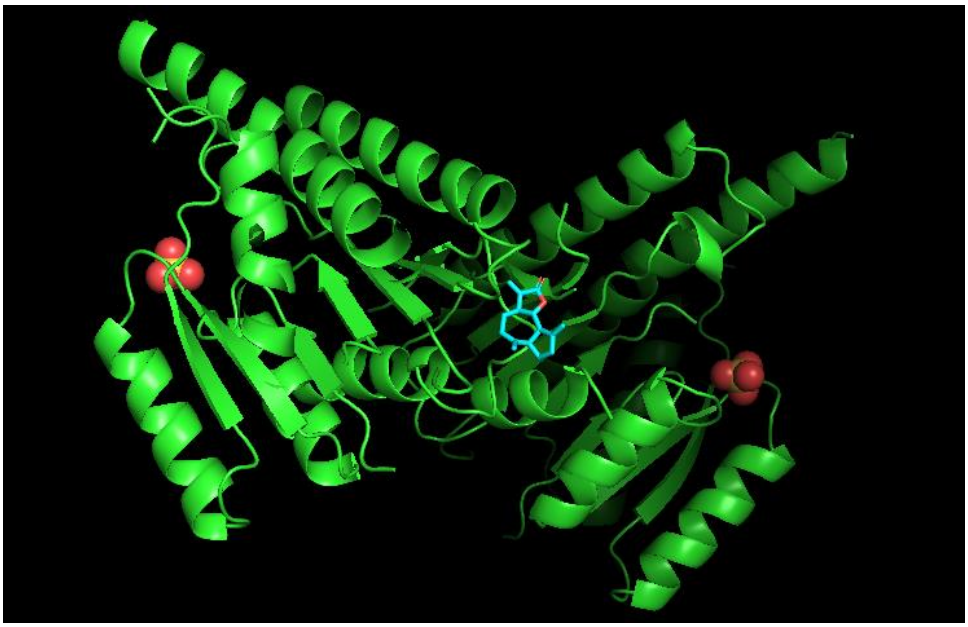

**S5 DEPT-135 spectrum of AS01**



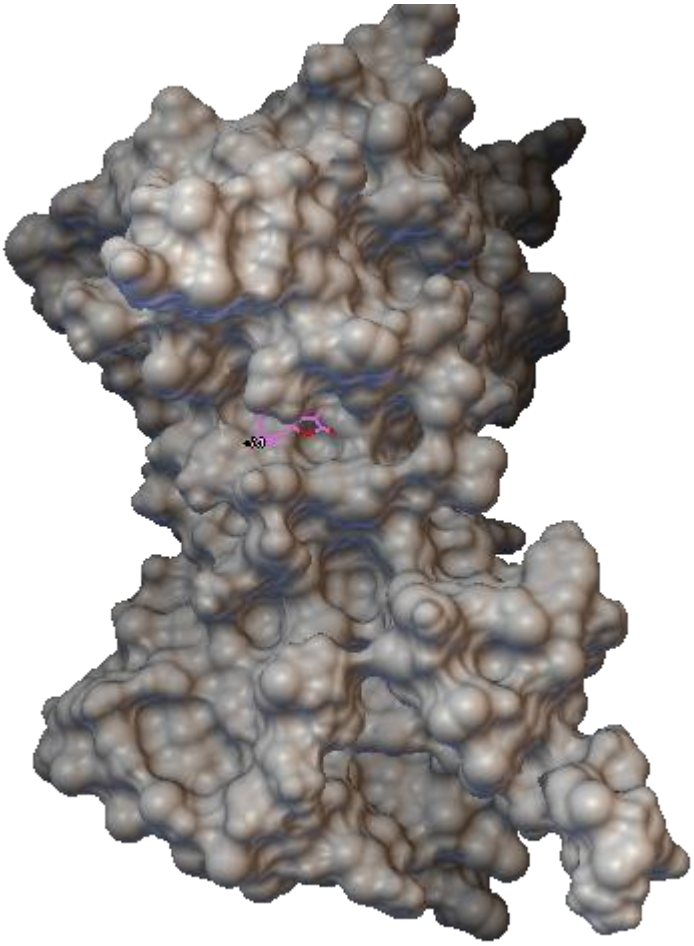



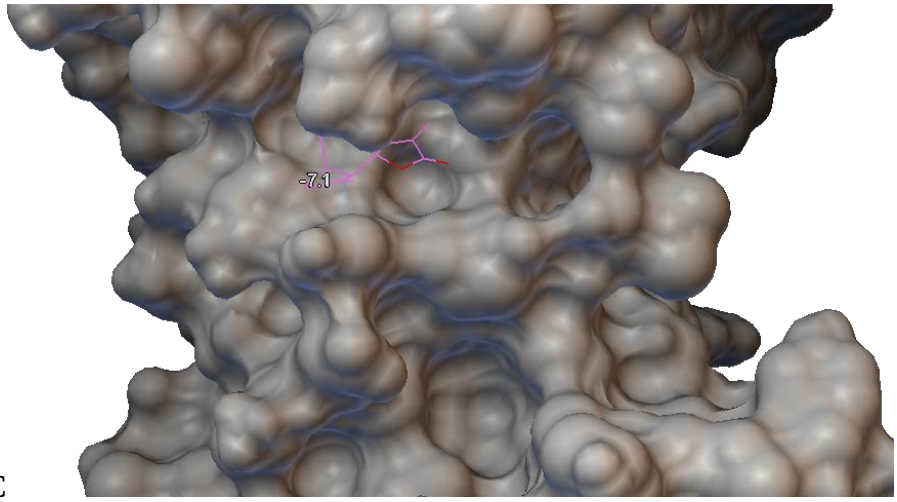

**S6 2DNMR of the compound AS01**

**A**

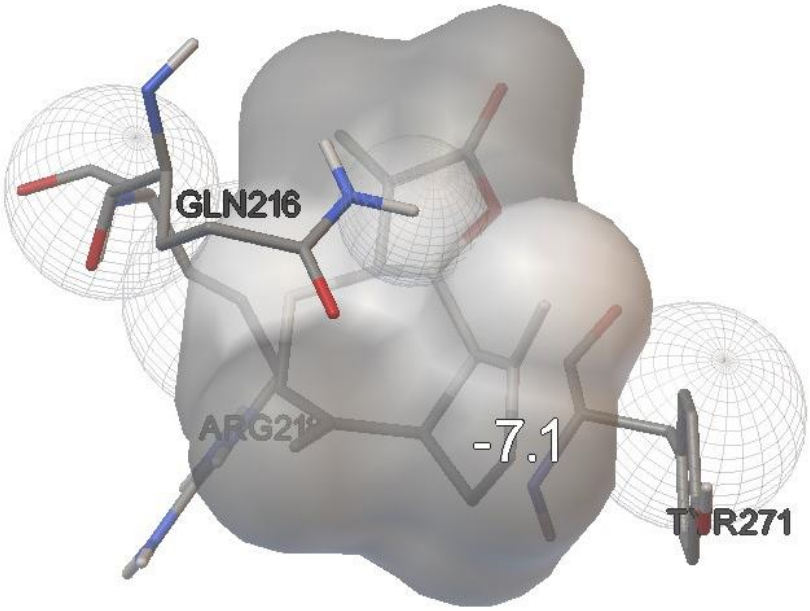


**B**






**C**




**D**

**S7 The binding modes of dehydrocostus lactone with enzyme pteridine reductase 1 (PTR1) (PDB ID: 2XOX).**

(A). Ribbon diagram of docked dehydrocostus lactone- PTR1 complex. (B). Surface representation showing dehydrocostus lactone in the binding site of PTR1. (C). A zoom view of diagram B. (D). Binding interaction of dehydrocostus lactone with amino acid residues of PTR1