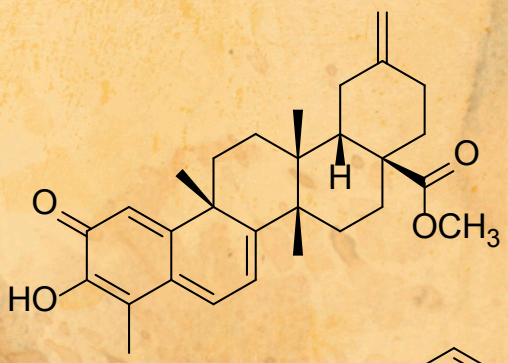
Secondary Metabolites from Mozambican Plants

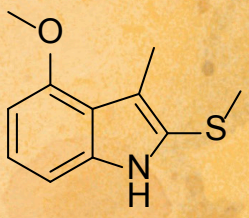
Monjane, Julião

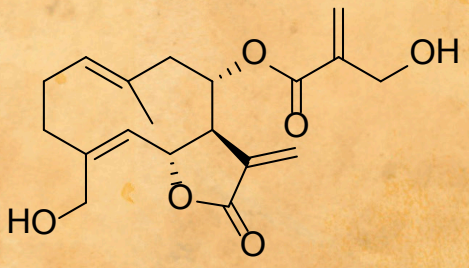
2017

Secondary Metabolites from Mozambican Plants

CENTRE FOR ANALYSIS AND SYNTHESIS | LUND UNIVERSITY
JULIÃO MONJANE







Secondary Metabolites from Mozambican Plants

Julião Armando Monjane

LUND
UNIVERSITY

DOCTORAL DISSERTATION
by due permission of the Faculty Science, Lund University, Sweden.
To be defended at Lecturer Hall F, Kemicentrum,
on Thursday 21st of December 2017 at 9:30 a.m.

Faculty opponent
Professor Anders Vik
School of Pharmacy, Department of Pharmaceutical Chemistry
University of Oslo, Norway

Organization LUND UNIVERSITY	Document name: DOCTORAL DISSERTATION
	Date of issue: 2017-11-27
Author(s) Julião Amando Monjane	Sponsoring organization Swedish International Development Agency (SIDA/Sarec)

Title and subtitle: Secondary Metabolites from Mozambican Plants – Isolation and Characterization

Abstract

Products derived from different natural sources have been used for thousands of years by human beings for their everyday needs. Out of these natural sources, plants were the most affordable. Plant-derived products were used for shelter, food, and as medicines. The medicinal properties of plant-derived products played an important role in ancient civilizations, and even in the present days they are useful due to their medicinal properties.

Based on the experience acquired by humans over the years by exploiting plant-derived products or secondary metabolites, this thesis was aimed to analyse the chemical components of some plants from the Mozambican flora used in the traditional medicine for the treatment of various ailments. Extracts of *Cadaba natalensis* (Capparaceae), *Clematis viridiflora* (Ranunculaceae), *Brachylaena discolor* (Asteraceae) and *Senna spectabilis* (Fabaceae) plant species were investigated for their chemical constituents.

By fractionation based on various chromatographic methods, fourty metabolites were isolated and their chemical structures were determined. Out of these, three were found to be novel metabolites, All in all, 40 secondary metabolites belonging to the three main classes of secondary metabolites were isolated and their sttructures were determined by high resolution NMR and MS techniques. The macrocyclic dibenzo-diazocyclodo-decanedione (**134**), (*S*)-2-ethyl-2-methyloxazolidin-5-one (**139a**), and 4-methoxy-3-methyl-2-(methylthio)-*1H*-indole (**141**) were the three novel metabolites, while the (*R*)-5-ethyl-5-methyloxazolidin-2-one (**140**) was isolated as natural product for the first time. The structures on these metabolites were determined by extensive use of the spectroscopic techniques of NMR (1D- and 2D-NMR), IR, as well as MS data. The structures of the known compounds were determined by the same techniques, and confirmed by the comparison of their spectroscopic data with those reported in the literature.

Isolated metabolites with interesting structural features were assayed for *in vitro* antiprotozoal activity towards two *Leishmania* strain, *Leishmania amazonensis clon 1* and *L. braziliensis*. The quinone methide triterpenoid (**29a**) showed a potent antileishmanial activity against both strain with the IC_{50} values 4.2 and 2.8 μM, respectively compared to the positive controle miltefone with the IC_{50} values 5.1 and 4.9 μM, respectively. Miltefone is a current used drug to treat *Leishmania*. **141** with the IC_{50} values 25.0 and 13.2 μM, respectively, and onopordopicrin (**153**) with the IC_{50} values 13.8 and 9.7 μM, respectively, also possessed interesting antileishmanial activity, although the germacranolide epoxy derivative **154**, derived from **153**, was inactive against both strain tested.

Keywords: Secondary metabolites, Mozambique, Medicinal plants, Isolation, Structural elucidation, Antileishmanial activity

Classification system and/or index terms (if any)

Supplementary bibliographical information		Language: English
ISSN and key title:		ISBN: 978-91-7422-554-9 978-91-7422-553-2
Recipient's notes	Number of pages 70	Price
	Security classification	

Secondary Metabolites from Mozambican Plants

Julião Armando Monjane

LUND
UNIVERSITY

Faculty of Science
Centre for Analysis and Synthesis

ISBN: 978-91-7422-553-2 (Print)
ISBN: 978-91-7422-554-9 (PDF)

Printed in Sweden by Media-Tryck, Lund University
Lund 2017

To my family

Popular summary

Plants, microbes, and invertebrates, i.e. all living organisms, produce a vast array of compounds known as natural products or simply secondary metabolites. The production of these secondary metabolites is not believed to promote the growth of the particular specie, instead they have other functions. It may, for example, be secondary metabolites that are useful for a chemical defence against predators, or for the protection and survival during environmental stress.

The secondary metabolites produced by living organisms are well known to possess a wide range of biological activities, which in some cases are useful to man. Antibiotics such as penicillin were isolated from fungi, and quinine was isolated from plants of the genus *Cinchona* and used medicinally as antimalarials. Also non-pharmaceutical green teas contain products derived from nature and used by humans for their health benefits. As the investigations of natural products have resulted in a remarkable number of compounds that benefit humans, the continued study of secondary metabolites and natural products has and continues to be of great importance.

List of papers

Publications by J.A. Monjane presented in his thesis

Thesis title: Secondary Metabolites from Mozambican Plants

1. Novel metabolites from the roots of *Cadaba natalensis*

J.A. Monjane, A. Uamusse, O. Sterner, *Phytochemistry Letters* **16**, 283-286 (2016)

J.A. Monjane has participated in the planning of the study and the selection of plant to be studied, he has carried out the extractions, the fractionations and the isolation of pure compounds, as well as the structure elucidation.

2. Novel sulfur-containing indole from the leaves of *Clematis viridiflora* Bertol.

J.A. Monjane, D. Capusiri, A. Giminez, O. Sterner, *International Journal of Research in Pharmacy and Biosciences* **4**, 10-14 (2017)

J.A. Monjane has participated in the planning of the study and the selection of plant to be studied, he has carried out the extractions, the fractionations and the isolation of pure compounds, as well as the structure elucidation. He has not performed the biological assays.

3. Onopordipicrin with Leishmanial Activity from the leaves of *Brachylaena discolor*

J.A. Monjane, D. Capusiri, A. Giminez, O. Sterner, in manuscript

J.A. Monjane has participated in the planning of the study and the selection of plant to be studied, he has carried out the extractions, the fractionations and the isolation of pure compounds, as well as the structure elucidation. He has not performed the biological assays.

4. Leishmanial Activity of a Quinone Methide Triterpenoid from the roots of *Senna spectabilis*

J.A. Monjane, D. Capusiri, A. Giminez, A. Sundin, O. Sterner, in manuscript

J.A. Monjane has participated in the planning of the study and the selection of plant to be studied, he has carried out the extractions, the fractionations and the isolation of pure compounds, as well as the structure elucidation. He has not performed the biological assays.

Abbreviations

AIDS	Acquired Immune Deficiency Syndrome
All	Allose
^{13}C-NMR	Carbon-13 Nuclear Magnetic Resonance
CH_2Cl_2	Dichloromethane
$CHCl_3$	Chloroform
COSY	Correlation Spectroscopy
δ	Chemical shift
DEPT	Distortionless Enhancement by Polarization Transfer
EtOAc	Ethylacetate
gal	Galactose
glc	Glycose
^{1}H-NMR	Proton Nuclear Magnetic Resonance
HIV	Human Immunodeficiency Virus
HMBC	Hetero Multiple Bond Coherence
HMQC	Hetero Multiple Quantum Coherence
IC_{50}	Inhibitory Concentration of 50%
Me	Methyl group
MeOH	Methanol
NOESY	Nuclear Overhauser Spectroscopy

Acknowledgement

I would like to express my sincerest thanks to:

- My supervisor Olov Sterner, for giving me the opportunity to carry out a phytochemical project in his research group for providing an extraordinary working environment, as well as for the open doors to discuss problems, most them related with the structural elucidation of the isolated compounds.
- My co-supervisor Amalia A. Uamusse, I am grateful for introducing me into the fascinating field of Natural Products, and encouraging discussions in this field with other Mozambican Institutions.
- SIDA/Sarec and Eduardo Mondlane University for the financial support, as well as, the ISP for supporting coordination between the Lund University and Eduardo Mondlane University.
- Maria Levin and Bodil Eliasson, for the help and support during all the 5 years spent at Lund.
- Karl-Erik Berquist and Anders Sundin for your help with NMR and computer problems, Sofia Essén by helping me with Mass Spectrometry, and Katarina Fredrikson with your help with chemicals.
- Past and present OS group members: Marcelo, Dan, Rodrigo, Zilma, Eira, Ivan, Maribel, Mariela thank for your collaboration and friendship during this period.
- To all CAS members thank you.
- Special thanks goes to my family for their unconditional support. Muito Obrigado.
- To all Mozambican friends, Obrigado.

Content

1 Secondary Metabolites

1.1 Introduction

Since the earliest days of civilization, humankind has exploited and relied on natural resources for food, shelter, clothing, and perhaps most important, the use of plants as major sources for medication to alleviate a number of ailments and prevention of diseases. The knowledge of the healing properties of plants was acquired during the development of the ancient civilizations, such as the Ayurveda, Chinese, Egyptian, European, and other civilizations. This knowledge was passed through generations, which formed the basis of a sophisticated traditional medicine system. The role in the history describing the use of plant-based products or natural products is well-documented in the literature.[1–4]

Although the long history of the use of plants, it was not until the 1800s with the isolation of the first active natural product morphine (**1**) from *Papaver somniferum* that it was understood that the useful properties of a plant depended on a substance. Morphine became the first commercially available pure active natural product. The ancient people of Mesopotamia used *P. somniferum* for the treatment of a wide range of ailments, and with the isolation of **1** products derived from plants captivated the attention of the chemists that continued to pursue these fascinating molecules both for their interesting chemical structures as well as their potential as therapeutic agents for the treatment of human diseases. Since then, natural products were extensively screened in order to identify novel medicines.[1,4]

Natural products chemistry has been fundamental for drug discovery and the development of new drugs. The chemotherapeutic drug paclitaxel (Taxol®, **2**) was originally isolated from the stem bark of the Western Yew tree, *Taxus brevifolia*.[5] **2** is an antitumor agent used for the treatment of ovarian, breast, lung, and prostate cancer. Interestingly, various *Taxus* species were used historically by several native American tribes as medicines.[1] The antimalarial drugs quinine (**3**) and artemisinin (**4**) are good examples of medicinally important and naturally plant-derived drugs. **3** was isolated from *Cinchona succiruba*, a plant that has been used over centuries for the treatment of malaria, fever, indigestion, throat diseases, and cancer in the Amazon region.[4,6] Another important antimalarial drug is **4**, isolated from *Artemisia*

annua (Qinghaosu), a plant used in the Chinese medicine for the treatment of malaria.[3,4]

The terrestrial plants were the major sources for obtaining naturally occurring drugs from living organisms, until the discovery and isolation of penicillin (**5**) from the fungus *Penicillin notarium* in the early 1940s. The success of **5** in treating infections led the screening of a large number of marine and terrestrial microorganisms for drug discovery. Terrestrial microorganisms produce a wide variety of active metabolites, and have provided important contributions to the discovery of various antibiotic agents such as penicillins, cephalosporins, aminoglycosides, tetracyclines, and polyketides.[7] The cephalosporins isolated from extracts of the fungus *Acremonium* are cholesterol-lowering agents, acarbose obtained from *Actynomyces* species is used in the treatment of diabetes millitus, and epirubicin is an anthracycline drug used as chemotherapeutic agent. They are some examples of naturally occurring drugs obtained from other sources than plants.[8,9]

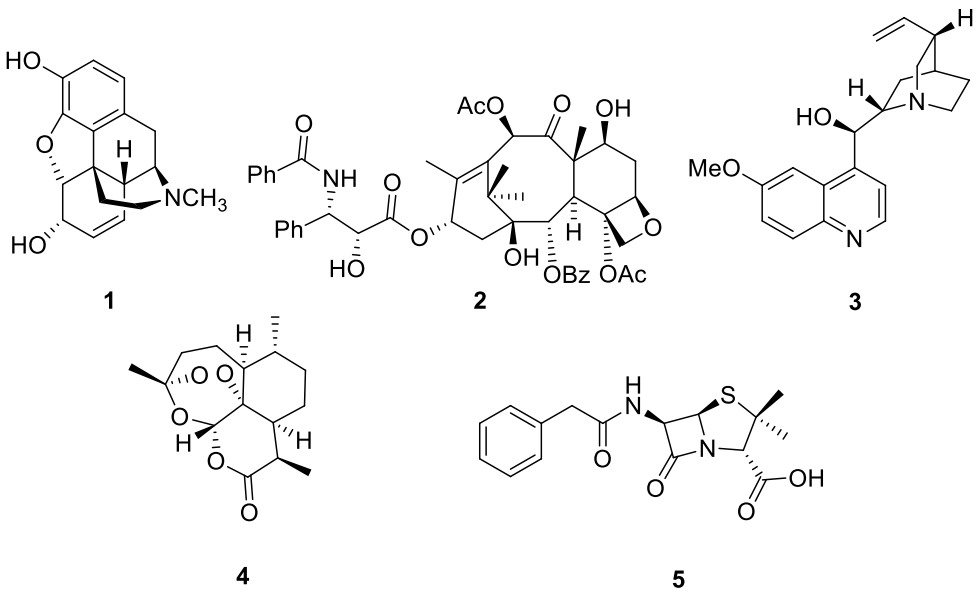


Figure 1. Examples of some important drugs isolated from natural sources.

While natural-derived biologically active compounds from living organisms, known as secondary metabolites or natural products, are not essential for life, as they are not required for the normal growth, development, or reproduction of an organism, they have been suggested to be waste products with a diversity of chemical structural features. However, these complex and frequently toxic

chemicals are today recognized as the means for interactions between organisms, for defence and signaling.[10]

Traditionally, secondary metabolites have a great economic importance as new chemical entities. They can be used as drugs or biocides, lead compounds in drug and biocide discovery, biological or pharmacological tools, raw materials for the production of drugs, like feedstock products, and as nutraceuticals, as insecticides, and pesticides. They are present in herbs, dietary supplements, spices, and foods. Most importantly, they still play a pivotal general role for drug discovery.[4,11]

1.2 Secondary Metabolites from African Plants

Plants have a long history of use in the African continent, for the treatment of various diseases and complaints. The vast majority of the African population still rely exclusively on plants as a source for medication.[12] The African traditional medicine is perhaps the oldest and the most diverse of all traditional medicine systems. The continent has a rich biological and cultural diversity marked by the regional differences in a way of healing practices. Unfortunately, the system of traditional medicines is poorly recorded, as the knowledge of these practices had been passed over generations orally.[13] However, some uses of these plants are currently available in the literature,[14–20] just to cite few examples.

The continent is highly endowed with diverse types of vegetation, constituting tropical rainforests, coastal and alpine forests, savannahs, and woodlands. These types of vegetation are considered as a reservoir for diverse natural products.[21] It has been verified that natural products derived from African plants have an enormous potential to be used as drugs or drugs leads. The plants from Africa have already provided useful natural products with important nutritional and therapeutical values. The plant *Coffee arabica* (Rubiaceae) is originated from Ethiopia. The beverage coffee is produced from the seeds of this specie and certain other varieties of the genus *Coffee*. The prepared coffee contains 1 to 2% of the purine alkaloid caffeine, the 1,3,7-trimethylanthine (**6**).[12]

African people have been using the rhizomes of *Hypoxis* spp. (Hypoxidaceae) for the treatment of urinary infections and internal cancer. From the Southern African *Hypoxis hemerocallidea* or *H. rooperi* and *H. obtuse*, the hypoxoside (**7**) was isolated.[22,23] Metabolite **7**, by enzymatic hydrolysis, yields rooperol (**8**), which inhibit the growth of certain cancer cells. Since **7** could act as a prodrug in humans, clinical trials on cancer patients and AIDS patients with dried methanol extracts of rhizomes are underway in South Africa.[12]

The Madagascar periwinkle, *Catharanthus roseus* or *Vinca rosea* (Apocynaceae) is one of the most quoted examples of African medicinal plants. In the folklore medicine, extracts of the leaves of the plant have been used in the treatment of diabetes, hypertension, tuberculosis, laryngitis, sore throat, dyspepsia, malaria, and for regulating menstruation, in several countries.[24] *C. roseus* is a perennial evergreen herb. In Mozambique, the plant is common known under the Portuguese name as '*Beijo-da-mulata*' and has been used to treat diarrhoea and venereal diseases.[25,26] Phytochemical investigations of the plant led to the isolation and characterization of 130 monomeric and dimeric indole alkaloids, most of them with considerable biological activity.[27–29] Among these alkaloids, two *bis*-indole alkaloids known as vinblastine (**9**) and vincristine (**10**) isolated from the leaves are well-known for their anticancer properties, and are commercially available.[27] Some structures of metabolites isolated from African plants are presented in Fig. 2.

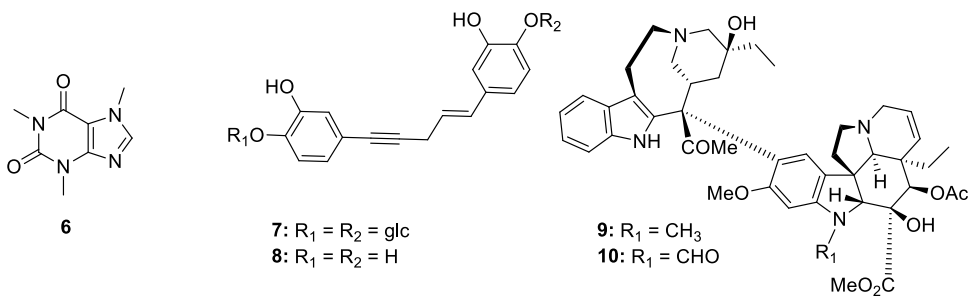


Figure 2. Important secondary metabolites derived from African plants.

The biological activities of the compounds derived from African plants have been reported in at least 2000 publications, and it is believed that the active principles for the development of drugs against some of the neglected tropical diseases may be found in the African flora.[30] In addition, a number of recent reviews had demonstrated the potential of natural products derived from African medicinal plants.[12] These reviews include naturals products with antimalarial,[31–33] anti-inflammatory and antioxidant,[34,35] infectious and parasitic diseases,[36,37] and antidiabetic properties,[38] Information regarding the ethnobotanical uses *versus* the bioactivities of the isolated compounds from African flora has been gathered.[31,39–42] Some biologically active natural products have been isolated and identified from the Southern African marine microorganisms, algae, sponges, ascidians, soft corals, and molluscs, [35,43] but the natural product chemistry of Southern Africa's unique marine flora and fauna is relatively unknown.

2 Secondary Metabolites from Mozambican sources

2.1 Introduction

Mozambique is located in the Southern region and on the East coast of Africa (Fig. 3), with more than 2,000 km long coastline extending from Tanzania to South Africa.[44] The dominant vegetation types of the country are savannah and secondary forests covering up to 70 % of the total area.[45] With 25 million inhabitants, the population is primarily working in agriculture. The sector of fishing also play an important role, and its main area is the Mozambique Channel, a branch of the Indian Ocean between Mozambique and Madagascar.[44]

Figure 3. Location of Mozambique in African Continent.

Modern healthcare systems are scarce and limited. Only 40 % of the population can access the public health system and the remaining still rely on traditional medicine and medicinal plants as primary source for their healthcare needs.[26,46] Even major diseases such as diarrhoea, malaria, sexual diseases, and respiratory complaints are treated with traditional medicine.[47]

The country has a high repository of biodiversity. About 5,500 plant species have been recorded in Mozambique, and it has been estimated that no more than 10 % are used for medicinal purposes.[26,48] However, the scientific validation of the efficacy of most of these plants has not been documented, so far. Tropical forests are classified as rich in terms of native plants,[49] and here there are no exceptions since the country has not explored its native plants.

There is a wide range of plant families comprising the traditional pharmacopeia. Some of these plants were compiled and edited, showing their exact location within the country.[50–54] Also, several investigators have gathered information about the use of medicinal plants in different regions of the country for the treatment of various ailments.[48,49,55,56] Medicinal plants are considered to play a key role in slowing down diseases such as diarrhoea, pneumonia, skin infections, and other infections related to the opportunistic HIV virus.

The roots and root bark of *Acacia nilotica*, *Elephantorhriza elephantine*, and *Scleocarya birrea* are frequently used by local people to treat diarrhoea. *Rauvolfia caffra* and *Bridelia cathartica* are traditionally used to treat headaches and pain in bone articulations associated with malaria.[47] The leaves of *B. cathartica* and *Salacia kraussii* have shown some antimalarial activity in scientific investigations.[55,57] The anthelmintic effect of *Melia azedarach* and *Trichilia claussenii* has been investigated.[58]

Most of the plants used as medicines in Mozambique have never been investigated with respect to their active ingredients. Screening programs have been carried out, mostly with the collaboration between different domestic and international research groups. These investigations include the search of antimycobacterial [59] and antibacterial[57,60,61] agents, and the characterization of essential oils.[62–65] A literature survey revealed that some secondary metabolites have been obtained from Mozambican sources. These sources, besides the medicinal plants, also include various marine sources.

Secondary metabolites could be divided in three main classes: terpenoids, phenolic compounds, and nitrogen-containing compounds.[66,67] All of these classes of metabolites have been isolated and characterized from Mozambican sources, and some of them showed biological activities.

2.2 Terpenoids

Terpenoids form a vast group of naturally occurring metabolites, and the majority of them occur in plants. Many terpenoids are volatile substances which give plants and flowers their fragrance.[67,68] Based on the number of building blocks, they are commonly classified as monoterpenes C10, sesquiterpenes C15, diterpenes C20, sesterterpenes C25, and triterpenes C30. Terpenoids display a wide range of biological activities against cancer, malaria, inflammation, and a variety of infectious diseases.[69]

Momordica balsamina (Cucurbitaceae), known as *cacana* in the Southern part of Mozambique, is a vegetable found in tropical and subtropical regions. Due to its nutritional value, the plant has been used largely as food [70] Also, the specie has been used to treat various diseases symptoms, mostly diabetes,[71] malaria,[26] liver, and measles.[25] Cucurbitacins are the main characteristic secondary metabolites found in *Momordica* spp. They are highly oxygenated triterpenes of interest due to their cytotoxic, hepatoprotective, anti-inflammatory, cardiovascular, and antidiabetic activities.[72,73] Phytochemical investigations on the aerial parts of *M. balsamina* led to the isolation of the cucurbitane-type triterpenoids **11**-**27** [70,74] The metabolites **23**, **26** and **27** have good antipalsmodial activity,[73,75] while the metabolite **27** was shown to be cytotoxic.[76,77] The secondary metabolites isolated from the aerial parts of *M. balsamina* are presented in Fig. 4.

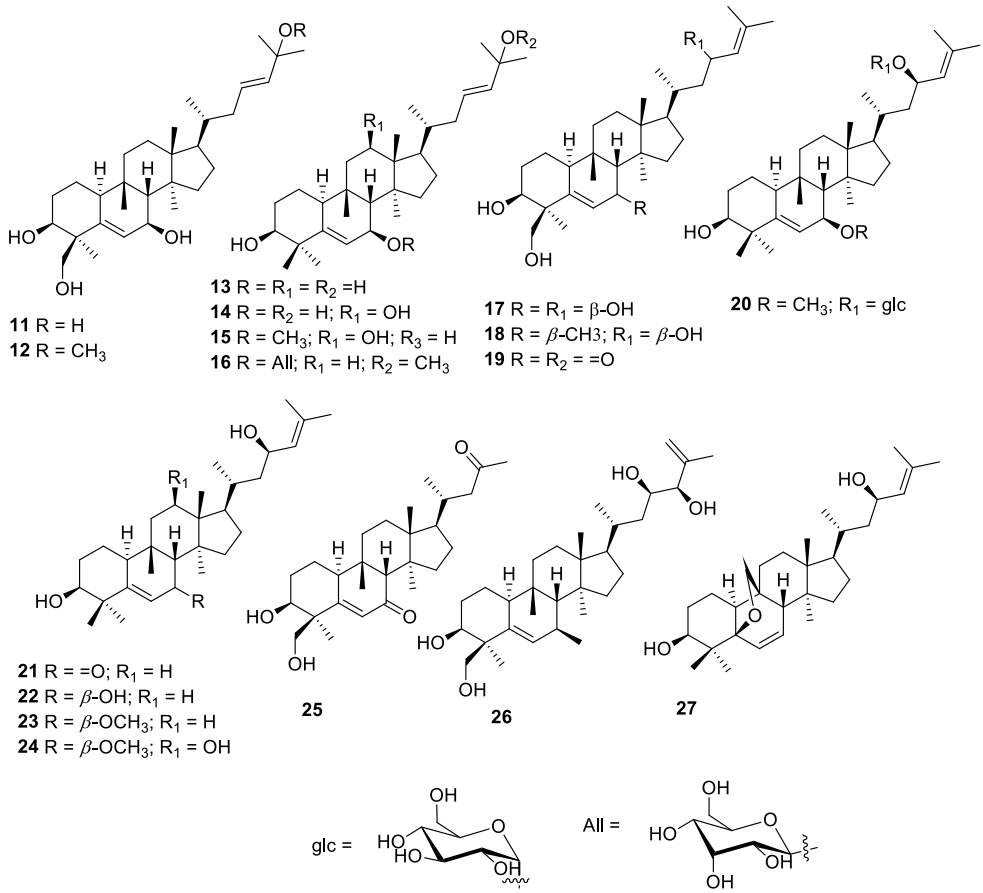
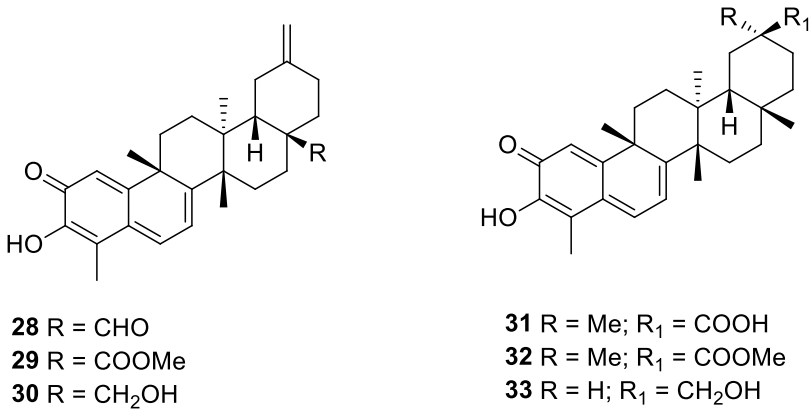


Figure 4. Cucurbitane-type triterpenoids isolated from the aerial parts of *M. balsamina*.

Salacia kraussii (Celastraceae), also known as *pswixa*, has been used for the treatment of bilharziasis, dysentery, malaria, and diarrhoea.[48,53] Figueiredo and co-workers isolated six quinone methides triterpenoids (**28**-**33**) from an extract of the roots, and their structures are shown in Fig. 5. The isolated compounds include the well-known celastrol (**31**), pristimerin (**32**), and isoiguesterol (**33**). All the isolated compounds showed antimalarial activity, and give an additive effect when combined with each other, and, therefore, these results could validate the traditional use of the plant for the treatment of malaria.[57]



28 R = CHO
29 R = COOMe
30 R = CH_2OH

31 R = Me; R_1 = COOH
32 R = Me; R_1 = COOMe
33 R = H; R_1 = CH_2OH

Figure 5. Structures of quinone methides isolated from *S. kraussii* with antimalarial activity.

Traditional healers have been used the roots, root bark, and leaves of *Crossopteryx febrifuga* (Rubiaceae), locally known as *chicombeque*, for the treatment of diarrhoea, stomachache, and wounds.[26] Two bisdesmosidic saponins (**34** and **35**) were isolated from the methanolic extract of the roots.[78]

Hoslundia opposita Vahl (Lamiaceae) is a small shrub which is widely distributed in West Africa, and is known as *buinite*, in the Northern part of the country. Various parts of the plant are remedies for snakebites, herpes, stomach troubles, conjunctivitis, epilepsy, chest pain, yellow fevers, and mental disorders. Infusions of its leaves have found a wide use in traditional medicine as purgative, diuretic, febrifuge, antibiotic, and antiseptic.[79] The crude extracts of the entire plant and the essential oil have been found to exhibit strong antibacterial activity. Mujovo and co-workers isolated euscaphic acid (**36**) from the leaves, which was found to be active against the *Mycobacterium tuberculosis* strains.[80]

The *munhongora*, *Cleistochlamys kirkii* (Annonaceae) is an African medicinal plant traditionally used in Mozambique for the treatment of wound infections, tuberculosis, and rheumatism,[46] stomachache, stomach virus, muscular pains, venereal diseases, and hernia,[26] and also, as food.[81] From the root bark was isolated a tetracyclic triterpene, polycarpol (**37**).[82]

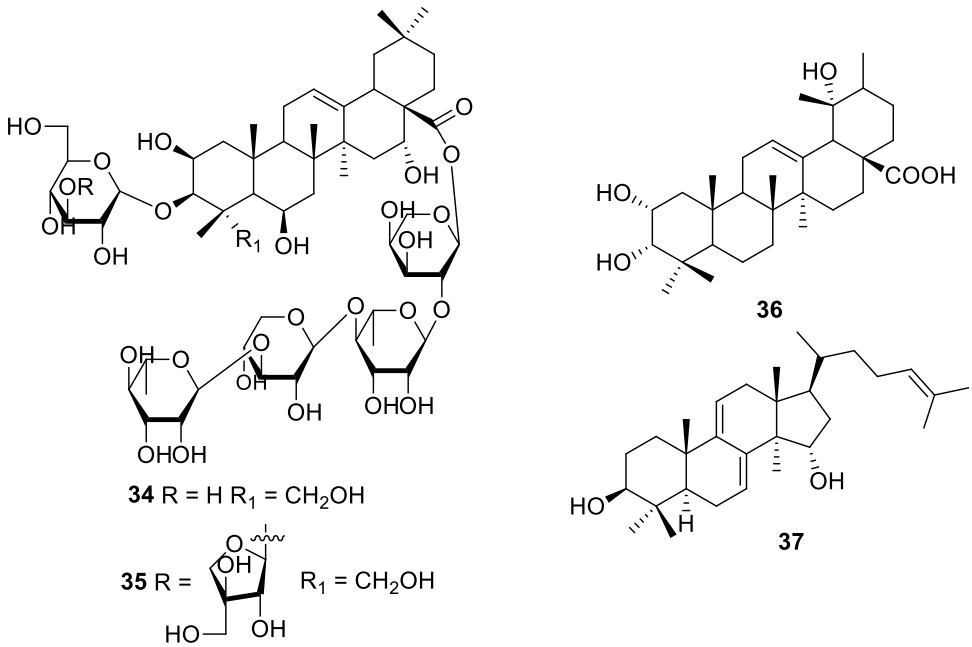


Figure 6. Structures of triterpenoids isolated from *C. febrifuga*, *H. opposita*, and *C. kirkii*.

Terminalia sericea (Combretaceae) is a shrub or medium-sized tree found abundantly in the tropical regions, and is characteristic of sandy savannah areas of Africa.[83] The plant is known locally as *gonono* or *miputua*. The dried fruits are used for the treatment of tuberculosis. A decoction of the roots is used for stomach troubles, diarrhoea, wounds, sexually transmitted diseases, female infertility, and renal diseases.[26,56,84] Bombardelli and co-workers based on the anti-inflammatory activity of the methanolic extract of the roots, isolated the terpenoid seric acid and its glycoside derivative, sericoside.[85]

Phytochemical investigation of the root extract of the shrub or tree *Olax dissiflora* (Olacaceae), known as *mucuti* or *nkondzomnhutana*, yielded four triterpenoids sapogenin, namely, 21-epimachaerimic acid, oleanolic acid, oleanolic-3-*O*-glucuronide, and hederagenin.[86] The leaves of the plants have been used in the treatment of wounds.[56]

The genus of *Cryptolepis* R. Br (Asclepiadaceae) includes about 20 species distributed throughout the tropical regions of Africa, Asia, Australia, and Papua New Guinea.[87] The aqueous extracts of the roots of *Cryptolepis obtusa* (*lellele*) are traditionally used as anti-abortive agent, to treat abdominal pains and gastro-intestinal complains.[51,88] From an extract of the roots, two steroidal alkaloids and a triterpene known as sitosteryl-3-*O*-*β*-glucopyranoside were isolated.[87]

In traditional medicine, roots extracts and dried powdered roots of *kanho-kanho*, *Euclea divinorum* (Ebenaceae) are used for the treatment of gastro-intestinal disturbances, cancer, ulcers, wounds, arthritis, miscarriage, jaundice, snakebites, and gonorrhoea. In the South-western Ethiopia, the plant is used to purify drinking water by adding branches to the gourds or pots and leaving them to soak in the water for several hours.[89] Costa and co-workers isolated naphthoquinones and terpenoids from the roots extract. The triterpenoids isolated from this plant were identified as lupeol and botulin [90]

The heartwood *teheteheratane*, *Ptaeroxylon obliquum* (Ptaeroxylaceae) grows only in Southern Africa, comprising South Africa, Zimbabwe, and Mozambique.[91] This plant is traditionally used in Southern Africa for the treatment of various diseases, ranging from headaches to tick control.[56,91] Agostinho and co-workers isolated five metabolites which include a meroterpenoid ptaerobliquol (**38**) from an extract of the roots [91]

Lippia javanica (Verbenaceae) is an erect wood shrub of up 2 m high, known as *mussumbe*, with strong aromatic leaves, which give off a lemon-like fragrance when crushed.[80] The plant is found in grasslands and woodlands, throughout the eastern and central parts of Southern Africa.[92] It is commonly used in Africa as a tea against various ailments like influenza, measles, malaria, stomachaches, fever, headaches, respiratory problems, and venereal diseases.[26,48,80] Numerous monoterpenoids have been identified from this plant. Mujovo and co-workers isolated three monoterpenoids: (*E*)-2(3)-tagentenone epoxide (**39**), myrcenone (**40**), and piperitenone (**41**) from an extract of the leaves. Metabolie **41** was found to inhibit the HIV-1 reverse transcriptase enzyme.[80]

The marine species are also good sources for secondary metabolites that are biologically active. From the Southern African soft coral *Cladiella kashmani*, collected off Ponta d'Ouro in Mozambique, two cembrane diterpenes were isolated, namely flaccidoxide (**42**) and (1*Z*,3*E*,7*E*,11*S*,12*S*,14*S*)-1,3,7-trien-14-ol (**43**). Both isolated metabolites were toxic to the brine shrimp *Artemia salina*. The occurrence of cembranoids is unusual for the genus of *Cladiella*.[93]

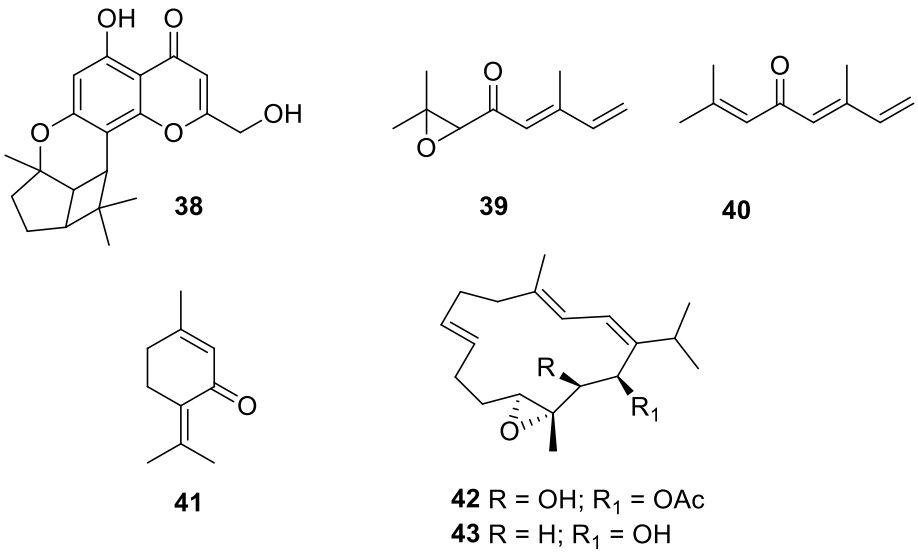


Figure 7. Terpenoids isolated from *P. obliquum*, *L. javanica*, and soft coral *C. kashimani*.

2.3 Phenolic compounds

The structure of phenolic compounds consists of an aromatic ring carrying one (phenol) or more hydroxyl (polyphenol) moieties. Several classes can be distinguished according to the number of phenol rings and the structural elements that join these rings. Two main groups of polyphenols, termed flavonoids and non-flavonoids have been adopted. The flavonoids group include flavones, flavanones, dihydroflavonols, flavonols, flavan-3-ols, isoflavonos, anthocyanidins, proanthocyanidins, and chalcones. The non-flavonoid polyphenols can be classified based on their carbon skeleton into the following subgroups: simple phenols, benzoic acids, hydrolysable tannins, acetophenones, phenylacetic acids, cinnamic acids, lignans, coumarins, benzophenones, xanthones, stilbenes, and secoiridoids.[94] The chemical structures and their natural occurrence throughout the plant, algal, bacterial, fungal, and animal kingdoms have been described in the literature. They exist in most plants tissues as secondary metabolites, and may play roles as antioxidants and for the interactions between the plant and its biological environment. Phenolics are also important components of the human diet due to their potential antioxidant activity,[95] their capacity to diminish oxidative stress induced tissue damage resulted from chronic diseases, as well as for their other biological activities.[96]

The genus of *Zanthoxylum* comprise approximately 250 species, it is well known for its ethnobotanical uses among the genera of the Rutaceae family. The plants belonging to this genus are rich sources of biologically active metabolites, such as alkaloids, aliphatic and aromatic amides, coumarins, as well as lignans.[97] *Zanthoxylum capense* is a medicinal plant indigenous to Mozambique, Zimbabwe, and South Africa. Its root bark has been used for the treatment of tuberculosis and paralysis, and to relieve toothache [98] and malaria[48]. From the root extract, the benzofuran neolignans zanthocapensol (**44**) and zanthocapensate (**45**),[99] the 2-arylbenzonfuran neolignans (-)-savinin (**46**) and (-)-episesamin (**47**),[100] the lignan (±)-syringaresinol (**48**), and the prenylpropanoid (+)-ailanthiodiol (**49**)[101] were isolated. Metabolite **45** showed inhibitory activity against *Staphylococcus aureus*, as well as the apoptosis-inducing activity in colon carcinoma cells. The structures of the phenolic compounds isolated from the roots of *Z. capense* are presented in the Fig. 8.

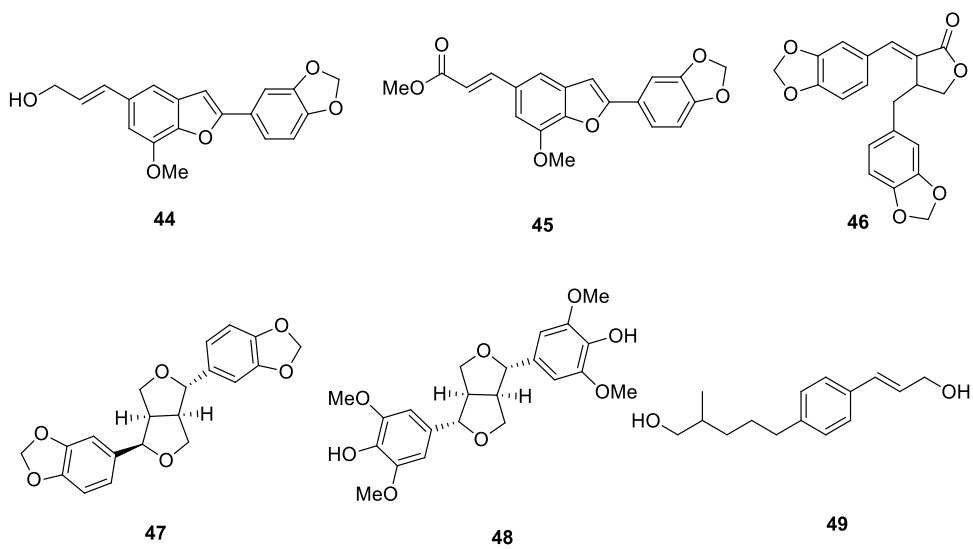


Figure 8. Phenolic compounds isolated from the roots of *Z. capense*.

From the root extracts of *E. divinorum* the naphtoquinones diosindigo A (**50**), 2-meyhlnaphtazarin (**51**), 7-methyljuglone (**52**), and diospyrin (**53**) were isolated.[90] Investigations of an extract of the leaves of *H. opposita* gave the two flavones 5,7-dimethoxy-6-methylflavone (**54**) and hoslunddiol (**55**), while the leaves of *L. javanica*, besides apegenin, yielded the metabolites cirsimaritin (**56**), 6-methoxyluteolin-4'-methyl ether (**57**), and 6-methoxy-luteolin-3',4'7-trimethyl ether.[80]

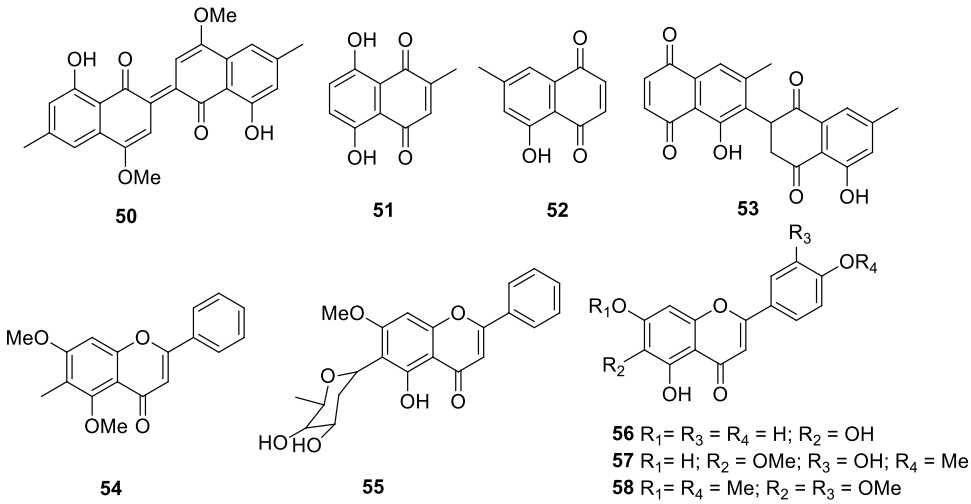


Figure 9. Phenolic compounds isolated from *E. divinorum*, *H. opposita*, and *L. javanica*.

The leguminous plant *citimbo* (local name), *Piliostigma thonnigii* (Fabaceae), belongs to a family with 133 genera. The bark of this plant has been used for the treatment of earache, toothache, diarrhoea, dysentery, intestinal problems, cough and respiratory problems throughout tropical Africa.[102] In Mozambique, the bark, leaves, and roots have been used to treat coughs, bilharziasis, venereal diseases, and backache.[26] From an extract of its leaves, the three flavonoids quercitrin, quercetin, and its 3-glucoside derivative were isolated.[103]

Landolphia kirkii (syn. *Cladostemon kirkii*, Apocynaceae), also known as *bingua*, is a specie growing in Mozambique.[104] Fresh and fermented fruits are used to make an alcoholic drink[105] and as food.[81] Bombardelli and co-workers isolated from an extract of the leaves three flavonoids: mearnsitrin (**59**), myricitrin (**60**), and kaempferol-5-rhamnoside.[106]

Brackenridgea zanguebarica (Ochnaceae), *bongo*, is a tree that can reach a height of up to 10 m. The specie occurs in dry lowland forests and woodland of Tanzania and other Southern and eastern Africa. The tree is believed to have magical powers in warding off evil spirits and in protecting from lightning strikes.[107] In Mozambican traditional medicine, the bark and roots of *B. zanguebarica* are used to treat diarrhoea, wounds, venereal diseases, and other ailments.[26] From the methanolic extract of the leaves the three flanones vitexin (**61**), iso-orientin (**62**), and sequojaflavone (**63**) were isolated.[108]

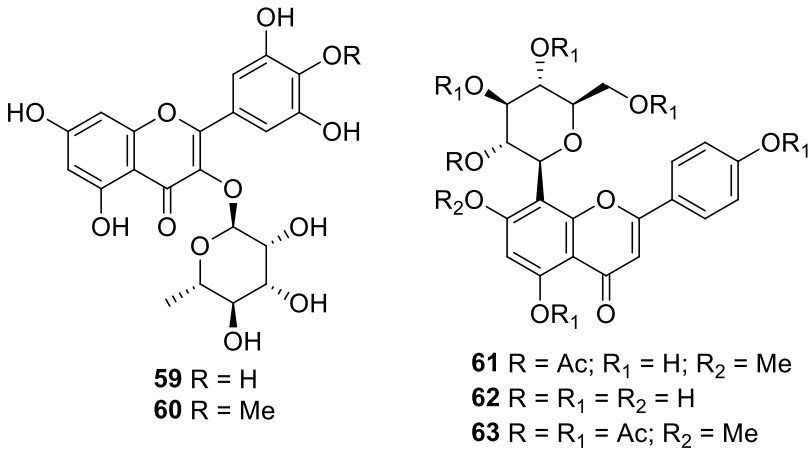


Figure 10. Phenolic compounds isolated from the leaves extracts of *L. kirkii* and *B. zanguebarica*.

Known as sources of chromones and coumarins, the two chromones ptaeroxylinol acetate (**64**) and peucenin (**65**) and the two coumarins prenyletin (**66**) and scopoletin were isolated from the roots of *P. obliquum*.[91]

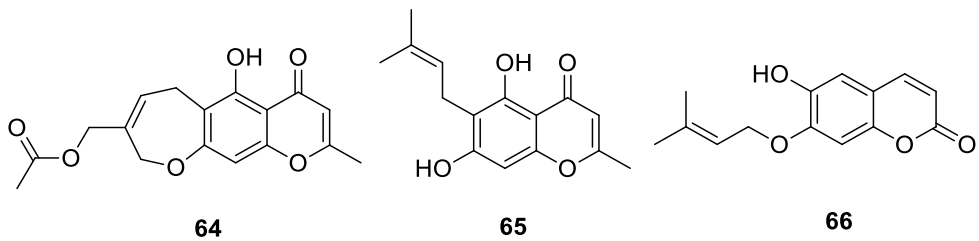


Figure 11. Phenolic metabolites isolated from the root extract of *P. obliquum*.

Through bioassay-guided fractionation of the methanol extract of the root bark of *C. kirkii*, six metabolites with different scaffolds, chamanetin (**67**), siochamanetin (**68**), dichaamanetin (**69**), (-)-cleistenolide (**70**), acetylmelodorinol (**71**), and benzophenone (**72**) were isolated.[82] The *C*-benzylated flavanone **69** exhibited strong bacteriostatic and bactericidal effects on all the Gram-positive bacteria tested.

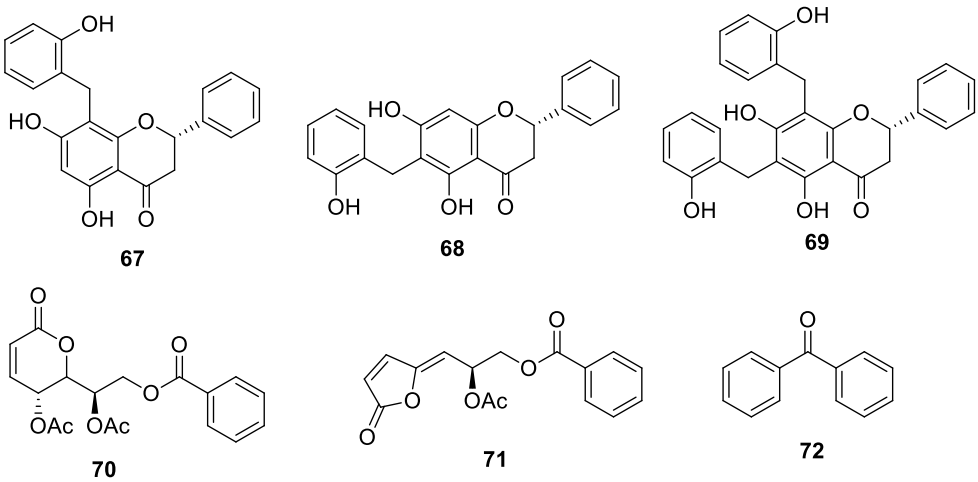


Figure 12. Structures of phenolic compounds isolated from the root bark of *C. kirkii*.

Pyrenacantha (Icacinaceae) species are used widely to treat infectious diseases such as ulcers, diarrhoea, herpes, and AIDS. *Pyrenacantha kaurabassana* (local name: *dema*) is found in Mozambique, Tanzania, and Zimbabwe. From an extract of the tubers of this plant, two heterodimers comprising anthraquinone and methylbenzoisocoumarin moieties (**73** and **74**) were isolated together with the two antraquinones emodin (**75**) and physicon (**76**). The metabolites **73** and **74** showed antibacterial activities against *S. aureus* and *Helicobacter pylori*.[109]

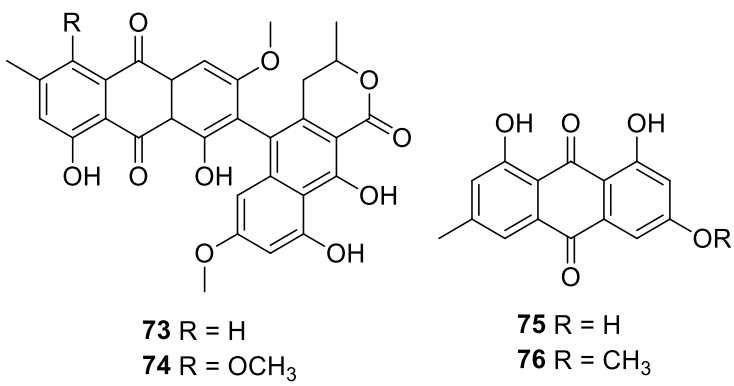


Figure 13. Heterodimers and antraquinones isolated from *P. kaurassana*.

The aqueous extracts of the latex of plant *Synadenium carinatum* (Euphorbiaceae) have been used in the traditional medicine to treat a number of inflammatory disorders.[110] From an extract of the leaves of this plant the three glycosyl derivatives of kaempferol 3-*O*-glucoside, 3-*O*-rhamnoside, and 3-*O*-galactoside, respectively, were isolated.[111] The glucoside of resveratrol was isolated from the roots of *T. sericea*.[112]

The roots of *Cladostomon kirkii* (Capparaceae) are used in Mozambican traditional medicine for the treatment of diarrhoea, stomachache, abdominal disorders, colds, sexual performance, and venereal diseases.[56,81] From an extract of the leaves of this plant, Bombardelli and co-workers isolated two kaempferol derivatives (kaempferin and kaempferol 3-gluco-7-rhamnoside) and two quercetin derivatives (quercetin 3,7-dirhamnoside and quercetin 3-gluco-7-rhamnoside).[106]

The herbal plant, *H. obtusa* is widespread in South East Africa. Its bulbiform rhizome is used for preparing infusions for urinary diseases. From the methanol extract of the ground rhizome the metabolite **7** was isolated.[23]

2.4 Nitrogen-containing compounds

More than 100,000 of secondary metabolites have been identified from natural sources, including nitrogen-free (such as terpenes, saponins, polyketides, phenolic, and polyacetylenes) and nitrogen-containing metabolites (such as alkaloids, amines, cyanogenic glycosides, non-protein amino acids, glucosinolates, alkamides, peptides, and lectins). The alkaloids are a highly diverse group of naturally chemical compounds containing one or several nitrogen atoms either in a ring structure (true alkaloids) or in a side chain (pseudoalkaloids). Chemically, they behave as bases. Depending on the ring structure and biosynthetic pathways, alkaloids are subdivided into pyrrolidine, piperidine, pyrrolizidine, quinolizidine, isoquinoline, protoberberine, morphinane, quinolone, acridone, indole, monoterpene indole, diterpene or steroid alkaloids. Several alkaloids exhibit significant biological activities.[113]

From an extract of the roots of *Z. capense*, the alkaloids, zanthocapensine (**77**), decarine (**78**), norchelerythrine (**79**), dihydrochelerythrine (**80**), 6-acetonyldihydrochelerythrine (**81**), tridecanonchelerythrine (**82**), 6-acetonyldihydronitidine (**83**), rutaecarpine (**84**), skimmianine (**85**),[98,99] oxychelerythrine (**86**), oxynitidine (**87**), arnottianamiden (**88**), and (+)-tembamide (**89**)[101] were isolated (Fig. 14). Metabolite **85** showed high activity against *M. tuberculosis*.[98] **77**-**81** and **83** showed inhibitory activity against *S. aureus*.[99] **77**, **78**, and **81** displayed cytotoxic to HCT116 cells.[100]

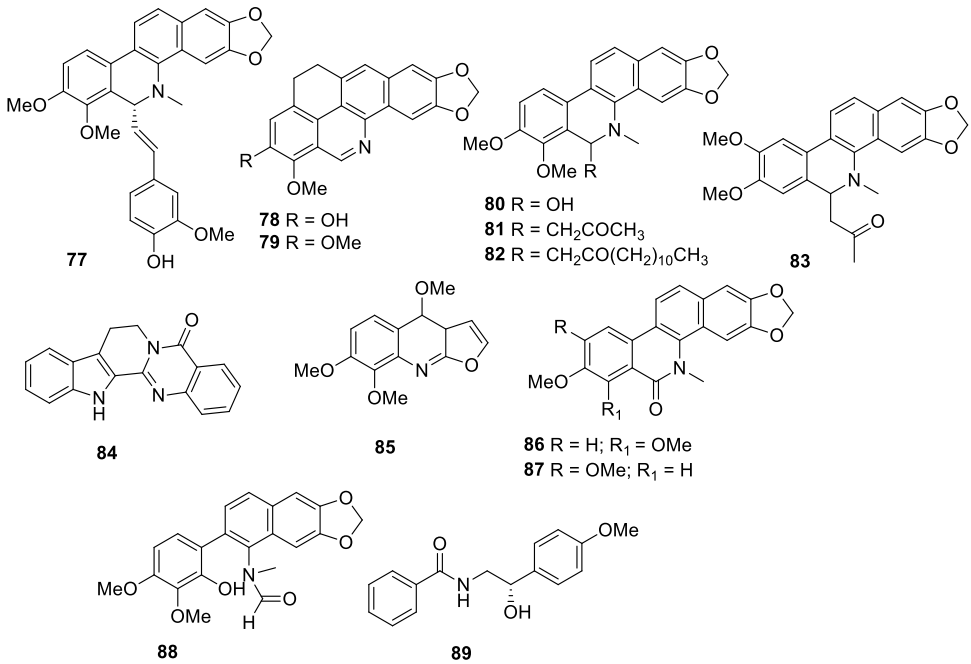


Figure 14. Alkaloids isolated from the roots of *Z. capense*.

Most of the *Tabernaemontana* species (Apocynaceae) have been used to treat tumors and cancer, and occur in tropical and subtropical parts of the world, including Africa.[114] The plants has been used for the treatment of malaria and respiratory complaints.[48] These species are characterized by producing large amounts and wide range of indole alkaloids, mainly corynanthe-type monoterpene alkaloids.[115] The phytochemical analysis performed with an extract of the leaves of *Tabernaemonta elegans* (*cacho*) led to the isolation and characterization of three indole alkaloids, belonging to a class of *β*-carbolines indole alkaloids, tabernines A-C (**90**-**92**) (**90**-**92**).[116]

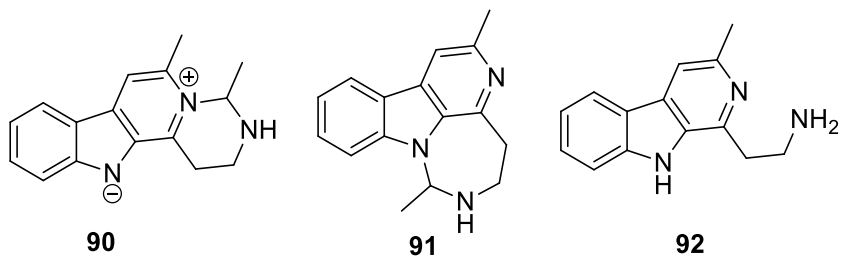


Figure 15. The *β*-carbolines alkaloids isolated from the leaves of *T. elegans*.

From an extract of the root bark of this plant the monomeric indole alkaloids dregamine (**93**) and tabermontanine (**94**), as well as the bisiindole alkaloids conoduramine (**95**), tabernaelegantines A-D (**96-99**), tabernaelegantinines A (**100**) and B (**101**) were isolated.[117,118]

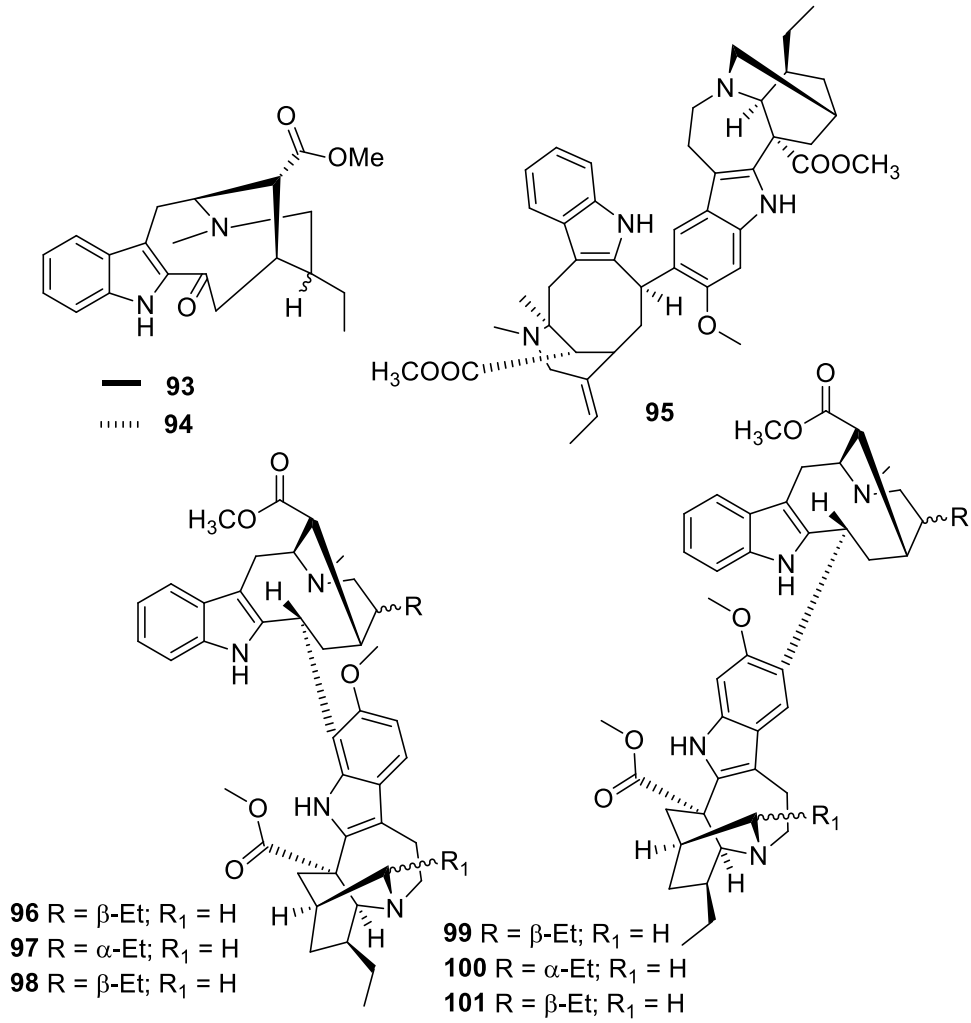


Figure 16. Indole alkaloids isolated from the roots bark of *T. elegans*.

From an extract of the root of *T. elegans*, monoterpene indole and bisindole alkaloids were isolated. Besides the isolation of metabolites **93**, **94**, **98**, and **101**, Mansoor and co-workers also isolated 16-epidreganine (**102**), voacangine (**103**), and

vobasine (**104**).[114] The bisindole alkaloids (19'*S*)-hydroxytabernaelegantine A (**105**), 3'-oxotabernaelegantine C (**106**), and 3'-oxotabernaelegantine D together with metabolites **96** and **108** were isolated by Paterna and co-workers, together with (3'*R*)-hydroxytabernaelegantine C.[119,120] Their structures are presented in the Fig. 17.

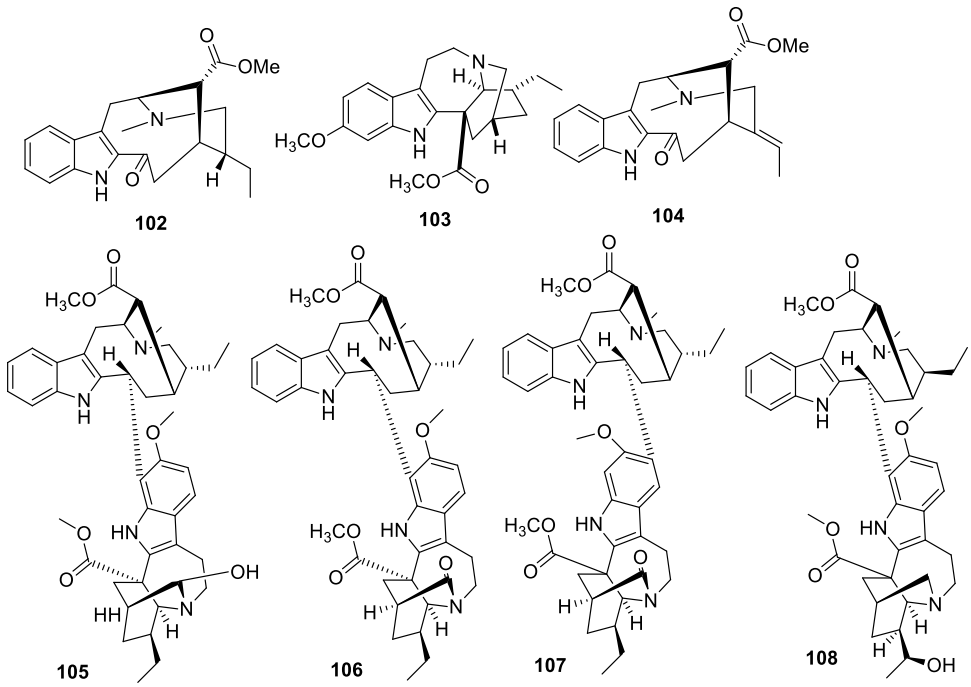


Figure 17. Indole alkaloids isolated from the roots of *T. elegans.*

From the extracts of a *T. elegans* callus culture, fourteen alkaloids were isolated. Besides the isolation of metabolites **94**, **96-98**, **100**, **101**, and **104**, the metabolites isovoacangine (**109**), vobasinol (**110**), apparicine (**111**), 16-hydroxy-16,22-dihydro-apparicine (**112**), tubotaiwine (**113**), isositsirikine (**114**), and 3(*R*/*S*)-hydroxy-conodurine (**115**), were also obtained.[121]

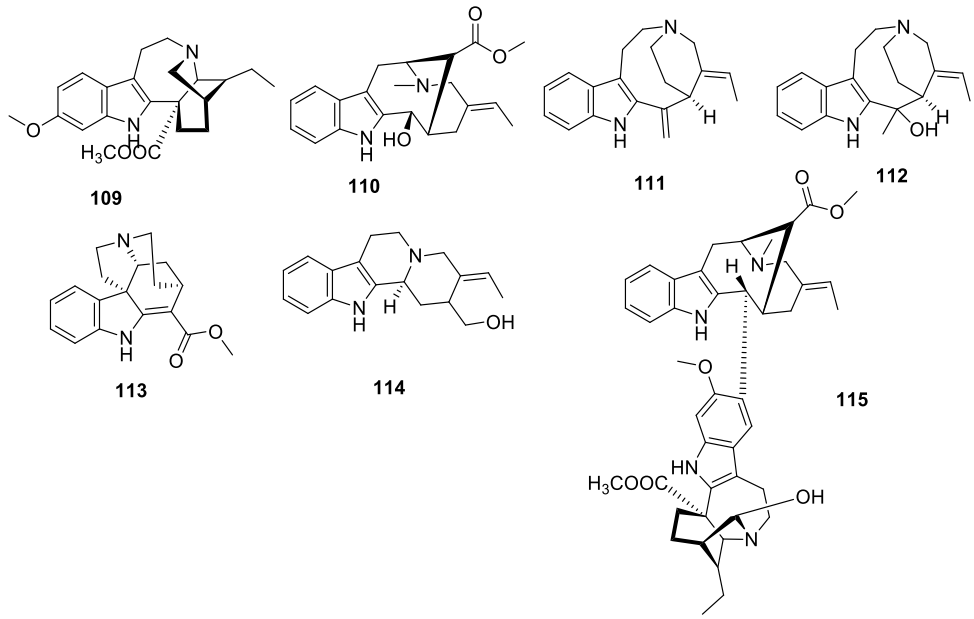


Figure 18. Indole alkaloids isolated from the *T. elegans callus* culture.

Gymnosporia arenicola (syn. *Maytenus heterophylla*, Celastraceae) is an African shrub or small tree, which naturally occurs in coastal sand dunes of Southern Mozambique and South Africa.[122] This plant is often employed in the African traditional medicine against infectious and inflammatory diseases.[53,122] In search of phytochemicals with promising cytotoxic activity from Mozambican plants, da Silva and co-workers isolated the spermidine macrocyclic alkaloid **116** from an extract of the leaves, which was shown to be non-cytotoxic towards the cell lines tested.[123]

The root of *C. obtusa* yielded two steroidal alkaloids with a 5Δ-pregnen nucleus (**117** and **118**),[87,88] while a chloroform extract of the root bark yielded the alkaloid echuinulin (**119**).[82] The structures of these compounds are presented in the Fig. 19.

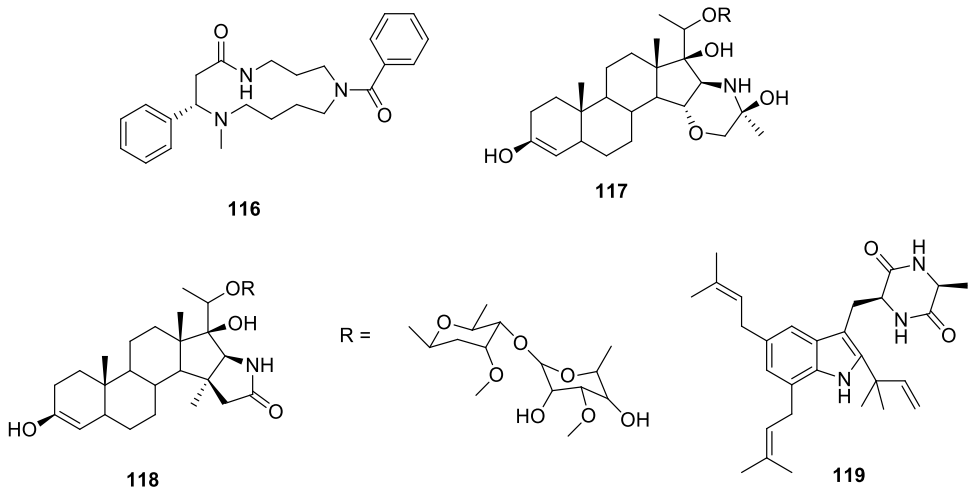


Figure 19. Structures of the alkaloid isolated fromt *G. arenicola*, *C. obtusa*, and *C. kirkii*.

Gorgonians produce a plethora of novel metabolites with diverse carbon skeletons and biological activities. In search for novel anti-oesophageal cancer agents from Southern African marine invertebrates, the methanol extract of the gorgonian *Leptogorgia gilchhristi* collected in Mozambique was investigated. The EtOAc partition fraction yielded three tetraprenylated alkaloids, the malonganenones A-C (**120**-**122**).[124]

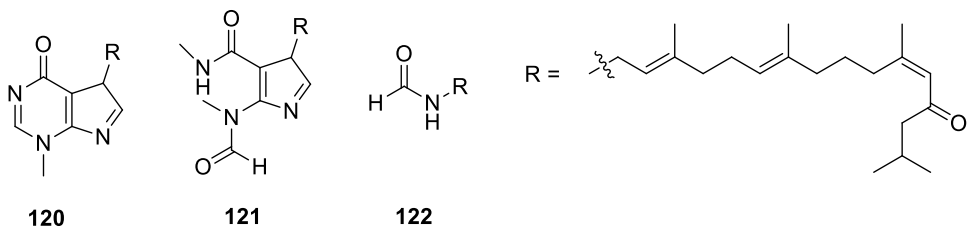


Figure 20. Tetraprenylated alkaloids isolated from the marine gorgonian *L. gilchhristi*.

Lissoclimum and *Didemnum* ascidians are prolific producers of cyclic peptides. A particularly intriguing family of marine cyclopeptides produced by *Lissoclimum patella* and *Didemnum molle* is the 18-, 21-, and 24- membered cyclopeptides characterized by an alternating sequence of thiazole, thiazoline or oxazoline heterocycles and hydrophobic amino acids.[125] *D. molle* is a common Indo-Pacific ascidian associated with endosymbiotic Prochloron algae. Different cyclic peptides were isolated from *D. molle* collected in different locations; the hexapeptides (18-

membered) comoramides and the heptapeptides (21-membered) mayotamides from the collection in Comoros Islands,[126] the heptapeptides cyclodidemnamide from Philippine Islands,[127] and mollamide.[128] The cycloheptapeptide, cyclodidemnamide B (**123**) was isolated from this marine ascidian collected at Ibo Island, Mozambique.[129]

Lithistid sponges have yielded a wide variety of bioactive marine natural products that include a diverse array of cyclic peptides, which are notable for their incorporation of structurally unusual amino acids and nonproteinorgenic D-amino acids.[130] Lithistid sponges identified as *Theonella* sp. are often superficially similar to *Theonella swinhoei* (Theonellidae), except that the interior tissue is colored orange or yellow due to the presence of aurantoside A (**124**). Schmdt and co-workers, besides isolating **124** they also isolated the two cyclic peptides mozamides A (**125**) and B (**126**) from a theonellid sponge collected in the Southern Mozambique.[131] Two other cyclic peptides known as swinholide A (**127**) and theopaulamide (**128**) were isolated from Mozambican specimens of the lithistid sponge *T. swinhoei*.[132]

The thiocoraline (**129**), a depsipeptide with antitumor activity, was isolated from the marine soft coral *Micromonospora marina* collected off the Mozambique coast.[133,134] The metabolite **129** demonstrated activity against a variety of subpanels in the NCI's 60 cell line screen, including breast, colon, renal, and melanoma, and was reported by PharmaMar scientists to have *in vivo* activity.[135]

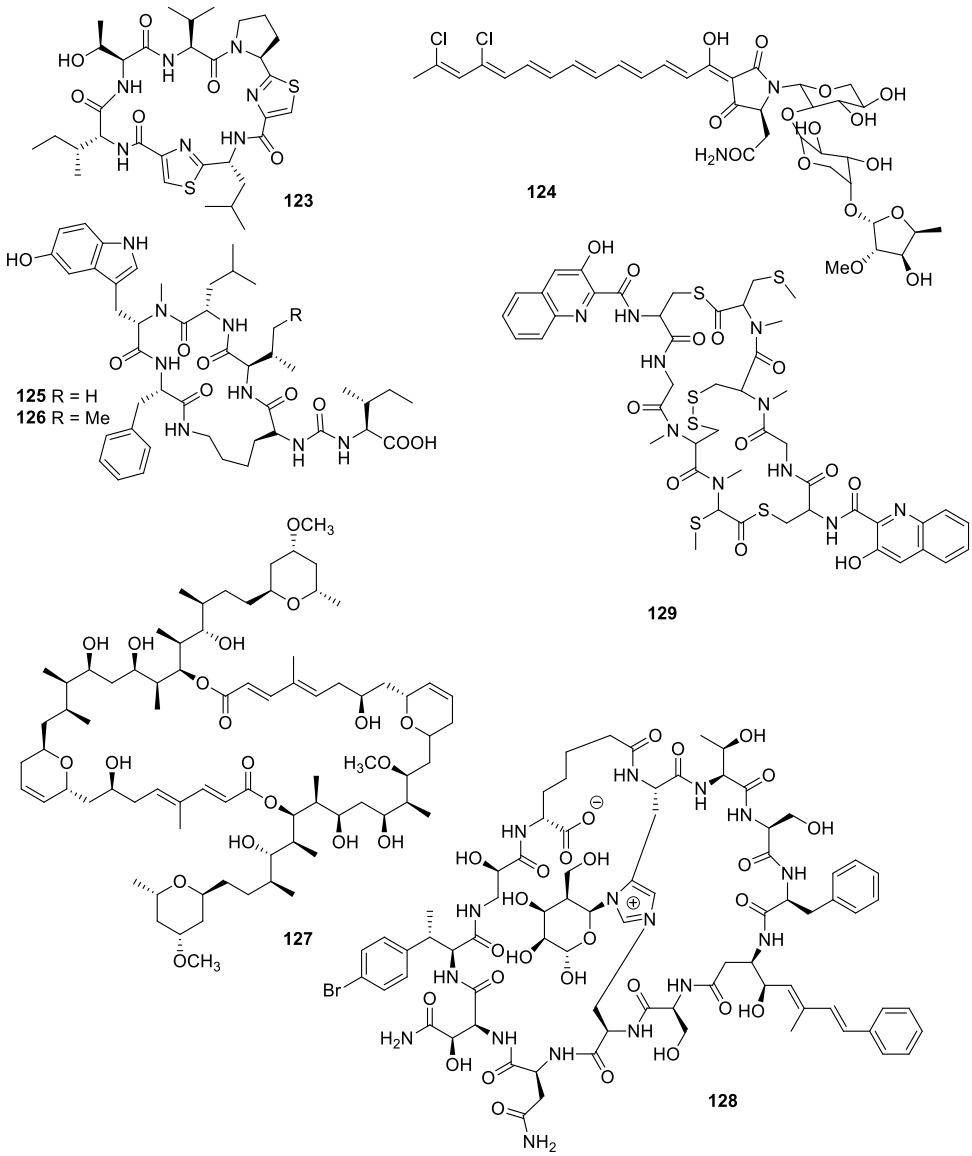


Figure 21. Secondary metabolites isolated from Mozambican marine sources

2.5 Other classes of metabolites from Mozambique

Afzelia cuanzenzis (Caesalpiniaceae) is a tropical plant found in Africa belonging to the genus of *Afzelia* which comprise fourteen plant species.[136] The roots and bark are used for treatment of venereal diseases, general sexual transmitted diseases.[26] From an extract of the seeds of this plant were isolated crepenynic acid (**130**) and dehydrocrepenynic acid (**131**).[136] The 4-ethyl-nonacosane (**132**) was isolated from the leaves of *L. javanica*[80] and from the root bark of *C. kirkii* was isolated the *cis*-solamin (**133**).[82]

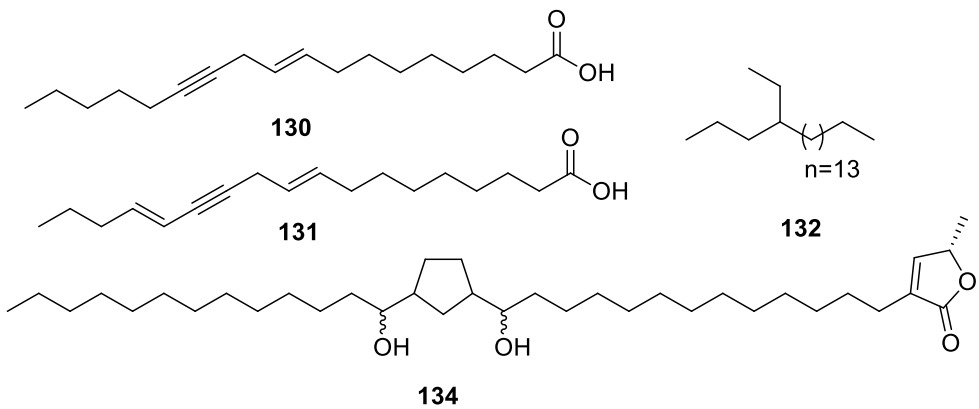


Figure 22. Other metabolites isolated from Mozambican plants

2.6 Aim of the thesis

Plants have been one of the most important sources of medicines throughout times. Recently, in spite of tremendous advances in the field of natural products chemistry, they still remain one of the major sources of drugs in the modern as well as traditional system of medicine. Throughout the world, over 74 % of all pharmaceuticals are plant-based.

In this work, four medicinal plants used in Mozambique for the treatment of various ailments were selected based on their traditional uses. The selected plants were from different families: *Cadaba natalensis* (Paper I), *Clematis viridiflora* (Paper II), *Brachylaena discolor* (Paper III), and *Senna spectabilis* (Paper IV) Extracts of these four plants were evaluated with the aim to characterize their

chemical constituents, as well as to assay the leishmanial activity of some of isolated metabolites.

In order to achieve the aim, the main tasks were:

- A review of the literature on the four selected plant from the aspect of chemistry and pharmacological properties.
- Collection and identification of the selected plant species.
- Preparation and fractionation of their crude extracts.
- Isolation of the chemical constituents by chromatographic techniques.
- Structural elucidation and characterization of the isolated metabolites using spectroscopic methods.
- Leishmanial activity assay for some isolated metabolites.

3 Plants Investigated

3.1 *Cadaba natalensis* (Paper I)

3.1.1 Introduction

The family of Capapraceae comprises approximately 40 to 45 genera with 700 to 900 species distributed in various habitats.[137] A number of medicinal plants belonging to this family are known, including the genus of *Cadaba*. *Cadaba* is a genus with 30 species widely distributed in southern Africa, India, Malaysia, and Australia.[138] Some species have been reported as toxic plants, while others are reputed for their medicinal values, such as purgative, antihelmithic, antispasmodic, antibacterial properties.[139–141] Alkaloids and sesqueterpene lactones are the most metabolites found in *Cadaba* species.[139] *Cadaba natalensis* Sond. (*tsatsassatana*) is a shrub found in Southern Mozambique, South Africa, and Swaziland.[52] Medicinally, the roots and leaves have been used to treat tuberculosis in Mozambique,[52,56] to induce vomiting and to treat chest pains in South Africa.[142] Prior to this study, no chemical investigations were done on this plant.

3.1.2 Results and discussions

The air-dried roots (300 g) were extracted sequentially by maceration with *n*-heptane, chloroform, EtOAc, and MeOH yielding four crude extracts weighing 8.9 g, 13.2 g, 35.1 g, and 57.7 g, respectively. By the employment of several chromatographic methods, from the EtOAc extract, the macrocyclic dibenzo-diazacyclodo- decanedione (**134**), thalifoline (**135**), *N*-methylcordaldine (**136**), and nepetin (**137**) were isolated, while the analysis of the *n*-heptane fraction yielded *β*-sitosterol (**138**), (*S*)-2-ethyl-2-methyloxazolidin-5-one (**139a**), and (*R*)-5-ethyl-5-methyloxazolidin-2-one (**140**). By the analysis of the spectroscopic and physical data of the isolated metabolites, **134** and **139a** were characterized as knew compounds, while the **140** was identified as natural product for the first time. **140** was obtained synthetically by halohydrin dehalogenase catalysed opening of the corresponding racemic terminal epoxide in the presence of cyanide.[143] The

structures of the know metabolites were established by comparison of their spectroscopic data with the reported data in the literature.

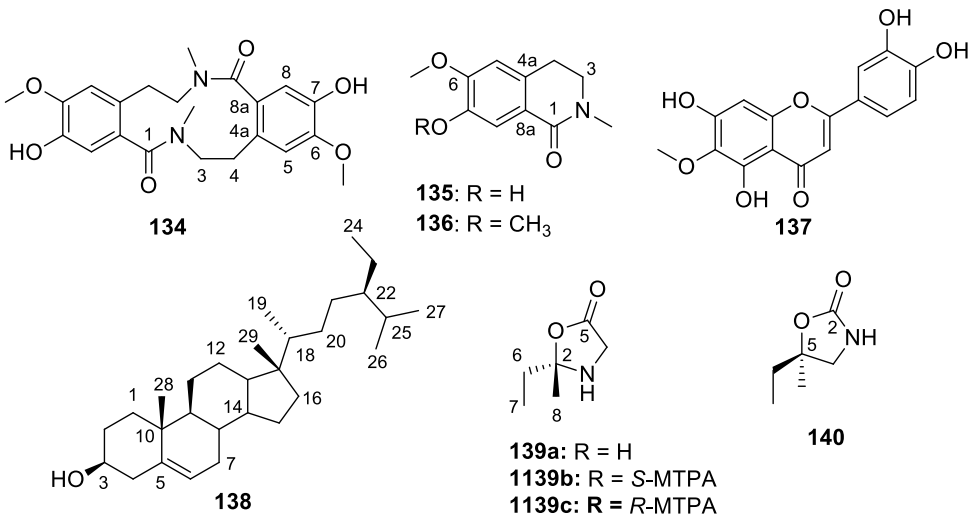


Figure 23. Structures of the metabolites isolated from the roots of *C. natalensis*.

The NMR data of metabolites **134-136** were very similar. It was found that they are related to thalifoline (**135**). **135** was obtained as colorless solid with molecular formula $C_{11}H_{13}NO_3$, based on its HR-ESMS (*m/z* 208.0972 $[M+H]^+$). Its ^{1}H-NMR showed resonances at δ_H 2.93 (2H, *t*, *J* = 6.7 Hz, H-4), 3.16 (3H, *s*, N-CH_3), 3.51 (2H, *t*, *J* = 6.7 Hz, H-3), 3.95 (3H, *s*, O-CH_3), resonances for aromatic protons at δ_H 6.63 (1H, *s*, H-5) and 7.72 (1H, *s*, H-8), and a sharp singlet at 6.05 for a phenolic hydroxyl group. The ^{13}C-NMR displayed eleven attributed for six aromatic carbons (C-4a, C-5, C-6, C-7, C-8), a carbonyl group (C-1), N-CH_3, O-CH_3, and two methylene groups (C-3 and C-4). The DEPT data revealed that C-5 and C-8 were hydrogenated carbons in aromatic system. Only the coupling between H-3 and H-4 was observed in COSY spectrum, suggesting that the aromatic ring is tetrasubstituted ring at positions 4a, 6, 7, and 8a. The final structure of **135** was deduced on the basis of HMBC correlations between N-CH_3 to C-1 and C-3; H-3 to C-1, C-4, and C-4a, and N-CH_3; H-5 to C-4, C-6, C-7, and C-8a; H-8 to C-1, C-4a, and C-6, and OCH_3 to C-6 confirmed the position of the methoxy group. The final structure was confirmed by NOESY correlation between OCH_3 to H-5, as well as by the presence of six degrees of unsaturation. The spectroscopic data were very similar to those reported to thalifoline.[144]

The molecular formula of **136** was deduced to be $C_{12}H_{15}NO_2$. The molecular formula suggested the presence on an extra methyl group compared to **135**, which

was confirmed by the presence of resonances for OCH_3 in both ^1H and ^{13}C-NMR spectra. The absence of the hydroxyl group on **136** it was possible to determine the position of the methoxy group at C-7, and it was confirmed by HMBC and NOESY correlations between OCH_3 toC-7. Thus **136** was identified as *N*-methylcorydaldine, and its data were similar with the reported in the literature.[145]

134 was isolated as a colorless solid with molecular formula $C_{22}H_{26}N_2O_6$, as suggested on the basis of its HR-ESMS (*m/z* 415.1865 $[M+H]^+$). An analysis of its NMR data showed a similar pattern to **135**. On the basis of MS data (molecular formula, molecular mass, and eleven degrees of unsaturation) it was concluded that **134** is a dimer of **135**. These observations were confirmed by the appearance of a fragment at *m/z* 208, corresponding to $[M+H]^+$ for **135**. **134** was found to be a novel macrocyclic dibenzo-diazocyclododecanedione.

Metabolites **134-136** belong to a class of compounds called isoquinolone alkaloids. The isoquinlone alkaloids are found as minor constituents in plants. Besides occurring in others family species, the *Thalictrum* spp. (Ranunculaceae) are the most common sources of these metabolites and they have been reported to possess a vasorelaxant effect.[146–148]

Metabolites **134** and **135** could share the same biogenetic pathway, although the route is not known. The isoquinolone alkaloids derive biogenetically by oxidative cleavage of a simple benzylisoqquinoline.[149] Dopamine is the precursor of isoquinoline alkaloids moiety, whereas the 4-hydroxyphenylacetaldehyde is incorporated as the benzyl component (Fig. 24). The (*S*)- norcoclaurine formed is transformed into (*S*)-*N*-methylcoclaurine, which probably could be the precursor of **134-136**.[150]

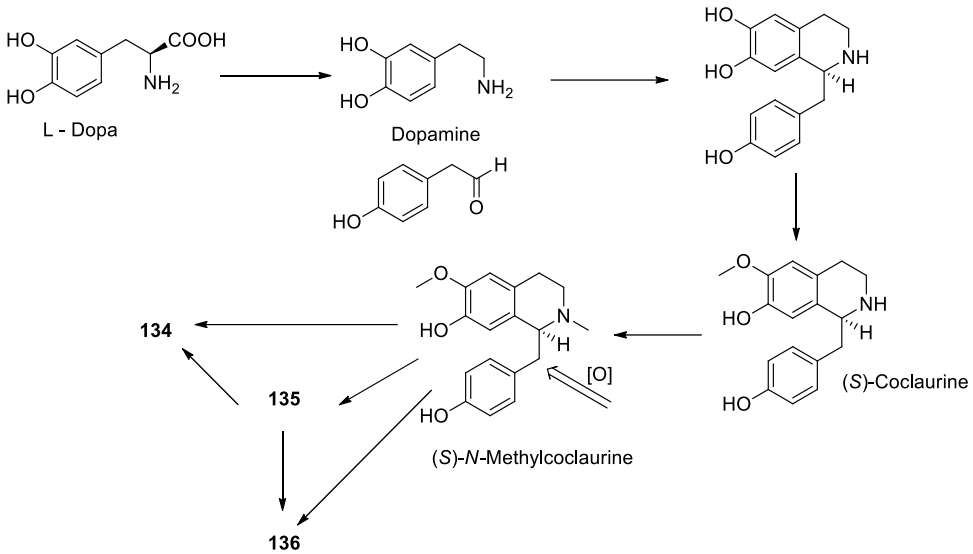


Figure 24. Proposed biogenesis route for metabolites **135-136**

137 was isolated as a yellowish solid. The HR-ESMS displayed the $[M]^+$ at *m/z* 316.0423, corresponding to the elemental composition $C_{16}H_{12}O_7$. The ^{1}H-NMR spectrum has the presence of two singlets at δ_H 6.60 (H-8) and 6.79 (H-3). The other aromatic protons for ring B were assigned as doublet at 7.38 (H-2'), doublet of doublets at 6.92 (H-6'), and doublet at 6.89 (H-5'). Moreover, the spectrum indicated the presence of OCH_3 at 3.60. In the COSY spectrum, the correlations between H-6' to H-2' and H-6' to H-5' were observed. In the ^{13}C-NMR spectrum, sixteen signals were observed. The most downfield ^{13}C signal at 181.3 was assigned as C-4. Based on ^{13}C-NMR and DEPT analysis six aromatic oxygenated carbons at δ_C 162.4, 157.0, 152.8, 152.3, 149.6, and 144.9 were assigned to C-2, C-7, C-5, C-9, C-4', and C-3', respectively. The three remaining quaternary carbons were found at 131.0, 121.5, and 104.0, for C-6, C-1', and C-10, respectively. From the HMBC spectrum the five methine aromatic signals were found at 119.2, 116.1, 113.3, 102.5, and 94.0 for C-6' C-5', C-2', C-3, and C-8, respectively. HMBC correlations observed between: OCH_3 to C-6; H-8 to C-4, C-6, C-7, C-9, and C-10; H-3 to C-1', C-2, C-4, and C-10; H-2' and H-6' to C-1', C-2, C-3', and C-4', made it possible to determine the structure of **137** as a flavone named nepetin.[151]

138 was isolated as colorless oil, and its mass spectral data suggested the molecular formula $C_{29}H_5O$. The ^{1}H-NMR spectrum showed the presence of six methyl groups as three doublets (Me-19, Me-26, and Me-27), one triplet (Me-24), and two singlets (Me-28 and Me-29), as well as, an olefinic proton (H-6) and an oxygenated hydrogen (H-3). The analysis of ^{13}C-NMR, together with COSY, HMQC, and DEPT data, twenty-nine carbons signals were assigned including six

methyl, eleven methylene, nine methine groups, and three quaternary carbons. The structure of **138** was elucidated as *β*-sitosterol by comparison of the spectroscopic data with those reported in the literature.[152] However, the key COSY correlations between H-2 and H-3, H-6 and H-7, H-9 and H-11, H-14 and H-15, H-21 and H-22, H-25 and H-26, H-25 and H-27; as well as the key HMBC correlations between H-6 to C-4, C-5, and C-10; H-28 to C-10; H-29 to C-14; H-17 to C-20; H-20 to C-22; and H-22 to C24, were observed. **138** is widely distributed in the plant kingdom. It is found in vegetable oils, nuts, and some prepared foods like salad dressings. It has the potential to reduce benign prostatic hyperplasia and high blood cholesterol levels. Its high concentrations in blood have been correlated with increased severity of heart disease in people that previously suffered from heart attack.[153]

The analysis of MS data of **139a** and **140** revealed that both metabolites were isomers and the ^{1}H-NMR data showed same features in terms of chemical shifts and multiplicity. In order to characterize each compound the ^{13}C-NMR was analysed. The chemical shift of the carbonyl groups were different. For **139a** was found at δ_C 188.6 corresponding to an ester while in **140** was found at δ_C 159.4 for a carbonyl group with two heteroatoms. These observation were confirmed by the analysis of the chemical shifts of the quaternary carbon atoms in both compounds. In **139a** the quaternary carbon appear at δ_C 91.1 for a carbon atom with two heteroatoms while in **140** is found at δ_C 83.6 for a carbon with an oxygen atom. Thus, both compounds could be identified, **139a** was a novel metabolite while **140** was isolated for the first time as natural product. The absolute configuration of **139a** was determined based on Mosher's method[154], which implies the preparation of 2*S*- and 2*R*- esters derivatives (**139b** and **139c**) for **139a**. Thus, the absolute configuration of **139a** was determined and the compound was named (*S*)-(+)-2-ethyl-2-methyloxazolidin-5-one. Due to lack of material, the absolute configuration of **140** was not determined. However, from the literature it was proposed to be (*R*)-5-ethyl-5-methyloxazolidin-2-one.[143]

The *Streptomyces* spp. are the only reported source for the oxazolidin-5-one. They are biosynthesized under specific nutrient and stress conditions by *Streptomyces venezuelae*. The formation of the five-membered oxazolidin-5-one arises through the reaction of the amino acid present in the culture medium with a biosynthesized precursor aldehyde or ketone. The pathway generates a reactive aldimine that undergoes series of transformations to furnish a cyclic product as outlined in Fig. 25.[155,156]

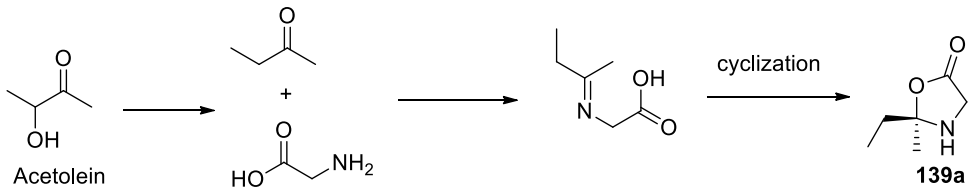


Figure 25. Proposed biosynthesis pathway of oxazolidin-5-one (**139a**)

Although rare in nature, stereoisomers of oxazolidin-2-one have been isolated from marine sponges belonging to the order of *Verongida*, mainly as dibrominated phenolic bis-oxazolidin-2-one. Their biogenesis has never been investigated. However, it is thought that they are derive biogenetically from the amino acid dibromotyrosine.[157–159] The metabolite **140** has an isoprene unity, and could be derived from an amino alcohol and a carbonate. The amino alcohol may be derived from an isoprenoid unity or from an unusual rare *β*-hydroxyamino acid (Fig. 26). The only known *β*-dialkyl-substituted amino acid is *β*-hydroxy valine. Although they have similar functionalities and ^{1}HNMR data, and the same molecular formula, **139a** and **140** could not share the same biogenesis.

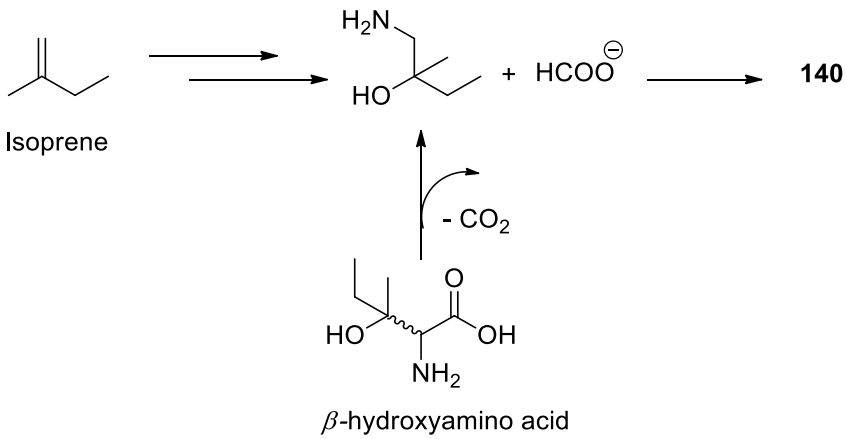


Figure 26. Proposed biogenesis of **140**

3.2 *Clematis viridiflora* Bertol. (Paper II)

3.2.1 Introduction

The family of the Ranunculaceae is composed by 62 genera with 2,450 species distributed all over the world except Antarctica. Many genera of the family have ornamental species that are used in gardens. A number of species are highly poisonous and some species have traditional medicinal uses,[160] most of them belonging to the genus of *Clematis*.

Clematis, a large genus within the dicotyledons, with approximately 350 and 355 species, is native in the temperate zone in both hemispheres and many species are horticultural interest, and some other are regarded as pharmaceutically important.[161–163] At least twenty six species of the genus have been traditionally used in various systems of medicine for the treatment of various ailments such as nervous disorders, syphilis, malaria, dysentery, rheumatism, asthma, and as analgesic, anti-inflammatory, diuretic, antitumor, antibacterial, and anticancer.[164–166] A survey of the literature revealed that triterpenes saponins, alkaloids, flavonoids, steroids, coumarins, macrocyclic compounds, phenolic glycosides, anemonin, and volatile oils constitute major classes of constituents of the genus.[164,165] There are 13 species growing in Mozambique and *Clematis viridiflora* Bertol. (*kwassakwassa*) is found in the Southern and Central parts of the country.[167] The roots and leaves of the plant have been medicinally used for the treatment of malaria and headache.[26,55] Prior to this study, no chemical investigations were done with this plant.

3.2.1 Results and discussions

In search for interesting secondary metabolites from the diverse flora of Mozambique an extract of the leaves of *C, viridiflora* was investigated for its chemical constituents. The air-dried powdered leaves were extracted with a mixture of MeOH:CH_2Cl_2 (1:1) to afford a crude extract which was then partitioned between *n*-heptane, CH_2Cl_2, and EtOAc, successively. After several chromatographic methods purifications, the metabolite **141** was isolated from the CH_2Cl_2 sub-fraction as a yellowish solid. The chromatographic analysis of the EtOAc sub-fraction led to the isolation of the following metabolites: prunassin (**142**), chlorogenic acid (**143**), 5-*p*-coumaricquinic acid (**144**), caffeic acid (**145**), umbelic acid (**146**), baicalin (**147**), quercetin-7-*O*-*β*-galactopyranside (**148**), astragalin (**149**), flindalatin-5-methyl ether (**150**), benzoic acid (**151**), 4-hydroxy-benzoic acid (**152**), 3-*O*-[2'-(tetracosyloxy)acetyl]lupeol ether (**153**). In addition **141** was assayed to leishmanial

activity towards to leishmanial strains *L. amazonensis clon 1* and *L. brasiliensis*. Their structures are presented in the Fig 27.

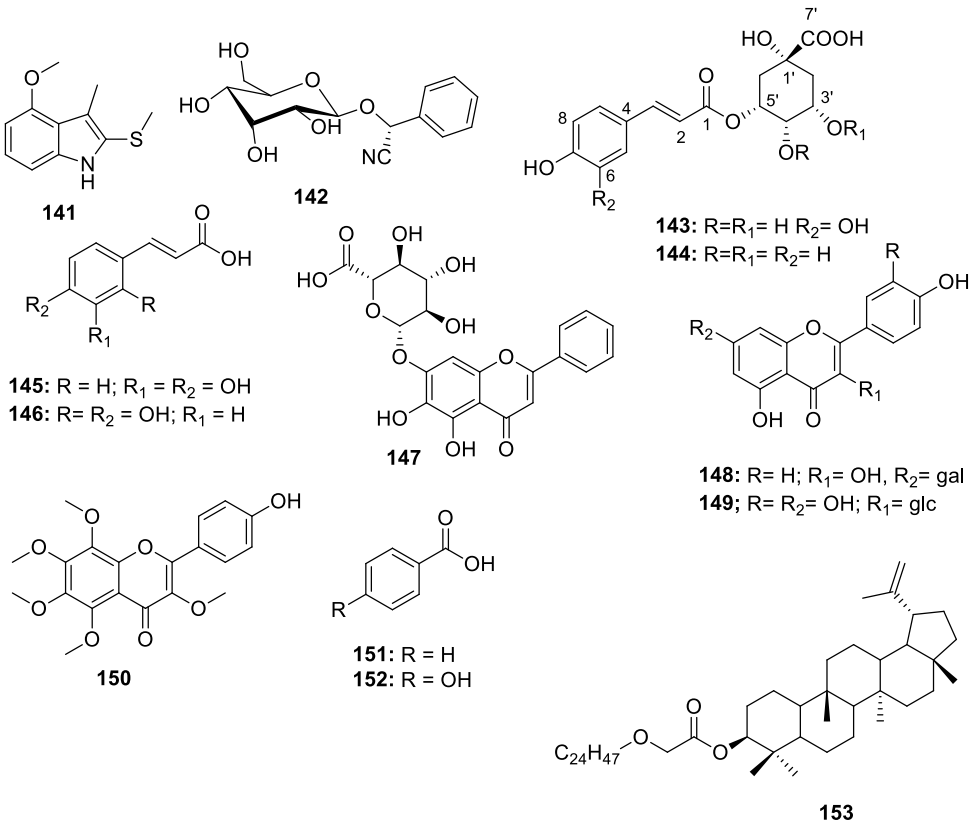


Figure 27. Secondary metabolites isolated from the leaves of C. viridiflora.

From the analysis of its spectroscopic analysis, it was deduced that **141** was a novel metabolite with a sulfur-containing indole nucleus. The final configuration and assignment was based on the analysis of NOESY correlations observed between protons S-CH_3 to CH_3, CH_3 to S-CH_3 and O-CH_3, as well as O-CH_3 to H-5 and CH_3. Thus, **141** besides being a novel metabolite, also, was identified as the first sulfur-containing indole nucleus isolated from any member of the Ranunculaceae family.

Most of studies related to sulfur-containing indoles were devoted to *Brassica* spp. (Cruciferae), which are the main source of these metabolites, in which they are found as minor constituents and are known as phytoalexins with a wide range of biological activities.[168–170] The main characteristic feature of these metabolites is the presence of one or two sulfur atoms, of which one of them is located at C-2.[170] The indole nucleus is biogenetically derived from *L*-Tryptophan and the sulfur atoms are

incorporated through Cysteine and Methionine. The brassicanal could be the biosynthetic precursor of **141**, as suggested in Fig. 28. The biogenesis of brassicanal A was studied in *Brassica campestis*.[168,171,172] **141** showed antileishmanial activity against both leishmania strains *L. amazonensis clon 1* and *L. brasiliensis*, with IC_{50} values 25.0 and 13.2 μM, respectively. The positive controle Miltefosine (a current drug used to treat leishmanial) was slightly more potent with IC_{50} values 5.1 and 4.9 μM, respectively.

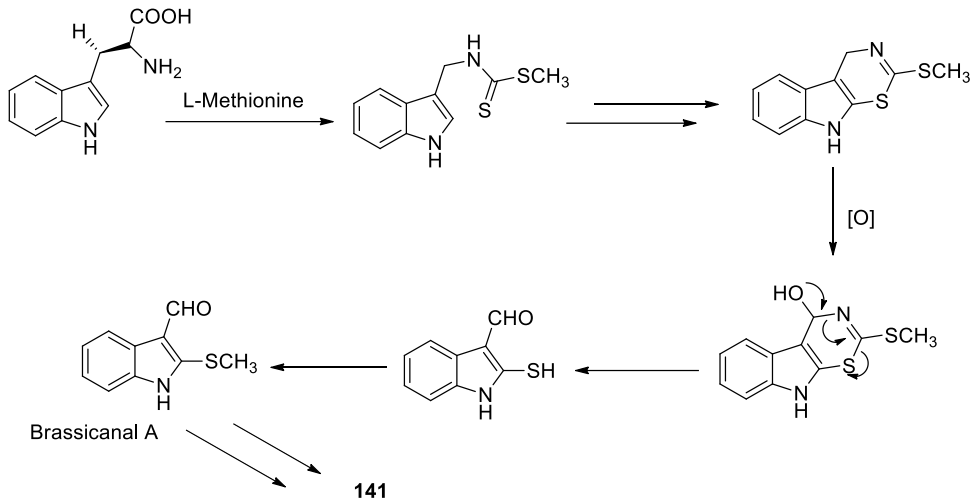


Figure 28. The biogenesis of Brassicanal A, a proposed precursor of **141.**

The ^{1}H-NMR spectrum of metabolite **142** showed one broad aromatic signal as a multiplet at δ_H 8.11-7.62, typically for a monosubstituted aromatic ring. A singlet was observed at 5.93, as well as a doublet at 4.30. The resonance at 3.94-2.83 suggested the presence of the sugar moiety. Based on HR-ESMS and NMR data, its molecular formula was deduced to be $C_{14}H_{17}NO_6$. However, the ^{13}C-NMR showed only twelve signal which confirmed the presence of a monosubstitued aromatic ring (two carbon atoms are missing). The anomeric carbon confirmed the presence of the sugar moiety. The final structure was determined by HMBC correlations of the H-α to the aromatic and sugar carbons, and **142** could be identified as a cyanogenic glycoside prunasin.

Isolation of **142** could indicate that *C. viridiflora* is a potentially toxic plant.[173] This also could be confirmed by the fact that some species of this genus are considered toxic plants. **142** is a cyanogenic glycoside, and cyanogenic glycosides

are secondary metabolites composed by α-hydroxynitrile and a sugar moiety, which after enzymatic hydrolysis release toxic hydrogen cyanide.[174]

The ^{1}H-NMR of **143** displayed two ortho-coupled doublets (H-8 and H-9), and a broad singlet (H-5), confirming the presence of a trisubstituted aromatic ring substituted; two doublets for olefinic protons (H-2 and H-3), indicating the presence of a *trans* di-substituted ethylene moiety. ^{13}C-NMR spectrum showed the presence of sixteen carbon signals, indicating two carbonyl groups at δ_C 175.2 and 167.3 corresponding to C-1 and C-7', respectively. Two oxygenated aromatic carbons (C-6 and C-7), two olefinic carbons (C-2 and C-3); three aromatic hydrogenated carbons (C-5, C-8, C-9), one aromatic non-hydrogenated carbon (C-4); three oxygenated methine carbons (C-3', C-4', and C-5'), one oxygenated quaternary carbon (C-1'), and two methylene groups identified as C-2' and C-6'. The H-C connectivities were assigned by the analysis of HMQC spectrum. In the COSY spectrum, besides, correlations between H-8 and H-9; H-5 and H-9, H-2 and H-3, were also observed the following correlations: H-5' and H-6'; H-3' and H-4'; and H-3' and H-2'. The final structure was elucidated on the basis of the following HMBC correlations: H-2 to C-4; H-3 to C-1, C-5 and C-9; H-8 to C-4 and C-6; H-9 to C-3 and C-7; H-5' to C-1 and C-1', C-4'; H-4' to C6'; H-3- to C-1' H-2' to C-7'. Thus, **143** was identified as chlorogenic acid.[175]

The analysis of ^{1}H- and ^{13}C-NMR data showed that **144** was structurally similar to **143**. However, in the aromatic region a 1,4-disubstituted ring was present. The two doublets confirmed the structure of **144** and identified as 5-*p*-coumarcquinic acid. Its data are comparable with those found in the literature.[176]

The ^{1}H-NMR spectrum of **145** displayed resonances for two doublets H-2 and H-3 for olefinic protons, doublet of doublets for H-8 and H-9, H-9 and H-5. In the ^{13}C-NMR spectrum, nine carbon signals were observed, which corresponded to a carbonyl group (C-1), two olefinic carbons (C-2 and C-3), three aromatic methines (C-5, C-8, and C-9), and three aromatic quaternary carbons (C-4, 6-3, and C-7). The HMBC connectivities between H-5 to C-3 and C-9; H-9 to C-3, C-5 and C-7; H-3 to C-1, helped to deduce the structure of **145** as caffeic acid.[177]

The structure of **146** showed the absence of one hydroxyl group in the aromatic system compared to **145**, which was very patent in the ^{1}H-NMR spectrum by the appearance of two doublets H-2/H-5 and H-4/H-6 and the metabolite was identified as umbellic acid.[178]

143 and **144** are chlorogenic acids widespread in the plant kingdom and they possess the antioxidant properties.[175] **143** is considered one of the most abundant polyphenol in the human diet, and is produced by certain species and is an important component of coffee. Chlorogenic acids are esters formed from cinnamic acid (caffeic acid) and quinic acid.[179] **145** is a well-known phenolic metabolite found in many food, including coffee. Recent studies suggested that **145** exert

anticancerinogenic effect, although little is known about the underlying molecular mechanism and specific target proteins.[180]

In general, **143-146** are cinnamic acid derivatives. They are naturally occurring compounds found in fruits, vegetables, flowers, and are consumed as dietary phenolic compounds. They play an important role for the production of different pharmaceutical ingredients. Some derivatives have been reported to have antitumor, antimicrobial, and anti-inflammatory properties, and others find application in perfumery and cosmetic industries.[181]

The HR-ESMS for **147** exhibited a $[M+H]^+$ peak at *m/z* 447.0525 and a fragment at *m/z* 271 was observed corresponding a $[M+H]^+$ for the aglycone after the cleavage of the glucuronic acid, and the molecular formula was found to be $C_{21}H_{18}O_{11}$. The ^{1}H-NMR indicated the presence of aromatic resonances of two singlets C-3 and C-8, and a multiplet at 8.06-7.60 corresponding to an aromatic ring monosubstituted. ^{13}C-NMR showed the typical fifteen carbon atoms for the flavone, one carbonyl group (C-6''), five methine carbons, corresponding to a sugar moiety. One on the methine carbons was found to be anomeric (C-1''). The HMQC helped to assign all H-C conectivities. The key HMBC: H-1'' to C-7; H-8 to C-1'', C-6, and C-10; and H-3 to C-1' and C-10, were used for the structural confirmation of baicalin and its data were compared with the reported in the literature.[182] Baicalin and its aglycone baicalein have been widely investigated in haematological malignancies due to their remarkable pharmacological properties as cancer targets.[183]

The structures of **148-150** have the same flavone backbone. So the correct assignment was performed on the basis of NMR data from the data analysed for metabolite **137**. They were identified as quercetin-7-*O*-*β*-galactopyraanoside (**148**),[184] astragalin (**149**),[185] and flindalatin-5-methyl ether (**150**).[186] **149** is known to have anti-tumor, anti-inflamatory and antioxidante activities.[187] No biological activities of **148** and **150** have been reported so far in the literature.

The ^{1}H-NMR analysis of **151** showed three aromatic signals at δ_H 8.12 as doublet (H-2/H-6), 7.62 as a multiplet (H-3/H-5), and another multiplet at 7.40 (H-4), which suggested the presence of a monosubstituted aromatic ring with unsaturated side chain. In the ^{13}C-NMR seven signals were detected, one attributed for a carbonyl group and other six for the aromatic ring. By the analysis of DEPT, COSY, HMQC, and HMBC data, the final structure of **151** was determined as benzoic acid. Its spectroscopic data match with the reported in the literature.[188]

152 was identified as 4-hydroxybenzoic acid based on the analysis of its NMR data. The ^{1}H-NMR showed two doublets in the aromatic region for H-2/H-6 and H-3/H-5. These protons were coupled together according to COSY spectrum. The ^{13}C-NMR displayed seven carbon signals attributed to one carbonyl group and six aromatic carbons. Two aromatic carbons were found to be quaternary by analysis of DEPT spectrum, and one of the carbons (C-4) was oxygenated. Based on HMBC analysis the structure of **152** was identified as a derivative of **151**. Its spectroscopic

data were compared with the reported in the literature.[189] **152** occurs naturally in a variety of plant species, and has been reported to have a wide range of biological activity, including the antimicrobial, antimutagenic, antiviral, and nematicidal. Besides these biological properties, is also used as preservative in many drugs, cosmetic products, pharmaceuticals, food, and beverages.[190]

Metabolite **153** was obtained as colorless solid. Its HR-ESMS showed a molecular peak at *m/z* 827.1290 $[M+Na]^+$, and the molecular formula was deduced to be $C_{55}H_{96}O_3$. A fragment observed at *m/z* 412 suggested the presence of lupeol moiety. The ^{1}H-NMR spectrum showed typical chemical shifts for lupeol, a pattern of signals attributed to a long-chain fatty acid, an overlapped triplet at δ_H 0.85 for a terminal methyl, and a large broad signal at 1.23 for a long-chain methylene groups. Also, from this spectrum, a broad singlet at 4.21 (H-2') and a triplet at 3.47 (H-1'') were observed. These observations were supported by the appearance of two oxygenated carbon signals at δ_C 69.3 (C-2') and 72.1 (C-1'') in the ^{13}C-NMR spectrum. The HMBC spectrum displayed correlations between H-2' to C-1' and C-1'', H-1' to one of the carbons of the long-chain, and H-3 to C-1'. The nature of long-chain moiety was deduced by the exhaustive analysis of the MS data. A small fragment observed at *m/z* 395 indicated the presence of $[CH_3(CH_2)_{23}OCH_2CO]^+$ group, and supported by the fragments found at *m/z* 367 and 295, for $[CH_3(CH_2)_{23}OCH_2]^+$ and $[CH_3(CH_2)_{20}]^+$, respectively. Thus, the structure of **153** was deduced as 3-*O*-[(2'-tetracosyloxy)acetyl]lupeol ester, and its spectroscopic data were very similar to these reported in the literature (Fotie et al., 2006). Lupeol and its esters derivatives are naturally occurring pentacyclic triterpenes found mostly in cabbage, green pepper, olive, and mangos. They have been reported to possess antimalarial, anti-inflammatory, antibacterial, and antitumor properties.[191–193]

3.3 *Brachylaena discolor* (Paper III)

3.3.1 Introduction

Asteraceae is the one of the largest and economically most important family of flowering plants and consists approximately 1,100 genera and 20,000 species, distributed worldwide, especially in temperate areas.[194] Several species of this family possess medicinal properties,[195] and sesqueterpene lactones are the commonest secondary metabolites found within the Asteraceae.[196]

The genus of *Brachylaena* is composed by 11 to 13 plant species, which five of them are endemic to Madagascar, while the others occur on the African mainland, mostly restricted to southern Africa.[197,198] These medicinal plants are reported to be

rich in sesqueterpene lactones, sesquitepenoids, terpenoids, tannins, and cardenolide glycosides.[198] *Brachylaena discolor* is a small tree with three known varieties: *discolor*, *transvaalensis*, and *rotundata*.[199] In traditional medicine, the roots and leaves have been used for the treatment of stomachache, tuberculosis, and diabetes.[53,71,200,201] Previous phytochemical analysis done its aerial parts led to the isolation of a sesqueterpene lactone, onopordopicrin (**153**).[202]

3.3.2 Results and discussion

In a continuing studies of phytochemical constituents with interesting structures isolated from Mozambican medicinal plants, antileishmanial activity was detected with metabolite **141** in a previous investigation. In this study, an extract of the leaves of *B. discolor* (var. *discolor*) was investigated. The methanolic crude extract was fractionated between *n*-heptane, $CHCl_3$, and EtOAc. Repeated chromatographic purifications of the EtOAc sub-fraction yielded thirteen metabolites. Two were identified as sesqueterpene lactones (**153**) and its gernacronolide epoxide, salonitelonide-8-*O*-2'-3'-isobutyrate (**154**) and eleven as phenolic compounds, hydroxytyrosol (**155**), dihydrosinapic acid (**156**), 6''-*O*-acetyl homoplantaginin (**157**), onoporidin (**158**), 3'-hydroxygenkwanin (**159**), luteolin (**160**), quercetin-3-*O*-glucoside-7,3',4'-trimethyl ether (**161**), quercetin-3-*O*-*β*-D-galactopyranoside (**162**), eupafolin (**163**), and **148**. Metabolites **153** and **154** were assayed for their antileishmanial activity against *L. amazonensis* and *L. brasiliensis* stains.

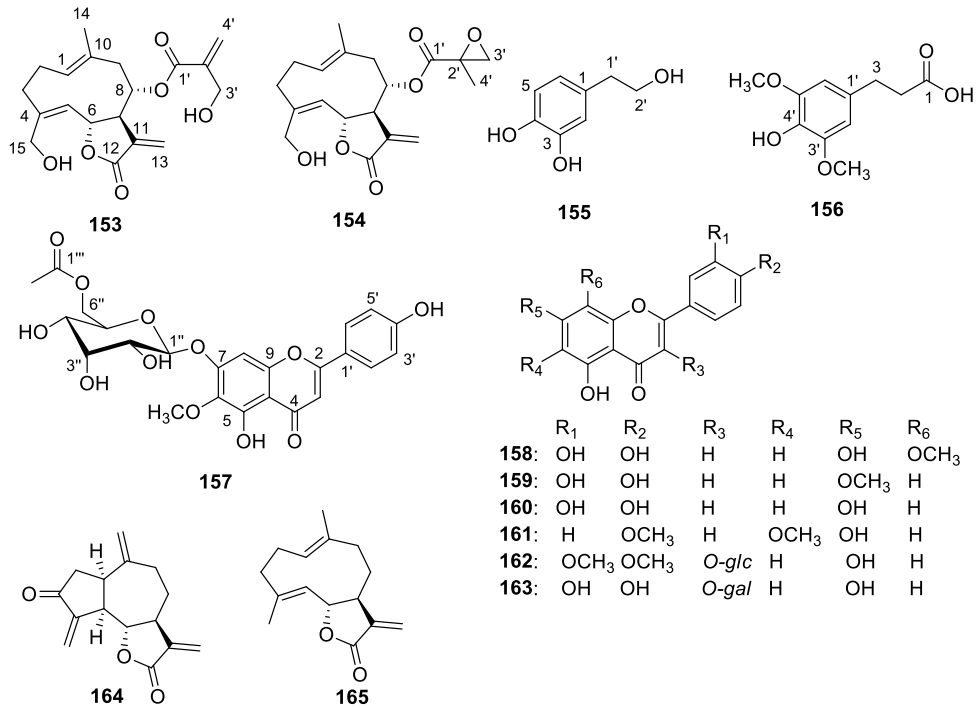


Figure 29. Structures of metabolites isolated, dehydrozaluzanin C (**164**) and costunolide (**165**).

Metabolite **154** was isolated as colorless oil, and its molecular formula was assigned to be $C_{19}H_{24}O_6$ according to its HR-ESMS analysis (*m/z*: 319.1650 $[M+H]^+$, calc. for $C_{19}H_{25}O_6$) and NMR results. The IR spectrum contained peaks at 3450, 2995, 1755, and 1705 cm^{-1}, corresponding to OH, methylene, γ-lactone, and unsaturated ester, respectively. The ^{1}H-NMR spectrum of **1** showed the presence of an olefinic methyl group (δ_H 1.58, *s*), oxygenated protons appeared at δ_H 5.16, 5.15, 4.29, 4.19, and 4.02. Also, from this spectrum six olefinic protons at δ_H 627, 6.26, 6.14, 5.96, 5.80, and 5.73, were observed. All the proton signals shown in the ^{1}H-NMR spectrum of **1** were assigned on the basis of HMQC and DEPT observations. The ^{13}C-NMR spectrum displayed 19 carbon signals. Two carbonyl groups (C-12 and C-16), six olefinic carbons (C-1, C-4, C-5, C-10, C-11, and C-13), three methine groups (C-5, C-7, and C-8), where the methines C-5 and C-8 were oxygenated. In addition, two oxygenated methylenes (C-15 and C-3̓), one methyl (C-14), and other three methylenes groups (C-2, C-3, and C-9), were observed. The analysis of ^{1}H and ^{13}C-NMR data gave an evidence that three double bonds were present in **1**, and according to IR data two of them were α, β-unsaturated carbonyl groups. The HMBC correlations between H-18 (α and β) to C-16 and C-3̓, and H-3̓to C-1̓helped to determine the presence of 2-(hydroxymethyl) acrylate group in the molecule, with unusual four carbon atoms. The extensive analysis of HMBC correlations between

H-13 (α, β) to C-12 and C-7; H-7 to C-12, H-8 to C-6 and C-1'; H-15 to C-3; H-2 to C-4 and C-14; H-1 to C-2; H-14 to C-9, the presence of a γ-lactone group identified from the IR data, and taking into account the number of the remaining 15 carbon atoms based on molecular formula, was concluded that the structure of **1** has compatibility with a germacrane-type sesqueterpene lactone, and its spectroscopic and physical data were very similar to these published for onopordipicrin.[203].

The basic germacrolide structure of **155** was found to be similar to **154** by the analysis of its spectroscopic data. However, the side chain at C-8 was different. NMR data suggested the presence on an epoxide (C-2' and C-3') than olefinic carbons as in the structure of **154**. This four carbon side chain was determined by the HMBC correlations and its spectroscopic data were similar to the reported in the literature for salotenolide-8-*O*-2',3'-isobutyrate.[205]

The metabolites **153** and **154** were found to be known germacronolide sesqueterpene lactone derivatives based on the analysis of their experimental spectroscopic and physical data with the reported in the literature. One interesting features of the structures of these two metabolites is the presence of an unusual and rare four carbon atom side chain at C-8 of the germacrane moiety, and both structures differ by the presence of two α,β-unsaturated groups in **153** compared to **154**. Metabolite **153** showed promising leishmanial activity against *L. amazonensis* and *L. brasiliensis* strains with IC_{50} values 13.8 and 9.7 μM, respectively, compared to the positive control Miltefosine (IC_{50} values 5.1 and 4.9 μM, respectively). **154** was inactive against both stains tested. These differences in activities of both sesqueterpene lactones isolated could be attributable to their structural features. **153** have an extra double bond compared to **154**. The extra α-methylene group has influence, since the methylene groups have been identified as responsible for the biological effects due to their electrophilic reactivities as Michael acceptors with thiol groups present in proteins.[206,207] The dihydrozaluzanin C (**164**) isolated from another Asteraceae member inhibits the growth of leishmanial promastigotes at concentrations ranging from 2.5 to 10 μM.[208] Zimmermann and co-workers reported that the lack of side chain at C-8, the antitrypanosomal activities lower drastically.[209]

Besides being found mainly in the family of Asteraceae, sesqueterpene lactones are also found in families members.[206] They are derived biogenetically from the mevalonic acid pathway via farnesyl diphosphate (FPP). Costunolide (**165**) has been proposed as the biosynthetic precursor of the germanocronolide-derived sesqueterpene lactones.[210] The proposed biogenesis of **153** and **154** is presented in Fig. 30.

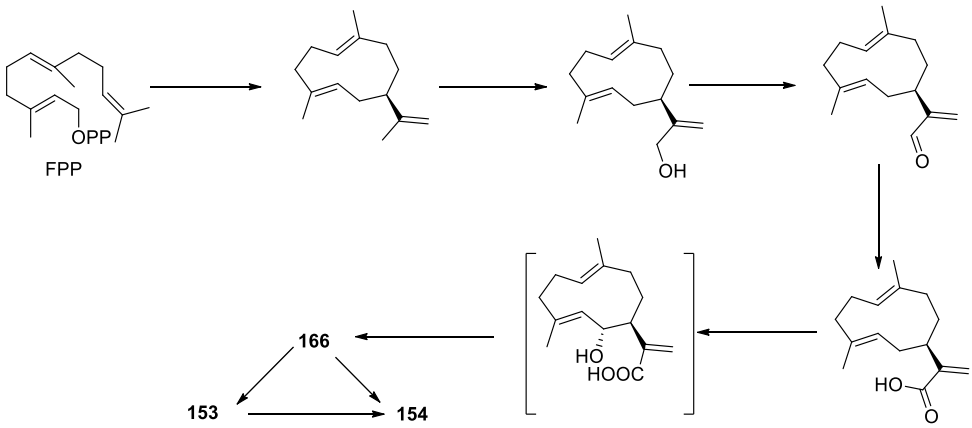


Figure 30. Presumed biosynthetic pathway of **153** and **154**.

^{1}H-NMR spectrum of **155** showed two triplets attributed to H-1' and H-2', which their coupling was confirmed by COSY data. In addition aromatic protons were observed as doublet of doublets (H-6), and two doublets (H-5 and H-2). From the ^{13}C-NMR, COSY, HMQC, and DEPT spectra the presence of a tri-substituted ring was confirmed. Obvious HMBC correlations were observed and **155** could be identified as hydroxytyrosol.[211] This metabolite is found in leaves and fruits of olive, and possess a broad range of biological activities including the anticancer, antiviral, and anti-inflammatory properties.[212]

The dihydrosinapic acid (**156**) was rapidly identified from the analysis its NMR data and compared to these reported in the literature.[213] Sinapic acid (3,5-dimethoxy-4-hydroxycinnamic acid) and its derivatives are found in spices, citrus, vegetables, cereals, and are known by exhibiting antioxidant, anti-inflammatory, anticancer, antimutagenic, antiglycemic, neuroprotective, and antibacterial activities.[214]

The basic structure of metabolites **157-163** was determined to be flavone derivatives. The structural elucidation of these type metabolites has been described in this thesis as well as their occurrence and medicinal properties.

3.4 Senna spectabilis (Paper IV)

3.4.1 Introduction

Fabaceae (Leguminosae) is the large and economically important family of the flowering plants with 700 to 751 genera and 19,000 to 20,000 known species, distributed over the world, with wood genera found mostly in the southern hemisphere and tropics, while the herbaceous genera is mostly found in temperate areas.[215,216] Most of the species belonging to this family have been used not only as food also for medicinal purposes, including the *Senna* or *Cassia* species.[216] The genus *Senna* is well-known and is distributed in tropical countries with 260 to 360 species, and they are reputed for their medicinal values.[217–219] Phenolic compounds and sennosides are the most common secondary metabolites found in this genus.[220]

Senna spectabilis is a plant used in Mozambique for the treatment of diarrhoea, stomachache, tuberculosis, and asthma.[26] Previous phytochemical investigations on this plant led to the isolation and characterization of a wide range of secondary metabolites, such as, piperidine alkaloids, triterpenoids, and phenolic compounds, [218,221–224] and some piperidine alkaloids have been reported to possess antileishmanial activity.[218]

3.4.2 Results and discussions

From the results obtained previously (Papers II and III) regarding with the leishmanial activity of the metabolites tested with interesting structures from Mozambican plants, it was proposed to analyse the extract of the roots of *S. spectabilis*. Leishmanial disease is considered one of the neglected tropical diseases and the drugs currently used miltifosine and Amphotericin B are limited due to their side-effects or high cost.[225]

A chemical investigation of an extract of the roots of *S. spectabilis* using various chromatographic purifications yielded eight known metabolites; a quinone methide triterpenoid 17-(methoxy-carbonyl)-28-*nor*-isoiguesterin (**29**), and it's biogenetically precursor, friedlin (**166**), as well as *β*-amyrin (**167**), octandronic acid (**168**), ursolic acid (**169**), lupeol (**170**), chrysophanol (**171**), and scopoletin (**172**). **29** is the first quinone methide terpenoid isolated from this plant, and its antileishmanial activity is reported along with the corrected relative stereochemistry (**29a**) (see Fig. 31). It was previously isolated from *Salacia kraussii* and was shown to possess *in vitro* antimalarial activity.[57]

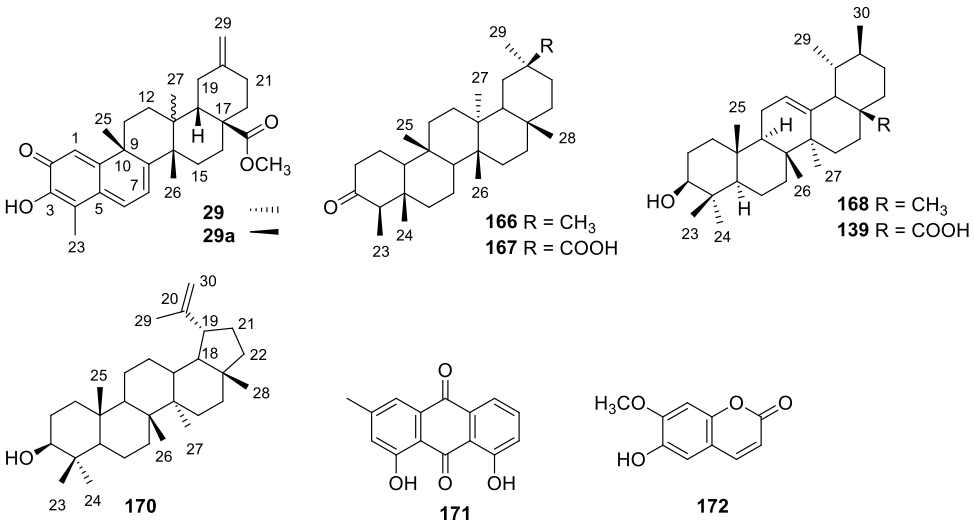


Figure 31. Metabolites isolated from *S. spectabilis.*

^{1}H-NMR of **29a** showed characteristic vinyl resonances for this type of triterpenic quinone methide at downfield, attributed for H-1, H-6, and H-7. The singlet observed at δ_H 2.21**(**Me-23) was due the quinoid methyl group. Other signals in the spectrum observed clearly were: the vinylic protons (H-29), the methyl groups (Me-25, Me-26, Me-27, and OMe). The ^{13}C-NMR displayed twenty nine signals, and classified by DEPT and HMQC data as five methyl groups (C-23, C-25, C-26, C-27, and OCH_3), eight methylene groups (C-11, C-12, C15, C-16, C-19, C-21, C-22, and a vinylic C-29), four methine groups (the vinylic C-1, C-6 and C-7, and aliphatic C-18), and eleven quaternary carbons (C-3, C-4, C-5, C-8, C-10, and C-20 as vinylic; C-9, C-13, C-14, and C-17 as aliphatics, and C-2 as carbonylic). The HMBC correlations between H-18 to C-14, C-16, C-20, and C-27; H-27 to C-12 and C-18; H-26 to C-8, C-16, and C-27; H-7 to C-5, C-9 and C-14; H-29 to C-19 and C-21, H-23 to C-5 and C-3; H-1 to C-3, C-5, and C-9; H-25 to C-7, C-10, and C-11; and H-6 to C-4, C-8, and C-10, led to determine the structure of **29.** However, by the analysis of NOESY spectrum, correlations between H_3-25, H_3-26, H_3-27, and H-18 revealed a different relative stereochemistry for Me-27 with the reported structure. These obervations were confirmed by the Mechanical Quantum calculations. The corrected structure **29a** is presented in the Fig. 31.

Quinone methide triterpenoids are secondary metabolites that occur mainly in the higher order plant families such as Celastraceae and Hyppocrateaceae. This class of metabolites display a wide range of biological activities including the anti-inflammatory, antioxidant, antifungal, antitrypanosomal, antimicrobial, and antitumor properties.[225,226]

The ^{13}C-NMR and DEPT analysis of **166** showed the presence of seven methyl groups (C-23, C-24, C-25, C26, C-27, C-28 and C-29); eleven methylene groups (C-1, C-2, C-6, C-7, C-11, 12, C-15, 16, C-19, C-21, and C-22), five methine groups (C-4, C-8, C-10, C-26, and C-18), and six quaternary carbons (C-2, as carbonyl; C-5, C-9, C-13, C-14, and C-20) accounting to the molecular formula $C_{29}H_{48}O$. The HMQC was used to determine the H-C connectivities. The HMBC correlations for H-23 to C-1 and C-5; H-24 to C-4, C-6, and C-10; H-25 to C-8, C-10, and C-11; H-2 to C-4 and C-10; H-27 to C-8, C-13 and C-15; and H-28/H-29 to C-19 and C-21, helped to find a backbone of the molecule, which its final structure was found by comparison of the reported data for friedelin.[227] Based on the same analysis done for **166**, the structure of **167** was determined as octandronic acid.[228]

The biological activities of **166** have been investigated. The molecule has shown to have antibacterial, vasodilation, anti-histaminic, anti-inflammatory, analgesic, and antipyretic properties. The gastro-protective, anti-oxidant, and liver protective activities and the ability to inhibit some cancer cell lines have been reported. **166** has been claimed to be the precursor of antitumoral quinone methide triterpenoids, including maytenin and pristemerin in Celasteraceae and Hippocraceae families.[229]

Based on its HR-ESMS, the molecular of **169** was deduced to be $C_{30}H_5O$. The ^{1}H-NMR showed the presence of eight methyl singlets (Me-23, Me-24, Me-25, Me-26, Me-27, Me-28, Me-29, and Me-30), one olefinic proton (H-12), and an oxygenated proton (H-3). The analysis of the ^{13}C-NMR, COSY, DEPT, and HMQC is was possible to assign all the protons and carbons. The backbone of the structure was done on the basis of HMBC correlations found in the clearly identified protons signals. So from spectrum the connectivities of H-3 to C-1 C-5, and C-23/C-24; H-23/H-24 to C-3 and C-5; H-25 to C-1, C-5, and C-9; H-26 to C-7, C-9, C-14; H-12 to C-9, C-14, and C-17; H-30 to C-16, C-18, and C-22; H-29 to C-19 and C-21; H-28 to C-20 and C-18; and H-27 to C-8, C-13, and C-15, were observed. The main structures was found to be similar to β-amyrin and the spectroscopic data were compared with the reported in the literature.[230] Thus metabolite **169** was characterized as usrsolic acid.[231] As most of the pentacyclic triterpenes, **168** and **169** are widely distributed in higher plants. They are known to possess the antimicrobial, anti-inflammatory, anticancer, antiviral, and antimycobacterial activities.[232] Metabolite **170** was identified as lupeol.[233] Its structural elucidation of **170** as well as the biological activities were described in this thesis for the lupeol derivative (**153**).

The ^{1}H-NMR spectrum of **171** showed five signal in the aromatic region (H-2, H-4, H-5, H-6, and H-7), a methyl group, and two chelated protons at δ_H 12.10 and 12.00. The analysis of ^{13}C-NMR and DEPT the C-9 and C-10 were found to be carbonyl groups. In the COSY spectrum, the correlations of H-2 and H-4, H-5 and H-6, H-6 and H-7, were observed. The final structure was determined by the

following HMBC correlations: H-2 to C-4, C-9, and C-11; H-4 to C-2, C-9, and C-10; H-5 to C-7, C-8a, and C-10; H-6 to C-8 and C-10a; and H-7 to C-8a. The structure of **171** was elucidated as chrysophanol.[234] **171** and its glucoside derivative have been reported to exhibit the anti-diabetic properties.[235]

The analysis of ^{1}H-NMR of **172** indicated the presence of two aromatic singlets (H-5 and H-8), two olefinic doublets (H-2 and H-3), a methoxy and an OH groups. The ^{13}C-NMR and DEPT data revealed the presence of a carbonyl group (C-1). The HMBC correlations between H-2 to C-4; H-3 to C-1 and C-9; H-5 to C-3, C-7, and C-9; and H-8 to C-4 and C-6, helped to determine the structure of **172** as a coumarin scopoletin,[236] and it is known to possess antioxidant, anti-inflammatory, and anticholinesterase activities.[237]

Conclusions and Remarks

Forty secondary metabolites were isolated from Mozambican plants, and characterized by spectroscopic as well as computational methods. The isolated compounds have various structural features and belong to different chemical classes, such as terpenoids, phenolic compounds, and nitrogen-containing compounds.

Three novel compounds were isolated and characterized, and all of them were nitrogen-containing compounds. A selection of the isolated compounds were assayed for antileishmanial activity, and several potent compounds were identified. This shows that it is possible to find novel lead compounds in Nature, for the remedies of illnesses such as a forgotten disease like leishmaniosis

However, before an extract or a compound can be used in man, the toxicity must be investigated, in order to provide not only an efficient but also a safe product. Still, the fact that this rather limited and academic investigation yielded several interesting metabolites is stimulating.

In summary, medicinal Mozambican plants can be assayed for the their content of secondary metabolites and their pharmacological properties. Besides plants, Mozambique has an incredible and largely untapped biodiversity, and for example, the marine sources along the long coast of Mozambique should also be investigated.

References

1. Dias, D. A.; Urban, S.; Roessner, U. *Metabolites* **2012**, *2* (2), 303–336.
2. Grothaus, P. G.; Cragg, G. M.; Newman, D. J. *Curr. Org. Chem.* **2010**, *14*, 1781–1791.
3. Newman, D. J.; Cragg, G. M.; Snader, K. M. *Nat. Prod. Rep.* **2000**, *17* (3), 215–234.
4. Cragg, G. M.; Newman, D. J. *Pure Appl. Chem.* **2005**, *77* (1), 7–24.
5. Wani, M. C.; Taylor, H. L.; Wall, M. E.; Coggon, P.; McPhail, A. T. *J. Am. Chem. Soc.* **1971**, *93* (9), 2325–2327.
6. McRae, J.; Yang, Q.; Crawford, R.; Palombo, E. *Environmentalist* **2007**, *27* (1), 165–174.
7. Chin, Y.-W.; Balunas, M. J.; Chai, H. B.; Kinghorn, A. D. *AAPS J.* **2006**, *8* (2), E239-53.
8. Butler, M. S. *J. Nat. Prod.* **2004**, *67* (12), 2141–2153.
9. Newman, D. J.; Cragg, G. M. *J. Nat. Prod.* **2016**, *79* (3), 629–661.
10. Wink, M. *Medicines* **2015**, *2* (3), 251–286.
11. David J. Newman and Gordon M. *J. Nat. Prod.* **2012**, *75* (3), 311–335.
12. Hostettmann, K.; Marston, a.; Ndjoko, K.; Wolfender, J. L. *Curr. Org. Chem.* **2000**, *4* (10), 973–1010.
13. Gurib-Fakim, A. *Mol. Aspects Med.* **2006**, *27* (1), 1–93.
14. van Wyk, B. E. *J. Ethnopharmacol.* **2008**, *119* (3), 342–355.
15. van Wyk, B.-E.; van Staden, J. *South African J. Bot.* **2002**, *68* (1), 1–13.
16. Corrigan, B. M.; Van Wyk, B. E.; Geldenhuys, C. J.; Jardine, J. M. *South African J. Bot.* **2011**, *77* (2), 346–359.
17. Van Wyk, B. E. *J. Ethnopharmacol.* **2015**, *176*, 118–134.
18. Atawodi, S. E. *J. Biotechnol.* **2005**, *4* (February), 128–133.
19. Maroyi, A.; Mosina, G. K. E. *Indian J. Tradit. Knowl.* **2014**, *13* (4), 665–672.
20. Semenya, S.; Maroyi, A.; Potgieter, M.; Erasmus, L. *Afr. J. Tradit. Complement. Altern. Med.* **2013**, *10* (2), 331–339.
21. Ntie-Kang, F.; Onguéné, P. A.; Fotso, G. W.; Andrae-Marobela, K.; Bezabih, M.; Ndom, J. C.; Ngadjui, B. T.; Ogundaini, A. O.; Abegaz, B. M.; Meváa, L. M. *PLoS One* **2014**, *9* (3), e90655.
22. Drewes, S. E.; Hall, A. J.; Learmonth, R. A.; Upfold, U. J. *Phytochemistry* **1984**, *23* (6), 1313–1316.

23. Bettolo, G. B. M.; Patamia, M.; Nicoletti, M. *Tetrahedron* **1982**, *38* (11), 1683–1687.
24. Shams, K. A.; Nazif, N. M.; Azim, N. S. A.; Shafeek, K. A. A.; Ismail, S. I.; Nasr, M. M. S. El. *African J. Tradit. Complement. Altern. Med.* **2009**, *6* (2), 118–122.
25. Matavele, J.; Habib, M. *Environ. Dev. Sustain.* **2000**, *2* (3), 227.
26. Bruschi, P.; Morganti, M.; Mancini, M.; Signorini, M. A. *J. Ethnopharmacol.* **2011**, *138* (2), 543–563.
27. Almagro, L.; Fernández-Pérez, F.; Pedreño, M. *Molecules* **2015**, *20*, 2973–3000.
28. Tiong, S. H.; Looi, C. Y.; Arya, A.; Wong, W. F.; Hazni, H.; Mustafa, M. R.; Awang, K. *Fitoterapia* **2015**, *102*, 182–188.
29. Wang, C. H.; Wang, G. C.; Wang, Y.; Zhang, X. Q.; Huang, X. J.; Zhang, D. M.; Chen, M. F.; Ye, W. C. *Fitoterapia* **2012**, *83* (4), 765–769.
30. Zofou, D.; Ntie-Kang, F.; Sippl, W.; Efange, S. M. N. *Nat. Prod. Rep.* **2013**, *30* (8), 1098–1120.
31. Ntie-Kang, F.; Lifongo, L. L.; Simoben, C. V; Babiaka, S. B.; Sippl, W.; Mbaze, L. M. *RSC Adv.* **2014**, *4* (67), 35348–35370.
32. Onguene, P. A.; Ntie-Kang, F.; Mbah, J. A.; Lifongo, L. L.; Ndom, J. C.; Sippl, W.; Mbaze, L. M. *Org. Med. Chem. Lett.* **2014**, *4* (1), 6.
33. Onguéné, A.; Ntie-Kang, P.; Lifongo, F. *Malar. J.* **2013**, *12* (1), 4–25.
34. Adebayo, S. A.; Dzoyem, J. P.; Shai, L. J.; Eloff, J. N. *BMC Complement. Altern. Med.* **2015**, *15* (1), 159.
35. Lawal, B.; Shittu, O. K.; Oibiokpa, F. I.; Berinyuy, E. B.; Mohammed, H. *Clin. Phytoscience* **2016**, *2* (1), 23–97.
36. Fabry, W.; Okemo, P. O.; Ansorg, R. *J. Ethnopharmacol.* **1998**, *60* (1), 79–84.
37. Gurib-Fakim, a; Mahomoodally, M. F. *Jordan J. Biol. Sci.* **2013**, *6* (2), 77–84.
38. Mohammed, A.; Ibrahim, M. A.; Africa, S.; Africa, N.; Africa, E.; Africa, W.; Africa, C.; Africa, S. *Planta Med* **2014**, *80*, 354–377.
39. Babiaka, S. B.; Ntie-Kang, F.; Lifongo, L. L.; Ndingkokhar, B.; Mbah, J. A.; Yong, J. N. *RSC Adv.* **2015**, *5* (54), 43242–43267.
40. Nwodo, J. N.; Ibezim, A.; Simoben, C. V; Ntie-Kang, F. *Anticancer. Agents Med. Chem.* **2015**, *50*, 1–58.
41. Ntie-Kang, F.; Njume, L. E.; Malange, Y. I.; Günther, S.; Sippl, W.; Yong, J. N. *Nat. Products Bioprospect.* **2016**, *6*, 63–96.
42. Ntie-kang, F.; Yong, J. N. *RSC Adv.* **2014**, *4* (1992), 61975–61991.
43. Davies-Coleman, M. T. *Stud. Nat. Prod. Chem.* **2005**, *38*, 61–107.
44 Alexandre, J.; Arroz, H. *Rev. Saude Publica* **2016**, *50* (5), 1–7.
45. Vasisht, K.; Kumar, V. *Compend. Med. Aromat. Plants* **2004**.
46. Verzar, R.; Petri, G. *J. Ethnopharmacol.* **1987**, *19*, 67–80.
47. Karan, V.; Kumar, V. *Ics-Unido* **2004**, *1*.
48. S.O. Bandeira; Gaspar, F.; Pagula, F. P. *Pharm. Biol.* **2001**, *39* (1), 70–73.

49. Magumisse, A. T.; Amade, D.; Faria, T. *African Crop Sci. Conf.* **2011**, *10* (2010), 109–113.

50. Jansen, P. C. M.; Mendes, O. *Plantas Medicinais-Seu Uso Tradicional Em Moçambique*, T1 ed.; Partido, I. do, Ed.; Ministério da Saúde Gabinete de estudos de Medicina Tradicional, 1982.

51. Jansen, P. C. M.; Mendes, O. *Plantas Medicinais-Seu Uso Tradicional Em Moçambique*, T2 ed.; Imprensa do Partido, Ed.; Ministério da Saúde Gabinete de estudos de Medicina Tradicional: Maputo, Mozambique, 1984.

52. Jansen, P. C. M.; Mendes, O. *Plantas Medicinais - Seu Uso Tradicional Em Moçambique*, T3 ed.; Imprensa do Partido, Ed.; Ministério da Saúde Gabinete de estudos de Medicina Tradicional: Maputo, Mozambique, 1990.

53. Jansen, P. C. M.; Mendes, O. *Plantas Medicinais - Seu Uso Tradicional Em Moçambique*, T4 ed.; Partido, I. do, Ed.; Ministério da Saúde Gabinete de estudos de Medicina Tradicional: Maputo, Mozambique, 1991.

54. Jansen, P. C. M.; Da Silva, C. M.; Mendes, O. *Plantas Medicinais - Seu Uso Tradicional Em Moçambique*, T5 ed.; Tradicional, D. de E. de P. M. e M., Ed.; Ministério da Saúde: Maputo, Mozambique, 2001.

55. Jurg, A.; Tomás, T.; Pividal, J. *J. Ethnopharmacol.* **1991**, *33* (1–2), 79–83.

56. Ribeiro, A.; Romeiras, M. M.; Tavares, J.; Faria, M. T. *J. Ethnobiol. Ethnomed.* **2010**, *6* (1), 33.

57. Figueiredo, J. N.; Räz, B.; Séquin, U. *J. Nat. Prod.* **1998**, *61* (6), 718–723.

58. Cala, A. C.; Chagas, A. C. S.; Oliveira, M. C. S.; Matos, A. P.; Borges, L. M. F.; Sousa, L. A. D.; Souza, F. A.; Oliveira, G. P. *Exp. Parasitol.* **2012**, *130* (2), 98–102.

59. Luo, X.; Pires, D.; Aínsa, J. A.; Gracia, B.; Mulhovo, S.; Duarte, A.; Anes, E.; Ferreira, M. J. U. *J. Ethnopharmacol.* **2011**, *137* (1), 114–120.

60. Kolaczkowski, M.; Kolaczkowska, A.; Sroda, K.; Ramalhete, C.; Michalak, K.; Mulhovo, S.; Ferreira, M. J. U. *Mycoses* **2010**, *53* (4), 305–310.

61. Madureira, A. M.; Ramalhete, C.; Mulhovo, S.; Duarte, A.; Ferreira, M.-J. U. *Pharm. Biol.* **2011**, *50* (4), 481–489.

62. da Cruz Francisco, J. *J. Agric. Food Chem.* **2001**, *49* (5), 2339–2342.

63. Pagula, F. P.; Baeckström, P. *J. Essent. Oil Res.* **2006**, *18* (1), 32–34.

64. Pagula, F. P.; Baser, K. H. C.; Kürkçüoglu, M. *J. Essent. Oil Res.* **2000**, *12* (3), 333–335.

65. Marrufo, T.; Nazzaro, F.; Mancini, E.; Fratianni, F.; Coppola, R.; De Martino, L.; Agostinho, A. B.; De Feo, V. *Molecules* **2013**, *18* (9), 10989–11000.

66. R. Croteau; Lewis, T. M.; N.G., K. y. *Biochem. Mol. Biol. Plants.* **2000**, *24*, 250–1318.

67. Zwenger, S.; Basu, C. *Biotechnol. Mol. Biol. Rev.* **2008**, *3* (February), 1–7.

68. Yadav, N.; Yadav, R.; Goyal, A. *Int. J. Pharmacol. Sci. Rev.* **2014**, *27* (45), 272–278.

69. Pattanaik, B.; Lindberg, P. *Life* **2015**, *5* (1), 269–293.

70. Ramalhete, C.; Mansoor, T. A.; Mulhovo, S.; Molnár, J.; Ferreira, M. J. U. *J. Nat. Prod.* **2009**, *72* (11), 2009–2013.
71. Deutschländer, M. S.; Lall, N.; van de Venter, M. *Pharm. Biol.* **2009**, *47* (4), 348–365.
72. Connolly, J. D.; Hill, R. A. *Nat. Prod. Rep.* **2008**, *25* (4), 794.
73. Ramalhete, C.; Molnár, J.; Mulhovo, S.; Rosário, V. E.; Ferreira, M. J. U. *Bioorganic Med. Chem.* **2009**, *17* (19), 6942–6951.
74. Ramalhete, C.; Lopes, D.; Mulhovo, S.; Molnár, J.; Rosário, V. E.; Ferreira, M. J. U. *Bioorganic Med. Chem.* **2010**, *18* (14), 5254–5260.
75. Ramalhete, C.; Da Cruz, F. P.; Lopes, D.; Mulhovo, S.; Rosário, V. E.; Prudêncio, M.; Ferreira, M. J. U. *Bioorganic Med. Chem.* **2011**, *19* (24), 7474–7481.
76. Spengler, G.; Ramalhete, C.; Martins, M.; Martins, A.; Serly, J.; Viveiros, M.; Molnár, J.; Duarte, N.; Mulhovo, S.; Ferreira, M. J. U.; Amaral, L. *Anticancer Res.* **2009**, *29* (10), 3989–3993.
77. Ramalhete, C.; Mulhovo, S.; Molnar, J.; Ferreira, M.-J. U. *Bioorg. Med. Chem.* **2016**, *24* (21), 5061–5067.
78. Gariboldi, Pierluigi; Verotta, Luisella; Gabetta, B. *Phytochemistry* **1990**, *29* (8), 2629–2635.
79. Akolade, J. O.; Muhammad, N. O.; Usman, L. A.; Odebisi, M. B.; Sulyman, A. O.; Akanji, F. T. *Br. J. Pharmacol. Toxicol.* **2014**, *5* (3), 109–114.
80. Mujovo, S. F.; Hussein, A. A.; Meyer, J. J. M.; Fourie, B.; Muthivhi, T.; Lall, N. *Nat. Prod. Res.* **2008**, *22* (12), 1047–1054.
81. Bruschi, P.; Mancini, M.; Mattioli, E.; Morganti, M.; Signorini, M. A. *J. Ethnobiol. Ethnomed.* **2014**, *10* (1), 1–22.
82. Pereira, F.; Madureira, A. M.; Sancha, S.; Mulhovo, S.; Luo, X.; Duarte, A.; Ferreira, M. J. U. *J. Ethnopharmacol.* **2016**, *178*, 180–187.
83. Eldeen, I. M. S.; Elgorashi, E. E.; Mulholland, D. A.; Van Staden, J. *J. Ethnopharmacol.* **2006**, *103* (1), 135–138.
84. Moshi, M. J.; Mbwambo, Z. H.; Nondo, R. S. O.; Masimba, P. J.; Kamuhabwa, A.; Kapingu, M. C.; Thomas, P.; Richard, M. *African J. Tradit. Complement. Altern. Med.* **2006**, *3* (3), 48–58.
85. Bombardelli, E.; Bonati, A.; Gabetta, B.; Mustich, G. *Phytochemistry* **1974**, *33*, 2559–2562.
86. Gabetta, B.; Martinelli, E. M.; Mustich, G. *Fitoterapia* **1974**, *4* (3), 3–5.
87. Paulo, A.; Jimeno, M. L.; Gomes, E. T.; Houghton, P. J. *Phytochemistry* **2000**, *53* (3), 417–422.
88. Paulo, M. A.; Comes, E. T.; Houchton, P. J.; Hart, S. *J. Pharm. Pharmacol.* **1998**, *50* (Suppl. 9), 223.
89. Ng'ang'a, M. M.; Hussain, H.; Chhabra, S.; Langat-Thoruwa, C.; Al-Harrasi, A.; Krohn, K.; Green, I. R. *Chinese Chem. Lett.* **2012**, *23* (5), 576–578.
90. Costa, M. A. C.; Lopes, M. H.; Paulo, M. I.; Ferreira, M. A.; Alves, A. C. *Phytochemistry* **1976**, *15*, 829.

91. Agostinho, D.; Boudesocque, L.; Thery-Kone, I.; Debierre-Grockiego, F.; Gueiffier, A.; Enguehard-Gueiffier, C.; Allouchi, H. *Phytochem. Lett.* **2013**, *6* (4), 560–566.
92. Mahlangeni, N. T.; Moodley, R.; Jonnalagadda, S. B. *Arab. J. Chem.* **2013**.
93. Davies-Coleman, M. T.; Gray, C. A.; Schleyer, M. H. *J. Nat. Prod.* **2000**, *63* (11), 1551–1553.
94. Stalikas, C. D. *J. Sep. Sci.* **2007**, *30* (18), 3268–3295.
95. Martin, K. R.; Appel, C. L. *Nutr. Diet. Suppl.* **2010**, *2*, 1–12.
96. Khadem, S.; Marles, R. J. *Molecules* **2010**, *15* (11), 7985–8005.
97. Dos Beirigo, P. J. S.; Torquato, H. F. V; Dos Santos, C. H. C.; De Carvalho, M. G.; Castro, R. N.; Paredes-Gamero, E. J.; De Sousa, P. T.; Jacinto, M. J.; Da Silva, V. C. *J. Nat. Prod.* **2016**, *79* (5), 1454–1458.
98. Luo, X.; Pires, D.; Aínsa, J. A.; Gracia, B.; Duarte, N.; Mulhovo, S.; Anes, E.; Ferreira, M. J. U. *J. Ethnopharmacol.* **2013**, *146* (1), 417–422.
99. Luo, X.; Pedro, L.; Milic, V.; Mulhovo, S.; Duarte, A.; Duarte, N.; Ferreira, M. J. U. *Planta Med.* **2012**, *78* (2), 148–153.
100. Mansoor, T. A.; Borralho, P. M.; Luo, X.; Mulhovo, S.; Rodrigues, C. M. P.; Ferreira, M. J. U. *Phytomedicine* **2013**, *20* (10), 923–929.
101. Cabral, V.; Luo, X.; Junqueira, E.; Costa, S. S.; Mulhovo, S.; Duarte, A.; Couto, I.; Viveiros, M.; Ferreira, M. J. U. *Phytomedicine* **2015**, *22* (4), 469–476.
102. Dasofunjo, K.; Ejoba, R. **2013**, *3* (4), 13–17.
103. Bombardelli, E.; Gabetta, B.; Mustich, G. *Fitoterapia* **1973**, *4* (2), 85–87.
104. Monteiro, L. G. S.; Zimba, T. F.; Mondlane, U. E. *UEM* **2015**, *1* (1), 7–13.
105. Magaia, T.; Uamusse, A.; Sjöholm, I.; Skog, K. *Sci. World J.* **2013**, *2013*, 1–6.
106. Bombardelli, E.; Gabetta, B.; Mustich, G. *Fitoterapia* **1973**, *44* (3), 91–92.
107. Drewes, S. E.; Hudson, N. A. *Phytochemistry* **1983**, *22* (12), 2823–2825.
108. Bombardelli, E.; Bonati, A.; Gabetta, B.; Mustich, G. *Phytochemistry* **1974**, *13* (1), 295–297.
109. Boudesocque-Delaye, L.; Agostinho, D.; Bodet, C.; Thery-Kone, I.; Allouchi, H.; Gueiffier, A.; Nuzillard, J. M.; Enguehard-Gueiffier, C. *J. Nat. Prod.* **2015**, *78* (4), 597–603.
110. Rogerio, A. P.; Cardoso, C. R.; Fontanari, C.; Souza, M. A.; Afonso-Cardoso, S. R.; Silva, É. V. G.; Koyama, N. S.; Basei, F. L.; Soares, E. G.; Calixto, J. B.; Stowell, S. R.; Dias-Baruffi, M.; Faccioli, L. H. *Glycobiology* **2007**, *17* (8), 795–804.
111. Bombardelli, E.; Gabetta, B.; Mustich, G. *Fitoterapia* **1974**, *45* (1), 6–8.
112. Bombardelli, E.; M.Martinelli, E.; Mustich, G. *Fitoterapia* **1975**, *46* (5), 199–200.
113. Aniszewski, T. *Alkaloids - Secrets of Life*; 2007.
114. Mansoor, T. A.; Borralho, P. M.; Dewanjee, S.; Mulhovo, S.; Rodrigues, C. M. P.; Ferreira, M. J. U. *J. Ethnopharmacol.* **2013**, *149* (2), 463–470.
115. Beek, T. A. Van; Verpoorte, R.; Svendsen, A. B.; Leeuwenberg, A. J. M.; Bisset, N. G. *J. Ethnopharmacol.* **1984**, *10* (1), 1–156.

116. Mansoor, T. A.; Ramalhete, C.; Molnár, J.; Mulhovo, S.; Ferreira, M. J. U. *J. Nat. Prod.* **2009**, *72* (6), 1147–1150.
117. Gabetta, B.; Martinelli, E. M.; Mustich, G. *Fitoterapia* **1975**, *46* (5), 195–198.
118. Bombardelli, E.; Bonati, A.; Gabetta, B.; Martinelli, E. M.; Mustich, G.; Danieli, B. *J. Chem. Soc. Perkin I* **1976**, 1432–1438.
119. Paterna, A.; Gomes, S. E.; Borralho, P. M.; Mulhovo, S.; Rodrigues, C. M. P.; Ferreira, M. J. U. *J. Ethnopharmacol.* **2016**, *194*, 236–244.
120. Paterna, A.; Gomes, S. E.; Borralho, P. M.; Mulhovo, S.; Rodrigues, C. M. P.; Ferreira, M. J. U. *J. Nat. Prod.* **2016**, *79* (10), 2624–2634.
121. van der Heijden, R.; Brouwer, R. L.; Verpoorte, R.; Wijnsma, R.; van Beek, T. A.; Harkes, A. A.; Svendsen, A. B. *Phytochemistry* **1986**, *25* (4), 843–846.
122. Da Silva, G.; Serrano, R.; Teixeira Gomes, E.; Silva, O. *Microsc. Res. Tech.* **2015**, *78* (11), 1001–1009.
123. Da Silva, G.; Martinho, A.; Soengas, R. G.; Duarte, A. P.; Serrano, R.; Gomes, E. T.; Silva, O. *Fitoterapia* **2015**, *106*, 7–11.
124. Keyzers, R. A.; Gray, C. A.; Schleyer, M. H.; Whibley, C. E.; Hendricks, D. T.; Davies-Coleman, M. T. *Tetrahedron* **2006**, *62* (10), 2200–2206.
125. Wipf, P. *Chem. Rev.* **1997**, *95* (6), 2115–2134.
126. Rudi, A.; Aknin, M.; Gaydou, E. M.; Kashman, Y. *Tetrahedron* **1998**, *54*, 13203–13210.
127. Toske, S. G.; Fenical, W. *Tetrahedron Lett.* **1995**, *36* (46), 8355–8358.
128. Carroll, A. R.; Bowden, B. F.; Coll, J. C.; Hockless, D. C. R.; Skelton, B. W.; White, A. H. *Aust. J. Chem.* **1994**, *47* (1), 61–69.
129. Arrault, A.; Witczak-Legrand, A.; Gonzalez, P.; Bontemps-Subielos, N.; Banaigs, B. *Tetrahedron Lett.* **2002**, *43* (22), 4041–4044.
130. Fusetani, N.; Matsunaga, S. *Chem. Rev.* **1993**, *93* (5), 1793–1806.
131. Schmidt, E. W.; Harper, M. K.; Faulkner, D. J. *J. Nat. Prod.* **1997**, *60* (8), 779–782.
132. Schmidt, E. W.; Bewley, C. A.; Faulkner, D. J. *J. Org. Chem.* **1998**, *63* (4), 1254–1258.
133. Romero, F.; Espliego, F.; Pérez Baz, J.; García de Quesada, T.; Grávalos, D.; de la Calle, F.; Fernández-Puentes, J. L. *J. Antibiot. (Tokyo).* **1997**, *50* (9), 734–737.
134. Baz, J. P.; Canedo, L. M.; Puentes, J. L. F. *J. Antibiot. (Tokyo).* **1997**, *50* (9), 738–741.
135. Newman, D. J.; Cragg, G. M. *J. Nat. Prod.* **2004**, *67*, 1216–1238.
136. Gussoni, M.; Greco, F.; Pegna, M.; Bianchi, G.; Zetta, L. *Magn. Reson. Imaging* **1994**, *12* (3), 477–486.
137. Hall, J. C.; Sytsma, K. J.; Iltis, H. H. *Am. J. Biol.* **2002**, *89* (11), 1826–1842.
138. Hall, J. C. *Botany* **2008**, *86*, 682–696.
139. Gohar, A. A. *Zeitschrift fur Naturforsch. - Sect. C J. Biosci.* **2002**, *57* (3–4), 216–220.

140. Ahmad, V. U.; Amber, A. ur R.; Arif, S.; Chen, M. H. M.; Clardy, J. *Phytochemistry* **1985**, *24* (11), 2709–2711.

141. Velmurugan, P.; Kamaraj, M.; Prema, D. *Int. J. Phytomedicine* **2010**, *2* (4), 379–384.

142. Schmidt, E., Lötter, M., McCleland, W. *Trees and Shrubs of Mpumalanga and Kruger National Park*, Jacana.; Johannesburg, 2002.

143. Elenkov, M. M.; Tang, L.; Meetsma, A.; Hauer, B.; Janssen, D. B. *Org. Lett.* **2008**, *10* (12), 2417–2420.

144. Hwan, L. J.; Won, L. B.; Suk, K. N.; Hwang, M. Y.; Suk, Y. M.; Hun, P. K. *J. Life Sci.* **2005**, *15* (3), 423–426.

145. Said, I. M.; Hamid, N. A. A.; Latif, J.; Din, L. B.; Yamin, B. M. *Acta Crystallogr.* **2005**, *E61*, 797–798.

146. Thuy, T. T.; Porzel, A.; Franke, K.; Wessjohann, L.; Sung, T. Van. *Pharmazie* **2005**, *60* (9), 701–704.

147. El-Sayyad, S. M.; Ross, S. A.; Sayed, H. M. *J. Nat. Prod.* **1984**, *47* (4), 708–710.

148. Shamma, M.; Foy, J. E. *J. Am. Chem. Soc.* **1974**, *96* (25), 7809–7811.

149. Krane, B. D.; Shamma, M. *J. Nat. Prod.* **1982**, *45*, 377.

150. Ziegler, J.; Facchini, P. J. *Annu. Rev. Plant Biol.* **2008**, *59* (1), 735–769.

151. Patel, K.; Patel, D. K. *J. Tradit. Complement. Med.* **2017**, *7* (3), 360–366.

152. Sai, V.; Chaturvedula, P.; Prakash, I. **2012**, *1* (9), 239–242.

153. Yinusa, I.; George, N. I.; Ayo, R. G.; Rufai, Y. *Glob. J. Pure Appled Chem. Res.* **2015**, *3* (3), 7–14.

154. Hoye, T. R.; Renner, M. K. *J. Org. Chem.* **1996**, *61*, 2056–2064.

155. Martinez-Farina, C. F.; Robertson, A. W.; Yin, H.; Monro, S.; Mcfarland, S. A.; Syvitski, R. T.; Jakeman, D. L. *J. Nat. Prod.* **2015**, *78* (6), 1208–1214.

156. Jakeman, D. L.; Bandi, S.; Graham, C. L.; Reid, T. R.; Wentzell, J. R.; Douglas, S. E. *Antimicrob. Agents Chemother.* **2009**, *53* (3), 1245–1247.

157. Borders, D. B.; Morton, G. 0.; Wetzel, E. R. *Tetrahedron Lett.* **1974**, *15* (31), 2709–2712.

158. Norte, M.; Rodriguez, M. L.; Fernandez, J. J.; Eguren, L.; Estrada, D. M. *Tetrahedron* **1988**, *44* (15), 4973–4980.

159. Kossuga, M. H.; Macmillan, J. B.; Rogers, E. W.; Molinski, T. F.; Nascimento, G. G. F.; Rocha, R. M.; Berlinck, R. G. S. *J. Nat. Prod.* **2004**, *67*, 1879–1881.

160. Jensen, U.; Hoot, S. B.; Johnasson, J. T.; Kosuge, K. *Plant Syst. Evol.* **1995**, *9*, 273–280.

161. Hai, W.; Cheng, H.; Zhao, M.; Wang, Y.; Hong, L.; Tang, H.; Tian, X. *Fitoterapia* **2012**, *83* (4), 759–764.

162. Zhao, M.; Ma, N.; Qiu, F.; Hai, W. L.; Tang, H. F.; Zhang, Y.; Wen, A. D. *Planta Med.* **2014**, *80* (11), 942–948.

163. Zhang, Z.; Wang, X.; Zhao, M.; Qian, K. *Carbohydr. Polym.* **2014**, *111*, 762–767.

164. Chawla, R.; Kumar, S.; Sharma, A. *J. Ethnopharmacol.* **2012**, *143* (1), 116–150.

165. Li, L.; Gou, M. L.; He, Y. X. *Phytochem. Lett.* **2013**, *6* (4), 570–574.
166. Khan, M. R.; Kihara, M.; Omoloso, A. D. *Fitoterapia* **2001**, *72* (5), 575–578.
167. Essig, F. B. *SIDA* **2004**, *21* (2), 861–877.
168. Pedras, M. S. C.; Okanga, F. I.; Zaharia, I. L.; Khan, A. Q. *Phytochemistry* **2000**, *53* (2), 161–176.
169. Pedras, M. S. C.; Loukaci, A.; Okanga, F. L. *Bioorganic Med. Chem. Lett.* **1998**, *8*, 3037–3038.
170. Chripkova, M.; Zigo, F.; Mojzis, J. *Molecules* **2016**, *21* (12), 1–15.
171. Pedras, M.; Jha, M.; Ahiahonu, P. *Curr. Org. Chem.* **2003**, *7* (16), 1635–1647.
172. Pedras, M. S. C.; Okinyo, D. P. O. *Org. Biomol. Chem.* **2008**, *6* (1), 51–54.
173. Nahrstedt, A.; Rockenbach, J. *Phytochemistry* **1993**, *34* (2), 433–436.
174. Vetter, J. *Toxicon* **2000**, *38*, 11–36.
175. Suárez-Quiroz, M. L.; Alonso Campos, A.; Valerio Alfaro, G.; González-Ríos, O.; Villeneuve, P.; Figueroa-Espinoza, M. C. *J. Food Compos. Anal.* **2014**, *33* (1), 55–58.
176. Lu, Y.; Sun, Y.; Foo, L. Y.; McNabb, W. C.; Molan, A. L. *Phytochemistry* **2000**, *55* (1), 67–75.
177. Jeong, C.-H.; Jeong, H. R.; Choi, G. N.; Kim, D.-O.; Lee, U.; Heo, H. J. *Chin. Med.* **2011**, *6* (1), 25.
178. Abdel-Kader, M. S. *J. Braz. Chem. Soc.* **2003**, *14* (1), 48–51.
179. Meng, S.; Cao, J.; Feng, Q.; Peng, J.; Hu, Y. *Evidence-based Complement. Altern. Med.* **2013**, *2013*.
180. Kang, N. J.; Lee, K. W.; Shin, B. J.; Jung, S. K.; Hwang, M. K.; Bode, A. M.; Heo, Y. S.; Lee, H. J.; Dong, Z. *Carcinogenesis* **2009**, *30* (2), 321–330.
181. Sharma, P. *J. Chem. Pharm. Res* **2011**, *3* (2), 403–423.
182. Pegg, R. B.; Amarowicz, R.; Oszmiañski, J. *POLISH J. FOOD Nutr. Sci. Pol. J. Food Nutr. Sci* **2005**, *1455* (1), 43–50.
183. Chena, H.; Gaoa, Y.; Wu, J.; Chen, Y.; Chen, B.; Hub, J.; Zhouc, J. *Csncer Lett.* **2015**, *354* (5), 5–11.
184. Güvenalp, Z.; Demirezer, L. O. *Turk. J. Chem.* **2005**, *29*, 163–169.
185. Saito, S.; Silva, G.; Santos, R. X.; Gosmann, G.; Pungartnik, C.; Brendel, M. *Int. J. Mol. Sci.* **2012**, *13* (3), 2846–2862.
186. Rashid, M. A.; Armstrong, J. A.; Gray, A. I.; Waterman, P. G. *Phytochemistry* **1992**, *31* (4), 1265–1269.
187. Cho, I.-H.; Gong, J.-H.; Kang, M.-K.; Lee, E.-J.; Park, J. H. Y.; Park, S.-J.; Kang, Y.-H. *BMC Pulm. Med.* **2014**, *14* (1), 122.
188. Waqas, M.; Khan, A. L.; Ali, L.; Kang, S. M.; Kim, Y. H.; Lee, I. J. *Molecules* **2013**, *18* (12), 15519–15530.
189. Makhmoor, T.; Choudhary, M. I. *Turk. J. Chem.* **2010**, *34* (1), 119–126.
190. Manuja, R.; Sachdeva, S.; Jain, A.; Chaudhary, J. *Int. jounal Pharm. Sci.* **2013**, *22* (2), 109–115.

191. Fotie, J.; Bohle, D. S.; Leimanis, M. L.; Georges, E.; Rukunga, G.; Nkengfack, A. E. *J. Nat. Prod.* **2006**, *69* (1), 62–67.

192. Titanji, V. P. K.; Zofou, D.; Ngemenya, M. N. *Afr. J. Tradit. Complement. Altern. Med.* **2008**, *5* (3), 302–321.

193. Wal, A.; Srivastava, R. .; Wal, P.; Rai, A.; Sharma, S. *Pharm. Biol. Eval.* **2015**, *2* (5), 142–151.

194. Jansen, R. K.; Palmer, J. D. *Proc. Natl. Acad. Sci. U. S. A.* **1987**, *84* (August), 5818–5822.

195. Singh, R.; Singh, G.; Tiwari, A. K.; Patel, S.; Agrawal, R. *Int. J. Zool.* **2015**, *1* (2), 137–167.

196. Modzelewska, A.; Sur, S.; Kumar, S. K.; Khan, S. R. *Curr. Med. Chem. - Anti-Cancer Agents* **2005**, *5* (5), 477–499.

197. Beentje, H. T. *Kew Bull.* **2000**, *55* (1), 1–41.

198. Razakarivelo, J. F.; Rafanomezantsoa, R. M.; Krebs, H. C.; Ravelonjato, B. A.; Randrianasolo, R.; Rakotondramanga, M. F. *J. Pharmacogn. Phytochem.* **2015**, *4* (1), 235–237.

199. Mellem, J. *Afr. J. Complement. Altern. Med.* **2015**, *12* (1), 38–44.

200. Maroyi, A. *J. Med. Plants Res.* **2013**, *7* (2), 53–60.

201. Mellem, J. *African J. Pharm. Pharmacol.* **2013**, *7* (12), 636–642.

202. Zdero, C.; Bohlmann, F. *Phytochemistry* **1987**, *26* (9), 2597–2601.

203. Lonergan, G.; Routsi, E.; Georgiardis, T.; Agelis, G.; Hondrelis, J.; Matsoukas, J.; Larsen, L. K.; Caplan, F. R. *J. Nat. Prod.* **1992**, *55* (5), 225–228.

204. Zdero, C.; Bohlmann, F. *Phytochemistry* **1987**, *26* (9), 2597–2601.

205. Bohlmann, F.; Zdero, C. *Phytochemistry* **1982**, *21* (3), 647–651.

206. Chadwick, M.; Trewin, H.; Gawthrop, F.; Wagstaff, C. *Int. J. Mol. Sci.* **2013**, *14* (6), 12780–12805.

207. Formisano, C.; Sanna, C.; Ballero, M.; Chianese, G.; Sirignano, C.; Rigano, D.; Millán, E.; Muñoz, E.; Taglialatela-scafati, O. *Fitoterapia* **2017**, *116*, 61–65.

208. Chan-Bacab, M. J.; Peña-rodríguez, L. M. *Nat. Prod. Rep.* **2001**, *18*, 674–688.

209. Zimmermann, S.; Fouché, G.; De Mieri, M.; Yoshimoto, Y.; Usuki, T.; Nthambeleni, R.; Parkinson, C. J.; Van Der Westhuyzen, C.; KaiserB, M.; Hamburger, M.; Adams, M. *Molecules* **2014**, *19* (3), 3523–3538.

210. Majdi, M.; Liu, Q.; Karimzadeh, G.; Malboobi, M. A.; Beekwilder, J.; Cankar, K.; Vos, R. De; Todorović, S.; Simonović, A.; Bouwmeester, H. *Phytochemistry* **2011**, *72* (14–15), 1739–1750.

211. Park, C. H.; Kim, K. H.; Lee, I. K.; Lee, S. Y.; Choi, S. U.; Lee, J. H.; Lee, K. R. *Arch. Pharm. Res.* **2011**, *34* (8), 1289–1296.

212. Granados-Principal, S.; Quiles, J. L.; Ramirez-Tortosa, C. L.; Sanchez-Rovira, P.; Ramirez-Tortosa, M. C. *Nutr. Rev.* **2010**, *68* (4), 191–206.

213. Lee, Y.-M.; Kwon, M.-J.; Kim, J.-K.; Suh, H.; Choi, J.-S.; Song, Y.-O. *Korean J. Food Sci. Technol.* **2004**, *36* (1), 129–133.

214. Chen, C. *Oxid. Med. Cell. Longev.* **2016**, *2016*.
215. Doyle, J. J. *Plant Physiol.* **2003**, *131* (3), 900–910.
216. Rahman, A. H. M. M.; Parvin, M. I. A. *Res. Plant Sci.* **2014**, *2* (1), 6–8.
217. Pansa Monkheang. *J. Med. Plants Res.* **2011**, *5* (26), 6173–6181.
218. De Albuquerque Melo, G. M.; Silva, M. C. R.; Guimarães, T. P.; Pinheiro, K. M.; Da Matta, C. B. B.; De Queiroz, A. C.; Pivatto, M.; Da Silva Bolzani, V.; Alexandre-Moreira, M. S.; Viegas, C. *Phytomedicine* **2014**, *21* (3), 277–281.
219. Viegas, C.; Silva, D. H. S.; Pivatto, M.; De Rezende, A.; Castro-Gambôa, I.; Bolzani, V. S.; Nair, M. G. *J. Nat. Prod.* **2007**, *70* (12), 2026–2028.
220. Shivjeet, S.; Singh, S. K. and; Yadav, A. *Am. J. Phytomedicine Clin. Ther.* **2013**, *3*, 291–312.
221. Christofidis, I.; Welter, A.; Jadot, J. *Tetrahedron* **1977**, *33* (22), 3005–3006.
222. Viegas, C.; Da S. Bolzani, V.; Furlan, M.; Barreiro, E. J.; Young, M. C. M.; Tomazela, D.; Eberlin, M. N. *J. Nat. Prod.* **2004**, *67* (5), 908–910.
223. Viegas, C.; Pivatto, M.; De Rezende, A.; Hamerski, L.; Silva, D. H. S.; Da Silva Bolzani, V. *J. Braz. Chem. Soc.* **2013**, *24* (2), 230–235.
224. Viegas, C.; Silva, D. H. S.; Pivatto, M.; Rezende, A. De; Castro-gambôa, I.; Bolzani, V. S. *J. Nat. Prod.* **2007**, *70*, 2026–2028.
225. Dos Santos, V. A. F. F. M.; Leite, K. M.; Da Costa Siqueira, M.; Regasini, L. O.; Martinez, I.; Nogueira, C. T.; Galuppo, M. K.; Stolf, B. S.; Pereira, A. M. S.; Cicarelli, R. M. B.; Furlan, M.; Graminha, M. A. S. *Molecules* **2013**, *18* (1), 1053–1062.
226. Thiem, D. A.; Sneden, A. T.; Khan, S. I.; Tekwani, B. L. *J. Nat. Prod.* **2005**, *68*, 251–254.
227. Sousa, G. F.; Duarte, L. P.; Alcântara, A. F. C.; Silva, G. D. F.; Vieira-Filho, S. A.; Silva, R. R.; Oliveira, D. M.; Takahashi, J. A. *Molecules* **2012**, *17* (11), 13439–13456.
228. Yeboah, E. M. O.; Majinda, R. R. T.; Kadziola, A.; Muller, A. *J. Fac. Pharm. Istanbul* **2010**, *73* (6), 1151–1155.
229. Souza-Moreira, T. M.; Alves, T. B.; Pinheiro, K. A.; Felippe, L. G.; De Lima, G. M. A.; Watanabe, T. F.; Barbosa, C. C.; Santos, V. A. F. F. M.; Lopes, N. P.; Valentini, S. R.; Guido, R. V. C.; Furlan, M.; Zanelli, C. F. *Sci. Rep.* **2016**, *6* (1), 36858.
230. Ogwuche, C.; Amupitan, J.; Ndukwel, G.; Ayo, R. *Med. Chem. (Los. Angeles).* **2014**, *4* (11), 729–733.
231. Keat, N. B.; Umar, R. U.; Lajis, N. H.; Chen, T. Y.; Li, T. Y.; Rahmani, M.; Sukari, M. A. *Malaysian J. Anal. Sci.* **2010**, *14* (1), 6–11.
232. Babalola, I. T.; Shode, F. O. *J. Pharmacogn. Phytochem.* **2013**, *2* (2), 214–222.
233. Al-Musayeib, N. M.; Mohamed, G. A.; Ibrahim, S. R. M.; Ross, S. A. *Med. Chem. Res.* **2013**, *22* (11), 5297–5302.
234. Zhang, H.; Guo, Z.; Wu, N.; Xu, W.; Han, L.; Li, N.; Han, Y. *Molecules* **2012**, *17* (1), 843–850.
235. Lee, M. S.; Sohn, C. B. *Biol. Pharm. Bull.* **2008**, *31* (11), 2154–2157.

236. Darmawan, A.; Kosela, S.; Kardono, L. B. S.; Syah, Y. M. *J. Appl. Pharm. Sci.* **2012**, *2* (12), 175–177.

237. Mogana, R.; Wiart, C. *Evidence-based Complement. Altern. Med.* **2013**, *2013*, 1–6.

Paper I

Phytochemistry Letters 16 (2016) 283–286

Contents lists available at ScienceDirect

Phytochemistry Letters

journal homepage: www.elsevier.com/locate/phytol

Novel metabolites from the roots of *Cadaba natalensis*

J.A. Monjane[a,b], A. Uamusse[a], O. Sterner[b,*]

[a] Chemistry Department, Eduardo Mondlane University, P.O. Box 257, Maputo, Mozambique
[b] Centre for Analysis and Synthesis, Lund University, P.O. Box 124, S-22100 Lund, Sweden

ARTICLE INFO

Article history:
Received 16 March 2016
Received in revised form 12 May 2016
Accepted 17 May 2016
Available online 28 May 2016

Keywords:
Cadaba natalensis
Diazacyclododecanedione
Oxazolidinone
Isoquiniline alkaloids
Isolation
Structure elucidation

ABSTRACT

From an extract of the roots of *Cadaba natalensis* Sond. the novel macrocyclic dibenzo-diazacyclododecanedione **1** was isolated together with (*S*)-2-ethyl-2-methyloxazolidin-5-one (**2a**). In addition, the five known compounds were obtained. Of these, (*R*)-5-ethyl-5-methyloxazolidin-2-one (**3**) is reported as a natural product for the first time. The structures of the isolated compounds were elucidated by analysis of the spectral data, 1D NMR (^{1}H, ^{13}C, and DEPT), 2D NMR (COSY, HMQC, HMBC, and NOESY), and HRESIMS, as well as by the comparison with previously reported data.

1. Introduction

The *Cadaba* genus belongs to the Capparaceae family and comprises about 30 species. It is widely distributed in southern Africa, India, Malaysia, and Australia (Hall, 2008). *Cadaba natalensis* Sond. is a 1–3 m high shrub, which in southern Africa grows in dry forests and on the savannah in southern Mozambique, South Africa, and Swaziland (Mendes and Mendes, 1990). The roots and leaves of the plant have been used in the traditional medicine, to treat tuberculosis in Mozambique (Mendes and Mendes, 1990; Ribeiro et al., 2010) and to induce vomiting and to treat chest pains in South Africa (Schmidt et al., 2002). Previous phytochemical studies with *Cadaba* species led to the isolation of a variety of secondary metabolites. The spermidine alkaloid cadabicine (Ahmad et al., 1985) was isolated from *Cadaba farinosa* together with terpenes, 12-aminododecanoic acid, stachydrine and its 3-hydroxylated derivative, and two flavonol glycosides (Al-Musayeib et al., 2013). The aerial parts of *Cadaba glandulosa* yielded flavonoids with anti-inflammatory actitivity (Mohamed et al., 2014), while the roots of *Cadaba trifoliata* proved to be a rich source of tannins, steroids, alkaloids, glycosides, and phenolic compounds (Velmurugan et al., 2010).

No previous phytochemical investigation of *C. natalensis* has been reported, and here we wish to report the isolation and structural characterization of the compounds present in its roots. Two novel metabolites were isolated and characterised chemically; the macrocyclic dibenzo-diazacyclododecanedione **1** was isolated as a minor metabolite from the ethyl acetate extract of the roots while the oxazolidine-5-one **2a** was obtained from the heptane extract. The isolation of (*R*)-5-ethyl-5-methyloxazolidin-2-one (**3**) is interesting, it has previously been prepared by halohydrin dehalogenase catalysed opening of the corresponding racemic terminal epoxide in the presence of cyanate (Elenkov et al., 2008), and this is the first time it has been isolated from a natural source. Compounds **1, 2a** and **3** represent structures that are relatively unusual in nature. In addition, the isoquinoline derivatives thalifoline (**4**), isolated also from, for example, *Phellodendrum amurense* belonging to *Rutaceae* family (Lee et al., 2005) and *N*-methylcorydaldine (**5**) (Said et al., 2005), as well as β-sitosterol (Chaturvedula and Prakash, 2012) and nepetin (Kabouche et al., 2011) were isolated. The structures of the known compounds were established by the analysis of the spectral data and, when possible, by comparing their spectroscopic data with those reported in the literature. The structures of compounds **1, 2a**, **3**, **4** and **5** are shown in Fig. 1.

Despite their rarity in nature, stereoisomers of oxazolidin-2-one have been isolated from marine sources. Phytochemical investigations of the sponges *Verongia lacunosa* (Borders et al., 1974), *Apysina aerophoba* (Norte et al., 1988), and *Clavelina oblonga*

* Corresponding author.
E-mail address: Olov.Sterner@science.lu.se (O. Sterner).

http://dx.doi.org/10.1016/j.phytol.2016.05.009
1874-3900/

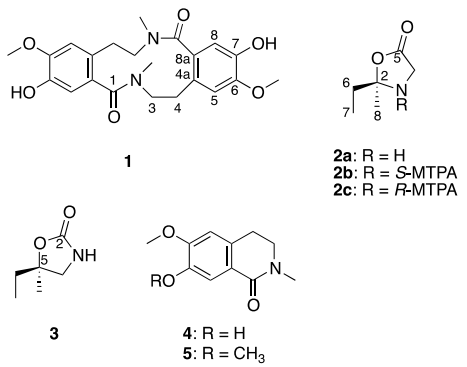


Fig. 1. Structures of the metabolites **1**, **2a**, **3**, **4** and **5** isolated from *C. natalensis*.

(Kossuga et al., 2004) led to isolation of a dibrominated phenolic bis-oxazolidinones (Lira et al., 2009). The bacterium *Streptomyces venuzuelae* is another reported source for natural oxazolidin-5-ones, and from extracts of *S. venuzuelae* grown under stress conditions in a particular amino acid medium (Martinez-Farina et al., 2015; Zheng et al., 2005; Doull et al., 1994; Ayer et al., 1991) a variety of jadomycins that possess antitumor and antibacterial activities were isolated. Isoquinolone derivatives can be found as minor constituents in plants, mainly in the Ranunculaceae family, and the *Thalictum* species are common sources of these metabolites. They are reported to possess a vasorelaxant effect, and may be prepared in nature by an oxidative degradation of benzylisoquinines (Krane and Shamma, 1982).

2. Results and discussion

Compound **1** was obtained as a yellowish solid. Its molecular formula was suggested to be $C_{22}H_{26}N_2O_6$ based on HRESI-MS data (*m/z* found: 415.1865 $[M+H]^+$; calc.: 415.1869 for $C_{22}H_{27}N_2O_6$). **1** consequently has eleven degrees of unsaturation. In the IR spectrum of **1**, absorption bands for hydroxyl and carbonyl groups at 3125 cm^{-1} and 1711 cm^{-1}, respectively, were observed. In addition, the characteristic absorption bands for carbon-carbon double bonds in an aromatic system were observed at 1680 and 1579 cm^{-1}. ^1H and ^{13}C NMR data (see Table 1) showed resonances similar to those of thalifoline (**4**), and the difference in elemental composition suggests that **1** is a dimer of something similar to **4**. The ^1H NMR spectrum of **1** reveals the presence of two methyl singlets at δ 3.94 and 3.15, for *O*-CH_3 and *N*-CH_3, and two singlets in the aromatic region at δ_H 7.67 (8-H) and 6.63 (5-H) (see Fig. 1 for the atom numbering of **1**) which suggests the presence of a 1,2,4,5-tetrasubstituted aromatic ring. In addition, two methylene triplets at δ_H 2.94 (3-H_2) and 3.56 (4-H_2), which couple strongly to each other in the COSY spectrum, were observed. This accounts for only 12 of the 26 hydrogens, and as only 11 signals are observed in the ^{13}C NMR spectrum the conclusion is that **1** is a symmetric compound with two identical parts and that each half has an exchangable proton. This proton was observed as a sharp singlet at δ_H 3.87, typical for phenolic hydroxyl protons. The 1D ^{13}C NMR data combined with the 2D HMQC data showed the presence of six hydrogenated carbons of which two were methyl groups (*O*-CH_3 at δ_C 56.0 and *N*-CH_3 at δ_C 35.2), two methylene groups (C-3 at δ_C 48.5 and C-4 at δ_C 27.6), and two methines assigned to the aromatic C-5 and C-8 (δ_C 108.7 and 114.4) (Table 1). Five carbons lack hydrogens, one was assigned as the carbonyl group C-1 (δ_C 165.0), and the other four as the substituted carbons of the benzene ring (δ_C 122.5, 130.9, 144.6 and 149.5). The complete structure was determined by HMBC and NOESY experiments. The HMBC correlation between the *N*-CH_3 and C-1 and C-3, the correlations between 3-H_2 and C-1, C-4, C-4a and NCH_3, as well as those between 4-H_2 and C-4a, C-5 and C-8a establish this part of the structure and identify the positions of two of the substituted carbons in the benzene ring. The expected HMBC correlations between 5-H and C-4, C-6, C-7 and C-8a, as well as between 8-H and C-1, C-4a, C-6 and C-7, were observed and confirmed the structure. The differentiation between C-6 and C-7 was made based on the HMBC correlation between *O*-CH_3 and C-7 as well as the NOESY correlation between *O*-CH_3 and 5-H. Compounds **1** and **4** display similar but significantly different NMR data, and the dimeric and macrocyclic nature of **1** is established by the MS experiments of **1** and **4** recorded under identical conditions. **4** only generate the molecular ion peak at *m/z* 208 $[M+H]^+$, while the base peak for **1** is also the molecular ion peak at *m/z* 415 $[M+H]^+$, with a small fragment at *m/z* 208. It is reasonable to suggest that **1** and **4** share the same biosynthetic pathway, although this remains unknown.

Compound **2a** was obtained as a white solid. Its molecular formula, $C_6H_{11}NO_2$ was deduced by HR-ESIMS experiments determining the exact mass of the quasi-molecular ion $[M+Na]^+$ (*m/z* found: 152.0684; calc.: 152.0687 for $C_6H_{11}NO_2Na$). This elemental composition is consistent with the 1D NMR data that suggested the presence of 11 protons and six carbons, and the number of unsaturations in **2a** is consequently 2. The IR spectrum showed absorptions bands at 3207, 2972, 2927, and 1733 cm^{-1}, typically for NH, aliphatic CH, and carbonyl groups, respectively. The ^1H NMR spectrum exhibited signals for two methyl groups (7-H_3 at δ_H 0.95 and 8-H_3 at δ_H 1.46), and two methylenes (4-H_2 at δ_H 3.53/3.41 and 6-H_2 at δ_H 1.77), and a broad singlet at δ_H 8.45 for NH. While 4-H_2 show no COSY correlations, 6-H_2 form an ethyl group with 7-H_3. 6-H_2, 7-H_3, and 8-H_3 all give HMBC correlations to C-2, as well as to each other, but not to any other carbons indicating that C-2 is not connected to another carbon. That C-2 is a saturated quaternary carbon with one oxygen and one nitrogen is supported by its ^{13}C NMR shift. 4-H_2 give HMBC correlations to C-2 as well as C-5, and its ^{13}C NMR shift indicate that C-4 is separated from C-2 by the nitrogen. In order to obey the requirement of two unsaturations, **2a** has to be a 2,2-disubstituted oxazolidin-5-one. The absolute configuration of **2a** was determined by Mosher's method (Hoye et al., 2007), where the N-H group was acylated with *S*- and *R*-α-methoxy-α-trifluoromethylphenylacetic acid chloride (MTPA-Cl) to give **2b** and **2c**. The differences in chemical shifts ($\Delta\delta^{SR} = \delta_S - \delta_R$) obtained by comparison of the analogous pairs of

Table 1
^{13}C and ^1H NMR spectroscopic data for compounds **1**, **2a** and **3** in $CDCl_3$ (δ_H in ppm, *J* in Hz).

C/H	1		2a		3	
	^{13}C	^1H	^{13}C	^1H	^{13}C	^1H
1	167.0; s	–	–	–	–	–
2	–	–	91.1; s	–	159.4; s	–
3	48.3; t	3.56; t; 6.6	–	–	–	–
4	27.6; t	2.94, t; 6.6	53.5; t	3.53; d; 8.2 3.45; d; 8.2	50.7; t	3.42; d; 9.7 3.30; d; 9.7
4a	130.9; s	–	–	–	–	–
5	108.7; d	6.63; s	188.6; s	–	83.6; s	–
6	149.5; s	–	32.6; t	1.77; q; 7.5	32.9; t	1.76; q; 6.9
7	144.6; s	–	7.8; q	0.95; t; 7.5	7.7; q	1.00; t; 6.9
8	114.4; d	7.67; s	24.7; q	1.46; s	25.1; q	1.49; s
8a	122.5; s	–	–	–	–	–
OCH3	56.0; q	3.94; s	–	–	–	–
NCH3	35.2; q	3.15; s	–	–	–	–
OH	–	3.87; s	–	–	–	–
NH	–	–	–	8.54; s	–	5.54; s

Table 2
^{1}H NMR chemical shifts data for *S*- and *R*-MTPA esters **2b** and **2c** in $CDCl_3$ (δ_H in ppm).

H	**2b** (*S*)	**2c** (*R*)	$\Delta\delta^{SR} = \delta_S - \delta_R$
4	4.05	3.43	+0.62
	3.94	3.33	+0.61
8	1.51	1.40	+0.11
6	1.61	1.75	−0.14
7	0.93	1.00	−0.07

protons for *S*- and *R*-MTPA esters (**2*S*** and **2*R***) are presented in Table 2, and suggest that **2a** has *S* configuration at C-2. Therefore, **2a** was identified as *(S)*-(+)-2-ethyl-2-methyloxazolidin-5-one.

The NMR and MS data analysis of **3** suggested that it is a known compound, *(R)*-5-ethyl-5-methyloxazolidin-2-one that previously has been reported as the product of a halohydrin dehalogenase catalysed opening of the corresponding racemic terminal epoxide in the presence of cyanate (Elenkov et al., 2008). However, it is new as a natural product and as the reported NMR data (Elenkov et al., 2008) are difficult to compare due to the use of different references, our NMR data are also given in Table 1. The optical rotation reported is identical to that determined for the product isolated here. Again, the HRESI-MS data for the molecular ion peak $[M+Na]^+$ (*m/z* found: 152.0687; calc.: 152.0687 for $C_6H_{11}NO_2Na$) suggested that the elemental composition is $C_6H_{11}NO_2$, which is consistent with the 1D NMR data. In the IR spectrum absorptions bands for NH, CH aliphatic and C=O groups at 3309, 2974, 2930, and 1740 cm^{-1}, respectively, were observed. The ^{1}H NMR spectrum showed signals for two methyl groups (7-H_3 at δ_H 1.00 and 8-H_3 at δ_H 1.49), and two methylenes (4-H_2 at δ_H 3.54/3.30 and 6-H_2 at δ_H 1.77), and a broad singlet at δ_H 5.54 for NH. Both the methylene protons in the ethyl group as well as 8-H_3 give HMBC correlations to C-4 as well as C-5, while HMBC correlations between 4-H_2 and C-2, C-5, C-6 and C-8 clearly demonstrate that **3** is a 5,5-disubstituted oxazolidin-2-one. This is consistent with the ^{13}C NMR differences observed between **2a** and **3**.

3. Experimental

3.1. General

A Bruker Alpha-P ART-IR instrument was used to record the infrared spectral data. The HRESI-MS data were determined with a Waters Q-TOF micromass spectrometer, using H_3PO_4 for calibration and as internal standard. 1D and 2D NMR data were obtained with Bruker Advance 400 spectrometer, and the NMR spectra were recorded in $CDCl_3$ (the solvent residual signals at δ_H 7.26 and δ_C 77.0 were used as reference). Chemical shifts (δ) are expressed in ppm and the constant couplings (*J*) are given in Hz. The optical rotations were measured by a Perkin-Elmer Model 341 Polarimeter (T = 20 °C and D = 589 nm). A Gallenkamp instrument was used for melting point measurements. Flash silica gel chromatography was performed on silica gel 60 (400–600 mesh, Merck), while the PTLC analyses were carried out using silica gel on TLC plates (20 × 20 cm, Sigma-Aldrich). TLC analyses were done with silica gel 60 F_{254} precoated plates. The chromatograms were visualized under UV lamp at 254 nm. The gel chromatographic separations were performed on Sephadex LH-20 (25–100 μm, GE Healthcare). All solvents used were analytical grade.

3.2. Plant material

The roots of *C. natalensis* were collected in Limpopo National Park, Gaza Province—Mozambique in August 2014. The plant was identified by Mr. Francisco Mapanga, a botanist of the Departamento de Ciencias Biologicas (Eduardo Mondlane University), where a voucher specimen with number 10.747 is kept.

3.3. Extraction and isolation

The air-dried and finely powdered roots (300 g) were subjected to sequential extraction with heptane, chloroform ($CHCl_3$), ethylacetate (EtOAc), and methanol (MeOH). All extracts were concentrated under reduced pressure on a rotary evaporator to yield four extracts, the heptane (8.9 g), chloroform (13.2 g), EtOAc (35.1 g), and MeOH (52.7 g) extracts. The MeOH extract was not further investigated. All extracts were found to contain mixtures of fatty acids and steroids that were not further fractionated.

The EtOAc extract (500 mg) was fractionated by column gel chromatography on Sephadex LH-20 eluted with MeOH:$CHCl_3$ (3:7) as the solvent system. Seven main fractions (F1 to F7) were collected based on their TLC profile. F2 and F3 were combined due their TLC similarities to afford fraction E (110 mg). Fraction E was further subjected to Sephadex LH-20 chromatography with MeOH:$CHCl_3$ (3:7) leading to collection of five sub-fractions (E1-E5). E2 (16.3 mg) was purified by PTLC eluted with DCM:EtOAc:MeOH (80:16:4) to yield compound **1** (3.3 mg). Purification of fraction F6 (18.3 mg) by the same technique yielded compounds **4** (5.5 mg) and **5** (4.2 mg). Fraction F7 (59.7 mg) afforded four sub-fractions by passing through Sephadex LH-20 eluted with MeOH:$CHCl_3$ (3:7). The sub-fraction F2-1 (71 mg) was further purified by Sephadex LH-20 eluted with MeOH:$CHCl_3$ (1:1) leading to the isolation of nepetin (16.7 mg).

The heptane extract (450 mg) was separated by column chromatography on silica gel using a stepwise gradient of heptane:EtOAc (4:1 to 1:1). Six fractions were collected (H1-H6). H6 was identified as pure β-sitosterol (25 mg), while fraction H2 (36.3 mg) was fractionated by Sephadex LH-20 chromatography with MeOH:$CHCl_3$ (3:7) to yield compounds **2a** (19.0 mg) and **3** (3.6 mg).

3.3.1. 3,11-dihydroxy-2,10-dimethoxy-6,14-dimethyl-7,8,15,16-tetrahydrodibenzo[c,i][1,7]diazacyclododecine-5,13(6H,14H)-dione (**1**)

Compound **1** was obtained as a yellowish solid (3.3 mg); m.p. 217–219 °C; IR in film ν_{max} (cm^{-1}) 3125 (OH), 1711 (C=O), 1680 and 1576 (C=C); ^{1}H ($CDCl_3$, 400 MHz) and ^{13}C NMR ($CDCl_3$, 100 MHz), see Table 1; positive HRESIMS *m/z* 415.1865 $[M+H]^+$ (calcd. for $C_{22}H_{27}N_2O_6$, 415.1869).

3.3.2. (S)-2-ethyl-2-methyloxazolidin-5-one (**2a**)

Compound **2a** was obtained as a white solid (19.0 mg); m.p. 66–68 °C; $[\alpha]_D^{20} = -11.4$ (c 1.9 $CHCl_3$); IR in film ν_{max} (cm^{-1}) 3250 (NH), 3000-2850 (CH), and 1710 (C=O); ^{1}H NMR ($CDCl_3$, 400 MHz,) and ^{13}C NMR ($CDCl_3$, 100 MHz), see Table 1; positive HRESIMS *m/z* 152.0684 $[M+Na]^+$ (calcd. for $C_6H_{11}NO_2Na$, 152.0687).

3.3.3. (R)-5-ethyl-5-methyloxazolidin-2-one (**3**)

Compound **3** was obtained as a white solid (3.6 mg); m.p. 69–71 °C; $[\alpha]_D^{20} + 9.2$ (c = 0.39 $CHCl_3$); IR in film ν_{max} (cm^{-1}) 3297 (NH), 2972 and 2927 (CH), and 1736 (C=O); ^{1}H NMR ($CDCl_3$, 400 MHz) and ^{13}C NMR ($CDCl_3$, 100 MHz), see Table 1; positive HRESIMS *m/z* 130.0867 $[M+H]^+$ (calcd. for $C_6H_{12}NO_2$, 130.0868);

3.4. Preparation of 2S and 2R oxazolidin-5-one esters **2b** and **2c**

To a stirred solution of **2a** (5.0 mg, 0.04 mmol) and dry pyridine (10 μl, 0.124 mml, 3.1 equiv.) in anhydrous $CHCl_3$ (1 ml) at room temperature, *S*-MTPA-Cl (14.2 μl, 0.076 mmol, 1.9 equiv., 99% purity) was added. The reaction mixture was left to stir overnight at room temperature and the progress was followed by TLC. The

reaction was quenched by addition of water (1 ml) and ether (3 ml). The aquous layer was extracted by two portion of ether (3 ml), and the combined organic layers were dried by anhydrous Na_2SO_4, filtered and evaporated *in vacuo*. The crude product mixture was purified by PTLC using the eluent system Heptan:EtOAc (1:1), to yield *S*-MTPA ester **2b** (8.9 mg, 67%) as white solid.

The similar procedure was used to prepare *R*-MTPA ester **2c** (7.4 mg, 55%) as white solid.

Acknowledgment

Financial support by Swedish International Development Agency is gratefully acknowledged.

References

Ahmad, V.U., Amber, A.-u.-R., Arif, S., Chen, M.H.M., Clardy, J., 1985. Cadabacine: an alkaloid from *cadaba Farinosa*. Phytochemistry 24, 2709–2711.

Al-Musayeib, N.M., Mohamed, G.A., Ibrahim, S.R.M., Ross, S.A., 2013. Lupeol-3-*O*-decanoate: a new triterpene ester from *cadaba farinosa* Forssk. growing in Saudi Arabia. Med. Chem. Res. 22, 5297–5302.

Ayer, S.W., McInnes, A.G., Thibault, P., Walter, J.A., 1991. Jadomycin, a novel 8*H*-benz[*b*]oxazolo[3,2-*f*]phenanthridine antibiotic from *Streptomyces venuzuelae* ISP5230. Tetrahedron Lett. 32, 6301–6304.

Borders, D.B., Morton, G.O., Wetzel, E.R., 1974. Structure of a novel bromine compound isolated from a Sponge. Tetrahedron Lett. 2709–2712.

Chaturvedula, V.S.P., Prakash, I., 2012. Isolation of stigmasterol and β-sitosterol from the dichloromethane extract of *Rubus suavissimus*. Int. Curr. Pharm. J. 1, 239–242.

Doull, J.L., Singh, A.K., Hoarel, M., Ayer, S.W., 1994. Conditions for the production of jadomycin B by *Streptomyces venezuelae* ISP5230: effects of heat shock, ethanol treatment and phage infection. J. Ind. Microbiol. 13, 120–125.

Elenkov, M.M., Tang, L., Meetsma, A., Hauer, B., Janssen, D.B., 2008. Formation of enantiopure 5-substituted oxazolidinones through enzyme-catalysed kinetic resolution of epoxides. Org. Lett. 10, 2417–2420.

Hall, J.C., 2008. Systematics of Capparaceae and Cleomaceae: an evaluation of the generic delimitations of Capparis and Cleome using plastid DNA sequence data. Botany 86, 682–696.

Hoye, T.R., Jeffrey, C.S., Shao, F., 2007. Mosher ester analysis for the determination of absolute configuration of stereogenic (chiral) carbinol carbons. Nat. Protoc. 2, 2451–2458.

Kabouche, A., Kabouche, Z., Touzani, R., Bruneau, C., 2011. Flavonoids from *Centaurea sulphurea*. Chem. Nat. Compd. 46, 966–967.

Kossuga, M.H., MacMillan, J.B., Rogers, E.W., Molinski, T.F., Nascimento, G.G.F., Rocha, R.M., Berlinck, R.G.S., 2004. (2S,3R)-2-Aminododecan-3-ol, a new antifungal agent from the Ascidian *Clavelina oblonga*. J. Nat. Prod. 67, 1879–1881.

Krane, B.D., Shamma, M., 1982. The isoquinolone alkaloids. J. Nat. Prod. 45, 377–384.

Lee, J.H., Lee, B.W., Kang, N.S., Moon, Y.H., Yang, M.S., Park, K.H., 2005. Alkaloids from the stem bark of *Phellodendron amurense* Rupr. J. Life Sci. 15, 423–426.

Lira, N.S., Montes, R.C., Tavares, J.F., Silva, M.S.D., Cunha, E.V., de Athayde-Filho, Petronio Filgueiras, Rodrigues, L.C., Dias, C.d.S., Barbosa-Filho, J.M., 2009. Brominated compounds from marine sponges of the genus Aplysina and a compilation of their ^{13}C NMR spectral data. Mar. Drugs 9, 2316–2368.

Martinez-Farina, C.F., Robertson, A.W., Yin, H., Monro, S., McFarland, S.A., Syvitski, R. T., Jakeman, D.L., 2015. Isolation and synthetic diversification of jadomycin 4-amino-L-phenylalanine. J. Nat. Prod. 78, 1208–1214.

Mendes, P. C. M. and Mendes, O., 1990. *Plantas Medicinais Seu Uso Tradicional em Mocambique*. Ministerio da Saude: Maputo, Mozambique ; Vol. Tomo 3, p 185.

Mohamed, G.A., Ibrahim, S.R.M., Al-Musayeib, N.M., Ross, S.A., 2014. New anti-inflammatory flavonoids from *Cadaba glandulosa* Forssk. Arch. Pharmacol. Res. 37, 459–466.

Norte, M., Rodriguez, M.L., Fernandez, J.J., Eguren, L., Estrada, D.M., 1988. Aplysinadiene and (R R)-5-[3,5-dibromo-4-[(2-oxo-5-oxazolidinyl)]-methoxyphenyl]-2-oxazolidinone. Two novel metabolites from *Aplysina aerophoba*. Synthesis of aplysinadiene. Tetrahedron 44, 4973–4980.

Ribeiro, A., Romeiras, M.M., Tavares, J., Faria, M.T., 2010. Ethnobotanical survey in Canhane village, district of Massingir, Mozambique: medicinal plants and traditional knowledge. J. Ethnobiol. Ethnomed. 6, 1–15.

Said, I.M., Hamid, N.A.A., Latif, J., Din, L.B., Yamin, B.M., 2005. 6,7-Dimethoxy-2-methyl-3,4-dihydro-isoquinolin-1(2H)-one. Acta Crystallogr. E61, 797–798.

Schmidt, E., Lötter, M., McCleland, W., 2002. Trees and Shrubs of Mpumalanga and Kruger National Park. Jacana, Johannesburg, pp. 124.

Velmurugan, P., Kamaraj, M., Prema, D., 2010. Phytochemical constituents of *Cadaba trifoliata* roxb. root extract. Int. J. Phytomed. 2, 379–384.

Zheng, J.-T., Rix, U., Zhao, L., Mattingly, C., Adams, V., Chen, Q., Rohr, J., Yang, K.-Q., 2005. Cytotoxic activities of new jadomycin derivatives. J. Antibiot. 58, 405–408.

Paper II

International Journal of Research in Pharmacy and Biosciences
Volume 4, Issue 2, 2017, PP 10-14
ISSN 2394-5885 (Print) & ISSN 2394-5893 (Online)
http://dx.doi.org/10.22259/ijrpb.0402003

Novel Sulfur-Containing Indole from the Leaves of Clematis Viridiflora Bertol

Julião Monjane[1,2], Efrain Salamanca[3], Alberto Giménez[3], Olov Sterner[2],*

[1]Department of Chemistry, Faculty of Science, Eduardo MondlaneUniversity, Maputo, Mozambique
[2]Centre for Analysis and Synthesis, Faculty of Science, Lund University, Lund, Sweden
[3]Instituto de Investigaciones Fármaco Bioquímicas, San Andrés University, La Paz, Bolivia

***Corresponding Author:** Olov Sterner, Centre for Analysis and Synthesis, Faculty of Science, Lund University, Lund, Sweden*

Received Date: 01-06-2017 ***Accepted Date: 08-06-2017*** ***Published Date: 22-06-2017***

ABSTRACT

A novel sulfur-containing indole, 4-methoxy-3-methyl-2-(methylthio)-1H-indole (1), together with twelve known metabolites, was isolated from an extract of the leaves of Clematis viridiflora. The structure of 1 was determined by spectral analysis including 1D- and 2D NMR, IR, and HR-MS spectra. The antiprotozoal activity of compound 1 toward topromastigotes of Leishmaniaamazonensisand L. braziliensis in vitrowas assayed, and 1showedpotent activity towards both strains tested (IC50, 25.0 µg mL-1 and 13.2 µg mL-1, respectively). This is the first report of the isolation of a sulfur-containing indolewithin the family of Ranunculaceae.

Keywords: *Clematis viridiflora, sulfur-containing indole, cyanogenic glycoside, antileishmanial activity*

INTRODUCTION

The genus *Clematis* comprises between 300and355 species, distributed worldwide. It is considered to be one of the largest among the flowering plants of the family of Ranunculaceae,[1] and 13 species of this genus grow in Mozambique.[2] Several *Clematis* species have been used in folk medicine for their analgesic, diuretic, anticancer, anti-inflammatory, and antibacterial properties.[3] At least 30 *Clematis* species have been characterized phytochemically, leading to the isolation of structurally diverse secondary metabolites with a wide range of biological activities.[4-5]*Clematis viridiflora*Bertol. is a green-flowered climbing shrub, growing on dunes in the Southern and Central parts of Mozambique.[2]The roots and leaves of thisplant have been used in Mozambique for the treatment of malaria and headache.[6-7] No phytochemical study of this plant has previously been reported, and as a part of our search for interesting secondary metabolites from the diverse flora of Mozambique an extract of the leaves of *C. viridiflora*was investigated for its chemical constituents.

MATERIALS AND METHODS

General Methods

1D and 2D NMR spectra were recorded at room temperature withBrukerAvance II 400 and AvanceIII 500 spectrometers. The chemical shifts (δ) are reported in ppm relative to the solvent signals (δ_H 7.26 and δ_C 77.0 for $CDCl_3$), while the coupling constants (*J*) are given in Hz. AWaters XEVO-G2 QTOF mass spectrometer was used for HR-ESI-MS measurements. The IR spectra data were recorded on a Bruker Alpha-P ART-IR spectrophotometer. The Waters Acquity Ultra Performace Liquid Chromatography UV-Detector was used to detect the UV light absorptions.The melting point measurements were carried out on Gallenkamp instrument. The column chromatography (CC) was performed using silica gel 60 (230-400 mesh, Merck) and gel permeation on Sephadex LH-20 (GE-Healthcare). Analytical TLC plates visualized under UV lamp at 254 nm and spraying with vanillin followed by heating. All solvents used were analytical grade.

Plant Material

The leaves of *c. viridiflora* were collected in Magude District, Maputo Province, Mozambique, in August 2014. The plant was identified locally by Mr. Francisco Mapanga (Eduardo Mondlane University) and a voucher specimen under accession number 351 is kept at the Herbarium of the Botanical Garden of Eduardo Mondlane University.

Extraction and Isolation

The air-dried powdered leaves (300 g) were extracted three times at room temperature in a mixture of MeOH:CH_2Cl_2 (1:1) for 48 hours. After filtration, the combined solutions were concentrated under reduced pressure to yield a dark greenish residue (37 g). The crude extract was suspended in a mixture of H_2O:MeOH(9:1), and then partitioned between *n*-heptane, CH_2Cl_2, and EtOAc, successively. Each fraction was concentrated under reduced pressure to give the *n*-heptane (1.450 g), CH_2Cl_2 (4.079 g), and EtOAc (5.324 g) fractions, respectively. The aqueous and *n*-heptane fractions were discarded.

Isolation of the metabolites from CH2Cl2subfraction

Chromatography column (*n*-heptane:EtOAc 2:1 and CH_2Cl_2:EtOAc 2:1 to 1:2) of the CH_2Cl_2 fraction (2.011 g) yielded five major fractions (A-E). The 3-*O*-[2'-(tetracosyloxy)acetyl] lupeol ether (77.5 mg) was obtained as a pure compound from sub-fraction A. The sub-fraction C (143.3 mg) was subjected to CC (CH_2Cl_2:EtOAc 2:1 to 1:4) which gave seven sub-fractions (C-1-C7). Subsequent purification of subfraction C-5 (35.6 mg) by CC (Et_2O:EtOAc 1:1 to 1:2) afforded compound **1** (3.9 mg, yellowish solid). Most of the compounds present in dichloromethane extract were found to be in trace amounts and could be not characterized by spectroscopic methods.

Isolation of the Metabolites from the EtOAc Subtraction

The EtOAc fraction (2 g) was fractionated by Sephadex LH-20 (100% MeOH) yielding eight sub-fractions (A – H). A CC on sub-fraction B (37.7 mg) yielded 4-hydroxibenzoic acid (8.9 mg). Sub-fraction C (400 mg) was subjected to Sephadex LH-20 (MeOH:$CHCl_3$ 1:1) to afford three other sub-fractions (C1-C3). Under the same conditions, sub-fraction C1 (115 mg) yielded chlorogenic acid (30.5 mg). Sub-fraction C2 (98.5 mg) was chromatographed on Sephadex LH-20 (100% MeOH) to give 5-*O*-*p*-coumaricquinic acid (46.0 mg), caffeic acid (13.8 mg), and umbellic acid (17.0 mg). Sub-fraction C3 (91.0 mg) was subjected Sephadex LH-20 (100% MeOH) to yield baicalin (31.0 mg). Sub-fraction E (63.2 mg) gave benzoic acid (6.2 mg) by passing over Sephadex LH-20 (100% MeOH). Sub-fraction F (22.0 mg) was purified by Sephadex LH-20 (100% MeOH) to yield astragalin (11.1 mg) and flindulatin-5-methyl ether (8.1 mg). From sub-fraction G (450 mg), quercetin-7-*O*-β-galactopyranoside (24.6 mg) was obtained as apure compound. Sub-fraction H (190 mg) was chromatographed using 100% MeOH over Sephadex LH-20 to afford 82.0 mg of **2**.

Spectroscopic Data of the Metabolite 1

4-methoxy-3-methyl-2-(methylthio)-1H-indole(**1**): yellowish solid; 3.9 mg; m.p. 195-197 ^oC; UV (MeOH) λ_{max} (log ε) 234 (2.9) nm and 280 (2.5) nm; IR (film): 3203, 2956, 2923, 1670, 1585, 1510, 1459 cm^{-1}; HR-ES-MS: *m/z* 207.0717 $[M+]^+$ (calc. for $C_{11}H_{13}NOS$, *m/z* 207.0718);^{1}H-NMR 500 MHz ($CDCl_3$) and ^{13}C-NMR 125 MHz ($CDCl_3$) see Table 1.

Table1. *^{13}C (125 MHz) and ^1H NMR (500 MHz) data for compound **1** in $CDCl_3$.*

Position	1	
	δ_C (ppm)	**δ_H (ppm), *J* (Hz)**
1	-	10.88 br *s*
2	129.6; *s*	-
3	116.5; *s*	-
3a	118.8; *s*	-
4	155.9; *s*	-
5	99.7; *d*	6.51; *dd*; 7.9, 0.4
6	125.8; *d*	7.24; *dd*; 7.9, 7.9
7	105.2; *d*	7.04; *dd*; 7.9, 0.4
7a	139.3; *s*	-
4-OCH_3	55.2; *q*	3.95; *s*
2-SCH_3	41.2; *q*	3.00; *s*
3-CH_3	11.2; *q*	2.60; *s*

Antileishmanial Assay

The activity was measured onin vitrocultures of Leishmania parasite in promastigote forms of complex *L. amazonensis* (clon 1: Lma, MHOM/BR/76/LTB-012) and complex *L. braziliensis* (strand M2904 C192 RJA), cultivated at 26 °C in Schneider medium (pH 6.8) supplemented with inactivated (56 °C x 30min) calf bovine serum (10%). Parasites in logarithmic phase of growth, at a concentration of $1x10^6$parasites/mL, were distributed on 96 micro well plates and different concentration of **1** (100, 50,and 25µg/mL) were added. The micro well plates were incubated for 72hrs at 26 °C after which a solution of XTT (1mg/mL) in

PBS (pH 7.0 at 37°C) with PMS (Sigma-Aldrich, 0,06mg/mL), was added (50µL/well) and the incubationcontinued for 4hrs at 26 °C.All assays were carried out as triplicates. DMSO (1%) and Miltefosine were used as negative and positive control. The optical density of each well was determined with a Synergy HT microplatereader, atλ 200-450nm. The IC_{50} values were calculated using the Gen5 program (BioTek).[24]

RESULTS AND DISCUSSION

The phytochemical analysis ofan extract of the leaves prepared with$MeOH:CH_2Cl_2$(50:50), led to the isolation of a novel sulfur-containing indole metabolite, 4-methoxy-3-methyl-2-(methylthio)-1*H*-indole (**1**). Besides the isolation of **1**, twelve known compounds were isolated and characterized. These are thecyanogenic glycoside prunasin (**2**),[8]chlorogenic acid (**3**),[9]5-*O*-*p*-coumaroylquinic acid (**4**),[10] flindulatin-5-methyl ether (**5**),[11]caffeic acid,[12]umbellic acid,[13] astragalin,[14] quercetin 7-*O*-*β*-galactopyranoside,[15] baicalin,[16]benzoic acid,[17] 4-hydroxibenzoic acid,[18] and 3-*O*-[2'-(tetracosyloxy)acetyl]lupeol ether.[19] The structures of the known metabolites were established by comparison of the experimental spectroscopic properties with those reported in the literature. The structures of some metabolites isolated from the leaves of *C. viridiflora*,as well asbrassicanal A (**6**), are presented in Figure 1.

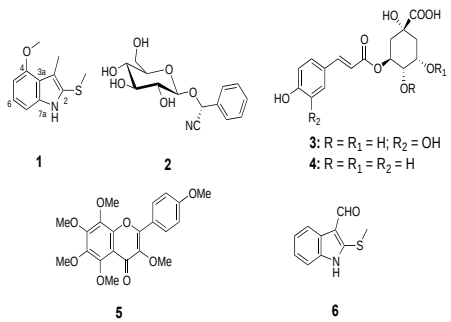


Figure1. *Structures of compounds**1-5** isolated from an extract of C. viridiflora,and brassicanal A (**6**).*

Compound **1** was obtained as a yellowish solid. The elementalcomposition of **1** was determined to be $C_{11}H_{13}NOS$, based on the molecular ion peak $[M]^+$ (*m/z*: found 207.0717; calc. 207.0718) of its HR-ESI-MS and by analysis of the 1D NMR spectra of **1** that display signals for 13 protons and 11 carbons. This gives an unsaturation of six, and as the ^{13}C NMR data suggest the presence of four carbon-carbon double bonds **1** should consist of two rings. The IR spectrum displayed absorption bands for a secondary anime (3203 cm^{-1}) and an aromatic system (1585, 1510, and 1459 cm^{-1}). The ^1H NMR spectrum analysis (Table 1) indicated the presence of an AMX spin system. This spin system was due to the presence of a trisubstituted benzene ring: [δ_H 7.24 (H, *dd*, *J*=7.9; 7.9 Hz),δ_H 7.04 (H, *dd*, *J*=7.9; 0.4 Hz), andδ_H 6.51 (H, *dd*, *J*=7.9; 0.4 Hz)]. In addition, four singletsthat were suggested to belong to amethoxy group (δ_H 3.95, OCH_3, *s*), a methylthiogroup (δ_H 3.00, SCH_3, *s*), a methyl (δ_H 2.60, CH_3, *s*), and an exchangeable proton at δ_H 10.88(1H, brs)were observed. The ^{13}C-NMR spectrum (Table 1) showed 11 carbon signals and based on DEPT analysis five of the unsaturated carbons were suggested to lack hydrogens while the remaining three each have one. The three saturated carbons are suggested to be methyl groups (vide supra), as their proton signals integrate for three protons each. The direct H-C connectivities were determinedwith HMQC experiments (see Table 1). The proton signal at δ_H 10.88 was not connected to a carbon atom and was attributed to the NH group (H-1), confirming the IR and ^1H-NMR data analysis discussed above. The COSY spectrum showed cross-peaks in the aromatic region between H-6 and H-5 as well asH-7, and the small coupling observed between H-5 andH-7 confirms the presence of a 1,2,3-trisubstituted benzene ring. HMBC correlations (see Figure 2a for pertinent correlations) from H-5 to C-3a, C-4 and C-7, from H-6 to C-4 and C-7a, from H-7 to C-3a and C-5, and from the methoxy protons to C-4 defines this ring and establish that C-3a and C-7a are part of the second ring as well as the position of the methoxy substituent. HMBC correlations (Figure 2a) from H_3-8 to C-2, C-3 and C-3a show that the tetrasubstituted double bond is conjugated with the benzene ring and attached to C-3a, and correlations from the methylthioprotons to C-2 show that the thiolether is linked to the other end of this double bond. This configuration is also supported by the NOESY correlation observed (see Figure 2). The only remaining part of the structure is an NH group that only can close the second ring between C-2 and C-7a. The HMBC correlationsfrom H-1 to C-3 and C-3a confirm this position of the NH group and establish that compound**1** is a novel sulfur-containing 1*H*-indolewith substituents at C-2,C-3and C-4 in the indole nucleus, named 4-methoxy-3-methyl-2-

(methylthio)-1*H*-indole. As far as we know, metabolite **1** is the first sulfur-containing indole nucleus to be isolated from any member of the Ranunculaceae family.

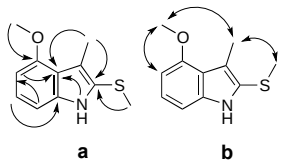


Figure2. *The key HMBC (a) and key NOESY (b) correlations of 1.*

A number of phytoalexins, secondary metabolites produced by plants inresponse to diverse forms of stress, with diverse structural features have been reported from different plant families.[20]Sulfur-containing indoles isolated from *Brassica* species (Cruciferae) have previously been identified asphytoalexins,[21]they have been reported as minor constituents and possess a wide range of biological activities.[22] The main structure of these metabolites is characterized by the presence of indole nucleus substituted with one or two sulfur atoms of which one is located at C-2.[20] The basic indole is biogenetically thought to be derived from *L*-tryptophan, and the sulfur atoms could be incorporated through cysteine and methione.[23]Compound**1**, containing one sulfur atom, could share the biosynthetic pathway with that ofbrassicanal A (**6**) reported from *Brassica campestris*.[23] It should be noted that besides **1**thecyanogenic glycoside prunasin (**2**)was isolated, indicating that*C. viridiflora*is a potentially toxic plant. Cyanogenic glycosides are secondary metabolites composed by α-hydroxynitrile and a sugar moiety, which after hydrolysis release hydrogen cyanide.

As we have a strong interest in the identification of new antileishmanial natural products, **1** was assayed in vitro against the two *Leishmania*strains *L. amazonensisclon 1* and *L. braziliensis*.**1** showed interestingantileishmanial activitytowards both strains,with the IC_{50} values 25.0 and 13.2 μg mL^{-1}, respectively. The positive control Miltefosine(a drug used to treat leishmaniasis) was only slightlymore potent with the IC_{50} values 5.1 and 4.9 μg mL^{-1}, respectively.

ACKNOWLEDGEMENTS

The authors are grateful to Swedish International Development Agency for financial support. We thank Sofia Essen from Lund University for the help with the HRES-MS spectra.

REFERENCES

[1] Hai W, Cheng H, Zhao M, Wang Y, Hong L, Tang H, Tian X, Two new cytotoxic triterpenoid saponins from the roots of *Clematis argentilucida*.Fitoterapia,83, 2012, 759-64.

[2] Essig FB,A Preliminary Analysis of *Clematis* (Ranunculaceae) in Sub-Saharan Africa. SIDA,21, 2004, 861-877.

[3] Lee TH, Huang NK, Lai TC, Yangd ATY, JaneWang G,Anemonin, from *Clematis crassifolia*, potent and selective inducible nitric oxide synthase inhibitor. Journal of Ethnopharmacology, 116,2008, 518–527.

[4] Hao DC, Gu XJ, Xiao PG, Peng Y,Chemical and biological research of *Clematis* medicinal resources.Chinese Science Bulletin, 58,2013, 1120-1129.

[5] Chawla R, Kumar S, Sharma A,The genus *Clematis* (*Ranunculaceae*): Chemical and pharmacological perspectives. Journal of Ethnopharmacology, 1432012, 116-150.

[6] Bruschi P, Morganti M, Mancini M, Signorini MA,Traditional healers and laypeople: A qualitative and quantitative approach to local knowledge on medicinal plants in Muda (Mozambique). Journal of Ethnopharmacology, 138,2011, 543-563.

[7] Jurg A, Tomás T, Pividal J,Antimalarial activity of some plant remedies in use in Marracuene, Southern Mozambique. Journal of Ethnopharmacology, 33, 1991, 79-83.

[8] Nahrstedt A, Rockenbach J,Occurrence of the cyanogenic glucoside prunasin and its corresponding mandelic acid amide glucoside in *Olinia* species (oliniaceae). Phytochemistry, 34,1993, 433-436.

[9] Han T, Li H, Zhang Q, Zheng H, Qin L,New thiazinediones and other components from *Xanthium strumarium*. Chemistry of NaturalCompounds, 42,2006, 567-570.

[10] Lu Y, Sun Y, Foo LY, McNabb WC, Molan AL, Phenolic glycosides of forage legume *Onobrychis viciifolia*.Phytochemistry, 55, 2000, 67-75.

[11] Rashid MA, Armstrong JA, Gray AI, Waterman PG,Alkaloids, flavonols, and coumarins from *Drummondita hassellii* and *D. calida*. Phytochemistry. 311992, 1265-1269.

[12] Saito N, Tatsuzawa F, Yazaki Y, Shigihara A, Honda T,7-Polyacylated delphinidin 3,7-diglucosides from the blue flowers of *Leschenaultia* cv. Violet Lena. Phytochemistry, 68,2007, 673–679.

[13] Abdel-Kader MS,New ester and furocoumarins from the roots of *Pituranthos tortuosus*. Journal

of the BrazilianChemical Society, 14, 2003, 48-51.

[14] Saito S, Silva G, Santos RX, Gosmann G, Pungartnik C, Brendel M,Astragalin from *Cassia alata*induces DNA adducts in vitro and repairable DNA damage in the yeast *Saccharomyces cerevisiae*. International Journal of Molecular Sciences. 13,2012, 2846-2862.

[15] Güvenalp Z, Demirezer LO,Flavonol glycosides from *Asperula arvensis*.Turkish Journal of Chemistry, 29, 2005, 163-169.

[16] Wu S, Sun A, Liu R,Separation and purification of baicalin and wogonoside from the Chinese medicinal plant *Scutellaria baicalensis* Georgi by high-speed counter-current chromatography. Journal of Chromatography A, 1066, 2005, 243–247.

[17] Waqas M, Khan AL, Ali L, Kang SM, Kim YH, Lee IJ,Seed germination-influencing bioactive secondary metabolites secreted by the endophyte *Cladosporium cladosporioides* LWL5. Molecules, 18,2013, 15519-15530.

[18] Azizuddin Makhmoor T, Choudhary MI,Radical scavenging potential of compounds isolated from *Vitex agnus-castus.* Turkish Journal of Chemistry, 34, 2010, 119-126.

[19] Tomosaka H, Koshino H, Tajika T, Omata S,Lupeol esters from the twig bark of Japanese pear (*Pyrus serotina* Rehd.) cv. Shinko. Bioscience, Biotechnology and Biochemistry, 65,2001, 1198-1201.

[20] Chripkova M, Zigo F, Mojzis J,Antiproliferative effect of indole phytoalexins. Molecules, 21, 2016, 1626-1640.

[21] Pedras MSC, Loukaci A, Okanga FL,The cruciferous phytoalxins brassinin and cyclobrassinin are intermediates in the biosynthesis of brassilexin.Bioorganic and Medicinal ChemistryLetters, 8, 1998, 3037-3038.

[22] Pedras MSC, Okanga FI, Zaharia IL, Khan AQ,Phytoalexins from crucifers: synthesis, biosynthesis, and biotransformation. Phytochemistry, 53,2000, 161-176.

[23] Pedras MSC, Jha M, Ahiahonu PWK,The synthesis and biosynthesis of phytoalexins produced by Cruciferous plants. Current Organic Chemistry, 7, 2003,1635-1647.

[24] Domínguez-CarmonaDB,Escalante-Erosa F, García-SosaFK,Ruiz-PinellG, Gutierrez-Yapu D,Chan-BacabMJ,Giménez-TurbaA,Peña-Rodríguez LM,Antiprotozoal activity of betulinic acid derivatives. Phytomedicine, 17, 2010, 379–382.

Citation: *Julião Monjane, Efrain Salamanca, Alberto Giménez, Olov Sterner. Novel Sulfur-Containing Indole from the Leaves of Clematis Viridiflora Bertol. International Journal of Research in Pharmacy and Biosciences. 2017;4(2):10-14.*

Paper III

Onopordopicrin with leishmanial activity from the leaves of *Brachylaena discolor*

Julião A. Monjane[1,2], Diandra Capusiri[3], Alberto Giménez[3], and Olov Sterner[2]

[1]Chemistry Department, Eduardo Mondlane University, P.O. Box 257, Maputo, Mozambique

[2]Centre for Analysis and Synthesis, Lund University, P.O. Box 22100, Lund, Sweden

[3]Instituto de Investigaciones Fármaco Bioquímicos, San Andrés University, La Paz, Bolivia

Abstract

The antileishmanial activity of the known sequesterpene lactone onopordopicrin (**1**), found in many *Brachylaena* species, and its germacronolide epoxide derivative (**2**), both isolated from an extract of the leaves of *Brachylaena discolor*, was assayed. **1** possess significant leishmanicidal activity against *Leishmania amazonensis* and *L. braziliensis* (IC_{50} values 13.8 and 9.7 μM, respectively) comparable with the positive control miltefosine (5.1 and 4.9 μM, respectively). However, **2** was found to be inactive against both strain tested. This is the first study reporting the above-mentioned activity of onopordopicrin (**1**).

Keywords: Asteraceae, *Brachylaena discolor*, Onopordopicrin, Germanocronolides, Antileishmanial activity

Introduction

Out of more than 5,500 plant species recorded in Mozambique, about 10 % are used for medicinal purposes, including the treatment of infections and other diseases.[1,2] Traditional medicine has remained as the most affordable and available source to solve health problems in rural communities all over the world. According to WHO, 80 % of the world's population still rely on such remedies for their basic healthcare needs. In addition, the knowledge provided by the traditional uses of plants to treat different illnesses has been helpful in the search of new biologically active metabolites.[3,4] Based on such knowledge, phytochemical studies on *Brachylaena* (Asteraceae) species has led to the identification of a large number of sesquiterpene lactones. The sesquiterpene lactones are considered the characteristic secondary metabolites of the genus, exhibiting mainly antibacterial activities.[5–9] The genus of *Brachylaena* is composed by 13 to 20 plant species, mostly distributed in the Southern part of Africa.[8,10] *Brachylaena discolor D.C.* (var. *discolor*) is an evergreen shrub or small tree usually 4 to 10 m height. In Mozambique, the plant is

found in the Southern part of the country, and grows in coastal woodland and bushland, and on the margins of evergreen forests. The roots and leaves have been used for medicinal purposes for the treatment of stomachache, tuberculosis, and diabetes.[4,11–13]

Previous phytochemical investigation on the aerial parts of *B. discolor* (var. *discolor*) led to the isolation of a sesquiterpene lactone, onopordopicrin (**1**).[6] In a continuing study of phytochemical constituents derived from Mozambican traditional medicinal plants, an extract of the leaves of *B. discolor* was investigated. In this paper, we wish to report the isolation and antileishmanial activity of metabolites **1** and **2** assayed towards *Leishmania amazonensis* and *L. braziliensis*, which is the first time **1** and **2** are assayed for antileishmanial activity. The structure of **1** was established by high resolution NMR and MS experiments, and confirmed by the comparison of its experimental spectroscopic and physical data with those reported in the literature.[14] Metabolite **1** has been found in larger amounts in various plants of the Asteraceae family[15–18] and has been reported to possess cytotoxic and antibacterial activities.[14,19]

Results and discussions

The leaves of *B. discolor* were air-dried at room temperature, and the dried leaves were macerated with MeOH. The crude methanolic extract was then fractionated between *n*-heptane, $CHCl_3$, and EtOAc. Repeated chromatographic purifications of the EtOAc extract yielded twelve known metabolites. Besides the isolation of **1**, the sesquiterpene lactone germacronolide epoxy derivative **2**,[5] was isolated, along with the following phenolic compounds: hydroxytyrosol (**3**),[20] dihydroxysinapic acid (**4**),[21] 6''-*O*-acetyl homoplantaginin (**5**),[22] onoporidin (**6**),[23] 3'-hydroxygenkwanin (**7**),[24] luteolin (**8**),[25] quercetin-3-*O*-glucoside-7,3',4'-trimethyl ether (**9**),[26] quercetin-3-*O*-β-D-galactopyranoside (**10**),[27] eupafolin (**11**),[28] quercetin-7-*O*-galactopyranoside (**12**).[29]

Sesquiterpene lactones are bioactive secondary metabolites, which are found in a variety of plants. The biological activities they possess could be useful for a range of conditions, from skin ulcer to atherosclerosis, neurodegeneration, and even cancer. They have also been proposed as lead compounds for the design of new anti-inflammatory drugs.[16,30] This class comprises a large group with more than 5000 known metabolites, most found in the family of Asteraceae.[31] Sesquiterpene lactones derive from the mevalonic acid pathway, and metabolites **1** and **2** have a germacronolide terpenoid skeleton. Costunolide (**14**) has been proposed as the common precursor of germacronolide-derived sesquiterpene lactones, and the first step in the biosynthesis of **14** is the cyclization of farnesyl diphosphate to

germacrene A via a germacrene A synthase.[32] Metabolites **1** and **2** could thereby share the same biosynthetic pathway, possessing an additional and unusual four carbon atom unit. Alas, the detailed biosynthetic mechanism is still not understood.

Metabolites **1** and **2** were evaluated for their antileishmanial activity. Metabolite **1** showed a promising antileishmanial activity against *L. amazonensis* and *L. braziliensis* strains with IC_{50} values of 13.8 and 9.7 μM, respectively. Miltefosine used to treat leishmaniosis, with IC_{50} values 5.1 and 4.9 μM, respectively, was used as positive control. Interestingly, metabolite **2** was inactive against both strain tested. The difference in activity of these two metabolites could be attributed to the presence of two *α,β*-unsaturated carbonyl functionality in **1**, while **1** has two of these functionalities **2** has only one, in addition to an epoxide. The presence of a second *α,β*-unsaturated carbonyl functionality in **1** could influence the activity, as such activated methylene groups are commonly identified as responsible for many biological effects as a consequence of their electrophilic reactivity as Michael acceptors, with can react with, for example, the thiol groups present in proteins.[16,31] This is also supported by the fact that dehydrozaluzanin C (**13**), another sesquiterpene lactone isolated from *Mummocia maronii* (Asteraceae) with two *α,β*-unsaturated carbonyl groups, inhibits the growth of leishmanial promastigotes at concentrations ranging between 2.5 to 10 μM.[33,34] The 2-(hydroxymethyl)acrylate side chain in **1**, as well as the number of *α,β*-unsaturated carbonyl groups play an important role, also for other biological activities. For example, in comparison of antitrypanosomal activities, the lack of the side chain of **1** drastically lowers its activity.[19]

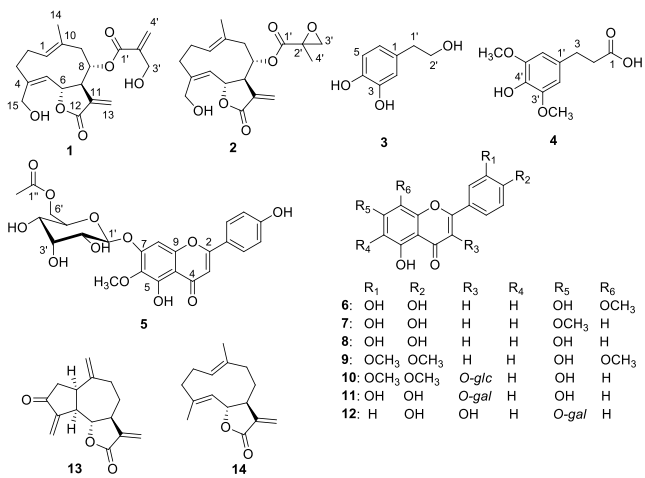


Figure 1 Structures of isolated metabolites, dehydrozaluzanin C (**13**) and costunolide (**14**).

Material and methods

General methods

1D and 2D NMR spectra were recorded at room temperature with a Bruker Advance II 400 spectrometer. The chemical shifts (δ) are reported in ppm relative to solvent residual signals (δ_H 7.26 and δ_C 77.0 for $CDCl_3$; δ_H 3.31 and δ_C 47.0 for CD_3OD; δ_H 2.50 and δ_C 39.5 for DMSO-d_6), while the coupling constants (J) are expressed in Hz. HRES-MS were performed with a Waters Acquity UPLC + Waters XEVO-G2 system spectrometer. The IR spectra data were recorded on a Bruker Alpha-P ART-IR spectrophotometer. The melting point measurements were carried out on Gallenkamp instrument. The column chromatography (CC) was performed using silica gel 60 (230-400 mesh, Merck) and gel permeation on Sephadex LH-20 (GE-Healthcare). Analytical TLC plates visualized under UV lamp at 254 nm and spraying with vanillin followed by heating. All solvents used were analytical grade.

Plant material

The leaves of *B. discolor* were collected in Magude District, Maputo Province, Mozambique, in August 2014. The plant was identified locally and a voucher specimen under accession number 225 is kept at the Herbarium of the Botanical Garden of Eduardo Mondlane University.

Extraction and isolation of compounds

The air-dried and powdered leaves of *B. discolour* (350 g) were extracted 3 times for 24 h with 200 ml MeOH. The combined extracts were evaporated to dryness under reduced pressure to give a crude MeOH extract (22.5 g). This was redissolved in a mixture of MeOH:H_2O (10:90) and then extracted with *n*-heptane, $CHCl_3$, and EtOAc to yield 10.5 g, 1.1 g, and 2.5 g, respectively, of organic subfractions.

The EtOAc subfraction (2.3 g) was subjected to column chromatography (CC) on silica gel eluted with DCM:EtOAc mixtures in different ratios (0-100 %) and then EtOAc:MeOH (95:5) to yield 5 fractions (A-E). Fraction D (697 mg) was subjected to Sephadex LH-20 chromatography eluted with a mixture of MeOH:$CHCl_3$ (1:1) to afford 5 subfractions (D1-D5). Subfraction D2 (304 mg) was passed through Sephadex LH-20 using the same solvent system as previously to afford two main subfractions (D21 and D22). The subfraction D22 (95.0 mg) was chromatographed on Sephadex LH-20 (100 % MeOH) giving hydroxytyrosol (**3**, 22.7 mg), dihydroxysinapic acid (**4**, 31.7 mg) and 6''-*O*-acetyl homoplantaginin (**5**, 26.7 mg). After passing the fraction D5 (180.1 mg) through Sephadex LH-20 (100 % MeOH), quercetin-7-*O*-galactopyranoside (**12**, 24.2 mg), quercetin-3-*O*-glucoside-7,3',4'-trimethyl ether (**9**, 13.9 mg), quercetin-3-*O*-*β*-D-galactopyranoside (**10**, 9.0 mg),

and eupafolin (**11**, 7.0 mg) were isolated. Fraction E (300 mg) was submitted to Sephadex LH-20 MeOH:$CHCl_3$ (1:1) to afford subfractions E1-E4. The Sephadex LH-20 (100 %) fractionation carried out with subfraction E3 (145.2 mg) resulted in the isolation of onoporidin (**6**, 10.2 mg). Fraction E4 (48.0 mg) was subjected on Sephadex LH-20 fractionation with a mixture of MeOH:$CHCl_3$ (1:1) to yield 3'-hydroxygenkwanin (**7**, 11.0 mg), and luteolin (**8**, 17.0 mg). Fraction B (198.7 mg) was submitted to CC over silica gel in a mixture of DCM:EtOAc (0-100 %) to give **1** (88.9 mg) and **2** (68.6 mg) as the main components.

Onopordopicrin (**1**): Colorless oil; $[\alpha]_D^{25}$ + 17.2^o (*c* 1.00, $CHCl_3$); m.p. 57-59^{o}C; HR-ESMS *m/z*: 349.1651 $[M+H]^+$ (calc. for $C_{19}H_{24}O_6$, 349.1650); IR film (cm-^{1}) 3450 (OH), 2925 (methylene), 1755 (C=O, γ-lactone), 1705 (C=O, unsaturated ester); ^{1}H-NMR (400 MHz, $CDCl_3$): 5.02 (1H, *dd*, *J* = 11.1, 4.0 Hz, H-1), 2.24 (1H, *m*, H-2α), 2.00 (1H, *m*, H-2β), 2.24 (1H, *m*, H-3α), 2.62 (1H, *m*, H-3β), 4.85 (1H, *d*, *J* = 10.0 Hz, H-5), 5.14 (1H, *dd*, *J* = 10.0, 10.0 Hz, H-6), 3.11 (1H, *m*, H-7), 5.19 (1H, *dd*, *J* = 9.4, 10.0 Hz, H-8), 2.62 (1H, *m*, H-9α), 2.00 (1H, *m*, H-9β), 6.29 (1H, *d*, *J* = 3.5 Hz, H-13α), 5.81 (1H, *d*, *J* = 3.5 Hz, H-13β), 1.51 (3H, *s*, H-14), 4.23 (1H, br *d*, H-15α), 4.07 (1H, br *d*, H-15β), 4.31 (2H, *s*, H-3'), 6.30 (1H, br *d*, H-4'α), 6.00 (1H, br *d*, H-4'β). ^{13}C-NMR (100 MHz, $CDCl_3$): 129.7 (C-1), 26.0 (C-2), 34.5 (C-3), 144.5 (C-4), 127.9 (C-5), 77.3 (C-6), 52.8 (C-7), 72.9 (C-8), 48.8 (C-9), 139.6 (C-10), 132.1 (C-11), 165.1 (C-12), 125.6 (C-13), 16.6 (C-14), 60.5 (C-15), 170.5 (C-1'), 135.2 (C-2'), 60.9 (C-3'), 125.7 (C-4').

Salonitelonide-8-O-2',3'-isobutyrate (**2**): Colorless oil; $[\alpha]_D^{25}$ + 16.4^o (*c* 1.00, $CHCl_3$); m.p. 69-71^{o}C; HR-ESMS *m/z*: 348.1570 $[M]^+$ (calc. for $C_{19}H_{24}O_6$, 348.1569); IR film (cm^{-1}): 3400 (OH), 1780 (C=O, γ-lactone), 1740 (C=O), ^{1}H-NMR (400 MHz, $CDCl_3$): 4.89 (1H, *dd*, *J* = 11.0, 4.2 Hz, H-1), 1.97 (1H, *m*, H-2α), 2.24 (1H, *m*, H-2β), 2.59 (1H, *m*, H-3α), 2.25 (1H, *m*, H-3β), 4.75 (1H, *d*, *J* = 10.0 Hz, H-5), 5.09 (1H, *d*, *J* = 10.0, 10.0 Hz, H-6), 3.01 (1H, *m*, H-7), 5.16 (1H, *dd*, *J* = 10.0, 9.0 Hz, H-8), 2.53 (1H, *d*, *J* = 13.1 Hz, H-9α), 2.44 (1H, *dd*, *J* = 13.1, 10.0 Hz, H-9β), 6.37 (1H, *d*, *J* = 3.6 Hz, H-13α), 6.10 (1H, *d*, *J* = 3.6 Hz, H-13β), 1.49 (3H, *s*, H-14), 4.30 (1H, br *d*, H-15α), 4.08 (1H, br *d*, H-15β), 3.11 (1H, *d*, *J* = 6.2 Hz, H-3'α), 2.80 (1H, *d*, *J* = 6.2 Hz, H-3'β), 1.57 (1H, *s*, H-4). ^{13}C-NMR (100 MHz, $CDCl_3$): 127.1 (C-1), 28.3 (C-2), 31.6 (C-3), 143.1 (C-4), 127.5 (C-5), 76.1 (C-6), 50.6 (C-7), 75.3 (C-8), 40.8 (C-9), 133.7 (C-10), 137.5 (C-11), 171.0 (C-12), 123.0 (C-13), 16.7 (C-14), 61.9 (C-15), 168.3 (C-1'), 55.2 (C-2'), 55.1 (C-3'), 19.0 (C-4').

Hydroxytyrosol (**3**): Brownish oil; m.p. 56-58^{o}C; HR-ESMS *m/z*: 154.0630 $[M]^{+}$ (calc. for $C_8H_{10}O_3$, 154.0632); IR film (cm^{-1}): 3375 (OH), 2946 (CH), 1606 (C=C, aromatic); ^{1}H-NMR (CD_3OD, 400 MHz): 6.59 (1H, *d*, *J* = 2.0 Hz, H-2), 6.67 (1H, *d*, *J* = 8.1 Hz, H-5), 6.52 (1H, *dd*, *J* = 8.1, 2.0 Hz, H-6), 2.66 (2H, *t*, *J* = 7.2 Hz, H-1'), 3.67 (2H, *t*, *J* = 7.2 Hz, H-2'); ^{13}C-NMR (CD_3OD, 100 MHz): 130.6 (C-1), 115.1 (C-2), 145.0 (C-3), 143.4 (C-4), 115.9 (C-5), 120.0 (C-6),38.5 (C-1'), 63.4 (C-2').

Dihydroxysinapic acid (**4**): Yellowish solid; m.p. 189-191^{o}C; HR-ESMS *m/z*: 226.0841 $[M]^{+}$ (calc. for $C_{11}H_{14}O_5$); IR film (cm^{-1}): 3450 (OH), 2915 (CH),1780 (C=O); ^{1}H-NMR ($CDCl_3$, 400 MHz): 2.58 (2H, *t*, *J* = 7.4 Hz, H-2), 2.86 (2H, *t*, *J* = 7.4 Hz, H-3), 6.34 (2H, *s*, H-2′/H-6), 3.01 (6H, *s*, 2 x OCH_3), 5.5 (1H, *s*, 4-OH), 11.01 (1H, *s*, COOH); ^{13}C-NMR ($CDCl_3$, 100 MHz): 177.5 (C-1), 35.9 (C-2), 31.1 (C-3), 131.2 (C-1), 105.1 (C-2′/C-6), 147.0 (C-3′/C-5), 133.2 (C-4), 56.3 (2 x OCH_3).

6''-O-acetyl homoplantaginin (**5**): yellowish powder; m.p. 258-260^{o}C; IR film (cm^{-1}); 3350 (OH), 1655 (C=O), 1593 (C=C aromatics); HR-ESMS *m/z*: 505.1330 $[M+H]^{+}$ (calc. for $C_{24}H_{25}O_{12}$, 505.1331); ^{1}H-NMR (DMSO-d_6, 400 Hz): 6.86 (1H, *s*, H-3), 6.97 (1H, *s*, H-8), 7.94 (2H, *d*, *J* = 8.6 Hz, H-2′/H-6′), 6.89 (2H, *d*, *J* = 8.6 Hz, H-3′/H-5′), 5.14 (H, *d*, *J* = 7.3 Hz, H-1''), 4.35 (1H, *d*, *J* = 10.1 Hz, H-6''α), 4.10 (1H, *dd*, *J* = 11.8, 7.3 Hz, H-6''β), 3.47-3.25 (4H, m, H-2'', H-3'', H-4'', H-5'')1.98 (3H, *s*, H-2''), 12.94 (1H, *s*, 5-OH), 3.79 (3H, *s*, OCH_3); ^{13}C-NMR (DMSO-d_6, 100 Hz): 164.5 (C-2), 102.4 (C-3), 182.0 (C-4), 152.4 (C-5), 131.9 (C-6), 157.3 (C-7), 95.0 (C-8), 153.0 (C-9), 106.0 (C-10), 121.2 (C-1′), 128.7 (C-2′,C-6′), 117.3 (C-3′, C-5′), 162.8 (C-4′), 100.1 (C-1''), 73.3 (C-2''), 77.3 (C-3''), 70.2 (C-4''), 76.6 (C-5''), 63.6 (C-6''), 171.4 (C-1'''), 20.6 (C-2'''), 60.4 (OCH_3).

Onopordin (**6**): yellowish solid, HR-ESMS *m/z*: 316.0580 $[M]^{+}$ (calc. for $C_{16}H_{12}O_7$, 316.0581); ^{1}H-NMR ($CDCl_3$, 400 MHz): 6.61 (1H, *s*, H-3), 6.30 (1H, *s*, H-6), 7.40 (1H, *d*, *J* = 2.0 Hz, H-2′), 6.89 (1H, *d*, *J* = 8.2 Hz, H-5′). 7.43(1H, *dd*, *J* = 8.2, 2.0 Hz, H-6′), 3.85 (3H, *s*, OCH_3), 12.15 (1H, *s*, 5-OH); ^{13}C-NMR: 162.9 (C-2), 101.7 (C-3), 180.4 (C-4), 155.9 (C-5), 98.9 (C-6), 157.3 (C-7), 127.1 (C-8), 148.7 (C-9), 103.5 (C-10), 121.0 (C-1′), 113.5 (C-2′), 145.1 (C-3′), 149.9 (C-4′), 116.3 (C-5′), 118.7 (C-6′), 61.0 (OCH_3).

3'-Hydroxygenkwanin (**7**): yellowish solid, HR-ESMS *m/z*: 284.0687 $[M]^{+}$ (calc. for $C_{16}H_{12}O_5$, 284.0683); ^{1}H-NMR (DMSO-d_6, 400 MHz) 6.65 (1H, *s*,H-3), 6.29 (1H, *d*, *J* = 1.9 Hz, H-6), 6.61 (1H, *d*, *J* = 1.9 Hz, H-8), 7.44 d (1H, *d*, *J* = 2.0 Hz, H-2′), 6.82 (1H, *d*, *J* = 8.7 Hz, H-5′), 7.41 (1H, *dd*, *J* = 8.7, 2.0 Hz, H-6′), 3.84 (3H, *s*, OCH_3); 12.95 (*s*, 5-OH); ^{13}C-NMR (DMSO-d_6, 100 MHz): 164.5 (C-2), 103.7 (C-3), 181.6 (C-4), 160.8 (C-5), 98.7 (C-6), 165.4 (C-7), 93.0 (C-8), 157.2 (C-9), 104.0 (C-10), 121.3 (C-1′), 129.0 (C-2′), 121.0 (C-3′), 161.0 (C-4′), 129.7 (C-5′), 128.5 (C-6′), 56.7 (OCH_3).

Luteolin (**8**): yellowish solid; HR-ESMS *m/z*: 286.0475 $[M]^+$ (calc. $C_{15}H_{10}O_6$, 286.0471); IR film (cm^{-1}) 3600 (OH), 2993 (CH), 1655 (C=O); ^{1}H-NMR (DMSO-d_6, 400 MHz): 6.65 (1H, *s*, H-3), 6.38 (2H, *d*, *J* = 2.0 Hz, H-6), 6.66 (1H, *d*, *J* = 2.0 Hz, H-8), 7.40 (1H, *d*, *J* = 2.2 Hz, H-2), 6.91 (1H, *d*, *J* = 9.1 Hz, H-5), 7.42 (1H, *dd*, *J* = 9.1, 2.2 Hz, H-6), 12.90 (1H, *s*, 5-OH); ^{13}C-NMR (DMSO-d_6, 400 MHz) 164.3 (C-2), 103.4 (C-3), 180.6 (C-4), 159.4 (C-5), 97.9 (C-6), 162.5 (C-7), 92.5 (C-8), 157.2 (C-9), 105.0 (C-10), 121.7 (C-1), 114.0 (C-2), 145.3 (C-3), 161.9 (C-4), 115.7 (C-5), 118.0 (C-6).

Quercetin-3-O-glucoside-7,3',4'-trimethyl ether (**9**): yellowish solid; HR-ESMS *m/z*: 511.3457 $[M+H]^+$ (calc. for $C_{24}H_{31}O_{12}$, 511.3453); ^{1}H-NMR (CD_3OD, 400 MHz): 6.61 (1H, *d*, *J* = 1.9 Hz, H-6), 6.33 (1H, *d*, *J* = 1.9 Hz, H-8), 6.77 (1H, *d*, *J* = 2.0 Hz, H-2), 6.50 (1H, *d*, *J* = 8.9 Hz, H-5), 7.41 (1H, *dd*, *J* = 8.9, 2.0 Hz, H-6), 4.53 (1H, *d*, *J* = 6.5 Hz, H-1'), 3.25 (1H, *m*, H-2'), 3.22 (1H, *m*, H-3'), 3.16 (1H, *m*, H-4'), 3.22 (1H, *m*, H-5'), 3.47 (1H, *m*, H-6'α), 3.55 (1H, *m*, H-6'β), 3.92 (3H, *s*, 8-OCH_3), 3.81 (3H, *s*, 3'-OCH_3), 3.84 (3H, *s*, 4'-OCH_3); ^{13}C-NMR (CD_3OD, 100 MHz): 156.4 (C-2), 133.6 (C-3), 177.0 (C-4), 161.7 (C-5), 101.2 (C-6), 164.6 (C-7), 93.5 (C-8), 154.9 (C-9), 105.3 (C-10), 121.5 (C-1), 117.8 (C-2), 149.0 (C-3), 147.2 (C-4), 116.7 (C-5), 118.5 (C-6), 101.7 (C-1'), 74.4 (C-2'), 75.1 (C-3'), 70.9 (C-4'), 78.2 (C-5'), 62.9 (C-6'), 56.9 (3H, *s*, 8-OCH_3), 56.5 (3H, *s*, 3'-OCH_3), 56.7 (3H, *s*, 4'-OCH_3).

Quercetin-3-O-D-galactoside (**10**): yellowish solid; HR-ESMS *m/z*: 465.1685 $[M+H]^+$(calc. for $C_{21}H_{21}O_{12}$, 465.1684); IR film (cm^{-1}): 3395 (OH), 1789 (C=O), 1655 (C=C, aromatic); ^{1}H-NMR (CD_3OD, 400 MHz): 6.69 (1H, *d*, *J* = 2.0 Hz, H-6), 6.64 (1H, *d*, *J* = 2.0 Hz, H-8), 8.44 (1H, *d*, *J* = 1.8 Hz, H-2), 7.18 (1H, *d*, *J* = 8.9 Hz, H-5), 8.12 (1H, *d*, *J* = 8.9, 1.8 Hz, H-6), 5.89 (1H, *d*, *J* = 7.66 Hz, H-1'), 4.8 (1H, *m*, H-2'), 4.23 (1H, *m*, H-3'), 4.30 (1H, *m*, H-4'), 4.17 (1H, *m*, H-5'), 4.32 (1H, *m*, H-6'α), 4.56 (1H, *m*, H-6'β); ^{13}C-NMR (CD_3OD, 100 MHz): 157.8 (C-2), 134.0 (C-3), 178.4 (C-4), 165.7 (C-5), 99.1 (C-6), 165.0 (C-7), 94.8 (C-8), 157.3 (C-9), 104.7 (C-10), 122.9 (C-1), 117.1 (C-2), 146.4 (C-3), 150.3 (C-4), 115.0 (C-5), 121.6 (C-6), 1005.2 (C-1'), 73.3 (C-2'), 75.0 (C-3'), 75.1 (C-4'), 77.3 (C-5'), 60.9 (C-6').

Eupafolin (**11**): yellowish solid; HR-ESMS *m/z*: 317.0583 $[M+H]^+$ (calc. for $C_{16}H_{13}O_7$, 317.0581); ^{1}H-NMR (CD_3OD, 400 MHz): 6.55 (1H, *s*, H-3), 6.57 (1H, *s*, H-8), 7.37 (1H, *s*, H-2), 6.91 (1H, *d*, *J* = 8.3 Hz, H-5), 7.37 (1H, *d*, *J* = 8.3 Hz, H-6), 3.87 (1H, *s*, OCH_3); ^{13}C-NMR (CD_3OD, 100 MHz): 166.0 (C-2), 103.8 (C-3), 184.2 (C-4), 154.7 (C-5), 132.9 (C-6), 153.9 (C-7), 95.2 (C-8), 158.8 (C-9), 105.6 (C-10), 123.9 (C-1), 114.5 (C-2) 147.0 (C-3), 151.1 (C-4), 116.7 (C-5), 120.3 (C-6); 60.9 (OCH_3).

Quercetin-7-O-galactopyranoside (**12**): orange solid; HR-ESMS *m/z*: 464.1021 $[M]^+$ (calc. for $C_{21}H_{20}O_{12}$, 464.1018); ^{1}H-NMR (CD_3OD, 400 MHz): 6.22 (1H, *d*, *J* = 2.0 Hz, H-6), 6.41 (1H, *d*, *J* = 2.0 Hz, H-8), 7.87 (1H, *d*, *J* = 2.0 Hz, H-2), 6.86 (1H, *d*, *J* = 8.2 Hz, H-5), 7.60 (1H, *dd*, *J* = 8.2, 2.0 Hz, H-6), 5.19 (1H, *d*, *J* = 7.5 Hz, H-1'), 3.42-3.76 (4H, *m*, H-2'', H-3'', H-4'', H-5''), 3.84 (1H, *m*, H-6'α), 4.24 (1H, *m*, H-6'β); ^{13}C-NMR (CD_3OD, 100 MHz): 150.1 (C-2), 136.0 (C-3), 177.4 (C-4), 163.3 (C-5), 100.2 (C-6), 164.9 (C-7), 95.1 (C-8), 158.1 (C-9), 105.3 (C-10), 126.9 (C-1), 115.9 (C-2), 145.5 (C-3), 148.0 (C-4), 116.9 (C-5), 123.0 (C-6), 105.4 (C-1'), 72.9 (C-2'), 75.2 (C-3''), 70.2 (C-4''), 77.3 (C-5''), 61.9 (C-6'').

Leishmanicidal detection assay

The colorimetric method - XXT was used for the assay of antileishmanial activities of metabolites **1** and **2**. It is described in the literature.[35]

Acknowledgements

The authors are grateful to Swedish International Development Agency for grants. We thank Sofia Essén from Lund University for the help with the HRES-MS spectra and Mr. Francisco Mapanga from Eduardo Mondlane University for identifying the plant.

References

1. S.O. Bandeira; Gaspar, F.; Pagula, F. P. *Pharm. Biol.* **2001**, *39* (1), 70–73.
2. Bruschi, P.; Morganti, M.; Mancini, M.; Signorini, M. A. *J. Ethnopharmacol.* **2011**, *138* (2), 543–563.
3. Hostettmann, K.; Marston, A.; Ndjoko, K.; Wolfender, J. L. *Curr. Org. Chem.* **2000**, *4* (10), 973–1010.
4. Maroyi, A. *J. Ethnobiol. Ethnomed.* **2013**, *9* (1), 31–48.
5. Bohlmann, F.; Zdero, C. *Phytochemistry* **1982**, *21* (3), 647–651.
6. Zdero, C.; Bohlmann, F. *Phytochemistry* **1987**, *26* (9), 2597–2601.
7. Zdero, C.; Bohlmann, F.; Wasshausen, D. C. *Phytochemistry* **1991**, *30* (11), 3810–3811.
8. Chaturvedula, V. S. P.; Schilling, J. K.; Miller, J. S.; Andriantsiferana, R.; Rasamison, V. E.; Kingston, D. G. I. *J. Nat. Prod.* **2002**, *65* (8), 1222–1224.
9. Vieira, P. C.; Himejima, M.; Kubo, I. *J. Nat. Prod.* **1991**, *45* (2), 416–420.
10. Beentje, H. T. *Kew Bull.* **2000**, *55* (1), 1–41.
11. Deutschländer, M. S.; Lall, N.; van de Venter, M. *Pharm. Biol.* **2009**, *47* (4), 348–365.
12. Jansen, P. C. M.; Mendes, O. *Plantas Medicinais - Seu Uso Tradicional Em Moçambique*, T4 ed.; Partido, I. do, Ed.; Ministério da Saúde Gabinete de estudos de Medicina Tradicional: Maputo, Mozambique, 1991.
13. Mellem, J. *African J. Pharm. Pharmacol.* **2013**, *7* (12), 636–642.
14. Lonergan, G.; Routsi, E.; Georgiardis, T.; Agelis, G.; Hondrelis, J.; Matsoukas, J.; Larsen, L. K.; Caplan, F. R. *J. Nat. Prod.* **1992**, *55* (5), 225–228.
15. Bach, S. M.; Fortuna, M. A.; Attarian, R.; Trimarco, J. T. de; Catalán, C. A. N.; Av-Gay, Y.; Bach, H. *Nat. Prod. Comm.* **2011**, *6* (11), 163–166.
16. Formisano, C.; Sanna, C.; Ballero, M.; Chianese, G.; Sirignano, C.; Rigano, D.; Millán, E.; Muñoz, E.; Taglialatela-scafati, O. *Fitoterapia* **2017**, *116*, 61–65.
17. Onguéné, A.; Ntie-Kang, P.; Lifongo, F. *Malar. J.* **2013**, *12* (1), 4–25.
18. Novello, C. R.; Teresinha, I.; Schuquel, A.; Sakuragui, C. M.; Luftmann, H.; Ueda-Nakamura, T.; Nakamura, C. V.; Mello, J. C. P. de. *Molecules* **2012**, *17*, 1852–1859.
19. Zimmermann, S.; Fouché, G.; De Mieri, M.; Yoshimoto, Y.; Usuki, T.; Nthambeleni, R.; Parkinson, C. J.; Van Der Westhuyzen, C.; KaiserB, M.; Hamburger, M.; Adams, M. *Molecules* **2014**, *19* (3), 3523–3538.
20. Park, C. H.; Kim, K. H.; Lee, I. K.; Lee, S. Y.; Choi, S. U.; Lee, J. H.; Lee, K. R. *Arch. Pharm. Res.* **2011**, *34* (8), 1289–1296.
21. Lee, Y.-M.; Kwon, M.-J.; Kim, J.-K.; Suh, H.; Choi, J.-S.; Song, Y.-O. *Korean J. Food Sci. Technol.* **2004**, *36* (1), 129–133.
22. Jin, M. R.; Xu, H.; Duan, C. H.; Chou, G. X. *Nat. Prod. Res.* **2015**, *29* (14), 1315–1322.
23. Wollenweber, E.; Dörr, M.; Roitman, J. N.; Schilling, E. *Z. Naturforsch.* **1995**, *50c*, 588–590.

24. Park, S.; Cui, X.; Ahn, D.; Lee, E. B.; Cha, D. S.; Jeon, H.; Zee, O. P.; Kim, Y.; Kim, D. K. *Nat. Prod. Sci.* **2014**, *20* (2), 80–85.
25. Miyazawa, M.; Hisama, M. *Biosci. Biotechnol. Biochem.* **2003**, *67* (10), 2091–2099.
26. Al-Youssef, H.; Hassan, W. H. B. *Biomed. Pharmacol. J.* **2012**, *5* (1), 9–18.
27. Kunert, O.; Haslinger, E.; Schmid, M. G.; Reiner, J.; Bucar, F.; Mulatu, E.; Abebe, D.; Debella, A. *Monatshefte für Chemie* **2000**, *131* (2), 195–204.
28. Wei, X.; Huang, H.; Wu, P.; Cao, H.; Ye, W. *Biochem. Syst. Ecol.* **2004**, *32* (11), 1091–1096.
29. Güvenalp, Z.; Demirezer, L. O. *Turk. J. Chem.* **2005**, *29*, 163–169.
30. Sinisi, A.; Millán, E.; Abay, S. M.; Habluetzel, A.; Appendino, G.; Muñoz, E.; Taglialatela-Scafati, O. *J. Nat. Prod.* **2015**, *24*, 1618–1623.
31. Chadwick, M.; Trewin, H.; Gawthrop, F.; Wagstaff, C. *Int. J. Mol. Sci.* **2013**, *14* (6), 12780–12805.
32. Majdi, M.; Liu, Q.; Karimzadeh, G.; Malboobi, M. A.; Beekwilder, J.; Cankar, K.; Vos, R. De; Todorović, S.; Simonović, A.; Bouwmeester, H. *Phytochemistry* **2011**, *72* (14–15), 1739–1750.
33. Chan-Bacab, M. J.; Peña-rodríguez, L. M. *Nat. Prod. Rep.* **2001**, *18*, 674–688.
34. Alain Fournet, Victoria Muñoz, François Roblot, Reynald Hocquemiller, A. C. and J.-C. G. *Phytother. Res.* **1993**, *7*, 111–115.
35. Capusiri, E. S.; Pinell, G. R.; Huallpara, J. C. T.; Turba, A. G. *Biofabro* **2008**, *16*, 21–27.

Paper IV

Leishmanial Activity of Quinone Methide Triterpenoid from the roots of *Senna spectabilis*

Julião A. Monjane[1,2], Diandra Capusiri[3], Alberto Giménez[3], Anders Sundin[2], and Olov Sterner[2]

[1]Chemistry Department, Eduardo Mondlane University, P.O. Box 257, Maputo, Mozambique

[2]Centre for Analysis and Synthesis, Lund University, P.O. Box 22100, Lund, Sweden

[3]Instituto de Investigaciones Fármaco Bioquímicos, San Andrés University, La Paz, Bolivia

Abstract

From an extract of the roots of *Senna spectabilis* (var. *excelsa*) eight known metabolites including the quinone methide triterpenoid 17-(methoxycarbonyl)-28-nor-isoiguesterin (**1**) and friedelin (**2**), a putative biosynthetic precursor of this class of metabolites, were isolated. The structures of the isolated metabolites were established by high resolution NMR and MS experiments, and confirmed by comparison of their experimental spectroscopic and physical data with data previously reported in the literature. The 2D structure and relative configuration of **1** were initially determined via analysis of NOESY data and confirmed by quantum mechanics-based ^{13}C NMR chemical shift calculations. **1** showed a potent antileishmanial activity against *Leishmania amazonensis* and *L. braziliensis*, with the IC_{50} values 4.3 and 2.8 μM, respectively, comparable with the positive control miltefosine (IC_{50} values 5.1 and 4.9 μM, respectively). This is the first example of the occurrence of a quinone methide triterpenoid in a species belonging to the Fabaceae family is reported.

Keywords: *Senna spectabilis*, Fabaceae, Quinone methide triterpenoid, *Leishmaniosis*, Relative configuration

Introduction

Protozoan parasites are among the most common pathogens in the world. They are recognized as the causative agents of some of the most serious tropical diseases in human beings as well as domestic animals. Malaria, amoebiasis, trypanosomiasis, and leishmaniosis are diseases caused by protozoan parasites that affect approximately 25 % of the world's population, mostly in developing countries. According to WHO, malaria, African trypanosomiasis, Chagas disease, and

leishmaniosis are considered the most important tropical diseases.[1–3] Leishmaniosis is a group of tropical diseases caused by a number of species of protozoan belonging to the genus of *Leishmania*. This ailment affects around 12 million people in 80 countries, and it is estimated that there is about 2 to 3 million new cases each year. In addition, leishmaniosis is considered one of the most neglected diseases.[1]

The first chemotherapy to treat leishmaniosis was based on the use of toxic heavy metals, particularly, compounds containing antimony. However, the emergence of resistant strains has limited their usefulness. The use of pentamidine isethionate, amphotericin B, miltefosine, and paramomycin as alternatives for the treatment of leishmaniosis has been limited due to their toxicity as well as the relatively high prices of these remedies.[4–6] There are a number of alternative products being developed, however, up to date none of these has been demonstrated to be fully effective against *Leishmania* parasites.[1]

In recent years, screenings for secondary metabolites of medicinal plants used for the treatment of leishmaniosis have been carried out. These studies have confirmed the importance of many plant species as potential sources for the isolation of novel compounds with antileishmanial or immunostimulant activities, as highlighted in a review about plant secondary metabolites with antileishmanial activity.[1] The drug resistance developed by *Leishmania* requires evaluation of alternatives to the drugs currently used, and their affordability and toxicity is also a problem.[7] In the continuing search for new bioactive secondary metabolites possessing antileishmanial activity from the plants of Mozambique, a root extract of *Senna spectabilis* was investigated.

Senna, or *Cassia*, is considered one of two the most representative genus of the *Fabaceae* family. The genus is widely distributed in tropical and subtropical areas of Africa, Asia, Australia, and South America. Several *Senna* species are used in the traditional medicine to treat a wide range of ailments.[8,9] *Senna spectabilis* var. *excelsa* (sin *Cassia spectabilis*) is a tree 7 to 10 m high.[10] The plant has been used traditionally in Mozambique for the treatment of diarrhoea, stomachache, tuberculosis, and asthma .[11]

Previous phytochemical studies of *S. spectabilis* led to the isolation and characterization of a number of metabolites, including alkaloids, in particular piperidine alkaloids, triterpenoids, and phenolic compounds.[9,12–14] The piperidine alkaloids present in the flowers were found to possess antileishmanial activity.[9,15] In this paper, we wish to report the isolation, the relative configuration, and the antileishmanial activity of a quinine methide triterpenoid, 17-(methoxycarbonyl)-28-nor-isoiguesterin (**1**), along with a putative biosynthetic precursor of this class of metabolites, friedelin (**2**), together with six other metabolites isolated from an extract of the roots of this plants. Despite the fact that **1** is a known metabolite previously isolated from *Salacia kraussii* and reported to possess antimalarial

activity,[16] this is the first report describing the isolation of a quinone methide triterpenoid from a species of the family Fabaceae.

Results and discussions

An extract of the roots of *S. spectabilis* was subjected to several chromatographic fractionations to yield eight known metabolites. Their chemical structures were determined by high resolution NMR and MS experiments, and confirmed by comparing the spectroscopic data with those previously reported. The metabolites are **1**,[16] friedelin (**2**),[17] octandronic acid (**3**),[18] *β*-amyrin (**4**),[19] ursolic acid (**5**),[20] lupeol (**6**),[21] chrysophanol (**7**),[22] and scopoletin (**8**).[23] Their structures are shown in Figure 1.

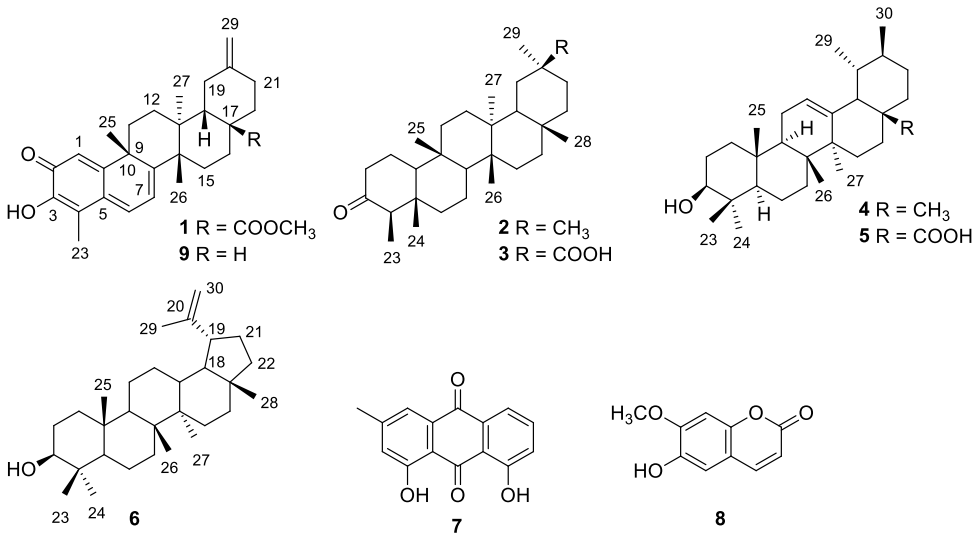


Figure 1 Chemical structures of metabolites isolated from *S. spectabilis* and isoiguesterin (**9**)

The spectroscopy data recorded for metabolite **1** were found to be very similar to those published in the literature.[16] However, despite the similarities, the relative configuration of **1** was found to be different from the reported structure, by careful analysis of NOESY data. Thus, the relative configurations of the stereogenic centers C-9, C-13, C-14, and C-18 were determined by the NOESY correlations observed between H_3-25, H_3-26, H_3-27, and H-18, as depicted in the Figure 2. The NMR-based initial structural assignments of **1** were reaffirmed by the comparison of the experimental and computed ^{13}C NMR chemical shift values. The Jaguar 7.9 software was used to optimize the molecular geometry and calculate the NMR

deshielding properties, the Isotropic Magnetic Shielding (IMS) constants at the Density Functional Theory (DFT), mPW1PW91 level of theory and 6-31G** basis set. The calculated chemical shift values for the ^{13}C nuclei (Table 1) were obtained from the equation (1). The predicted chemical shift values at the above referred basis set for **1** were plotted with the experimental data (see Figure 3). The statistical analysis of the experimental and calculated chemical shift values generated a slope with r^2 0.993, verifying the 2D and relative configurations assignments at the C-9, C-13, C-14, and C-18 stereogenic centers. These conclusions were also supported by plotting the scaled ^{13}C chemical shift values obtained by the linear fit of the calculated shift values, versus experimental data (see Figure 4). The slope obtained with r^2 0.923 supported the relative configurations indicated above.

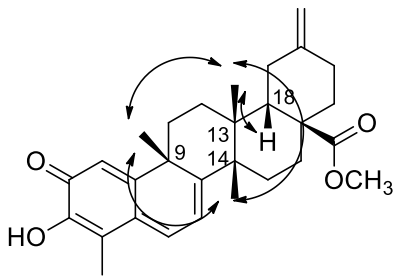


Figure 2 Corrected structure of **1** and key NOESY correlations.

1 is a quinone methide triterpenoid structurally related to isoiguesterin (**9**). They are secondary metabolites that occur mainly in the higher order plant families such as Celastraceae and Hyppocrateaceae.[24,25] This class of metabolites display a wide range of biological activities, including anti-inflammatory, antioxidant, antifungal, antitrypanosomal, antimicrobial, and antitumor properties.[4] The biosynthesis of quinone methide triterpenoids has not yet been investigated, only a few preliminary studies have been reported. The mevalonate pathway in *Peritassa lavigata* is responsible for the synthesis of the quinone methide scaffold, via friedelin, which is considered as primary precursor of maytenin and pristimerin.[26,27] **1** could share the same biosynthetic pathway as **2** by the incorporation of a methyl group at the carboxylic site after the oxidation of Me-28 to a carboxylic acid.

The antileishmanial activity of **1** was assayed against *Leishmania amazonensis* and *L. braziliensis*. The results showed that **1** has the IC_{50} values 4.3 and 2.8 μM, respectively, and is more potent towards both strains tested than miltefosine (IC_{50} values 5.1 and 4.9 μM, respectively) which is a current drug used for the treatment for leishmaniosis. The quinone methide triterpenoid, 20-*epi*-isoiguesterinol, isolated from *S. madagascariensis* together with **9**, was found to be more potent against *L. donovani* and *L. mexicana* compared to amphotericin B.[25] Thus the quinone

methide triterpenoids show a great potential for future development as drugs for the treatment of *Leishmania*.

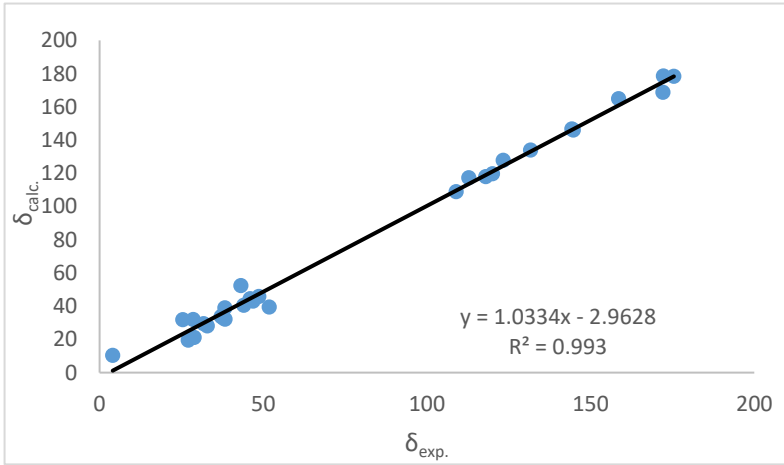


Figure 3 Plot of calculated chemical shifts vs experimental

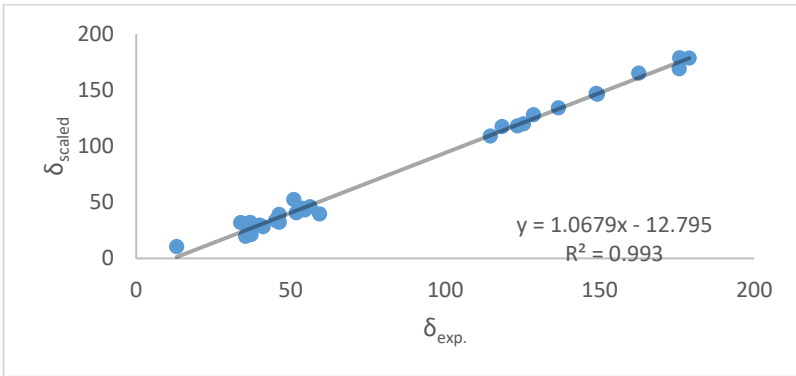


Figure 4 Plot of scaled chemical shifts vs experimental

Table 1 - Chemical shifts – the calculated and experimental

Carbon	IMS (σ)	$\delta_{calc.}$	δ_{scaled}	$\delta_{exp.}$	Carbon	IMS (σ)	$\delta_{calc.}$	δ_{scaled}	$\delta_{exp.}$
1	76.2	120.0	125.3	119.7	16	170.7	25.4	33.8	31.8
2	23.9	172.2	175.8	178.5	17	147.5	48.6	56.2	45.8
3	51.9	144.3	148.8	146.7	18	144.3	51.8	59.3	39.4
4	83.3	112.8	118.3	117.3	19	157.8	38.3	46.3	32.1
5	72.8	123.3	128.5	127.8	20	51.4	144.7	149.2	146
6	64.5	131.7	136.6	133.9	21	167.6	28.6	36.8	31.9
7	78.6	118.01	123.4	117.9	22	158.4	37.7	45.7	32.9
8	24.1	172.1	175.7	168.7	23	192.6	4.0	13.0	10.3
9	149.3	46.9	54.5	43.0	25	157.8	38.2	46.2	39
10	37.6	158.5	162.6	164.9	26	167.5	28.9	37.1	21.1
11	158.9	37.1	45.1	33.8	27	169.8	27.0	35.4	19.5
12	164.4	31.7	39.9	29.4	28	20.7	175.4	178.9	178.3
13	152.1	44.4	51.8	40.5	29	87.2	108.9	114.6	108.9
14	150.2	46.0	53.7	44.4	30	152.8	43.1	51.0	52.3
15	163.3	32.3	41.0	28.1					

Experimental

General methods

1D and 2D NMR spectra were recorded at room temperature with a Bruker Avance II 400 spectrometer. The chemical shifts (δ) are reported in ppm relative to solvent residual signals (δ_H 7.26 and δ_C 77.0 for $CDCl_3$). The HRESI-MS data were recorded with a Waters Q-TOF micromass spectrometer, using H_3PO_4 for calibration and as internal standard. The IR spectra data were recorded on a Bruker Alpha-P ART-IR spectrometer. The melting point measurements were carried out on Gallenkamp instrument. The optical rotations were measured by a Perkin-Elmer Model 341 Polarimeter (T = 20 ^oC and D = 589 nm). The column chromatography (CC) was performed using silica gel 60 (230-400 mesh, Merck) and gel permeation on Sephadex LH-20 (GE-Healthcare). Analytical TLC plates were visualized under a UV lamp at 254 nm and by spraying with vanillin followed by heating. All solvents used were analytical grade.

Plant material

The roots of *S. spectabilis* were collected in Moamba District, Maputo Province in Mozambique in June 2013, and were dried in the shade until use. A voucher specimen with number C/157 is kept in the herbarium of Departamento de Ciências Biológicas at Eduardo Mondlane University (Maputo, Mozambique).

Extraction and isolation

The air-dried roots (300 g) of *S. spectabilis* were grinded into a fine power. The powder was extracted sequentially with heptane, chloroform, EtOAc, and mixture of MeOH:H_2O (7:3). The chloroform extract (500 mg) was fractionated by column chromatography using a mixture of heptane:ethylacetate (2:1 to 1:2) to yield three main fractions F1, F2, and F3. F1 (46.6 mg) was subjected to Sephadex LH-20 MeOH:$CHCl_3$ (3:7) and three main fractions (F1A, F1B, and F1C) were collected. F1A (26.3 mg) was then subjected to column chromatography over silica gel in a mixture of heptane:EtOAc (2:1) to yield compound **1** (12.5 mg) as redish solid. F2 (36 mg) was fractionated by silica gel column chromatography (eluted with heptane:EtOAc (2:1)) which led to the isolation of *β*-amyrin (**4**) (7.4 mg) and lupeol (**6**, 6.1 mg). F3 yielded pure octandronic acid (**3**, 15.3 mg)

4.0 g of the EtOAc extract were dissolved in a mixture of MeOH:H_2O (9:1) and then extracted with $CHCl_3$. The chloroform soluble part was concentrated and fractionated by CC eluted with heptane:EtOAc (4:1 to 1:1) to yield friedelin (**2**, 24.6 mg) and ursolic acid (**5**, 14.2 mg). One of subfraction was subjected to PTLC using the eluent solvent heptane:EtOAc (4:1) to yield, chrysophanol (**7**, 7.2 mg) and scopoletin (**8**, 5.2 mg).

17-(Methoxycarbonyl)-28-nor-isoiguesterin (**1**): redish solid; 12.5 mg; m.p 193 - 195; $[\alpha]_D^{25}$ = +84.4 (*c* 1.0 $CHCl_3$); IR (film): 3367, 2947, 2871, 1724, 1596, 1439 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz): *δ* 7.04 (1H, *br s*, OH), 7.00 (1H, *d*, *J* = 6.8 Hz, H-6), 6.54 (1H, *s*, H-1), 6.30 (1H, *d*, *J* = 6.8 Hz, H-7), 4.61 (2H, *s*, H-29), 3.75 (3H, *s*, H-30), 2.62 (1H, *d*, *J* = 6.7 Hz, H-18), 2.49 (1H, *d*, *J* = 15.6 Hz, H-19), 2.30 - 2.06 (6H, *m*, H-11, H-16, H-19, H-21, H-22), 2.21 (3H, *s*, H-23), 2.04-1.94 (2H, *m*, H-11, H-22), 1.90-1.84 (2H, *m*, H-12), 1.82-1.72 (1H, *m*, H-15), 1.72-1.54 (2H, *m*, H-15, H-16), 1.48 (3H, *s*, H-25), 1.36 (3H, *s*, H-26), 0.74 (3H, *s*, H-27); ^{13}C NMR ($CDCl_3$, 100 MHz): *δ* 119.7 (*d*, C-1); 178.5 (*s*, C-2), 146.7 (*s*, C-3), 117.3 (*s*, C-4), 127.8 (*s*, C-5), 133.9 (*d*, C-6), 117.9 (*d*, C-7), 168.7 (*s*, C-8), 43.0 (*s*, C-9), 164.9 (*s*, C-10), 33.8 (*t*, C-11), 29.4 (*t*, C-12), 40.5 (*s*, C-13), 44.4 (*s*, C-14), 28.1 (*t*, C-15), 31.8 (*t*, C-16), 45.8 (*s*, C-17), 39.4 (*d*, C-18), 32.1 (*t*, C-19), 146.0 (*s*, C-20), 31.9 (*t*, C-21), 32.9 (*t*, C-22), 10.3 (*q*, C-23), 39.0 (*q*, C-25), 21.1 (*q*, C-26), 19.5 (*q*, C-27), 178.3 (*s*, C-28), 108.9 (*d*, C-29), 52.3 (*q*, C-30); HRESMS: m/z 449.2692 $[M+H]^+$ (calc. for $C_{29}H_{37}O_4$).

Friedelin (**2**): colorless solid (24.6 mg); ^{1}H-NMR (400 MHz; $CDCl_3$) δ_H 0.71 (3H, *s*, Me-24), 0.87 (3H, *s*, Me-25), 0.88 (3H, *d*, *J* = 6.1 Hz, Me-23), 0.93 (3H, *s*, Me-29), 0.95 (1H, *m*, H-22α), 0.97 (6H, *s*, Me-26, Me-30), 1.02 (3H, *s*, Me-27), 1.16 (3H, *s*, Me-28), 1.28 (1H, *m*, H-6α), 1.33 (1H, *m*, H-7α), 1.35 (1H, *m*, H-8), 1.42 (1H, *m*, H-7β), 1.48 (1H, *m*, H-22β), 1.54 (2H, *m*, H-10), 1.52 (1H, *m*, H-18), 1.68 (1H, *m*, H-1α), 1.74 (1H, *m*, H-6β), 1.99 (1H, *m*, H-1β), 2.22 (1H, *d*, *J* = 6.1 Hz, H-4), 2.23 (2H, *m*, H-2α), 2.39 (1H, *m*, H-2β); ^{13}C-NMR (100 MHz, $CDCl_3$) δ_C 22.3 (C-1), 41.2 (C-2), 210.1 (C-3), 59.0 (C-4), 42.1 (C-5), 41.5 (C-6), 17.9 (C-7), 53.1 (C-8), 37.6 (C-9), 60.2 (C-10), 35.6 (C-11), 32.4 (C-12), 38.7 (C-13), 40.2 (C-14), 30.6 (C-15), 36.0 (C-16), 29.6 (C-17), 43.1 (C-18), 35.7 (C-19), 28.2 (C-20), 33.1 (C-21), 39.2 (C-22), 6.8 (C-23), 14.2 (C-24), 17.6 (C-25), 18.7 (C-26), 20.4 (C-27), 32.1 (C-28), 31.7 (C-29), 35.1 (C-30).

Octandronic acid (**3**): colorless solid (15.3 mg); ^{1}H-NMR (400 MHz; $CDCl_3$) δ_H 0.73 (3H, *s*, Me-24), 0.87 (3H, *s*, Me-25), 0.86 (3H, *d*, *J* = 6.7 Hz, Me-23), 1.34 (3H, *s*, Me-29), 1.07 (1H, *m*, H-22α), 1.01 (3H, *s*, Me-26), 1.07 (3H, *s*, Me-27), 1.09 (3H, *s*, Me-28), 1.76 (1H, *m*, H-6α), 1.40 (1H, *m*, H-7α), 1.39 (1H, *m*, H-8), 1.52 (1H, *m*, H-7β), 1.49 (1H, *m*, H-22β), 1.33 (2H, *m*, H-10), 1.62 (1H, *m*, H-18), 2.01 (1H, *m*, H-1α), 1.76 (1H, *m*, H-6β), 1.94 (1H, *m*, H-1β), 2.26 (1H, *q*, *J* = 6.1 Hz, H-4), 2.29 (2H, *m*, H-2α), 2.36 (1H, *m*, H-2β); ^{13}C-NMR (100 MHz, $CDCl_3$) δ_C 22.6 (C-1), 41.4 (C-2), 212.3 (C-3), 59.0 (C-4), 42.1 (C-5), 41.4 (C-6), 18.3 (C-7), 53.1 (C-8), 36.8 (C-9), 60.1 (C-10), 36.2 (C-11), 30.5 (C-12), 40.1 (C-13), 38.3 (C-14), 33.2 (C-15), 35.9 (C-16), 29.6 (C-17), 42.6 (C-18), 31.4 (C-19), 40.1 (C-20), 29.3 (C-21), 38.2 (C-22), 6.8 (C-23), 14.4 (C-24), 17.7 (C-25), 20.3 (C-26), 17.3 (C-27), 32.1 (C-28), 31.9 (C-29), 184.3 (C-30).

β-Amyrin (**4**): colorless solid (7.4 mg); ^{1}H-NMR (400 MHz; $CDCl_3$) δ_H 0.98 (3H, *s*, Me-24), 0.93 (3H, *s*, Me-25), 0.75 (1H, *m*, H-5), 0.77 (3H, *s*, Me-23), 0.89 (3H, *s*, Me-29), 1.07 (1H, *m*, H-22α), 0.94 (3H, *s*, Me-26), 1.11 (3H, *s*, Me-27), 0.82 (3H, *s*, Me-28), 1.30 (1H, *m*, H-6α), 1.49 (1H, *m*, H-22β), 1.89 (1H, *m*, H-18), 1.51 (1H, *m*, H-1α), 1.53 (1H, *m*, H-6β), 1.56 (1H, *m*, H-1β), 1.53 (2H, *m*, H-2α), 1.52 (1H, *m*, H-2β), 1,59 (1H, *m*, H-19), 5.16 (1H, *t*, *J* = 3.5 Hz, H-12); ^{13}C-NMR (100 MHz, $CDCl_3$) δ_C 38.9 (C-1), 27.4 (C-2), 79.6 (C-3), 40.2 (C-4), 55.3 (C-5), 18.6 (C-6), 32.8 (C-7), 40.2 (C-8), 47.5 (C-9), 37.0 (C-10), 23.8 (C-11), 121.9 (C-12), 145.3 (C-13), 41.7 (C-14), 26.3 (C-15), 27.2 (C-16), 32.5 (C-17), 48.6 (C-18), 47.3 (C-19), 30.9 (C-20), 38.0 (C-21), 35.2 (C-22), 15.9 (C-23), 28.3 (C-24), 16.7 (C-25), 17.1 (C-26), 26.1 (C-27), 28.2 (C-28), 33.6 (C-29), 23.7 (C-30).

Ursolic acid (**5**)*:* colorless solid (14.2 mg); ^{1}H-NMR (400 MHz; $CDCl_3$) δ_H 5.14 (1H, *dd*, *J* = 13.8; 3.5 Hz; H-12); 3.00 (1H, *dd*, *J* = 5.0; 9.5 Hz, H-3); 2.10 (1H, *d*; *J* = 11.1 Hz, H-18); 1.90 (2H, *dd*, *J* = 13.8; 3.5 Hz, H-11); 1.59 (2H, *m*, H-16); 1.57 (1H, *s*, H-9); 1.56 (2H, *m*, H-1); 1.55 (2H, *m*, H-22); 1.52 (1H, *m*, H-20); 1.46 (1H, *m*, H-6α); 1.44 (2H, *m*, H-2); 1.31 (1H, *m*, H-19); 1.29 (1H, *m*, H-6β); 1.29 (2H, *m*,

H-21); 1.25 (2H, *m*, H-7); 1.15 (3H, *s*, H-27); 1.01 (2H, *m*, H-15); 0.92 (3H, *d*, *J* = 6.7 Hz, H-30), 0.91 (3H, *s*, H-23); 0.87 (3H, *s*, H-25); 0.81 (3H, *d*, *J* = 5.6 Hz, H-29); 0.69 (3H, *s*, H-26), 0.68 (3H, *s*, H-24), 0.67 (1H, *s*, H-5); ^{13}C-NMR (400 MHz; $CDCl_3$) δ_C 178.2 (C-28); 138.2 (C-13); 124.6 (C-12); 76.9 (C-3); 55.2 (C-5); 52.3 (C-18); 47.1 (C-9); 46.8 (C-16); 42.0 (C-14); 39.1 (C-8); 38.5 (C-20); 38.3 (C-19); 37.9 (C-4); 37.7 (C-1); 36.5 (C-10); 36.3 (C-22); 32.6 (C-15); 30.8 (C-7); 28.2 (C-23); 27.5 (C-21); 26.7 (C-2); 23.8 (C-11); 23.3 (C-27); 23.0 (C-16); 21.2 (C-30); 17.9 (C-6); 16.9 (C-29); 16.8 (C-24); 16.0 (C-25); 15.2 (C-26).

Lupeol (**6**)*:* colorless solid (6.1 mg); ^{1}H-NMR (400 MHz; $CDCl_3$) δ_H 4.70, 4.55 (2H, *s*, H-29α, H-29β), 3.2 (1H, *m*, H-3), 0.77, 0.79, 0.85, 0.94, 0.97, 1.05, 1.65 (each 3H, *s*); ^{13}C-NMR (100 MHz; $CDCl_3$) δ_C 100MHz): δ 151.2 (C-20), 108.0 (C-29), 78.9 (C-3), 55.5 (C-5), 51.5 (C-9), 48.3 (C-18), 48.1 (C-19), 43.7 (C-17), 42.9 (C-14), 40.9 (C-8), 41.3 (C-22), 38.9 (C-4), 38.7 (C-1), 38.1 (C-13), 37.2 (C-10), 36.5 (C-16), 34.2 (C-7), 29.9 (C-21), 28.0 (C-23), 27.3 (C-2), 27.1 (C-15), 25.4 (C-12), 21.0 (C-11), 19.5 (C-30), 18.5 (C-6), 18.1 (C-28), 16.1 (C-25), 16.0 (C-26), 15.5 (C-24), 14.8 (C-27).

Chrysophanol (**7**), orande solid (7.2 mg); ^{1}H-NMR (400 MHz; $CDCl_3$) δ_H 2.47 (3H, *s*, H-11), 7.11 (3H, *d*, *J* = 1.2 Hz, H-2), 7.31 (1H, *dd*, *J* = 8.4 Hz, 1.2 Hz, H-7), 7.65 (1H, *d*, *J* = 1.2 Hz, H-4), 7.67 (1H, *d*, *J* = 8.4 Hz, H-6), 7.83 (1H, *dd*, *J* = 8.4 Hz, 1.2 Hz, H-5), 12.1 (1H, *s*, 1-OH), 12.2 (1H, *s*, 8-OH); ^{13}C-NMR (100 MHz; $CDCl_3$) δ_C 23.0 (C-11), 109.3 (C-9a), 115.8 (C-4a), 116.0 (C-8a), 118.9 (C-5), 122.3 (C-4), 124.3 (C-2), 124.8 (C-7), 135 (C-10a), 135.9 (C-6), 149.5 (C-3), 163.5 (C-8), 162.9 (C-1), 183.0 (C-10), 192.1 (C-9).

Scopoletin (**8**), orange solid (5.2 mg); ^{1}H-NMR (400 MHz; $CDCl_3$) δ_H 3.90 (3H, *s*, OMe), 6.24 (1H, *d*, *J* = 9.8 Hz, H-2), 6.81 (1H, *s*, H-8), 7.20 (1H, *s*, H-5), 7.88 (1H, *d*, *J* = 9.8 Hz, H-3); ^{13}C-NMR (400 MHz; $CDCl_3$) δ_C 55.9 (OMe), 103.7 (C-8), 109.9 (C-5), 112.7 (C-4), 113.6 (C-2), 144.5 (C-3), 146.0 (C-6), 151.3 (C-9), 151.5 (C-7), 160.5 (C-1),

Computational analysis

The conformational analysis of **1** was performed using the Maestro 9.2 software using Macromodel interface. For conformational search, the Monte Carlo Multiple Minimum (MCMM) method was used with a maximum number of 2000 steps in gas phase. The lowest energy conformer based on Boltzmann distribution was selected for the calculation of Isotropic Magnetic Shielding (IMS) (σ). The Jaguar 7.9 software was used to optimize the molecular geometry and calculate the ^{13}C NMR shielding constants at the Density Functional Theory (DFT), mPW1Pw91 level of theory and 6-31G** basis set. The chemical shift values were calculated via equation (1) where $\delta^{x}_{calc.}$ is the calculated NMR chemical shift for nucleus *x*, σ^0 is

the shielding tensor for the carbon nuclei in benzene calculated at the above-mentioned basis set.[28,29]

$$\delta^{x}_{calc.} = \frac{\sigma^{0} - \sigma^{x}}{1 - \frac{\sigma^{x}}{10^{6}}} \quad (1)$$

Antileishmanial assay

The antileishmanial activity of **1** was evaluated against *Leishmania amazonensis clon1: Lma (MHOM/BR/76/LTB-012 and Leishmania braziliensis: M2904 C192 RJA*, using the Colorimetric method-XXT.[30] The miltifosine was used as positive control.

Acknowledgements

The authors are grateful to Swedish International Development Agency for the grants. We thank Sofia Essén from Lund University for the help with the HRES-MS spectra.

References

1. Chan-Bacab, M. J.; Peña-rodríguez, L. M. *Nat. Prod. Rep.* **2001**, *18*, 674–688.
2. Gallagher, R.; Marx, J.; Hines, P. *Science (80-.).* **1994**, *264* (5167), 1827–1827.
3. Borst, P.; Ouellettt, M. *Ann. Rev. Microbiol.* **1995**, *49*, 427–460.
4. Dos Santos, V. A. F. F. M.; Leite, K. M.; Da Costa Siqueira, M.; Regasini, L. O.; Martinez, I.; Nogueira, C. T.; Galuppo, M. K.; Stolf, B. S.; Pereira, A. M. S.; Cicarelli, R. M. B.; Furlan, M.; Graminha, M. A. S. *Molecules* **2013**, *18* (1), 1053–1062.
5. Chawla, B.; Madhubala, R. *Am. J. Trop. Med. Hyg.* **2010**, *84*, 1–13.
6. Berman, J. D.; Lee, L. S. *Am. J. Trop. Med. Hyg.* **1983**, *32* (5), 947–951.
7. Sen, R.; Chatterjee, M. *Phytomedicine* **2011**, *18* (12), 1056–1069.
8. Viegas, C.; Silva, D. H. S.; Pivatto, M.; Rezende, A. De; Castro-gambôa, I.; Bolzani, V. S. *J. Nat. Prod.* **2007**, *70*, 2026–2028.
9. Gabriela Muniz de Albuquerque Melo, Marcela Campelo Rodrigues Silva, Thaís Pereira Guimarães, Kátia Mantovani Pinheiro, Carolina Barbosa Brito da Matta, Aline Cavalcanti de Queiroz, Marcos Pivatto, Vanderlan da Silva Bolzani, Magna Suzana Alexandre-Moreir, C. V. J. *Phytomedicine* **2014**, *21* (3), 277–281.
10. Karau, G. M.; Nyagah, E.; Njagi, M.; King, A.; Wangai, L. N. **2013**, *2* (2), 51–59.
11. Bruschi, P.; Morganti, M.; Mancini, M.; Signorini, M. A. *J. Ethnopharmacol.* **2011**, *138* (2), 543–563.
12. Viegas, C.; Pivatto, M.; De Rezende, A.; Hamerski, L.; Silva, D. H. S.; Da Silva Bolzani, V. *J. Braz. Chem. Soc.* **2013**, *24* (2), 230–235.
13. Da Silva, K. A. B. S.; Manjavachi, M. N.; Paszcuk, A. F.; Pivatto, M.; Viegas, C.; Bolzani, V. S.; Calixto, J. B. *Neuropharmacology* **2012**, *62* (2), 967–977.
14. Viegas, C.; Silva, D. H. S.; Pivatto, M.; De Rezende, A.; Castro-Gambôa, I.; Bolzani, V. S.; Nair, M. G. *J. Nat. Prod.* **2007**, *70* (12), 2026–2028.
15. Muniz, G.; Melo, D. A.; Campelo, M.; Silva, R.; Pereira, T.; Mantovani, K.; Barbosa, C.; Cavalcanti, A.; Queiroz, D.; Pivatto, M.; Bolzani, S.; Alexandre-moreira, M. S.; Viegas, C. *Eur. J. Integr. Med.* **2014**, *21* (3), 277–281.
16. Figueiredo, J. N.; Räz, B.; Séquin, U. *J. Nat. Prod.* **1998**, *61* (6), 718–723.
17. Sousa, G. F.; Duarte, L. P.; Alcântara, A. F. C.; Silva, G. D. F.; Vieira-Filho, S. A.; Silva, R. R.; Oliveira, D. M.; Takahashi, J. A. *Molecules* **2012**, *17* (11), 13439–13456.
18. Yeboah, E. M. O.; Majinda, R. R. T.; Kadziola, A.; Muller, A. *J. Fac. Pharm. Istanbul* **2010**, *73* (6), 1151–1155.
19. Abbas, M.; Disi, A.; Al-Khalil, S. *Jordan J. Pharm. Sci.* **2009**, *2* (2), 131–139.
20. Lai, J. S.; Huang, J. Y.; Huang, K. F. *Chin. Pharm. J.* **1996**, *48*, 451–458.
21. Al-Musayeib, N. M.; Mohamed, G. A.; Ibrahim, S. R. M.; Ross, S. A. *Med. Chem. Res.* **2013**, *22* (11), 5297–5302.
22. Zhang, H.; Guo, Z.; Wu, N.; Xu, W.; Han, L.; Li, N.; Han, Y. *Molecules* **2012**, *17* (1), 843–850.

23. Darmawan, A.; Kosela, S.; Kardono, L. B. S.; Syah, Y. M. *J. Appl. Pharm. Sci.* **2012**, *2* (12), 175–177.
24. Murayama, T.; Eizuru, Y.; Yamada, R.; Sadanari, H.; Matsubara, K. *Antivir. Chem. Chemother.* **2007**, *18* (3), 133–139.
25. Thiem, D. A.; Sneden, A. T.; Khan, S. I.; Tekwani, B. L. *J. Nat. Prod.* **2005**, *68*, 251–254.
26. Pina, E. S.; Silva, D. B.; Teixeira, S. P.; Coppede, J. S.; Furlan, M.; França, S. C.; Lopes, N. P.; Pereira, A. M. S.; Lopes, A. A. *Sci. Rep.* **2016**, *6* (November 2015), 22627.
27. Corsino, J. *Phytochemistry* **2000**, *55*, 741–748.
28. Lodewyk, M. W.; Siebert, M. R.; Tantillo, D. J. *Chem. Rew.* **2012**, *112* (3), 1839–1862.
29. Smith, S. G.; Goodman, J. M. *J. Am. Chem. Soc.* **2010**, *132* (37), 12946–12959.
30. Capusiri, E. S.; Pinell, G. R.; Huallpara, J. C. T.; Turba, A. G. *Biofabro* **2008**, *16*, 21–27.

Plants, microbes, and invertebrates, i.e. all living organisms, produce a vast array of compounds known as natural products or simply secondary metabolites. The production of these secondary metabolites is not believed to promote the growth of the particular specie, instead they have other functions. It may, for example, be secondary metabolites that are useful for a chemical defence against predators, or for the protection and survival during environmental stress.

The secondary metabolites produced by living organisms are well known to possess a wide range of biological activities, which in some cases are useful to man. Antibiotics such as penicillin were isolated from fungi, and quinine was isolated from plants of the genus Cinchona and used medicinally as antimalarials. Also non-pharmaceutical green teas contain products derived from nature and used by humans for their health benefits. As the investigations of natural products have resulted in a remarkable number of compounds that benefit humans, the continued study of secondary metabolites and natural products has and continues to be of great importance.

Printed by Media-Tryck, Lund 2017 NORDIC ECOLABEL, 3041 0903

LUND UNIVERSITY

ISBN 978-91-7422-553-2

Centre for Analysis and Synthesis
Department of Chemistry
Faculty of Science
Lund University