UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA

# Antimicrobial and antimalarial activity of *Tabernaemontana elegans* Stapf. (Apocynaceae)

by

**Zimasa Mkatshane**

Submitted in partial fulfilment of the requirements for the degree

**Magister Scientiae**

**(Option: Medicinal Plant Science)**

In the Faculty of Natural & Agricultural Sciences

University of Pretoria

August 2019

**Supervisor: Dr. M.J Bapela**

**Co-supervisor: Dr. T.E Tshikalange**

# DECLARATION

I, Zimasa Mkatshane declare that the thesis/dissertation, which I hereby submit for the degree Magister Scientiae (Option: Medicinal Plant Science) at the University of Pretoria, is my own work and has not previously been submitted by me for a degree at this or any other tertiary institution.

**Signature: ……....**

**Date: ……...**

# ACKNOWLEDGEMENTS


- First and foremost, I am thankful to God for the strength and guidance in completing this research study.
- I am grateful to my family and friends for always being supportive of my studies and for their unconditional love.
- My gratitude goes to my supervisor Dr. M.J. Bapela for giving me the opportunity to conduct this study. I am thankful for her support, guidance, inspiration and suggestions throughout the course of this study. Her help in preparation of this dissertation is acknowledged.
- I express my gratitude to my co-supervisor Dr. T.E. Tshikalange for his assistance with plant collection, support, guidance and suggestions throughout the study.
- I would like to thank Dr. Marcel for his help with *in vitro* antiplasmodial assay conducted in this study.
- Dr. Selepe is acknowledged for her help with NMR experiments.
- I am thankful to Vuyokazi Matshaya for her help with isolation.
- Postgraduate students at the Department of Plant and Soil Sciences, especially Sewes Alberts, Precious Ramontja and Mcebisi Mabuza, they are all thanked for their help and support.
- National Research Fund (NRF) is thanked for funding this study.

# ABSTRACT

Medicinal plants play a crucial role in the healthcare treatment of many infections and also serve as the basis for the synthesis of novel chemotherapeutic agents. Included in these plants is the *Tabernaemontana* genus (Apocynaceae) comprising of about 18 species in Africa of which two (*Tabernaemontana elegans* and *T. ventricosa*) are indigenous to South Africa. These plant species are used by indigenous people to treat various diseases including venereal diseases, malaria cancer, tuberculosis, stomachache and infertility. The aim of the study was to validate some of the ethnomedicinal uses of *T. elegans* as well as to isolate and identify the compounds responsible for their activity.

In this study, different plant parts of *T. elegans* were collected based on their medicinal uses for venereal diseases and malaria by Vha-Venda people residing in Mutale Municipality, Limpopo Province. The plant parts were extracted sequentially using hexane, dichloromethane, methanol, water and decoctions were prepared separately. The recovered crude extracts were tested against *Neisseria gonorrhoeae*, *Candida albicans*, human immunodeficiency virus (HIV) and *Plasmodium falciparum*. Additionally, the plant extracts were subjected to an antiproliferative bioassay against mammalian skeletal myoblast cells. Based on the *in vitro* screening results, the plant extracts were then subjected to $^{1}$H-NMR-based metabolomics in an attempt to discriminate between selective and non-selective antiplasmodial plant extracts. The best antiplasmodial selective plant extract (stem bark) was further subjected to phytochemical analysis using conventional chromatographic and spectroscopic techniques.

For the antigonoccocal assay, the positive control was ciprofloxacin with MIC value <0.05 mg/ml. The best antigonoccocal activity was observed from the methanol extract of roots with MIC value of 0.10 ± 0.04 mg/ml and methanol extract of the stem bark with MIC value of 0.20 ± 0.06 mg/ml. Root decoction also inhibited bacterial growth with MIC value of 0.80 ± 0.37 mg/ml. An activity of 6 mg/ml was observed in the water extract of the stem bark. This was the first study to report on significant antigonoccocal activity of *T. elegans*. Ciprofloxacin was also used as the positive control for the anticandidal assay and displayed an MIC value of <0.05 mg/ml. The extracts displayed an activity of >6 mg/ml for the anticandidal assay. The root extracts (water and dichloromethane) showed a moderate inhibition of 60.78 ± 0.16% and 50.24 ± 0.11% when tested against HIV 1 reverse transcriptase (RT) enzyme. The decoction and water extracts of leaves showed moderate inhibition activity of 58.32 ± 0.05% and 54.00 ± 0.12% and the stem bark also showed moderate inhibition activity in the decoction and water extracts with activity of 57.06 ± 0.03% and 51.29 ± 0.13%, respectively. This was the first study to report on the anti-HIV activity of *T. elegans*. The positive control (doxorubicin) displayed 95.83 ± 0.005% inhibition of the tested enzyme. Six plant extracts exhibited significant antiplasmodial activity ranging from 0.16 ± 0.01 – 4.62 ± 0.55 µg/ml with methanol extracts of stem bark ($IC_{50}$ = 0.31 ± 0.01 µg/ml) and methanol root ($IC_{50}$ = 0.16 ± 0.01 µg/ml) being the extracts with the best activity. The positive control, chloroquine, displayed an antiplasmodial activity of 0.004 ± 0.002 µg/ml.

The plant extracts were subjected to $^{1}H$ NMR-based metabolomics to discriminate between the selective and non-selective antiplasmodial plant extracts. An activity profile that could be used to predict activity of unknown samples was observed. Phytochemical analysis was conducted on the combined non-polar

extracts (hexane and dichloromethane) of the stem bark which were the most active plant extracts. Four compounds were isolated and were subjected to further NMR analysis in an attempt to predict antiplasmodial activity and for the corroboration of their respective structures. Two of the compounds (**2** and **3**) were predicted to have significant antiplasmodial activity based on activity profile generated from metabolomics. The study supported some (malaria and gonorrhoea) of the ethnomedicinal uses of *T. elegans* by indigenous South African people and demonstrated that metabolomics could be used in predicting antiplasmodial activity of unknown samples. Further studies will focus on identification and *in vitro* screening of the four compounds as well as isolating more compounds.

# TABLE OF CONTENTS

## LIST OF ABBREVIATIONS

**AIDS**: Acquired Immunodeficiency Syndrome

**ABTS:** 2,2' – azino-bis(3-ethylbenzothiazoline-6-sulphonic acid)

**CC**: Column Chromatography

**CFU**: Colony forming units

**$^{13}$C NMR:** Carbon Nuclear Magnetic Resonance

**COSY:** Correlated Spectroscopy

**DCM**: Dichloromethane

**DMSO**: Dimethyl sulfoxide

**INT**: Iodonitrotetrazolium

**HIV**: Human Immunodeficiency Virus

**HMBC:** Heteronuclear Multiple Bond Coherence

**$^{1}$H NMR:** Proton Nuclear Magnetic Resonance

**HSQC:** Heteronuclear Single Quantum Coherence

**MIC:** Minimum Inhibitory Concentration

**OPLS-DA:** Orthogonal Projections to Latent Structures – Discriminant Analysis

**PCA:** Principal Component Analysis

**RT**: Reverse Transcriptase

**SI**: Selective Index

**STIs:** Sexually Transmitted Infections

**TLC**: Thin Layer Chromatography

**WHO**: World Health Organization

## LIST OF FIGURES

## LIST OF TABLES

# CHAPTER 1: INTRODUCTION

## 1.1. Problem Statement

Medicinal plants are plants that have medicinal properties and are one of the crucial agricultural economy crops (Yu et al., 2018; Jamshidi-Kia et al., 2018). They have been used worldwide since ancient times and the traditional knowledge has been passed orally from one generation to the next (Hoffman, 2003; Omolewa, 2007). These plants are very rich in compounds that can be used for the development of novel drug candidates. Different plant parts such as roots, seeds, leaves, fruits, flowers or even whole plant may be used. In most parts of the medicinal plants, the active compounds have direct or indirect therapeutic effects and are used as medicinal agents. Bioactive compounds that are synthesized and accumulated in various plant parts have different therapeutic effects in living organisms (Jamshidi-Kia et al., 2018).

Traditional herbal medicines are getting significant attention in global health debates. In Africa, the use of plants as medicine by indigenous people may account for 70% or more of basic healthcare treatments (Bandeira et al., 2001). World Health Organisation (WHO) encourages the administration of herbal remedies, however, as with all medicines; herbal remedies are expected to have side effects, which may be of adverse nature (WHO, 2018). The reported adverse events are often due to poor quality of source material as well as manufacturing and processing factors. There are crucial measures that need to be considered such as quality assurance and control measures (WHO, 2018). These are important measures to ensure safety and efficacy of herbal remedies. In addition, evidence-based proof of bioactivity must be provided. Scientific and systemic evaluation of herbal remedies is needed and the basis for such studies is provided by ethnopharmacology (WHO, 2018).

Ethnopharmacology continues to constitute the scientific backbone for developing active therapeutics based on traditional medicines of various ethnic groups (Mukherjee et al., 2010). The knowledge of traditional medicine is rapidly disappearing due to change in culture and decreasing access to sources of natural medicine, in both rural and urban areas (Alves and Rosa, 2007). Wild populations of many species are overharvested around the world. In southern Africa there are over 3400 plant species that are used for medicinal purposes. About 10% (2062 taxa) of the total flora have been specifically cited as used and /or traded in South Africa (Williams et al., 2013). It has been determined that 82% species (0.4% of the total national flora) are threatened with extinction at a national level (Williams et al., 2013). A further 100 species are of conservation concern, which includes two species that are already extinct in the wild. One of the causes of overharvesting is the high demand of traditional medicine, which results in declining of indigenous plants (Williams et al., 2013).

Additionally, agricultural expansion, trade, urban development, high levels of poverty and an increasing human population are the causes of the drastic decline of medicinal plants (Williams et al., 2013). Significant changes in forests and other vegetation types result in loss of habitats for many species of plants. The loss of habitat means loss species and therefore loss of biodiversity (Bennett, 2017). In many parts of the world, rural and indigenous communities are facing these huge changes, which contribute to weaken indigenous knowledge (Aswani et al., 2018). Although many South African medicinal plants are used traditionally against various diseases, most of them have not been validated for their efficacy. Additionally, most of those that have been screened still need to be subjected to further phytochemical analyses. There is a need for further studies on the bioactivity of South African

medicinal plants, identification of their active constituents and the determination of their modes action.

*Tabernaemontana* genus belongs to the Apocynaceae family and consists of about 100 species that are widely distributed throughout the tropical regions (Mansoor et al., 2009). *Tabernaemontana elegans* is used by South African natives for various ethnomedicinal purposes including tuberculosis, cancer, infertility, stomach ache and some venereal disease (Watt and Breyer-Brandwijk, 1962; Arnold and Gulumian, 1984). In Mozambique, the stem bark of *T. elegans* is used for the treatment of malaria (Bandeira, 2001). It has been previously reported that root extract of *T. elegans* has antibacterial activity against *Staphylococcus aureus* and antimycobacterial activity against *Mycobacterium smegmatis* as well as antifungal activity against *Candida albicans* (Pallant et al., 2012). In a study conducted by Steenkamp et al. (2007), the aqueous root extract showed significant activity against *C. albicans*. In another study, polar and DCM extracts of the stem bark showed significant antiplasmodial activity (Bapela et al., 2014). Indole alkaloids, dregamine and voacangine were isolated as the active antibacterial components of the plant. Bisindole alkaloids tabernaelegantinine B and tabernalegantine C, which were also isolated from the methanol root extract of this plant were found to be potent apoptosis inducers in HCT116 human colon carcinoma cells and possible leads for anticancer drug development (Mansoor et al., 2013). Some of the compounds that are responsible for the mentioned biological activities were not identified. This plant has not been tested against all the pathogens known to cause the mentioned diseases. Previous studies have shown *T. elegans* to be rich in monoterpenoid indole alkaloids, of which most of them have been isolated (Van der Heijden et al., 1986; Mansoor et al., 2009; Pallant et al., 2012;). The modes of action and activity of

the isolated compounds still need investigation. Due to the need of novel agents which may be used for the treatment of malaria, bacterial and fungal infections, further studies need to be conducted on the chemical properties of this plant.

### 1.2 Hypothesis

*Tabernaemontana elegans* has compounds that are active against venereal diseases and malaria.

### 1.3 Objectives

The objectives of this study are to:

- Collect fruits, roots, stem bark and leaves of *Tabernaemontana elegans* used for the treatment of malaria and venereal diseases by the local people of Venda.
- Investigate the antibacterial, antifungal, antiviral and antimalarial activity of each plant part.
- Conduct $^{1}$H NMR-based metabolomics on screened plant extracts.
- Conduct $^{1}$H NMR-based metabolomics to identify the classes of compounds that are attributable to activity.
- Isolate compounds responsible for the observed bioactivity.

### 1.4 Structure of the thesis

All chapters in this thesis have been written according to the format of the South African Journal of Botany.

**Chapter 1:** A problem statement highlighting the ethnomedicinal uses of *Tabernaemontana elegans* and the studies that have been performed on this plant species as well as its research gaps are presented in his chapter. The formulated hypothesis, objectives for the study and structure of the thesis are stated in this chapter.

**Chapter 2:** This chapter outlines a review on southern African *Tabernaemontana* species. The taxonomy, distribution, ethnomedicinal uses as well as pharmacological activity of the plant species are presented in this chapter.

**Chapter 3:** A study on the *in vitro* screening against venereal diseases, malaria and cytotoxicity of four plant parts of *Tabernaemontana elegans* Stapf. (Apocynaceae),

**Chapter 4:** $^{1}$H NMR-based metabolomics study of antiplasmodial plant extracts as well as the identification of the classes of compounds and isolation of compounds attributable to antiplasmodial activity are described in this chapter.

**Chapter 5:** A general discussion based on the results of the study is presented in this chapter. Future prospects are also highlighted.

**Chapter 6:** The appendix includes all the additional data of this study.

## 1.5 References

Alves, R.R.N., Rosa, I.M.L. Biodiversity, traditional medicine and public health: where do they meet? 2007. Journal of Ethnobiology and Ethnomedicine 3(14), 1 – 9.

Arnold, H.J., Gulumian, M. 1984. Pharmacopoeia of traditional medicine in Venda. Journal of Ethnopharmacology 12, 35 – 74.

Aswani, S., Lemahieu, A., Sauer, W.H.H. 2018. Global trends of local ecological knowledge and future implications. PLoS ONE 13(4), e0195440. https://doi.org/10.1371/journal.pone.0195440.

Bandeira, S.O., Gaspar, F., Pagula, F.P. 2001. African ethnobotany and healthcare: emphasis on Mozambique. Pharmaceutical Biology 39, 70 – 73.

Bapela, M.J., Meyer, M.J.J., Kaiser, M. 2014. *In vitro* antiplasmodial screening of ethnopharmacologically selected South African plant species used for the treatment of malaria. Journal of Ethnopharmacology 156, 370 – 373.

Bennet, L. 2017. Deforestation and climate change. The Climate institute, 1 – 16.

Hoffman, D. 2003. Medical herbalism. The Science and Practice of Herbal Medicine. Rochester, VT: Inner Traditions/Bear & Co.

Jamshidi-Kia, F., Lorigooini, Z., Amini-Khoei, H. 2018. Medicinal plants: past history and future perspective. Journal of Herbmed Pharmacology 7(1), 1 – 7.

Mansoor, T.A., Ramalho, R.T., Mulhovo, S., Rodrigues, C.M.P., Ferreira, M.J.U. 2009. Induction of apoptosis in HuH-7 cancer cells by monoterpene and β-carboline indole alkaloids isolated from the leaves of *Tabernaemontana elegans*. Bioorganic and Medicinal Chemistry Letters 19, 4255 – 4258.

Mukherjee, P.L., Venkatesh, P., Ponnusankar, S. 2010. Ethnopharmacology and intergrative medicine – let the history tell the future. Journal of Ayruveda and Intergrative Medicine 1, 100 – 109.

Omolewa, M. 2007. Traditional African modes of education in the modern world. International Review of Education 53, 593 -612.

Pallant, C.A., Cromarty, A.D., Steenkamp, V. 2012. Effect of an alkaloidal of *Tabernaemontana elegans* (Stapf.) on selected micro-organisms. Journal of Ethnopharmacology 140, 398 – 404.

Semenya, S.S., Potgieter, M.J., Erasmus, L.J.C. 2013. Indigenous plant species used by Bapedi healers to treat sexually transmitted infections: Their distribution, harvesting, conservation and threats. South African Journal of Botany 87, 66 – 75.

Steenkamp, V., Fernandes, A.C., Van Rensburg, C.E.J. 2007. Screening of Venda medicinal plants for antifungal activity against *Candida albicans*. South African Journal of Botany 73, 256 – 258.

Watt, J.M., Breyer-Brandwijk, M.G. 1962. The medicinal and poisonous plants of southern and eastern Africa, second edition. Livingstone, London.

Williams, V.L., Victor, J.E., Crouch, N.R. 2013. Red listed plants of South Africa: Status, trends, and assessment challenges. South African Journal of Botany 86, 23 - 35.

World Health Organisation. 2018. WHO guidelines on good herbal processing practices for herbal medicines.

World Health Organisation. 2004. Guidelines on safety monitoring of herbal medicines in pharmacovigilance systems.

Yu, N., Wei, Y., Zhu, Y., Zhu, N., Wang, Y., Zhang, H., Sun, A. 2018. Integrated approach for identifying and evaluating the quality of *Marsdenia tenacissima* in the medicine market. PLoS ONE 13(4), e0195240. https://doi.org/10.1371/journal.pone.0195240.

# CHAPTER 2: SOUTHERN AFRICAN TABERNAEMONTANA GENUS: A REVIEW

## 2.1 Introduction

Plants have been a source of medicines to humans since ancient times and continue to play an important role as therapeutic remedies to date (Fabricant and Farnsworth, 2001; Yuan et al., 2016). They are used to treat physical symptoms of diseases as well as their spiritual origins (Cheikhyoussef et al., 2011). Ethnobotanists study traditional medicine in an attempt to discover and record herbal remedies that are used by traditional practitioners and indigenous people at large. They do these studies to screen for plants that are used to improve human health (WHO, 2002; Popović et al., 2016). The research on traditional medicine escalated in the 20$^{th}$ century with the aim to find new cures for different health conditions (Popović et al., 2016). Today, approximately 80% of cardiovascular, antimicrobial, immunosuppressive and anticancer drugs originate from plants (Pan et al., 2013). It is accepted widely that more than 80% of drugs are either derived directly from natural products or developed from a natural compound (Pan et al., 2013). An example of the role of ethnomedicine in guiding discovery and development of drugs is that of antimalarial drugs, specifically quinine and artemisinin (Cragg and Newman, 2013).

Plants consist of many different compounds, which include secondary metabolites that can be classified into four major classes including phenolic compounds, terpenoids, alkaloids, sulphur-containing compounds and terpenoids (Watchel-Galor and Benzie, 2011, Guerriero et al., 2018). These constituents function alone or in conjunction with one another to produce the desired pharmacological effect (Yuan et al., 2016). They have shown a wide range of benefits in managing different challenging diseases which include cancer, human

immunodeficiency virus / acquired immunodeficiency syndrome (HIV/AIDS), malaria, sickle cell disease and other infectious as well as non-infectious diseases (Sofowora et al., 2013; Builders, 2018). In most instances the secondary metabolite activities vary depending on factors such as plant species, plant part, time of collection, geographic origin, preparation method and the amount administered (Builders, 2018; Yang et al., 2018). Ethnobotanicals are important for pharmacological research and drug development, not only when plant constituents are used directly as therapeutic agents, but also as starting material for the synthesis of drugs or as models for pharmacologically active compounds (Watchel-Galor and Benzie, 2011).

In the plant kingdom, the Apocynaceae family is one of the most medicinally diverse families. The Apocynaceae family consists of 424 genera with more than 4 600 species distributed in five subfamilies (Bhadane et al., 2018). Plants belonging to the Apocynaceae family are a rich source for drugs that have found use both in conventional and traditional medicine. Their medicinal activity is mainly attributed to the presence of alkaloids, which are either steroidal alkaloids or indole alkaloids (Islam and Lucky, 2019). Indigenous people use Apocynaceae species to treat fever, gastrointestinal ailments, malaria, diabetes, inflammations ecto-parasitic diseases and many other diseases (Arnold et al., 2002; Wong et al., 2011; Mongalo and Makhafola, 2018; Islam and Lucky, 2019). The *Tabernaemontana* genus is a very large genus consisting of about 100 species which belongs to the Apocynaceae family and is characterized by novel structured indole alkaloids (Mansoor, 2009; Athipornchai, 2018). The alkaloids of this genus were shown to exhibit biological activities which include antimalarial, anticancer, and antiarrhythmic agents (Athipornchai, 2018). *Tabernaemontana* species continue to be of great interest to ethnopharmacologists because of the anecdotal evidence associated with them. This

review aimed to discuss the ethnomedicinal uses, phytochemical constituents and pharmacological activities of the two southern African *Tabernaemontana* species which are *T*. *elegans* and *T*. *ventricosa*. Additionally, the study intended to highlight some of the research gaps that are associated with the indigenous South African *Tabernaemontana* species.

## 2.2 Taxonomy, plant description and distribution

*Tabernaemontana* genus (Apocynaceae) is distributed throughout the tropical and subtropical areas (Zhu et al., 1990). Its species are used in traditional medicine and for other purposes (van der Heijden et al., 1986). The genus *Tabernaemontana* can be found throughout tropical America, tropical and subtropical Africa, as well as Madagascar. The genus consists of 25 species and 18 of these species occur in Africa. Out of the 18 species that occur in Africa only two of them, *Tabernaemontana elegans* (Stapf.) (Figure 2.1) and *T. ventricosa* Hochst. ex. A.DC. (Figure 2.2) are indigenous to South Africa, (Schmidt et al., 2002). *Tabernaemontana elegans* (Stapf.) (Syn. *Conopharyngia elegans* Stapf.) is commonly known as a toad tree in English because of its brown fruit and wart-like skin. The toad tree is indigenous to tropical east Africa through to South Africa and Swaziland and is the most abundant and harvested of the two species (Mansoor et al., 2009; Mansoor et al., 2013). It is most commonly encountered along riverbanks and in coastal savanna woodlands. It is a semi-deciduous tree or shrub that grows 1.5 m to 5 m tall, but can reach a height of 12 m found in evergreen river fringes at low altitudes and in coastal scrub forests (Coats Palgrave et al., 2003). The leaves are opposite, leathery and dark glossy green above and paler beneath (Figure 2.1). Each inflorescence is a corymb of 5 – 20 cm, two are together in the forks of the branches, many-flowered and is borne on

a peduncle which is 1 – 8.5 cm long (Omino, 2002; Schmelzer and Gurib-Fakim, 2008). The fruits are leathery to woody and are in pairs, with a green skin that is covered in pale warts (Schmelzer and Gurib-Fakim, 2008).

**Figure 2.1:** *Tabernaemontana elegans* (Apocynaceae) (https://www.randomharvest.co.za).

*Tabernaemontana ventricosa* (Figure 2.2), synonymously known as *Tabernaemontana usambarensis,* is a small tree known as a forest toad tree which occurs in open or secondary forests and can grow up to 15 m tall but averages between 4 and 8 m. The tree grows in riverine forests and forest under storeys in the coastal areas of Kwazulu Natal and is also found in Zimbabwe as well as Mozambique and marginally into Swaziland and Mpumalanga (Schmidt 2002; Schmelzer and Gurib-Fakim, 2008). Leaves are simple, opposite, leathery, glossy dark green above and paler below. Flowers are white, trumpet-shaped to 25 mm wide with narrow and twisted petals and are borne in branched clusters at the ends of branches (Schmidt, 2002). The fruits are pods, usually paired and joined at the

base. In contrast to the fruits of *T. elegans*, they are smooth and dark-green but sometimes may have wrinkles and light-green markings. The fruits split open to reveal a fleshy orange pulp with embedded seeds (Schmidt, 2002; Schmelzer and Gurib-Fakim, 2008).

**Figure 2.2:** *Tabernaemontana ventricosa* (Apocynaceae) (https://www.tropical.theferns.info/viewtropical.php?id=Tabernaemontana+ventricos.)

## 2.3 Ethnomedicinal uses

*Tabernaemontana elegans* is gathered by South African natives for a variety of ethnomedicinal purposes including tuberculosis, cancer, infertility, stomach ache and some venereal disease (Watt and Breyer-Brandwijk, 1962; Arnold and Gulumian, 1984). In Mozambique, the stem bark of *T. elegans* is commonly used for the treatment of malaria (Mansoor et al., 2009). Root decoction is applied as a wash to wounds and drunk for chest pains and pulmonary diseases by the Zulu and Vha-Venda people of South Africa and is also said to have aphrodisiac properties (Watt and Breyer-Brandwijk, 1962; Arnold and Gulumian, 1984). A powdered whole fruit is

boiled to treat venereal diseases (Mabogo, 1990). Other uses include treatment of malaria and heart diseases with a decoction prepared from the stem bark (Pooley, 1998). A potpourri of plant material that has roots of *T. elegans* and *Ipomoea batatas* is also used to treat venereal diseases (Arnold and Gulumian 1984; Mabogo, 1990). The leaves are used in combination with *I. batatas* to treat gonorrhoea (Arnold and Gulumian 1984; Mabogo, 1990). The endocarp is dried, pulverised, boiled in water and the water is filtered and taken orally to treat cancer (Arnold et al., 2002). The coagulated milky sap is used as a styptic (Arnold et al., 2002). The root infusion is used as remedy for stomach ache and lung ailments (Arnold and Gulumian, 1984). Menorrhagia is treated by exposing the vulva to the smoke of burned roots (Arnold and Gulumian, 1984). The stem bark and root decoctions are used for febrifuge (Watt and Breyer-Brandwijk, 1962). *Tabernaemontana ventricosa* has fewer reported ethnomedicinal uses than *T. elegans*. The bark decoction of *T. ventricosa* is used by Zulu people to reduce fever and the latex can be used to treat sore eyes and wounds (Pooley 1993; Schmelzer and Gurib-Fakim, 2008). In Natal the seeds, bark and roots are used to treat nervous problems and high blood pressure. Some people use the latex of this tree as birdlime (Schmelzer and Gurib-Fakim, 2008).

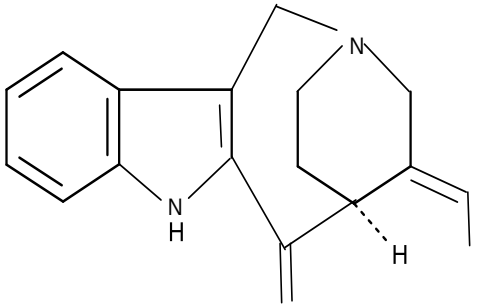
### 2.4 Phytochemical constituents

Phytochemical studies of *Tabernaemontana elegans* have revealed that it is particularly rich in monoterpene alkaloids. Alkaloids are secondary compounds derived from amino acids or via transamination process. They are classified according to the amino acids that provide their nitrogen atom and part of their skeleton. The alkaloids of *Tabernaemontana* contain L-tryptophan-derived alkaloids (Aniszewski, 2007). Alkaloids are used for chemotaxonomic classification which

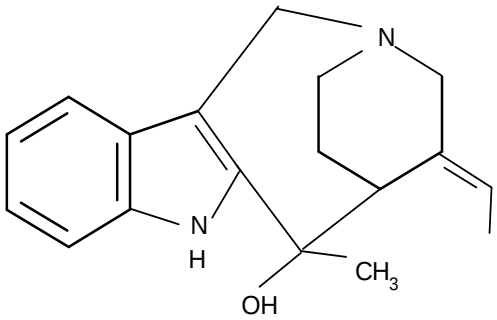
depends upon the type of parent base compound present in the alkaloid. Indole alkaloids contain indole as the parent base (Singh, 2016). This genus is characterised by indole alkaloids of unusual structures, which are endowed with novel bioactivities and are considered chemotaxanomically important (Zhu et al., 1990). The *Tabernaemontana* genus is known to produce a variety of skeletal types of indole alkaloids, including aspidosperma-type, tetrakis-aspidosperma-type, iboga-type and vobasinyl-ibogan-type (Vanbeek et al., 1984, 1985; Schmelzer, 2006). Indole alkaloids are natural products with pharmacological activities which have been shown to have many biological activities including antiviral, cytotoxicity, enzyme inhibitory, antimicrobial, reversal vincristine-resistance and antifungal activities (Vanbeek et al., 1985; Achenbach et al., 1997; Kam et al., 1998; Chaturvedula et al., 2003; Andrade et al., 2005; Almagro et al., 2015; Rocha et al., 2018).

There are 32 alkaloids that were isolated from *T. elegans* and eight from *T. ventricosa* (Figure 2.3). *Tabernaemontana elegans* synthesizes and accumulates both monoterpene indole and bisindole alkaloids. Monoterpene indole alkaloids are from a tryptamine unit and a $C_9/C_{10}$ terpenoid unit and bisindole alkaloids consists of two monomeric indole alkaloid units (Balsevich, 1988; Rahman et al., 2016). Heijden et al. (1986) isolated five new and 13 known indole alkaloids from a whole plant extract of *T. elegans* with 96% alcohol. The five new alkaloids were identified as dregminol (**7**), dregaminol-methylether (**10**), tabernaemontaninol (**9**), 3-*R/S*-hydroxy-tabernaelegantine B (**17**) and 3-*R/S*-methoxy-tabernaelegantine C (**18**) and the 13 known alkaloids are apparicine (**1**), 16-*S*-hydroxy-16,22-dihydro-apparicine (**2**), tubotaiwine (**3**), vobasine (**4**), vobasinol (**5**), isovoacongine (**11**), tabernaelegantine A, B, C, D (**13**, **14**, **15**, **16**), 3-*R/S*-hydroxy-conodurine (**12**), dregamine (**6**) and tabernaemontanine (**8**). The known alkaloids were previously isolated by Bombardelli

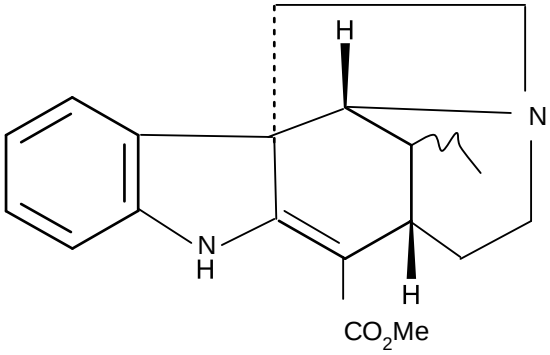
et al. (1976), Gabetta et al. (1975) and Danieli et al. (1980). Mansoor et al. (2009) isolated three *β*-carboline indole alkaloids known as tabernine A, B and C (**21**, **22**, and **23**) as well as a novel monoterpene known as eleganine A (**24**), from a methanol extract of leaves. Mansoor et al. (2013) isolated four monomeric indole and two bisindole alkaloids from a methanol extract of the roots. The four monomeric indole alkaloids include tabernaemontanine (**8**), dregamine (**6**), 16-epidregamine (**26**) as well as voacangine (**25**) and the two bisindole alkaloids include tabernaelegantinine C (**28**) and tabernaelegantinine B (**29**) (Mansoor et al., 2013). Three new and two known bisindole alkaloids of the vobasinyl-iboga type were isolated by Paterna et al. (2016) from the methanol extract of roots. The three new indole alkaloids are (19'*S*)-hydroxytabernaelegantine A (**38**), 3'oxotabernaelegantine C (**39**) and 3'-oxotabernaelegantine D (**40**) and the two known are tabernaelegantine A (**13**) and tabernaelegantine D (**16**). The major indole alkaloids of *T. ventricosa* which were isolated from the stem bark include 10-hydroxyheyneanine (**31**) and akuammicine (**32**). There were minor amounts of apparicine (**1**), tubotaiwine (**3**), norfluorocurarine (**33**), akuammicine N4-oxide (**34**), 16-epi-isositsirikine (**35**) and 10-hydroxycoronaridine (**36**) (Schmelzer and Gurib-Fakim , 2008). Isolation of the stem bark extract (1:1 MeOH in $CH_2Cl_2$) by Munayi, (2016) in *T. ventricosa* yielded four compounds which were identified as friedelin (**42**), *trans*-triacontyl-4-hydroxy-3-methoxycinnamate (**43**), betulinic acid (**44**) and catechin (**45**). The root bark extract (1:1 MeOH in $CH_2Cl_2$) was also isolated by Munayi, (2016) and yielded two compounds which were identified as stigmasterol (**46**) and *β*-sitosterol (**47**).



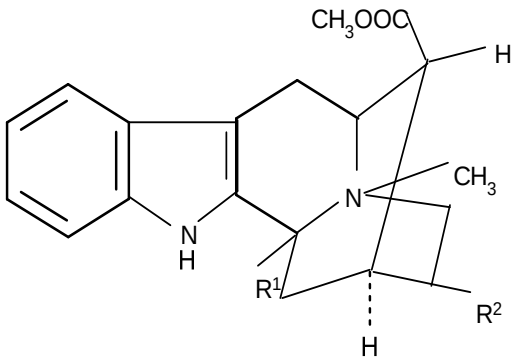

**(1)** Apparicine




**(2)** 16-*S*-hydroxy-16, 22-dihydro-apparicine




**(3)** Tubotaiwine



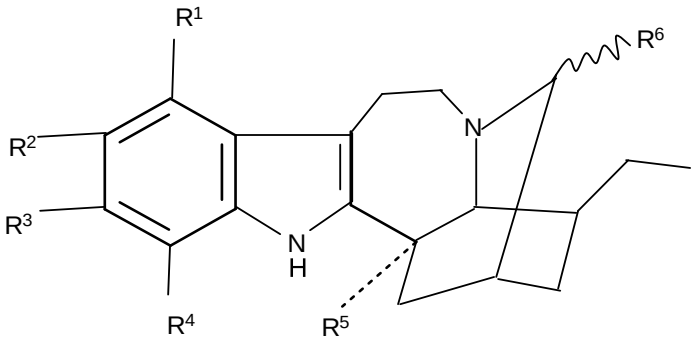

**(4)** Vobasine : $R^1$ = O, $R^2$ = CH-$CH_3$

**(5)** Vobasinol : $R^1$ = α-OH, $R^2$ = CH-$CH_3$
**(6)** Dregamine : $R^1$ = O, $R^2$ = α-$C_2H_5$
**(7)** Dregaminol : $R^1$ = α-OH, $R^2$ = α-$C_2H_5$

**(8)** Tabernaemontanine : $R^1$ = O, $R^2$ = β-$C_2H_5$

**(9)** Tabernaemontaninol : $R^1$ = α-OH, $R^2$ = β-$C_2H_5$

**(10)** Dregaminol-methylether : $R^1$ = $OCH_3$, $R^2$ = α-$C_2H_5$




**(11)** Isovoacangine: $R^1 = R^2 = R^4 = H$, $R^3 = OCH_3$, $R^5 = COOCH_3$, $R^6 = H$

**(12)** 3-*R*/*S*-hydroxy-conodurine: $R^1 = R^2 = H$, $R^3 = OCH_3$, $R^4$ = 3'-vobasinyl, $R^5$ = COOCH3, $R^6$ = OH

**(13)** Tabernaelegantine A: $R^1 = R^2 = H$, $R^3 = OCH_3$, $R^4$ = 3'-tabernaemontanyl, $R^5 = COOCH_3$, $R^6 = H$

**(14)** Tabernaelegantine B: $R^1 = H$, $R^2$ = 3'-tabernaemontanyl, $R^3 = OCH_3$, $R^4 = H$, $R^5 = COOCH_3$, R6 = H

**(15)** Tabernaelegantine C: $R^1 = R^2 = H$, $R^3 = OCH_3$, $R^4$ = 3'-dregaminyl, $R^5 = COOCH_3$, $R^6 = H$

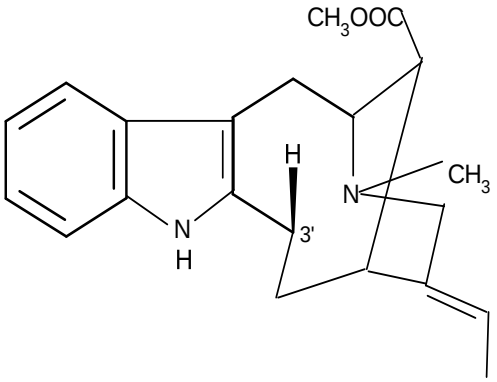
**(16)**Tabernaelegantine D: $R^1 = H$, $R^2$ = 3'-dregaminyl, $R^3 = OCH_3$, $R^4 = H$, $R^5 = COOCH_3$, $R^6 = H$

**(17)** 3-*R*/*S*-hydroxy-tabernaelegantine B: $R^1 = H$, $R^2$ = 3'-tabernaemontanyl, $R^3 = OCH_3$, $R^4 = H$, $R^5 = COOCH_3$, $R^6 = OH$

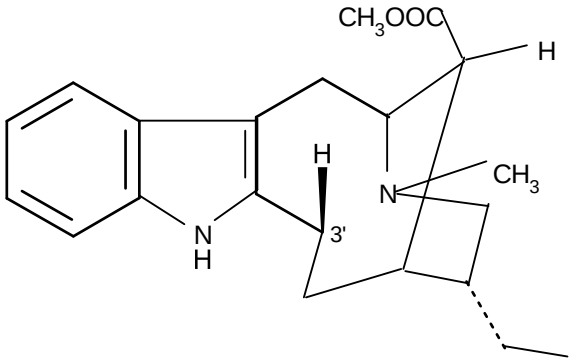
**(18)** 3-*R*/*S*-methoxy-tabernaelegantine C: $R^1 = R^2 = H$, $R^3 = OCH_3$, $R^4$ = 3'-dregaminyl, $R^5 = COOCH_3$, $R^6 = OCH_3$

**(19)** Conopharyngine: $R^1 = R^4 = R^6 = H$, $R^2 = R^3 = OCH_3$, $R^5 = COOCH_3$

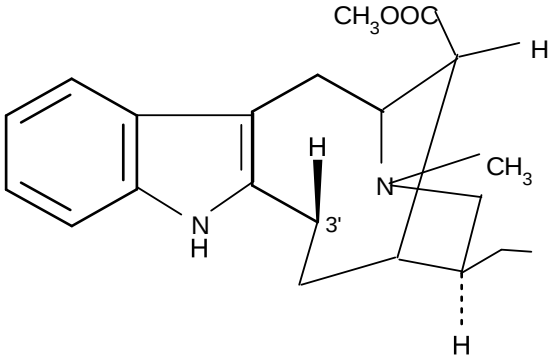
**(20)** Conoduramine: $R^1 = R^4 = R^6 = H$, $R^2$ = 3'-vobasinyl, $R^3 = OCH_3$, $R^5 = COOCH_3$



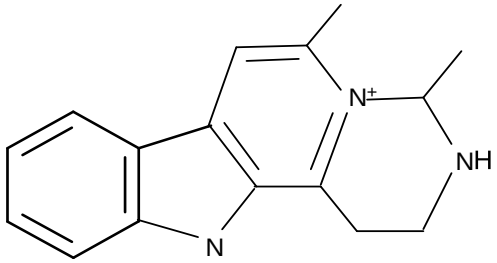

3'-vobasinyl



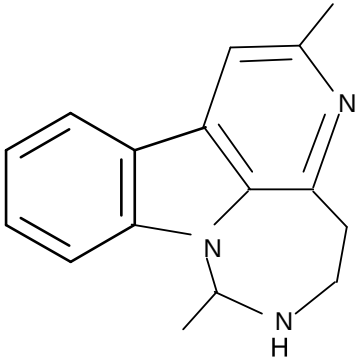

3'-dregaminyl



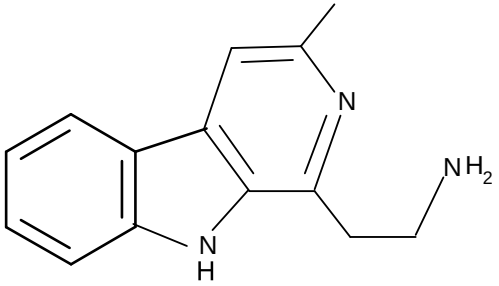

3'-tabernaemontanyl



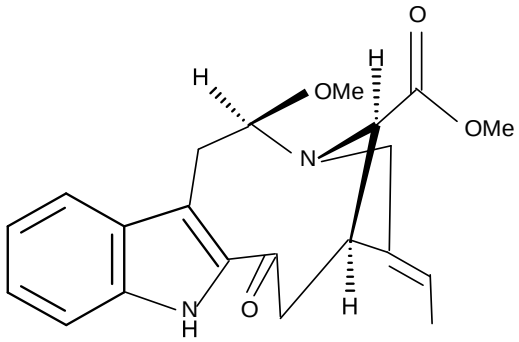

**(21)** Tabernine A



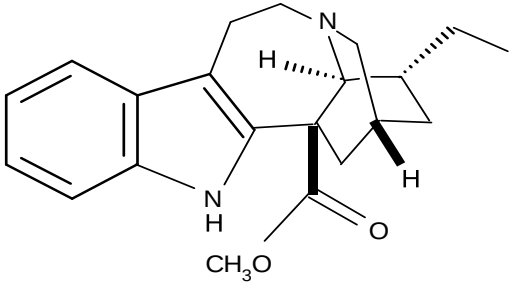

**(22)** Tabernine B



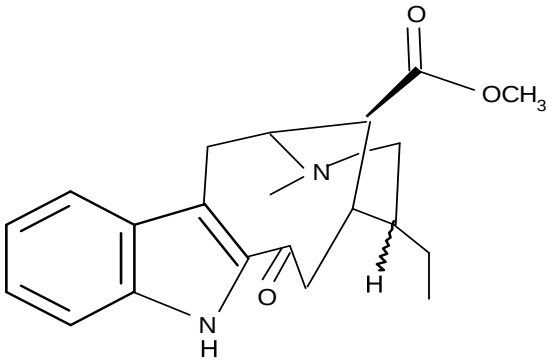

**(23)** Tabernine C



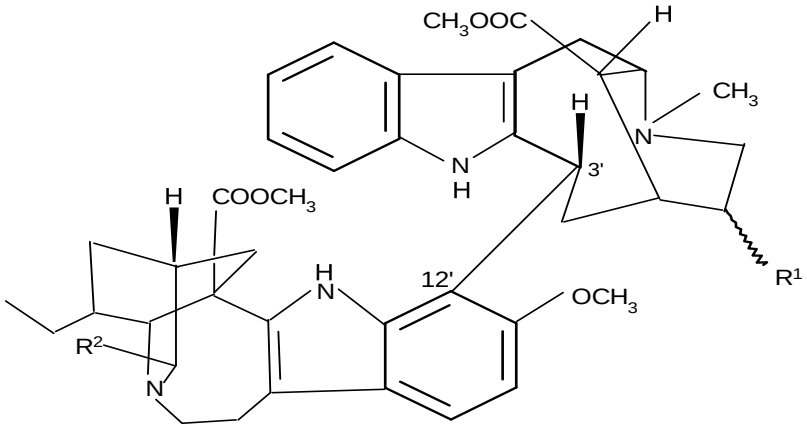

**(24)** Eleganine A




**(25)** Voacangine

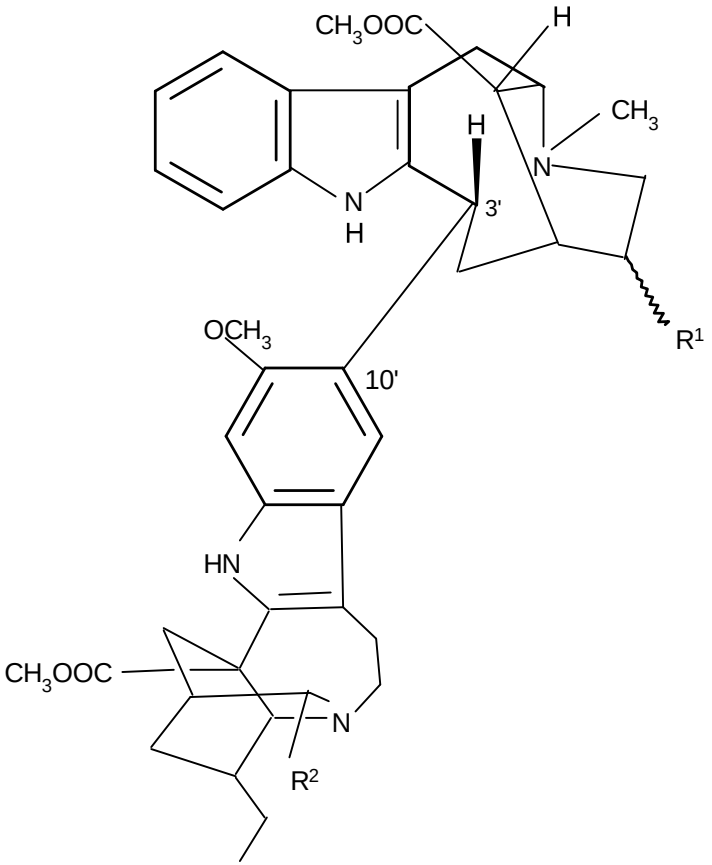



**(26)** 16-Epidregamine



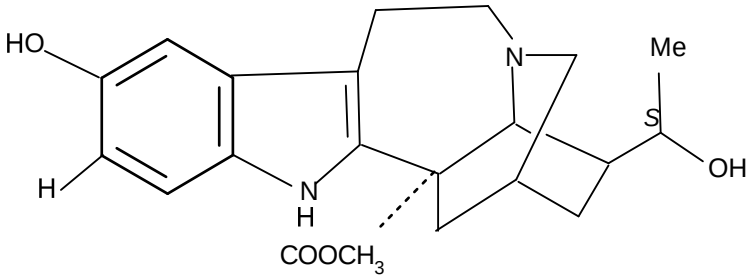

**(27)** Tabernaelegantinine A: $R^1$ = β - Et, $R^2$ = $CH_2COCH_3$

**(28)** Tabernaelegantinine C: $R^1$ = α – Et, $R^2$ = CN

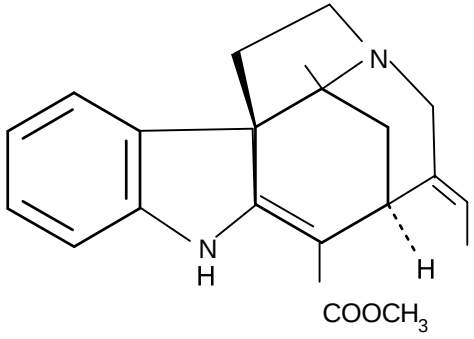



**(29)** Tabernaelegantinine B: $R^1$ = β – Et, $R^2$ = $CH_2COCH_3$

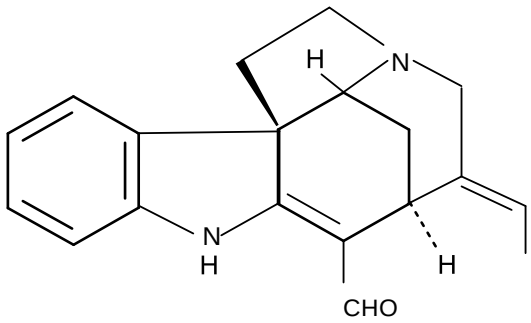
**(30)** Tabernaelegantinine D: $R^1$ = α – Et, $R^2$ = CN



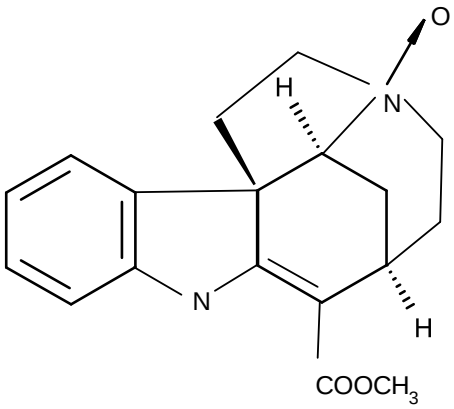

**(31)** 10-Hydroxyheyneanine



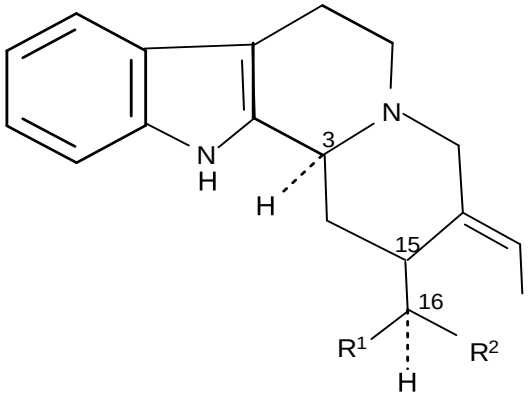

**(32)** Akuammicine



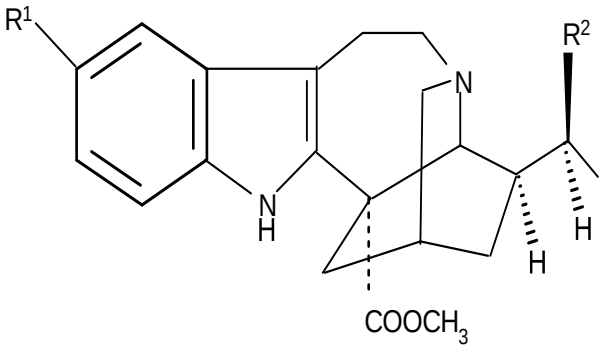

**(33)** Norfluorocurarine



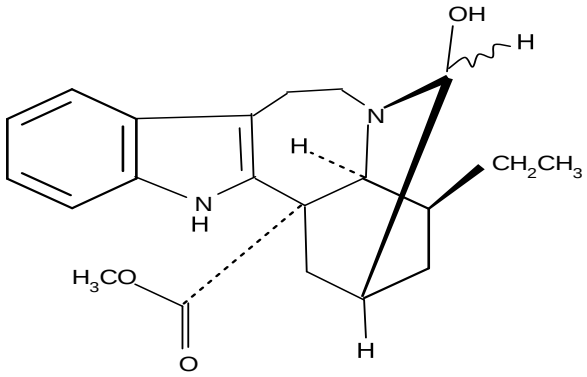

**(34)** Akuammicine N4-oxide



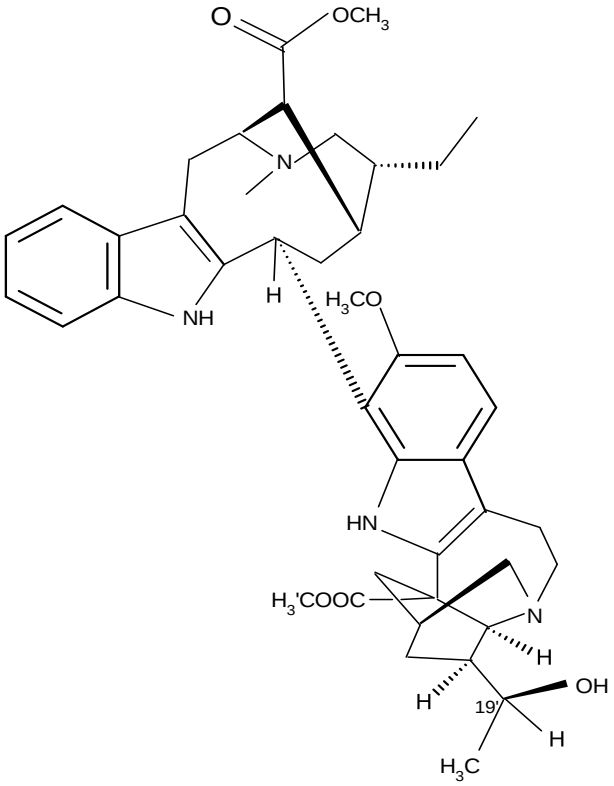

**(35)** 16-Epi-isositsirikine: $R^1 = COOCH_3$, $R^2 = CH_2OH$



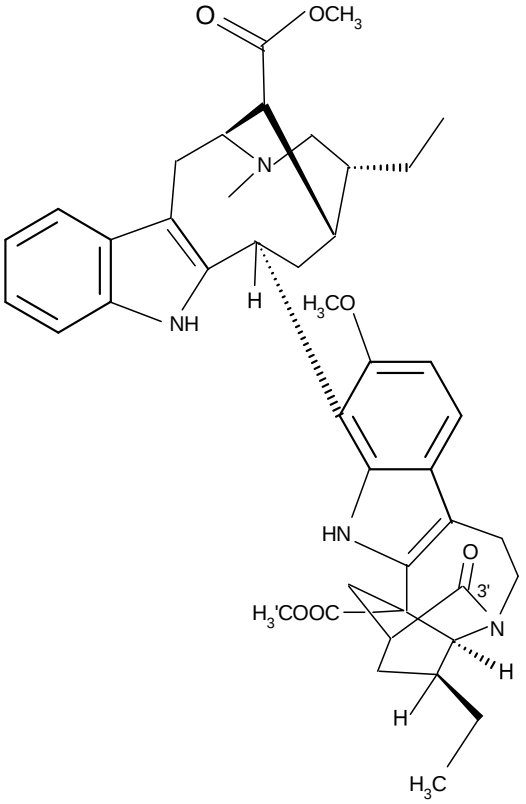

**(36)** 10-Hydroxycoronaridine: $R^1 = OH$, $R^2 = H$



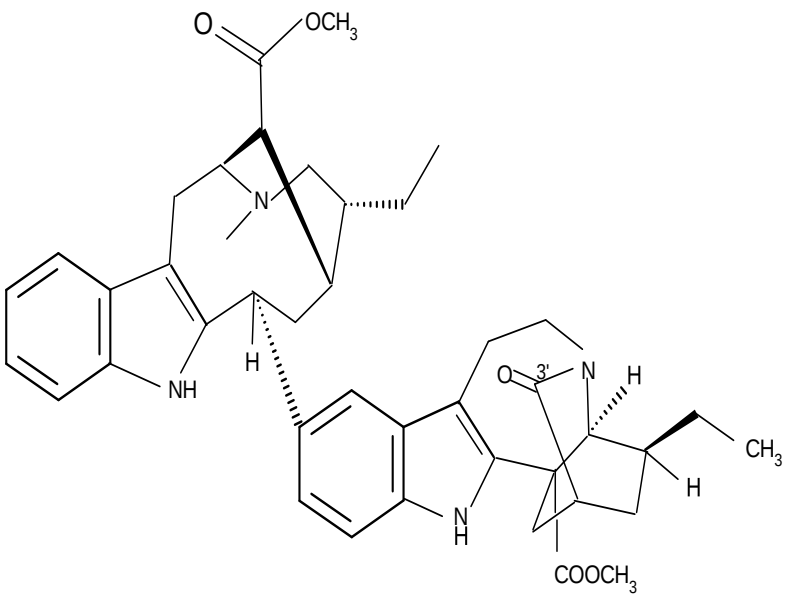

**(37)** 3-Hydroxycoronaridine



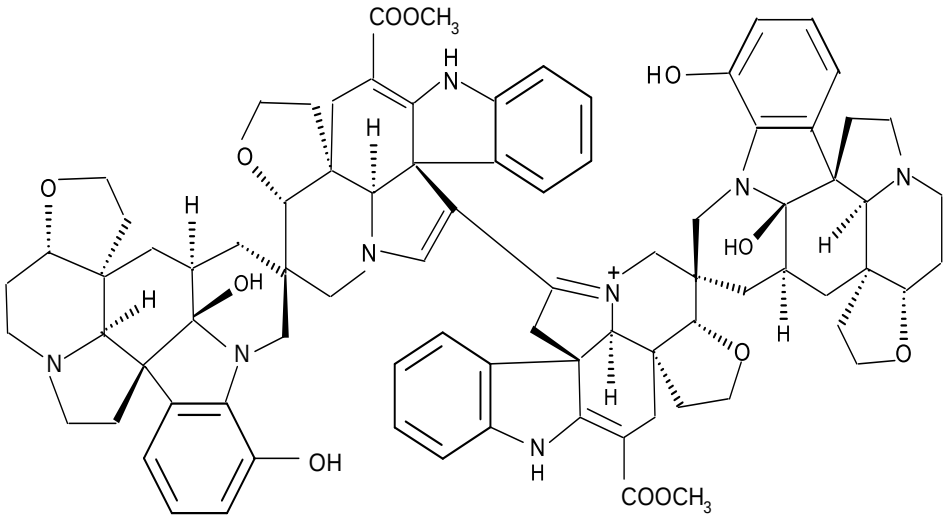

**(38)** (19’*S*)-hydroxytabernaelegantine A



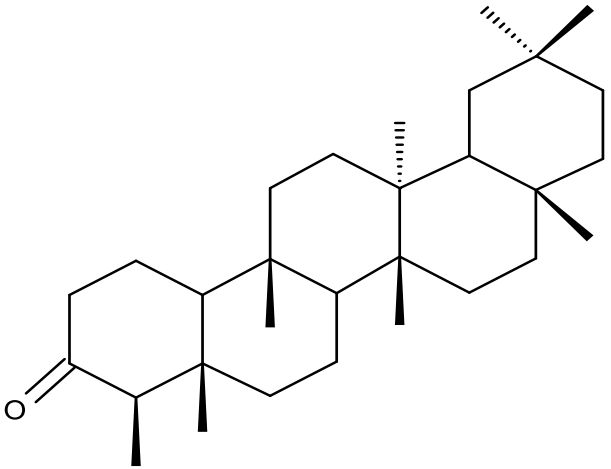

**(39)** 3’-Oxotabernaelegantine C



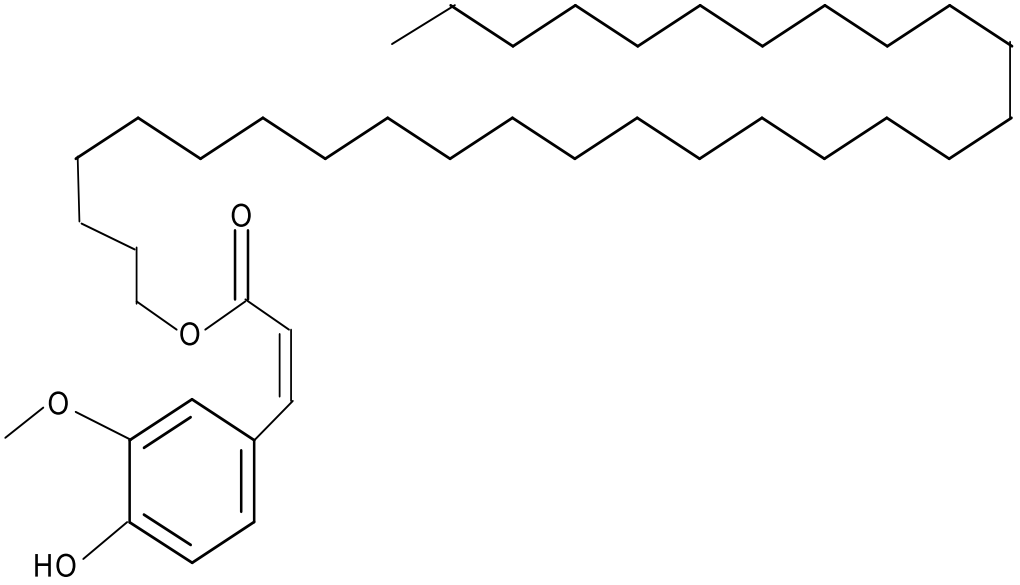

**(40)** 3’-Oxotabernaelegantine D



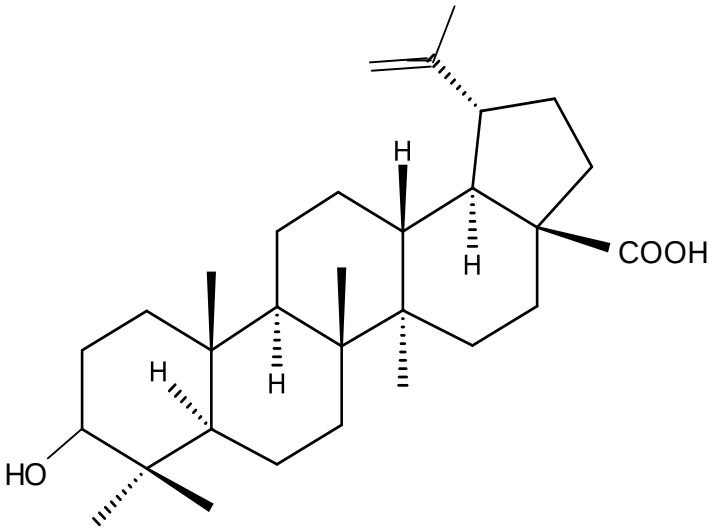

**(41)** Alasmontamine A



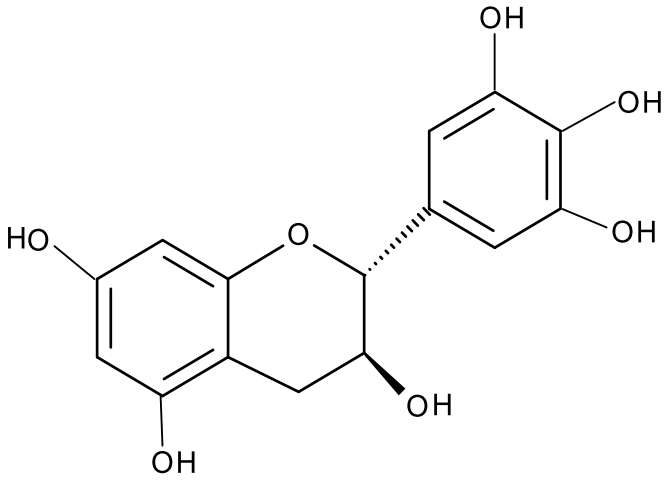

**(42)** Friedelin



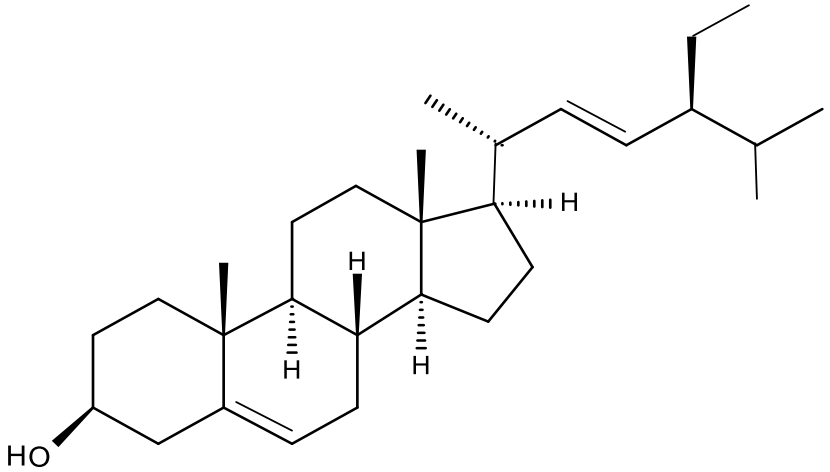

**(43)** *Trans*-tricontyl-4-hydroxy-3-methoxycinnamate



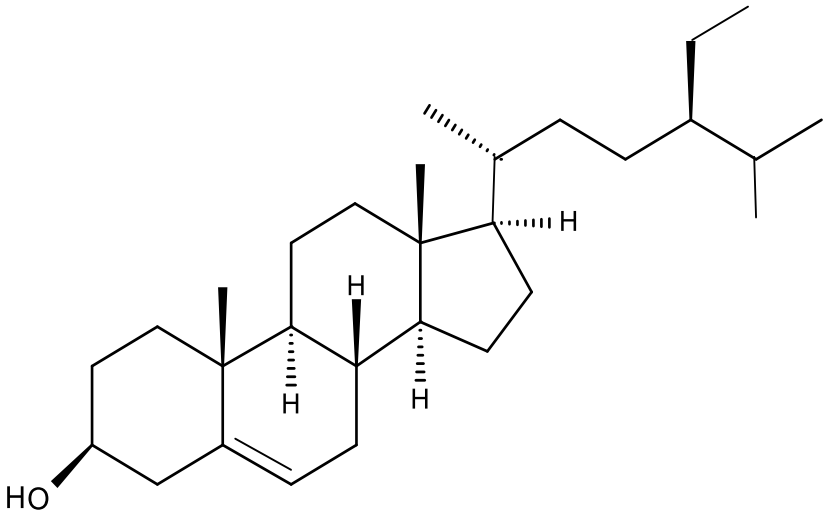

**(44)** Betulinic acid




**(45)** Catechin




**(46)** Stigmasterol




**(47)** *β*-sitosterol

**Figure 2.3:** Indole alkaloids from *Tabernaemontana elegans* (Bombardelli, 1976; Danieli, 1980; Van der Heijden et al., 1986; Mansoor et al., 2009; Mansoor et al., 2013; Hirasawa et al., 2009) and *T*. *ventricosa* (Schmelzer and Gurib-Fakim, 2008; Munayi, 2016).

## 2.5 Pharmacological activities

### 2.5.1 Antibacterial activity

Bacterial infections remain a significant threat to human health. There are new resistance mechanisms that are emerging and spreading globally, threatening the ability to treat common infectious diseases. It is becoming harder and sometimes impossible to treat infections such as tuberculosis, gonorrhoea, pneumonia, foodborne diseases and blood poisoning as antibiotics become less active (World Health Organisation (WHO), 2018). Development of novel antibiotics is needed to ensure that effective treatment remains available. Several studies have been performed on the antibacterial activity of *Tabernaemontana elegans*. Pallant and Steenkamp (2008) have previously identified *T. elegans* as having antibacterial activity against *Staphylococcus aureus* and antimycobacterial activity against *Mycobacterium smegmatis* by using a colorimetric, tetrazolium-based broth microdilution assay. The aqueous root extract showed antibacterial activity against *S. aureus* and *M. smegmatis* at 0.5 mg/ml and 1 mg/ml, respectively. In a study conducted by Maduriera et al. (2012), the dichloromethane (DCM) and ethyl acetate extracts of leaves showed antibacterial activity against *Enterococcus faecalis* and *S. aureus* at 62.5 and 12.5 µg/ml, respectively. The ethyl acetate (EtoAc) extract of roots showed activity of 15.6 µg/ml against *Mycobacterium tuberculosis* H37Rv and 31.2 µg/ml against *M*. *tuberculosis* H37Ra in a study conducted by Luo et al. (2011). The 70% ethanol (EtOH) and water ($H_2O$) extracts of roots both showed moderate activity of 125 µg/ml against *M. tuberculosis* (Luo et. al., 2011). There was significant activity in the root EtoAc extract of 15.6 µg/ml against *M. bovis* BCG and moderate activity of 125 µg/ml against *M. smegmatis* (Luo et. al., 2011). *Tabernaemontana elegans* was identified by Van Beek et al. (1984) as one of the eight

*Tabernaemomtana* species to have antibacterial activity against Gram-positive bacteria. Dregamine (**6**) was reported to possess convulsant and respiration-stimulant activities and has been used in treatment of muscular and nervous asthma, respiratory depression as well as type III poliovirus (HPV-3). Conoduramine (**20**), 3-hydroxyconodurine (**12**), 3-hydroxycoronaridine (**37**) and apparicine (**1**) showed strong inhibitory against a range of gram-positive and gram-negative bacteria (Schmelzer and Gurib-Fakim, 2008). Tabernaemontanine (**8**) has vasodilatory effect and can be used in cases of cerebral trauma, arteriosclerosis and circular irregularities. It also shows antibacterial activity against several human pathogenic bacterial strains and is cytotoxic to human nasopharyngeal epidermoid carcinoma cells *in vitro* (Schmelzer and Gurib-Fakim, 2008). Indigenous people orally administer a combination of *T. elegans* and *Ipomoea batatas* leaves decoction to treat gonorrhoea (Arnold and Gulumian, 1984; Mabogo, 1990). A powdered whole fruit decoction as well as a decoction combination of *T. elegans* roots with those of *I. batatas* are used to treat venereal diseases (Arnold and Gulumian, 1984; Mabogo, 1990). The fruits of *T. elegans* and the root combinations (*T. elegans* and *I. batatas*) have not been tested against venereal diseases. *Tabernaemontana ventricosa* bark extracts do not show antibacterial activity *in vitro*. In opiate receptor studies, akuammicine (**32**) showed opioid activity. (Schmelzer and Gurib-Fakim, 2008).

### 2.5.2 Antifungal activity

Over a billion of people are affected by fungal infections and more than 150 million have serious fungal infections which have major impact in their lives or are fatal (Bongomin, 2017). Although there has been a great change in the epidemiology of fungal infections in the past decade, *Candida*, *Aspergillus*, *Cryptococcus* species,

*Pneumocystis jirovecii*, *Histoplasma capsulatum* and mucormycetes remain the main fungal pathogens responsible for most fatal fungal infections (Bongomin, 2017). The increase in fungal infection incidences has exacerbated the need for new antifungal agents since many of the currently used drugs have undesirable side effects, are ineffective against new or re-emerging fungi and/or lead to the rapid development of resistance (Santos, 2017). Studies conducted by Steenkamp et al. (2007) revealed antifungal activity of the aqueous root extract of *T. elegans* against *Candida albicans* at <1 mg/ml. *Candida albicans* is the main agent responsible for mucosal diseases. *Tabernaemontana elegans* significant activity against *C. albicans* reveals that it could be a useful lead in the search for new anticandidal drugs. Indigenous people use powdered whole fruit decoction and a combination of *T. elegans* and *I. batatas* roots to treat venereal diseases (Arnold and Gulumian, 1984; Mabogo 1990). *Tabernaemontana ventricosa* did not show antifungal activity *in vitro* (Schmelzer and Gurib-Fakim, 2008). There are no antifungal studies conducted on the isolated compounds of the two plant species.

### 2.5.3 Antiviral activity

Influenza is a viral pathogen that imposes a health burden of the central nervous system. It remains a major threat for humans which require effective antiviral remedies. It is known to be a resistant virus with frequent mutations, causing severe respiratory diseases in the upper respiratory system and the clinical efficacy of the used drugs is ambiguous (Mehrbod, 2014; Mehrbod et al., 2018). The methanol extract of leaves of *Tabernaemontana ventricosa* was tested against influenza A virus (IAV) (Mehrbod et al., 2018). The extract had a good selectivity

index (SI) value (>3) but was weakly active against IAV with no significant decrease in IAV hemagglutination assay (HA) titer ($P \geq 0.05$).

### 2.5.4 Antiparasitic activity

Intestinal parasitic infections and protozoan infections are recognized as major causes of illness and disease in disadvantaged communities. There are about 450 million to 840 million cases that occur worldwide and 95% of these are in developing countries (M'bondoukwe et al., 2018). In sub-Saharan Africa, *Plasmodium* is the protozoan associated with the highest disease burden (M'bondoukwe et al., 2018). Drug resistance associated with the treatment of human parasites is widespread and is considered to be one of the foremost concerns in health and disease management (Pramanik et al., 2019). Previous studies have shown that *T. elegans* could be a useful lead in the search for new antiparasitic drugs. Studies conducted by Bapela et al. (2014) of DCM and polar extracts from the stem bark of this species also showed significant antiplasmodial activity at $IC_{50}$ values of 0.33 and 0.83 µg/ml with respective SI values of 14 and 46. The SI values were calculated by dividing the $IC_{50}$ value obtained for the L-6 skeletal myoblast cells by the $IC_{50}$ value of the protozoal species under study (Bapela et al., 2014). The study conducted by Bapela et al. (2014) was the first one to document significant antiplasmodial activity of *T. elegans.* There are no data published on the antiplasmodial activity of indole alkaloids isolated from *T. elegans* (Bapela et. al., 2019). However, the indole alkaloids are also found in other closely related plant species. Girardot et al. (2012) conducted an antiplasmodial study on indole alkaloids isolated from *Muntafara sessilofolia* which included dregamine (**6**) and tabernaemontanine (**8**). Dregamine (**6**) and tabernaemontanine (**8**), also found in *T.*

*elegans* showed significant antiplasmodial activity when tested against chloroquine-resistant strain of *P. falciparum*. In a study conducted by Ramalhete et al. (2008) on antimalarial activity of some plants, leaves DCM extract of *T. elegans* showed moderate activity antiplasmodial activity ($IC_{50}$ = 26.9 ± 5.3 µg/mL). Indigenous people use a root infusion as well as a stem bark decoction to treat malaria and febrifuge by orally administering them (Watt and Breyer-Brandwijk, 1962; Pooley, 1998; Ngarivhume et al., 2015). The DCM extract of the stem bark was tested against *Leishmania donovani* and showed no significant leishmanicidal activity (Bapela et al., 2017). The acetone extract of leaves was tested against anthelmintic activity of *Haemonchus contortus* and only showed an inhibitory activity of 47 ± 7 % (Fouche et. al., 2016). *Tabernaemontana ventricosa* did not show antimalarial activity *in vitro* (Schmelzer and Gurib-Fakim, 2008). A leaf extract of *T. ventricosa* showed anti-amoebic activity (Schmelzer and Gurib-Fakim, 2008).

### 2.5.5 Anticancer activity

The global cancer burden is estimated to have increased to 18.1 million new cases and 9.6 million deaths in 2018 (World Health Organisation, 2018). The increase is due to a number of factors including ageing and population growth as well as the changing prevalence of certain causes of cancer linked to social and economic development (WHO, 2018). Mansoor et al. (2013) isolated indole alkaloids from roots of *T. elegans* to evaluate *in vitro* cytotoxicity against HCT116 human colon carcinoma cells. The bisindole alkaloids tabernaelegantine C (**28**) and tabernaelegantinine B (**29**) were found to be cytotoxic to HCT116 cells at 20 µM, with tabernaelegantinine B being the most cytotoxic. The cytotoxicity of the most promising compounds was corroborated by Guava-ViaCount flow cytometry assays

and were further studied for apoptosis induction activity in HCT116 cells by evaluation of nuclear morphology following Hoechst staining as well as by caspase-3 like activity assays (Mansoor et al., 2013). The two compounds (**28** and **29**) showed characterised patterns of apoptosis in the HCT116 cancer cells and suggests their potential as possible leads for anticancer drug development. This study supports the ethnomedicinal use of *T. elegans* for treatment of cancer (Mansoor et al., 2013). The *β* – carbolines known as tabernines A, B and C (**21**, **22**, **23**) isolated from methanol leaves extract were evaluated for the ability to modulate multidrug resistance in mouse lymphoma cell lines. Tabernine A and C showed a weak activity at high concentrations (Mansoor et al., 2009). In a study conducted by Paterna et al. (2016), the three new isolated compounds, namely, (19'*S*)-hydroxytabernaelegantine A (**38**), 3'-oxotabernaelegantine C (**39**) and 3'-oxotabernaelegantine D (**40**) and two known compounds, namely, tabernaelegantine A (**13**) and tabernaelegantine D (**16**) were evaluated for their cytotoxicity against HCT116 colon cancer cells and HepG2 liver carcinoma cells. Compounds **38-40** and **16**, were found to be cytotoxic to HCT116 colon cancer cells, at $IC_{50}$ values ranging from 8.4 to > 10 µM and none of the compounds showed significant cytotoxicity against HepG2 cancer cells (Paterna et al., 2016). The cytotoxicity of the compounds was corroborated using a lactate dehydrogenase assay. Hoechst staining and morphology assessment as well as caspase-3/7 activity assays were also performed for investigating the activity of the compounds. The induced inhibition of proliferation of HCT116 cells by compounds **38** and **39** was associated with G1 phase arrest, while compound **40** and **16** were linked to G2/M cell cycle arrest (Paterna et al., 2016). Alasmontamine A (**41**) isolated from the methanol extract of *T. elegans* showed moderate cell growth inhibitory activity against HL-60 cells at $IC_{50}$ value of 31.7 µM (Hirasawa et al.,

2009). The compounds that were isolated from the stem bark and root bark extract (1:1 MeOH in $CH_2Cl_2$) of *T. ventricosa* which include friedelin (**42**), betulinic acid (**44**) and catechin (**45**) showed poor activity when tested for anticancer activity against drug sensitive leukemia cells while *trans*-tricontyl-4-hydroxy-3-methoxycinnamate (**43**) showed an interesting cell viability of 31.13 % at 1 µg/ml (Munayi, 2009). A decoction of fruit endocarps of *T. elegans* is administered orally by indigenous people to treat cancer (Arnold et al., 2002). The fruits have not been tested against cancer.

## 2.6 Conclusions and future prospects

In this review, ethnomedicinal uses, phytochemical constituents and pharmacological uses of southern African *Tabernaemontana* species were discussed. The diverse uses of *Tabernaemontana elegans* by indigenous people and the scientific evidence of its phytochemistry and pharmacological properties indicate therapeutic potential of this species. The ethnomedicinal uses of *T. ventricosa* need to be more explored and scientifically validated. An ethnomedicinal study on the venereal diseases that indigenous people treat with *T. elegans* needs to be performed to know which pathogens can be tested for scientific validation as well as isolation and characterisation of the compounds responsible for the activity. The biological activities of some of the isolated compounds still need to be evaluated. The ethnomedicinal uses of *T. ventricosa* need to be scientifically validated and further isolation and identification of its compounds are required.

## 2.8 References

Achenbech, J., Benirschke, M., Torrenegra, R. 1997. Alkaloids and other compounds from seeds of *Tabernaemontana cymose*. Phytochemistry 45, 325 – 335.

Almagro, L., Fernández-Pérez, F., Pedreño, M.A. 2015. Indole alkaloids from *Catharanthus roseus*: bioproduction and their effect on human health. Molecules 20, 2973 – 3000.

Aniszewski, T. 2007. Alkaloids – secrets of life. Alkaloid chemistry, biological significance, applications and ecological role. Elsevier. Amsterdam, Netherlands.

Arnold, H.J., Gulumian, M. 1984. Pharmacopoeia of traditional medicine in Venda. Journal of Ethnopharmacology 12, 35 – 74.

Arnold, T.H., Prentice, C.A., Hawker, L.C., Snyman, E.E., Tomalin, M., Crouch, N.R., Pottas-Bircher, C. 2002. Medicinal and magical plants of southern Africa: an annotated checklist. Strelitzia, 13. National Botanical Intsitute, Pretoria.

Athipornchai, A. 2018. A review on *Tabernaemontana* spp: multipoential medicinal plant. Asian Journal of Pharmaceutical and Clinical Research 11, 45 – 53.

Andrade, M.T., Lima, J.A., Pinto, A.C., Rezende, C.M., Carvalho, M.P., Epifanio, R.A. 2005. Indole alkaloids from *Tabernaemontana australis* (Muell. Arg) Miers that inhibit acetylcholinesterase enzyme. Bioorganic Medicinal Chemistry 13, 4092 – 4095.

Balsevich, J. 1988. Monoterpene indole alkaloids from Apocynaceae other than *Catharanthus roseus*. Phytochemicals in Plant Cell Cultures, 371 -384.

Bapela, M.J., Meyer, M.J.J., Kaiser, M. 2014. *In vitro* antiplasmodial screening of ethnopharmacologically selected South African plant species used for the treatment of malaria. Journal of Ethnopharmacology 156, 370 – 373.

Bapela, M.J., Kaiser, M., Meyer, J.J.M. 2017. Antileishmanial activity of selected South African plant species. South African Journal of Botany 108, 342 – 345.

Bapela, M.J., Heyman, H., Senejoux, F., Meyer, M.J.J. 2019. $^{1}$H NMR-based metabolomics of antimalarial plant species traditionally used by Vha-Venda people in Limpopo Province, South Africa and isolation of antiplasmodial compounds. Journal of Ethnopharmacology 228, 148 – 155.

Bhadane, B.S., Patil, M., Maheshwari, V.L., Patil, R.H. 2018. Ethnopharmacology, phytochemistry, and biotechnology of family Apocynaceae: A review. Phytotherapy Research 32, 1181 – 1210.

Bombardelli, E., Bonati, A., Gabetta, B., Martinelli, E.M., Mustich, G. 1976. Structures of tabernaelegantine A – D and tabernaelegantinines A and B, new indole alkaloids from *Tabernaemontana elegans*. Journal of the Chemistry Society, Perkin Transactions 1, 1432 – 1438.

Bongomin, F., Gago, S., Oladele, R.O., Denning, D.W. 2017. Global and multinational prevalence of fungal diseases – estimate precision. Journal of Fungi 3, 57.

Builders, P.F. 2018. Introductory chapter: Introduction to herbal medicine. [Online]. Available from: https://www.intechopen.com/books/herbal-

medicine/introductory-chapter-introduction-to-herbal-medicine. Accessed 18 June 2019.

Chaturvedula, V.S.P., Sprague, S., Schilling, J.K., Kingston, D.G.I. 2003. New cytotoxic indole alkaloids from *Tabernaemontana calcarean* from Madagascar Rain-forest. Journal of Natural Products 66, 528 – 531.

Cheikhyoussef, A., Mapaure, I., Shapi, M. 2011. The use of some indigenous plants for medicinal and other purposes by local communities in Namibia with emphasis on Oshikoto region: a review. Research Journal of Medicinal Plants 5, 406 – 419.

Coats Palgrave, K.C., Drummond, R.B., Moll, E.J., Coats Palgrave, M. 2003. Trees of Southern Africa, 3rd ed. New Holland Publishers, Cape Town.

Danieli, B., Palmisano, G., Gabetta, B., Martinelli, M. 1980. Tabernaelegantinines C and D, two new bisindole alkaloids containing a cyano group from *Tabernaemontana elegans* Stapf. Part 2. Journal of the Chemistry Society, Perkin Transactions 1, 601 – 606.

Fabricant, D.S., Fransworth, N.R. 2010. Study on natural medicinal chemistry and new drug discovery. Health Perspect 109, 69 – 75.

Fouche, G., Sakong, B.M.,Adenubi, O.T., Pauw, E., Leboho, T., Wellington, K.W. Eloff, J.N. 2016. Anthelmintic activity of acetone extracts from South African plants used on egg hatching of *Haemonchus contortus*. Onderstepoort Journal of Veterinary Research 83, 1 – 7.

Gabetta, B., Martinelli, E.M., Mustich, G. 1975. Plants of Mozambique. VIII. Alkaloids of *Tabernaemontana elegans*. Fitoterapia 46, 195 – 198.

Girardot, M., Deregnaucourt, C., Deville, A., Dubost, L., Joyeau, R., Allorge, L., Rasoanaivo, P., Mambu, L. 2012. Indole alkaloids from *Muntafara sessifolia* with antiplasmodial and cytotoxic activities. Phytochemistry 73, 65 – 73.

Guerriero, G., Berni, R., Muñoz-Sanchez, J.A., Apone, F., Abdel-Salam, E.M., Qahtan, A.A., Alatar, A.A., Cantini, C., Cai, G., Hausman, J., Siddiqui, K.S., Hernández-Sotomayor, S.M.T., Faisal, M. 2018. Production of plant secondary metabolites: examples, tips and suggestions for biotechnology. Genes 9, 309.

Hirasawa, Y., Miyama, S., Hosoya, T., Koyama, K., Rahman, A., Kusumawati, I., Zaini, N.C., Morita, H. 2009. Alasmontamine A, a first tetrakis monoterpene indole alkaloid from *Tabernaemontana elegans*. Organic Letters 11, 5718 – 5721.

Islam, M.S., Lucky, R.A. 2019. A study on different plants of Apocynaceae family and their medicinal uses. Universal Journal of Pharmaceutical Research 4, 42 – 46.

Kam, T.S., Sim, K.M., Koyano, T., Toyoshima, M., Hayashi, M., Komiyama, K. 1998. Conodiparines A-D, new bisindoles from *Tabernaemontana*. Reversal of vincristine-resistance with cultured cells. Bioorganic and Medicinal Chemistry Letters 8, 1693 – 1696.

Luo, X., Pires, D., Aínsa. J.A., Gracia, B., Mulhovo, S., Duarte, A., Anes, E., Ferreira, M.U. 2011. Antimycobacterial evaluation and preliminary phytochemical investigation of selected medicinal plants traditionally used in Mozambique. Journal of Ethnopharmacology 1 – 7.

Mabogo, D.E.N. 1990. The ethnobotany of Vhavenda (MSc thesis). University of Pretoria, Pretoria. [Online]. Available from

https://repository.up.ac.za/handle/2263/28881?show=full. Accessed 20 February 2017.

Madureira, A.M., Ramalhete, C., Mulhovo, S., Duarte, A., Ferreira, M.U. 2012. Antibacterial activity of some African medicinal plants used traditionally against infectious diseases. Pharmaceutical Biology 50, 481 – 489.

Mansoor, T.A., Ramalhete, C., Molná, J., Mulhovo, S., Ferreira, M.J.U. 2009. Tabernines A – C, *β*-Carbolines from the leaves of *Tabernaemontana elegans*. Journal of Natural Products 72, 1147 – 1150.

Mansoor, T.A., Borralho, P.M., Dewanjee, S., Mulhovo, S., Rodrigues, C.M.P., Ferreira, M.U. 2013. Monoterpene bisindole alkaloids, from the African medicinal plant *Tabernaemontana elegans*, induce apoptosis in HCT116 human colon carcinoma cells. Journal of Ethnopharmacology 149, 463 – 470.

Mehrbod, P., Omar, A.R., Hair-Bejo, M., Haghani, A., Ideris, A. 2014. Mechanisms of action and efficacy of statins against influenza. BioMed Research International, 1 – 8.

Mehrbod, P., Abdalla, M.A., Njoya, E.M., Ahmed, A.S., Fotouhi, F., Farahmand, B., Gado, D.A., Tabatabaian, M., Fasanmi, O.G., Eloff, J.N.E., McGaw, L.J., Fasina, F.O. 2018. South African medicinal plant extracts active against influenza A virus. BMC Complementary and Alternative Medicine 18, 112.

Munayi, R.R. 2016. Phytochemical investigation of *Bridelia micrantha* and *Tabernaemontana ventricosa* for cytotoxic principles against drug sensitive leukemia cell Lines (MSc) Thesis, University of Nairobi, Nairobi, Kenya. [Online]. Available from https://www.semanticscholar.org/paper/Phytochemical-Investigation-Of-Bridelia-

Micrantha-Munayi/53a898367a4247d336575c45bf49d6ab1e4cd643. Accessed 10 June 2019.

Mongalo, N.I., Makhafola, T.J. 2018. Ethnobotanical knowledge of the lay people of Blouberg area (Pedi tribe), Limpopo Province, South Africa. Journal of Ethnobiology and Ethnomedicine 14, 1 – 23.

Neuwinger, H.D. 1996. African Ethnobotany. Poisons and Drugs. Chapman and Hall, Weinheim.

Ngarivhume, T., van't Klooster, C.I.E.A., de Jong, J.T.V.M., Van der Westhuizen, J.H. 2015. Medicinal plants used by traditional healers for the treatment of malaria in the Chipinge district in Zimbabwe. Journal of Ethnopharmacology 159, 224 – 237.

Omino, E.A. 2002. Flora of tropical east Africa – Apocynaceae. A.A.Balkema Publishers. Netherlands.

Pallant, C.A., Steenkamp, V. 2008. In vitro bioactivity of Venda medicinal plants used in the treatment of respiratory conditions. Human and Experimental Toxicology 27, 859 – 866.

Pallant, C.A., Cromarty, A.D., Steenkamp, V. 2012. Effect of an alkaloidal fraction of *Tabernaemontana elegans* (Stapf.) on selected micro-organisms. Journal of Ethnopharmacology 140, 398 – 404.

Pan, S., Zhou, S., Gao, S., Yu, Z., Zhang, S., Tang, M., Sun, J., Ma, D., Han, Y., Fong, W., Ko, K. 2013. New perspectives on how to discover drugs from herbal medicines: CAM's outstanding contribution to modern therapeutics. Evidence-based Complementary and Alternative Medicine, 1 – 25.

Paterna, A., Gomes, S.E., Borralho, P.M., Mulhovo, S., Rodrigues, C.M.P., Ferreira, M.U. 2016. Vobasinyl-iboga alkaloids from *Tabernaemontana elegans*: cell cycle arrest and apoptosis-inducing activity in HCT116 colon cancer cells. Journal of Natural Products 79, 2624 – 2634.

Pooley, E. 1993. The complete field guide to trees of Natal, Zululand and Transkei. Natal Flora Publications Trust, Durban, South Africa.

Pooley, E., 1998. A field guide to wild flowers of KwaZulu-Natal and the Eastern Region. Natal Flora Publications Trust, Durban.

Popović, Z., Matić, R., Bojović, S., Stefanović, M., Vidaković, V. 2016. Ethnobotany and herbal medicine in modern complementary and alternative medicine: An overview of publications in the field of integrative and complementary medicine 2001 – 2013. Journal of Ethnopharmacology 181, 182 – 192.

Pramanik, P.K., Alam, M.N., Chowdhury, D.R., Chakraborti, T. Drug resistance in protozoan parasites: an incessant wrestle for survival. Journal of Global and Antimicrobial Resistance 18, 1 – 11.

Rahman, M.T., Tiruveedhula, V.V., Cook, J.M. 2016. Synthesis of bisindole alkaloids from the Apocynaceae which contain macroline or sarpagine unit: a review. Molecules 21, 1525.

Ramalhete, C., Lopes, D., Mulhovo, S., Rosario, V.E., Ferreira, M.J.U. 2008. Antimalarial activity of some plants traditionally used in Mozambique. In: Workshop Plantas Medicinais e Fitoterapeuticas nos Tropicos. [Online]. Available from https://pdfs.semanticscholar.org/0b51/a5f8271167aadf6d631706e34185eb5359b4.pdf. Accessed 18 March 2019.

Random harvest. [Online]. Available from https://www.randomharvest.co.za/en-us/South-African-Indigenous-Plants/Show-Plant/PlantId/107?Plant=Tabernaemontana%20elegans. Accessed 17 June 2019.

Rocha, M.P., Campana, P.R.V., Scoaris, D.O., Almeida, V.L., Lopes, J.C.D., Silva, A.F., Pieters, L., Silva, C.G. 2017. Biological activities of extracts from *Aspidosperma subincanum* Mart. And in silico prediction for inhibition of acetylcholinesterase. Phytotherapy Research 32, 2021 – 2033.

Santos, A.L.S., Branquinha, M.H., Kneipp, L.F. 2017. Fungal infections: a veritable worldwide problem. Journal of Infectious Diseases and Medical Microbiology 1, 1 – 2.

Schmelzer, G.H., 2006. *Tabernaemontana elegans* Stapf. In: Schmelzer, G.H. and Gurib-Fakim, A. (Editors). Prota 11(1): Medicinal plants/Plantes medicinales 1. [CD-Rom]. PROTA, Wageningen, Netherlands.

Schmelzer, G.H., Gurib-Fakim, A. 2008. Plant Resources of Tropical Africa 11 (2). Medicinal plants 2. PROTA Foundation / CTA. Wageningen, Netherlands.

Schmelzer, G.H., Gurib-Fakim, A., 2008. Plant Resources of Tropical Africa 11 (1) PROTA Medicinal Plants 1. PROTA Foundation/Backhuys Publishers/CTA Wageningen, Netherlands, p. 592.

Schmidt, E., Lötter, M., McCleland, W. 2002. Trees and shrubs of Mpumalanga and Kruger National Park. Jacana, Johannesburg.

Singh, R. 2016. Chemotaxonomy: a tool for plant classification. Journal of Medicinal Plant Studies 4, 90 – 93.

Sofowora, A., Ogunbodede, E., Onayade, A. 2013. The role and place of medicinal plants in the strategies for disease prevention. African Journal of Traditional, Complementary and Alternative Medicines 10, 210 – 229.

Tropical Plants Database, Ken Fern. tropical.theferns.info. [Online]. Available from https://www.tropical.theferns.info/viewtropical.php?id=Tabernaemontana+ventricosa. Accessed 20 May 2019.

Tshikalange, T.E., Meyer, J.J.M., Hussein, A.A. 2004. Antimicrobial activity, toxicity, and isolation of bioactive compound from plants used to treat sexually transmitted disease. Journal of ethnopharmacology 96, 515 – 519.

Van der Heijden, R., Brouwer, R.L., Verpoorte, R., Van Beek, T.A., Harkes, P.A.A., Baerheim Svendsen, A. 1986. Indole alkaloids from *Tabernaemontana elegans*. Planta Medica 2, 144 – 147.

Van Beek, T.A., Deedler, A.M., Verpoorte, R., Baerheim Svendson, A. 1984. Antimicrobial, antiamoebic and antiviral screening of some *Tabernaemontana* species. Planta Medica 50, 180 – 185.

Vanbeek, T.A., Verpoorte, R., Svendsen, A.B. 1985. Pharmacognostical studies of *Tabernaemontana* species. 11. Antimicrobially active alkaloids from *Tabernaemontana chippii*. Journal of Natural Products 48, 400 – 423.

Wachtel-Galor, S., Benzie, I.F.F. 2011. Herbal medicine: an introduction to its history, usage, regulation, current trends, and research needs. In: Benzie IFF, Wachtel-Galor S (eds) Herbal medicine: biomolecular and clinical aspects, second edition. CRC Press, Boca Raton (FL).

Watt, J.M., Breyer-Brandwijk, M.G. 1962. The medicinal and poisonous plants of southern and eastern Africa, second edition. Livingstone, London.

Wilder-Smith, A. 2017. Recovery from serious fungal infections should be realisable to everyone. 17, 1111 – 1113.

Wong, S.K., Lim, Y.Y., Abdullah, N.R., Nordin, F.J. 2011. Antiproliferative and phytochemical analysis of leaf extracts of ten Apocynaceae species. Pharmacognosy Research 3, 100 – 106.

World health organisation (WHO). 2002. The world health report. Reducing risks, promoting healthy life. Available from: https://www.who.int/whr/2002/en/. Accessed 10 June 2019.

World Health Organisation (WHO). 2018. Antibiotic resistance. [Online] Available from: https://www.who.int/news-room/fact-sheets/detail/antibiotic-resistance. Accessed 16 July 2019.

World Health Organisation (WHO). 2018. Latest global cancer burden. International Agency for Research on Cancer, Geneva.

Yang, L., Wen, K.S., Ruan, X., Zhao, Y.X., Wei, F., Wang, Q. 2018. Response of plant secondary metabolites to environmental factors. Molecules 23, 762.

Yuan, H., Ma, Q., Ye, L., Piao, G. 2016. The traditional medicine and modern medicine from natural products. Molecules 21, 559.

Zhu, J.-P., Guggisberg, A., Kalt-Hadamowsky, M., Hesse, M. 1990. Chemotaxanomic study of the genus *Tabernaemontana* (Apocynaceae) based on their indole content. Plant Systematics and Evolution 172, 13 – 34.

# CHAPTER 3: IN VITRO ANTIGONOCCOCAL, ANTICANDIDAL, ANTI-HIV AND ANTIMALARIAL ACTIVITY OF TABERNAEMONTANA ELEGANS (APOCYNACEAE)

## 3.1 Introduction

While infections and associated mortality have declined, they remain a significant threat to human worldwide (Bloom and Cadarette, 2019). The drugs that have helped in declining of infectious disease mortality are now beginning to lose their effectiveness (Bloom and Cadarette, 2019). Medicinal plants have attracted the attention of pharmaceutical and scientific communities as sources of novel drugs (Romulo et al., 2018). Included in these plants is *Tabernaemontana elegans* which is used by indigenous people to treat venereal diseases, cancer, malaria and pulmonary diseases. The success of drug discovery depends largely on a chosen set of relevant *in vivo* and *in vitro* assays as well as phytochemical screening which are based on ethnomedicinal data (Brusotti et al., 2014; Atanasov et al., 2015; Romulo et al., 2018).

It has been previously reported that *T. elegans* has antibacterial activity against *Staphylococcus aureus* and antimycobacterial activity against *Mycobacterium smegmatis* as well as anti-fungal activity against *Candida albicans.* (Pallant et al., 2012). Studies on phytochemical analysis have shown that *T. elegans* is particularly rich in monoterpenoid indole alkaloids (Pallant et al., 2012). Indole alkaloids, dregamine and voacangine were identified as the active antibacterial components of the plant (Pallant et al., 2012). Bisindole alkaloids tabernaelegantinine B and tabernalegantine C which were also isolated from the methanol root extract of this plant were found to be potent apoptosis inducers in HCT116 human colon carcinoma cells and proved to be possible leads for anticancer drug development (Mansoor et al., 2013). Studies conducted by Bapela et. al. (2014) on the stem bark (DCM extract) of *T. elegans* showed antiplasmodial

activity. Most of the compound(s) that are responsible for the mentioned biological activities were not identified. The alkaloids in *T. elegans* are structurally diverse and are also found in plant species of the same genus. Girardot et al. (2012) isolated four bisindole and one monomeric indole alkaloids as well as 11 known compounds from *Muntafara sessifolia* that are closely related to those isolated from *T. elegans*. Dregamine and tabernaemontanine also found in *T. elegans* demonstrated significant antiplasmodial activity (Girardot et al., 2012). These alkaloids were also isolated in a study conducted by Bapela et al. (2019) from *T. elegans* but were not tested for antiplasmodial activity since they had already been tested by Girardot et al. (2012).

This study focuses on *Tabernaemontana elegans* (Apocynaceae), which is traditionally used by the local people of Mutale municipality in Venda to treat infectious diseases, namely, venereal diseases and malaria. *Tabernaemontana elegans* has been tested against *Candida albicans* (roots) and malaria (stem bark) and compounds were isolated but were not tested to see which ones are responsible for the activity (Steenkamp et al., 2007; Bapela et al., 2019). Due to the clear need of new agents which may be used for the treatment of malaria and other bacterial and fungal infections, further studies will be conducted on the properties of this plant. The aim of this study is to validate some of the ethnomedicinal uses of *T. elegans* and to also isolate, identify and test the activity of the isolated compounds. The new lead compounds need to have new modes of action that will combat challenges of resistance.

## 3.2 Materials and methods

### 3.2.1 Plant collection

Thirty grams of roots, stem bark, leaves and fruits were collected in February 2017 based on their traditional uses against sexually transmitted infections (STIs) and malaria by Vha-Venda people in Mutale Municipality, Limpopo Province (Table 3.1). The GPS coordinates of the place of collection are 22°40’26.5’’S 30°34’30.9’’E. Mr. Ndou T.A assisted with collection and identification of the plant. The fruits were used as the botanical morphological character for the identification of the plant. A voucher specimen (120337) of the plant species was prepared with the assistance of Jason Sampson and kept at the H.G.W.J Schweickerdt Herbarium of the University of Pretoria. .

**Table 3.1:** Ethnomedicinal uses of *Tabernaemontana elegans* Stapf. (Apocynaceae) by southern African indigenous people.

| Plant part | Method of preparation and administration | Traditional use |
|---|---|---|
| **Fruits** | Endocarps isolated from the fruits are dried, pulverized and boiled in water. The decoction is filtered and administered orally. | Cancer (Arnold et al., 2002). |
| | Powdered whole fruit is boiled in water. | **Venereal diseases** (Mabogo, 1990) |
| **Latex** | Latex drawn from fresh leaves and fruits is applied to wounds. | Styptic agent (Arnold et al., 2002). |
| **Leaves** | The leaves are used in combination with *Ipomoea batatas* leaves. A decoction is prepared from equal amounts of ground leaf powder of *I. batatas* and *T. elegans* and then administered three times a day. | **Gonorrhoea** (Arnold and Gulumian, 1984; Mabogo, 1990). |
| **Roots** | The roots of *T. elegans* are used in combination with those of *I. batatas*. | **Venereal disease** (Arnold and Gulumian, 1984; Mabogo, 1990). |
| | A hot infusion is prepared and is orally administered. | **Malaria** (Ngarivhume et al., 2015), lung ailment, stomachache (Arnold and Gulumian, 1984). |
| | A decoction is prepared and is taken orally. It is used as a wash to wounds. | Pulmonary diseases, chest pains, wounds (Watt and Breyer-Brandwijk, 1962; Arnold and Gulumian, 1984), aphrodisiac (Neuwinger, 1966), **febrifuge** (Watt and Breyer-Brandwijk, 1962). |
| | An infusion is prepared and is orally administered. | Aphrodisiac, stomach ache (Arnold and Gulumian, 1984). |
| | Root is burned and vulva is exposed to smoke. | Menorrhagia (Arnold and Gulumian, 1984). |
| **Stem** | A decoction prepared from stem bark is orally administered. | **Malaria**, heart diseases (Pooley, 1998), **febrifuge** (Watt and Breyer-Brandwijk, 1962). |

### 3.2.2 Extraction of plant samples

The harvested plant parts (Figure 3.1) were air dried at room temperature and then ground into fine powder. About 30 g of each powdered plant material was weighed and then sequentially extracted with hexane, dichloromethane (DCM), methanol and water (Figure 3.2).

**Figure 3.1:** *Tabernaemontana elegans* parts (fruits, stem bark, leaves and roots) that were collected (https://cjmgrowers.co.za).

Each extraction was performed three times with each solvent. Decoction which is a method of extraction used by indigenous people that involves boiling plant material was also performed for all the plant parts. The extracts were then filtered under vacuum through Whatman No. 1 filter paper. Organic extracts were evaporated in the rotavapor and the water extracts as well as decoction extracts were then freeze dried (Brusotti et al., 2014). The percentage yield of the obtained extracts is the weight of extract divided by the weight of plant material and multiplied by a hundred.

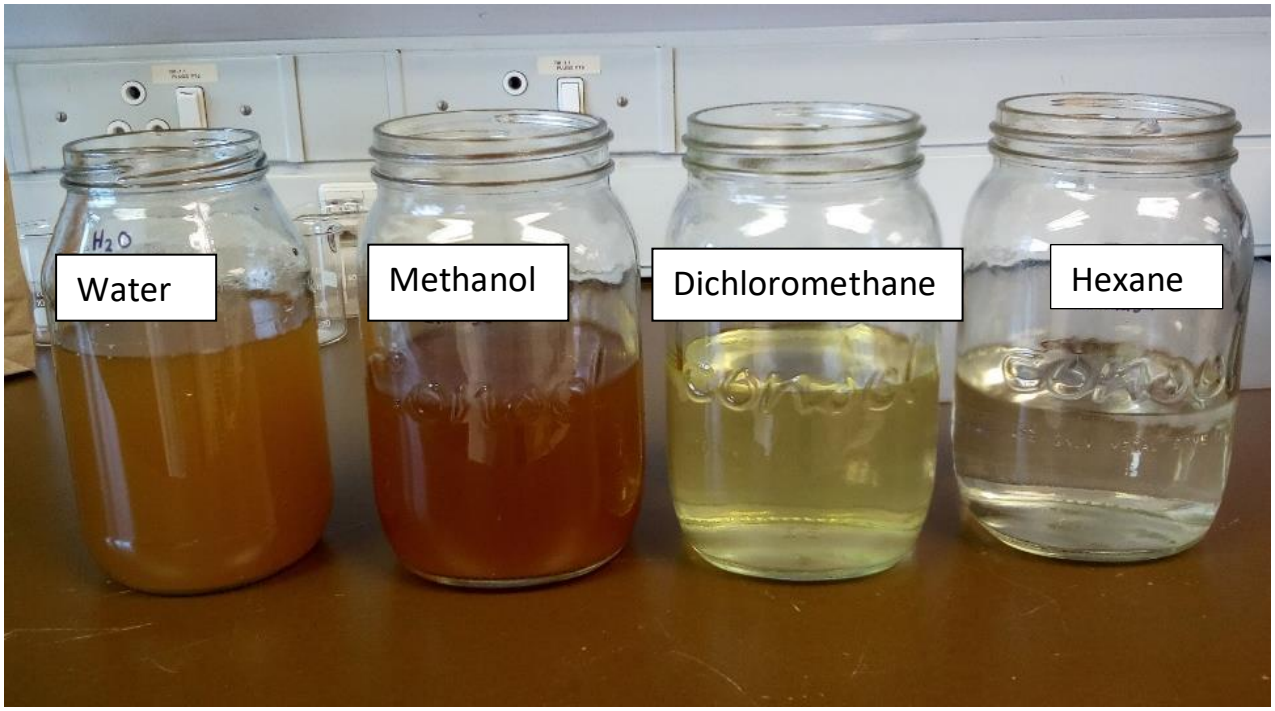



**Figure 3.2:** Plant parts were sequentially extracted in four solvents of increasing polarity.

### 3.2.3 Bioassays

Twenty plant extracts were assayed for antibacterial activity, antifungal activity, reverse transcriptase activity, antiplasmodial activity and cytotoxicity.

#### 3.2.3.1 Antibacterial and antifungal assay

*Neisseria gonorrhoea* was cultured on Mueller-Hinton agar (Merck SA (Pty) Ltd.) and grown in Mueller-Hinton broth and *Candida albicans* was cultured on Tryptone soy agar (Merck SA (Pty) Ltd.) and grown in Tryptone soy broth, both at 37°C for 24 hours. Extract plates were inoculated with *N. gonorrhoea* strains (ATCC 19424) and *C. albicans* strains (ATCC 10231) and were incubated at 37°C. The plate with *N. gonorrhoea* strain was incubated in anaerobic condition. Minimum inhibitory concentration (MIC) was determined after 24 hours of incubation. The MIC value

was determined as the lowest extract concentration with no visible growth (Eloff, 1998). The MIC assay is discussed in section 3.2.3.2.

### 3.2.3.2 Minimum inhibitory concentration assay

The minimum inhibitory concentration (MIC) was used to determine the antigonococcal and anticandidal activity of plant extracts. The minimum inhibitory concentration is determined by using the broth microdilution technique in 96-well microtitre plates (Eloff, 1998). Each of the 20 plant extracts (12 mg) was dissolved in 100 µl of 10% DMSO and 900 µl of broth was used to make a final concentration of 12 mg/ml. Ciprofloxacin was used as the positive control while DMSO was the negative control (Figure 3.3). Samples were tested in triplicates in 96-ELISA well plates. Microbial strains were inoculated in sterile broth and prepared to a density of 1.5 x $10^8$ colony forming units per mL (CFU/mL), which correspond with the 0.5 McFarland Standard. Two-fold serial dilutions were performed. The microplates were covered and incubated for 24 hours at 37°C. Microbial growth was determined by addition of 40 µl of p-iodonitrotetrazolium (INT) violet dye to microplate wells and incubated for 30 minutes. A pink colour change indicated microbial growth. The MIC was defined as the lowest concentration that inhibited colour change of INT.

### 3.2.3.3 Reverse transcriptase (RT) assay

Plant extracts were screened for anti-HIV activity against recombinant HIV-1 enzyme by using a non-radioactive HIV-1 RT colorimetric ELISA kit (Roche). Six milligrams of each plant extract was weighed and dissolved in 1 ml of DMSO to make a final concentration of 6 mg/ml stock solution. The stock solution (10 µl) was added to the lysis buffer (90 µl) to make a final solution of 0.6 mg/ml. The enzyme was prepared to a stock solution of 0.764 mg/ml and 0.327 µl was added to 1000 µl

of the lysis buffer. Twenty microliters of enzyme, 20 µl of the diluted extract and 20 µl of the reaction mixture were added together into the wells of a normal microtiter plate. The positive controls were (i) 20 µl of the lysis buffer, reaction mixture and enzyme (ii) 20 µl doxorubicin, reaction mixture and enzyme. For the negative control, only the lysis buffer and the reaction mixture were added. The plates were incubated at 37 °C for an hour and the samples were then transferred into wells of microplate modules and covered with foil. The plates were incubated at 37 °C for an hour and then washed five times with washing buffer (250 µl). Two hundred microliters of anti-digoxigenin-peroxidase working solution was then added to plates. The plates were incubated for an hour at 37°C and then washed five times with 250 µl of washing buffer. Two hundred microliters of 2,2' – azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) substrate solution was added in each well and allowed to stand for 10 minutes. The absorbance was read on a microtiter plate reader at 405 nm with a reference wavelength of 490 nm. The percentage inhibition was calculated by using the equation below:

HIV – RT inhibition(%) = {100 – ($Abs_{405\ nm}$ sample/ $Abs_{405\ nm}$ positive control)} x 100

where the absorbance of the sample is compared to that of the positive control (Fonteh et al., 2009).

### 3.2.3.4 Antiplasmodial assay

The *in vitro* antiplasmodial activity of the acquired 20 plant extracts was determined against *Plasmodium falciparum* using [$^3$H]hypoxanthine incorporation assay (Desjardins et al., 1979; Matile and Pink, 1990). The chloroquine sensitive NF54 strain was the test organism and chloroquine (Sigma) was used as a standard drug. Plant extracts (1 mg) were dissolved and then added to parasite cultures. The

cultures were incubated in RPMI 1640 medium with no hypoxanthine, supplemented with 5.94 g/l of HEPES, 2.1g/l of $NaHCO_3$, 100 U/ml of neomycin, 5 g/l of AlbumaxR as well as washed human red cells $A^+$ at 25% haematocrit (0.3% parasitaemia). A preparation of serial drug dilutions of eleven 3-fold dilution steps that cover a range from 100 to 0.002 µ/ml was made. Incubation (48 hours) of the 96-well plates was done in a humidified atmosphere at 37 °C, in the presence of $CO_2$ (4%), $O_2$ (2%) and $N_2$ (93%). After incubation, [$^3$H]hypoxanthine (50 µl) was added to each well. Further incubation of 24 hours was done under the same conditions and cells were harvested with a Betaplate™ cell harvester (Wallac). The harvested red blood cells were washed with distilled water after being transferred into glass fibre filters. The filters were dried and then inserted into a plastic foil with of scintillation fluid (10 ml) and counted in a Betaplate™ liquid scintillation counter (Wallac). The sigmoidal inhibition curves were used to calculate the 50% inhibitory concentration ($IC_{50}$) values by linear regression using Microsoft Excel (Huber and Koella, 1993).

### 3.2.3.5 Cytotoxicity assay

The cytotoxicity of crude plant extracts was determined by using the antiproliferative bioassay (Huber and Koella, 1993). The bioassay determines the plant extract's potential lethality or safe therapeutic application against mammalian cells. Bioassays were performed in 96-well microtiter plates, each well containing of RPMI 1640 (100 µl) medium supplemented with 200mM 1% L-glutamine, fetal bovine serum (10%) and 4000 rat skeletal myoblast L6 cell line (Ahmed et al., 1994). A preparation of serial drug dilutions of eleven 3-fold dilution steps that cover a range from 100 to 0.002 µg/ml was done. Podophyllotoxin was used as a control. After an incubation of 70 hours, the plates were viewed under an inverted

microscope to assure growth of the controls and sterile conditions. Alamar Blue (10 µl) was added to each well and the plates were incubated for 2 hours. A Spectramax Gemini XS microplate fluorometer (Molecular Devices Cooperation) was used to read the plates with an excitation wavelength of 536 nm and an emission wavelength of 588 nm. The sigmoidal dose inhibition curves were used to calculate the $IC_{50}$ values by linear regression with a SoftmaxPro software (Molecular Devices Cooperation) (Huber and Koella, 1993).

### 3.3 Results and discussion

Diverse southern African ethnic groups harvest various plant parts of *Tabernaemontana elegans* for various ethnomedicinal uses. The yield of the obtained extracts ranged from 0.20 to 4.70 g. The highest yield was obtained from methanol and water extracts. The percentage yields are illustrated in Table 3.2.

*Antigonococcal and anticandidal activity*

Antigonococcal and anticandidal activities of the 25 plant extracts were determined using the microdilution assay and the results are expressed as MIC values (Table 3.2). The highest concentration that was tested was 6 mg/ml and the lowest concentration 0.05 mg/ml. Ciprofloxacin was used as a positive control and showed an MIC value of <0.05 mg/ml.

The plant parts that showed significant activity against *N. gonorrhoea* were the stem bark (methanol extract) and roots (methanol and decoction extracts).

**Table 3.2:** The MIC (mg/ml) and $IC_{50}$ (µg/ml) values of plant extracts that were tested against *Neisseria gonorrhoea*, *Candida albicans*, Human Immunodeficiency Virus and *Plasmodium falciparum* with their cytotoxicity and selective index values.

| Plant part | Solvent | % Yield | [1]Antigonoccocal activity(mg/ml) | [2]Anti*candidial* activity(mg/ml) | Reverse transcriptase activity (% inhibition) | [3]Antiplasmodial activity (µg/ml) | [4]Cytotoxicity (µg/ml) | [5]Selectivity index |
|---|---|---|---|---|---|---|---|---|
| **Fruits:** | Hexane | 5.00 | >6.00 | >6.00 | 36.61 ± 0.24 | 16.55 ± 3.75 | 65.95 ± 1.55 | 4 |
| | DCM | 1.00 | >6.00 | >6.00 | 43.57 ± 0.08 | 6.60 ± 0.56 | 37.15 ± 11.75 | 6 |
| | Methanol | 4.00 | >6.00 | >6.00 | 8.80 ± 0.02 | 28.95 ± 1.05 | 57.65 ± 0.95 | 2 |
| | Water | 5.30 | >6.00 | >6.00 | 29.30 ± 0.24 | ND | 65.80 ± 14.80 | 1 |
| | Decoction | 5.70 | >6.00 | >6.00 | 17.17 ± 0.20 | 48.50 | 39.05 ± 4.05 | 1 |
| **Leaves:** | Hexane | 2.00 | >6.00 | >6.00 | 26.51 ± 0.11 | 2.30 ± 0.14 | 18.95 ± 0.45 | 8 |
| | DCM | 2.30 | >6.00 | >6.00 | 24.56 ± 0.18 | 2.67 ± 0.48 | 19.10 ± 0.40 | 7 |
| | Methanol | 6.00 | >6.00 | >6.00 | 49.56 ± 0.08 | 9.92 ± 0.39 | 48.65 ± 3.55 | 5 |
| | Water | 14.0 | >6.00 | >6.00 | **54.00 ± 0.12** | 22.10 ± 1.30 | 43.95 ± 6.65 | 2 |
| | Decoction | 11.0 | >6.00 | >6.00 | **58.32 ± 0.05** | 22.05 ± 1.55 | 63.55 ± 2.35 | 3 |
| **Stem bark:** | Hexane | 1.00 | >6.00 | >6.00 | 32.63 ± 0.04 | 5.15 ± 0.45 | 49.55 ± 1.65 | 10 |
| | DCM | 1.00 | >6.00 | >6.00 | 16.49 ± 0.03 | **3.25 ± 0.33** | 52.00 ± 1.30 | 16 |
| | Methanol | 2.30 | **0.20 ± 0.06** | >6.00 | 14.56 ± 0.17 | **0.31 ± 0.01** | 24.25 ± 0.05 | 79 |
| | Water | 1.70 | 6.00 | >6.00 | **51.29 ± 0.13** | **2.38 ± 0.02** | 56.90 ± 2.70 | 24 |
| | Decoction | 0.70 | >6.00 | >6.00 | **57.06 ± 0.03** | **4.02 ± 0.82** | 49.75 ± 0.25 | 12 |
| **Roots:** | Hexane | 3.70 | >6.00 | >6.00 | 26.64 ± 0.02 | 32.15 ± 2.25 | >100 | >3 |
| | DCM | 3.70 | >6.00 | >6.00 | **50.24 ± 0.11** | 2.66 ± 0.41 | 16.75 ± 2.05 | 6 |
| | Methanol | 15.7 | **0.10 ± 0.04** | >6.00 | 30.42 ± 0.03 | **0.16 ± 0.01** | 2.56 ± 0.11 | 16 |
| | Water | 7.70 | >12.50 | >6.00 | **60.78 ± 0.16** | 10.77 ± 0.93 | 26.15 ± 6.45 | 2 |
| | Decoction | 5.00 | **0.80 ± 0.37** | >6.00 | 28.40 ± 0.10 | **4.62 ± 0.55** | 91.50 ± 0.00 | **20** |
| **Ciprofloxacin** | | | **<0.05** | **<0.05** | | | | |
| **Doxorubicin** | | | | | **95.83 ± 0.005** | | | |
| **Chloroquine** | | | | | | **0.004 ± 0.002** | | |

[1]*Neisseria gonorrhea* strain

[2]*Candida albicans* strain

[3]Chloroquine sensitive NF54 strain

[4]Rat skeletal myoblast L6 cell line

[5]Selectivity index for antiplasmodial activity

ND: not determined

The antigonococcal activity is presented in Table 3.2 as minimum inhibitory concentration (MIC) values. Only the stem bark and roots showed significant activity for this pathogen. The best antigonoccocal activity was observed in the methanol extract of roots with MIC value of 0.10 ± 0.04 mg/ml and methanol extract of the stem bark with MIC value of 0.20 ± 0.06 mg/ml. The root decoction also inhibited bacterial growth with MIC value of 0.80 ± 0.37 mg/ml. An activity of 6.00 mg/ml was observed in the water extract of the stem bark. There was none of the active extracts that had the same activity as the positive control, however there was a huge difference when compared to the negative control which is DMSO with activity greater than 6 mg/ml. Hexane, DCM and decoction extracts of the stem bark were not active at the tested concentrations (MIC >6.00 mg/ml) as well as the hexane DCM and water extracts of roots. The extracts obtained from the fruits and leaves were all not active at the tested concentrations against *N*. *gonorrhoea* (MIC >6.00 mg/ml). There are no previous studies on the antigonococcal activity of *T. elegans*. All the tested plant extracts showed MIC values greater than 6 mg/ml for *Candida albicans,* which means none of them was active at the assayed concentrations. However, studies conducted by Steenkamp et al. (2007) on roots of *T. elegans* against *C. albicans* showed significant activity by aqueous extracts, which could not be supported by our current study. The discrepancy of the results could be due to different areas of plant collection and the seasons at which the plant was collected.

*Reverse transcriptase activity*

The results of the reverse transcriptase (RT) assay are presented in Table 3.3 as % inhibition of the RT enzyme and are expressed as mean values ± standard deviation. In this experiment, the aim was to determine the plant extracts that inhibit

viral replication of the HIV-1 RT enzyme by using a non-radioactive HIV-1 RT colorimetric ELISA kit. Due to its vital role in the viral life cycle, HIV-RT is the target of considerable interest for anti-viral drug development (Zhang et al., 2010). In this assay, the ability of RT to synthesize DNA from a primer/template hybrid poly (A) x oligo $(dT)_{15}$ is manipulated and uses digoxigenin (DIG) and biotin labelled nucleotides (Zhang et al., 2009). The anti-digoxigenin-peroxidase binds to the digoxigenin-labelled nucleotides. The DNA binds to the surface of the microplate modules coated with streptavidin. The ABTS substrate solution is cleaved by the peroxidase enzyme and produces a coloured reaction product (Figure 3.5) (Kapewangolo et al., 2016; Fonteh et al., 2009). The results were compared to the positive control doxorubicin, which indicated 95.83 ± 0.01% inhibitory activity. Plant extracts with inhibition activity below 20% were considered to be insignificant, 20 – 39% low, 40 – 69% moderate, and 70 -100% high inhibition activity.

Extracts with activity all showed moderate inhibition (50 – 61%) of HIV-1 RT. The root water extract showed a moderate inhibition of 60.78 ± 0.16%. There was moderate inhibition activity of 50.24 ± 0.11% in the DCM extract of roots. Inhibition was mostly seen in aqueous extracts. The decoction and water extracts of leaves .11showed moderate inhibition activity of 58.32 ± 0.05% and 54.00 ± 0.12% and the stem bark also showed moderate inhibition activity in the decoction and water extracts with activity of 57.06 ± 0.03% and 51.29 ± 0.13%, respectively. The leaves extracts (hexane, DCM and methanol), stem bark extracts (hexane, DCM and methanol), root extracts (hexane, methanol and decoction) as well as fruit extracts showed low or no significant inhibition activity of HIV-1 RT. There are no previous reports on the inhibition of HIV-1 RT by extracts from *T. elegans*. This plant is used by indigenous people for treating venereal diseases but was not specified to be used

for HIV. The moderate to low activity observed in the results could mean that southern African natives use the plant to treat other venereal diseases and not HIV-AIDS. An ethnomedicinal study on the specific venereal diseases that are treated with this plant needs to be performed. Studies conducted on a member of the Apocynaceae family (*Hoodia gordonii*) by Kapewangolo et al. (2016) showed significant to moderate inhibition against HIV-1 RT for ethanol and ethyl acetate extracts with inhibition values of 73.55 ± 0.04 and 69.81 ± 9.45 µg/ml, respectively.

*Antiplasmodial activity*

The antiplasmodial activity of plant extracts is presented in Table 3.3 as $IC_{50}$ values. The selectivity index (SI) was calculated by dividing the $IC_{50}$ value obtained for the L-6 rat skeletal myoblast cells by the $IC_{50}$ value of the *Plasmodium* species. In this study, a plant extract was considered to have significant activity when the concentration that inhibits 50 % of the *Plasmodium* growth ($IC_{50}$) was ≤ 5 µg/ml and has an SI value of ≥ 10. It is considered that the antiplasmodial efficacy of a given plant extract is not due to the *in vitro* cytotoxicity when the SI ≥ 10, thus displaying antiplasmodial activity (Vonthron-Senecheau et al., 2003). The results were compared to the positive control chloroquine which displayed an inhibition activity of 0.004 ± 0.002 µg/ml.

Twenty plant extracts were assayed for antiplasmodial activity and only 6 of them exhibited significant inhibition ranging from 0.16 ± 0.01 – 4.62 ± 0.55 µg/ml. The 6 extracts were from the stem bark and roots. Methanol stem bark and root extract exhibited the highest antiplasmodial activity ($IC_{50}$ = 0.31 ± 0.013 µg/ml and $IC_{50}$ = 0.16 ± 0.01 µg/ml, respectively). The methanol stem bark extract was considered to be the best candidate since it had a higher SI value (79) than that of

the methanol root extract (SI = 16). Dichloromethane (DCM), water and decoction extracts of the stem bark significantly inhibited the *P. falciparum* strain at $IC_{50}$ values 3.25 ± 0.33, 2.38 ± 0.02 and 4.02 ± 0.82 µg/ml, respectively. These extracts were considered to be of safe therapeutic use when evaluated on rat skeletal myoblast cells, with SI values ranging from 16 to 12. Even though the activity of the extracts mentioned above was significant, none of them had an inhibition activity close to that of the positive control ($IC_{50}$ = 0.004 ± 0.002 µg/ml). The reason for this could be due to the presence of other compounds not responsible for activity in the crude extract which may retard the efficacy of the compound attributable to antiplasmodial activity. Non-polar extracts of leaves (hexane and DCM) and roots (DCM) inhibited plasmodial growth at $IC_{50}$ values of 2.30 ± 0.14 , 2.67 ± 0.48 and 2.66 ± 0.41 µg/ml but are not considered as potential hits for drug discovery since their SI values (8, 7 and 6, respectively) are less than 10, meaning that they are toxic to rat myoblast L6 cells. Root decoction showed significant activity ($IC_{50}$ = 4.62 µ/ml; SI = 20). Extracts from fruit did not show any inhibitory effects ($IC_{50}$ ≥ 5 µg/ml; SI ≤ 10). Studies conducted by Bapela et al. (2014) of DCM and polar extracts from the stem bark of this species also showed significant antiplasmodial activity at $IC_{50}$ values of 0.33 and 0.83 µg/ml with respective SI values of 14 and 46. The study conducted by Bapela et al. (2014) was the first one to document significant antiplasmodial activity of *T. elegans* and validates the ethnomedicinal uses of this plant species. Ramalhete et al. (2008) conducted a study on the leaves of the same plant species which showed moderate or no significant activity of polar leaf extracts.

Previous studies on phytochemical constituents of *T. elegans* revealed that it is rich in monoterpenoid indole alkaloids (Pallant et al., 2012). These alkaloids are structurally diverse and are also found in plant species of the same genus. Girardot

et al. (2012) isolated dregamine and tabernaemontanine from *Muntafara sessifolia* which demonstrated significant antiplasmodial activity. Dregamine showed activity of 62.0 ± 2.4 µM while tabernaemontanine showed activity of 12.0 ± 0.8 µM (Girardot et al., 2012). These alkaloids were also isolated in a study conducted by Bapela et al. (2019) from *T. elegans* but were not tested for antiplasmodial activity since they had already been tested by Girardot et al. (2012).

### 3.4 Conclusion

This study validates some of the ethnomedicinal uses (malaria and gonorrhoea) of the plant parts of *Tabernaemontana elegans* used by Vha-Venda people. The stem bark and root extracts showed significant activities against the tested pathogens and have the potential to be developed as the antigonococcal, anti-HIV and anti-malarial agents. Further studies are required to explore other pharmacological activities as well as identifying the other chemical constituents that are attributable to the observed activity.

## 3.5 References

Ahmed, H.M. 2016. Ethnopharmacobotanical study on the medicinal plants used by herbalists in Sulaymaniya Province, Kurdistan, Iraq. Journal of Ethnobiology and Ethnomedicine 12, 8.

Arnold, H.J., Gulumian, M. 1984. Pharmacopoeia of traditional medicine in Venda. Journal of Ethnopharmacology 12, 35 – 74.

Arnold, T.H., Prentice, C.A., Hawker, L.C., Snyman, E.E., Tomalin, M., Crouch, N.R., Pottas-Bircher, C. 2002. Medicinal and magical plants of southern Africa: an annotated checklist. Strelitzia, 13. National Botanical Intsitute, Pretoria.

Atanasov, A.G., Waltenberger, B., Pferschy-Wenzig, E., Linder, T., Wawrosch, C., Uhrin, P., Temml, V., Wang, L., Schwaiger, S., Heiss, E.H., Rollinger, J.M., Schuster, D., Breuss, J.M., Bochkove, V., Mihovilovic, M.D., Kopp, B., Bauer, R., Dirsch, V.M., Stuppner, H. 2015. Discovery and resupply of pharmacologically active plant-derived natural products: A review. Biotechnology Advances 33(8), 1582 – 1614.

Bapela, M.J, Meyer, M.J.J., Kaiser, M. 2014. *In vitro* antiplasmodial screening of ethnopharmacologically selected South African plant species used for the treatment of malaria. Journal of Ethnopharmacology 156, 370 – 373.

Bapela M.J., Heyman, H., Senejoux, F., Meyer, J.J.M. 2019. $^{1}$H NMR-based metabolomics of antimalarial plant species traditionally used by Vha-Venda people in Limpopo Province, South Africa and isolation of antiplasmodial compounds. Journal of Ethnopharmacology 228, 148 – 155.

Bloom, D.E., Cadarette, D. 2019. Infectious disease threats in the twenty-first century: strengthening the global response. Frontiers in Immunology 10, 549.

Bradley, W., Powers, R. 2013. Multivariate analysis in metabolomics. Current Metabolomics 1, 92 – 107.

Brusotti, G., Cesari, I., Dentamaro, A., Caccialanza, G., Massolini, G. 2014. Isolation and characterization of bioactive compounds from plant resources: The role of analysis in the ethnopharmacological approach. Journal of Ethnopharmacology 87, 218 – 228.

De Wet, B. 2011. Medicinal plants and human health. South African Pharmaceutical Journal, 78, 6.

Eloff, J.N. 1998. A sensitive and quick microplate method to determine the minimal inhibitory concentration of plant extracts for bacteria. Planta Medica 64, 711 -713.

Fonteh, P.N., Keter, F.K., Meyer, D., Guzei, I.A., Darkwa, J. 2009. Tetra-chloro-(bis-(3,5-dimethylpyrazolyl)methane)gold(III) chloride: An HIV-1 reverse transcriptase and protease inhibitor. Journal of Biochemistry 103, 19 -194.

Girardot, M., Deregnaucourt, C., Deville, A., Dubost, L., Joyeau, R., Allorge, L., Rasoanaivo, P., Mambu, L. 2012. Indole alkaloids from *Muntafara sessilifolia* with antiplasmodial and cytotoxic activities. Phytochemistry 73, 65 – 73.

Huber, W., Koella, J.C., 1993. A comparison of the three methods of estimating $EC_{50}$ in studies of drug resistance of malaria parasites. Acta Tropica 55, 257–261.

Kapewangolo, P., Knott, M., Shithigona, R.E.K., Uusiku, S.L., Kandawa-Schuls, M. 2016. In vitro anti-HIV and antioxidant activity of *Hoodia gordonii* (Apocynaceae), a commercial plant product. BMC Complementary and Alternative Medicine 16, 411.

Karou, D., Nadembega, W.M.C., Outattara, L., Ilboudo, D.P., Canini, A., Nikiéma, J.B., Simpore, J., Colizzi, V., Traore, A.S. 2007. African ethnopharmacology and new drug discovery. Medicinal and Aromatic Plant Science and Biotechnology, 1, x – y.

Mabogo, D.E.N. 1990. The ethnobotany of Vhavenda (MSc thesis). University of Pretoria, Pretoria. [Online]. Available from https://repository.up.ac.za/handle/2263/28881?show=full. Accessed 20 February 2017.

Marinho, F.F., Simoes, A.O., Barcellos, T., Moura, S. 2016. Brazilian *Tabernaemontana* genus: Indole alkaloids and phytochemical activities. Fitoterapia, 114, 127 – 137.

Mathur, S., Hoskins, C. 2017. Drug development: Lessons from nature (Review). Biomedical Reports 6, 612 – 614.

Mugisha, M.K., Origa, H.O., Odyek, O and Makawiti, D.W. 2008. Medicinal plants used in the treatment of fungal and bacterial infections in and around Queen Elizabeth Biosphere Reserve, western Uganda. African Journal of Ecology. 46, 90-97.

Ngarivhume, T., van't Klooster, C.I.E.A., de Jong, J.T.V.M., Van der Westhuizen, J.H. 2015. Medicinal plants used by traditional healers for the treatment

of malaria in the Chipinge district in Zimbabwe. Journal of Ethnopharmacology 159, 224 – 237.

Nisha, M., Kalyanasundaram, M., Paily, K.P., Abidha, Vanamil, P., Balaraman, K. 2007. *In vitro* screening of medicinal plants extracts for macrofilaricidal activity. Parasitology Research 100, 575 – 579.

Pallant, C.A., Cromarty, A.D., Steenkamp, V. 2012. Effect of an alkaloidal fraction of *Tabernaemontana elegans* (Stapf.) on selected micro-organsims. Journal of Ethnopharmacology 140, 398 – 404.

Pooley, E., 1998. A field guide to wild flowers of KwaZulu-Natal and the Eastern Region. Natal Flora Publications Trust, Durban.

Ramalhete, C., Lopes, D., Mulhovo, S., Rosário, V.E., Ferreira, M.J.U., 2008. Antimalarial activity of some plants traditionally used in Mozambique. In: Workshop Plantas Medicinais e Fitoterapêuticas nos Trópicos.

Romulo, A.,Zuhud, E.A.M., Rondevaldova, J., Kokoska, L. 2018. Screening of in vitro antimicrobial activity of plants used in traditional Indonesian medicine. Pharmaceutical Bioloy 56, 287 – 293.

Shakya, A.K. 2016. Medicinal plants: Future source of new drugs. International Journal of Herbal Medicine, 4, 59 – 64.

Steenkamp, V., Fernandes, A.C., Van Rensburg, C.E.J. 2007. Screening of Venda medicinal plants for antifungal activity against *Candida albicans*. South African Journal of Botany 73, 256 – 258.

Stuppner, H. 2015. Discovery and resupply of pharmacologically active plant-derived natural products: A review. Biotechnology Advances 33, 1582 – 1614.

The largest selection of indigenous species in any Nursery in KZN. Trees, shrubs, aloes, grasses and ground covers. [Online]. Available from https://cjmgrowers.co.za/tabernaemontana-elegans/. Accessed 13 April 2019.

Vonthron-Senecheau, C., Weniger, B., Quattara, M., Tra, Bi, F., Kamenan, A., Lobstein, A., Brun, R., Anton, R. 2003. *In vitro* antiplasmodial activity and cytotoxicity of ethnobotanically selected Ivorian plants. Journal of Ethnopharmacology 87, 221 – 225.

Wang, W., Chen, J., Sheng, H., Wang, N., Yang, P., Zhou, X., Bergquist, R. 2017. Infectious disease of poverty, the first five years. Infectious Disease of Poverty 6, 96.

Watt, J.M., Breyer-Brandwijk, M.G. 1962. The medicinal and poisonous plants of southern and eastern Africa, second edition. Livingstone, London.

Zhang, C., Wu, Y., Sun, Y., Hong, C., Xiang, K., Guo, Y., Bartlam, M., Lou, Z. 2010. A novel non-radioactive assay for HIV-RT (RdDp) based on pyrosequencing for high-throughput drug screening. Protein Cell, 1, 284 – 290.

Zhu, J.-P., Kalt-Hadamowsky, Hesse,M. 1990. Chemotaxanomic study of the genus *Tabernaemontana* (Apocynaceae) based on their indole content. Plant Systematics and Evolution 172, 13 – 34.

# CHAPTER 4: $^{1}$H NMR-BASED METABOLOMICS AND ISOLATION OF COMPOUNDS

## 4.1 Introduction

Malaria is still one of the major tropical parasitic diseases in the world, more especially in sub-Saharan Africa (World Health Organisation (WHO), 2019). Malaria is caused by parasites of the *Plasmodium* genus which include *Plasmodium falciparum*, *P. ovale*, *P. vivax*, and *P. malariae*. *Plasmodium falciparum* is the most widespread and virulent causative agent for human malaria (Ramlhete et al., 2008). Globally, there were 92% cases of malaria infections and 93% of malaria deaths in the African region in 2017 (WHO, 2019). Drug-resistance of *P. falciparium* and the resistance of *Anopheles* mosquitoes to insecticides are widespread, and the search for new antimalarial drugs is becoming increasingly important (Ngrivhume et al., 2015; Haldar et al., 2018; Menard and Dondorp, 2017). Documentation of traditional medicine could facilitate future research on the safety and efficacy of medicinal plants and could provide a starting point for identifying single and chemical entities with antimalarial activity which could lead to the development of standardized phytomedicines. Metabolomics is a technique that can provide information of activity for possible novel drugs and drug scaffolds, and therefore accelerate the process of drug discovery (Cuperlovic-Culf and Culf, 2016; Tuyiringire et al., 2018; Steuer et al., 2019). Studies on medicinal plants have demonstrated that metabolomics could be used in discriminating the bioactive fractions and also in identifying classes of compounds that are responsible for the observed bioactivity (Bapela et al., 2019; Borges et al., 2019). *Tabernaemontana elegans* has demonstrated potential in being a source of antiplasmodial lead compounds (Chapter 3). The aim of this study was to conduct $^{1}$H NMR-based metabolomics on selective and non-selective antiplasmodial plant extracts from *T. elegans* as well as to identify the classes of compounds and to

isolate compounds from the most active plant extract that are attributable to antiplasmodial activity by using $^{1}$H NMR-based metabolomics profile.

## 4.2 Materials and methods

### 4.2.1 $^{1}$H NMR analysis

$^{1}$H NMR analysis was performed on 18 plant extracts that were tested for antiplasmodial activity. Twelve of the 18 plant extracts were further subjected to $^{1}$H NMR based metabolomics. Selection was based on the pathogen in which the plant extracts showed the most activity. The analysis was performed on a Varian 400 MHz spectrometer (Department of Chemistry, University of Pretoria) that operates at 600.13 MHz. Each plant extract was reconstituted in deuterated dimethyl sulfoxide (DMSO) at a concentration of 12 mg/ml. From each dissolved plant extract, 600 µL was transferred into a 5 mm NMR tube and then analyzed on a spectrometer at 30°C. The $^{1}$H NMR spectrum of each plant extract was obtained with 64 scans, a 14 ppm spectral width and at a constant temperature of 25°C.

### 4.2.2 Multivariate data analysis

The acquired $^{1}$H NMR spectra were reduced to MestReNova 10.0. All the spectra were referenced to DMSO (3.30), phased manually, baseline corrected with Whittaker smoother and integrated to bins of 0.02 ppm width. The generated ASCII files were then imported to Microsoft excel for secondary variable labelling, after which the files were imported to SIMCA-P 14.00. The acquired data were Pareto scaled before being subjected to principal component analysis (PCA) and orthogonal projections to latent structures-discriminant Analysis (OPLS-DA) algorithms. The Pareto scaling method reduces the influence of intense peaks while emphasizing the weak peaks (Emwas et al., 2018). A PCA model was generated to detect natural

clustering patterns and for identifying outliers. An OPLS-DA model was further applied to differentiate selective plant extracts from non-selective plant extracts in the analyzed dataset. A contribution plot was generated from the OPLS-DA plot to determine the spectral domains associated with the antiplasmodial metabolites of the analyzed plant extracts.

### 4.2.3 Isolation of compounds

The powdered plant material (1.00 kg) was sequentially extracted with hexane, dichloromethane (DCM), methanol and water as discussed in Chapter 3, section 3.2.2. The obtained hexane and DCM extracts (23.00 g) were combined because they showed similar chemical profiles and to increase the amount of the extract for isolation.

Isolation was performed on the non-polar extracts (23.00 g) of the stem bark based on the preliminary results of the *in vitro* screening. The extract was subjected to column chromatography (CC) on silica gel, eluting with hexane: ethyl acetate: methanol as an eluent mixture starting at 9.5:0.4:0.1 with increasing polarity up to 0:0:10 (1000 ml of each polarity step). The collected eluates were combined into 14 (A – N) fractions based on thin layer chromatograph (TLC) analysis. Fraction A was further chromatographed on silica gel with hexane: ethyl acetate (8:2). The recovered fractions were further subjected to Sephadex LH-20 (Sigma, USA) chromatography eluting with 100% DCM to yield compounds **1** (42 mg). Further chromatography of fraction B was done on silica gel column using hexane: ethyl acetate (8:2) and was re-chromatographed with hexane: DCM followed by DCM: methanol starting with 100 % hexane with increasing polarity up to 100 % methanol to yield compounds **2** (570 mg) and **4** (8.5 mg). Fraction C was further

chromatographed on silica gel column using hexane: ethyl acetate (8:2) and then re-chromatographed using hexane: DCM and DCM: methanol starting with 100 % hexane with increasing polarity up to 100 % methanol to yield compound **3** (35 mg). A simplified flow chart for the isolation of the four compounds is shown in Figure 4.1. The four isolated compounds were reconstituted in deuterated chloroform ($CDCl_3$) and subjected to NMR experiments, which included $^{1}H$ NMR, $^{13}C$ NMR, COSY, HSQC and HMBC (Appendix, section 6.2).

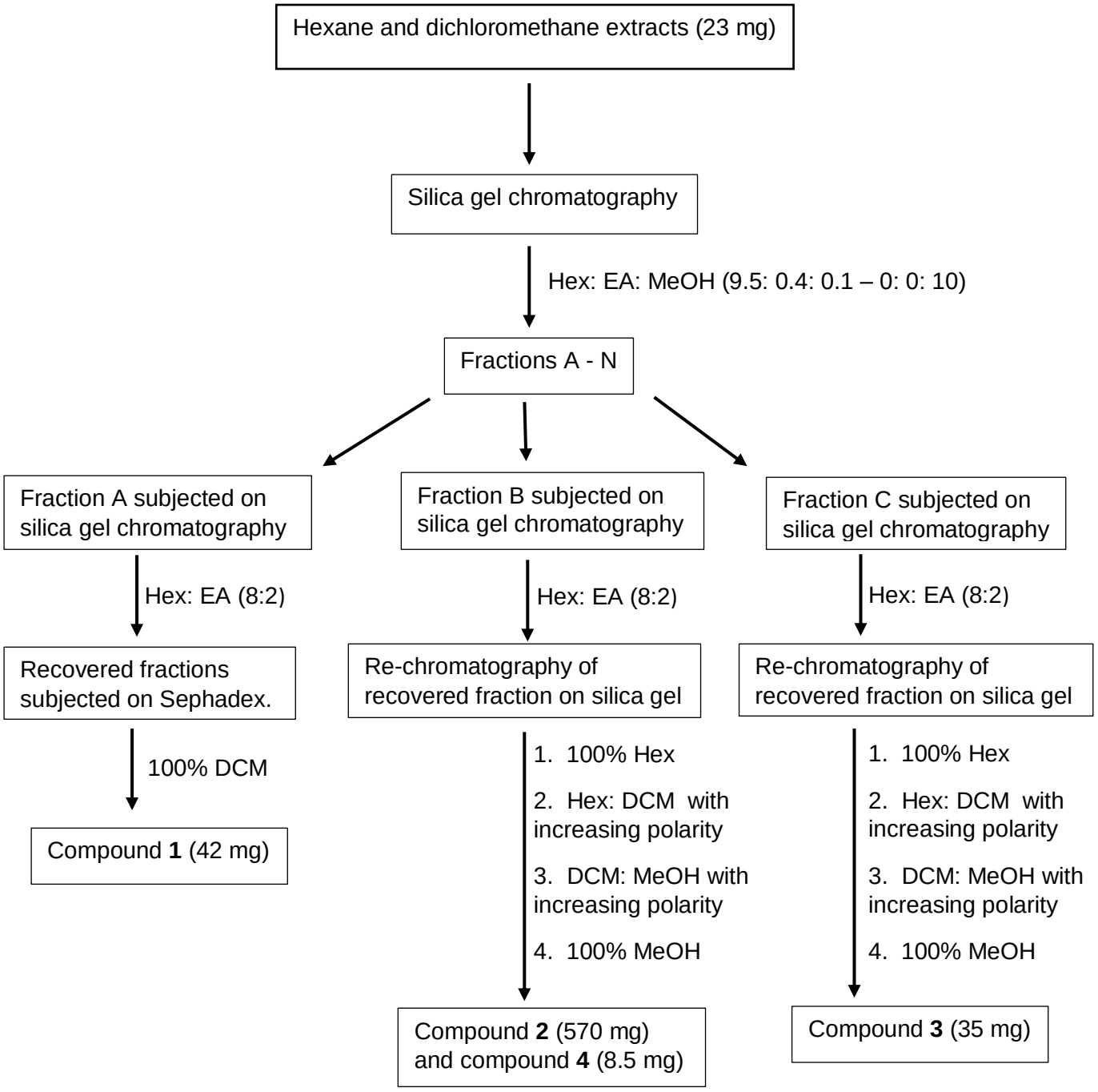



**Figure 4.1:** A simplified flow chart for the isolation of the four compounds.

## 4.3 Results and discussion

*$^{1}$H NMR-based metabolomics*

$^{1}$H NMR-based metabolomics was conducted on 12 plant extracts based on antiplasmodial selectivity. Figure 4.2 shows the stacked $^{1}$H NMR spectra of the

selective plant extracts (D03 and C03), where D03 is the methanol extract of roots and C03 is the methanol extract of stem bark, and the non-selective plant extracts (A01 and A02) where A01 is the hexane extract of fruits and A02 is the dichloromethane extract of fruits plant extracts. The spectra of these plant extracts shared many spectral features which could mean that they share closely related compounds which made it difficult to tell which spectral regions are responsible for antiplasmodial activity and this led to the $^{1}$H NMR-based metabolomics. However, the peaks of the spectra differ, more peaks were observed in the active extracts which could mean that the quantity of the related compounds present differs.

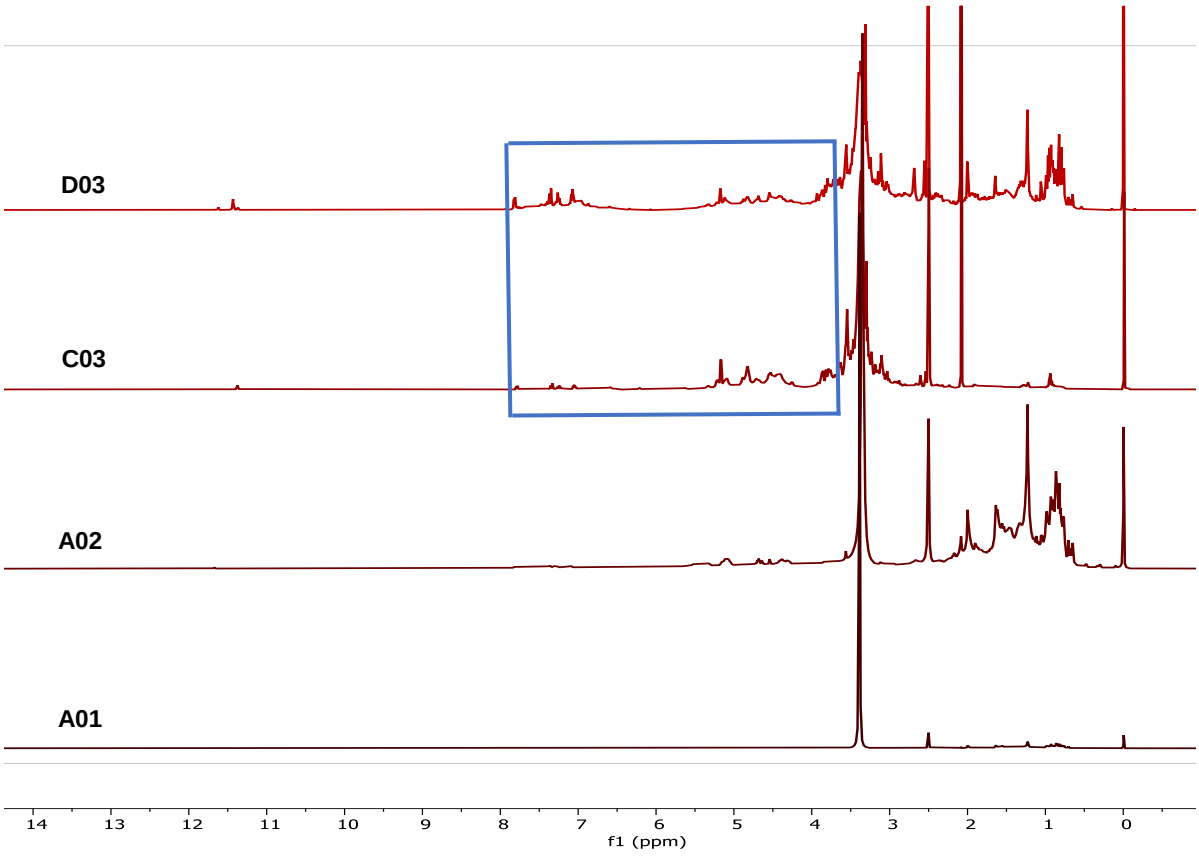



**Figure 4.2:** Stacked $^{1}$H NMR spectra of active (D03 and C03) and non-active (A01 and A02) plant extracts from *Tabernaemontana elegans*.

The *in vitro* antiplasmodial screening of 20 plant extracts showed that 6 of the extracts were active against *Plasmodium* with no cytotoxicity. Eighteen of these extracts were subjected to $^{1}$H NMR analysis. Out of 18 extracts 12 were subjected to NMR-based metabolomics based on antiplasmodial selectivity where the selective ones have a selective index of ≥ 10 and the non-selective ones have a selective index less than 10 (Table 4.1). The selective extracts include the stem bark extracts (hexane, DCM, methanol and water) as well as root extracts (methanol and water) and the non-selective extracts include fruit extracts (methanol, water and decoction), leaves extracts (water and decoction) plus root extract (water).

**Table 4.1:** Selective and non-selective antiplasmodial extracts of *Tabernaemontana elegans* that were subjected to $^{1}$H NMR-based metabolomics.

| Plant part | Solvent | Code | Antiplasmodial activity (µg/ml) | Cytotoxicity µg/ml | Selective Index (SI) |
|---|---|---|---|---|---|
| **Fruit** | Methanol | A03 | 28.95 ± 1.05 | 57.65 ± 0.95 | 2 |
| | Water | A04 | ND | 65.80 ± 14.80 | 1 |
| | Decoction | A05 | 48.50 | 39.05 ± 4.05 | 1 |
| **Leaves** | Water | B04 | 22.10 ± 1.30 | 43.95 ± 6.65 | 2 |
| | Decoction | B05 | 22.05 ± 1.55 | 63.55 ± 2.35 | 3 |
| **Stem bark** | **Hexane** | **C01** | **5.15 ± 0.45** | **49.55 ± 1.65** | **10** |
| | **DCM** | **C02** | **3.25 ± 0.33** | **52.00 ± 1.30** | **16** |
| | **Methanol** | **C03** | **0.31 ± 0.01** | **24.25 ± 0.05** | **79** |
| | **Water** | **C04** | **2.38 ± 0.02** | **56.90 ± 2.70** | **24** |
| **Root** | **Methanol** | **D03** | **0.16 ± 0.01** | **2.56 ± 0.11** | **16** |
| | Water | D04 | 10.77 ± 0.93 | 26.15 ± 6.45 | 2 |
| | **Decoction** | **D05** | **4.62 ± 0.55** | **91.50 ± 0.00** | **20** |

The acquired spectra were subjected to PCA to naturally differentiate between the selective and non-selective plant extracts (Figure 4.3). The PCA results did not show any distinct grouping that correlated to the observed antiplasmodial activity of the extracts. The lack of PCA clustering that correlates to activity could possibly be attributable to chemical variation within the different plant parts as well as extraction of the plant material using different solvents. Interpretation of multivariate PCA data is mostly done by means of $R^2$ and $Q^2$ values where $R^2$ signifies the variability in the data that is accounted for by the model and indicates the goodness of fit, while $Q^2$ gives the measure of the cumulative predictive capability of the model (Bhushan and Rathore, 2011; Worley and Powers, 2013). The $R^2$ and $Q^2$ values of 0.666 and 0.134 were obtained in the PCA analysis. These low $Q^2$ value indicate that this model was not significant in discriminating the plant extracts based on the factors mentioned above and antiplasmodial selectivity.

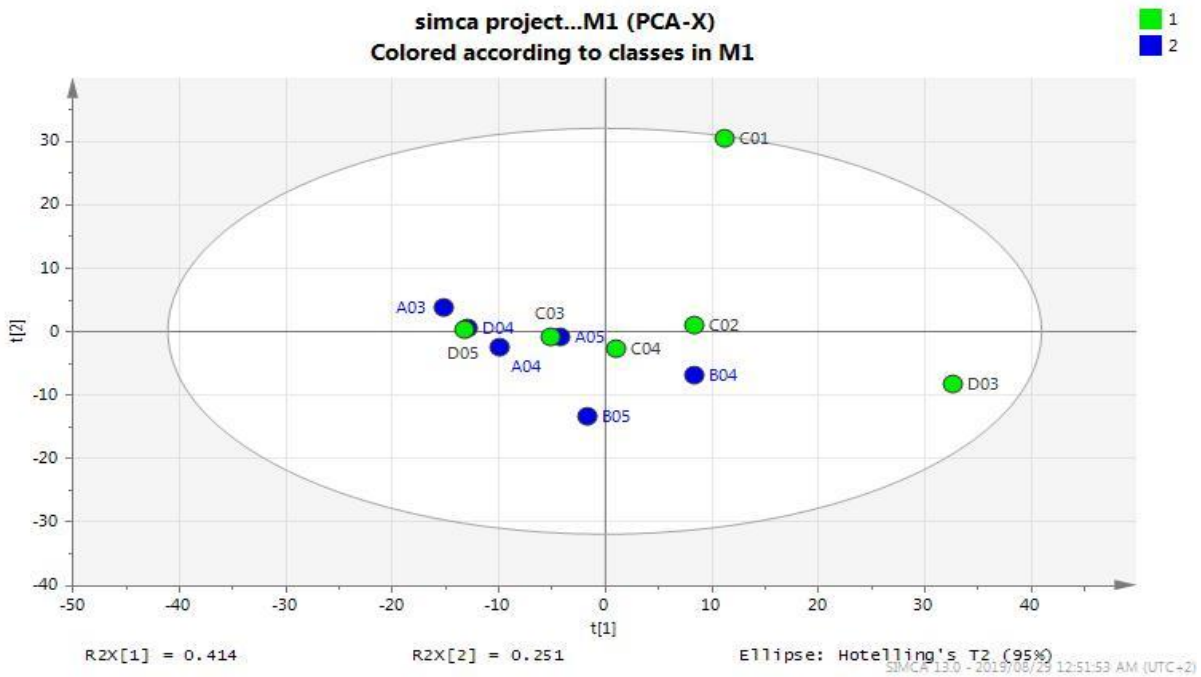



**Figure 4.3:** Principal component analysis score plot of the antiplasmodial selective (1) and non-selective (2) plant extracts from *Tabernaemontana elegans*.

The lack of a clustering pattern by the PCA algorithm led to the application of a supervised OPLS-DA to the $^{1}$H NMR data to enhance the poor clustering. In Figure 4.4, the OPLS-DA scatter plot shows a clear discrimination between the selective (1) and non-selective (2) antiplasmodial plant extracts indicating that there is a phytochemical difference between the two groups. The OPLS-DA plot is also interpreted by means of $R^2$ and $Q^2$. The $R^2$ value is 0.757 which means that the model was able to differentiate between selective and non-selective plant extracts the value of $Q^2$ (0.467) means that the predictability of the model was significant, and can be used to predict antiplasmodial activity of unknown plant samples. Models that explain data well and are well fitted usually have $R^2$ and $Q^2$ values close to 0.5 and highly disparate values of $R^2$ and $Q^2$ are an indicator of overfitting in a supervised analysis (Worley and Powers, 2013; Blasco et al., 2015).

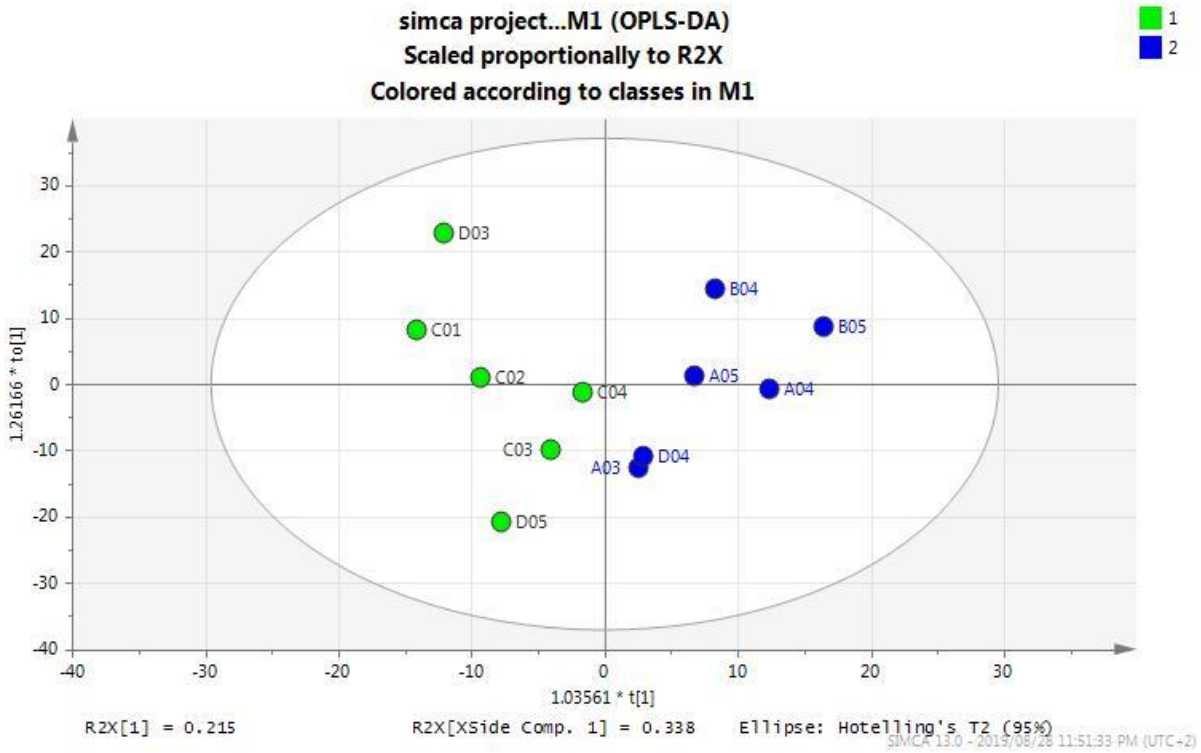



**Figure 4.4:** OPLS-DA scores plot of the antiplasmodial selective (1) and non-selective (2) plant extracts from *Tabernaemontana elegans*.

A contribution plot was generated from the OPLS-DA scoring plot to observe which spectral regions of the $^{1}$H NMR spectra are responsible for the differences in the selective and non-selective plant extracts (Figure 4.5). The contribution plot indicates that the major positive bars are chemical shifts that are present in the selective plant extracts and are attributable to the observed antiplasmodial activity of *T. elegans*. The prominent spectral regions include 3.07 - 3.9 ppm, 4.59 – 4.67 ppm, 6.15 – 6.25 ppm, 6.43 – 6.47 ppm and 7.89 – 8.13 ppm. The specified spectral regions correspond to aryl-$NH_2$, amide-NH, $NH_2$ and aromatic H chemical shifts, which are characteristic of indole alkaloids isolated from *T. elegans*. Based on the contribution plot, antiplasmodial activity in *T. elegans* may be attributable to indole alkaloids. Studies conducted on DCM and polar extracts from the stem bark of this species showed significant antiplasmodial activity (Bapela et al., 2014). Additionally, indole alkaloids from a related plant species *Muntafara sessilofolia* which include dregamine and tabernaemontanine also found in *T. elegans* showed significant antiplasmodial activity when tested against chloroquine-resistant strain of *P. falciparum* (Girardot et al., 2012).

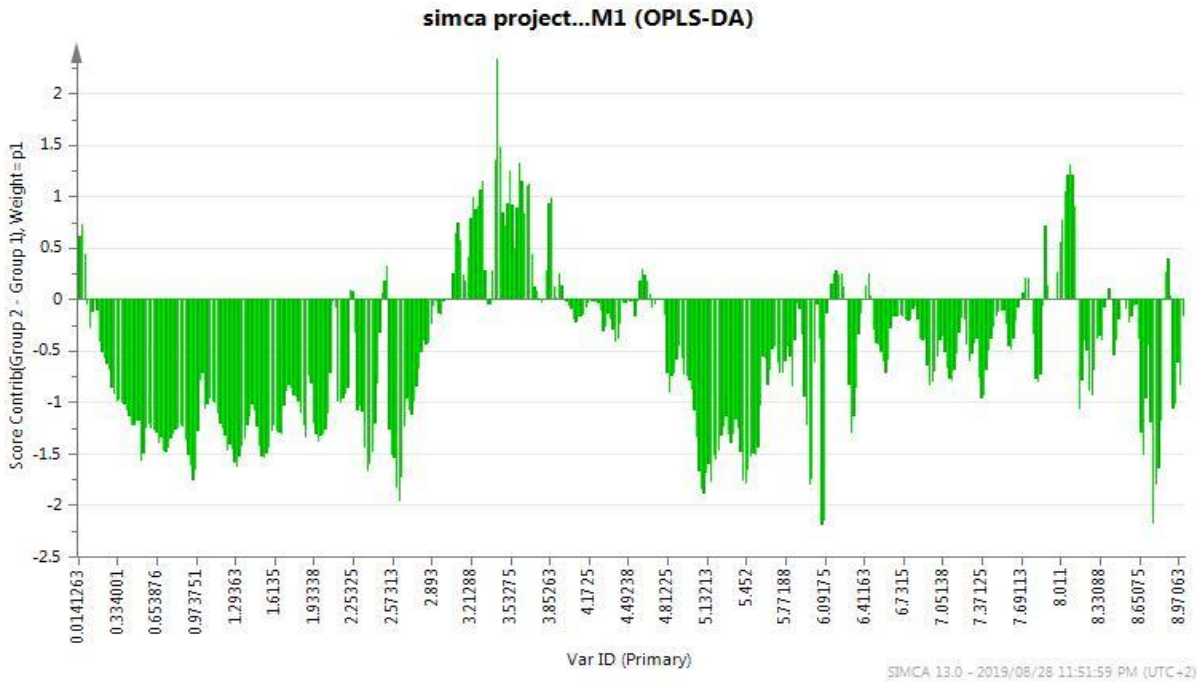



**Figure 4.5:** Contribution plot of antiplasmodial selective and non-selective plant extracts. (Selective – positive bars and non-selective – negative bars).

*Isolation of compounds*

Phytochemical analysis was performed based on the preliminary *in vitro* antiplasmodial results presented in Chapter 3, Table 3.2. The stem bark of *Tabernaemontana elegans* showed high selective antiplasmodial activity and was subjected to column chromatography. It was further fractionated and purified to yield four compounds **1 – 4**. The four compounds were then subjected to $^{1}$H NMR experiments in attempt to predict their antiplasmodial activity. The $^{1}$H NMR spectra of the four compounds were compared to the contribution plot profile that was generated during $^{1}$H NMR-based metabolomics (Figure 4.5). Compounds **2** and **3** have many spectral regions that are present in the selective extracts of the contribution plot and are therefore predicted to have significant antiplasmodial activity than compounds **1** and **4** (Figure 4.6). The regions in the squares, which denote antiplasmodial activity are only present and prominent in compounds **2** and **3**.

Compound **1** was identified as a terpenoid while compounds **2**, **3**, and **4** were identified as alkaloids. The four compounds were subjected to two dimensional NMR experiments, namely, $^{1}H$, $^{13}C$, COSY, HSQC and HMBC (Appendix, section 7.2). These compounds still need to be identified and validated *in vitro* for their activities.

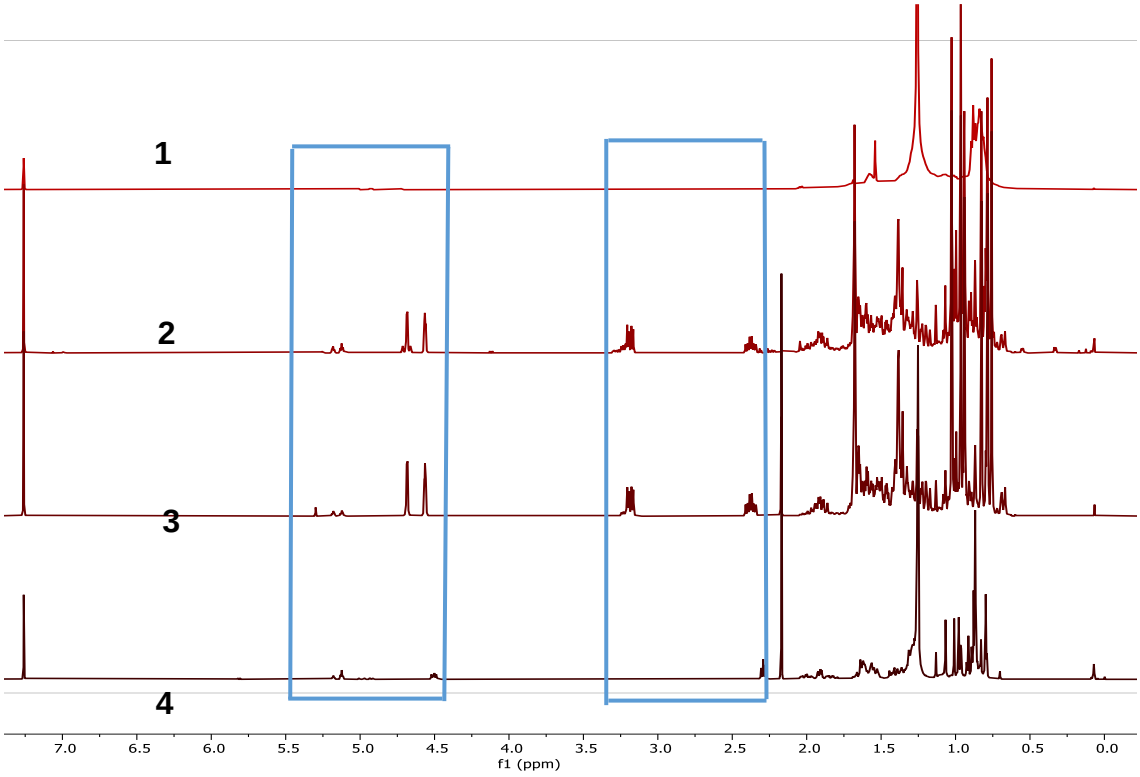



**Figure 4.6:** Stacked $^{1}H$ NMR spectra of isolated compounds.

## Conclusion

The $^{1}H$ NMR-based metabolomics technique in this study was successfully used to discriminate selective and non-selective plant extracts of *Tabernaemontana elegans*. The predictability of the generated model was significant and could be used to predict antiplasmodial activity of unknown samples. Phytochemical analysis led to isolation of four compounds and $^{1}H$ NMR spectroscopy was used to predict their antiplasmodial activity where two of the compounds were predicted to have significant antiplasmodial activity. The classes of compounds that are attributable to

antiplasmodial activity were identified to be alkaloids. Identification, further purification experiments and antiplasmodial *in vitro* screening of the isolated compounds are underway as well as isolation of more compounds.

## 4.5 References

Bapela, M.J., Meyer, M.J.J., Kaiser, M. 2014. *In vitro* antiplasmodial screening of ethnopharmacologically selected South African plant species used for the treatment of malaria. Journal of Ethnopharmacology 156, 370 – 373.

Bapela M.J., Heyman, H., Senejoux, F., Meyer, J.J.M. 2019. $^{1}$H NMR-based metabolomics of antimalarial plant species traditionally used by Vha-Venda people in Limpopo Province, South Africa and isolation of antiplasmodial compounds. Journal of Ethnopharmacology 228, 148 – 155.

Bhushan, N., Rathore, A.S. 2011. Use of multivariate data analysis (MVDA) for generating process understanding from manufacturing data of biotech process. 2011. Proceedings of the Indian National Science Academy 77, 133 – 142.

Blasco, H., Blaszczyński, J., Billaut, J.C., Nadal-Desbarats, L., Pradat, P.F., Devos, D., Moreau, C., Andres, C.R., Emond, P., Corcia, P., Slowiński, R. 2015. Comparative analysis of targeted metabolomics: dominance-based rough set approach versus orthogonal partial least square-discriminant analysis. Journal of Biomedical Informatics 53, 291 – 299.

Borges, D.G.L., Echeverria, J.T., de Oliveira, T.L., de Freitas, M.G., Dramasceno-Junior, G.A., Carollo, C.A., de Almeida Borges, F. 2019. Drug discovery potential of ovicidal natural products using metabolomics. PLOS One 14(1).

Cuperlovic-Culf, M., Culf, A.S. 2016. Applied metabolomics in drug discovery. Expert Opinion on Drug Discovery 11, 759 – 770.

Girardot, M., Deregnaucourt, C., Deville, A., Dubost, L., Joyeau, R., Allorge, L., Rasoanaivo, P., Mambu, L. 2012. Indole alkaloids from *Muntafara sessifolia* with antiplasmodial and cytotoxic activities. Phytochemistry 73, 65 – 73.

Menard, D., Dondorp, A. 2017. Antimalarial drug resistance: a threat to malaria elimination. Cold Spring Harbor Perspectives in Medicine 7, 1 – 24.

Emwas, A., Saccenti, E., Gao, X., McKay, R.T., Martins dos Santos, V.A.P., Roy, R., Wishart, D.S. 2018. Recommended strategies for spectral processing and post-processing of 1D $^{1}$H-NMR data of biofluids with a particular focus on urine. Metabolomics 14, 31.

Haldar, K., Bhattacharjee, S., Safeukui, I. 2018. Drug resistance of *Plasmodium*. Nature Reviews Microbiology 16, 156 – 170.

Ramalhete, C., Lopes, D., Mulhovo, S., Rosario, V.E., Ferreira, M.J.U. 2008. Antimalarial activity of some plants traditionally used in Mozambique. In: Workshop Plantas Medicinais e Fitoterapeuticas nos Tropicos. [Online]. Available from https://pdfs.semanticscholar.org/0b51/a5f8271167aadf6d631706e34185eb5359b4.pdf. Accessed 18 March 2019.

Tuyiringire, N., Tusubira, D., Munyampundu, J., Tolo, C.U., Muvunyi, C.M., Ongwang, P.E. 2018. Application of metabolomics to drug discovery and understanding the mechanisms of action of medicinal plants with anti-tuberculosis activity. Clinical and Translational Medicine 7, 29.

Steuer, A.E., Brockbals, L., Kraemer, T. 2019. Metabolomic strategies in biomarker research – new approach for indirect identification of drug consumption

and sample manipulation in clinical and forensic toxicology. Frontiers in Chemistry 7, 319.

# CHAPTER 5: GENERAL DISCUSSION

## 5.1 General discussion

Infectious diseases are caused by pathogenic microorganisms, such as bacteria, parasites, viruses or fungi, which can be spread directly or indirectly from one human to another (World Health Organisation (WHO), 2019). These infectious diseases remain a major health concern globally, more especially in developing countries, where human immunodeficiency virus (HIV) and human immunodeficiency virus/acquired immunodeficiency syndrome (HIV/AIDS), tuberculosis, malaria and viral hepatitis kill millions of people around the world each year (Wang et al., 2017; Gupta and Kaushal, 2018; Bloom and Cadarette, 2019; WHO, 2019). The rate of new infections is continuing to rise due to the development of resistant strains. This is one of the reasons why people seek alternative medicines, such as herbal remedies, to cure these infections (Mugisha et al., 2008; Gupta and Birdi, 2017; Baars et al., 2019).

Plants have been a source of medicines to humans for various infectious diseases since ancient times and continue to play an important role as templates for the synthesis of drugs (Cox and Balick, 1994; Tshikalange et al., 2005; Cragg and Newman, 2013; Shakya, 2016). Drug discovery from medicinal plants needs an approach that is multidisciplinary in which the success depends largely on a chosen set of relevant *in vivo* and *in vitro* assays (Brusotti et al., 2014; Atanasov et al., 2015). A large number of modern drugs have been discovered since the history of ethnobotany paying a distinct importance to the documentation of traditional information of medicinal plants. Many ethnopharmacological studies validated the bioactivity of medicinal plants against microbial and parasitic infections (Mustafa et al., 2017).

Ethnopharmacological studies are conducted in an attempt to determine the safety and efficacy of herbal remedies, and to also discover novel chemotherapeutic agents (Etkin and Elisabetsky, 2005; Karou et al., 2007). Medicinal plants consist of diverse secondary metabolites that constitute a rich source of bioactive substances and this has increased the scientific interest in bioprospecting plants for novel chemical entities (Nisha et al., 2007). Most of the drugs that are active against infectious agents are derived from natural products or structures that are suggested by natural products. Conventional drugs that are derived from plants or modelled on natural products include aspirin from willow tree bark, quinine an alkaloid isolated from the Cinchona bark as well as chloroquine a synthetic analogue of quinine. Additionally, digoxin is from *Digitalis lanata* flower, artemisinin was isolated from *Artemisia annua* L., artemether is a semisynthetic derivative of artemisinin with less side effects and morphine is from opium, which demonstrate that natural products can provide efficient therapeutic agents (Nisha et al., 2007; Mathur and Hoskins, 2017). The recognition of clinical, pharmaceutical and economic value of herbal products is still growing although it differs between countries. There are major challenges in their evaluation and ensuring their safety and efficacy through registration and regulation. Traditional medicinal knowledge and practices in many developing countries such as South Africa which has a rich plant biodiversity, have not been adequately studied, exploited or documented (De Wet, 2011; Ahmed, 2016). Intensive harvesting of medicinal plants has resulted in overexploitation due to increased demand and is a threat to the biodiversity in South Africa (Street and Prinsloo, 2013; Le Roux et al., 2019). Many plant species are becoming endangered and may be lost without being extensively evaluated for their medicinal properties.

In the current study, different plant parts namely, fruits, leaves, stem bark and roots of *Tabernaemontana elegans* were tested for their activity against *Neisseria gonorrhoea*, *Candida albicans*, human immunodeficiency virus (HIV), and *Plasmodium falciparum*. Selection of this plant and the pathogens was based on its use by the local people of Vha-Venda in treating various diseases. The selected pathogens cause infections that are a major health concern globally. These infections are continuing to rise because of development of resistant strains to the currently used drugs. This has led to the need for new drugs from sources such as herbal remedies. Plant extracts of medicinal plants could serve as an alternative source of resistance modifying agents owing to the wide variety secondary metabolites (Verpoorte, 1998; Gupta and Birdi, 2017). The aim of this study was to find the active plant extracts against the four pathogens and to also isolate the compounds responsible for the activity.

The twenty extracts recovered from the plant parts of *T. elegans* were active against *Neisseria gonorrhoea*, HIV as well as malaria and none of them was active against *C. albicans*. This study has reported for the first time the antimicrobial activity of *T. elegans* against *N. gonorrhoea* and HIV. The stem bark and the root extracts showed the best activities against the three pathogens. For gonorrhoea only the stem bark extract (methanol) and root extracts (methanol and decoction) extract showed significant activity, the leaf extracts (water and decoction), stem bark extracts (water and decoction) as well as root extracts (DCM and water) showed moderate activity in HIV and for malaria the stem bark extracts (DCM, methanol, water and decoction) and root extracts (methanol and decoction) showed significant activity. The activity of the decoctions validates the mode of administration and the ethnopharmacological significance of the plant by indigenous people of Vha-Venda.

There was most activity for the malaria pathogen. Plant extracts were then subjected to $^{1}$H NMR analysis to observe spectral regions that were responsible for the antiplasmodial activity. They acquired spectra shared many spectral regions which made it difficult to see which ones are responsible for activity and this led to the application of $^{1}$H NMR-based metabolomics. The $^{1}$HNMR-based metabolomics models showed the potential in discriminating the selective and non-selective plant extracts. The predictability of the generated model was significant and could be used to predict antiplasmodial activity of unknown samples. Phytochemical analysis of *T. elegans* showed that it has many compounds. The stem bark non-polar extract was subjected to column chromatography and was further fractionated and purified to yield four compounds that will be further identified and subjected to *in vitro* screening for activity. The $^{1}$H NMR spectra of the compounds were used to predict the antiplasmodial activity by fitting to the contribution plot profile of metabolomics and two of the compounds (**2** and **3**) were predicted to be active.

## 5.2 Conclusion

The diverse uses of *Tabernaemontana elegans* by indigenous people and the scientific evidence of its phytochemistry and pharmacological properties indicate therapeutic potential of this species. The study supported some of the ethnomedicinal uses (malaria and gonorrhoea) of the plant parts of *T. elegans* used by Vha-Venda people. $^{1}$H NMR-based metabolomics showed potential in discriminating between antiplasmodial selective and non-select extracts. Further studies should focus on identifying the four compounds, their respective pharmacological activities and the isolation of more compounds from *T. elegans*.

## 5.3 References

Ahmed, H.M. 2016. Ethnopharmacobotanical study on the medicinal plants used by herbalists in Sulaymaniya Province, Kurdistan, Iraq. Journal of Ethnobiology and Ethnomedicine 12, 8.

Atanasov, A.G., Waltenberger, B., Pferschy-Wenzig, E., Linder, T., Wawrosch, C., Uhrin, P., Temml, V., Wang, L., Schwaiger, S., Heiss, E.H., Rollinger, J.M., Schuster, D., Breuss, J.M., Bochkove, V., Mihovilovic, M.D., Kopp, B., Bauer, R., Dirsch, V.M., Stuppner, H. 2015. Discovery and resupply of pharmacologically active plant-derived natural products: A review. Biotechnology Advances 33, 1582 – 1614.

Baars, E.W., Zoen, Belt-van Zoen, E., Breitkreuz, T., Martin, D., Matthes, H., von Schoen-Angerer, T., Soldner, G., Vagedes, J., van Wietmarschen, H., Patijin, O., Willcox, M., von Flotow, P., Teut, M., von Ammon, K., Thangavelu, M., Ursula, W., Hummelsberger, J., Nicolai, T., Hartemann, P., Szőke, H., McIntyre, M., van der Werf, E.T., Huber, R. 2019. The contribution of complementary and alternative medicine to reduce antibiotic use: a narrative review of health concepts, preventions, and treatment strategies. https://doi.org/10.1155/2019/5365608.

Bloom, D.E., Cadarette, D. 2019. Infectious disease threats in twenty-first century: strengthening the global response. Frontiers in Immunology 10, 549. doi: 10.3389/fimmu.2019.00549.

Brusotti, G., Cesari, I., Dentamaro, A., Caccialanza, G., Massolini, G. 2014. Isolation and characterization of bioactive compounds from plant resources: The role of analysis in the ethnopharmacological approach. Journal of Pharmaceutical and Biomedical Analysis 87, 218-228

Cox, P.A., Balick, M.J., 1994. The ethnobotanical approach to drug discovery. Scientific American 270, 82-87

Cragg, G.M., Newman, D.J., 2013. Natural products: a continuing source of novel drug leads. Biochimica et Biophysica Acta 1830, 3670–3695

De Wet, B. 2011. Medicinal plants and human health. South African Pharmaceutical Journal 78, 6.

Etkin, N.L., Elisabetsky, E., 2005. Seeking a transdisciplinary and culturally germane science: The future of ethnopharmacology. Journal of Ethnopharmacology 100, 23-26.

Gupta, P.D., Birdi, T.J. 2017. Development of botanicals to combat antibiotic resistance. Journal of Ayruveda and Integrative Medicine 8, 266 – 275.

Gupta, M., Kaushal, K. 2018. An update on newer vaccines in development phase for malaria, tuberculosis, and human immunodeficiency virus/acquired immune deficiency syndrome. International Journal of Noncommunicable Diseases 3, 20 – 24.

Karou, D., Nadembega, W.M.C., Outattara, L., Ilboudo, D.P., Canini, A., Nikiéma, J.B., Simpore, J., Colizzi, V., Traore, A.S. 2007. African ethnopharmacology and new drug discovery. Medicinal and Aromatic Plant Science and Biotechnology 1, x – y.

Le Roux, J.J., Hui, C., Castillo, M.L., Iriondo, J.M., Keet, J., Khapugin, A.A., Médail, F., Rejmánek, M., Theron, G., Yannelli, F.A., Hirsch, H. Recent anthropogenic plant extinctions differ in biodiversity hotspots and coldspots. Current Biology. doi: 10.1016/j.cub.2019.07.063.

Mathur, S., Hoskins, C. 2017. Drug development: Lessons from nature (Review). Biomedical Reports 6, 612 – 614.

Mugisha, M.K., Origa, H.O., Odyek, O., Makawiti, D.W. 2008. Medicinal plants used in the treatment of fungal and bacterial infections in and around Queen Elizabeth Biosphere Reserve, western Uganda. African Journal of Ecology 46, 90-97.

Mustafa, G., Arif, R., Atta, A., Sharif, S., Jamil, A. 2017. Bioactive compounds from medicinal plants and their importance in drug discovery in Pakistan. Matrix Science Pharma 1, 17 – 26.

Nisha, M., Kalyanasundaram, M., Paily, K.P., Abidha, Vanamil, P., Balaraman, K. 2007. *In vitro* screening of medicinal plants extracts for macrofilaricidal activity. Parasitology Research 100, 575 – 579.

Street, R., Prinsloo, G. 2013. Commercially important medicinal plants of South Africa: A review. Journal of Chemistry, 1 – 16.

Shakya, A.K. 2016. Medicinal plants: Future source of new drugs. International Journal of Herbal Medicine 4, 59 – 64.

Tshikalange, T.E., Meyer, J.J.M., Hussein, A.A. 2005. Antimicrobial activity, toxicity and the isolation of a bioactive compound from plants used to treat sexually transmitted diseases. Journal of Ethnopharmacology 3, 515 – 519.

Verpoorte, R. 1998. Exploration of nature's chemodiversity: the role of secondary metabolites as leads in drug development. Drug Discovery Today 3, 232–238.

Wang, W., Chen, J., Sheng, H., Wang, N., Yang, P., Zhou, X., Bergquist, R. 2017. Infectious disease of poverty, the first five years. Infectious Disease of Poverty 6, 96.

World Health Organisation. 2019. Infectious diseases. [Online]. Available from: https://www.who.int/topics/infectious_diseases/en/. Accessed 15 July 2019.

# CHAPTER 6: APPENDIX

## 6.1 $^{1}$H NMR spectra of plant extracts

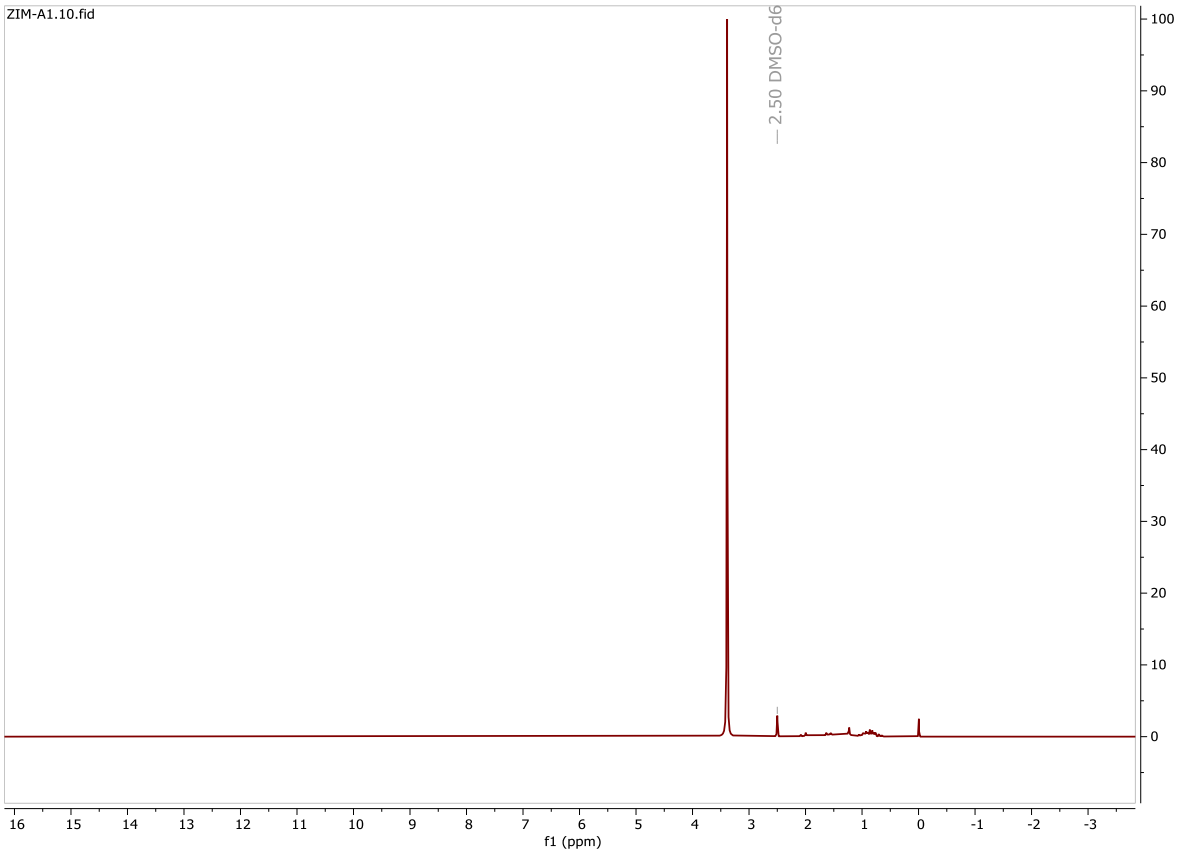



**Figure 6.1.1:** $^{1}$H NMR spectrum of hexane fruit extract.

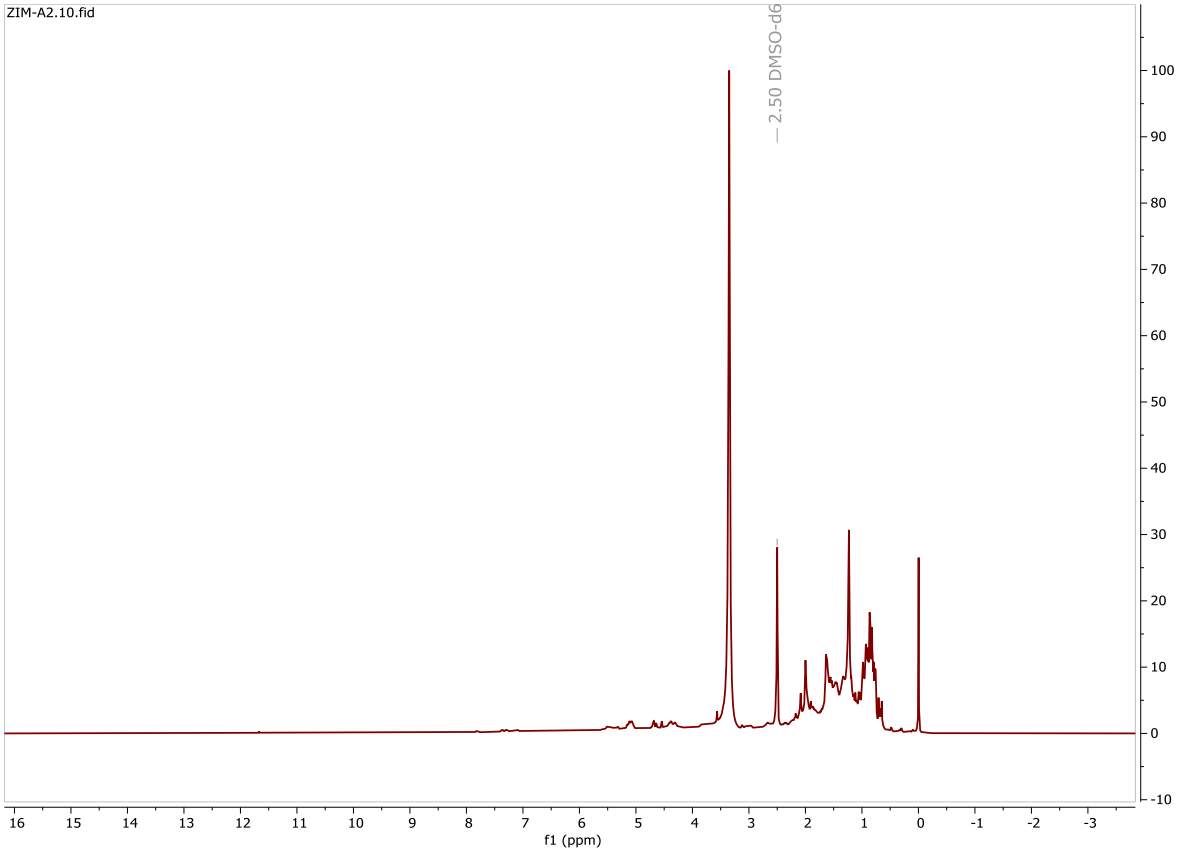



**Figure 6.1.2:** $^{1}$H NMR spectrum of dichloromethane fruit extract.

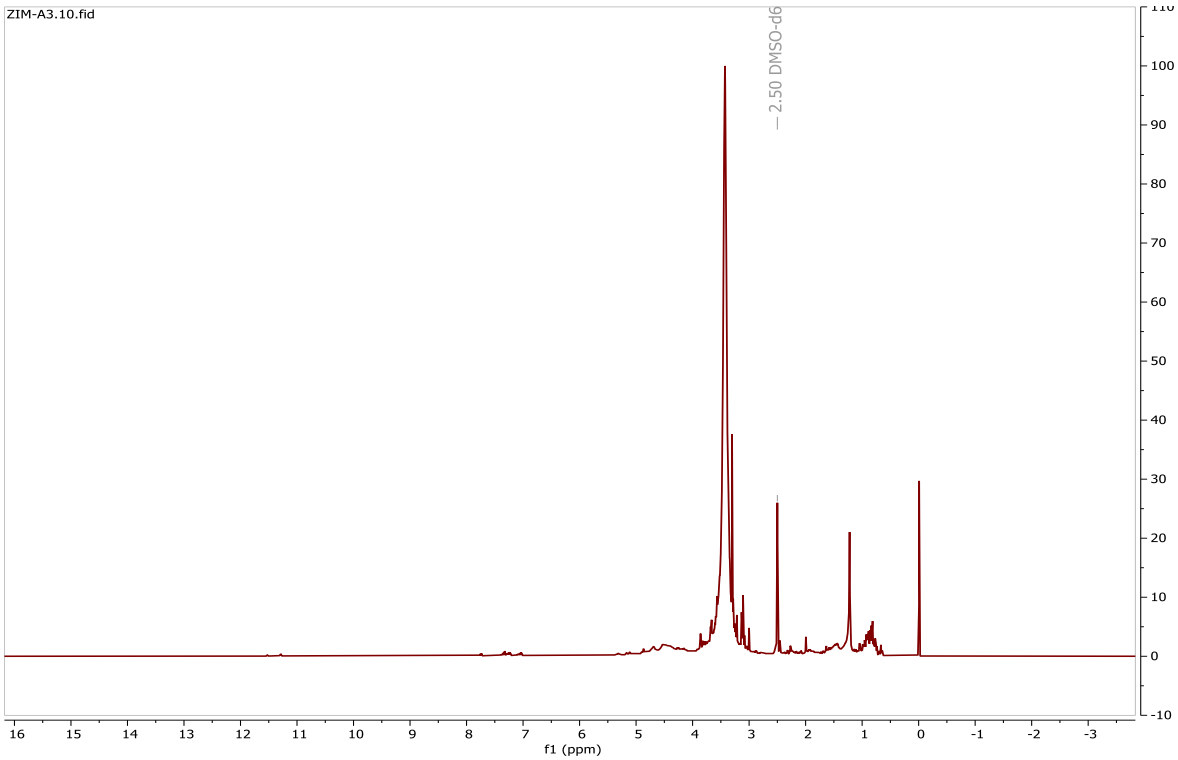



**Figure 6.1.3:** $^{1}$H NMR spectrum of methanol fruit extract.

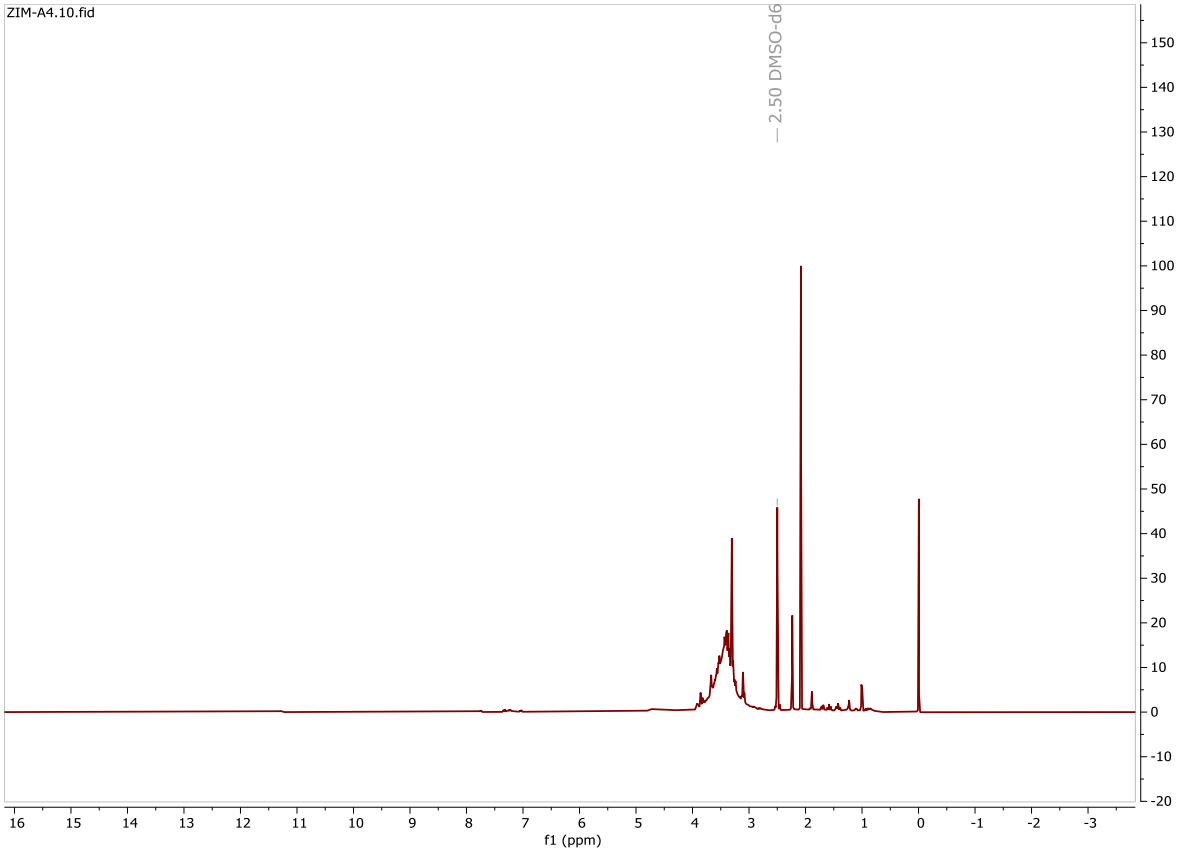



**Figure 6.1.4:** $^{1}$H NMR spectrum of water fruit extract.

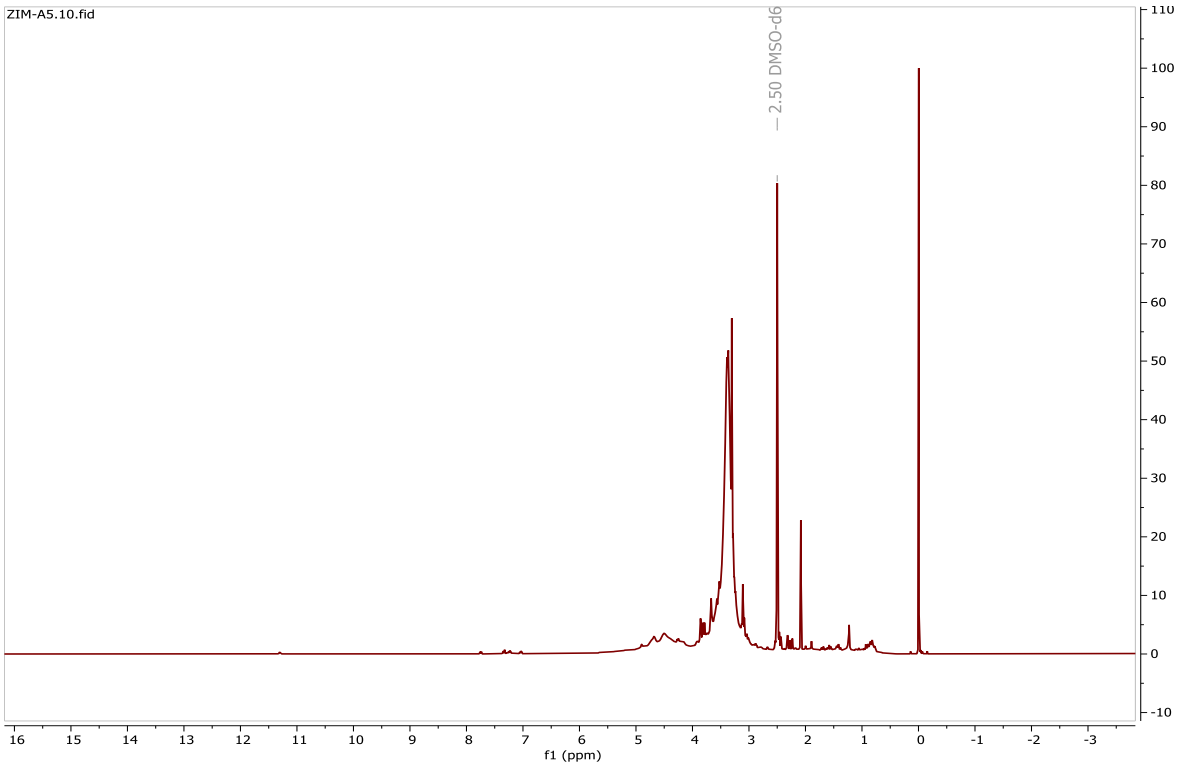



**Figure 6.1.5:** $^{1}$H NMR spectrum of decoction fruit extract.

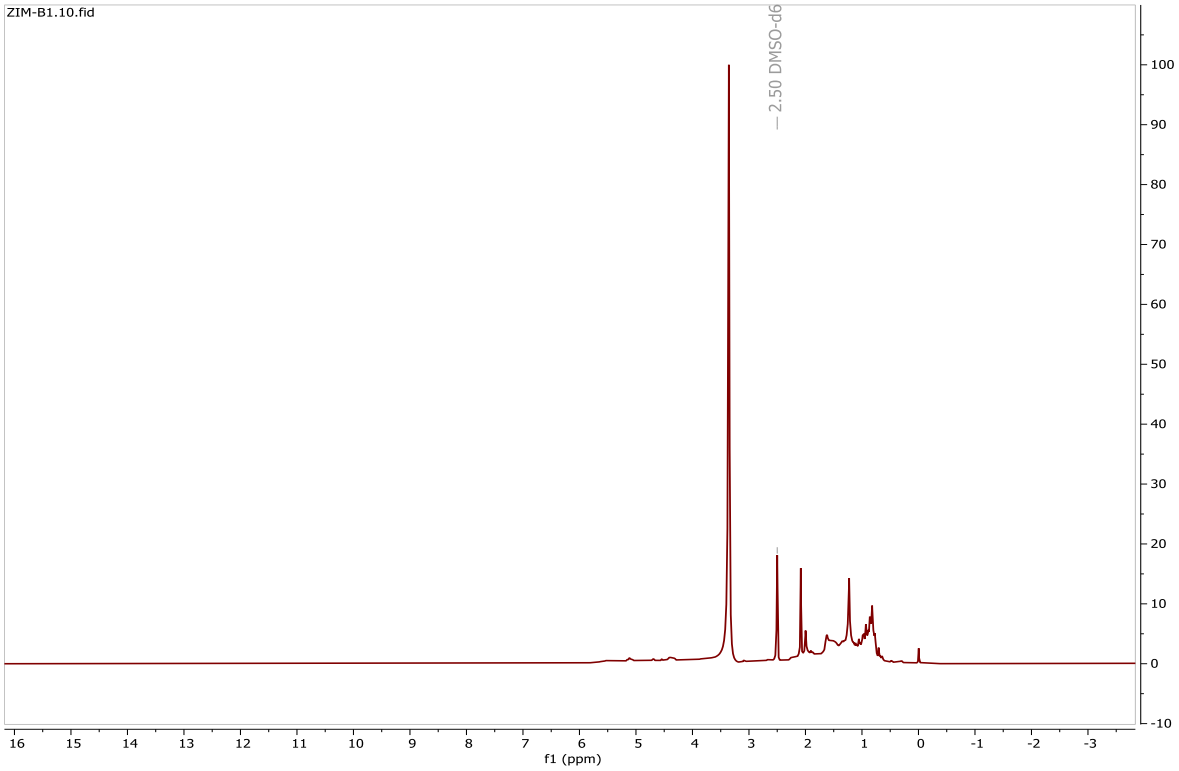



**Figure 6.1.6:** $^{1}$H NMR spectrum of hexane leaf extract.

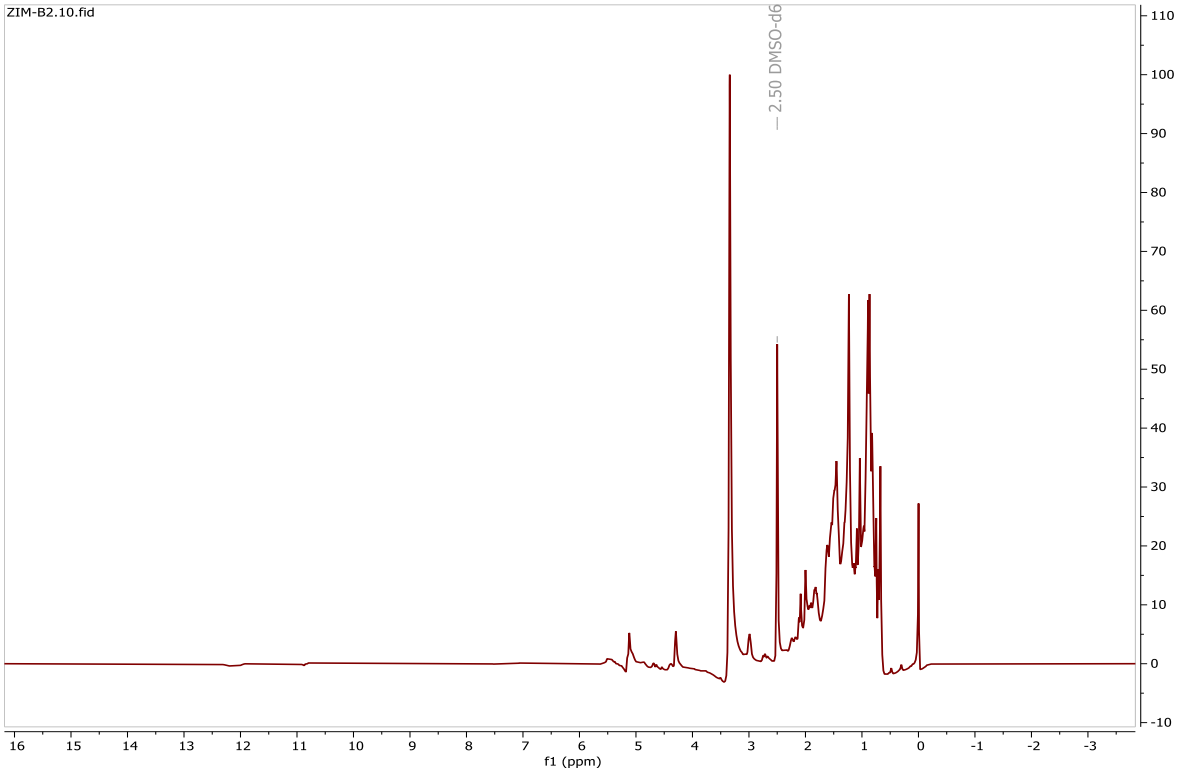



**Figure 6.1.7:** $^{1}$H NMR spectrum of dichloromethane leaf extract.

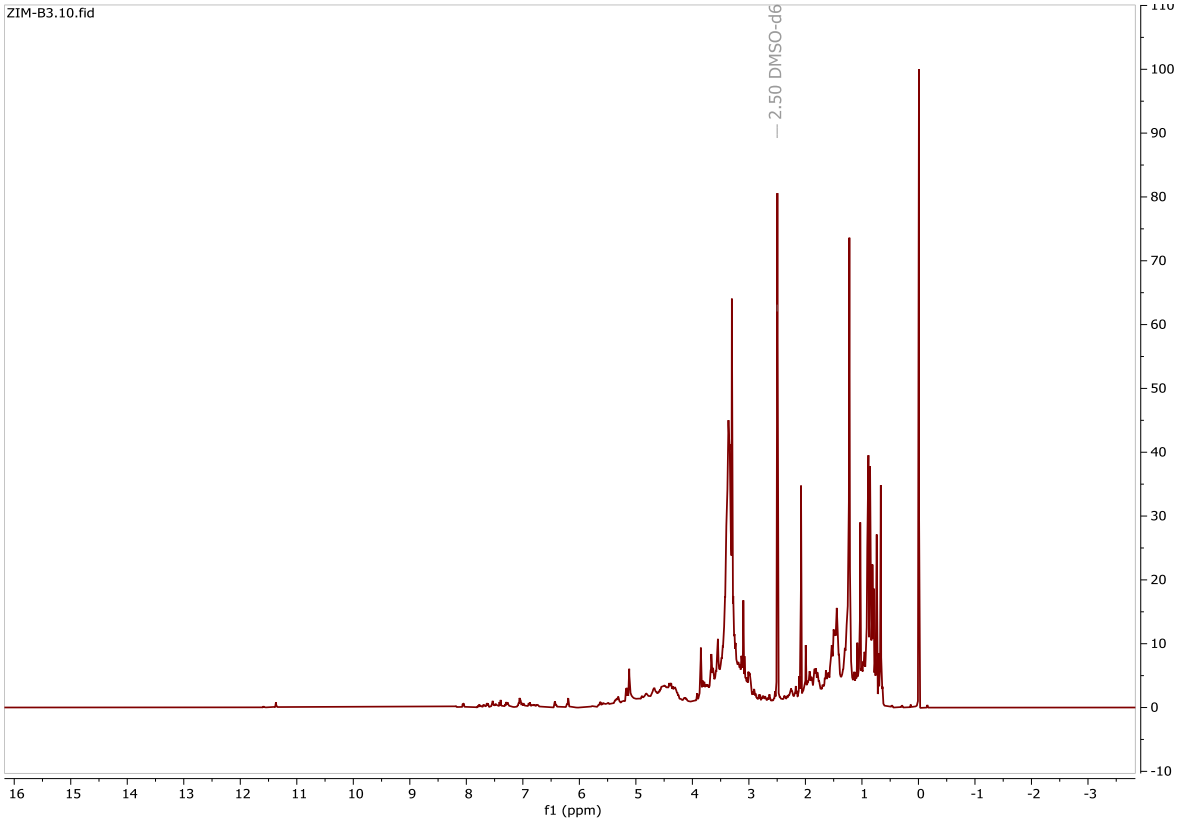



**Figure 6.1.8:** $^{1}$H NMR spectrum of methanol leaf extract.

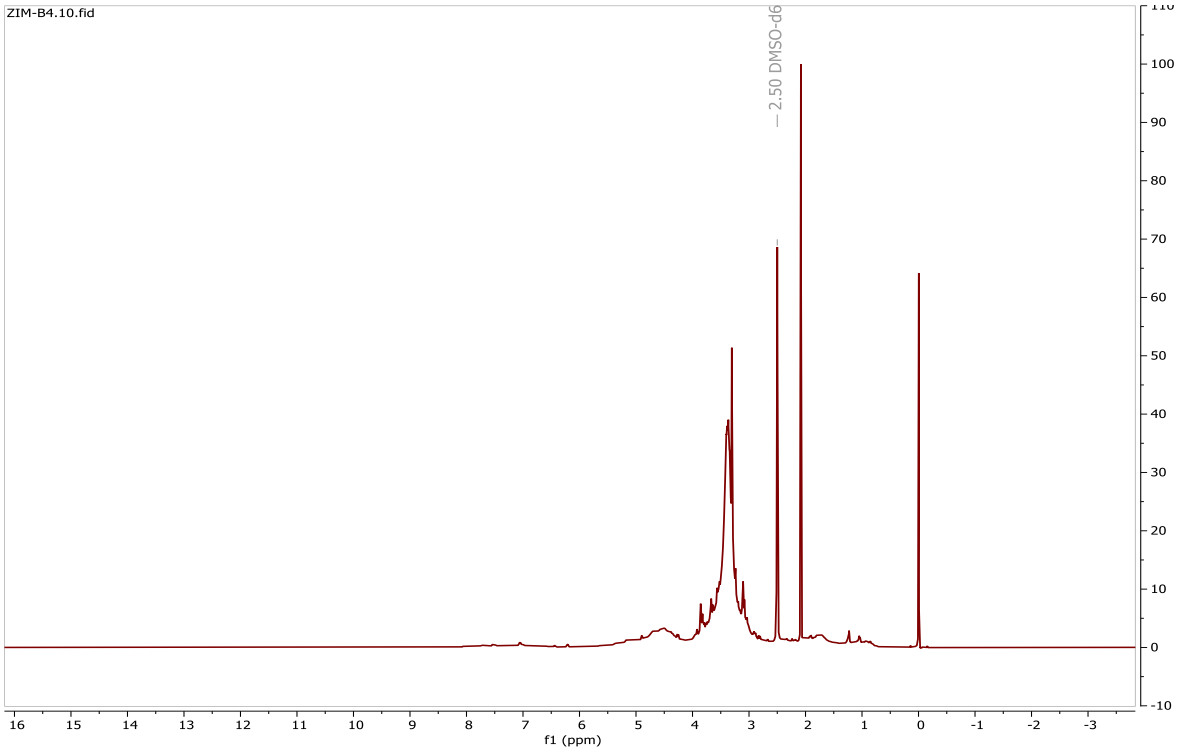



**Figure 6.1.9:** $^{1}$H NMR spectrum of water leaf extract.

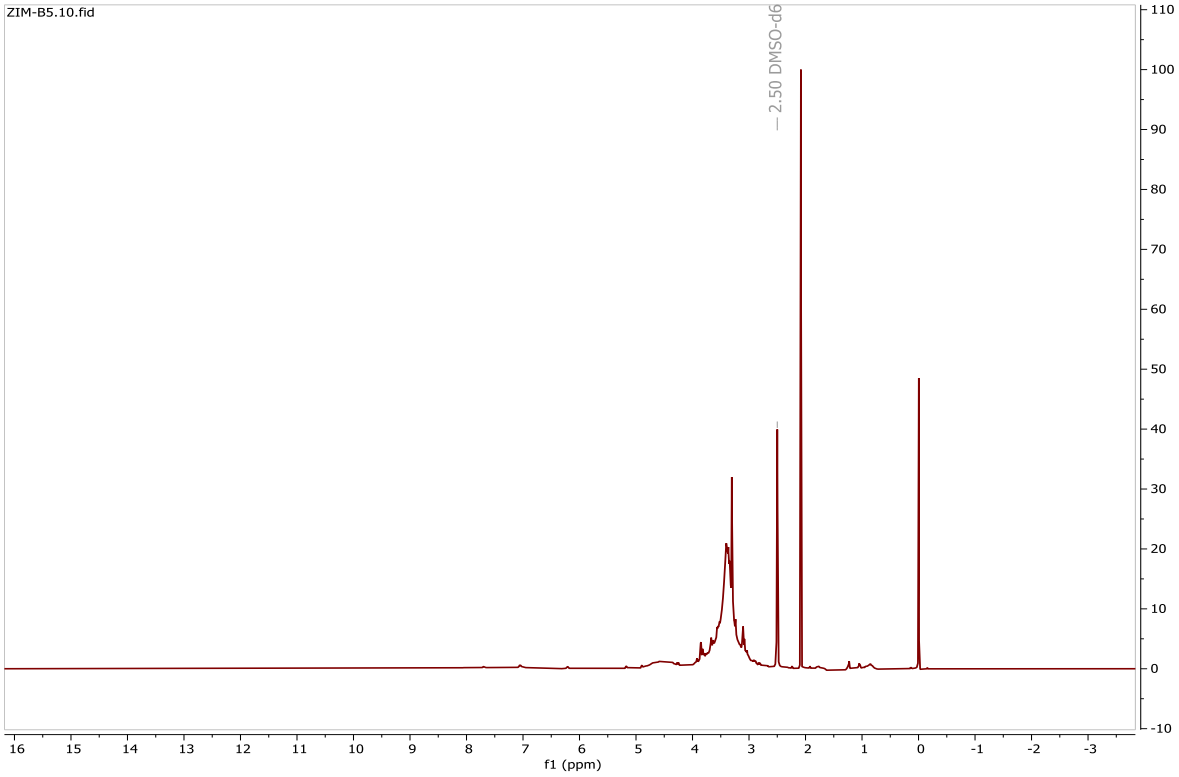



**Figure 6.1.10:** $^{1}$H NMR spectrum of decoction leaf extract.

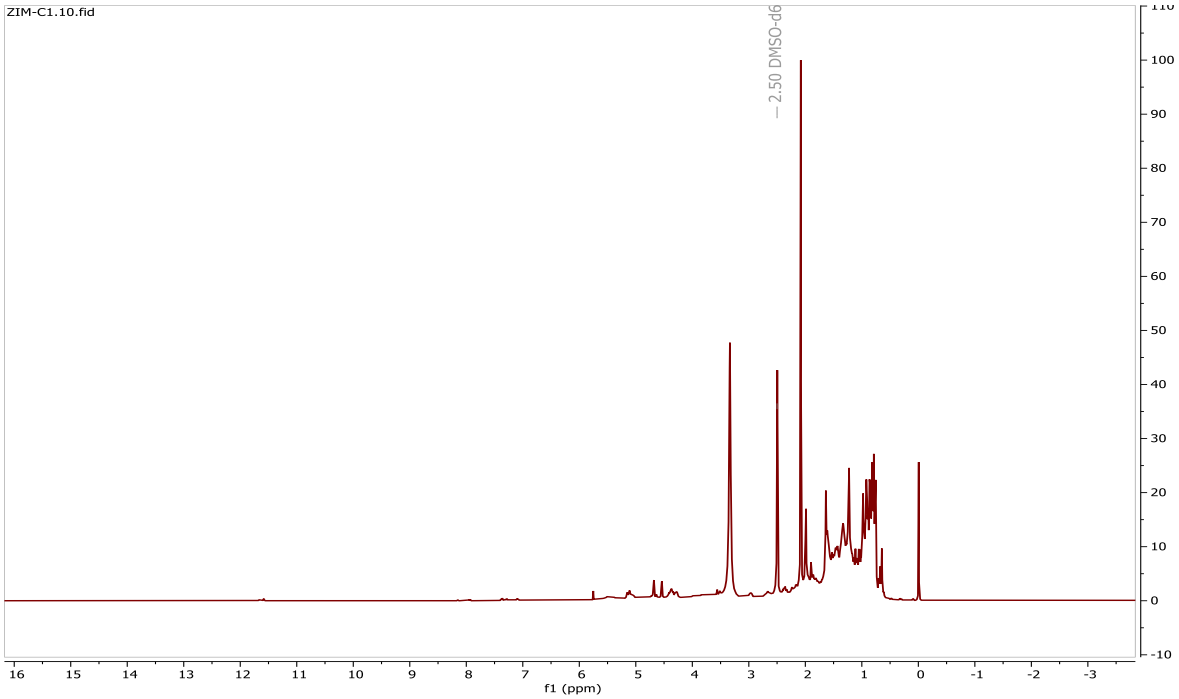



**Figure 6.1.11:** $^{1}$H NMR spectrum of hexane stem bark extract.

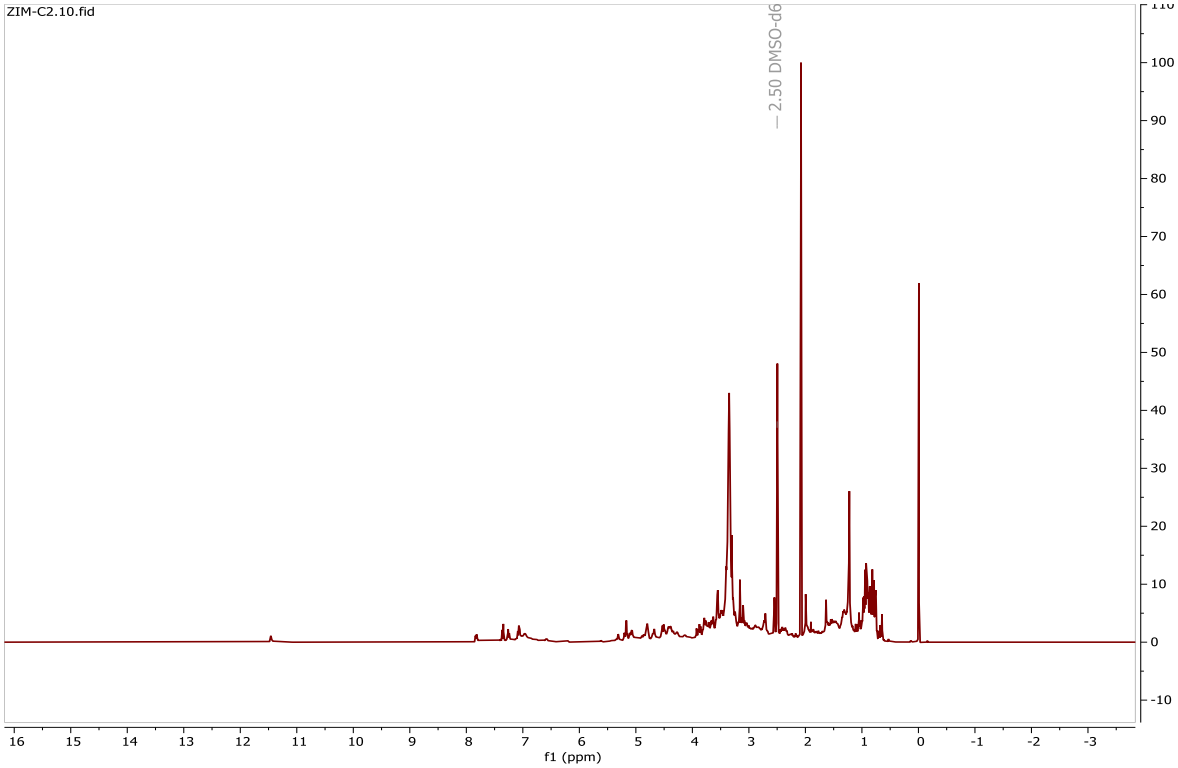



**Figure 6.1.12:** $^{1}$H NMR spectrum of dichloromethane stem bark extract.

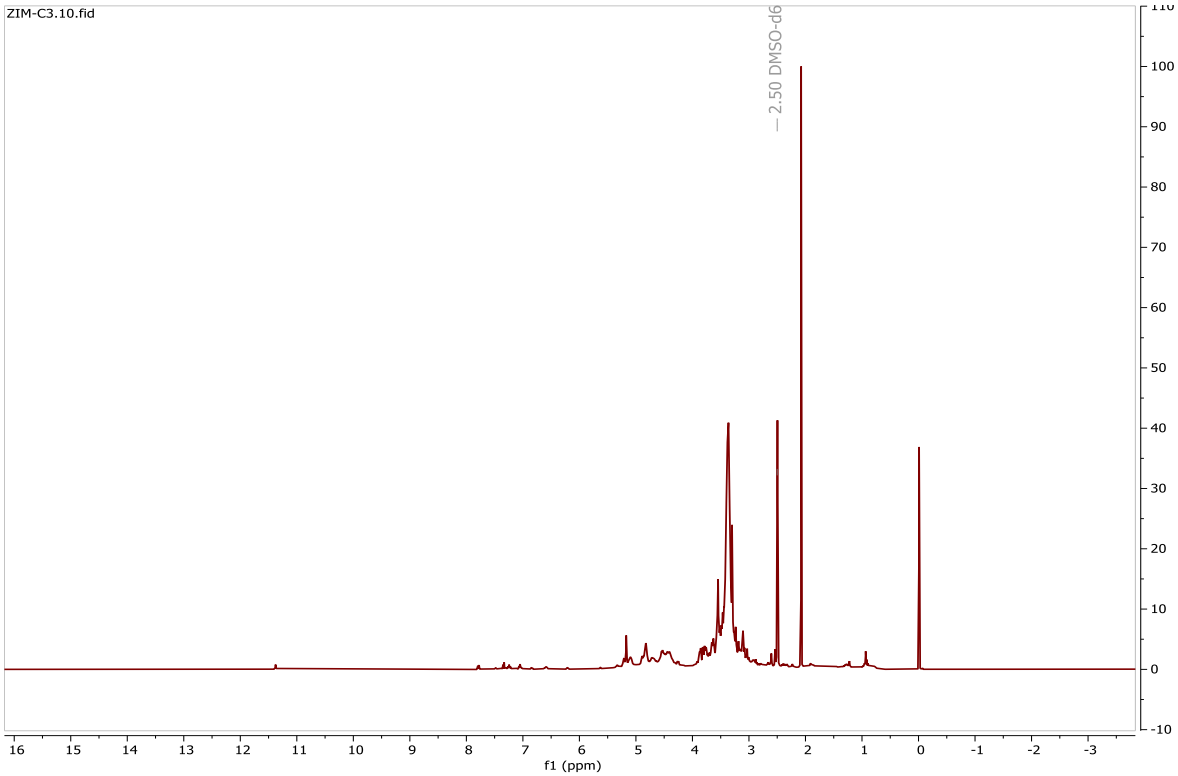



**Figure 6.1.13:** $^{1}$H NMR spectrum of methanol stem bark extract.

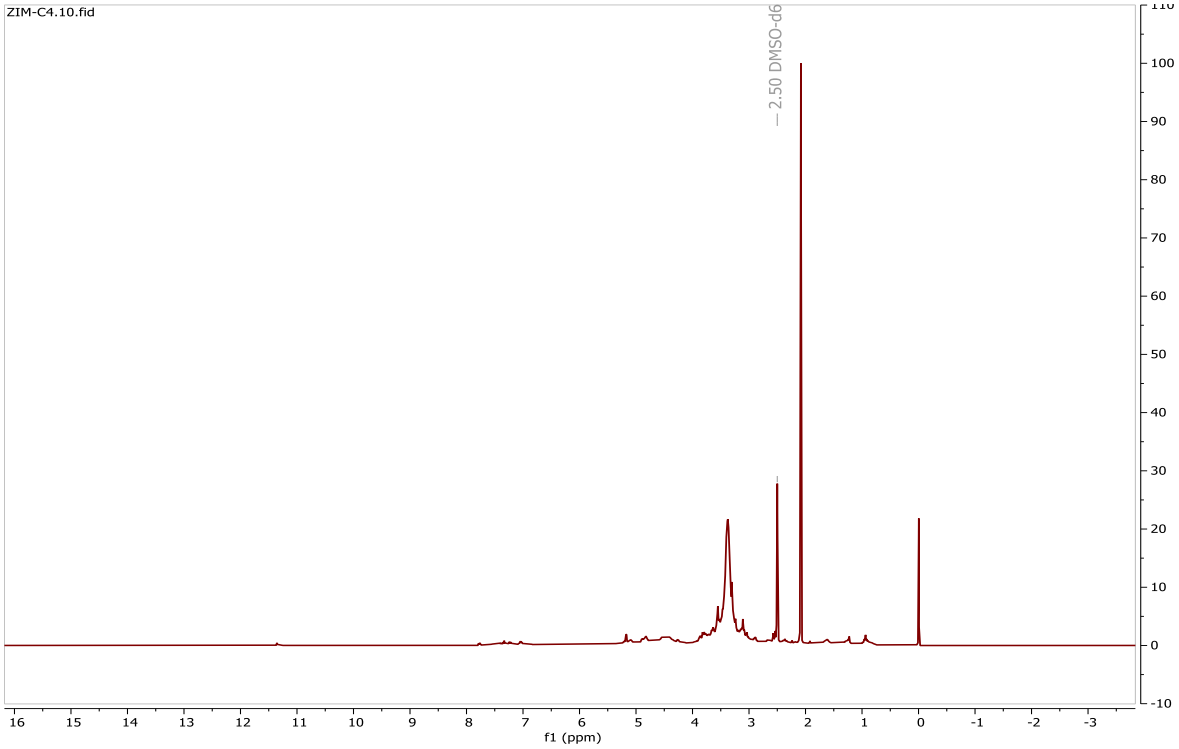



**Figure 6.1.14:** $^{1}$H NMR spectrum of water stem bark extract.

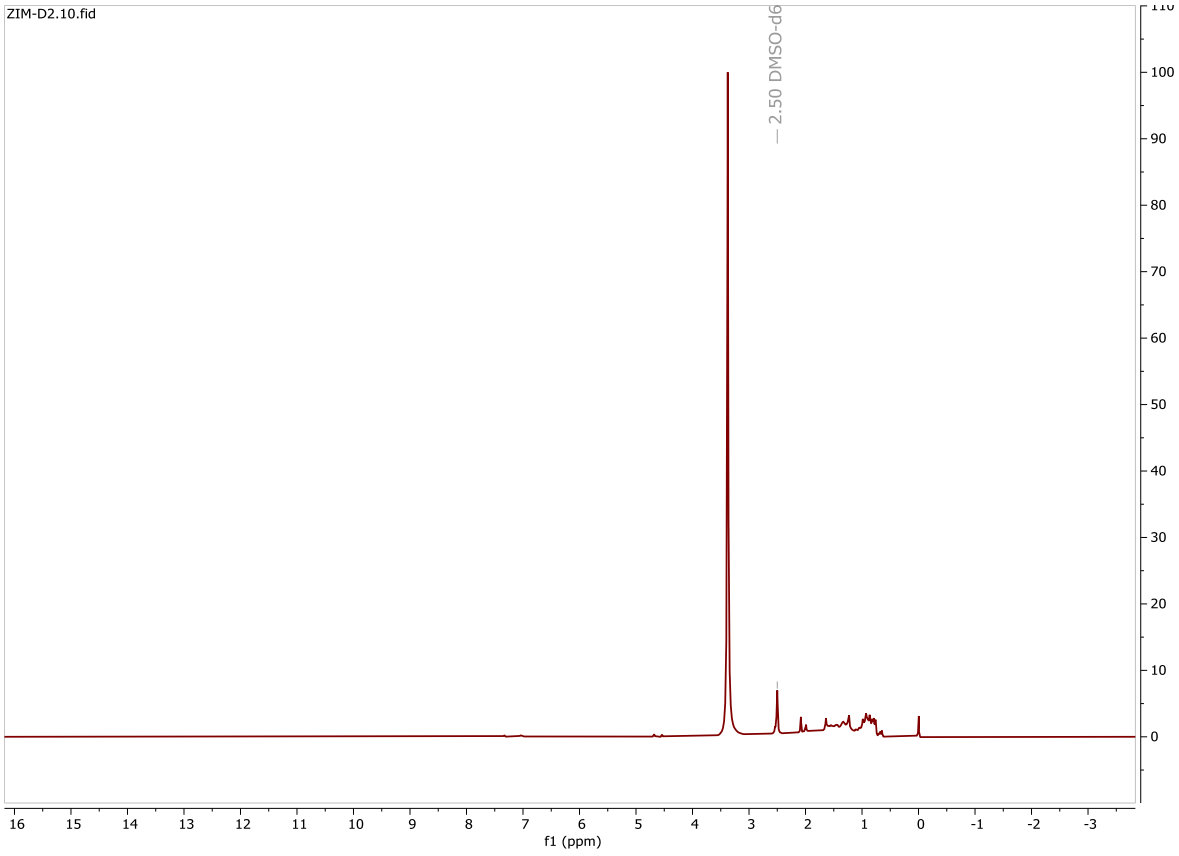



**Figure 6.1.15:** $^{1}$H NMR spectrum of dichloromethane root extract.

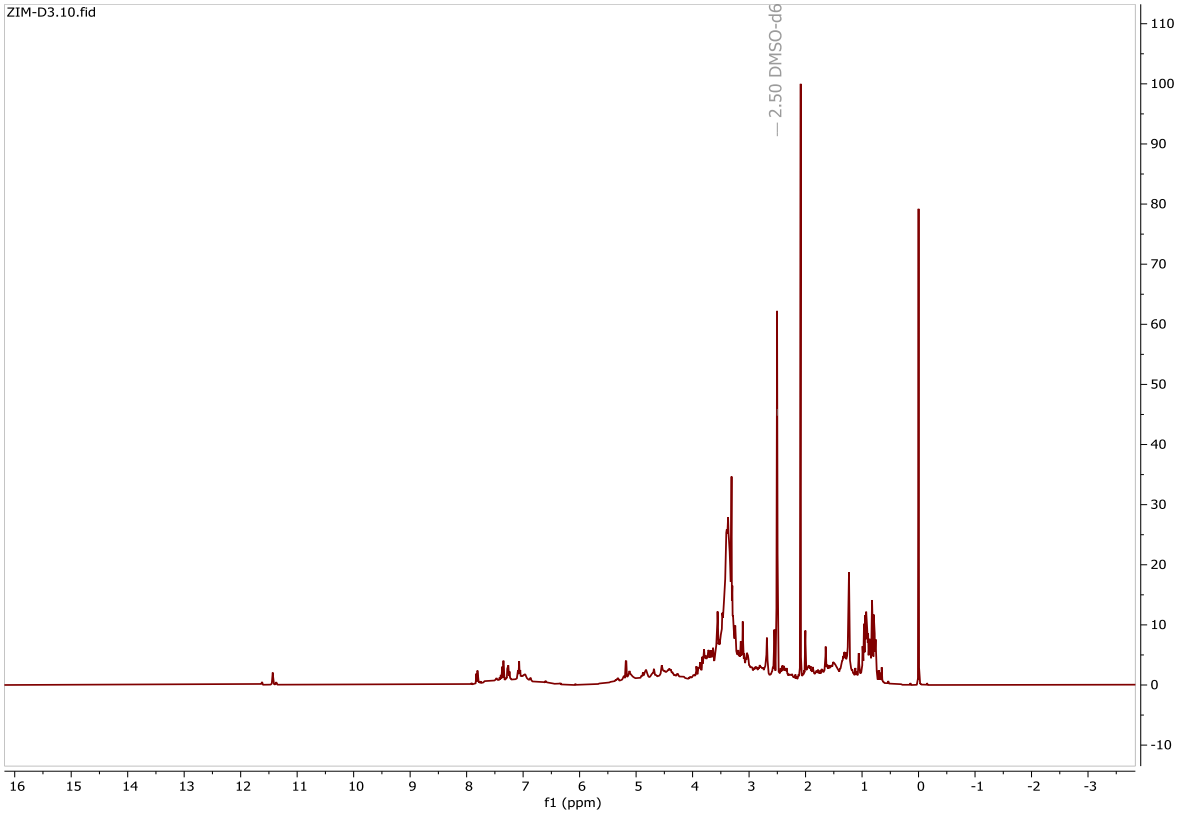



**Figure 6.1.16:** $^{1}$H NMR spectrum of methanol root extract.

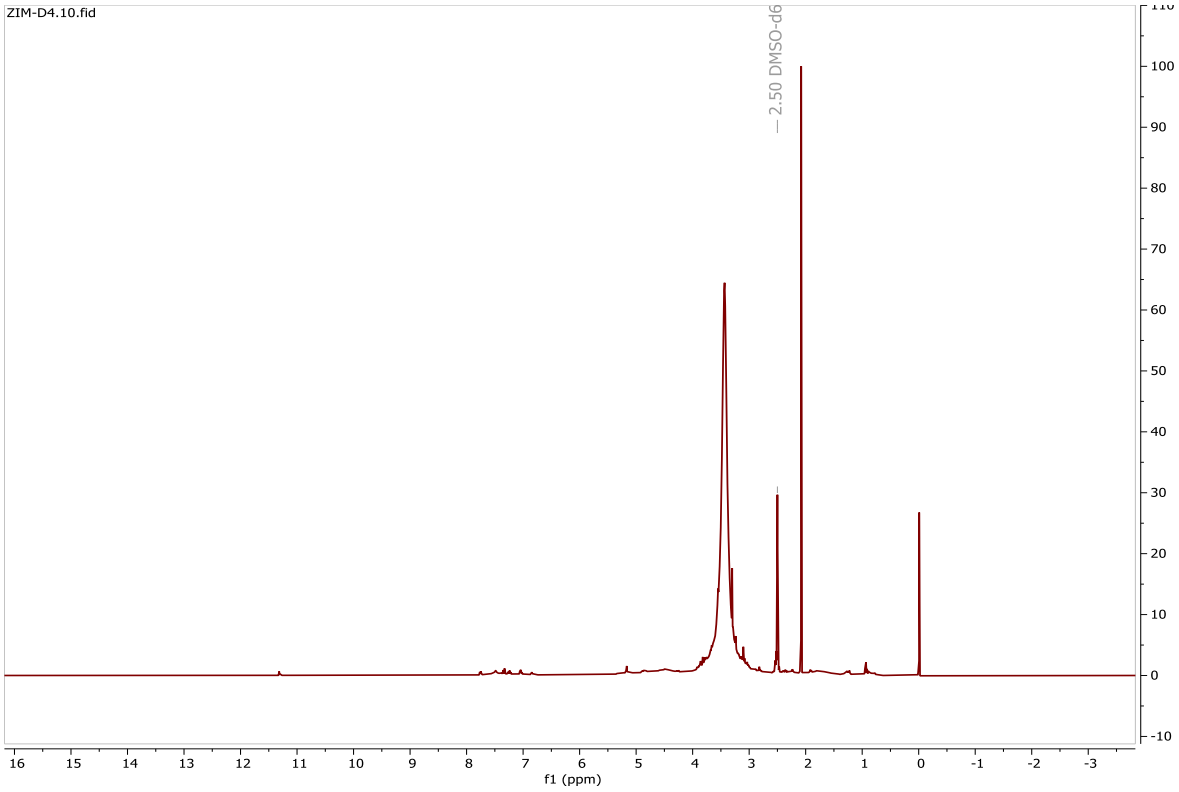



**Figure 6.1.17:** $^{1}$H NMR spectrum of water root extract.

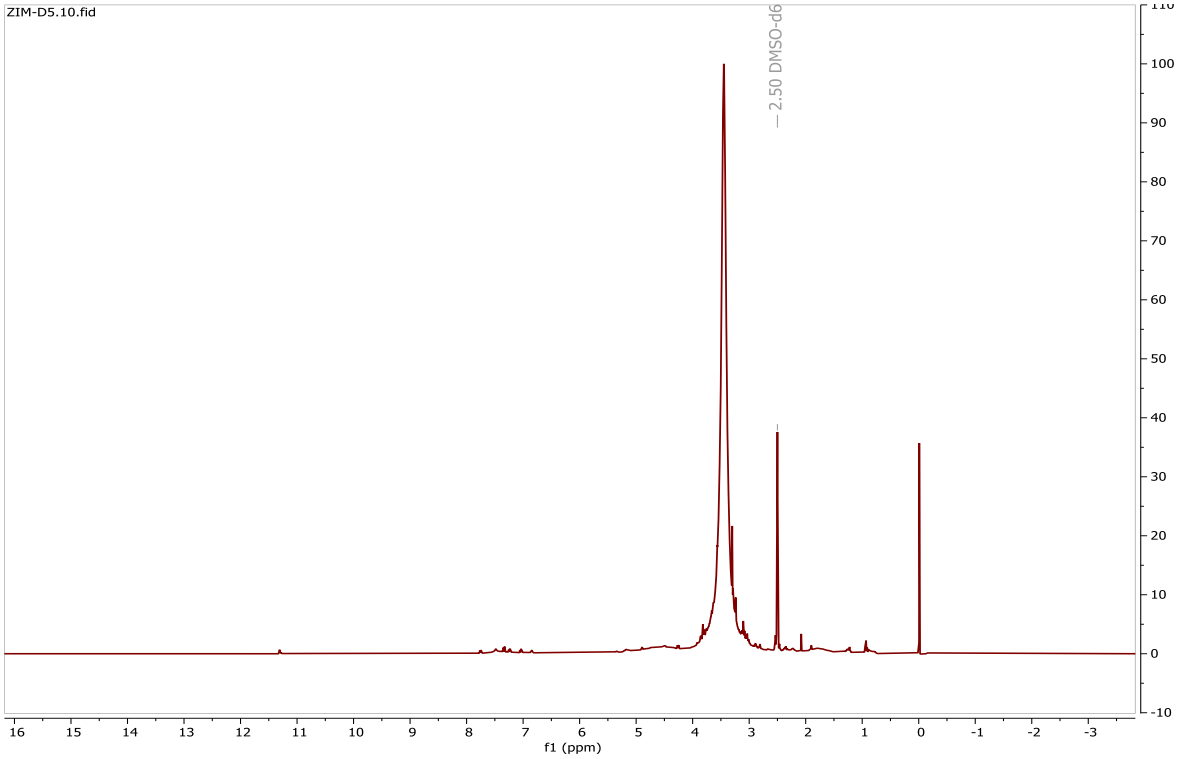



**Figure 6.1.18:** $^{1}$H NMR spectrum of decoction root extract.

## 6.2 NMR spectra of isolated compounds

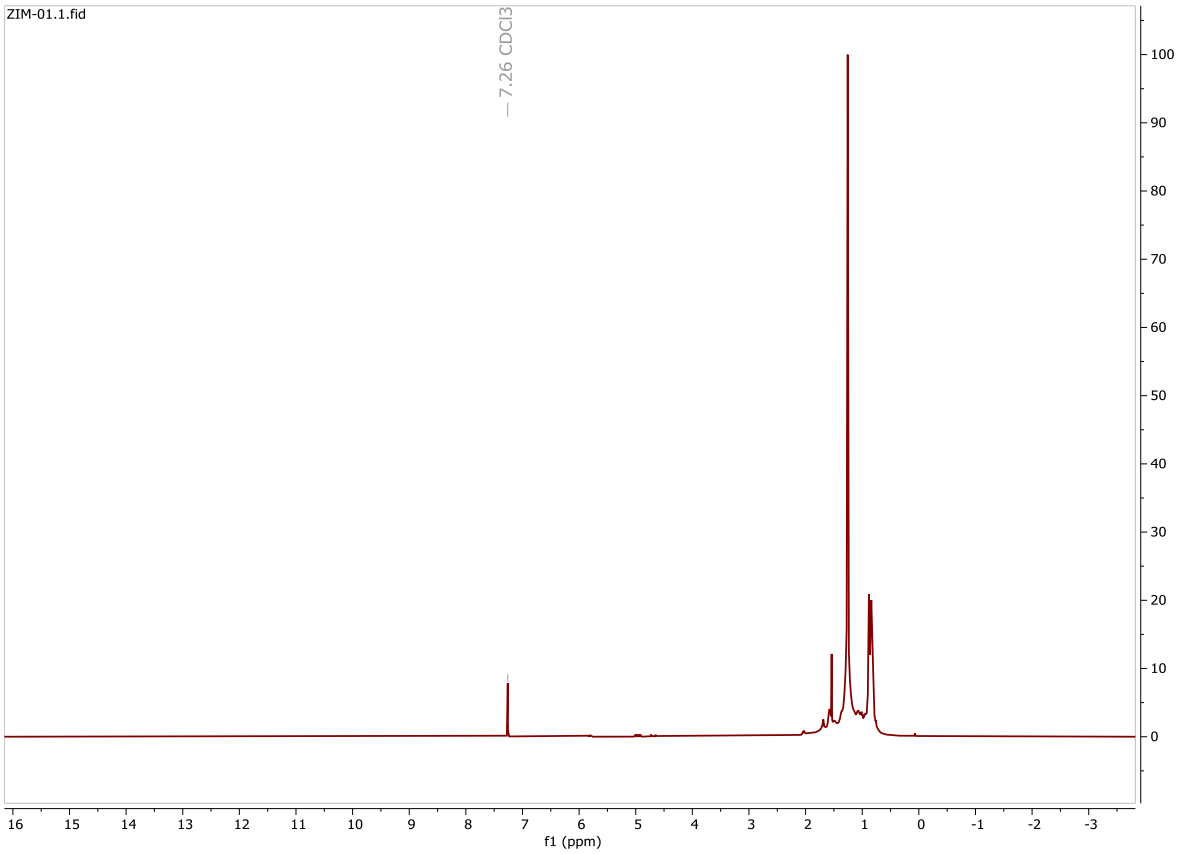



**Figure 6.2.1:** $^{1}$H NMR spectrum of compound **1**.

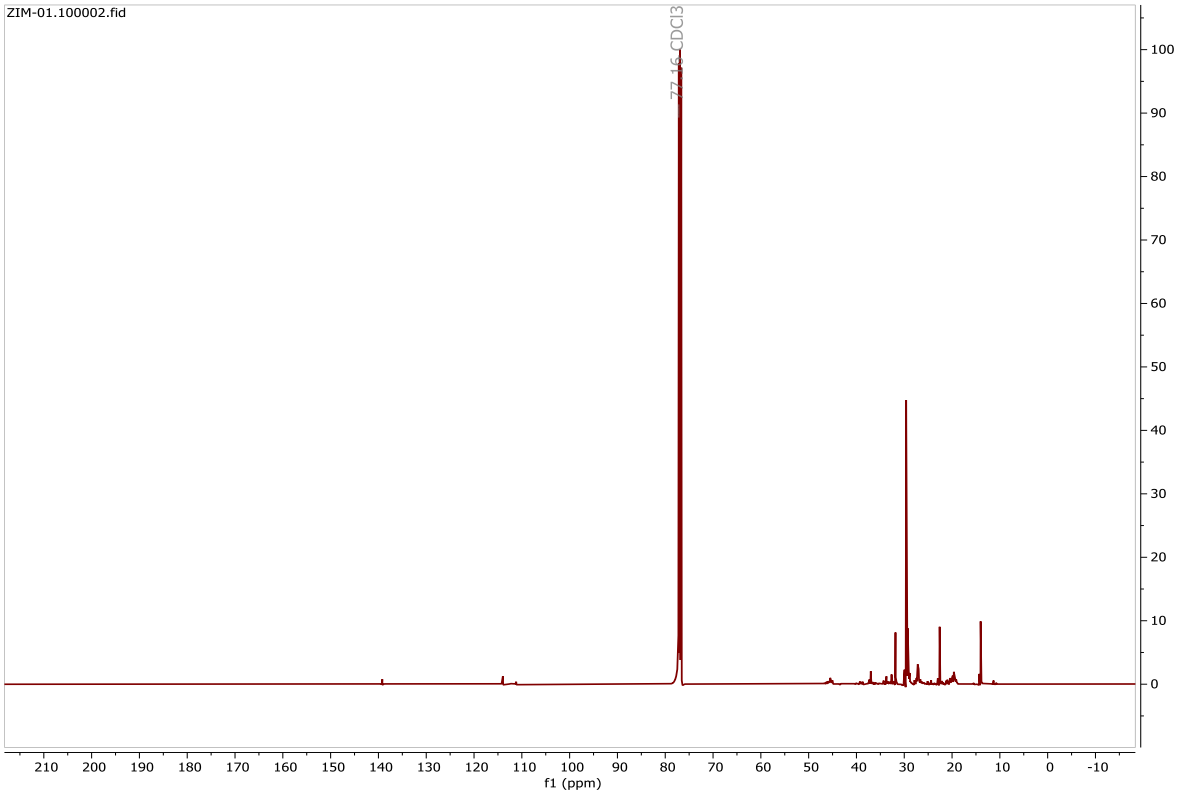



**Figure 6.2.2:** $^{13}C$ NMR spectrum of compound **1**.

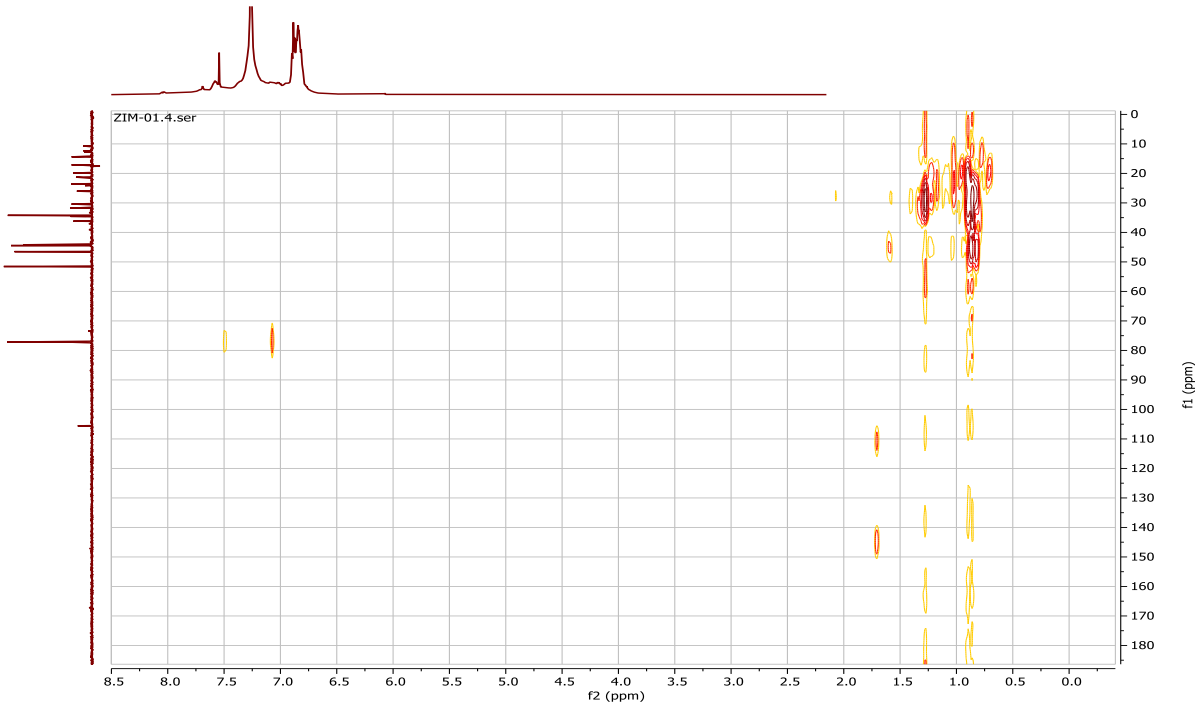



**Figure 6.2.3:** HMBC spectrum of compound **1**.

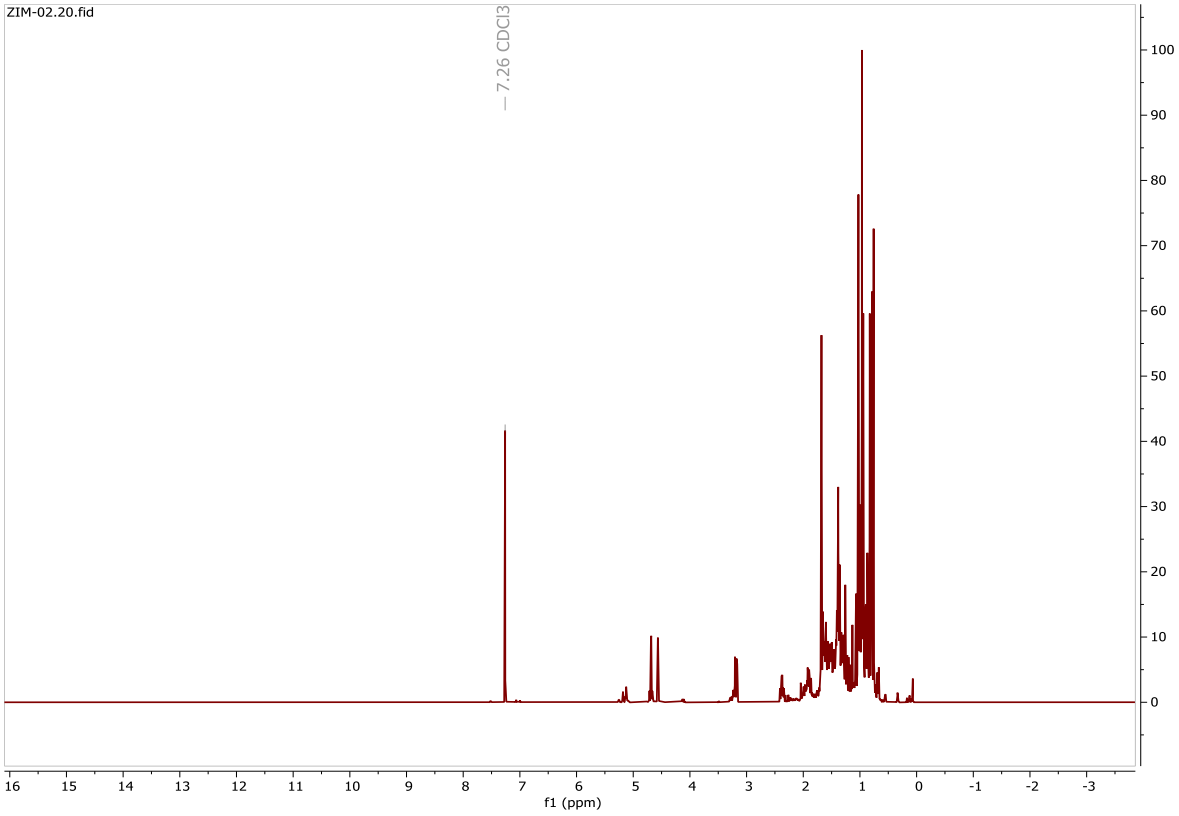



**Figure 6.2.4:** $^{1}$H NMR spectrum of compound **2**.

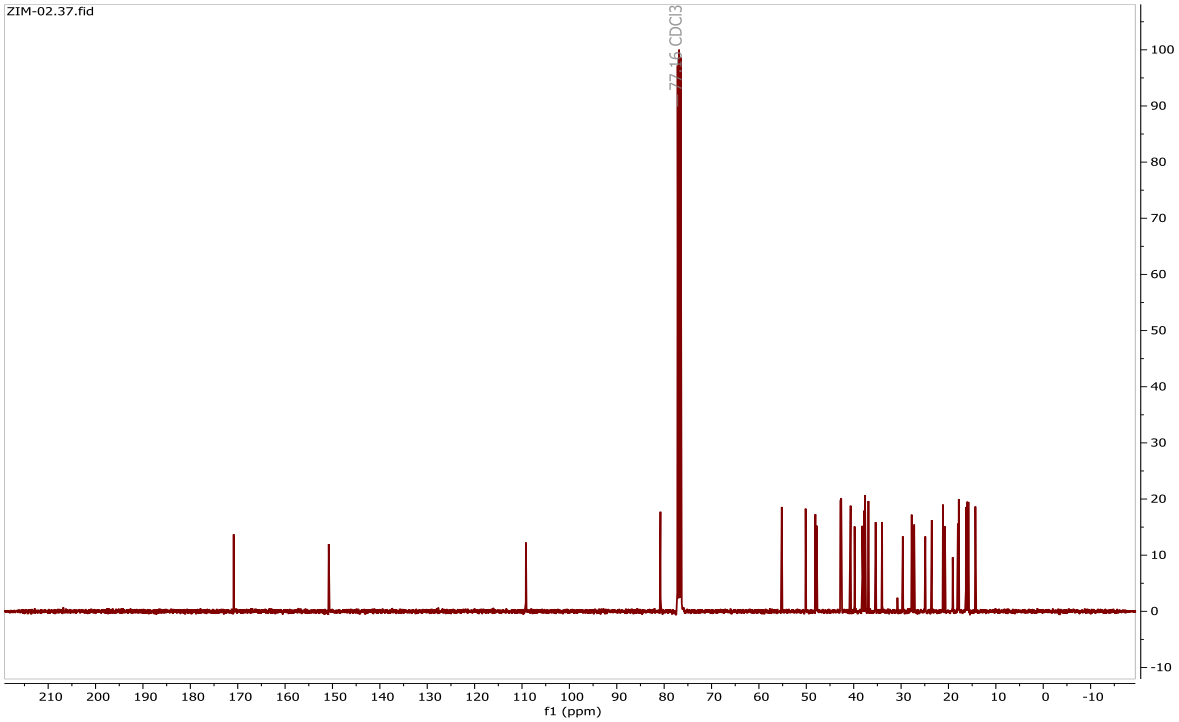



**Figure 6.2.5:** $^{13}$C NMR spectrum of compound **2**.

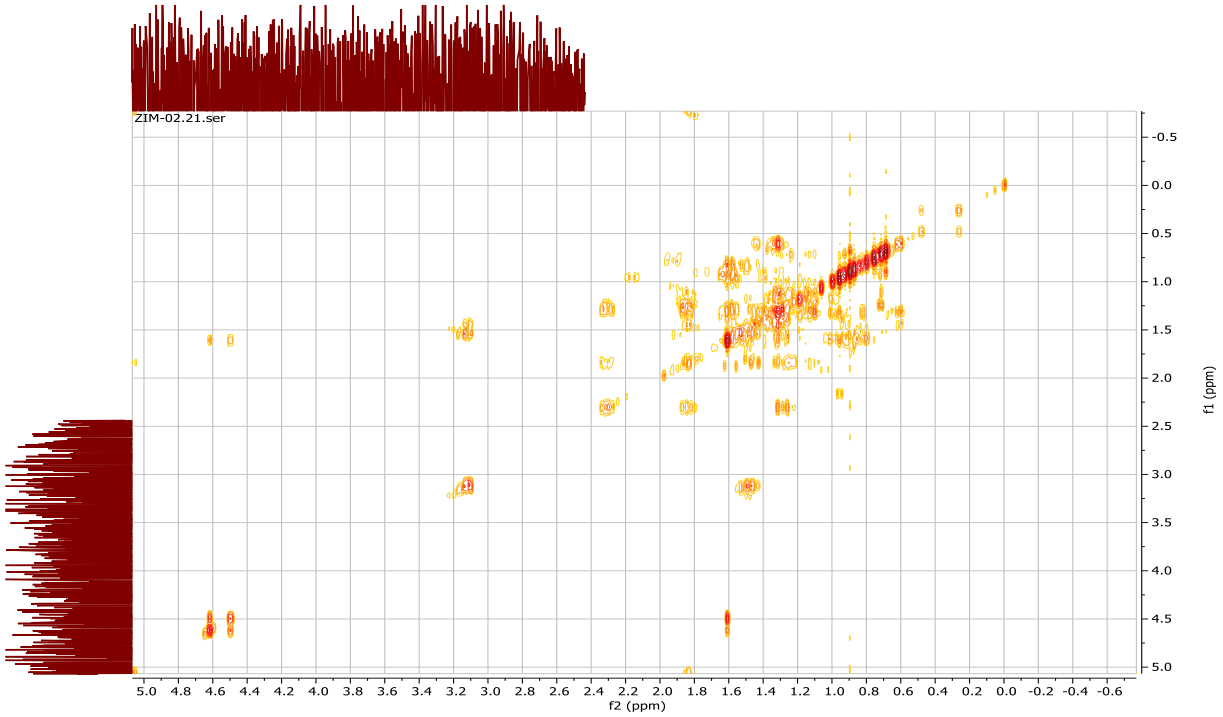



**Figure 6.2.6:** H-H COSY spectrum of compound **2**.

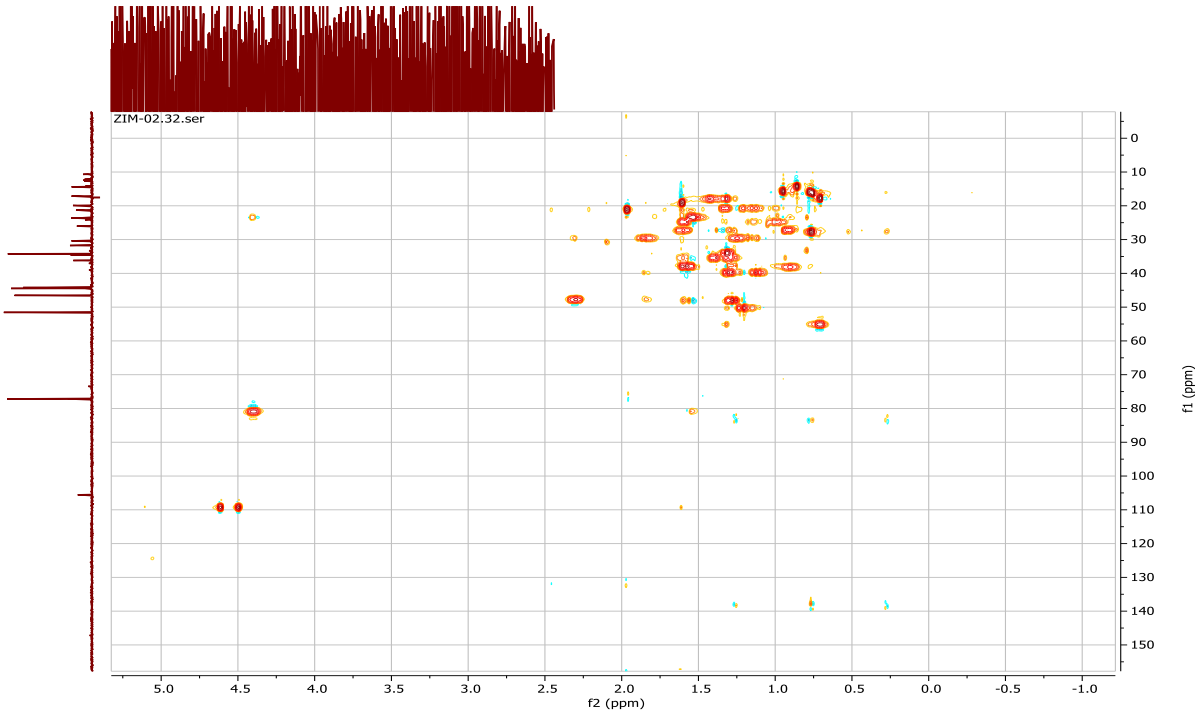



**Figure 6.2.7:** HMBC spectrum of compound **2**.

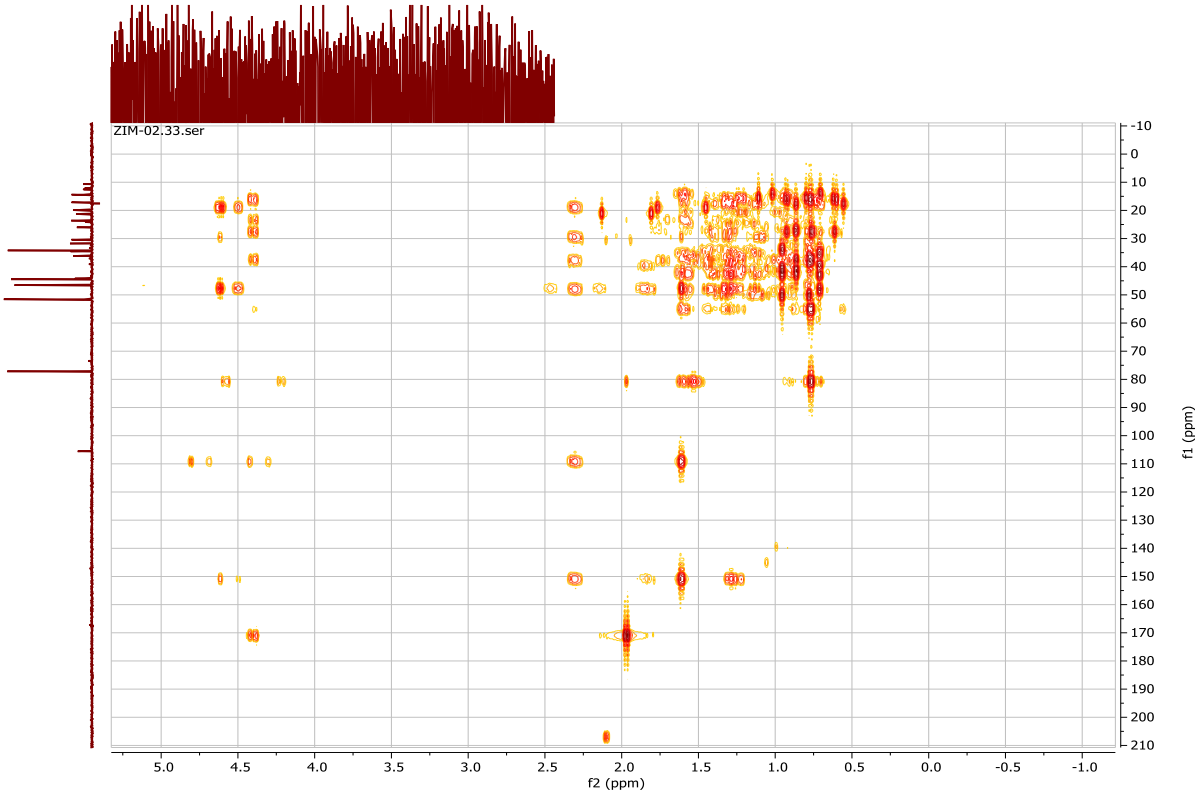



**Figure 6.2.8:** HSQC spectrum of compound **2**.

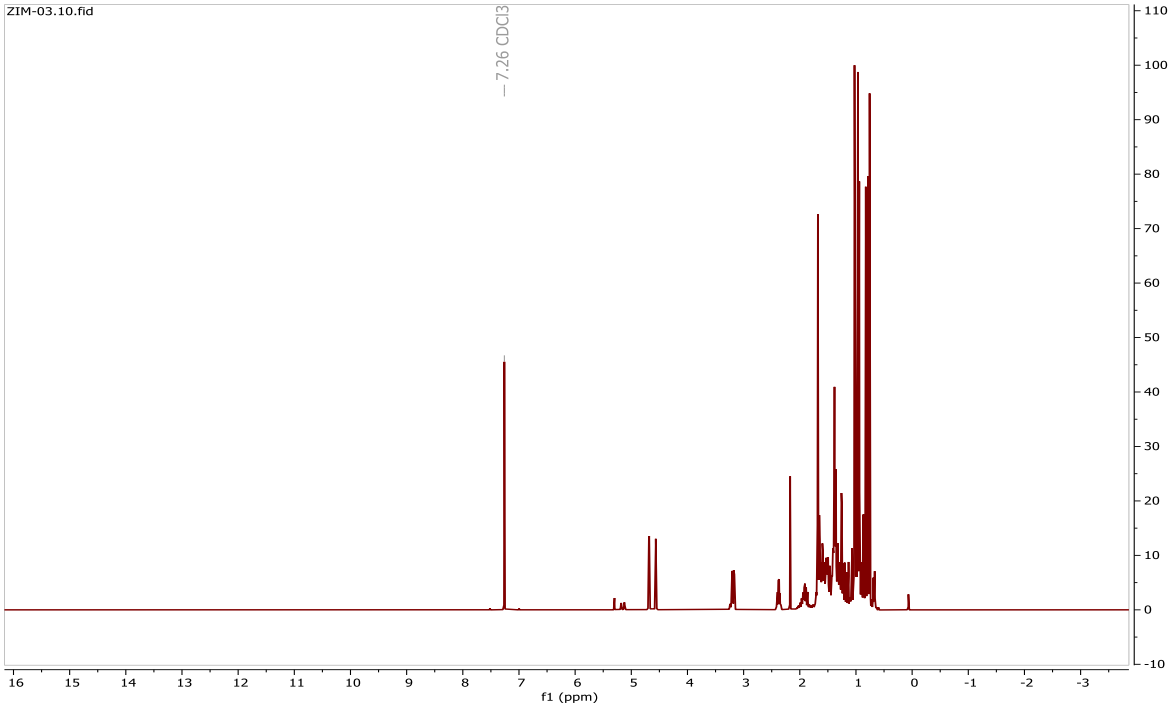



**Figure 6.2.9:** $^{1}$H NMR spectrum of compound **3**.

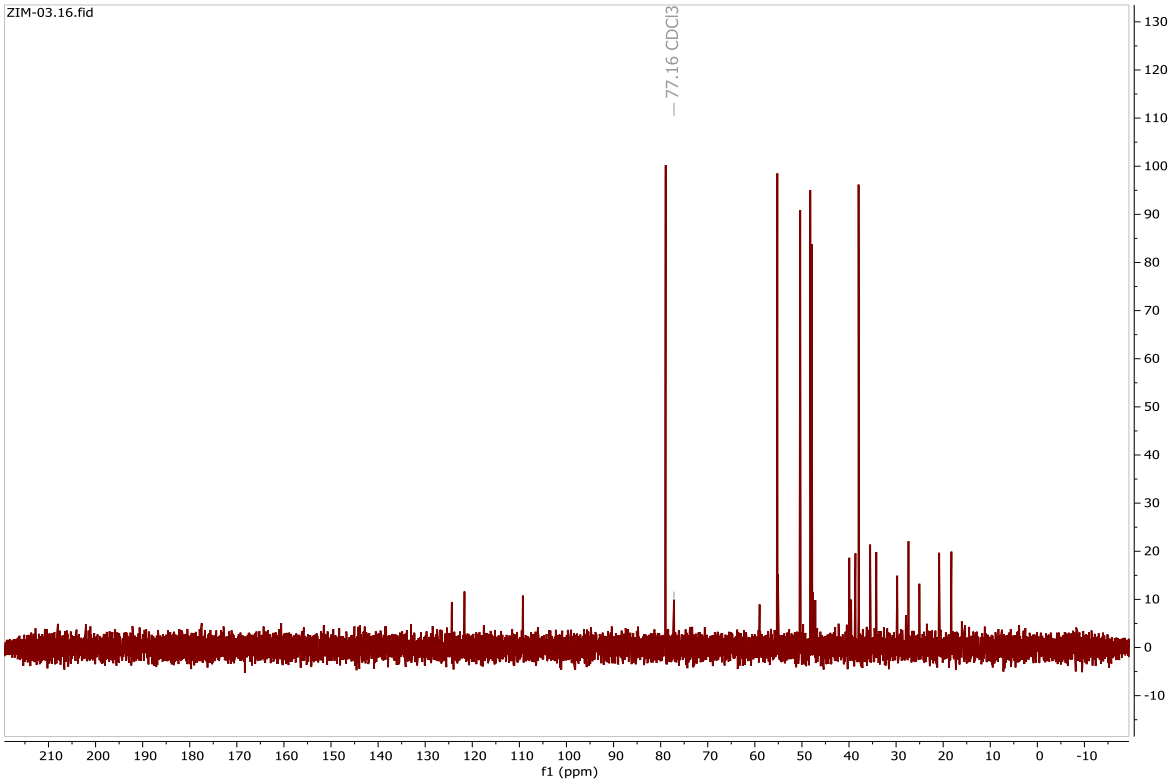



**Figure 6.2.10:** $^{13}$C NMR spectrum of compound **3**.

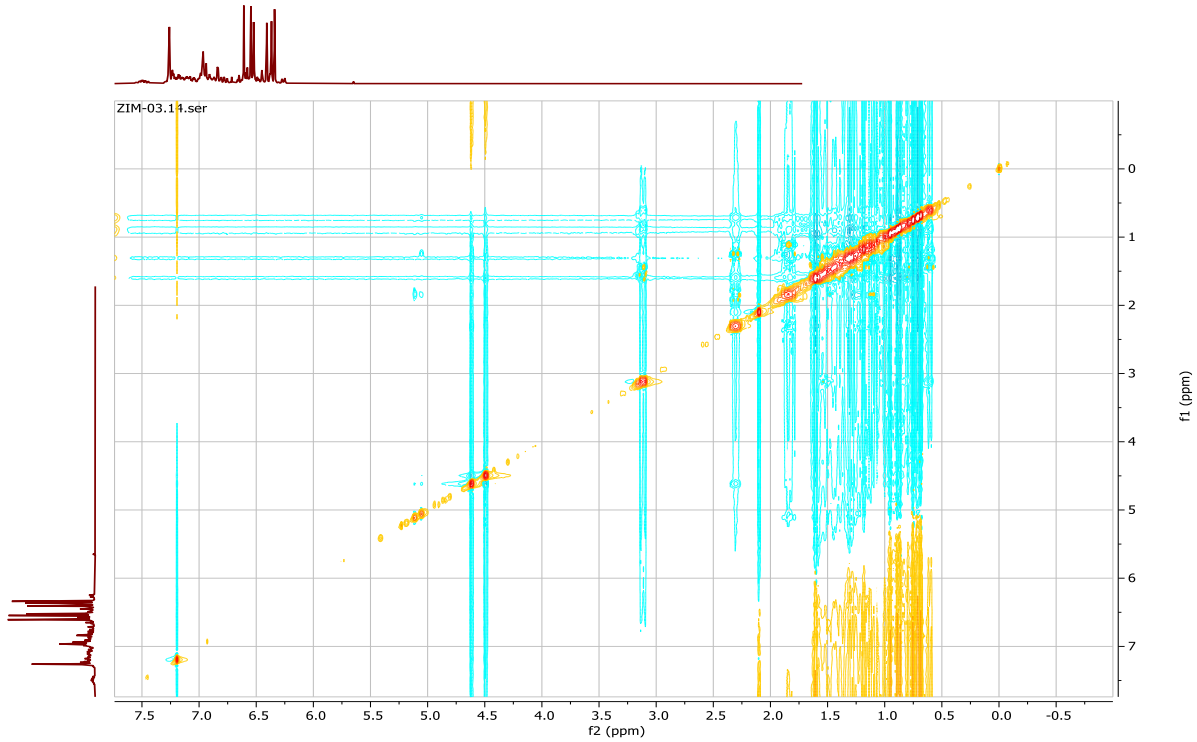



**Figure 6.2.11:** H-H COSY spectrum of compound **3**.

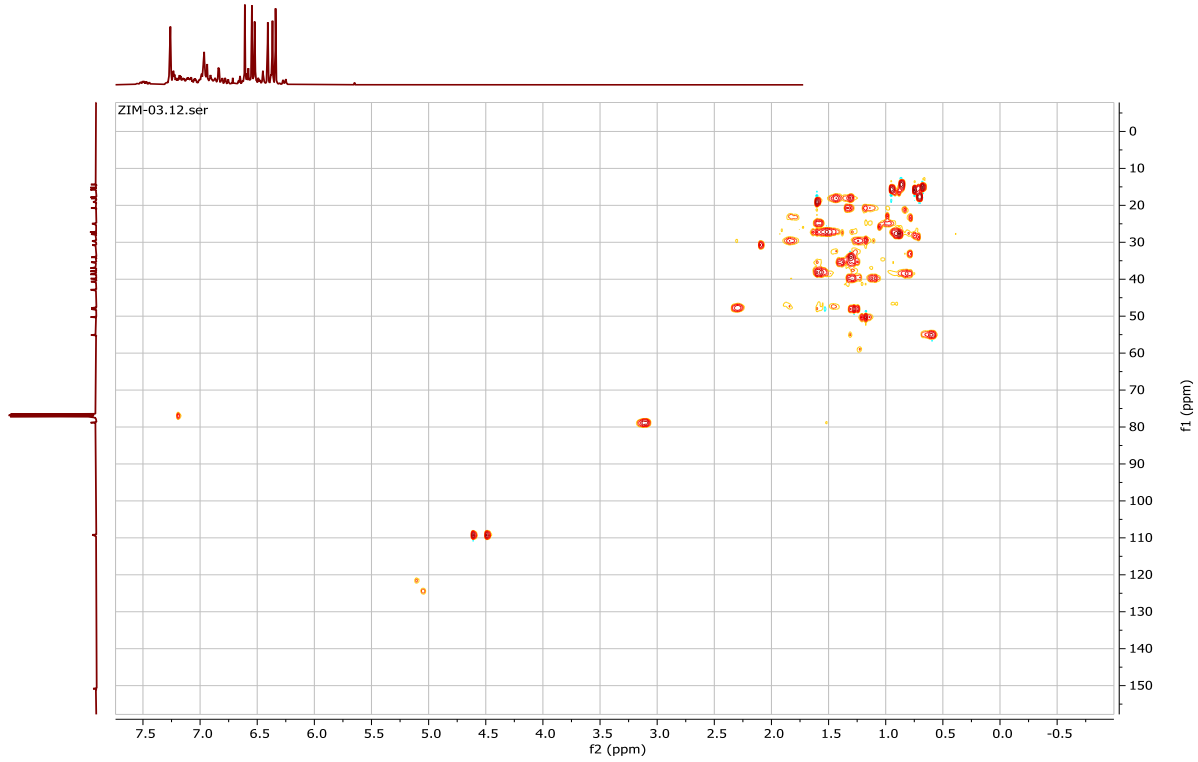



**Figure 6.2.12:** HMBC spectrum of compound **3**.

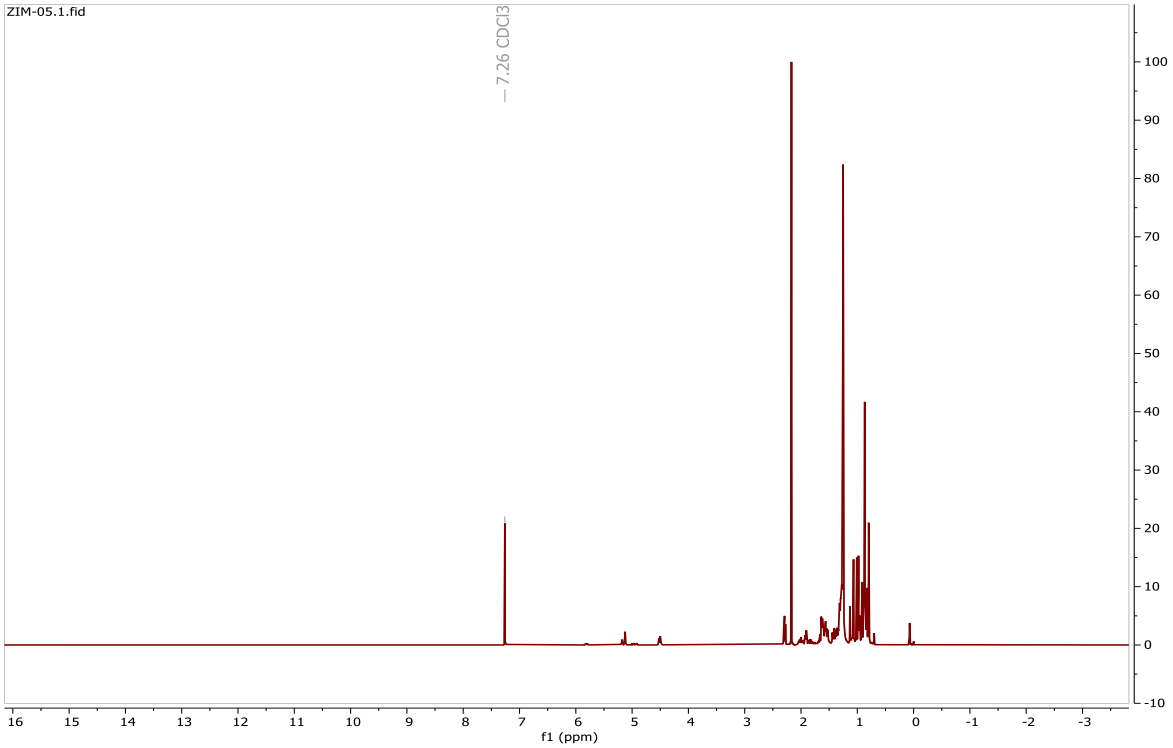



**Figure 6.2.13:** $^{1}$H NMR spectrum of compound **4**.

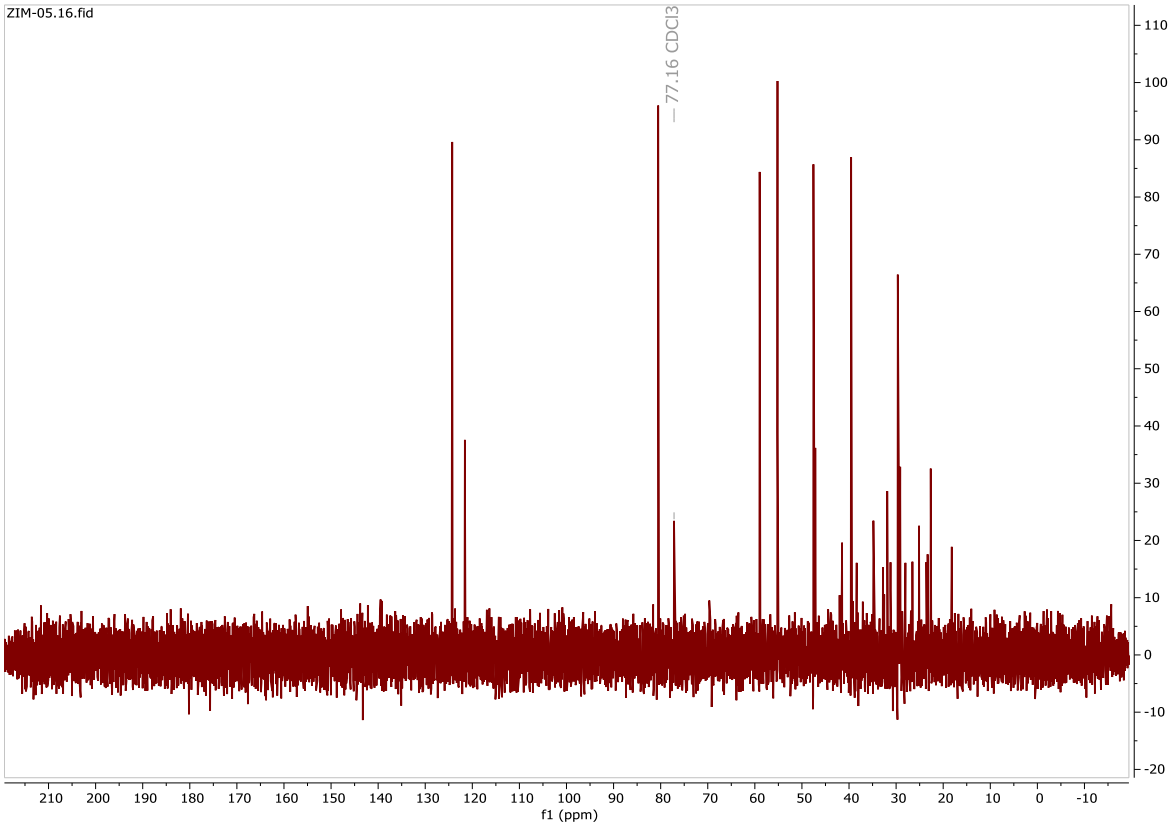



**Figure 6.2.14:** $^{13}$C NMR spectrum of compound **4**.

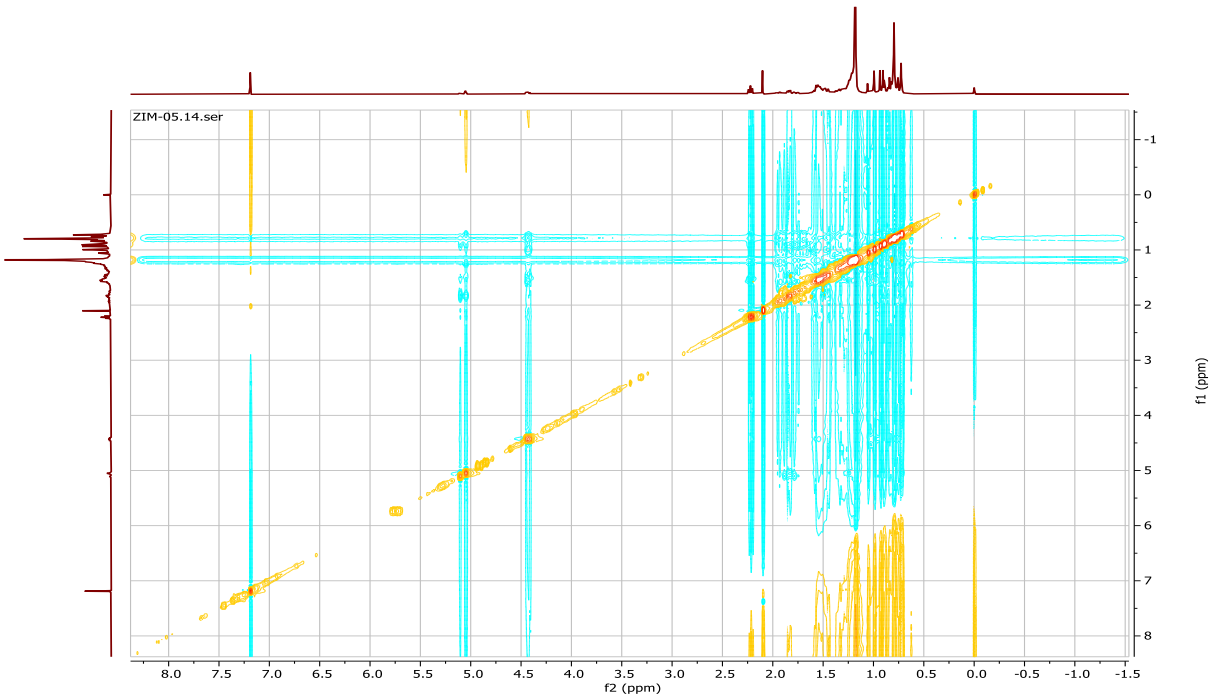



**Figure 6.2.15:** H-H COSY spectrum of compound **4**.


**Figure 6.2.16:** HMBC spectrum of compound **4**.