JIMMA UNIVERSITY

COLLEGE OF NATURAL SCIENCES

DEPARTMENT OF CHEMISTRY

PHYTOCHEMICAL INVESTIGATION OF THE STEM BARK OF *Dracaena steudneri* engl AND EVALUATION FOR ITS ANTIBACTERIAL ACTIVITY

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JIMMA, ETHIOPIA

PHYTOCHEMICAL INVESTIGATION OF THE STEM BARK OF *Dracaena steudneri* engl AND EVALUATION FOR ITS ANTIBACTERIAL ACTIVITY

BY: AYANA JALETA

A RESEARCH THESIS SUBMITTED TO THE SCHOOL OF GRADUATE STUDIES, JIMMA UNIVERSITY, IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE IN CHEMISTRY (ORGANIC)

ADVISORS: NEGERA ADDISSA (PhD)

TEMESGEN MITIKU (MSc)

April 27, 2023

JIMMA, ETHIOPIA

Declaration

I declare that this is my original work, except where the reference is made and has never been submitted anywhere for award of any degree in any university.

_____

Ayana Jaleta

This MSc thesis has been submitted for examination with our approval as supervisors

_____

Negera Abdissa (PhD)

Department of Chemistry

Jimma University

_____

Temesgen Mitiku (M.Sc)

Department of Chemistry

Jimma University

SCHOOL OF GRADUATE STUDIES

JIMMA UNIVERSITY

COLLEGE OF NATURAL SCIENCES

MSc THESIS APPROVAL SHEET

We, the undersigned, member of the Board of Examiners of the final open defense by **Ayana Jaleta** have read and evaluated his/her thesis entitled "**Phytochemical Investigation of the Stem bark of *Dracaena steudneri* Engl and Evaluation for its Antimicrobial**" and examined the candidate. This is therefore to certify that the thesis has been accepted in partial fulfillment of the requirements for the degree Master of Science in **Chemistry (Organic)**.

Mr. Mudin Jemal Name of the Chairperson	Signature	April 27, 2023 Date
Dr. Negera Abdissa Name of Major Advisor	Signature	April 27, 2023 Date
Dr. Tsegaye Girma Name of the Internal Examiner	Signature	April 27, 2023 Date
Dr. Bekele Lemma Name of the External Examiner	Signature	April 27, 2023 Date

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Abbreviations/Acronyms

AIDS/HIV ----------Human immunodeficiency virus/ acquired immunodeficiency syndrome

COSY----------Correlated Spectroscopy

1D NMR----------One-dimension nuclear magnetic resonance

2D NMR----------Two-dimension nuclear magnetic resonance

DMSO ----------Dimethyl sulfoxide

G----------Gram

HMBC----------Heteronuclear Multiple Bond Correlation

HSQC---------- Heteronuclear Single Quantum Correlation

IC_{50}----------Half-maximal inhibitory concentration

Mg----------Milligram

Rf----------Retardation factor

TB ----------Tuberculosis

TCM ----------Chinese medicine

TLC----------Thin-layer chromatography

XDR-TB ----------Extensive resistant tuberculosis

µM----------Micrometer

µg----------Microgram

WHO----------World Health Organization

Abstract

Medicinal plants are considered as a good resource for novel and effective pharmaceuticals. *Dracaena. steudneri* is widely used for cough treatment of labor, splenomegaly, hernia, asthma and related chest problems in children, fibroids and infertility in women. The stem bark of the same plant is used to treat tuberculosis in Kenyan and Ugandan. However, phytochemical and bioactivity information from the stem bark of this plant is limited. Therefore, the study was aimed at investigating phytochemicals from stem bark of *D. steudneri* and evaluating their antibacterial activities. The phytochemicals were extracted using maceration. The extract was subjected to column chromatography using solvent gradients. NMR techniques (1D & 2D NMR) were used for the structural elucidation of isolated compounds. Disk diffusion method was used to evaluate anti-bacterial activity of the crude extract and isolated compounds. Three compounds (**323**, **324** and **325**) were isolated from the stem bark of the study plant and they were elucidated as droserone (**323)**, 5-hydroxy-2, 3-dimethyl-7-methoxychromone **(324)** and ergosterol (**325)**. The crude (100 mg/ml) has shown marginal activity against the four test strains (*S. aureus, S. typhi, E. coli and B. cereus*), and the isolated compounds showed little antibacterial activity against the tested bacterial strains. Further study is required to search for antimicrobial compounds against bacterial and fungi strains. Moreover, the assessment of the relationship between *D. steudneri* species and fungi is also needed.

Key Words: Antibacterial activity, *Dracaena steudneri*, nuclear magnetic resonance, stem bark, structural elucidation

1. INTRODUCTION

1.1. Background

Medicinal plant consumption is a common practice for the treatment of both human and animals ailments since pre-historic time. Plants remain good sources of drugs that are being used in modern medicines. This can be either as starting materials for the partial synthesis of useful compounds or models for the synthesis of new drugs. Since over 60% of antimicrobial drugs are of natural origin, plants play a major role in drug discovery and development[1]. This is very important for the control of infectious diseases, where death burden due to pneumonia, influenza and tuberculosis alone caused 60.1% during the 20 centuries[2]. Natural products are of huge structural diversity and great biodiversity. This makes natural products an outstanding and challenging subject to drug discovery and development. In fact, the advent of phytochemistry and pharmaceutical chemistry has enhanced the ability to utilize active compounds isolated from the plants, or their synthetic equivalents in medicine. Antimalarial agents like quinine (1), artemisinin (2), caffeine (3), nicotine (4), codeine (5), atropine (6), colchicine (7), cocaine (8), and capsaicin (9) [3] are among the natural product -derived drugs[4](Figure **1**). This is a momentum that new lead compounds may emerge from plants, especially from those with recognized traditional uses.

In addition to plants and animals, microbes those live in mutual association with plants can also serve as source of natural products to serve either as drugs or as template for drug synthesis. Particularly, microbes (bacteria or fungi) that colonize inter- and/or intracellular locations of plants, called endophytes, are receiving more attention of researchers to be used as source of natural products or drugs nowadays[5]. They colonize different parts of the plants such as in the stem, roots, petioles, leaf segments, inflorescences of weeds, fruit, buds, seeds and dead and hollow hyaline cells of plants. The secondary metabolites released by these endophytes can enhance the growth of the plant and nutrient gain. They can also enable their hosts to tolerate harsh conditions of various type of biotic or abiotic stresses; also enhance their resistance to pests and their preys. They are known produce a number of bioactive metabolites in a single plant or microbe, which served as an excellent source of drugs with potential applications in agriculture, food, medicine and cosmetics industries[6].

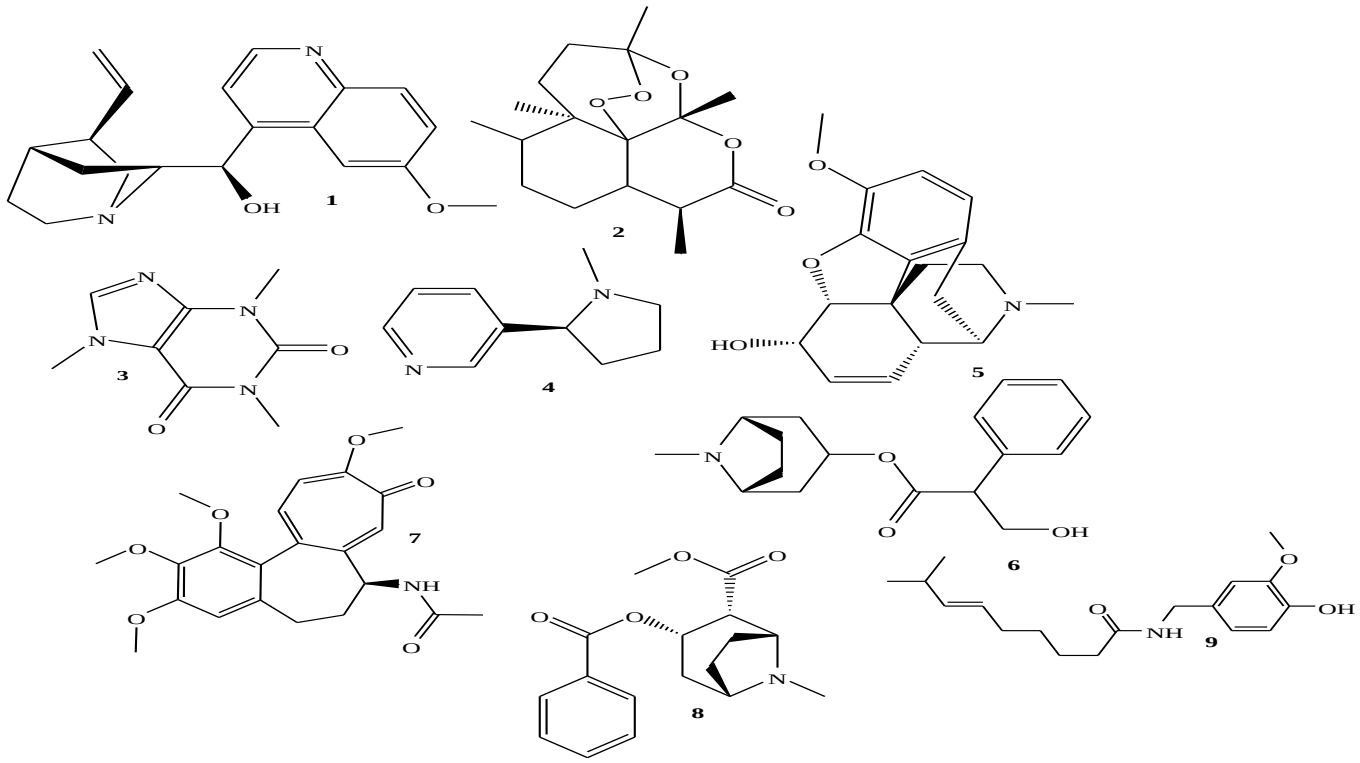


Figure 1: Examples of natural product derived drugs

Dracaena steudneri is one of the medicinal plants commonly used to treat infectious diseases like blackleg, rabies, for emergency (dingetegna), evil spirit, wound[7]. Its bark is used to treat Cryptococcal meningitis, Tuberculosis, Oral candidiasis in Tanzanian[8]. Despite the plant has a wider traditional use, it's phytochemical and bioactivity information is limited. Therefore, this study was aimed at investigating phytochemicals from stem bark of *D. steudneri* and evaluating their antibacterial activities

1.2. Statement of the problem

There is huge progress in understanding, preventing and treating diseases in the past century. However, the human and economic cost of infectious diseases was stuck somewhere between staggering and incalculable. As per a report by the Institute of Labor Economics[9], eight major

diseases causes namely HIV/AIDS, malaria, measles, hepatitis, dengue fever, rabies, tuberculosis and yellow fever) causes up to US $8 trillion costs and more than 156 million life years were lost for the year 2016 alone.

Our globe is facing antimicrobial resistance while antimicrobial discovery is hesitated by the manufacturers. This antimicrobial resistance has great economic impact as well life loss. Since the microbes are developing resistance to the existing antimicrobials, new antimicrobial discovery is urgently needed. As per WHO 2016 report, more than 7000 annual deaths are reported due to unsuccessful treatment of antimicrobial resistance. It is guessed that by 2050, about 10 million people may die from antimicrobial resistance if action is not taken and this will cost about 100 trillion US dollars[10]. Particularly, six microbes (bacteria) grouped as 'ESKAPE' are the very challenging and responsible for life threatening nosocomial infections. The 'ESKAPE' is an acronym for bacterial pathogens associated with multidrug resistance: *Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter spp*. These microbes develop resistance to one or more of the existing antimicrobials[11,12]. As a result, there is a paramount need for searching for new drugs to overcome these problems of our globe.

Dracaena steudneri is one of the *Dracaena* species used for different by purposes by people of different cultures. Its whole part is used to treat cough treatment while the stem bark is used to induce labor and to make painless labor. The decoction of the plant is used for the treatment of malaria and to ease delivery in Uganda and Tanzania respectively[13]. The stem bark of *D. steudneri* is used to treat tuberculosis by Kenyan and Ugandan people[14]. In Ethiopian folk of medicine, *D. steudneri* is claimed to be used for the treatment of blackleg, rabies, for emergency (dingetegna), evil spirit, wound[7]. Its bark is used to treat cryptococcal meningitis, Tuberculosis, Oral candidiasis in Tanzanian folk medicine[8]. From the seeds of this plant, eight compounds were isolated and elucidated and showed significant cytotoxicity against the leukemia cell lines with IC_{50} less than 10 µM[15].

Up to the best of our knowledge, there is no study conducted on the *D. steudneri* for its antibacterial and phytochemical investigation of its stem bark. Therefore, the present study

aimed at phytochemical investigation and evaluation of antibacterial activities of *D. steudneri* stem bark.

1.3. Objective of the study

1.3.1. General objective

- To identify phytochemicals from the stem bark extract of *D. steudneri* and evaluate their antibacterial activity.

1.3.2. Specific objectives

- To extract secondary metabolites from the stem bark of *D. steudneri* using methanol/dichloromethane (1:1) solvents,
- To isolate and purify secondary metabolites from the stem bark extract of *D. steudneri* using chromatographic techniques
- To elucidate the structures of the isolated compounds using ^{1}H NMR, ^{13}C NMR, COSY, HSQC, HMBC and NOESY spectroscopic techniques,
- To evaluate antibacterial activity of the crude extract and isolated compounds against selected bacteria: *Escherichia coli*, *Staphylococcus aureus*, *Bacillus cereus* and *Salmonella typhi* strains.

1.4. Significance of the study

This study contributes to the existing body of knowledge by generating current information on the phytochemistry of *D. steudneri* and its biological activities against bacteria. It also generates information that can be used by scientific communities for further studies on the *D. steudneri* and other related *Dracaena* species. This also has a profound contribution for the community since the study may prove or disprove the traditional claim of the medicinal plant. It also paves the way for scientific formulation where the claim is confirmed scientifically.

2. REVIEW OF RELATED LITERATURE

2.1. Infectious Diseases

Infectious diseases are caused by organisms that are unseen by our naked eyes but can be detected by microscopes. In addition to being a leading cause of death of infectious diseases worldwide, the increase of antibiotic resistance has become the concern of our globe today [16]. There is also a major global health problem attributable to diseases, such as tuberculosis (TB), whose treatment became complicated due to drug resistances developed to existing drugs. Mycobacterial drug resistance is coupled with the persistent nature of mycobacteria. This drug resistance problem highlights the need of developing new antibiotics for infectious diseases including tuberculosis. Tuberculosis treatment is a lengthy therapy, which takes at least six months and the drugs exhibits toxicity. This creates compliance or adherence problems in the patients. This is a cause for drug resistance in TB patients and results in deadly MDRTB bacteria and extensive resistant tuberculosis (XDR-TB). Such drugs should be active against drug-resistant bacteria as well as against persistent bacteria and shorten the duration of treatment [17]. This study aims at phytochemical investigation and evaluation of the antibacterial activity of *D. steudneri*.

2.2. Botanical Information

Dracaenas' are one of the foliage plants that come from the Agavaceae (Liliaceae) family. Different species of Dracaena are distributed in the tropic and subtropics regions of the world [18]. There are more than 60 Dracaena species, which are mainly found in tropical and subtropical parts of Africa. Dracaena species are utilized for different purposes. Either for instance, Dracaena species such as *Dracaena loureiri, Dracaena augustifolia, Dracaena fragrans, Dracaena marginate* and *Dracaena deremensis* are used for medicinal purposes or as ornaments as indoor plants. Some species are used as a diet for animals. Among them few are *Dracaena cinnabari, Dracaena afromontana, Dracaena ombet, dracaena fragrans, Dracaena ellenbeckiana, Dracaena laxxisima, Dracaena cochincheinensis, Dracaena manni, Dracaena arboria* and many others [19].

Many species of genus *Dracaena* are used in human folk medicine to treat several diseases mainly infectious diseases [20]. Researchers have characterized secondary metabolites of some *Dracaena* species and found flavanones and flavans [21], chalcones and dihy-drochalcones [22-23],

homoisoflavonoids [24][25][26], polymeric flavonoids [27], steroids and steroidal saponins [28], lignans [29] and phenolic amides [30]. These classes of compounds are known to exhibit anti-inflammatory[31], antimicrobial[32], and cytotoxic [33] activities. *Dracaena* is also a source of flavonoids and homoisoflavonoids[34] a class of compounds which are classified into five subclasses based on their basic nucleus structure[34]. They are known for their wide range of biological activities such as anti-diabetic, anti-inflammatory, anti-microbial, anti-viral, hypocholesterolemic, anti-mutagenic, anti-estrogenic, anti-oxidant and cytotoxic activities[35]. Another class of compounds characterized from the *D. usambarensis* is Retro-di-hydrochalcones. They are flavonoids characterized from the root of this plant species. They differ by their phloroglucinol-like oxygenation pattern on ring A of flavonoids[36]. They are flavonoids formed through carbonyl transposition of the corresponding normal chalcones, so that ring A and C3-unit are derived from cinnamoyl CoA and ring B, from the acetate malonate pathway[36]. They are also known of their interesting biological activities. For example, the study by Risinger et al. (2013) revealed the cytotoxic potency of taccabulin A, a retro-dihydrochalcone with the ability to overcome resistance mechanisms mediated by ATP-dependent efflux transporters and βIII-tubulin[37]

2.3. Ethnomedicinal uses of *Dracaena*

2.3.1. The Genus Dracaena

People of different cultures use the species among the Dracaena Genus for different purposes. For instance, *D. afromontana* and *D. steudneri* are used to make field boundaries and graves and for ornamental purposes in Rwanda[38]. The roots and bark of *D. afromontana* are variably used as medicine for chest pains and rheumatism treatment in East African countries like Rwanda, Uganda, Tanzania and Burundi. The whole part *of D. steudneri* is used in Uganda for cough treatment while the stem bark is used to induce labor and to make painless labor. In addition, its leaves are used to treat splenomegaly, hernia, asthma and related chest problems in children, fibroids and infertility in women. The decoction of the plant is used for the treatment of malaria and to ease delivery in Uganda and Tanzania respectively[39], [40]. Boiled roots of *D. fragrans* (L.) are taken with a glass of water to increase the level of CD4 in HIV/AIDS patients in Tanzania [14]. The stem bark of *D. steudneri* is used to treat tuberculosis by the Kenyan and Ugandan people[41]. In Ethiopian folk of medicine, *D. steudneri* is claimed to be used for the treatment of blackleg, rabies, for emergency (dingetegna), evil spirit, wound[7]. Its bark is used to treat cryptococcal

meningitis, Tuberculosis, Oral candidiasis in Tanzanian folk medicine[8]. In folk medicine of Yemeni, Dracaena is used for the treatment of treating dysentery, diarrhea, hemorrhage and external ulcers. It was also used by early Romans and Arabs to treat different ailments such as diarrhea, wounds, dysentery diseases, ulcers of mouth, throat, intestines, fevers and stomach, respiratory disease and eczema [42]. *D. cinnabari* also called Dragon's blood; a red resin extract has many applications such as coloring and local medicinal uses in Arabs. It is used to treat different ailments of Dermal and dental, eye, internal and external bleeding, and gastrointestinal tract [43]. The species has also non-medicinal uses such as cosmetic and dye uses, serving as food for animals; women use it as lipstick, nail varnish and face smoothening and softening agent.

2.3.2. Ethnomedicinal uses of *Dracaena steudneri*

Dracaena steudneri is one of the *Dracaena* species used for different by purposes by people of different cultures. For example, it is used to make field boundaries and graves and for ornamental purposes in Rwanda [44],. The whole part *of D. steudneri* is used in Uganda for cough treatment while the stem bark is used to induce labor and to make painless labor. In addition, its leaves are used to treat splenomegaly, hernia, asthma and related chest problems in children, fibroids and infertility in women. The decoction of the plant is used for the treatment of malaria and to ease delivery in Uganda and Tanzania respectively[13]. The stem bark of *D. steudneri* is used to treat tuberculosis by Kenyan and Ugandan people [14]. In Ethiopian folk of medicine, *D. steudneri* is claimed to be used for the treatment of blackleg, rabies, for emergency (dingetegna), evil spirit, wound [7]. Its bark is used to treat Cryptococcal meningitis, Tuberculosis, Oral candidiasis in Tanzanian folk medicine[8]. From the seeds of this plant, eight compounds were isolated and elucidated. Two of them(188,190) showed significant cytotoxicity against the leukemia cell lines with IC_{50} less than 10 µM [15](Figure11).

Figure 2: Picture of *D. steudneri Engl* taken during from study area

2.4. Phytochemistry of Genus *Dracaena*

Intense investigations have been conducted on some *Dracaena* species of different regions of the world. These include phytochemical screenings, structural elucidations of isolated compounds and evaluation of their biological activities. More investigations have been conducted on species such as *D. cinnabari*, *D. cochinchinensis* red resin, *D. Cambodiana*, *D. draco L.* These studies revealed the presence of compound classes such as saponin, flavonoids, tannins, quinone, terpene, glacial glucosides, and phenols. For instance, flavonoids are the major compounds found in constituents of dragon's blood like *D. colchinchinensis*[45]. Chalcones and dihydrochalcones are dominant flavonoid compounds in the dragon's blood in addition to phenolic constituents while few flavanones and flavans in *D. cochinchinensis* are found in *D. colchinchinensis (*look at the following structures of compounds illustrated on figures 3-11)[45].

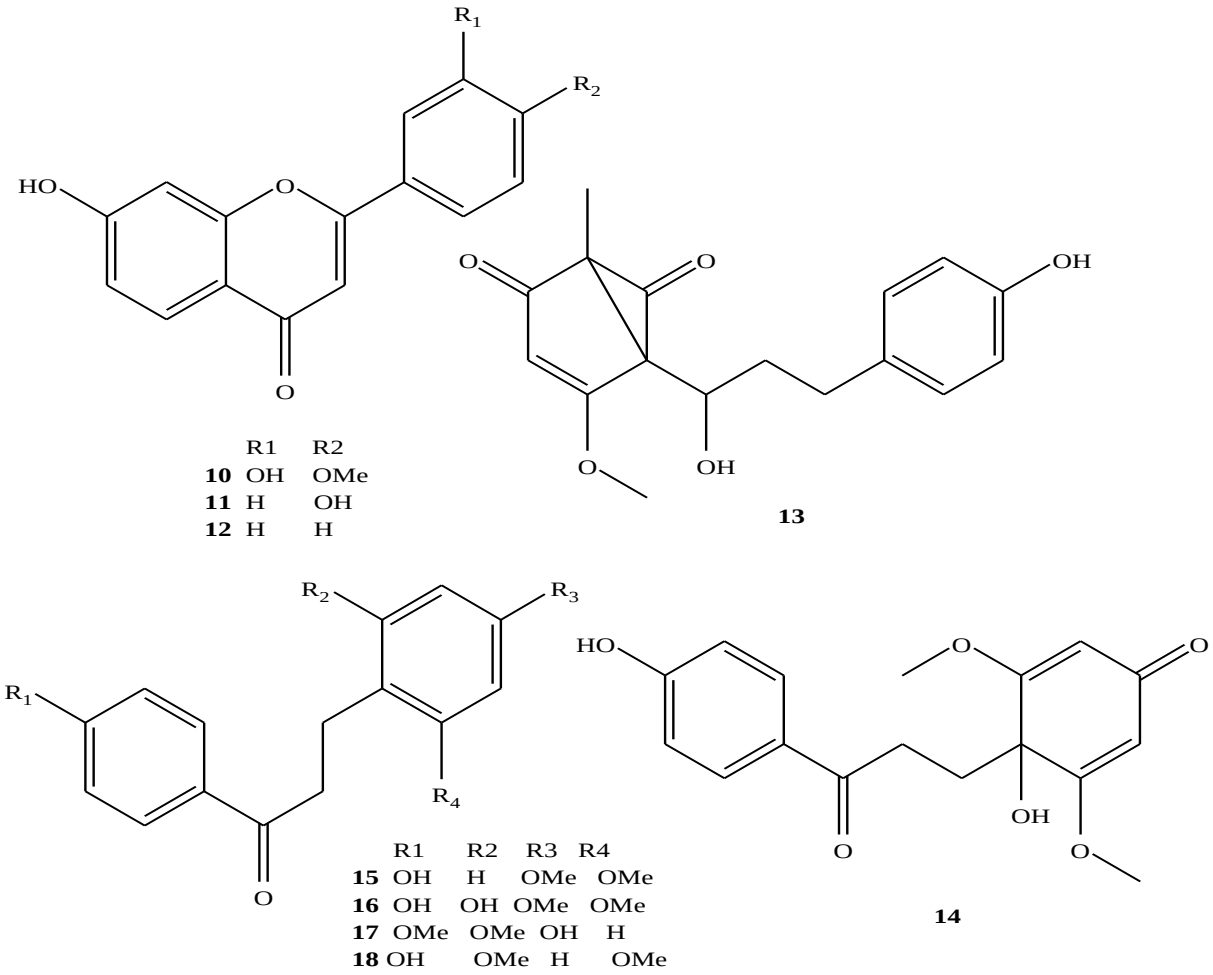


Figure 3: Flavones and dihydrochalcone derivatives from *D. cochinchinensis red resin*

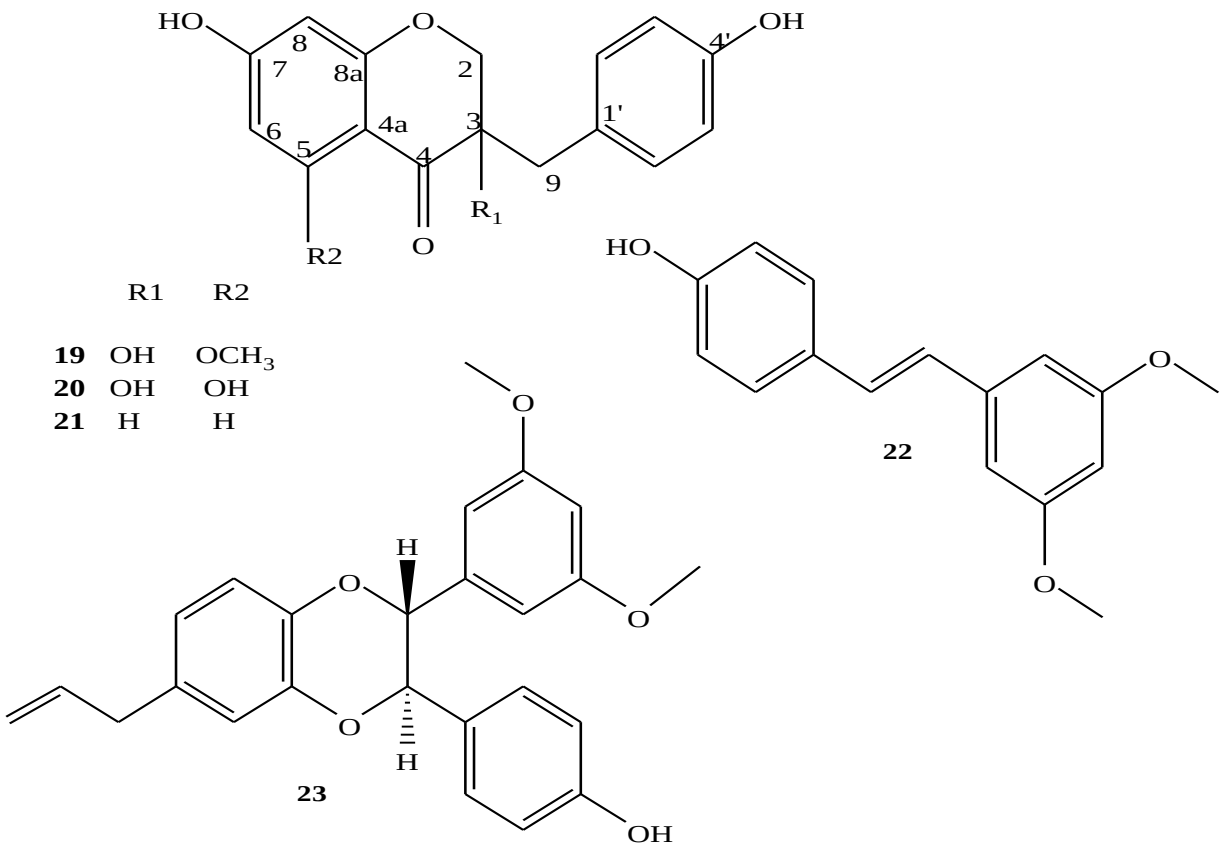
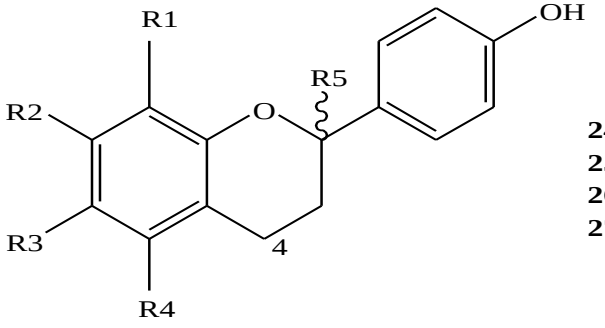
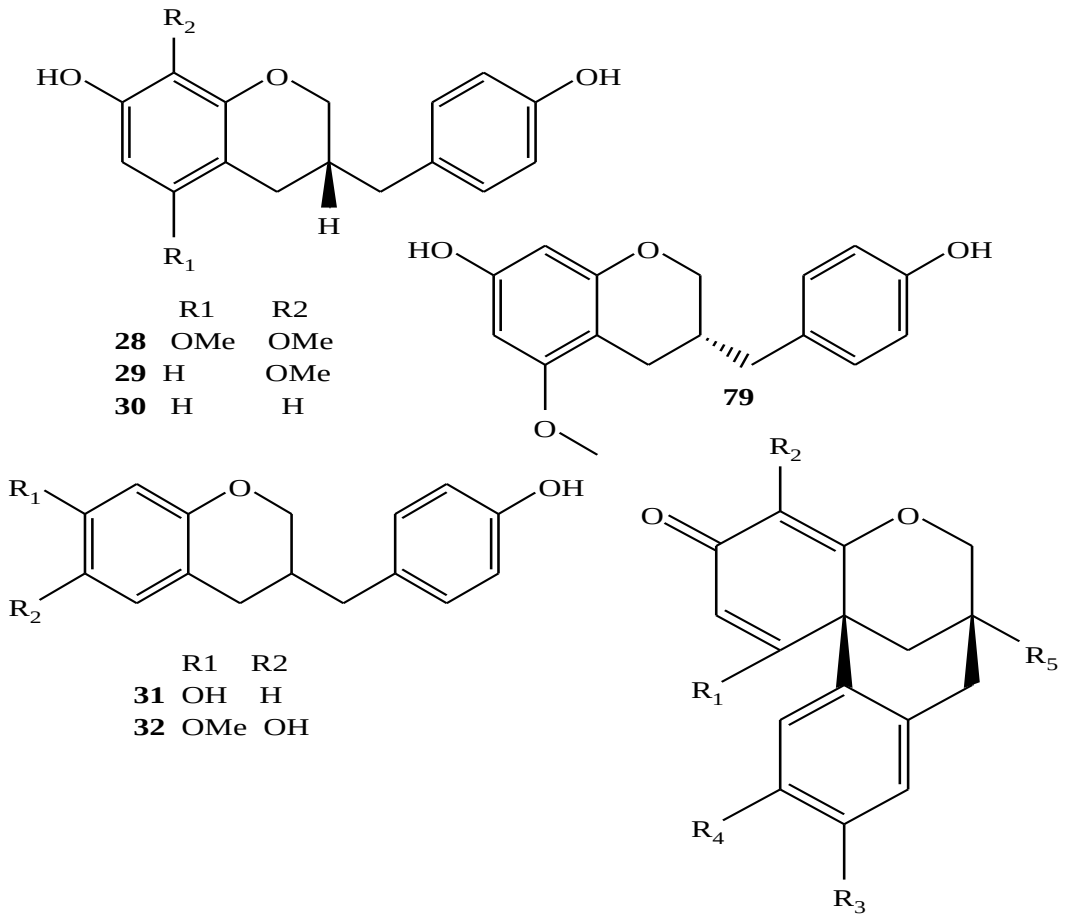


Figure 4: Homoisoflavanones and stilbenoids from *D. cochinchinensis red resin*



	R1	R2	R3	R4	R5
24	Me	OH	H	H	H
25	H	OMe	Me	OH	H
26	H	OH	H	H	H
27	Me	OH	H	H	H(beta)

Figure 5: Flavans from *D. cochinchinensis red resin*



	R1	R2
28	OMe	OMe
29	H	OMe
30	H	H

	R1	R2
31	OH	H
32	OMe	OH

	R1	R2	R3	R4	R5
33	H	OMe	OH	OMe	OH
34	OMe	H	OMe	OH	H
35	OMe	H	OH	OH	H
36	H	H	OH	OMe	H
37	H	H	OH	OH	H

Figure 6: Homoisoflavans and meta-homoisoflavans from *D. cochinchinensis red resin*

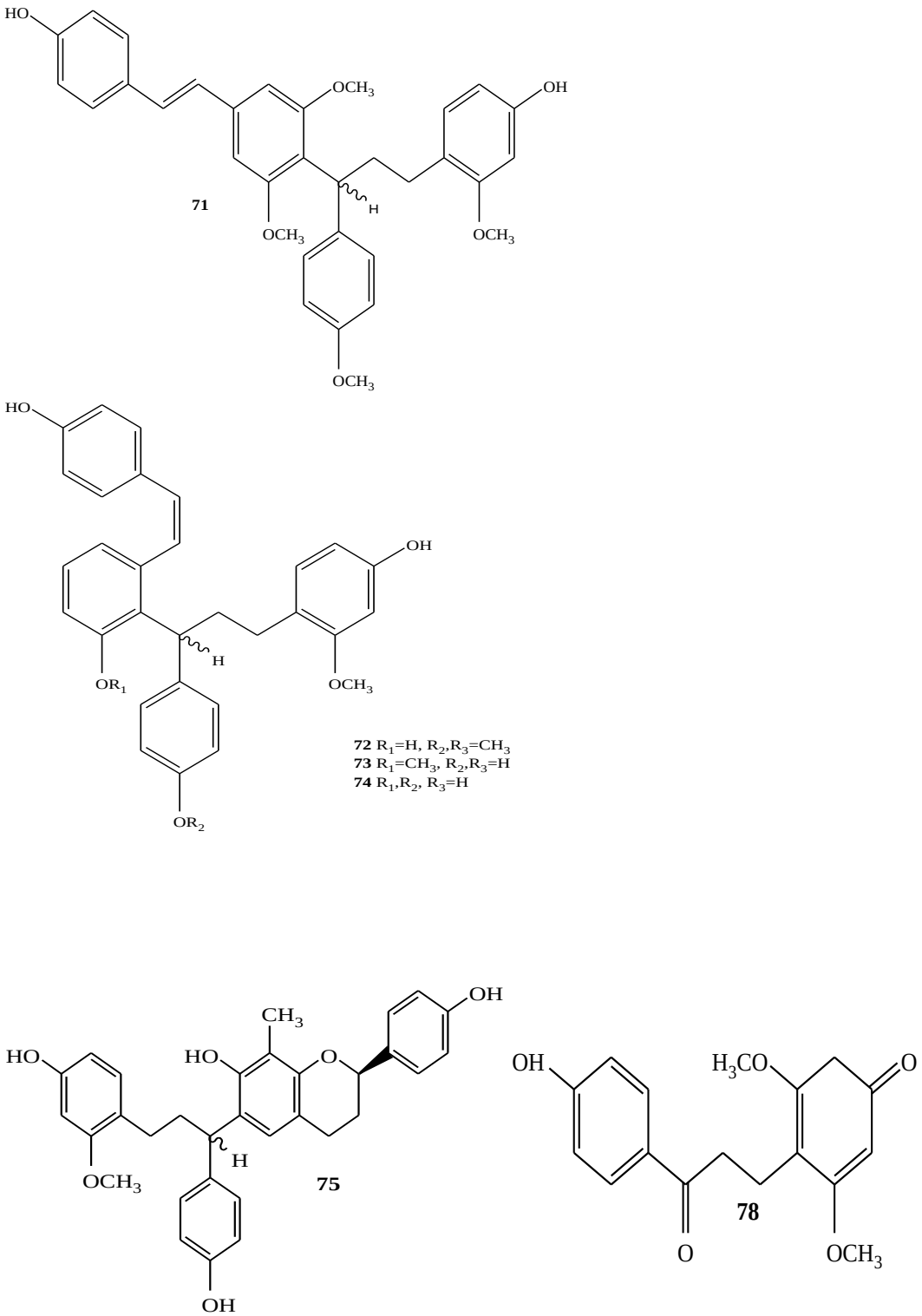
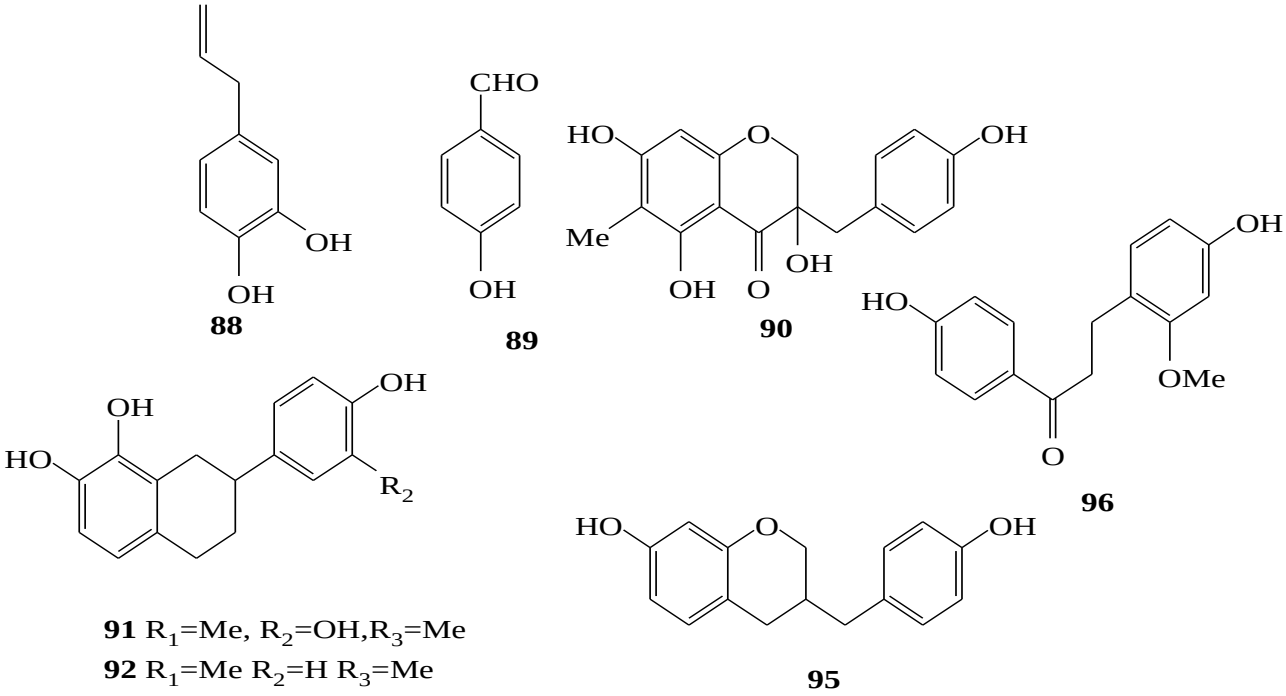


Figure 7: Polymeric flavonoid compounds isolated from stem of *D. cochinchinensis*



91 R_1=Me, R_2=OH,R_3=Me
92 R_1=Me R_2=H R_3=Me
93 R_1=H R_2=OMe R_3=H
94 R_1=H R_2=H R_3=H

Figure 8: Flavonoid Compounds isolated from *D. cambodiana*[21]

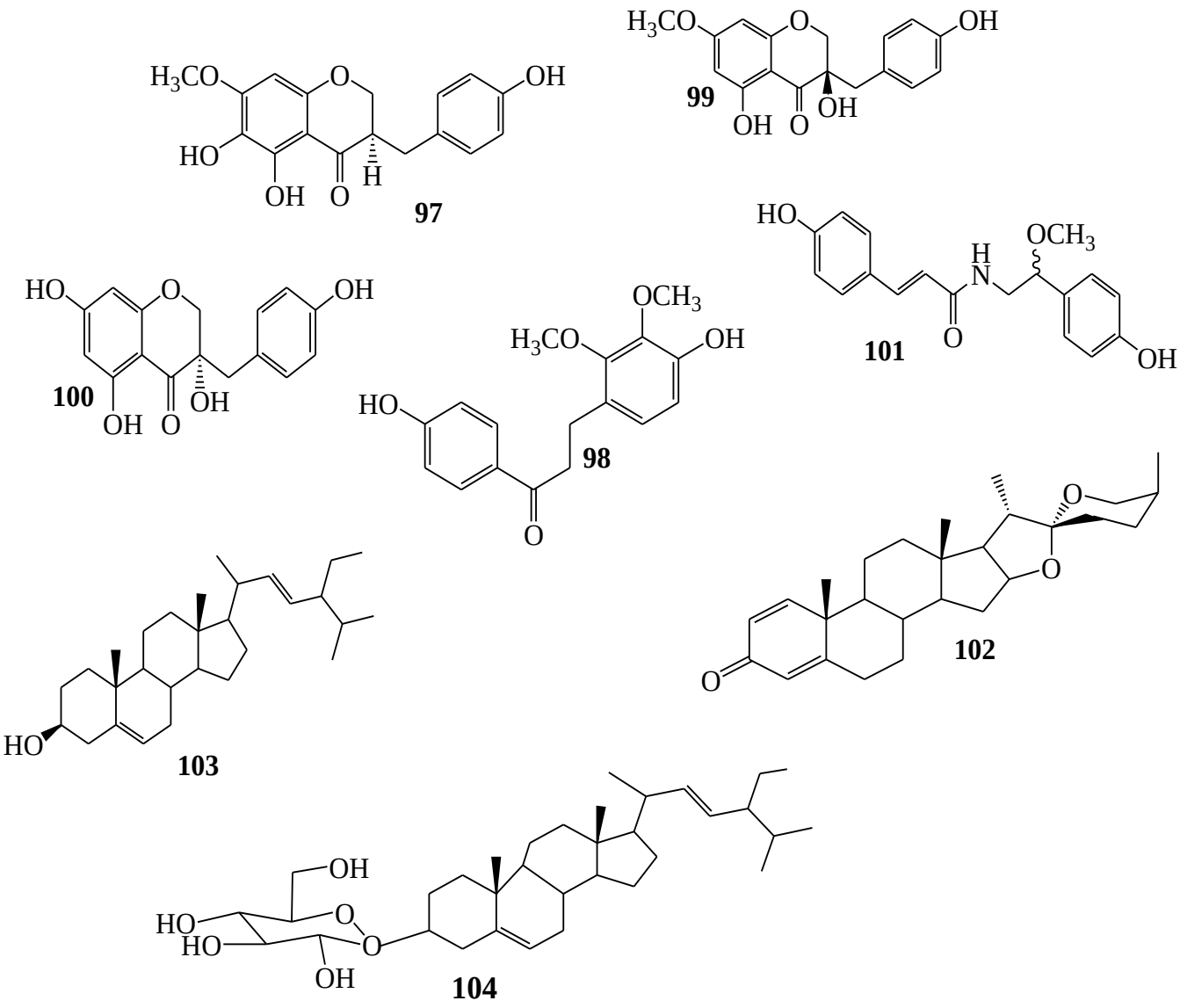


Figure 9: Flavonoid compounds isolated from *Dracaena usambarensis Engl*[46]

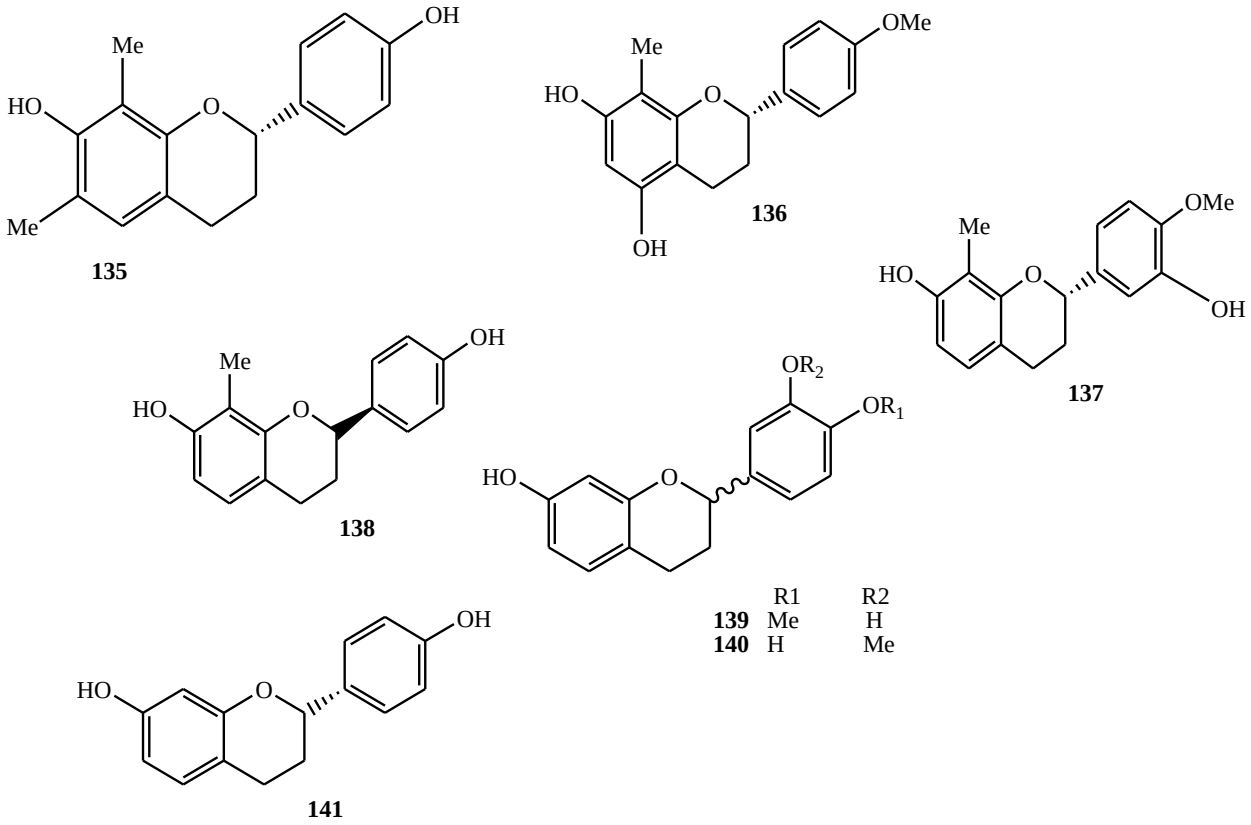


Figure 10: Compounds isolated from dragon's blood of *D. cambodiana* [47]

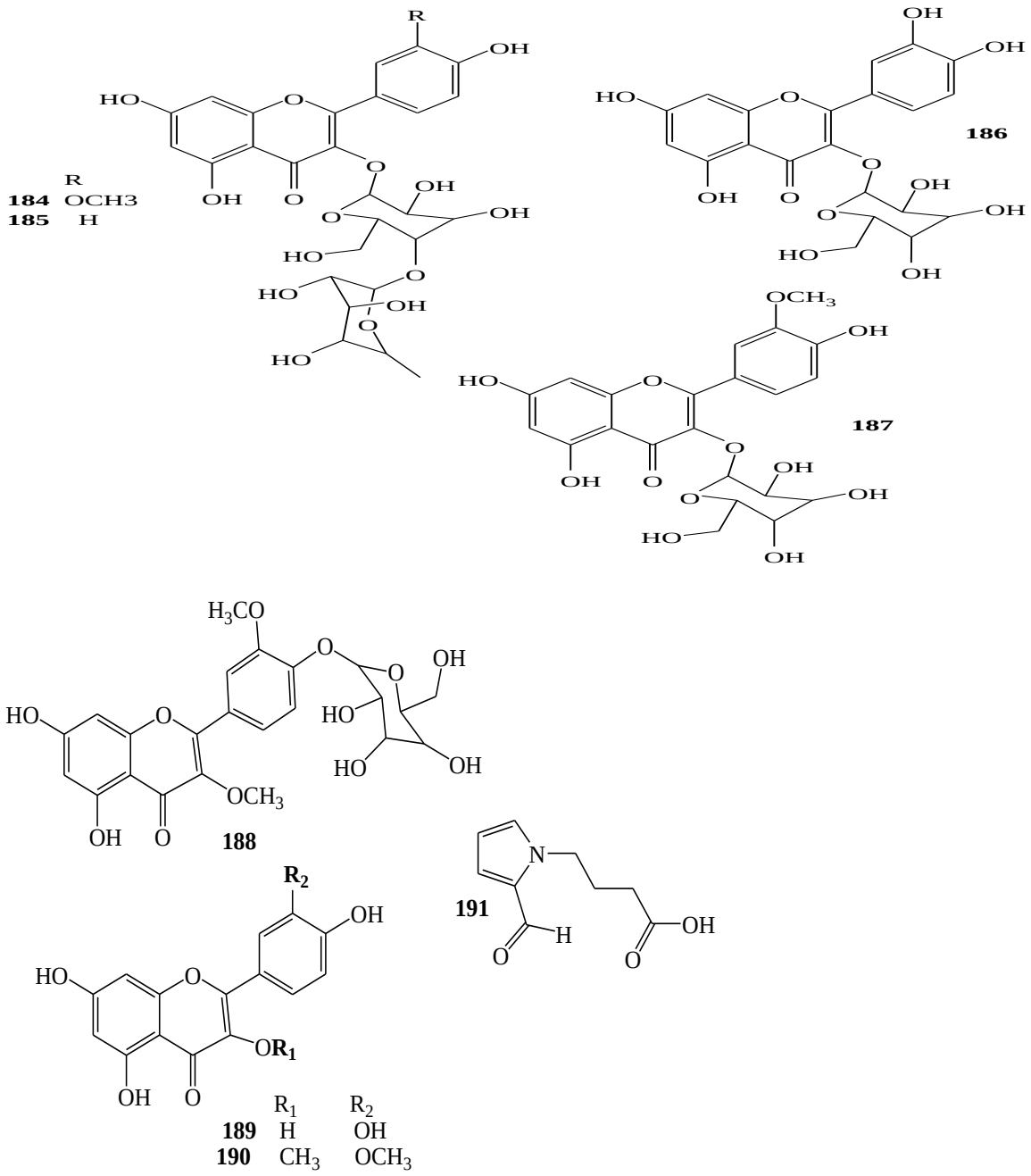


Figure 11: Structures of compounds isolated from seed of *D. steudneri Egl*[15]

2.5. Chromones

2.5.1. Occurrence and biological activities

Chromones are naturally occurring class of compounds with a benzoannelated gamma pyrone ring, whose chemical and biological aspects studied in detail and isolated from various plant species[48]. They are found within chemical structure of flavonoids (phenolic compounds). Compounds containing chromone skeleton have broad-spectrum biological activities such as anti-fungal, Phosphatidylinositol-3-Kinase (PI3K) inhibitors, anti-hypertensive, anti-viral, anti-allergenic, antitubilin, and anticancer agents. They are traditionally categorized as 'mast stabilizers' since they have anti-inflammatory effect through inhibiting the release pro-inflammatory agents from mast cells. For instance, cromoglycate (192) also known as, disodium cromoglycate, a derivative of the first clinically used chromone khellin(193), was found to have protective effect against allergen challenge without any bronchodilator effect. Egyptians and the Eastern Mediterranean countries were using Ammi visnaga seeds for the treatment of respiratory disorders in ancient times from which Khelin the first chromone was extracted. Even though cromoglycate and its derivatives are safe drug for the treatment of asthma, they have shorter half-lives and they need to be administered repeatedly. They are no more being used for the treatment of asthma as better drugs such as inhalational corticosteroids are arrived. However, interests continue in this class of compounds as downregulators of mast cell activity[49].

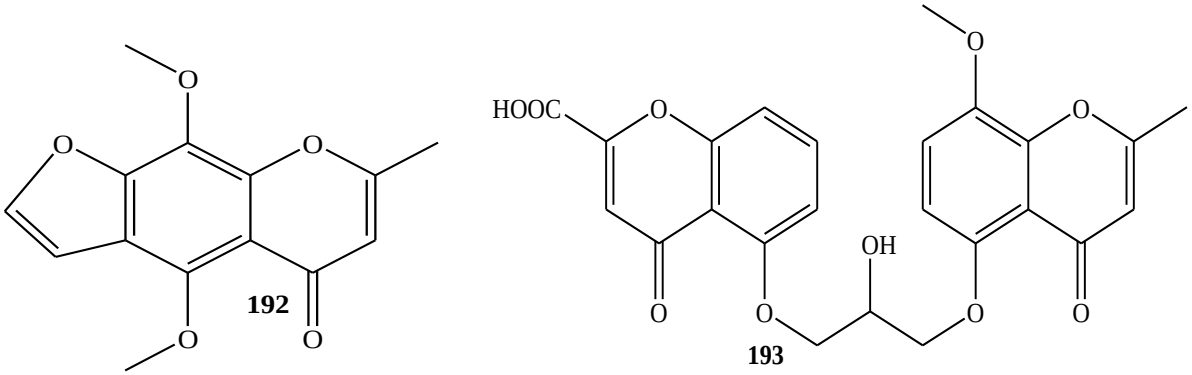


Figure 12: Structures of initial chromones used histamine stabilizers for asthma treatment

Cassiarins D (**294**), a chromone was isolated from flowers of Cassia siamea was found having antiplasmodial activity against Plasmodium falciparum 3D7 at IC50 =2.6 μM. Another chromone(**295**) isolated from roots of Spathelia excelsa was found active against epimastigotes forms of Trypanosoma cruzi at IC50= 11 μg mL-1[50].

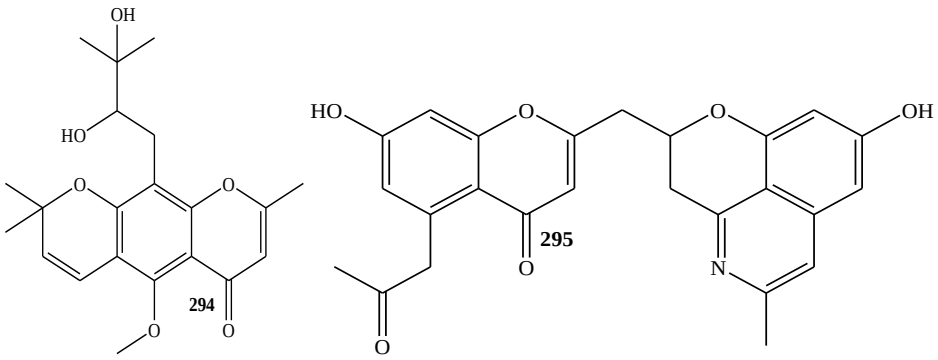


Figure 13: Structures of chromones having antiprozotoa(antiparasitic) activity

A Chromone artorigidusin (**296**) isolated from *Artocarpus rigidus* indicated antimycobacterial activity at a MIC of 12.5 µg/mL. The same compound also showed cytotoxicity the gastric carcinoma cell NCI-H187 with IC50 15.63 µg/mL[51]. Compounds (**297)**[52] and (**298)**[53], purified from the endophytic fungus Chalara sp., strain 6661, isolated from Artemisia vulgaris, showed strong antibacterial activity against Bacillus subtilis with an inhibition zone of 23 and 22 mm per 15 µg, respectively[5].

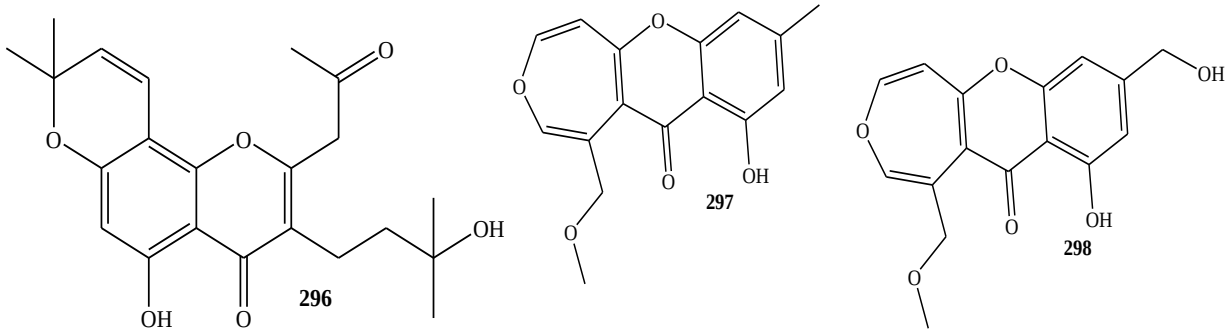


Figure 14: Structure of chromones with anti-tumor activities

Chromone scaffold containing compounds may also act as anti-inflammatory agents those act through different molecular mechanisms like inhibition of NO production, or IL-5 effects and inhibition of COX and LOX activities. For example, heterocarpin (**299**) decreased the production of NO by inhibiting iNOS protein expression. Another chromone, isoeugenin (**300**), isolated from the rhizomes of Imperata cylindrical (L.) P. Beauv., exhibited the capacity to inhibit iNOS expression and inhibit COX-2 expression.

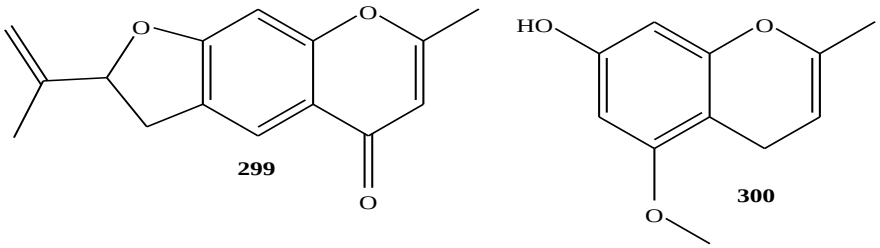


Figure 15: Structure of chromones with anti-inflammatory activities

2.5.2. Biosynthesis pathway of Chromones

Liao et al has hypothesized the biosynthesis of 2-(2-phenylethyl)chromone(**306**) in agarwood via type III polyketide synthase which uses Cinnamoyl-CoA(**301**) and Malonyl-CoA(**302**) as starting materials[54]. The procedure is indicated in the following figure:

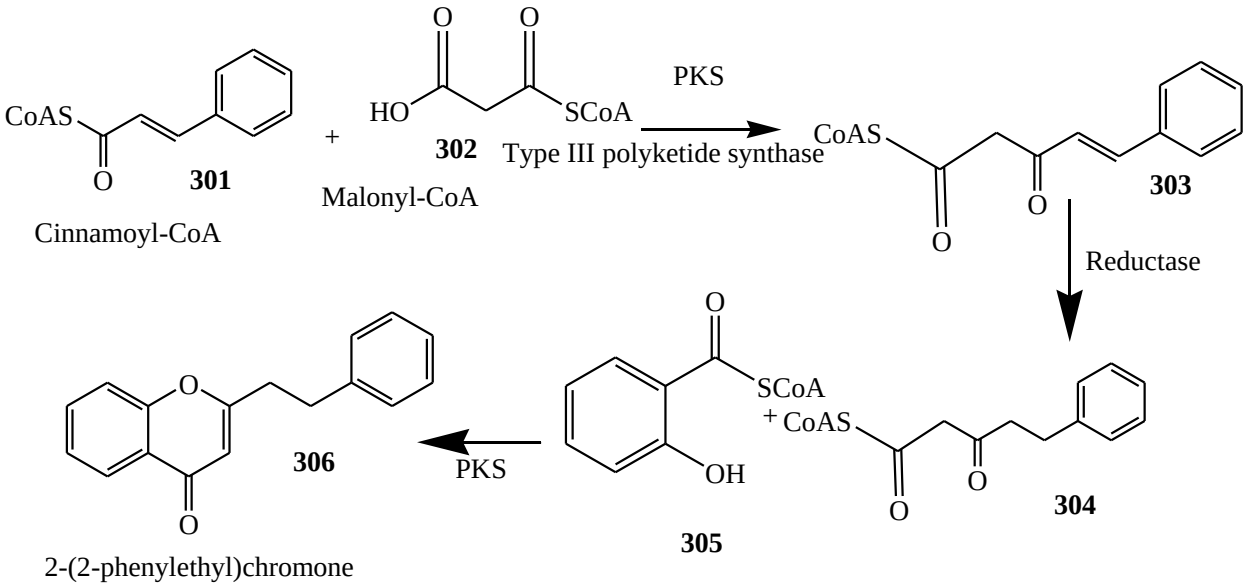


Figure 16: Hypothetic biosynthesis pathway of 2-(2-phenylethyl) chromone in agarwood via polyketide synthase

2.6. Naphthoquinones

2.6.1. Occurrences, classes and biological activities

Quinones are chemically classified into three classes based on the type of aromatic system that supports the quinone ring. Anthraquinones (an anthracene ring, linear or angular) (**307**), benzoquinones (a benzene ring) (**308a** and **308b**) and naphthoquinones (a naphthalene ring) (**309a** and **309b**). Both benzoquinones and naphthoquinones have two isomers except

anthraquinones[55]. Based on the position of the carbonyls within the ring system, it is possible to have different quinones. For instance, naphthoquinones have two different arrangements for their carbonyl groups: 1, 2-naphthoquinones (**309a**) with neighboring functional groups or 1, 4-naphthoquinones (**309b**) with a space of two carbons between carbonyls. They are structurally related to naphthalene (**310**). These isomers have notably different pharmacological actions due to the difference in their physicochemical properties. Quinones are highly reactive compounds with an application as natural or synthetic dyes[56].

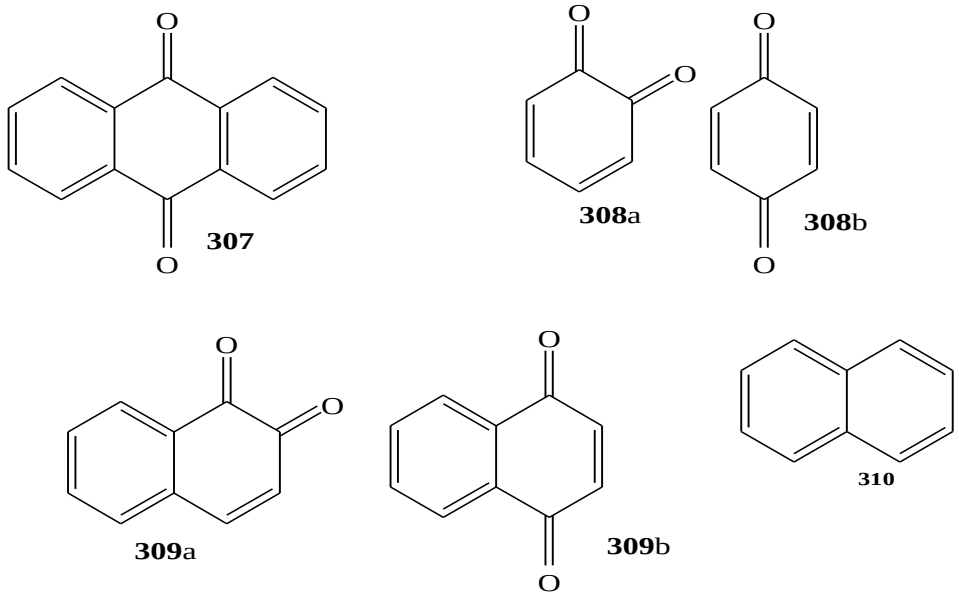


Figure 17: Structures of different Quinone classes

Naphthoquinones are widely distributed in several families of higher plants such as Verbenaceae, Proteaceae, Bignoniaceae and also found in microorganisms[56]. Their unique structure, biological, and functional properties (NQs) have attracted the enormous consideration, particularly from a medicinal chemistry perspective. Their derivatives have exceptional biological activities due to their bearing hydroxyl, methyl, nitrogen, sulfur, halide, phenylamino phenylthio, or sulphide functional groups. Particularly, derivatives bearing hydroxyl groups are seeking more consideration because of their broad-spectrum pharmacological properties such as antibacterial, antifungal, antiparasitic, antimalarial and antiviral. They have the capacity to produce reactive oxygen species (ROS) which make them potential anticancer agents. Natural products like lawsone (**311**), plumbagin (**312**), juglone (**313**), lapachol (**314**) and shikonin (**315**) are naphthoquinone derivatives isolated from plant sources have fascinated researchers for their

abundance, structural diversity, and broad-spectrum therapeutic potential[57]. Their multiple roles offer them a promising armory to fight microbial pathogens ESKAPE (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter spp*.) and even MDR pathogens.

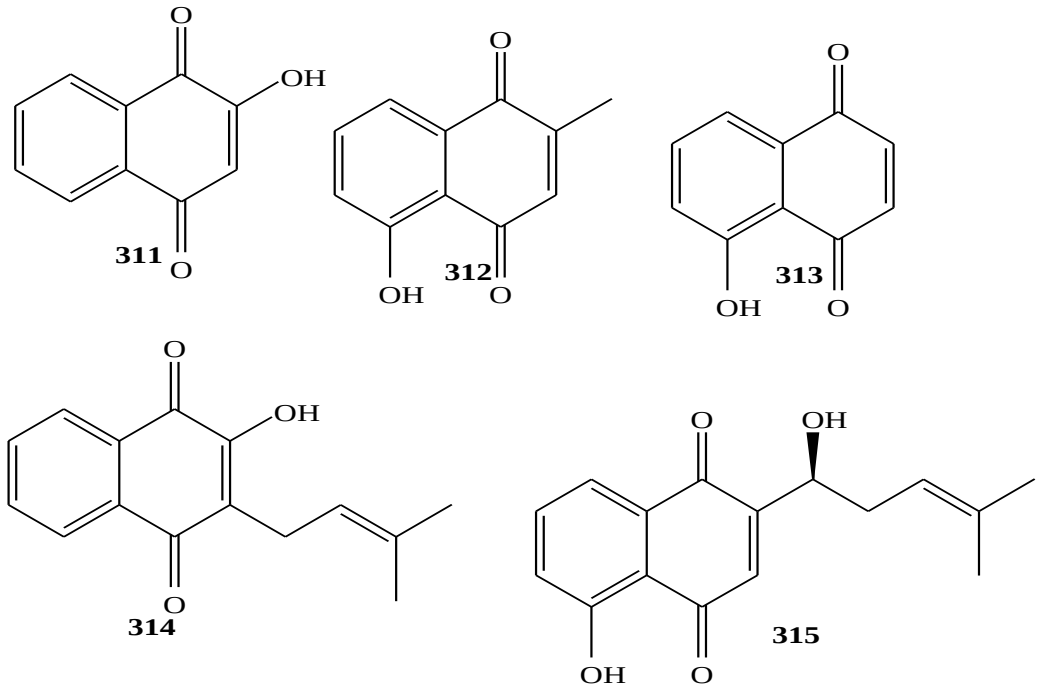


Figure 18: Structures of naphthoquinone derivatives isolated from plant sources

2.6.2. Biosynthesis of Naphthoquinones

Plumbagin (**318**), a naphthoquinone of the Asphodelaceae, is formed through the polyketide pathway through condensation of six acetyl-CoA units (**316**). The following biosynthetic scheme is taken from the work of Brinngman et al work, which elucidated biosynthesis of droserone that was determined using labeled precursors to sterile plant cultures, which unambiguously showed its acetogenic origin[58].

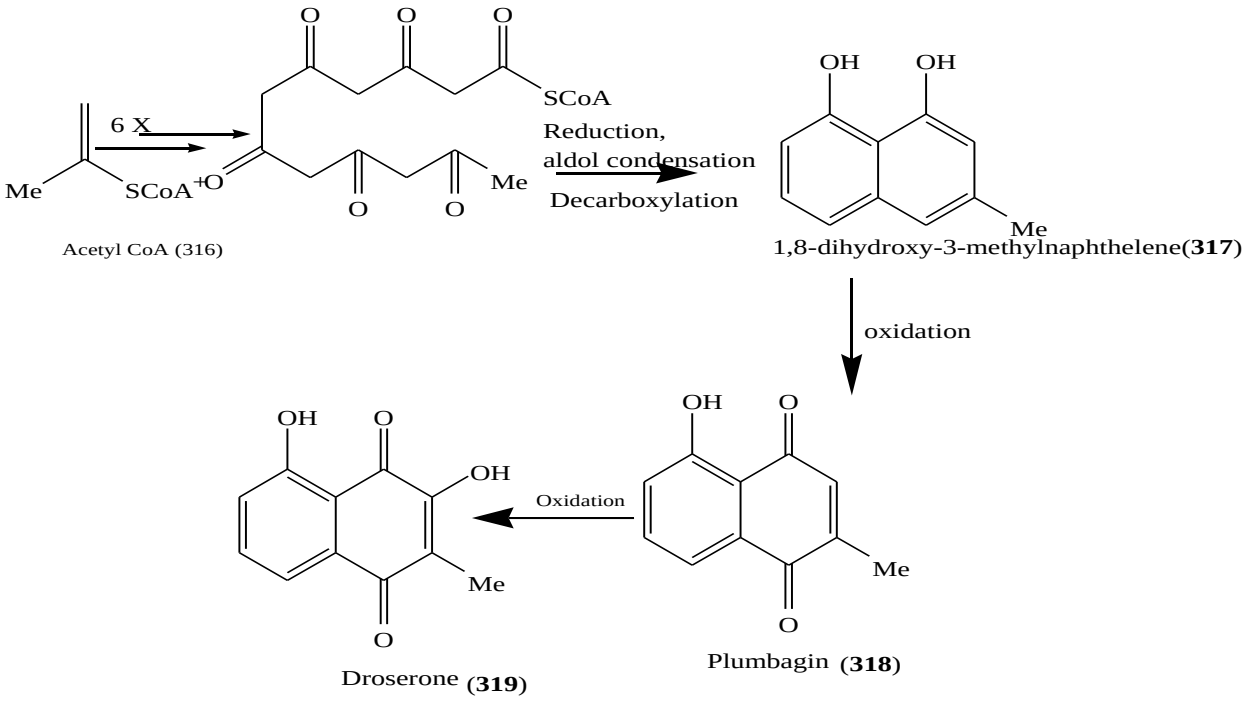


Figure 19: Biosynthetic pathway of Plumbagin (318) through polyketide pathway

2.7. Natural products from Microbial endophytes

'Endophytes are the microbes that populate in the plant tissues beneath the outer layer (epidermal cell) and interact differentially with the host like symbiotic, mutuality, commensalism, and trophobiotic.' They exist in different parts of plants such as roots, stems and leaves. Their density decrease from roots to stems to leaves[59–61].

Endophytic fungi produce an array of secondary metabolites, which are beneficial effect for the host plant[62]. They produce prominent and novel secondary metabolites from various phytochemical classes namely, terpenoids, alkaloids, steroids, phenols, lactones, and isocoumarins. Compounds with antimicrobial, antiviral, antioxidant, cytotoxic, immunosuppressive, and anticancer activities have successfully isolated from endophytic fungi in the last two decades[63–65].

Paclitaxel (**320**), a tetracyclic diterpenoid belongs to a class called taxane, is one of the best-selling anticancer drug isolated from the bark of pacific yew tree (*Taxus brevifolia*). FDA approved it for the treatment of several types of tumors including breast, ovary, and Kaposi's sarcoma. It was reported that an endophytic fungus called *Taxomyces andreanae* produces it[66].

The endophytic fungus *Chaetomium globosum*, which was isolated from marine green alga *Ulva pertusa*, was found responsible for the production of bioactive molecules cytoglobosins C ***(321)*** and D ***(322)*** as potent anticancer alkaloids towards A549 cells at IC50 values of 2.26 and 2.55 µM, respectively[67].

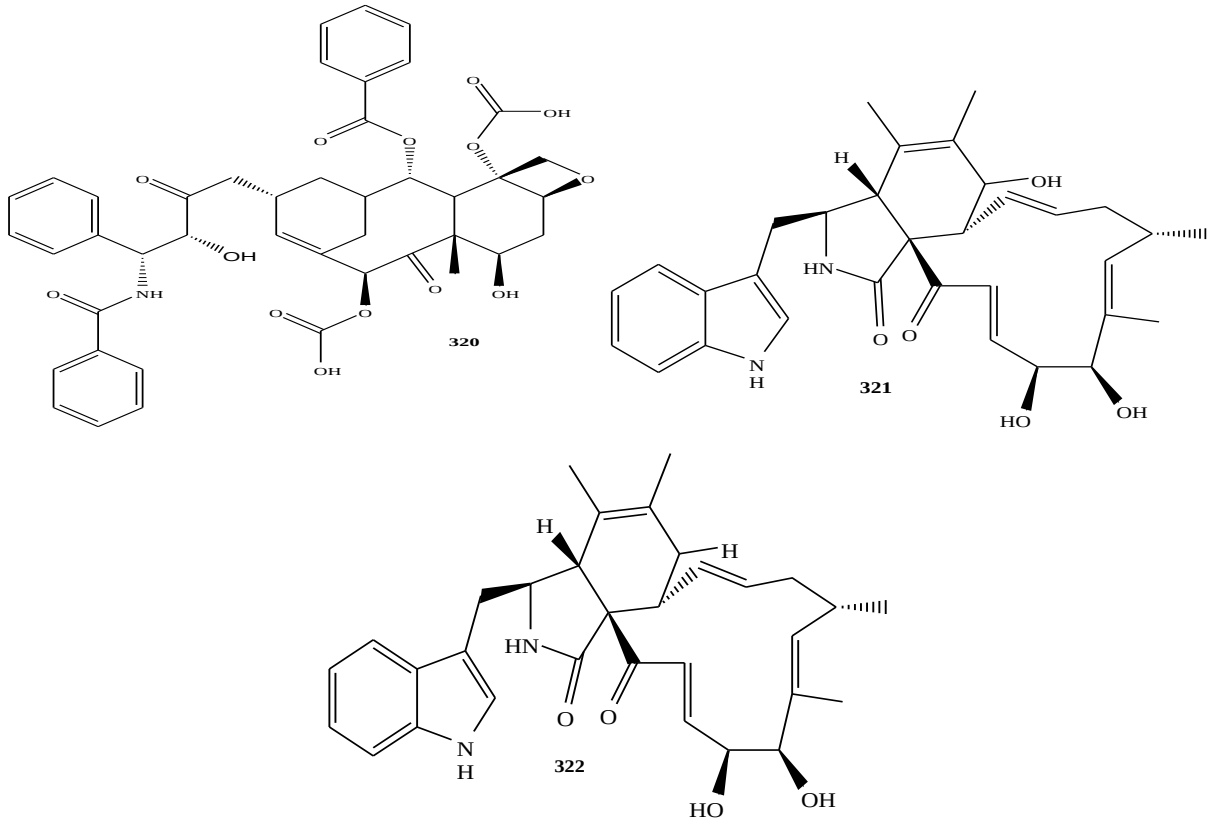


Figure 20: Structures of anticancer bioactive molecules produced by endophytic fungi

2.8. Biosynthesis of ergosterol(tetracyclic triterpenoid)

Cell membrane of eukarotic cells contains an essential molecule called sterol for its organization and structure. There are three main kinds of it in different eukaryotic cells: phytosterol (sitosterol, stigmasterol, campesterol) (in plants), cholesterol in animal cells (in vertebrates) and ergosterol in fungi cells. Each of them have different biosynthetic pathway even though they have common initial pathways from acetyl CoA to squalene epoxide (Figure **21**)[68].

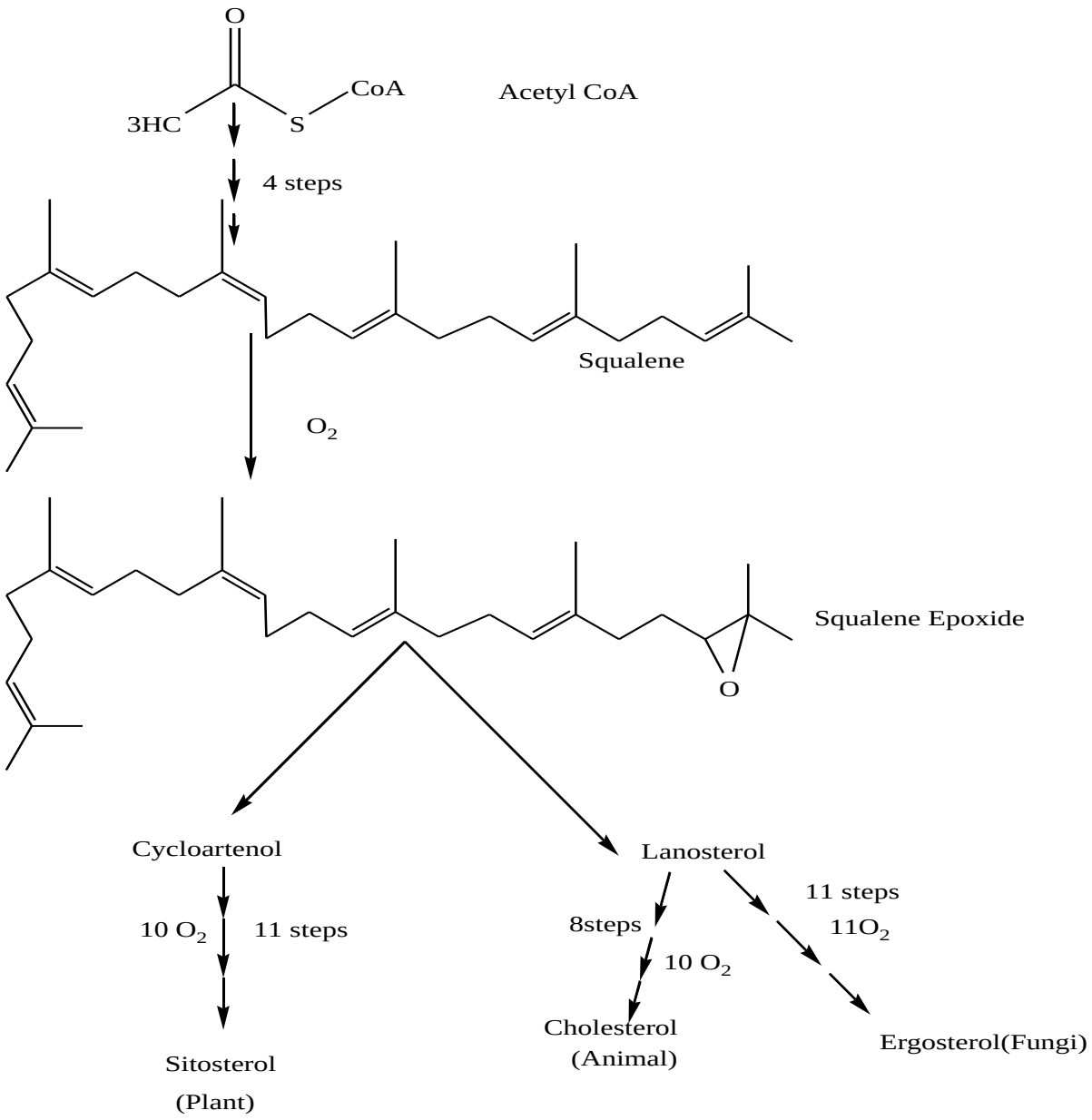


Figure 21: Simplified generalized synthetic pathway of sterols in plants, animals, and fungi

3. MATERIALS AND METHODS

3.1. Chemicals

In this study, analytical grade solvents (dichloromethane, methanol, Hexane, ethyl acetate) and Silica gel (60–120 mesh size) were used for extraction and isolation purpose. Dimethylsulfoxide (DMSO), gentamycin, Müeller-Hinton Agar were used for antibacterial activity test.

3.2. Apparatus and equipment

Mortar and pestle, rotary evaporator (Laborota, 4000, Heidolph, USA), silica gel coated TLC plate, UV-254 and 365 nm chamber (UV-Tec), Whatmann filter paper No. 42 (125 mm), 0.5 McFarland, Müeller-Hinton Agar medium plates, Sterile paper discs (6 mm diameter, Whatmann No.3) and column chromatography (300 mm (B-14/23, B-19/26) and 500 mm, B-34/35) apparatuses and equipments were used.

3.3. Collection of Plant Materials

The stem bark of *D. steudneri* was collected from Konta Special Woreda, which is located in South-Western Ethiopia in Southwest Ethiopia Peoples' region. It was collected in April 2022. Botanist at Department of Biology, Jimma University, authenticated it and voucher specimen was stored in the Jimma University herbarium

3.4. Extraction, isolation of pure compounds and their characterization

3.4.1. Extraction

The collected plant material (the stem bark of the *D. steudneri*) was shade-dried for one month at Jimma University chemistry laboratory and grounded using a mortar and pestle. The 128g sample was weighed and added into a 2500ml conical flask. First, 800ml of methanol was added and was shaken slightly. Second, 800ml of dichloromethane was added and shaken again slightly. Then it was shaken gently for 10 minutes and kept at room temperature for 72 hours three times[69]. Finally, the extracts were filtered through a Whatmann filter paper No. 42 (125mm) and concentrated using a rotary in a water bath set at 40°C[70]. Thirteen and half-gram (13.5g) crude extract was obtained and 13g was subjected to column chromatography for isolation while 0.5g was kept for antibacterial activity.

3.4.2. Isolation of pure compounds and their characterization

The crude extract of the stem bark of *D. steudneri* (13g) was subjected to column chromatography (500mm diameter) filled with silica gel (150g) then eluted with hexane in increasing amounts of ethyl acetate gradient to give different fractions. The fractions were combined based on their TLC profile (fractions of the same Rf values). Accordingly, 83 fractions were collected from column chromatography and fractions 1-26 were obtained by 2% ethyl acetate and 98% hexane and these fractions were having clearly spotted compounds with better yield. Fractions 4 and 23 had four spots in common (fraction 23 shares similar spots with fraction 4 in addition to other spots). These fractions were merged to increase the concentration of the similar spots and then subjected to mini-column, which gave fraction 17 that yielded a pure compound coded as **324** (packed into vial for characterization) (figure **22)**. Fractions 11-18 were merged based on their TLC spots (five spots) and re-columned. Then from the resulting 16 fractions, fractions 8 (compound **325**) and 12 were collected pure and vialed for characterization by NMR and for antibacterial activity. Since both compounds **325** (4mg) and fraction 12(1mg) showed similar Rf values (0.560), fraction 12 was used for bacterial activity (figure **22)**. Fractions 25 and 26 were merged since they had six spots and subjected mini column chromatography. Fractions 1-23 from this chromatographic separation having different spots were collected and fractions 11 and 12 (with more than four spots) were merged and re-subjected to column chromatography. This chromatographic separation gave 20 fractions, and fraction 15(**323**, 20mg) was found pure while others were having more than three spots (figure **22** below and appendix 1). The purified compounds were elucidated using both proton and carbon NMR techniques. Literatures were also used to compare the spectra of the compounds with previously reported compounds.

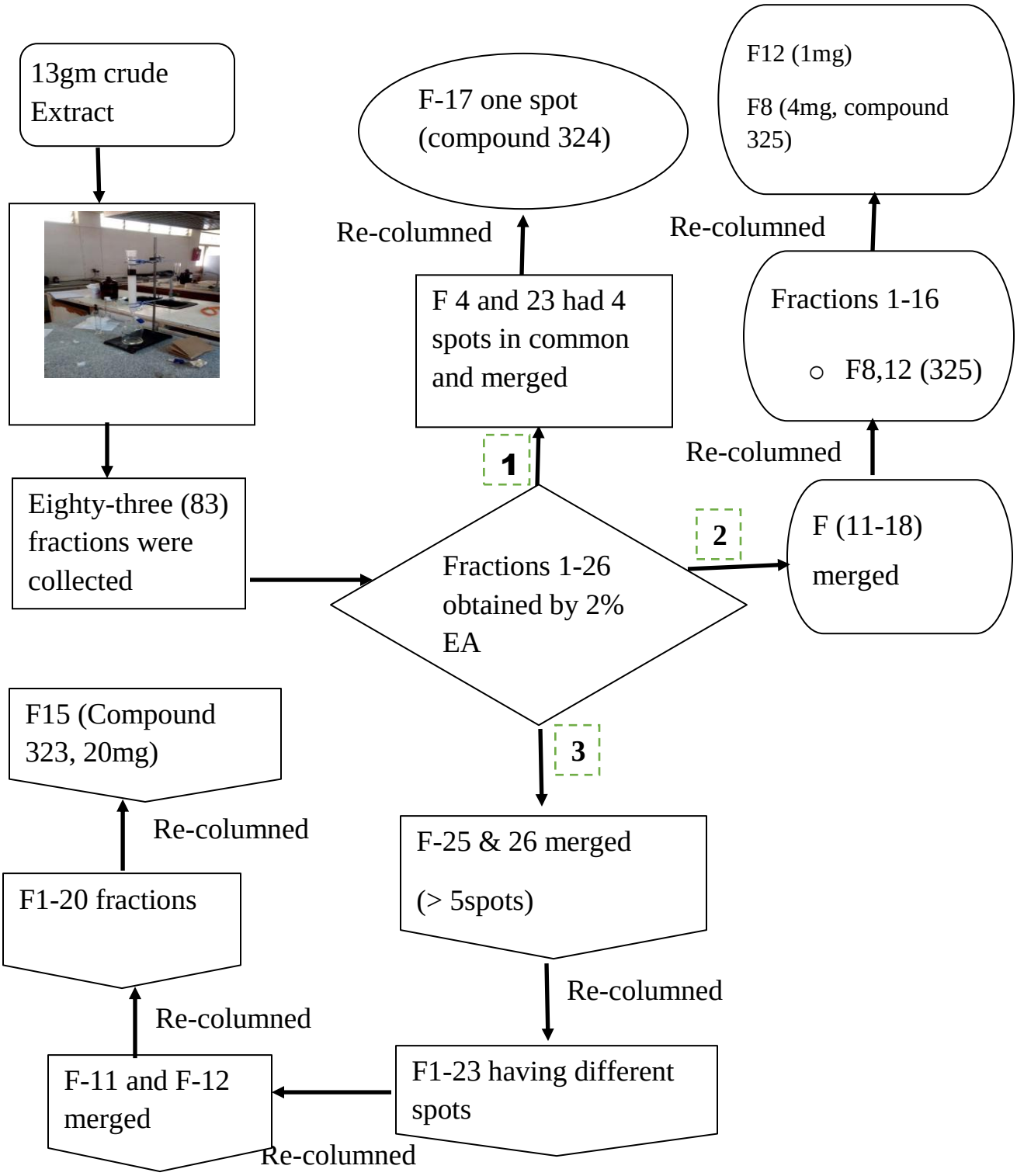


Figure 22: Flowchart of isolation of bioactive compounds from *Dracaena steudneri*

3.5. *Test strains* and Antibacterial activity test

Staphylococcus aureus ATCC 25923, *Escherichia coli* ATCC 35218, *Salmonella typhi (*ATCC 27870**)**, *and Bacillus cereus* (ATCC 14579). The strains were used for antibacterial evaluation of both crude extract and isolated compounds.

The antibacterial activities of the crude extract and the isolated compounds (**323**, **324** and **325**) were evaluated using Agar disk diffusion method against the four bacterial test strains (two gram-positive and two gram negative bacteria strains). The bacteria stock culture was maintained on the nutrient agar slants, which was stored at 40 °C. To prepare the test solutions 100mg of crude extract, 1mg of compound **324**, 500 µg of compound **325** and **323** were dissolved in 1mL of DMSO to achieve final stock concentrations of 100mg/ml, 1mg/ml and 500 µg/ml, respectively. The test pathogen solutions of freshly grown liquid culture having similar turbidity with 0.5 McFarland were seeded over the Müeller-Hinton Agar medium plates. Sterile paper discs (6 mm diameter, Whatmann No.3) were separately soaked in the above stock solutions (samples and standards) and then applied over the seeded plates at equidistance. Gentamycin (0.3w/v (3mg/ml)) was used as a standard drug (positive control) while DMSO was used as a negative control. The plates were inverted and incubated at 37 °C for 24 hours. After 24 hours of incubation period, the plates were taken out to measure inhibition zone around disks. The inhibition zones formed around the discs were the indication of antibacterial activity and measured in millimeter. Each experiment was carried out in duplicates. The mean of the inhibition zone of each test sample was taken for evaluating the antibacterial activity. The inhibition zones produced by crude extract, isolated compounds were compared with the inhibition zones produced by positive control (standard drug) [71].

4. RESULTS AND DISCUSSION

4.1. Structural elucidation of the isolated compounds

4.1.1. Characterization of Compound 323

Compound **323** was obtained as yellowish solid with Rf value of 0.60. It was soluble in chloroform and characterized by one dimension (^{1}H NMR, ^{13}C NMR) and two dimension (COSY, HSQC, and HMBC) NMR spectroscopic techniques (see appendices **3-10** and table **1**).

Table 1: NMR spectroscopic data of compound 323 and **Comparison with previous reports**

Compound 323			**Reported compound (Droserone)** [72,73]	
Position	**^{1}H ppm (multiplicity) ($CDCl_3$, 500 M Hz)**	**^{13}C ppm (CDCl3, 125 M Hz)**	**^{1}H ppm (multiplicity) ($CDCl_3$, 500 M Hz)**	**^{13}C ppm (CDCl3, 125 M Hz)**
1	-	184.4	-	184.5
2	-	121.7	-	155.5
3	-	152.7	-	136.1
4	-	184.1	-	183.9
4a	-	112.9	-	113.6
5	-	161.1	-	160.0
6	7.20(1H, d)	123.1	7.23 (1H, d)	122.7
7	7.62 (1H, dd)	137.5	7.61 (1H,dd)	136.8
8	7.66 (1H, d)	119.6	7.29 (1H, d)	118.2
8a	-	121.7	-	132.4
9	2.10 (3H, s)	8.7	2.08 (1H,s)	8.7
3-OH	3-OH not detected		not detected	-
5-OH	11.10 (1H,s)		not detected (11.43)[a]	-

[a] detected when d_6 DMSO was used

The ^{1}H NMR spectrum (Table 1) indicated highly down field shifted proton signals at δ_H 11.10 for hydroxyl proton involved in hydrogen bonding. The shielded *singlet* signal integrated for three protons is for methyl group resonating at δ_H 2.10 in the ^{1}H NMR spectrum and there are two most down shielded carbon signals at δ_C 184.4 and 184.1 for two carbonyl carbons in the ^{13}C NMR spectrum[74]. The ^{13}C NMR also indicated that there are aromatic carbon peaks at δ_C 112.9, 161.1, 123.1, 137.5, 119.6 and 132.6 ppm. There are also two additional carbon peaks of alkene functional groups at δ_C 121.7 and 152.7 ppm. The ^{1}H NMR spectrum further showed signals for three aromatic protons δ_H 7.20, 7.62 and 7.66 ppm. The methyl protons (δH 2.10) showed HMBC correlation with the carbonyl carbon C-4 (δC 184.1) and oxygenated quaternary carbon (δC 152.7), indicating that ring A was substituted with hydroxyl and methyl groups at C-2 and C-3, respectively. The ^{13}C NMR spectrum also further showed the presence of seven quaternary carbons, three methine carbon signals constituting the naphthoquinone skeleton, and a methyl group as a substituent. Based on the above spectroscopic data and exhaustive 2D NMR spectrum(table **1**,appendices **1-10**), the compound **323** was identified as 3,5-dihydroxy-2-methyl-1,4- naphthoquinone, trivial name droserone (see figure 23 below), which had previously been isolated from plants such as *Diospyros maritima* Blume[75], *Dionaea muscipula*[73], *Drosera erythrohiza subspecies magna*[72].

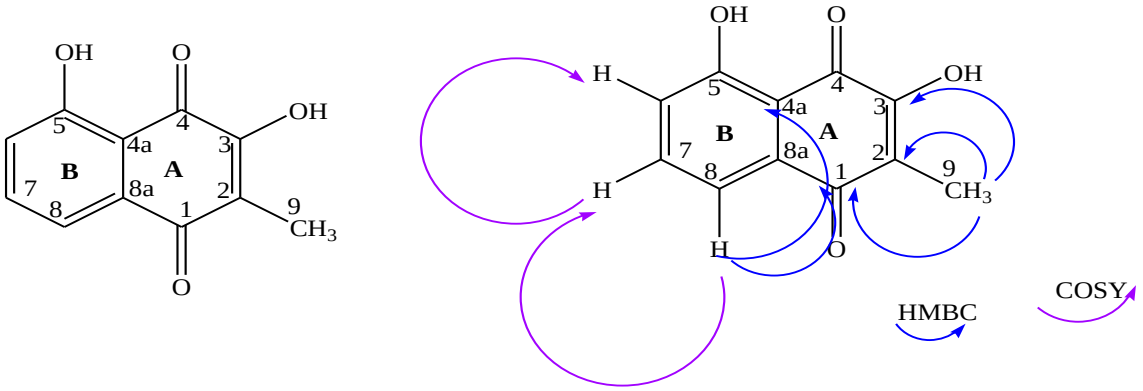


Figure 23: Proposed structure of compound 323, HMBC and COSY correlation.

4.1.2. Characterization of Compound 324

Compound 324 was isolated as yellowish red solid with Rf value of 0.49. In ^{1}H NMR spectrum, there are four proton singlet peaks at chemical shifts of 2.00, 2.38, 3.85, and 12.95 ppm and the peak at 6.31 ppm is the peak of doublet of doublets. The first three peaks have three protons each, which indicates they are the peaks of three methyl groups, which are singlets and the one at

3.85 ppm is the peak of a methyl group attached to an electronegative element particularly oxygen. As a result, this peak is the peak of a methoxy group. The other two methyl proton peaks are the peaks of allylic protons. In other words, they are next to the olefin carbons. This is also confirmed by the presence of two allylic carbons in ^{13}C NMR at chemical shifts of 9.2 and 18.5ppm. The proton peak at 6.31 ppm is the position of protons on benzene ring. It is the peak of two protons those split each other. Since the coupling constant is less than 6Hz, the protons must be on *meta* position for each other. The other proton peak is at chemical shift of 12.95 ppm. It the phenolic hydroxyl proton peak[76].

The ^{13}C NMR of compound **324** indicates the presence of 12 resonance chemical shifts. The first two chemical shifts at 9.2 and 18.5ppm are the peaks methyl groups directly attached to olefinic carbons. The methyl group at 18.55ppm is slightly de-shielded compared to the methyl group at 9.25 ppm. This is because it is directly attached to an olefinic Carbon that is directly attached to electronegative element oxygen. The peak at 55.8ppm is the peak of methoxy group. The chemical shifts at 91.8, 97.6, 104.7, 162.0, 165.1, 157.6 ppm are aromatic carbon peaks.

The HMBC correlation spectra also showed that H-6 is correlated with C-4a (104.8ppm), C-5(162.0ppm), 8(91.8ppm) and H-8→ C-4a (104.8ppm), C-6(97.7ppm), C-7(165.1ppm), C-8a(157.6ppm) and 7-OMe with C-7(165.1ppm); 10-CH_3 with C-3(115.2ppm),C-2(162.0 ppm), C-4(182.0ppm) and 9-CH3 with C-3(115.2ppm), C-2(162.0 ppm) and C-8a(157.6ppm). This is in line with what is reported in previous study[80].

Based on the NMR experiments (appendices **11-18,** table 2,) and literature report, the structure of compound **324** is proposed as 5-hydroxy-2, 3-dimethyl-7-methoxychromone (**324**) as shown in figure **24** below. It is not for the first time for this compound to be isolated from natural products as it was isolated from *Lichen Graphis scripta*[77]. Even though chromones like 2,5-dimethyl-7-hydroxychromone and 2,5-dimethyl-7-methoxychromone were *isolated from Cassia fistula bark*, the present chromone compound seems the first time to be isolated from plants[78]. It could be due to endophytic fungi leaving in plant cells. It was also isolated from a *Rhinocladiella sp*, a fungus obtained from the sponge *Ircinia oros* and a fungus *Trichoderma harzianum*[79,80].

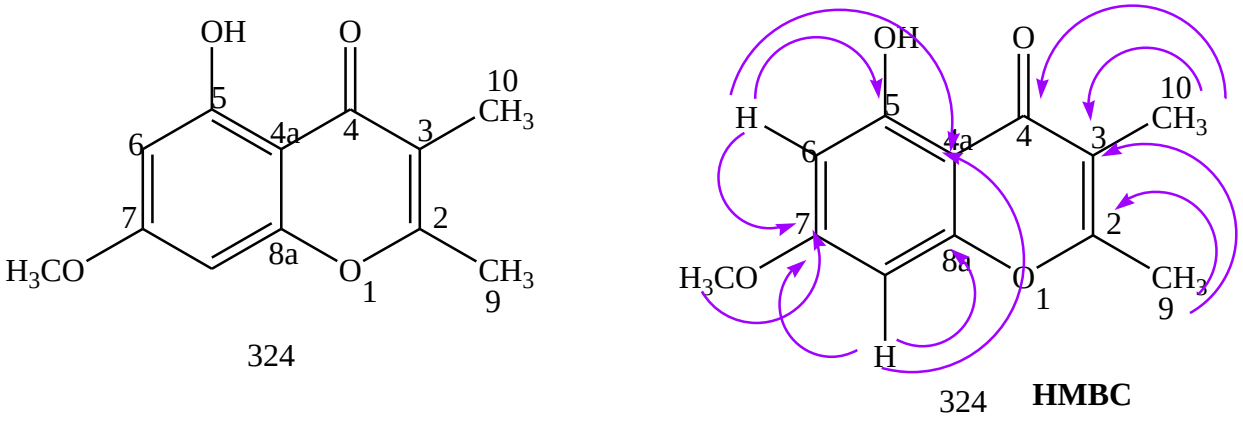


Figure 24: Proposed structure of compound 324 and HMBC correlation.

Table 2: NMR spectroscopic data for compound 324 and Comparison with previous reports

	Compound 324		**Reported compound**[77]	
Position	**1H ppm (multiplicity), (CDCl3, 500 M Hz)**	**δ C(ppm) (CDCl3, 125 M Hz)**	**δ H (multiplicity, J_{HH}) (CDCl3, 500 M Hz)**	**δ C(ppm) (CDCl3, 125 M Hz)**
1	--	--	--	--
2	--	162.0	--	162.5
3	--	115.2	--	115.1
4	--	182.0	--	181.9
4a	--	104.7	--	104.7
5	--	162.0	--	162.0
6	6.32(1H, d, J=2.0Hz)	97.7	6.32(1H, d, J=2.0Hz)	97.6
7	--	165.1	--	165.0
8	6.31(1H, d, J=2.0Hz)	91.8	6.31 (1H, d, J=2.0Hz)	91.8
8a	---	157.6	---	157.5
9	2.38(3H,s)	18.5	2.38 (3H, s)	18.4
10	2.00(3H,s)	9.2	2.00 (3H, s)	9.1
5-OH	12.95(1H,s)	--	12.95 (1H, s)	--
7-OMe	3.85(3H,s)	55.7	3.84(3H, s)	55.7

4.1.3. Characterization of Compound 325

Compound **325** was obtained as reddish solid with Rf value of 0.560. Its ^{1}H NMR spectra indicate the presence of 14 peaks of protons. There are four olefinic protons at δ (5.22ppm, 5.41ppm and 5.6ppm) which is also confirmed by the ^{13}C NMR spectra, which indicate the presence of six olefinic carbons at δ (141.4ppm, 139.8ppm, 135.6ppm, 132.ppm, 119.6 and

116.3ppm). The proton peak at δ 5.22ppm is the peak of two olefinic protons directly attached to carbons whose δ is 132 and 135.6 ppm as indicated in HSQC spectra. ^{1}H NMR spectra also indicate the presence of six methyl groups. The proton with chemical shift of 3.65 represents a chemical shift of hydroxyl hydrogen (- OH), on a carbon of 70.48-ppm chemical shift. This proton has no direct correlation with any carbon as seen from HSQC experiment. The peak at 7.26ppm is the peak of the solvent[81].

The ^{13}C NMR spectra of compound 325 shows the presence of 28 total carbons at δ (12.1, 16.3, 17.6, 19.7, 19.8, 21.1, 23.0, 28.3, 32.0, 33.1, 37.0, 38.4, 39.1, 40.4, 40.8, 42.8, 42.8, 46.3, 54.6, 55.7, 70.5, 116.3, 119.6, 132, 135.6, 139.8, 141.4) ppm. The δC at 12.1 ppm represents the peak of two carbons. This is clearly indicated by the DEPT-135 spectra (see appendices **24**&**25**). The peak at $^{\delta}$C 70.5 ppm is the peak of alkyl group attached to electronegative element oxygen. This is confirmed by the proton $^{\delta}$H at 3.65 ppm.

Table 3: NMR spectroscopic data for compound 325 and Comparison with previous reports

	Compound 325		**Reported compound (ergosterol)82**	
Position	**$^{\delta}$H (multiplicity) (CDCL3, 500Hz)**	**δ C(ppm) (in CDCL3, 125Hz)**	**δ H (multiplicity) (CDCl3, (400Hz))**	**δ C(ppm) (in CDCL3, 100Hz)**
1		38.4	-	38.3
2		32.0	-	32.0
3	3.65(1H, m)	70.5	3.63 (1H, m)	70.4
4	-	40.8	-	40.8
5		139.8	-	139.7
6	5.60(1H, d)	119.6	5.57 (1H,d)	119.5
7	5.41(1H, d)	116.3	5.38 (1H, t)	116.3
8	-	141.4		141.3
9	-	46.3		46.2
10	-	37.0		37.0
11	-	21.1		21.1
12		39.1		39.1
13		42.8		42.8
14		54.6		54.5
15		23.0		23.0
16		28.3		28.2
17		55.7		55.7
18	0.66(3H, s)	12.1	0.63 (3H, s)	12.0
19	0.95(3H,s)	16.3	0.94 (3H, s)	16.2

20		40.4		40.3
21	1.05(3H, d)	19.7	1.03(3H,d)	19.6
22	5.22(1H, m)	132.0	5.16 (1H, m)	132.0
23	5.22(1H, m)	135.6	5.24 (1H, m)	135.5
24		42.8		42.8
25		33.1		33.1
26	0.85(3H, s)	21.1	0.83 (3H, s)	21.1
27	0.83(3H, d)	19.8	0.82 (3H, d)	19.9
28	0.94(3H, d)	17.6	0.91(3H,d)	17.5

The DEPT-135 experiment indicates the presence of 6 methyl groups at δ (12.1, 16.3,17.6, 19.7, 19.9 and 21.1ppm), 11 methine groups at δ (135.6, 132.0, 119.6, 116.3, 70.5, 55.7, 54.6, 46.3, 42.8, 40.4, 33.1), 7 methylene groups at δ(21.1, 23.0, 28.4, 32.0, 37.0, 38.4 and 40.8ppm) and quaternary carbons at 141.4, 139.8, 42.8 and 39.1 ppm. These quaternary carbons on the DEPT-135 are in line with information given on HSQC experiment (appendices **28** & **29**) as they are seen as non-protonated carbons.

Based on these experiments (HSQC, DEPT-135, HMBC, proton and carbon 13 NMR) (appendices **19-33**, table **3**) and literature reports[83], the structure of compound **325** is proposed as shown in the following figure **25**. It is a tetracyclic triterpenoid class of compound. The structure of this compound seems ergosterol. The compound was not reported from plants at all but its peroxide derivative[84]. It is almost found in fungi and isolated from them. This does not mean that it is never found in plants but rarely. Plants rarely synthesize this compound[85] and it is the first report that this compound is isolated from plants. Other sterols such as stigmasterol, campesterol and sitosterol have been isolated from plants and also reported to be found in Draceana species like *Dracaena cinnabari,*[86]. Qualitative phytochemical screening also indicated the presence of this class of compounds(triterpenoids) in *Draceana reflexa leaves*[87]. Even though, this compound was not isolated from plants previously, its peroxide derivative (Ergosterol Peroxide) was isolated from *Euphorbia species*[84]. The other probable reason will be that there might be mutual relationship between *D. steudneri* and fungi strain that is responsible for the production of this compound. *Particularly*, endophytic fungi are important sources of antimicrobial agents i.e they enhance the production of secondary metabolites that protect the plant against pathogens. They have the capacity to produce secondary metabolites and compounds those exhibit antimicrobial activities. A well-known anticancer agent called Taxol is reported that it is produced in plants by the help of *Pestalotiopsis microspore* endophytic fungi. The level of taxol

production was related to the presence of endophytic fungi strains[66,88,89]. A previous study indicated that the stem of *Draceana cambodiana* contained 60 endophyte fungi species while about 24 in the root of the plant[90]. These endophytic fungi may also enhance the production of rarely produced secondary metabolites like ergostrol[91].

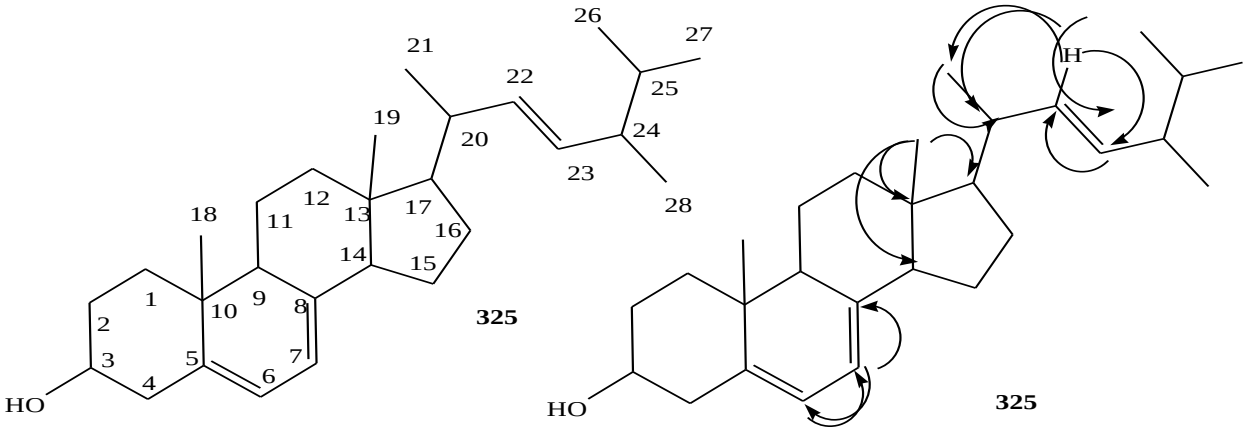


Figure 25: Proposed structure of compound 325 with MHBC

4.2. Antibacterial activity of the compounds and extracts

The isolated compounds and the crude extract (methanol and dichloromethane 1:1) of the same plant were screened for their antibacterial activities against four bacteria strains, two-gram positive (*S. aureus* and *B. cereus*) and two-gram negative (*E. coli* and *S. typhi*) bacteria (see appendix **2** and table **4**). Accordingly, the crude extract and the isolated compounds have not shown good biological activity against the tested strains. From the isolated compounds, compound **323** has shown some activity even though it is not so good. As proposed structure, this compound resembles the structure of droserone, a well-known naphthoquinone isolated from plants. It did not show a promising antibacterial activity against the tested bacteria strains. This is in agreement with previous report, as it did not show activity against *S. aureus*[92].

Compound ***325*** did not show antibacterial activity against bacteria strains aforementioned at 500µg/ml concentration. This result is in line with previous study that indicated both ergosterol and cholesterol do not inhibit bacteria at concentrations below 1024µg/ml, even though they can modify the activity of aminoglycoside antibacterial agents[93]. The peroxide derivative of ergosterol, which was isolated from *Euphorbia species*, had showed activity against

mycobacteria using bactec 960 system while it lacks activity using bactec 460 system at 0.625 to 50 mg/L. The same derivative compound of ergosterol was not active against *S. aureus* at 100mg/L[94].

Compound ***324*** also did not show good antibacterial activity. As per report of Li et al.(2019)[80], it has antibacterial activity. There is no much study is done about this compound on its biological activities.

Table 4: Antibacterial activity of isolated compounds and crude extracts

Bacteria strain	**Compound(sample) and their zone of inhibition in mm**					
	^{15A}**323** (500µg/ml)	^{17A}**324** (1mg/ml)	^{8A}**325** (500µg/ml)	Crude (100mg/ml)	Gentamycin [b] (3mg/ml)	DMSO (0.01%)[a]
S. aureus	7.9±0.14	7±0.14	7±0.00	7±0.00	25±0.00	-
E.coli	6.95±0.07	7±0.00	7±0.00	10±0.14	25±0.07	-
S. typhi	7.95±0.07	-	-	11±0.21	24±0.00	-
B. cereus	7.90±0.14	-	-	7±0.00	25±0.00	-

a negative control**, b** positive control, 15A**=** label for compound **323** on the disk**,** 17A =label for compound **324** on the disk**,** 8A =label for compound **325** on the disk

5. Conclusion and Recommendations

5.1. Conclusion

In the present study, three compounds **(323, 324 and 325)** were isolated from stembark of *D. steudneri*. They were isolated and purified using column chromatographic separation techniques using hexane and ethyl acetate solvent systems as mobile phase. Their structures were characterized using 1D NMR and 2D NMR techniques. They were of the naphthoquinone, chromones and triterpene classes of compounds. These three compounds were not isolated from the dracaena species, genus and family. This is the first report for their isolation from the asparageanacea or daracaenacea family, genus Dracaena and *steudneri* species. The pure compounds were identified as droserone (naphthoquinone), ergosterol (tetracyclic triterpene) and *2, 3-dimethyl-5-hydroxy-7-methoxychromone (chromone).*

The in-vitro antibacterial activities of the compounds, the crude extract of the stem bark revealed that the three compounds were of little/ no activity while the crude had showed marginal antibacterial activities against the tested bacteria strains.

5.2. Recommendations

Based on the present findings, further studies on the same plant are recommended as:

- ✓ Further isolation and purification of bioactive phytochemical constituents from the same plant since we did not isolate all the compounds.
- ✓ Assessing the relationship between *D. steudneri* and *endophytic* fungi as ergosterol can also be due to endophytic fungi living in plant cells
- ✓ Since it is claimed to treat wound by the community, antifungal activity and further antibacterial activities with other bacterial strains is needed.

6. References

(1) Newman, D. J.; Cragg, G. M. Natural Products as Sources of New Drugs over the Nearly Four Decades from 01/1981 to 09/2019. *J. Nat. Prod.* **2020**, *83* (3), 770–803. https://doi.org/10.1021/acs.jnatprod.9b01285.

(2) Armstrong, G. L.; Conn, L. A.; Pinner, R. W. Trends in Infectious Disease Mortality in the United States during the 20th Century. *Jama* **1999**, *281* (1), 61–66. https://doi.org/10.1001/jama.281.1.61.

(3) Atanasov, A. G.; Waltenberger, B.; Pferschy-Wenzig, E. M.; Linder, T.; Wawrosch, C.; Uhrin, P.; Temml, V.; Wang, L.; Schwaiger, S.; Heiss, E. H.; Rollinger, J. M.; Schuster, D.; Breuss, J. M.; Bochkov, V.; Mihovilovic, M. D.; Kopp, B.; Bauer, R.; Dirsch, V. M.; Stuppner, H. Discovery and Resupply of Pharmacologically Active Plant-Derived Natural Products: A Review. *Biotechnol. Adv.* **2015**, *33* (8), 1582–1614. https://doi.org/10.1016/j.biotechadv.2015.08.001.

(4) Vaníčková, L.; Pompeiano, A.; Maděra, P.; Massad, T. J.; Vahalík, P. Terpenoid Profiles of Resin in the Genus Dracaena Are Species Specific. *Phytochemistry* **2020**, *170* (October 2019), 112197. https://doi.org/10.1016/j.phytochem.2019.112197.

(5) Lösgen, S.; Magull, J.; Schulz, B.; Draeger, S.; Zeeck, A. Isofusidienols : Novel Chromone-3-Oxepines Produced by the Endophytic Fungus Chalara Sp., *Eur. J. Org. Chem.* 2008, 698–703. https://doi.org/10.1002/ejoc.200700839.

(6) Strobel, G.; Daisy, B.; Castillo, U.; Harper, J. Natural Products from Endophytic Microorganisms ⊥. **2004**, 257–268. https://doi.org/10.1021/np030397v.

(7) Amsalu, N.; Bezie, Y.; Fentahun, M.; Alemayehu, A.; Amsalu, G. Use and Conservation of Medicinal Plants by Indigenous People of Gozamin Wereda, East Gojjam Zone of Amhara Region, Ethiopia: An Ethnobotanical Approach. *Evidence-based Complement. Altern. Med.* **2018**, *2018*. https://doi.org/10.1155/2018/2973513.

(8) Kisangau, D. P.; Lyaruu, H. V. M.; Hosea, K. M.; Joseph, C. C. Use of Traditional Medicines in the Management of HIV/AIDS Opportunistic Infections in Tanzania: A Case

in the Bukoba Rural District. *J. Ethnobiol. Ethnomed.* **2007**, *3*, 1–8. https://doi.org/10.1186/1746-4269-3-29.

(9) Armitage, E. C.; Crew, B.; Dargie, R.; Analysis, P.; Wu, B.; Art, C.; Hutchinson, M.; Rayment, P. J.; Pope, I.; Bruni, N.; Edenbach, B.; Marketing, G.; Kimberly, P. R.; Kyprianou, F.; Baker, K.; Phillips, S.; Takeda, C.; Truong, L.; Hasegawa, S.; Sugita, Y.; Lubey, S.; Grayson, M.; Valiente, M.; Sun, G.; Yu, N.; Ma, S.; Pan, R.; Shi, J.; Zhang, P.; Jones, P. R.; Swinbanks, D.; Armitage, C. Index.

(10) By, C.; Neill, J. I. M. O. TACKLING DRUG-RESISTANT INFECTIONS GLOBALLY : FINAL REPORT AND RECOMMENDATIONS THE REVIEW ON. **2016**, No. May.

(11) Kamal, O.; Elmahi, O.; Uakkas, S.; Olalekan, B. Y.; Damilola, I. A.; Adedeji, O. J.; Hasan, M. M.; Carla, A.; Ahmad, S.; Essar, M. Y.; Thomson, D. J. Antimicrobial Resistance and One Health in the Post COVID‐19 Era : What Should Health Students Learn ? *Antimicrob. Resist. Infect. Control* **2022**, 1–4. https://doi.org/10.1186/s13756-022-01099-7.

(12) León-buitimea, A.; Garza-cárdenas, C. R.; Garza-cervantes, J. A. The Demand for New Antibiotics : Antimicrobial Peptides , Therapies as Future Strategies in Antibacterial Agent Design. **2020**, *11* (July), 1–10. https://doi.org/10.3389/fmicb.2020.01669.

(13) Ntaganda, J.; Habarurema, G.; Habinshuti, J.; Rutikanga, A.; Ndayambaje, J. B. Phytochemical Screening and in Vitro Antimicrobial Activity of Dracaena Afromontana Leaves. *Discov. Phytomedicine* **2020**, *7* (1), 7. https://doi.org/10.15562/phytomedicine.2020.112.

(14) Moshi, M. J.; Otieno, D. F.; Weisheit, A. Ethnomedicine of the Kagera Region, North Western Tanzania. Part 3: Plants Used in Traditional Medicine in Kikuku Village, Muleba District. *J. Ethnobiol. Ethnomed.* **2012**, *8* (1), 14. https://doi.org/10.1186/1746-4269-8-14.

(15) Nchiozem-Ngnitedem, V.-A.; Omosa, L. K.; Derese, S.; Efferth, T.; Spiteller, M. Cytotoxic Flavonoids from the Seeds of Dracaena Steudneri Engl against Leukemia Cancer Cell Lines. *Phytomedicine Plus* **2022**, *2* (2), 100234. https://doi.org/10.1016/j.phyplu.2022.100234.

(16) Parekh, J.; Chanda, S. V. In Vitro Antimicrobial Activity and Phytochemical Analysis of Some Indian Medicinal Plants. *Turkish J. Biol.* **2007**, *31* (1), 53–58.

(17) Packman, P. F.; Schulz, K. C. Mixed-Mode Fracture Model for Multi-Layered Composite Plates. *Am. Soc. Mech. Eng. Press. Vessel. Pip. Div. PVP* **1992**, *248*, 1–7.

(18) Wotoyitide, T. L. Antibacterial Activity of the Root Extracts of Draceana Laxissima and Draceana Fragrans on Selected Urinary Tract Pathogens. **2012**, No. January, 1–57.

(19) PER-01/PJ/2017, N. Phytochemical Investigation and Antibacterial Activities of Roots Extracts of Aloe Debrana. *Occup. Med. (Chic. Ill).* **2017**, *53* (4), 130.

(20) Omara, T. East African Quintessential Plants Claimed to Be Used as Blood Purifiers, Cleansers, Detoxifiers and Tonics: An Appraisal of Ethnobotanical Reports and Correlation with Reported Bioactivities. *Bull. Natl. Res. Cent.* **2021**, *45* (1). https://doi.org/10.1186/s42269-021-00637-4.

(21) Cambodiana, D. Antioxidant Phenolic Compounds Of. **2010**, 8904–8914. https://doi.org/10.3390/molecules15128904.

(22) Su, X. Q.; Song, Y. L.; Zhang, J.; Huo, H. X.; Huang, Z.; Zheng, J.; Zhang, Q.; Zhao, Y. F.; Xiao, W.; Li, J.; Tu, P. F. Dihydrochalcones and Homoisoflavanes from the Red Resin of Dracaena Cochinchinensis (Chinese Dragon's Blood). *Fitoterapia* **2014**, *99*, 64–71. https://doi.org/10.1016/j.fitote.2014.09.006.

(23) González, A. G.; León, F.; Sánchez-Pinto, L.; Padrón, J. I.; Bermejo, J. Phenolic Compounds of Dragon's Blood from Dracaena Draco. *J. Nat. Prod.* **2000**, *63* (9), 1297–1299. https://doi.org/10.1021/np000085h.

(24) Hernández, J. C.; León, F.; Estévez, F.; Quintana, J.; Bermejo, J. A Homo-Isoflavonoid and a Cytotoxic Saponin from Dracaena Draco. *Chem. Biodivers.* **2006**, *3* (1), 62–68. https://doi.org/10.1002/cbdv.200690008.

(25) Liu, J.; Mei, W. L.; Wu, J.; Zhao, Y. X.; Peng, M.; Dai, H. F. A New Cytotoxic Homoisoflavonoid from Dracaena Cambodiana. *J. Asian Nat. Prod. Res.* **2009**, *11* (2), 192–195. https://doi.org/10.1080/10286020802674962.

(26) Masaoud, M.; Ripperger, H.; Himmelreich, U.; Adam, G. Cinnabarone, a Biflavonoid from Dragon's Blood of Dracaena Cinnabari. *Phytochemistry* **1995**, *38* (3), 751–753. https://doi.org/10.1016/0031-9422(94)00718-9.

(27) Dai, H. F.; Wang, H.; Liu, J.; Wu, J.; Mei, W. L. Two New Biflavonoids from the Stem of Dracaena Cambodiana. *Chem. Nat. Compd.* **2012**, *48* (3), 376–378. https://doi.org/10.1007/s10600-012-0256-7.

(28) Zheng, Q.; Li, H.; Zhang, Y.; Yang, C. Dracaenogenins A and B , New Spirostanols from the Red Resin of Dracaena Cochinchinensis. **2005**, *1*, 160–164. https://doi.org/10.1016/j.steroids.2005.09.007.

(29) Luo, Y.; Dai, H. F.; Wang, H.; Mei, W. L. Chemical Constituents from Dragon's Blood of Dracaena Cambodiana. *Chin. J. Nat. Med.* **2011**, *9* (2), 112–114. https://doi.org/10.3724/SP.J.1009.2011.00112.

(30) Hu, L.; Wang, F. F.; Wang, X. H.; Yang, Q. S.; Xiong, Y.; Liu, W. X. Phytoconstituents from the Leaves of Dracaena Cochinchinensis (Lour.) S. C. Chen. *Biochem. Syst. Ecol.* **2015**, *63*, 1–5. https://doi.org/10.1016/j.bse.2015.09.012.

(31) Huang, H.; Lin, M.; Hwang, S.; Hwang, T.; Kuo, Y.; Chang, C.; Ou, C.; Kuo, Y. Two Anti-Inflammatory Steroidal Saponins from Dracaena Angustifolia Roxb. **2013**, 8752–8763. https://doi.org/10.3390/molecules18088752.

(32) Luo, Y.; Shen, H. Y.; Zuo, W. J.; Wang, H.; Mei, W. L.; Dai, H. F. A New Steroidal Saponin from Dragon's Blood of Dracaena Cambodiana. *J. Asian Nat. Prod. Res.* **2014**, *17* (4), 409–414. https://doi.org/10.1080/10286020.2014.967229.

(33) Shen, H. Y.; Zuo, W. J.; Wang, H.; Zhao, Y. X.; Guo, Z. K.; Luo, Y.; Li, X. N.; Dai, H. F.; Mei, W. L. Steroidal Saponins from Dragon's Blood of Dracaena Cambodiana. *Fitoterapia* **2014**, *94*, 94–101. https://doi.org/10.1016/j.fitote.2014.01.020.

(34) Du Toit, K.; Drewes, S. E.; Bodenstein, J. The Chemical Structures, Plant Origins, Ethnobotany and Biological Activities of Homoisoflavanones. *Nat. Prod. Res.* **2010**, *24* (5), 457–490. https://doi.org/10.1080/14786410903335174.

(35) Reanmongkol, W.; Subhadhirasakul, S. Antinociceptive and Antipyretic Activities of Extracts and Fractions from Dracaena Loureiri in Experimental Animals. No. April 2003.

(36) Fleischer, T. C.; Waigh, R. D.; Waterman, P. G. A Novel Retrodihydrochalcone from the Stem Bark of Uvaria Mocoli. *Phytochemistry* **1998**, *47* (7), 1387–1391. https://doi.org/10.1016/S0031-9422(98)80009-5.

(37) Risinger, A. L.; Li, J.; Bennett, M. J.; Rohena, C. C.; Peng, J.; Schriemer, D. C.; Mooberry, S. L. Taccalonolide Binding to Tubulin Imparts Microtubule Stability and Potent in Vivo Activity. *Cancer Res.* **2013**, *73* (22), 6870–6892. https://doi.org/10.1158/0008-5472.CAN-13-1346.

(38) Wilkin, P.; Sohmer, S. H.; The, A. P. D.; Psychot, G. Review Reviewed Work (s): Flora of Tropical East Africa . Dracaenaceae by G . Mwachala , P . K . Mbugua , H . J . Beentje and S . A . Ghazanfar Review by : Paul Wilkin Published by : Springer on Behalf of Royal Botanic Gardens , Kew Stable URL : Https:/. **2022**, *63* (2).

(39) Tugume, P.; Kakudidi, E. K.; Buyinza, M.; Namaalwa, J.; Kamatenesi, M.; Mucunguzi, P.; Kalema, J. Ethnobotanical Survey of Medicinal Plant Species Used by Communities around Mabira Central Forest Reserve, Uganda. *J. Ethnobiol. Ethnomed.* **2016**, *12* (1), 1–28. https://doi.org/10.1186/s13002-015-0077-4.

(40) Stanley, T. Research Paper. *10 Performance-Based Proj. Sci. Classr.* **2021**, *4*, 107–115. https://doi.org/10.4324/9781003232506-9.

(41) Bunalema, L.; Obakiro, S.; Tabuti, J. R. S.; Waako, P. Knowledge on Plants Used Traditionally in the Treatment of Tuberculosis in Uganda. *J. Ethnopharmacol.* **2014**, *151* (2), 999–1004. https://doi.org/10.1016/j.jep.2013.12.020.

(42) Italiano, I. Dragon's Blood in East and West Africa , Arabia And The Canary Islands: Mark Milburn Source : Africa : Rivista Trimestrale Di Studi e Documentazione Dell ' Istituto Italiano per l ' Africa e l ' Oriente , Settembre 1984 , Anno 39 , No . 3 (. **1984**, *3* (3), 486–493.

(43) Ong, M. G.; Mat Yusuf, S. N. A.; Lim, V. Pharmacognostic and Antioxidant Properties of

Dracaena Sanderiana Leaves. *Antioxidants* **2016**, *5* (3), 1–9. https://doi.org/10.3390/antiox5030028.

(44) Damen, T. H. J.; van der Burg, W. J.; Wiland-Szymańska, J.; Sosef, M. S. M. Taxonomic Novelties in African Dracaena (Dracaenaceae). *Blumea J. Plant Taxon. Plant Geogr.* **2018**, *63* (1), 31–53. https://doi.org/10.3767/blumea.2018.63.01.05.

(45) Fan, J. Y.; Yi, T.; Sze-To, C. M.; Zhu, L.; Peng, W. L.; Zhang, Y. Z.; Zhao, Z. Z.; Chen, H. B. A Systematic Review of the Botanical, Phytochemical and Pharmacological Profile of Dracaena Cochinchinensis, a Plant Source of the Ethnomedicine Dragon's Blood. *Molecules* **2014**, *19* (7), 10650–10669. https://doi.org/10.3390/molecules190710650.

(46) Nchiozem-Ngnitedem, V. A.; Omosa, L. K.; Bedane, K. G.; Derese, S.; Brieger, L.; Strohmann, C.; Spiteller, M. Anti-Inflammatory Steroidal Sapogenins and a Conjugated Chalcone-Stilbene from Dracaena Usambarensis Engl. *Fitoterapia* **2020**, *146* (July), 104717. https://doi.org/10.1016/j.fitote.2020.104717.

(47) Luo, Y.; Wang, H.; Zhao, Y. X.; Zeng, Y. B.; Shen, H. Y.; Dai, H. F.; Mei, W. L. Cytotoxic and Antibacterial Flavonoids from Dragon's Blood of Dracaena Cambodiana. *Planta Med.* **2011**, *77* (18), 2053–2056. https://doi.org/10.1055/s-0031-1280086.

(48) Badoni, R.; Deepak, S.; Semwal, K.; Combrinck, S. Health Benefits of Chromones : Common Ingredients of Our Daily Diet. *Phytochem. Rev.* **2020**, *19* (4), 761–785. https://doi.org/10.1007/s11101-020-09681-w.

(49) Edwards, A. M.; Howell, J. B. L. The Chromones : History , Chemistry and Clinical Development . A Tribute to the Work of Dr R . E . C . Altounyan. **2000**, *30*.

(50) Sharma, S. K.; Kumar, S.; Chand, K.; Kathuria, A.; Gupta, A.; Jain, R. An Update on Natural Occurrence and Biological Activity of Chromones #. **2011**, 3825–3852.

(51) Aenboonrueng, J. S.; Rjchomphu, W. A.; Uksamrarn, A. S. Bioactive Constituents of the Root Bark of Artocarpus Rigidus Subsp . Rigidus. **2006**, *54* (October).

(52) Plant, M.; Raw, L.; Kim, Y. A.; Kong, C.; Park, H. H.; Lee, E.; Jang, M. Anti-Inflammatory Activity of Heterocarpin from the Salt Marsh Plant Corydalis Heterocarpa

in LPS-Induced RAW 264.7 Macrophage Cells. **2015**, *2*, 14474–14486. https://doi.org/10.3390/molecules200814474.

(53) Silva, C. F. M.; Pinto, D. C. G. A.; Silva, A. M. S. Chromones : A Promising Ring System for New Anti- Inflammatory Drugs. **2016**, 1–10. https://doi.org/10.1002/cmdc.201600359.

(54) Chromones, B. Monitoring the Chemical Profile in Agarwood. https://doi.org/10.3390/molecules23061261.

(55) Una, D.; Estructurada, R.; Itzel, L.; López, L.; Daniel, S.; Flores, N.; Yesenia, S.; Belmares,. Naphthoquinones : Biological Properties and Synthesis of Lawsone and Derivatives — A Structured Review. **2014**, 248–258.

(56) Pereyra, C. E.; Dantas, R. F.; Ferreira, S. B.; Gomes, L. P.; Paes, F.; Jr, S. The Diverse Mechanisms and Anticancer Potential of Naphthoquinones. *Cancer Cell Int.* **2019**, 1–20. https://doi.org/10.1186/s12935-019-0925-8.

(57) Mone, N. S.; Bhagwat, S. A.; Sharma, D.; Chaskar, M.; Patil, R. H.; Zamboni, P.; Nawani, N. N.; Satpute, S. K. Naphthoquinones and Their Derivatives : Emerging Trends in Combating Microbial Pathogens. **2021**.

(58) Bringmann, G.; Rischer, H.; Wohlfarth, M.; Schlauer, J. Droserone from Cell Cultures of Triphyophyllum Peltatum (Dioncophyllaceae) and Its Biosynthetic Origin P. **2000**, *53*.

(59) Ryan, R. P.; Germaine, K.; Franks, A.; Ryan, D. J.; Dowling, D. N. Bacterial Endophytes : Recent Developments and Applications. **2008**, *278*, 1–9. https://doi.org/10.1111/j.1574-6968.2007.00918.x.

(60) Khare, E.; Mishra, J.; Arora, N. K. Multifaceted Interactions Between Endophytes and Plant : Developments and Prospects. **2018**, *9* (November), 1–12. https://doi.org/10.3389/fmicb.2018.02732.

(61) Taylor, P.; Sun, X.; Guo, L. Mycology : An International Journal on Fungal Biology Endophytic Fungal Diversity : Review of Traditional and Molecular Techniques. **2012**, No. July 2013, 37–41.

(62) Tanaka, A.; Christensen, M. J.; Takemoto, D.; Park, P.; Scott, B. Reactive Oxygen Species Play a Role in Regulating a Fungus – Perennial Ryegrass Mutualistic Interaction. **2006**, *18* (April), 1052–1066. https://doi.org/10.1105/tpc.105.039263.1.

(63) Chen, L.; Zhang, Q.; Jia, M.; Ming, Q.; Yue, W.; Qin, L.; Han, T.; Chen, L.; Zhang, Q.; Jia, M.; Ming, Q.; Yue, W.; Rahman, K.; Qin, L. Critical Reviews in Microbiology Endophytic Fungi with Antitumor Activities : Their Occurrence and Anticancer Compounds Endophytic Fungi with Antitumor Activities : Their Occurrence and Anticancer Compounds. **2016**, *7828* (December). https://doi.org/10.3109/1040841X.2014.959892.

(64) Li, S.; Zhang, X.; Wang, X.; Zhao, C. European Journal of Medicinal Chemistry Novel Natural Compounds from Endophytic Fungi with Anticancer Activity. *Eur. J. Med. Chem.* **2018**, *156*, 316–343. https://doi.org/10.1016/j.ejmech.2018.07.015.

(65) Cheng, T.; Kolařík, M.; Quijada, L.; Stadler, M. IMA Fungus A Re‐Assessment of Taxomyces Andreanae , the Alleged Taxol‐Producing Fungus , Using Comparative Genomics. **2022**. https://doi.org/10.1186/s43008-022-00103-4.

(66) Uzma, F.; Mohan, C. D.; Hashem, A.; Konappa, N. M.; Salomone, S. Endophytic Fungi — Alternative Sources of Cytotoxic Compounds : A Review. **2018**, *9* (April), 1–37. https://doi.org/10.3389/fphar.2018.00309.

(67) Cui, C.; Li, X.; Li, C.; Proksch, P.; Wang, B. Cytoglobosins A - G , Cytochalasans from a Marine-Derived Endophytic Fungus , Chaetomium Globosum QEN-14. **2010**, *85* (Figure 1), 729–733.

(68) Dupont, S.; Lemetais, G.; Ferreira, T.; Cayot, P.; Gervais, P.; Beney, L. Ergosterol Biosynthesis : A Fungal Pathway For Life On Land ? **2012**, 1–8. https://doi.org/10.5061/dryad.pd28pm7n.

(69) Mohammed, B.; Hindustan, I.; Ahad, A. Phytochemical Investigation.

(70) Edeoga, H. O.; Okwu, D. E.; Mbaebie, B. O. Phytochemical Constituents of Some Nigerian Medicinal Plants. *African J. Biotechnol.* **2005**, *4* (7), 685–688.

https://doi.org/10.5897/AJB2005.000-3127.

(71) Razmavar, S.; Abdulla, M. A.; Ismail, S. B.; Hassandarvish, P. Antibacterial Activity of Leaf Extracts of Baeckea Frutescens against Methicillin-Resistant Staphylococcus Aureus. **2014**, *2014*.

(72) Anthony Timmers, M.; Anthony Dias, D.; Urban, S. HPLC-NMR Chemical Profiling of the Australian Carnivorous Plant, Drosera Erythrohiza Subspecies Magna. *Nat. Prod. J.* **2013**, *3* (1), 35–41. https://doi.org/10.2174/2210315511303010008.

(73) Kreher, B.; Neszmélyi, A.; Wagner, H. Naphthoquinones from Dionaea Muscipula. *Phytochemistry* **1990**, *29* (2), 605–606. https://doi.org/10.1016/0031-9422(90)85125-Y.

(74) Bringmann, G.; Rüdenauer, S.; Irmer, A.; Bruhn, T.; Brun, R.; Heimberger, T.; Stühmer, T.; Bargou, R.; Chatterjee, M. Antitumoral and Antileishmanial Dioncoquinones and Ancistroquinones from Cell Cultures of Triphyophyllum Peltatum (Dioncophyllaceae) and Ancistrocladus Abbreviatus (Ancistrocladaceae). *Phytochemistry* **2008**, *69* (13), 2501–2509. https://doi.org/10.1016/j.phytochem.2008.06.019.

(75) Taylor, P.; Diospyros, L.; Nematollahi, A.; Aminimoghadamfarouj, N.; Wiart, C. Journal of Asian Natural Products Reviews on 1 , 4-Naphthoquinones From. *J. Asian Nat. Prod. Res.* **2012**, *14* (1), 37–41.

(76) Charisiadis, P.; Kontogianni, V. G.; Tsiafoulis, C. G.; Tzakos, A. G.; Siskos, M.; Gerothanassis, I. P. 1H-NMR as a Structural and Analytical Tool of Intra- and Intermolecular Hydrogen Bonds of Phenol-Containing Natural Products and Model Compounds. **2014**, 13643–13682. https://doi.org/10.3390/molecules190913643.

(77) Takenaka, Y.; Tanahashi, T.; Nagakura, N.; Hamada, N. 2,3-Dialkylchromones from Mycobiont Cultures of the Lichen. **2000**, *53* (7), 1589–1593.

(78) Article, R. A Phytopharmacological Evaluation of Cassia Fistula . A Comprehensive Review. **2020**, *62* (09), 45–53.

(79) Chromone, N.; Alkaloid, I. NPC Natural Product Communications. **2016**, *2* (Table 1), 9–12. https://doi.org/10.1177/1934578X1601100927.

(80) Li, M, Li, M, Zhang, K., Metabolites, N. Non-Volatile Metabolites from Trichoderma Spp. 2019, 9, 58; https://doi.org/10.3390/metabo9030058.

(81) Hoffman, R. E. Standardization of Chemical Shifts of TMS and Solvent Signals in NMR Solvents. **2006**, No. March, 606–616. https://doi.org/10.1002/mrc.1801.

(82) Li, X.; Wu, Q.; Xie, Y.; Ding, Y.; Du, W. W.; Sdiri, M.; Yang, B. B. Ergosterol Purified from Medicinal Mushroom Amauroderma Rude Inhibits Cancer Growth in Vitro and in Vivo by Up-Regulating Multiple Tumor Suppressors. **2000**, *6* (19).

(83) Woo, J.; Lermer, L.; Chilton, J.; Klingeman, H. G.; Towers, G. H. N. Antitumor Sterols from the Mycelia of Cordyceps Sinensis. **1999**, *51*, 4–11.

(84) Duarte, N.; Ferreira, M. U.; Martins, M.; Viveiros, M. Antibacterial Activity of Ergosterol Peroxide against Mycobacterium Tuberculosis : Dependence upon System and Medium Employed. **2007**, *604*, 601–604. https://doi.org/10.1002/ptr.

(85) Brumfield, K. M.; Laborde, S. M.; Moroney, J. V; Brumfield, K. M.; Laborde, S. M.; A, J. V. M. A Model for the Ergosterol Biosynthetic Pathway in Chlamydomonas Reinhardtii. *Eur. J. Phycol.* **2017**, *52* (1), 64–74. https://doi.org/10.1080/09670262.2016.1225318.

(86) Masaoud, M.; Schmidt, J.; Adam, G. J. Sterols and triterpenoids from Dracaena cinnabari. *Planta Medica*, **1995**, *38* (3), 795–796.

(87) Shukla, A.; Vats, S.; Shukla, R. K. Phytochemical Screening, Proximate Analysis and Antioxidant Activity of Dracaena Reflexa Lam. Leaves. *Indian J. Pharm. Sci.* **2015**, *77* (5), 640–644. https://doi.org/10.4103/0250-474X.169035.

(88) Soliman, S. S. M.; Trobacher, C. P.; Tsao, R.; Greenwood, J. S.; Raizada, M. N. A Fungal Endophyte Induces Transcription of Genes Encoding a Redundant Fungicide Pathway in Its Host Plant. *BMC Plant Biol.* **2013**, *13* (1), 1. https://doi.org/10.1186/1471-2229-13-93.

(89) Stierle, A.; Strobel, G.; Stierle, D. Of Pacific Yew. **1978**, No. 11, 2–4.

(90) Gong, L.; Guo, S. Endophytic Fungi from Dracaena Cambodiana and Aquilaria Sinensis and Their Antimicrobial Activity. **2009**, *8* (5), 731–736.

(91) Andhale, N. B.; Shahnawaz, M.; Ade, A. B. Fungal Endophytes of Plumbago Zeylanica L . Enhances Plumbagin Content. *Bot. Stud.* **2019**, 1–9. https://doi.org/10.1186/s40529-019-0270-1.

(92) Krychowiak, M.; Grinholc, M.; Banasiuk, R.; Krauze-Baranowska, M.; Głód, D.; Kawiak, A.; Królicka, A. Combination of Silver Nanoparticles and Drosera Binata Extract as a Possible Alternative for Antibiotic Treatment of Burn Wound Infections Caused by Resistant Staphylococcus Aureus. *PLoS One* **2014**, *9* (12), 1–20. https://doi.org/10.1371/journal.pone.0115727.

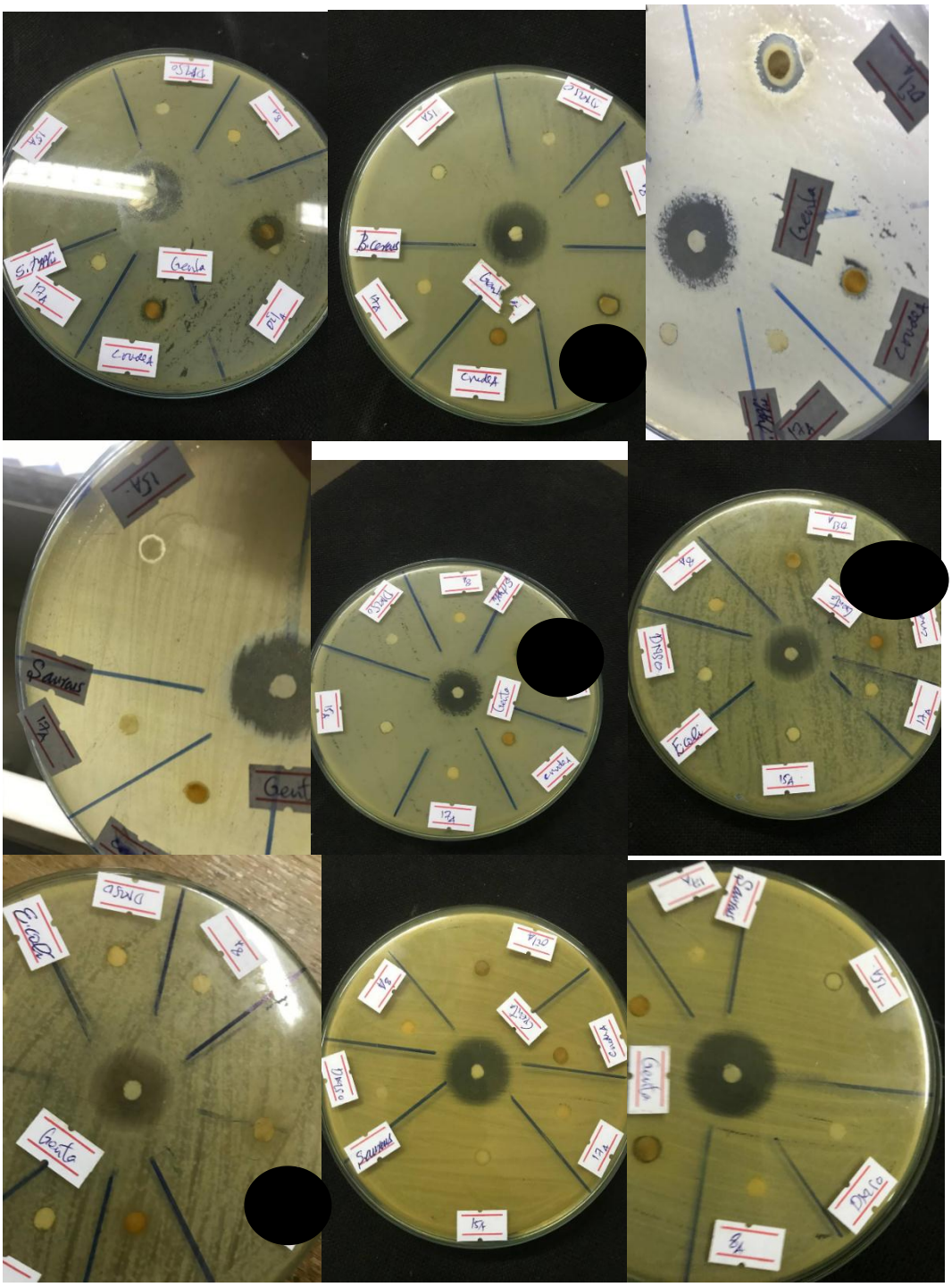
(93) Jacqueline, Q.; Andrade, C.; Morais-braga, M. F. B.; Guedes, G. M. M.; Tintino, S. R.; Freitas, M. A.; Menezes, I. R. A.; Coutinho, H. D. M. ScienceDirect Enhancement of the Antibiotic Activity of Aminoglycosides by Alpha-Tocopherol and Other Cholesterol Derivates. *Biomed. Pharmacother.* **2014**. https://doi.org/10.1016/j.biopha.2014.10.011.

(94) Jang, M. H.; Piao, X. L.; Kim, J. M.; Kwon, S. W.; Park, J. H. Inhibition of Cholinesterase and Amyloid-&bgr; Aggregation by Resveratrol Oligomers from Vitis Amurensis. *Phyther. Res.* **2008**, *22* (4), 544–549. https://doi.org/10.1002/ptr.

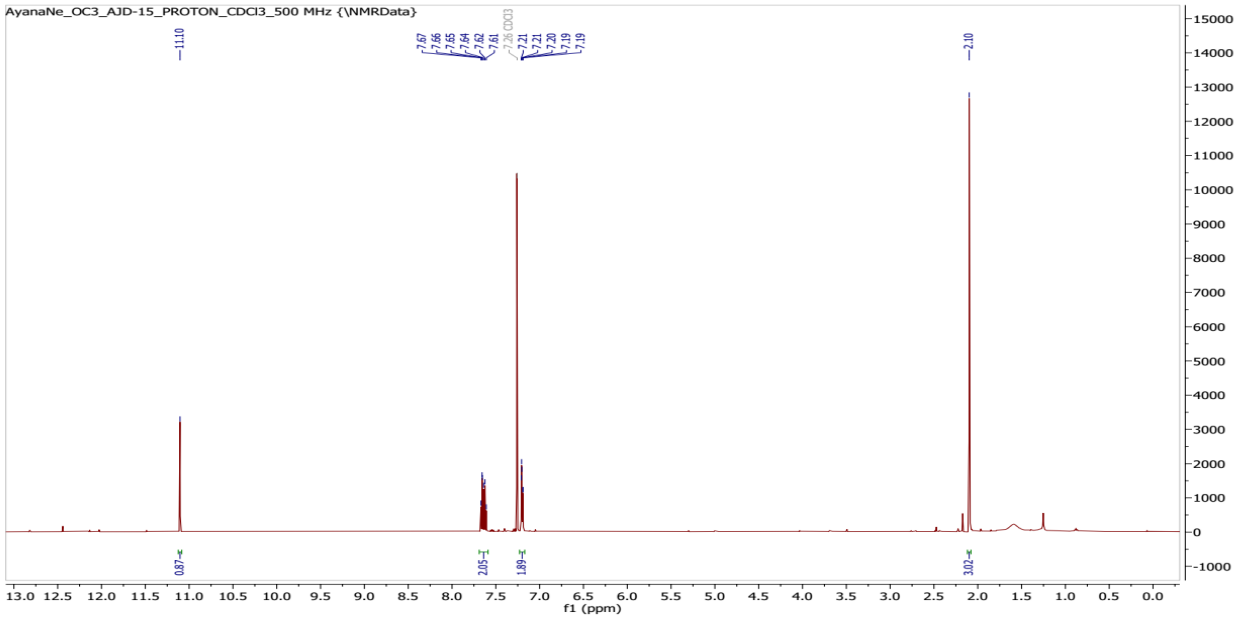
7. Appendices

Appendix1: Compound isolation using column chromatography

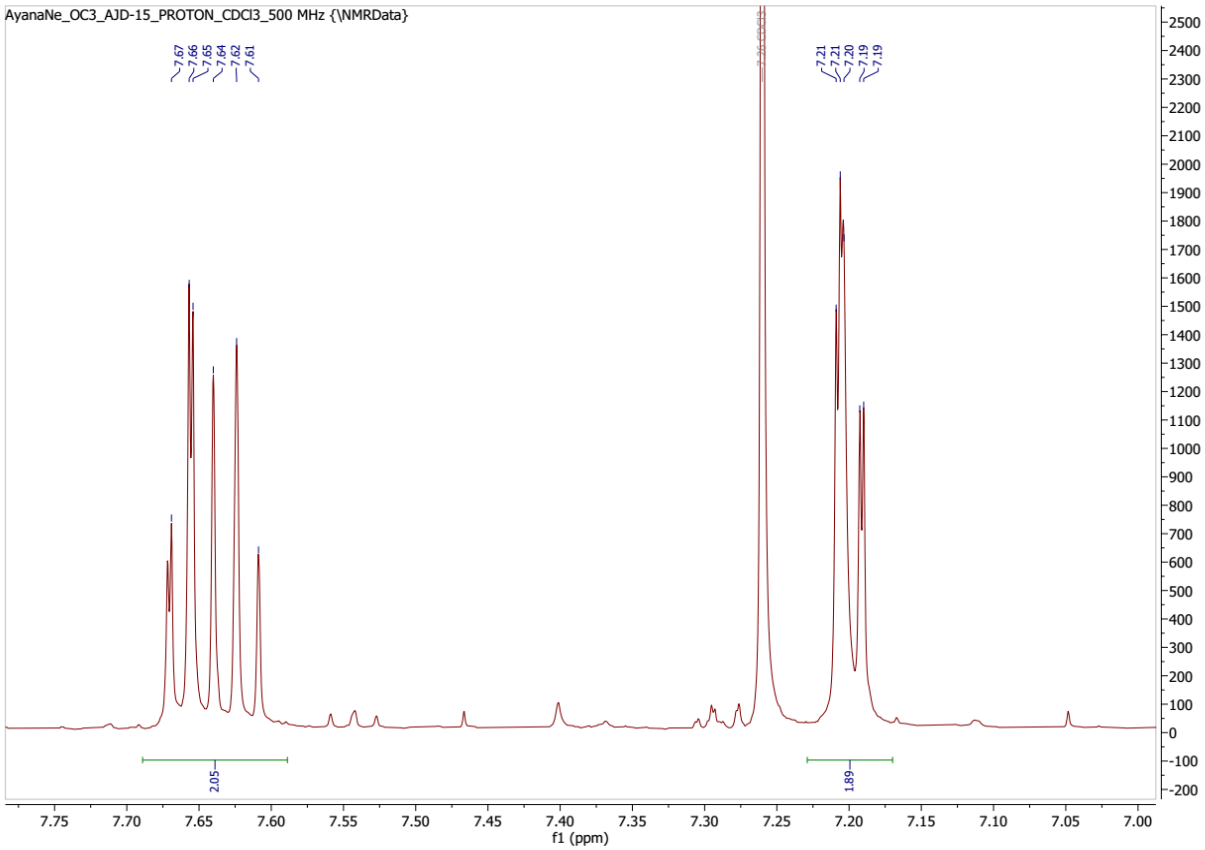
Appendix2: Invitro antibacterial activities of crude extracts and isolated compounds



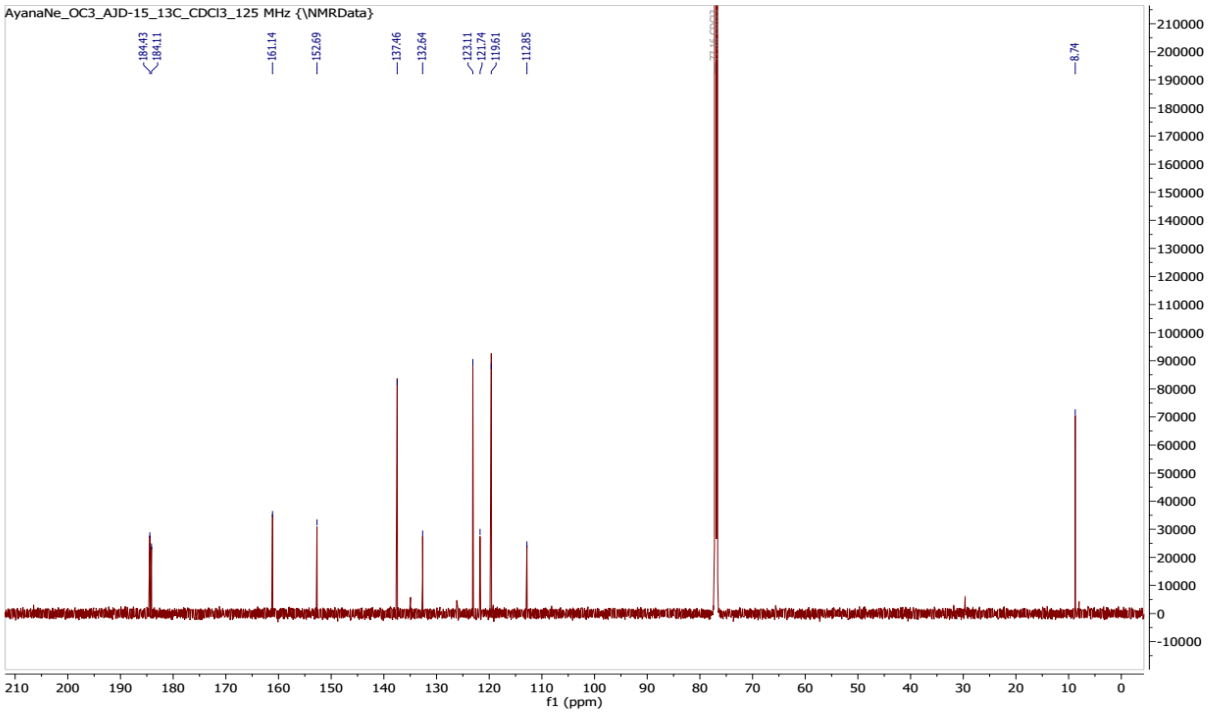
Appendix3: ^{1}H NMR of compound 323 in $CDCl_3$



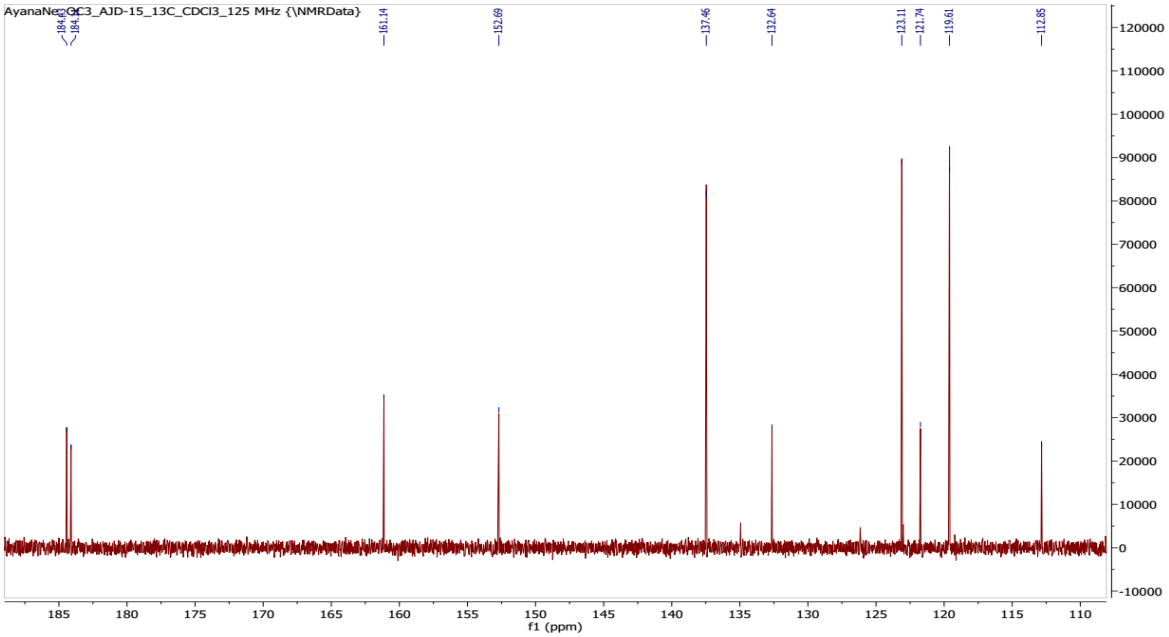
Appendix4: Expanded ^{1}H NMR of compound 323 in $CDCl_3$



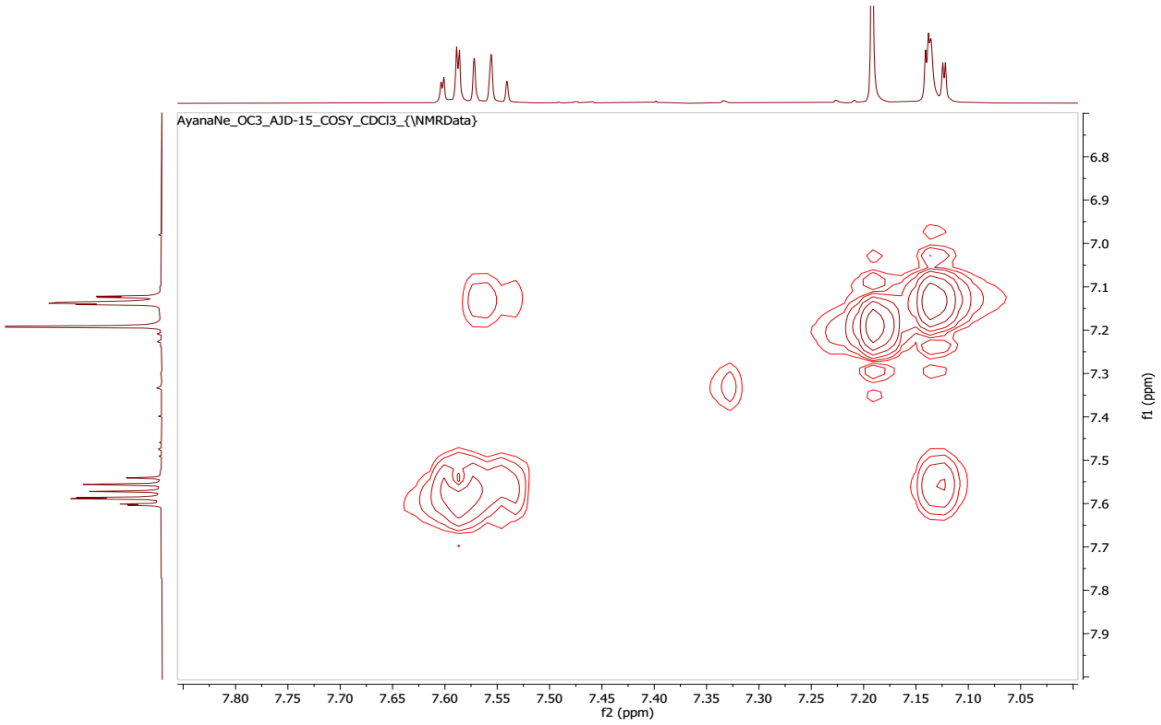
Appendix5: ^{13}C NMR spectra of compound 323 in $CDCl_3$



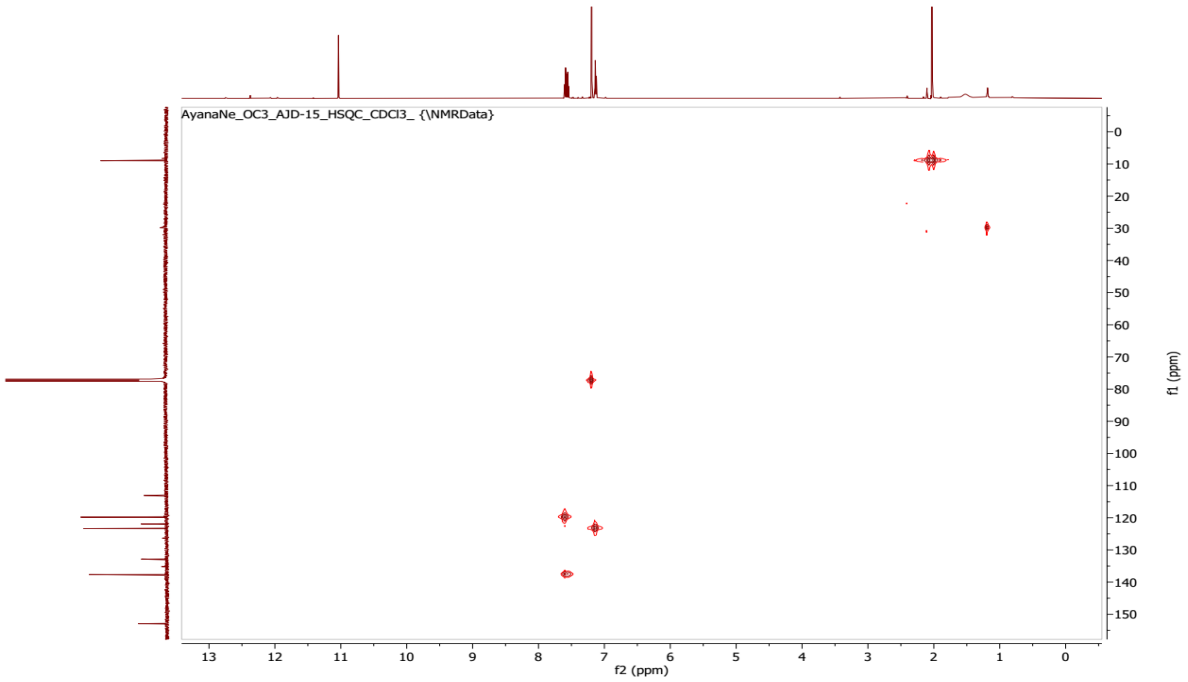
Appendix6: Expanded ^{13}C NMR spectra of compound 323 in $CDCl_3$



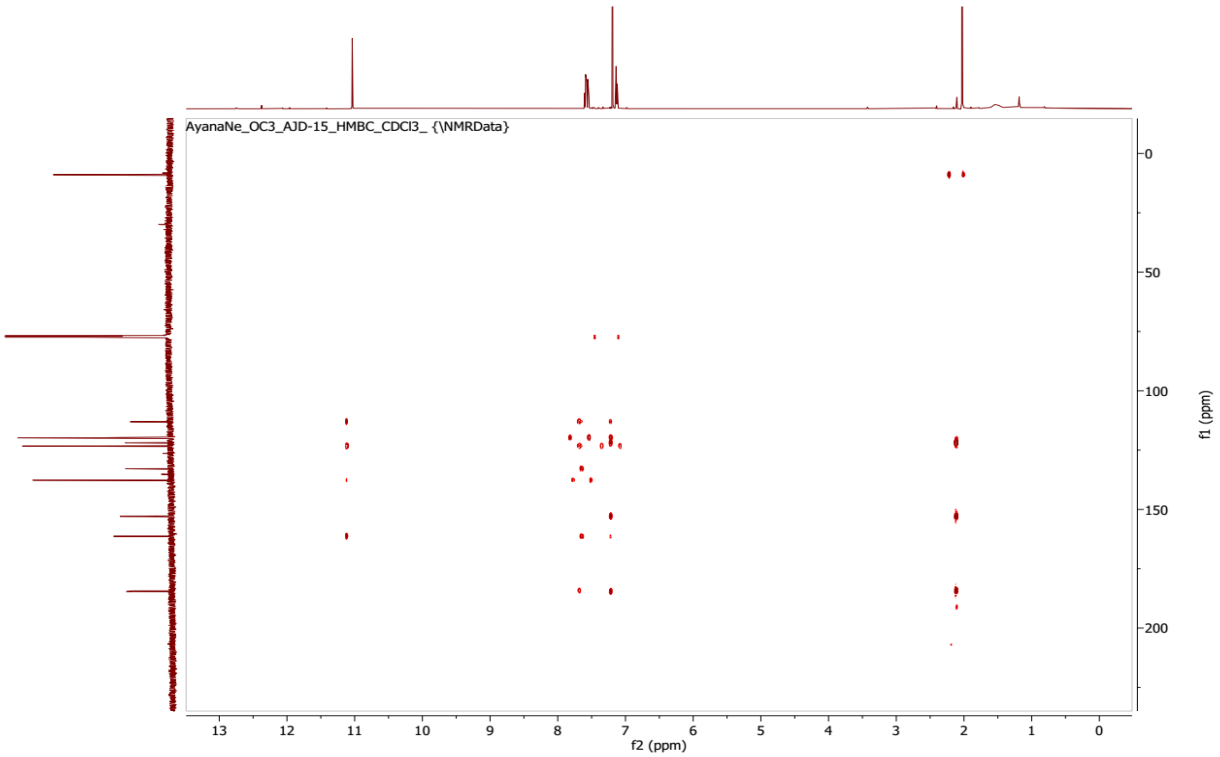
Appendix7: COSY spectra of compound 323 in $CDCl_3$



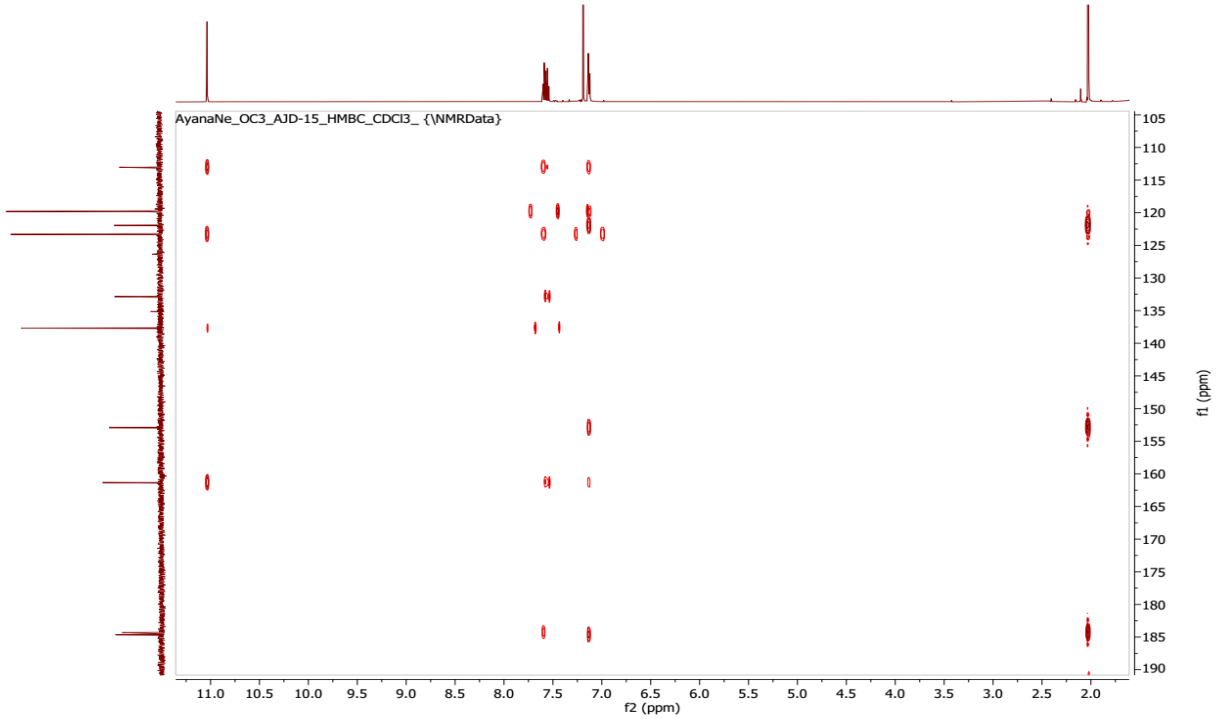
Appendix8: HSQC spectra of compound 323 in $CDCl_3$



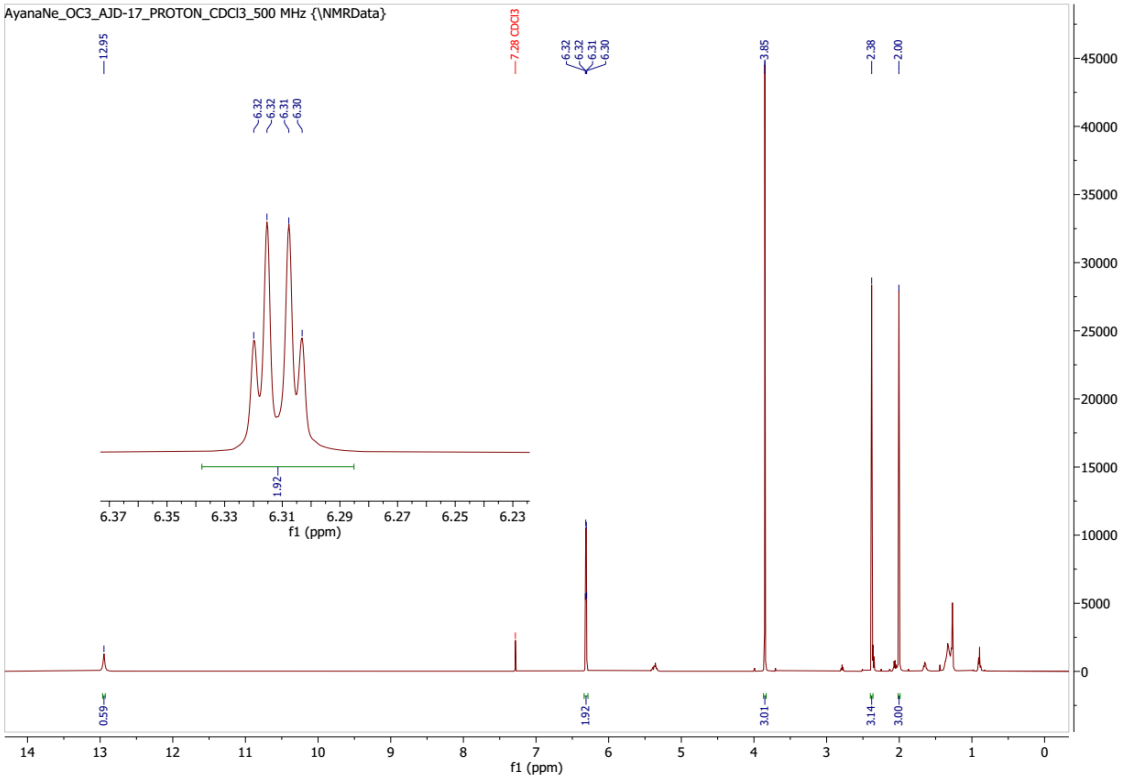
Appendix9: HMBC spectra of compound 323 in $CDCl_3$



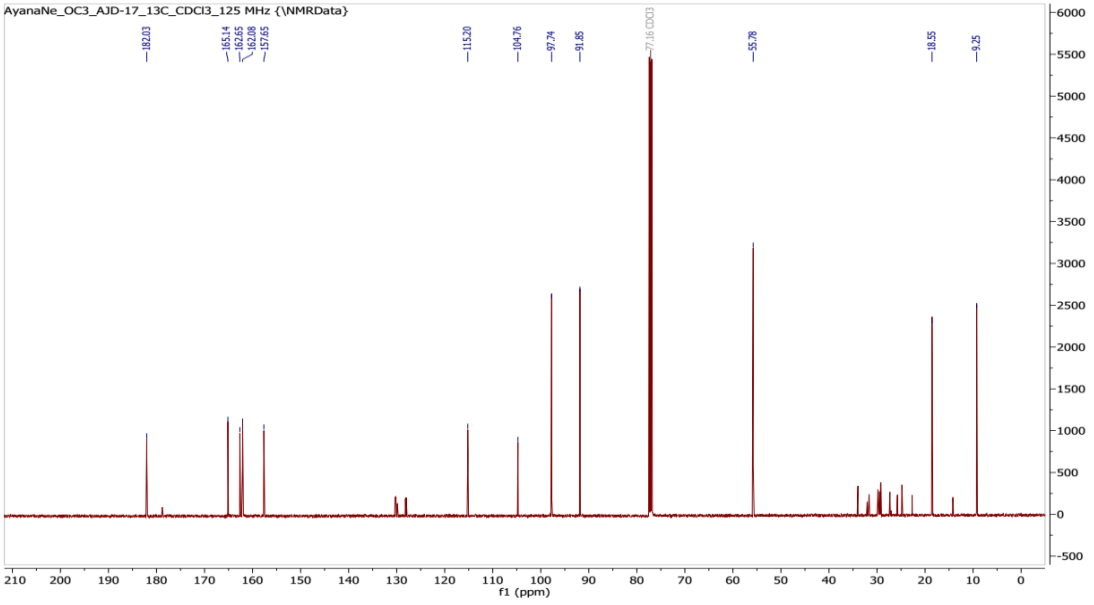
Appendix10: Expanded HMBC spectra of compound 323 in $CDCl_3$



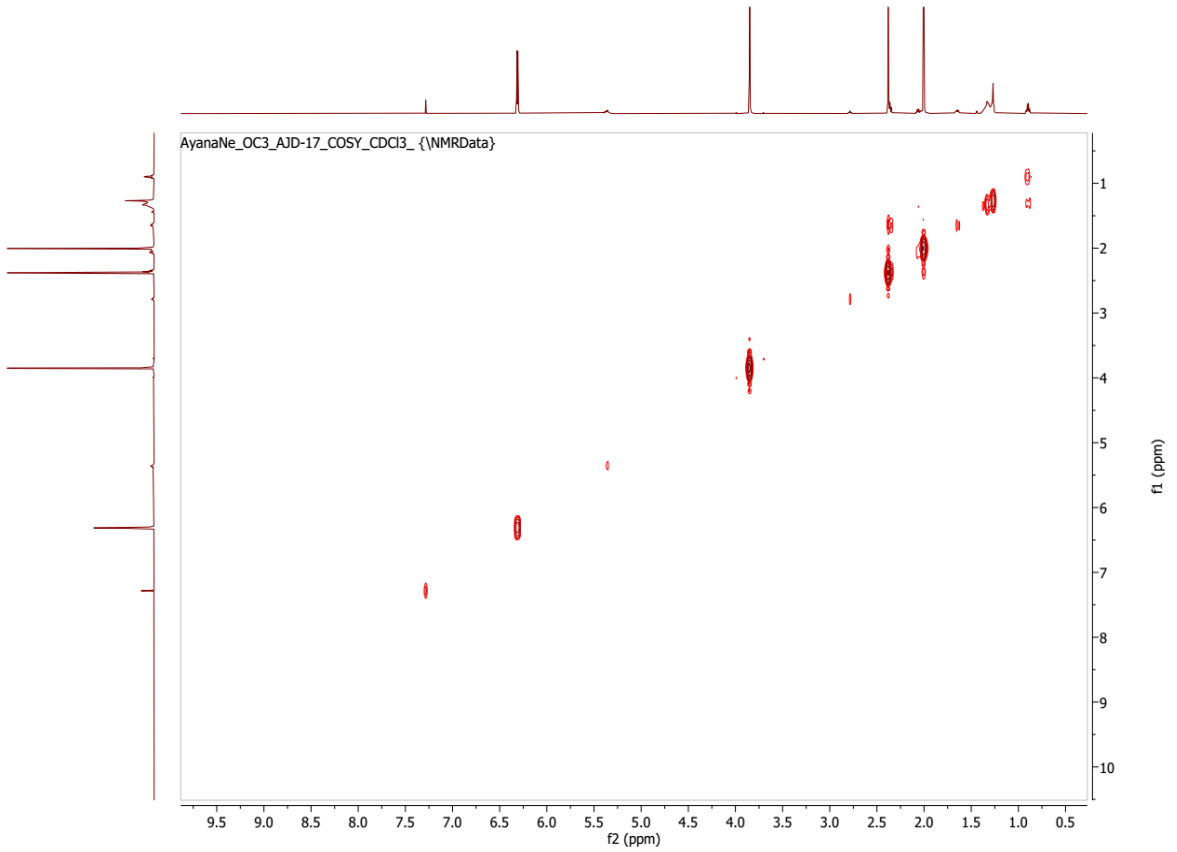
Appendix11: ^{1}H NMR spectra of compound 324 in $CDCl_3$



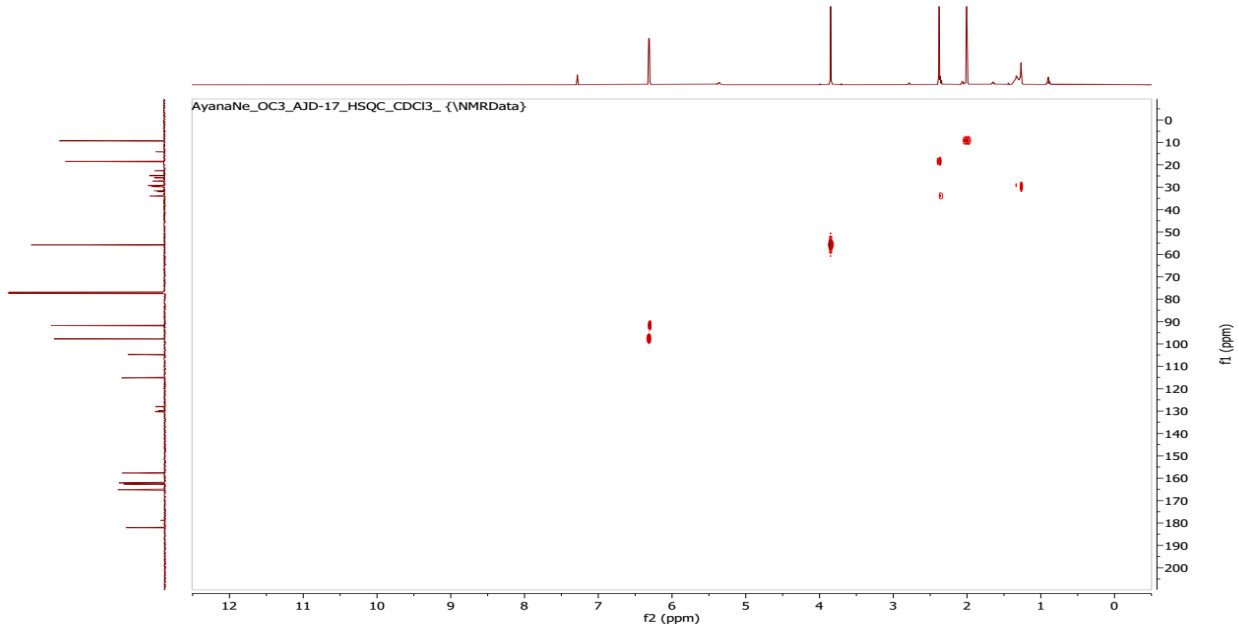
Appendix12: ^{13}C NMR spectra of compound 324 in $CDCl_3$



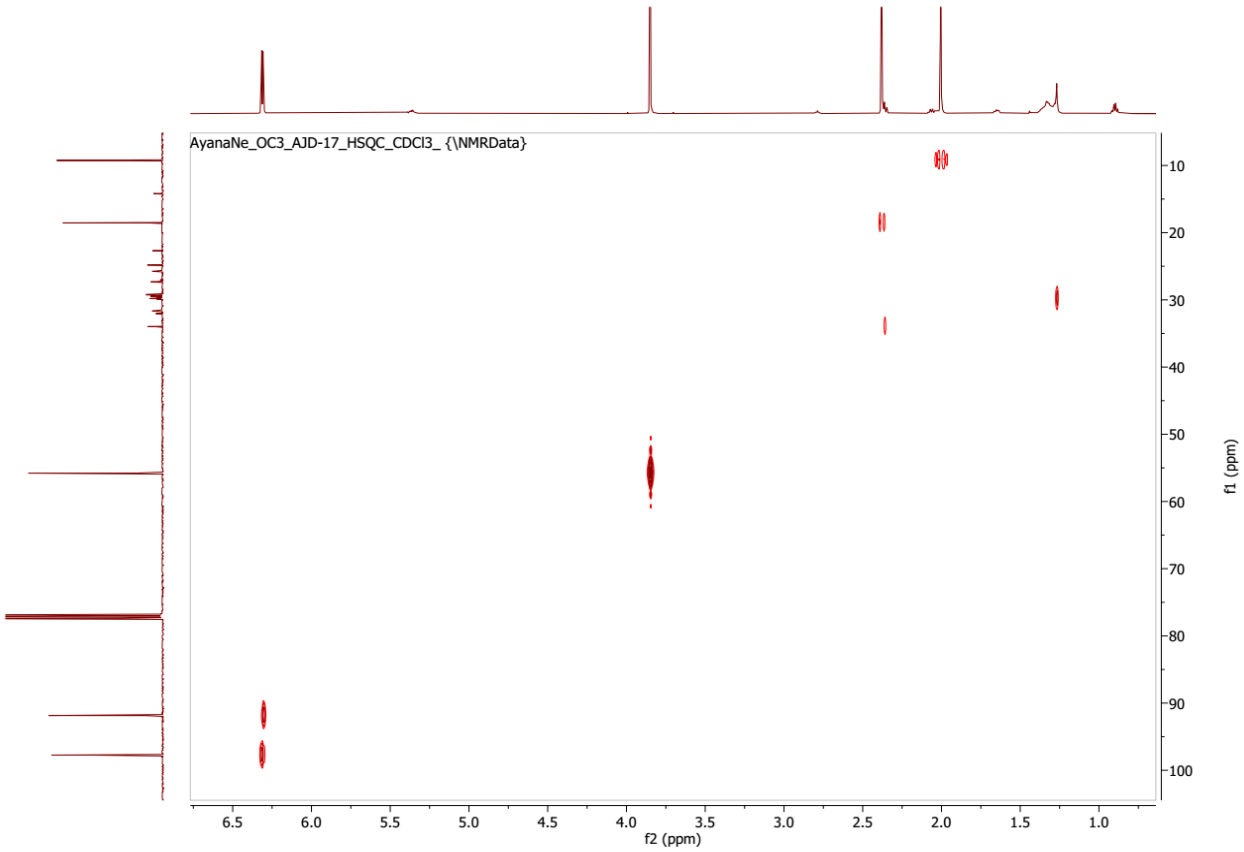
Appendix13: COSY spectra of compound 324 in $CDCl_3$



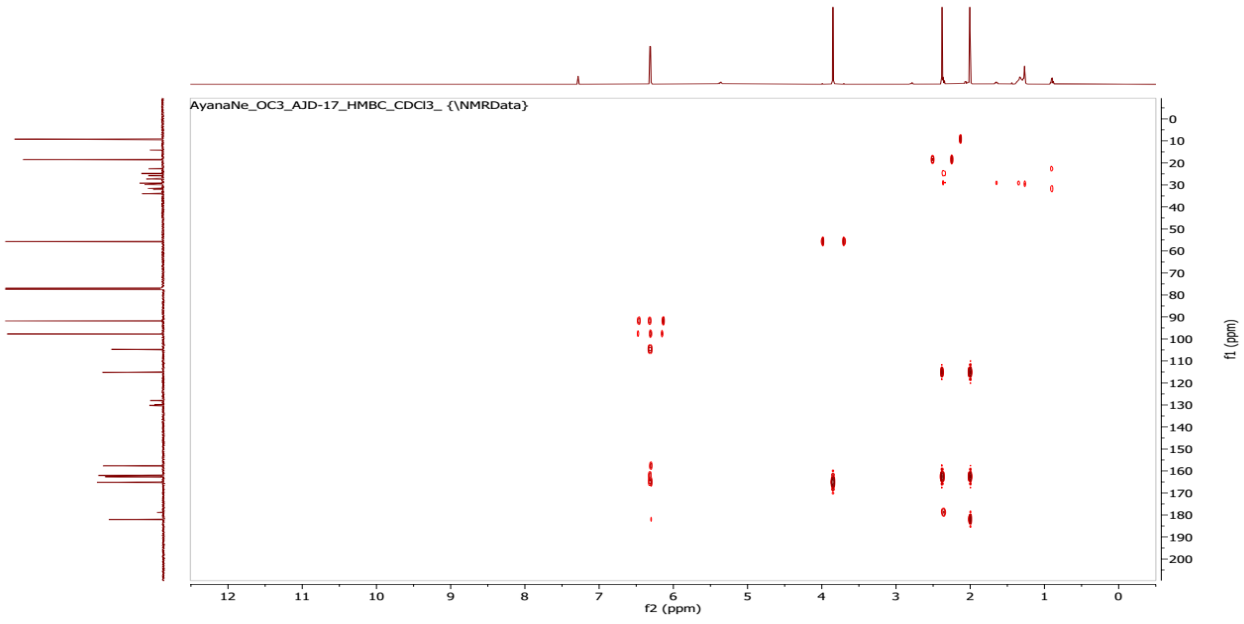
Appendix14: HSQC spectra of compound 324 in $CDCl_3$



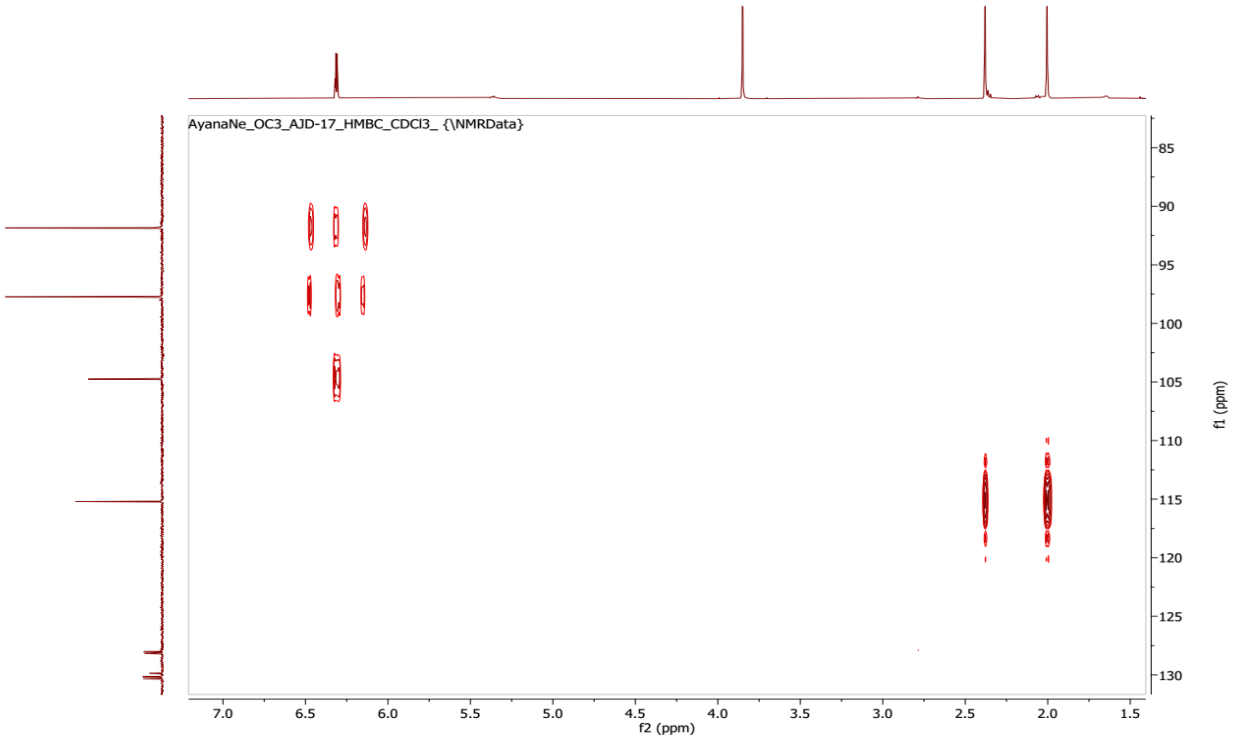
Appendix15: Expanded HSQC spectra of compound 324 in $CDCl_3$



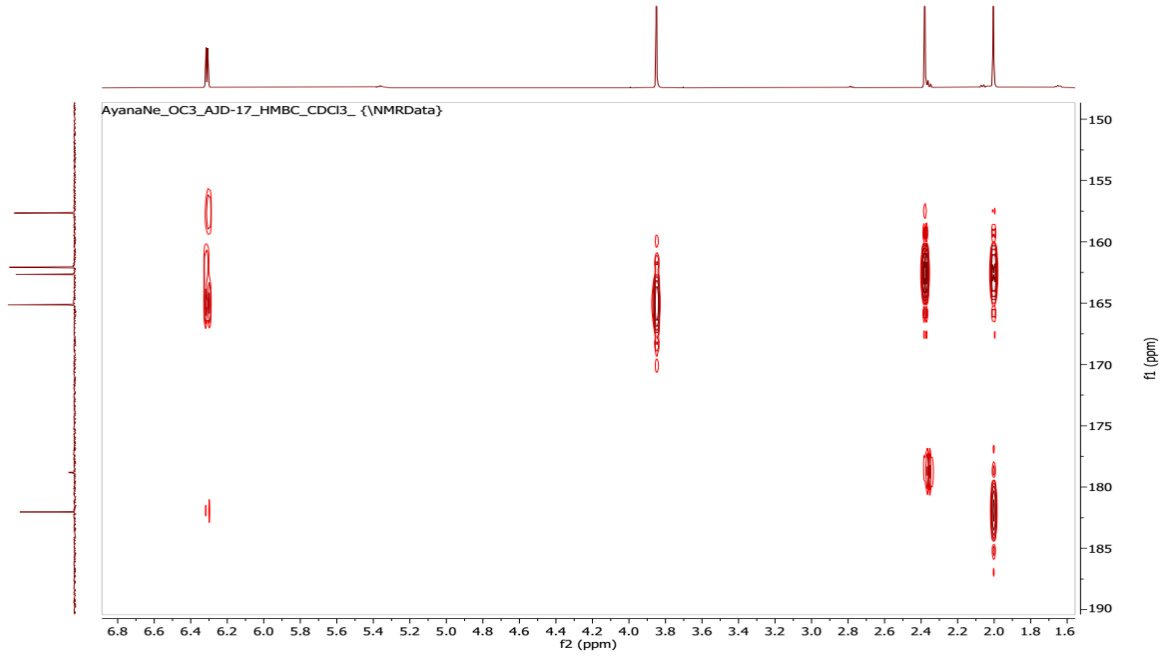
Appendix16: HMBC spectra of compound 324 in CDCl3



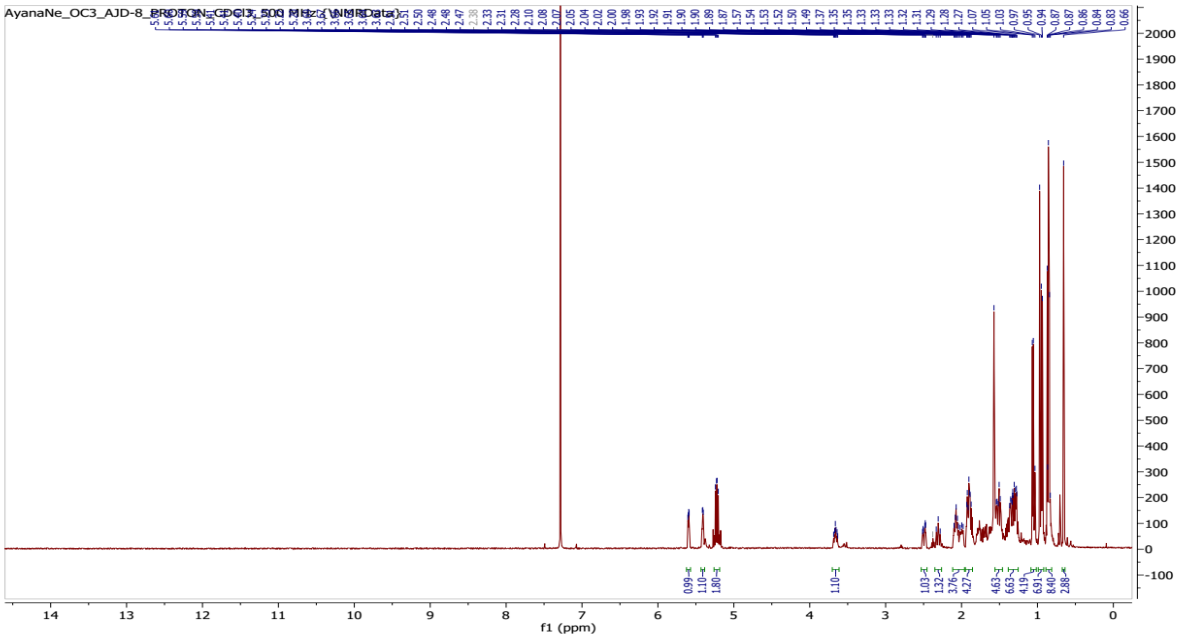
Appendix17: Expanded HMBC spectra of compound 324 in $CDCl_3$ (1)



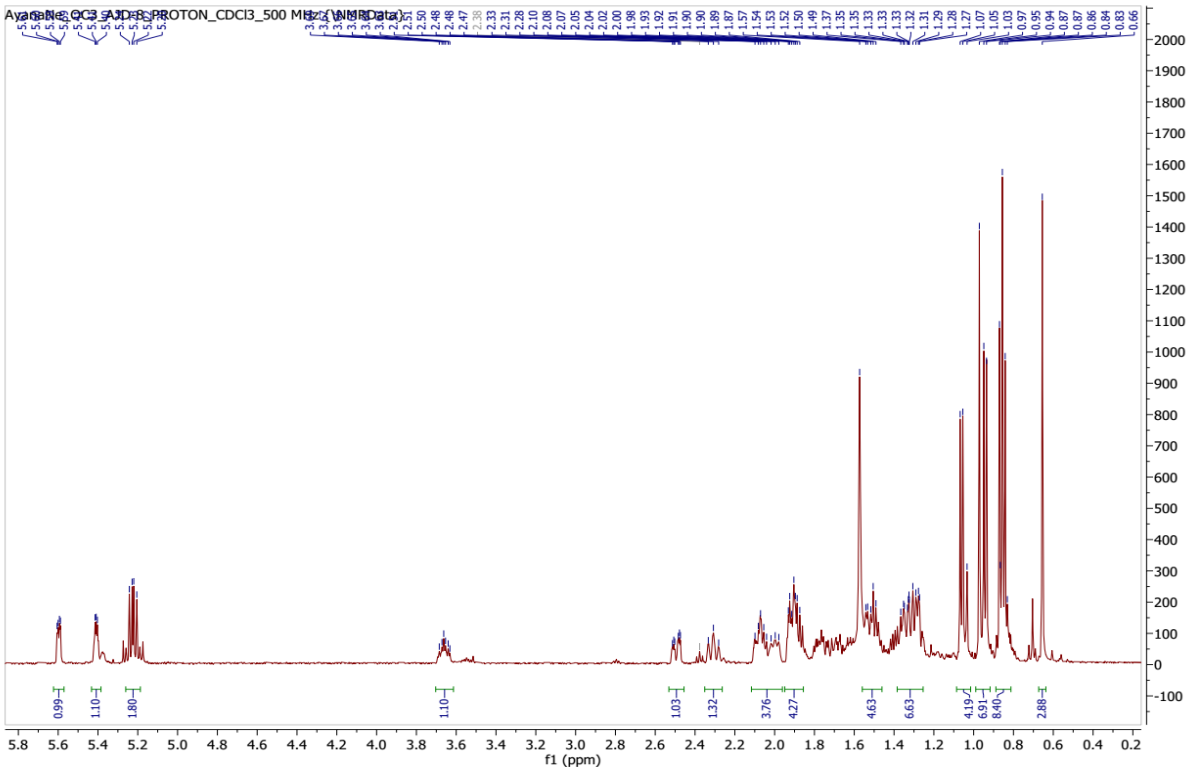
Appendix18: Expanded HMBC spectra of compound 324 in $CDCl_3$ (2)



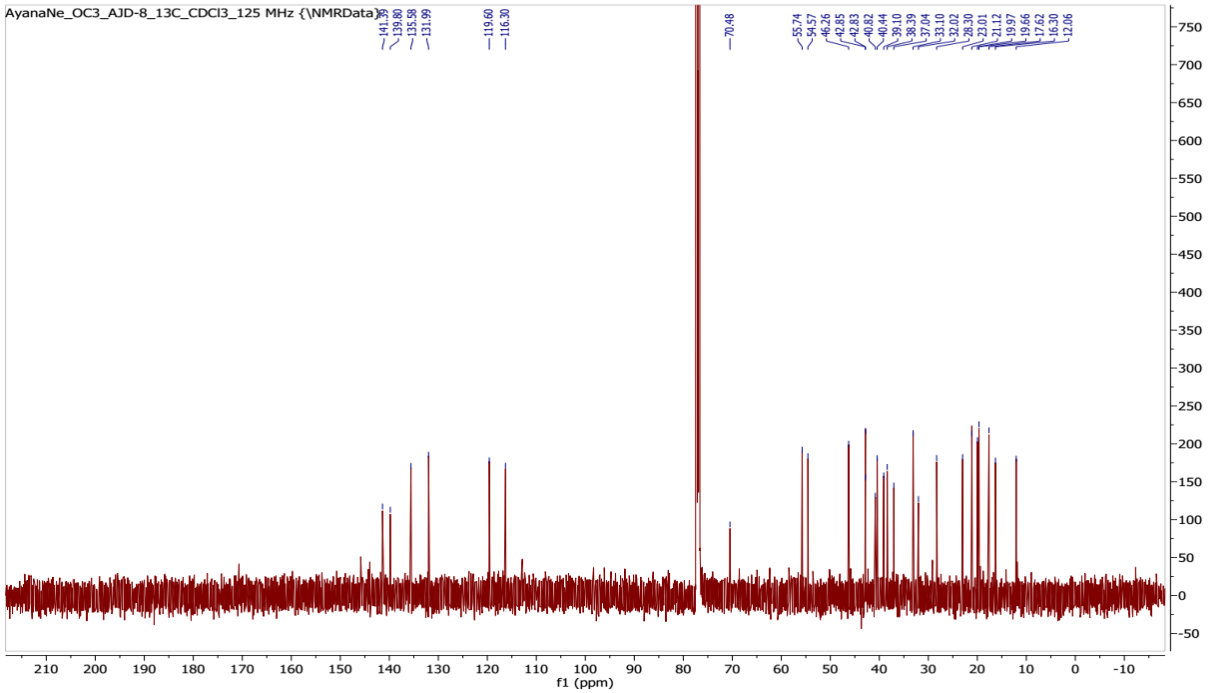
Appendix19: ^{1}H NMR spectra of compound 325 in $CDCl_3$



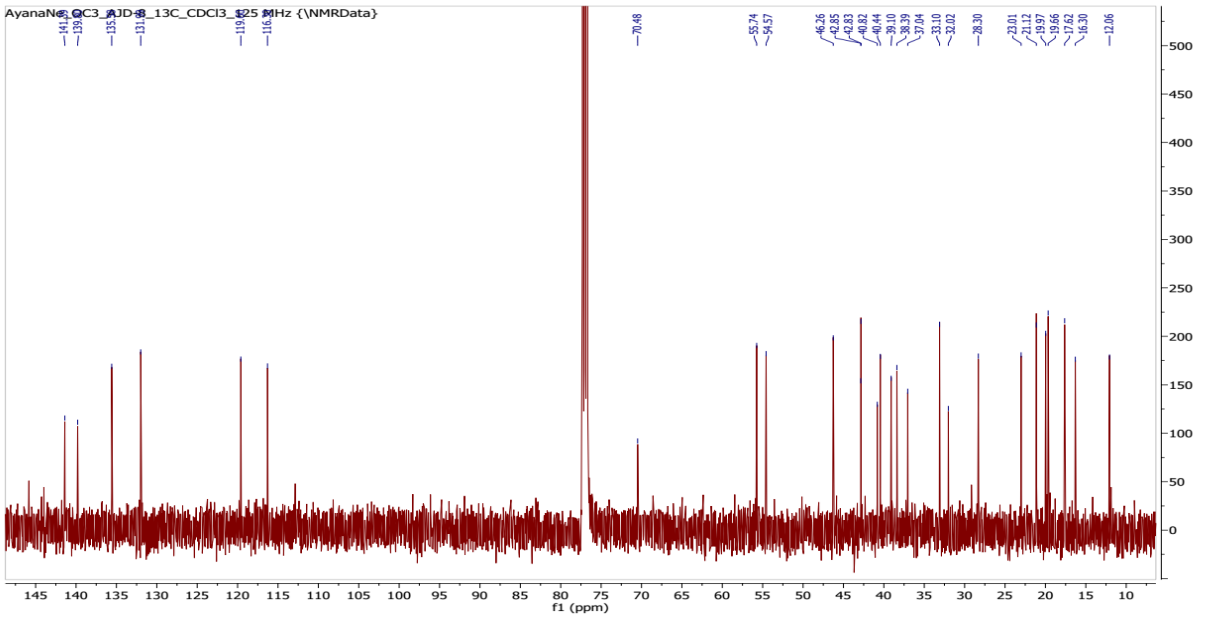
Appendix20: Expanded ^{1}H NMR spectra of compound 325 in $CDCl_3$



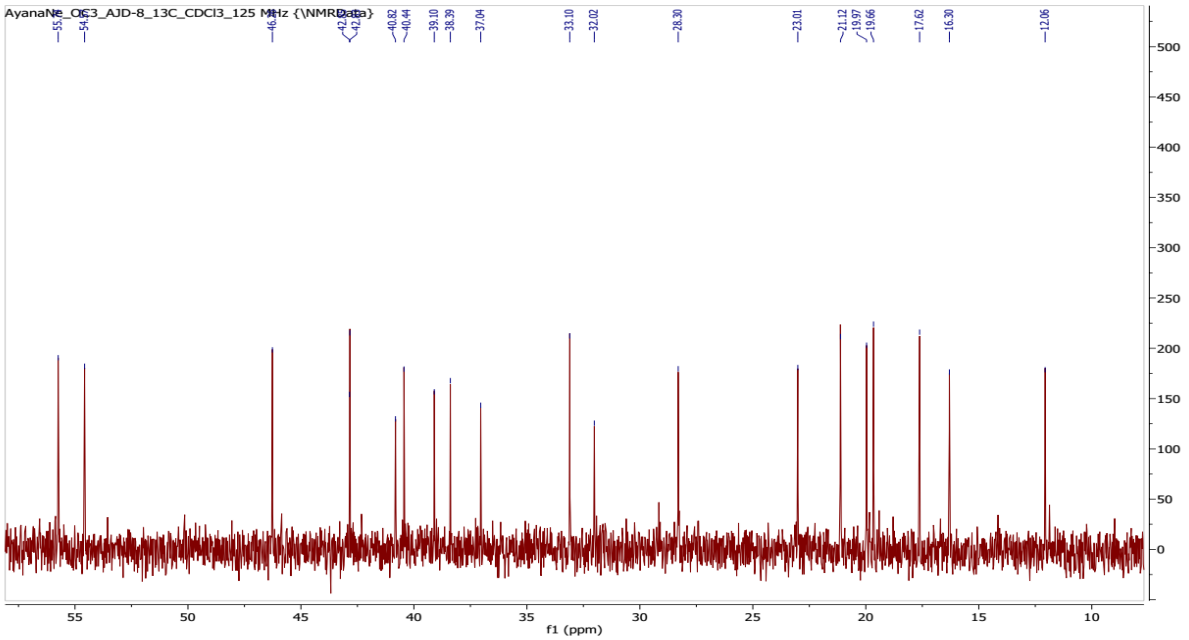
Appendix21: ^{13}C NMR spectra of compound 325 in $CDCl_3$



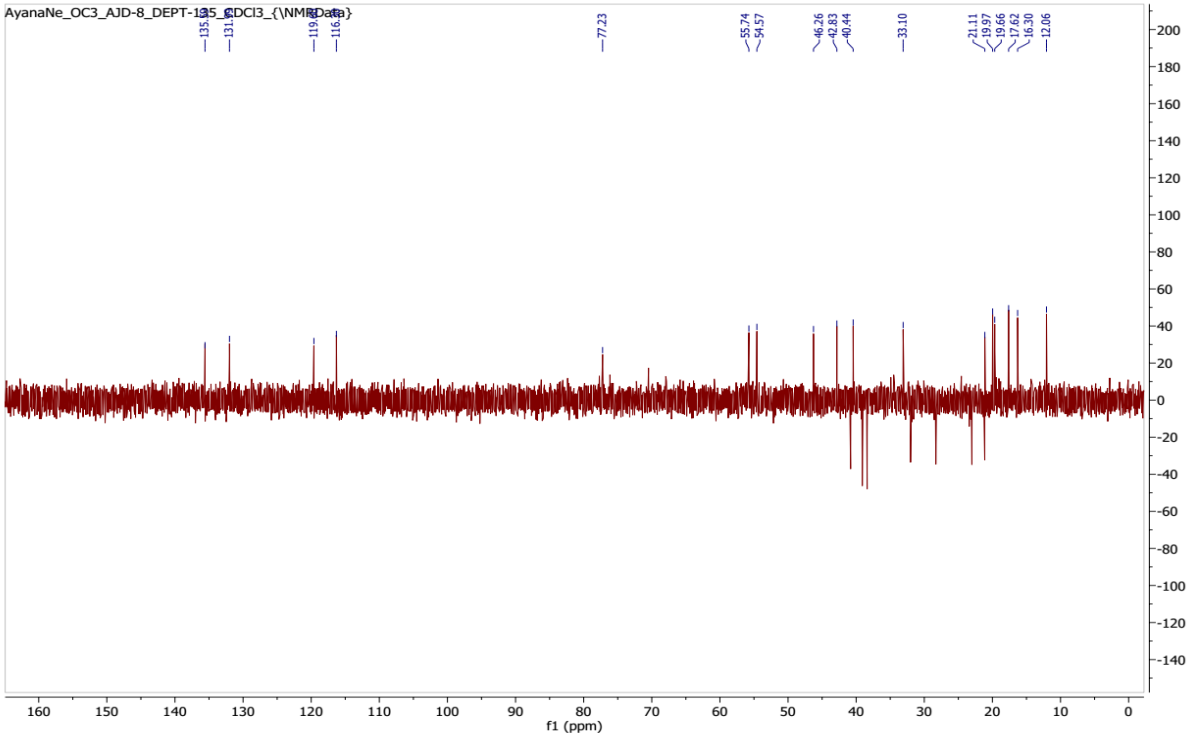
Appendix 22: Expanded ^{13}C NMR spectra of compound 325 in $CDCl_3$ (1)



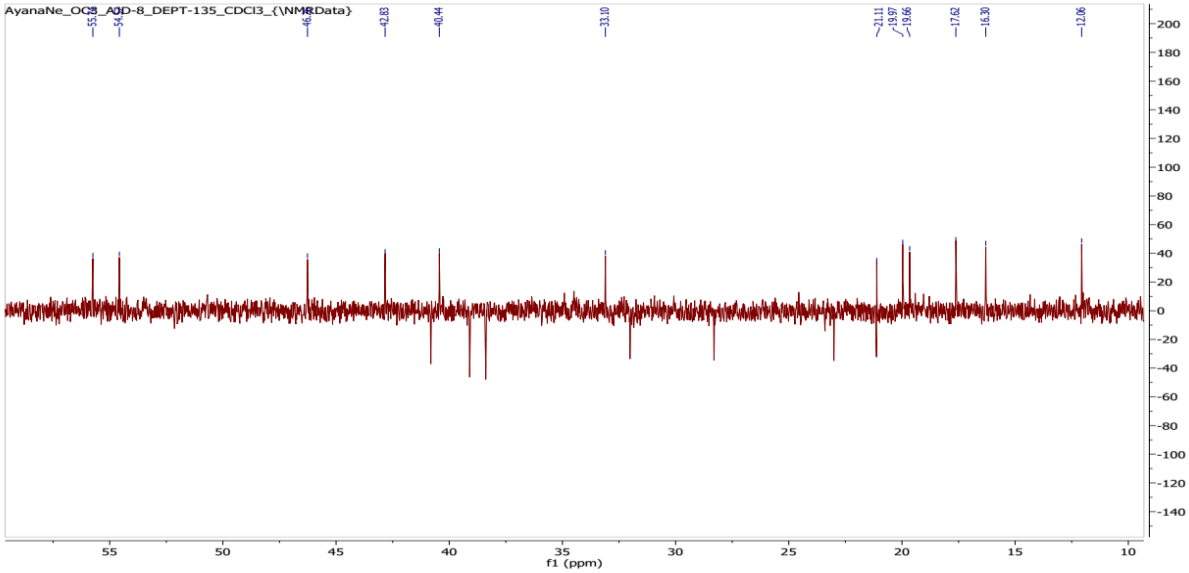
Appendix23: Expanded ^{13}C NMR spectra of compound 325 in $CDCl_3$ (2)



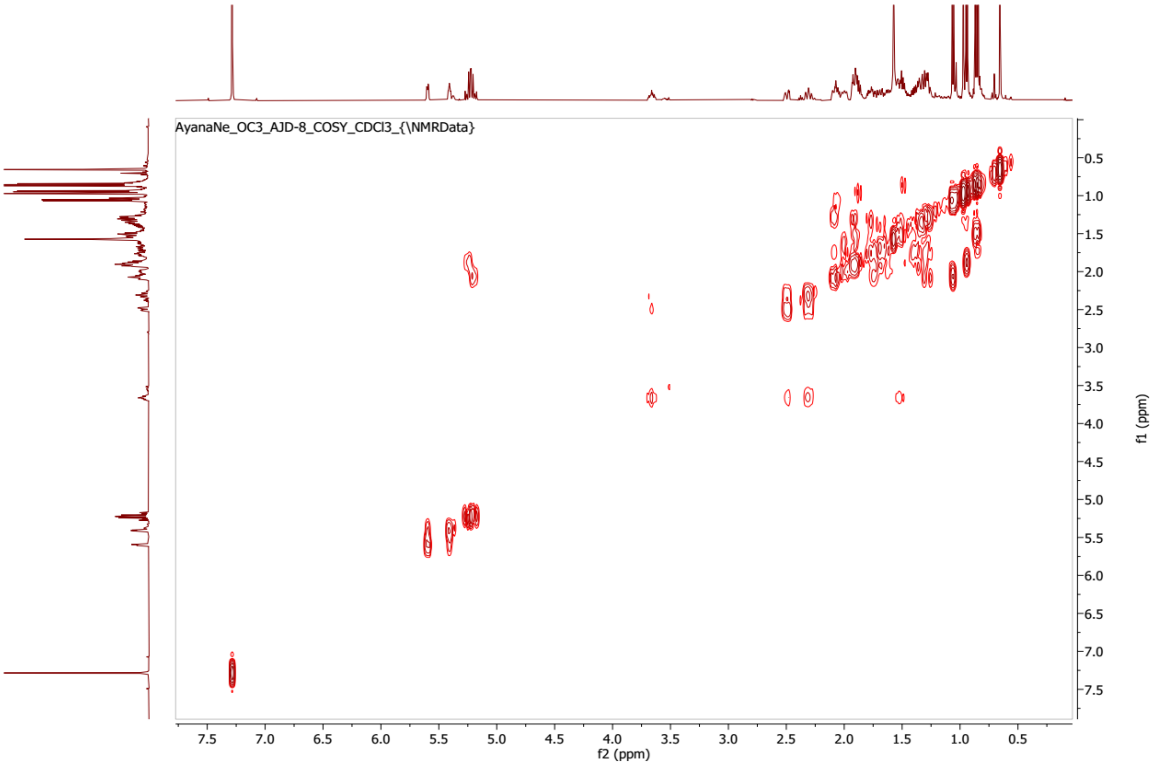
Appendix24: DEPT-135 spectra of compound 325 in $CDCl_3$



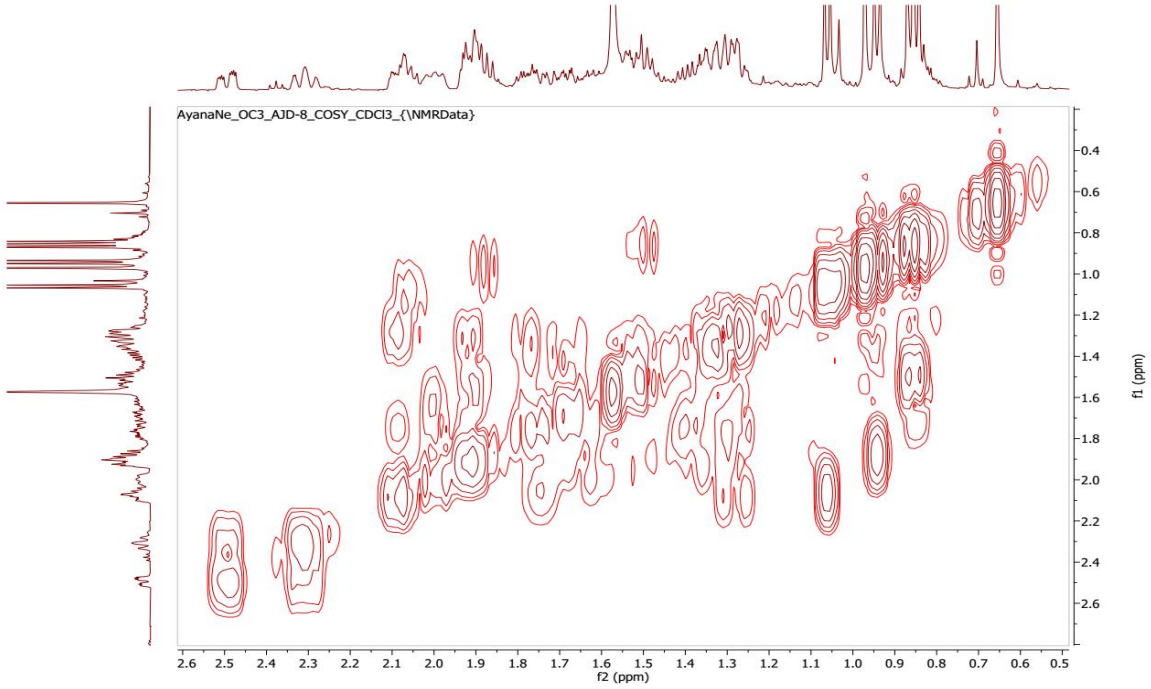
Appendix25: Expanded DEPT-135 spectra of compound 325 in $CDCl_3$



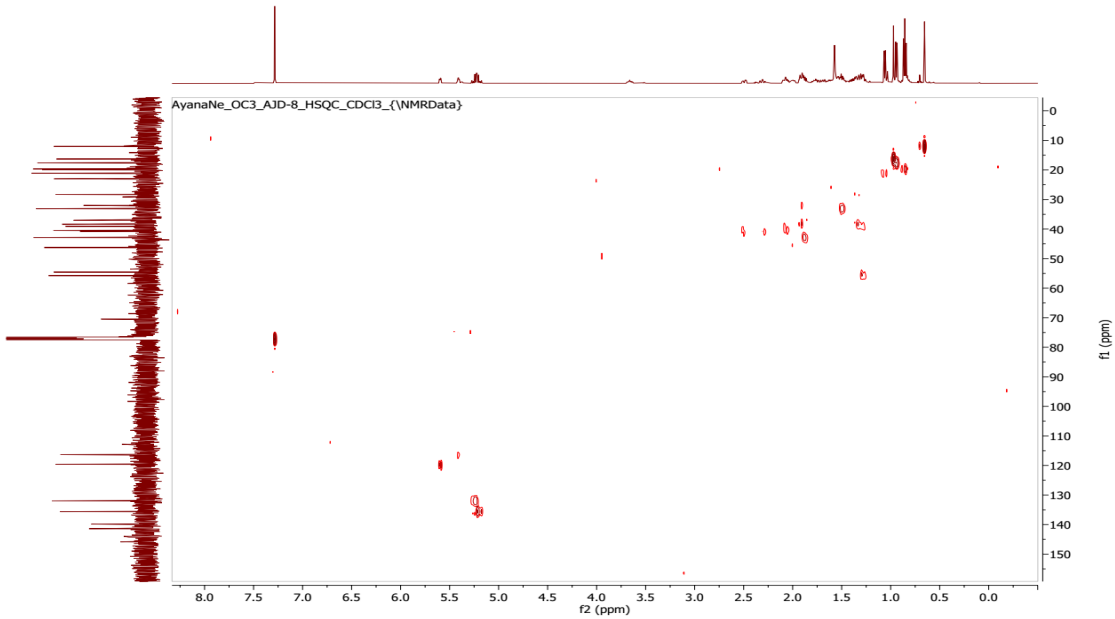
Appendix26: COSY spectra of compound 325 in $CDCl_3$



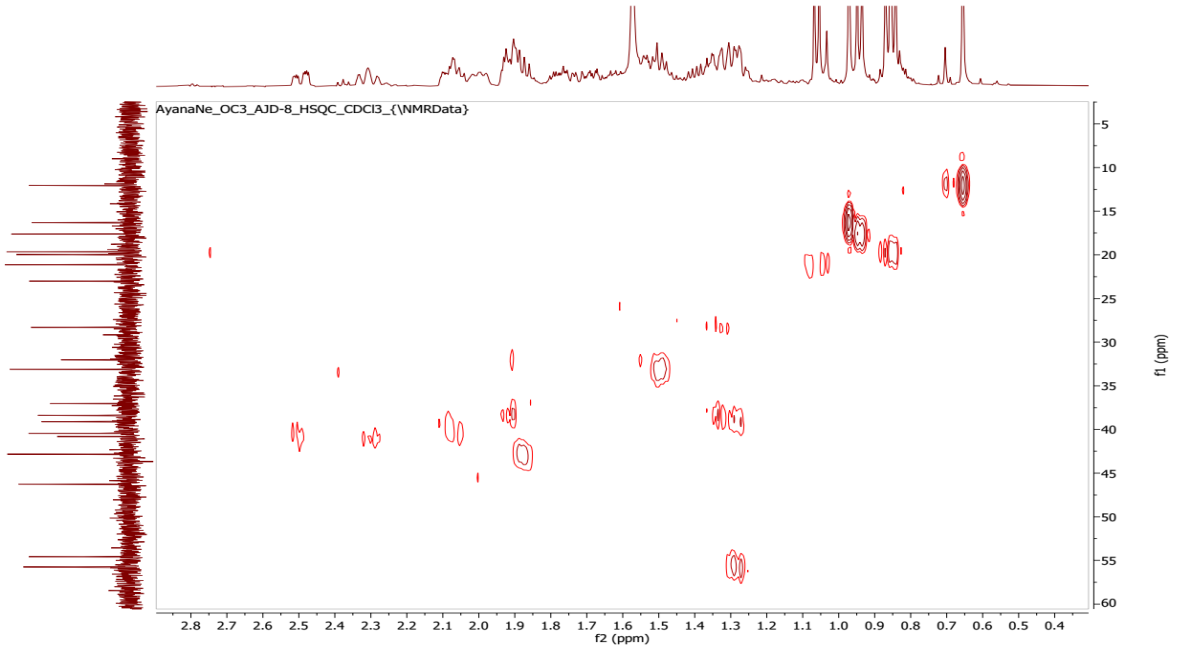
Appendix27: Expanded COSY spectra of compound 325 in $CDCl_3$



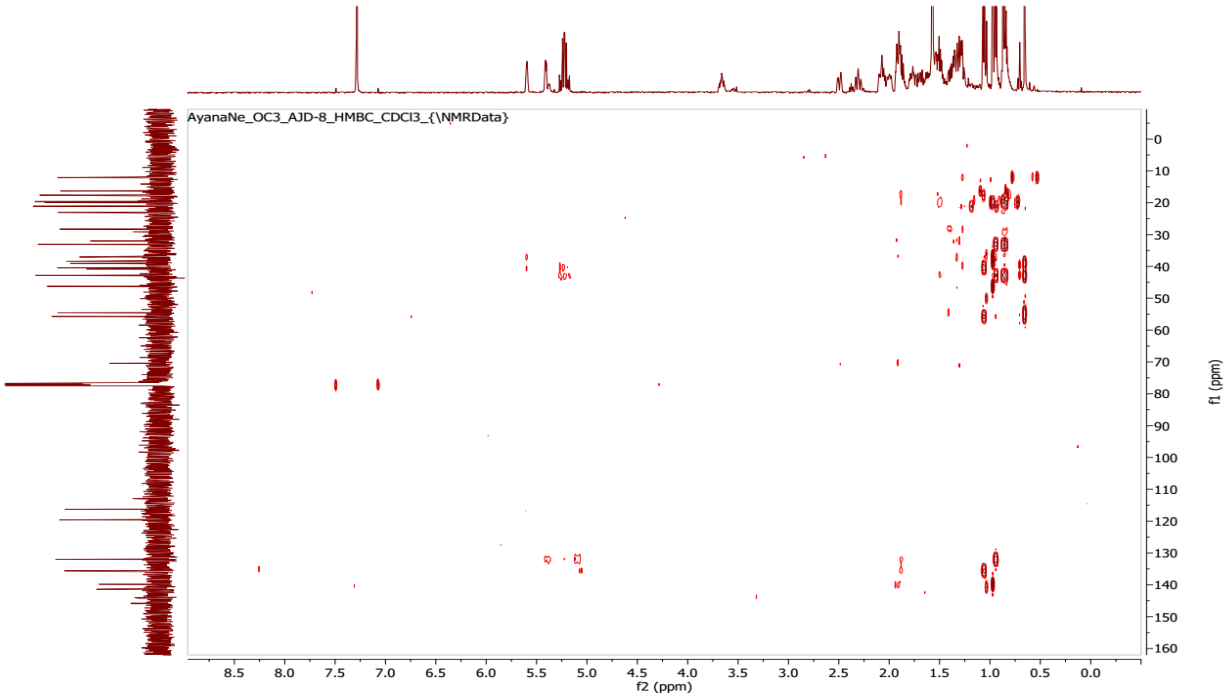
Appendix28: HSQC spectra of compound 325 in $CDCl_3$



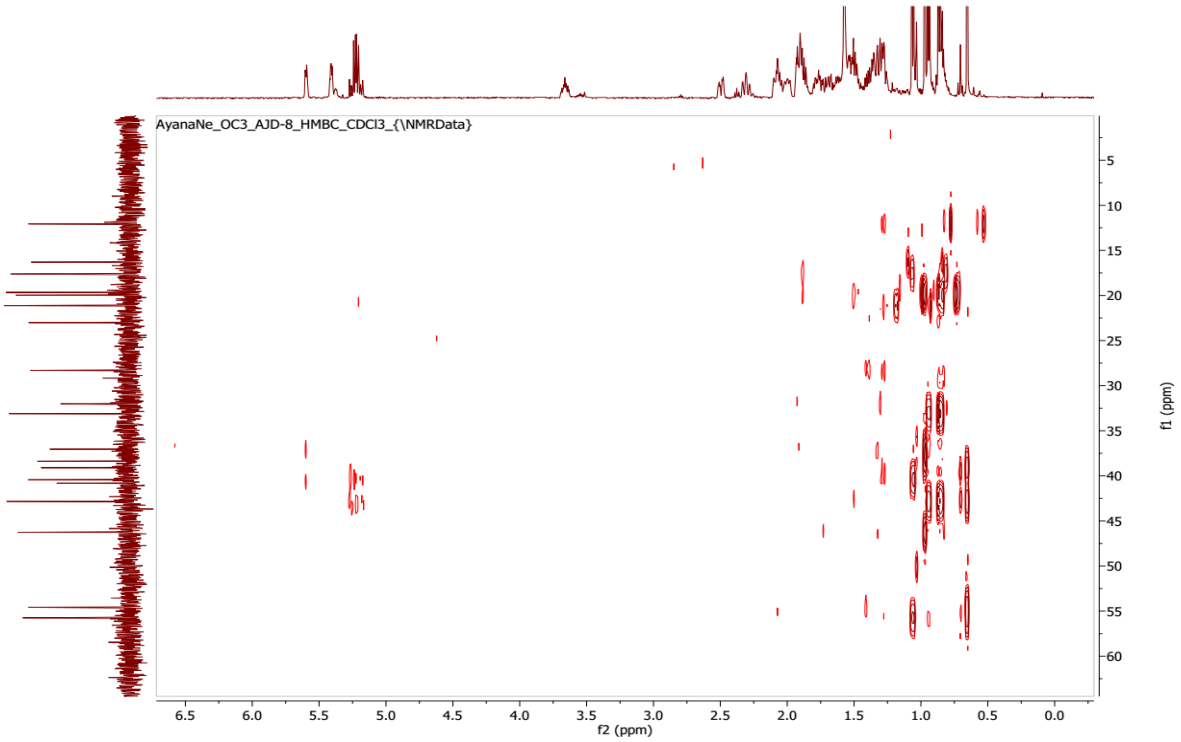
Appendix29: Expanded HSQC spectra of compound 325 in $CDCl_3$



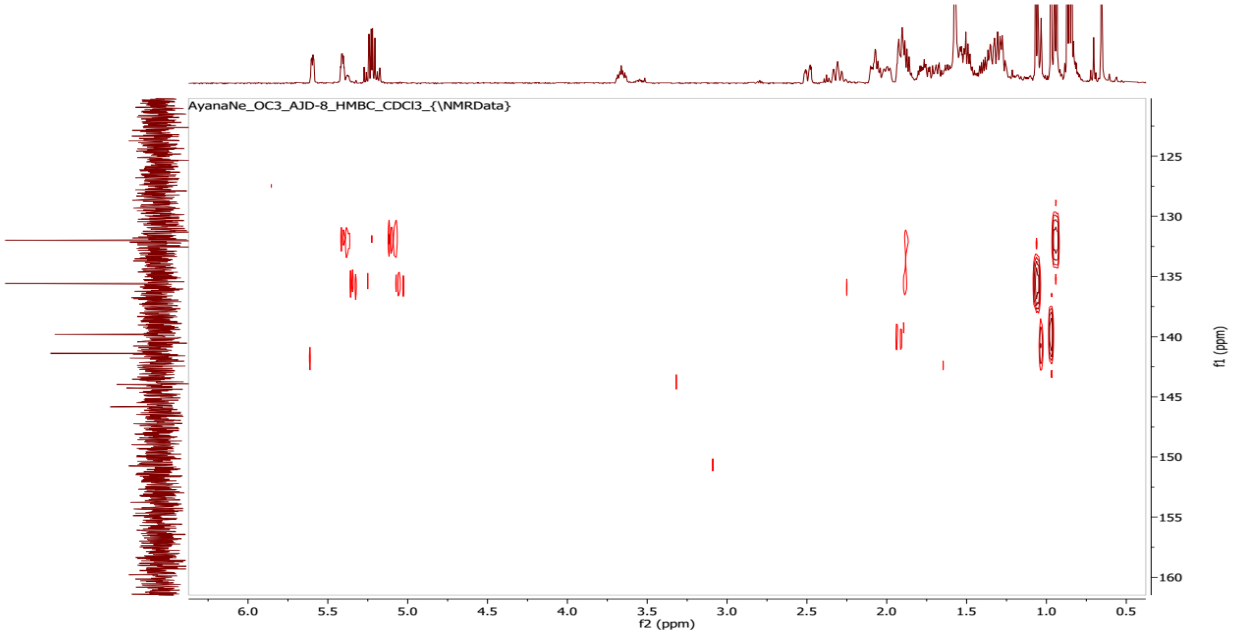
Appendix30: HMBC spectra of compound 325 in $CDCl_3$



Appendix31: Expanded HMBC spectra of compound 325 in $CDCl_3$ (1)



Appendix32: Expanded HMBC spectra of compound 325 in $CDCl_3$ (2)



Appendix33: NOE spectra of compound 325 in $CDCl_3$