

UNIVERSITY *of the* WESTERN CAPE

**SECONDARY METABOLITES OF THE *UROMYCLADIUM
TEPPERIANUM* MacAlpine EPIPHYtic FUNGUS.**



UNIVERSITY *of the*
WESTERN CAPE

MASTERS THESIS 2021

**FACULTY OF NATURAL SCIENCE
CHEMISTRY DEPARTMENT**

By

Kelly Luana Viegas Correia

Student No. 3458642

Prof W.T. Mabusela

Abstract

Research on natural products and medicinal plants has been conducted more with each passing year due to the great interest in isolating bioactive compounds and secondary metabolites from natural products such as plants, fungi, and many other naturally occurring products. To our knowledge, the *Uromycladium tepperianum* MacAlpine fungus has not been studied in depth before thus, its organic characterization was unknown, but related species have been studied, and these have shown compounds that may be used as medicine and as health benefits. Crude and macerated extracts of the *Uromycladium tepperianum* fungus have been fractionized using various chromatographic techniques such as solvent-solvent extraction followed by dry column chromatography to achieve the required separations. Thirty-nine compounds have been isolated from the hexane(*n*-hex) extract, Ethylacetate (EtOAc) extract, and n-butanol (BuOH) extract, and they belong to the following groups, steroids, terpenoids, amino-acids, flavonoids, alkaloids, phenolics, fatty-acids, flavones, and others. Nuclear magnetic resonance (NMR) characterization of the compounds was carried based on proton NMR (¹H-NMR), carbon NMR (¹³C-NMR), distortion enhancement by polarization transfer NMR (DEPT-NMR), Heteronuclear single quantum coherence spectroscopy NMR (HSQC-NMR), Heteronuclear multiple bond correlation NMR (HMBC-NMR) and Homonuclear correlation Spectroscopy NMR (COSY-NMR). All four compounds found in the hexane extract have been isolated previously from other known plants such as spinach, argan oil, cactus pear seed oil, and others, but to our knowledge, the four compounds isolated from the hexane extract were found in the *Uromycladium tepperianum* fungus for the first time. The compounds found in the other extracts, were carried out by Liquid chromatography-mass spectroscopy (LC-MS) in order to analyze compounds that where isolated, but their yields were to low for NMR detection. The compounds were then matched with databases and programs: Global natural products social molecular network, FoodDB, STOFF (environment), KNAPSAcK (Natural product), PubChem (biomolecules), PlantCyc (plant), ChBI (Biomolecules), NANPDB (Natural product), UNPD (Natural Product), Elemental composition, Pubchem, Chemspider Science direct, Synapt G2 qTOF, MZmine, Chem Calc, and Science finders Scholar.

Declaration

I, Kelly Luana Viegas Correia, hereby declare that this dissertation entitled **secondary metabolites from the epiphytic fungus *Uromycladium tepperianum* MacAlpine** is my original and of my knowledge. This research has not been submitted anywhere else for the award of a degree at any other institution or University. Other literature sources have been quoted and referenced, but the words have been re-written and rephrased.



Date 07/11/2021

Signed Kelly Correia

Acknowledgments

I would like to express my sincere gratitude to my supervisor and Head of the Department (HOD) of the department of chemistry, Prof. Wilfred T. Mabusela, who gave me the opportunity and encouragement to continue with my work in the field of organic chemistry and for allowing me to be part of his team.

I would like to sincerely thank my parents for always guiding me, believing in me, loving and supporting me in all I have wanted to accomplish, and financing my studies.

I would like to thank the Department of Chemistry for the opportunity they have provided for me to be part of the Master's program.

A special thanks to my fellow post-graduate students Masande Yalo, Masixole, Sazi, Franck, and Ndikho for always being there when I needed guidance, patience, and endless support, and for the belief they have in me as an organic chemist.

Lastly, I would like to thank my family and friends for their support, love, and guidance.

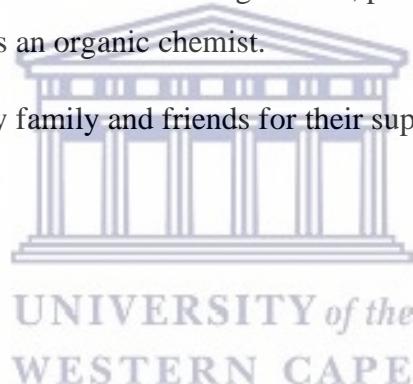


Table of Contents

Abstract	I
Declaration.....	II
Acknowledgments	III
List of abbreviations	VI
List of Figures	VII
List of Spectra.....	VIII
List of Tables.....	IX
Scheme table.....	X
List of Isolated compounds.....	XI
Chapter 1.....	1
1.1 Introduction: Secondary Metabolites From the Epiphytic fungus <i>Uromykladium Tepperianum</i>	1
1.2 Problem statement.....	3
1.2.1 Hypothesis.....	3
1.3 Aims and Objectives	4
Chapter 2.....	5
Literature review.....	5
2.1 Introduction to fungi	5
2.1.1 Natural Products and potential drug development	14
2.1.2 Mushrooms	22
2.1.3 Mushrooms and health benefits	23
2.1.4 Kinds of mushrooms.....	24
2.1.5 Mushrooms in cancer research.....	26
2.1.6 Rust fungi.....	31
2.1.7 Application of LC-MS and MS in plant extract analysis.....	32
Chapter 3.....	33
Materials and methods	33
3.1 Reagents and General methods	33
3.2 Spectroscopy	33
3.3 Chromatography	33
3.4 Mass spectrometry (MS)	34
3.5 <i>Uromykladium tepperianum</i> MacAlpine preparation	34
3.6 Isolation and purification of compounds	37
Chapter 4.....	41
4.1 Results and Discussion	41
.....	42
4.1.1 Reagent tests and results	42
4.1.1 Structure elucidation of Spinasterol (compound 1) and Schottenol (compound 2) found in the	

hexane extract.....	43
4.1.2 Structure elucidation of Palmitic Acid (compound 3) and linoleic Acid (compound 4) found in the hexane extract.....	45
4.1.3 Compounds found in EtOAc and BuOH extracts	47
4.1.4 Literature review on compounds obtained through LC-MS	49
Chapter 5.....	56
5.1 Conclusion.....	56
Appendix.....	58
References.....	67



List of abbreviations

EtOAc	Ethyl Acetate
DCM	Dichloromethane
BuOH	Butanol
GC-MS	Gas Chromatography – Mass Spectroscopy
MeOH	Methanol
NMR	Nuclear Magnetic Resonance
¹ H-NMR	Proton Nuclear Magnetic Resonance
¹³ C-NMR	Carbon-13 Nuclear Magnetic Resonance
CDCl ₃	Deuterated Chloroform
COSY	Correlation Spectroscopy
HMBC	Heteronuclear Multiple Bond Correlation
HSQC	Heteronuclear Single Quantum Coherence
TLC	Thin Layer Chromatography
IR	Infra-red spectroscopy
Hex 5	Hexane extract fractions 16-26 (mix linoleic acid and palmitic acid)
Flakes	Hexane extract fractions 35-55 (mix Spinasterol and schottenol)
STD	Standard solution
n-Hex	Hexane
LC-MS	Liquid chromatography- Mass spectroscopy

List of Figures

Figure 1: Uromycladium tepperianum MacAlpine fungus and material in powder form	34
Figure 2: solvent-solvent extraction, layer separation	36
Figure 3: Difference in chemical composition between the 3 samples	42
Figure 4:Presence of carbohydrates.....	42



List of Spectra

spectra 1:Spectra C13 compound 1 and 2.....	58
spectra 2:Spectra DEPT compound 1 and 2	58
spectra 3:Spectra Proton compound 1 and 2.....	58
spectra 4:Spectra HMBC compound 1 and 2.....	59
spectra 5:Spectra Cosy compound 1 and 2.	59
spectra 6:GC-MS Spectra visualization of both compound 1 and 2. (mixture).....	60
spectra 7:GC-MS Spectra molar mass of Spinasterol compound 1	60
spectra 8:GC-MS Spectra molar mass of Schottenol compound 2.....	60
spectra 9:Spectra C13 compounds 3 and 4	61
spectra 10:Spectra DEPT compounds 3 and 4	61
spectra 11:Spectra Proton compounds 3 and 4	61
spectra 12:Spectra HMBC compounds 3 and 4	62
spectra 13:Spectra COSY compounds 3 and 4	62
spectra 14:GC-MS Spectra visualization of compounds 3 and 4 mixture.	63
spectra 15: GC-MS Spectra molar mass of linoleic Acid compound 4.	63
spectra 16:GC-MS Spectra molar mass of Palmitic acid compound 3.	63
spectra 17: compound 37 peaks	64
spectra 18: compound 38 peak.....	65
spectra 19: compound 39 spectra.....	66



UNIVERSITY *of the*
WESTERN CAPE

List of Tables

Table 1:NMR interpretation of compounds 1 and 2.....	55
Table 2:NMR interpretation of compounds 3 and 4.....	57
Table 3: EtOAc and BuOH fractions sent to LC-MS.....	58
Table 5: LC-MS on EtOAc and BuOH extract compounds	48
Table 6: Global Natural products social molecular network compound finding	60

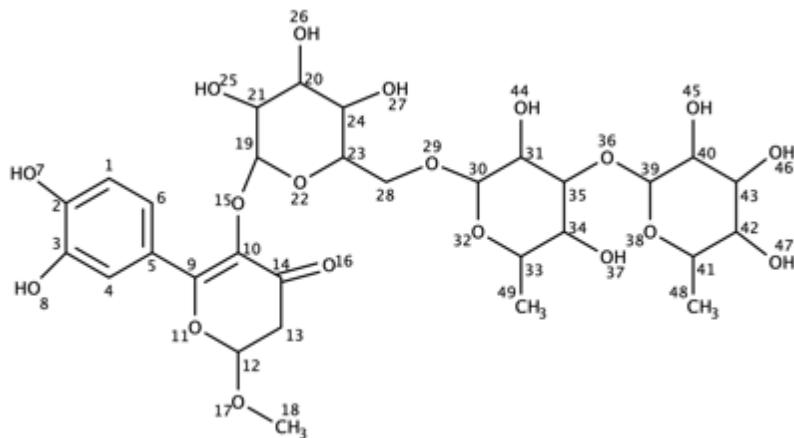


Scheme table

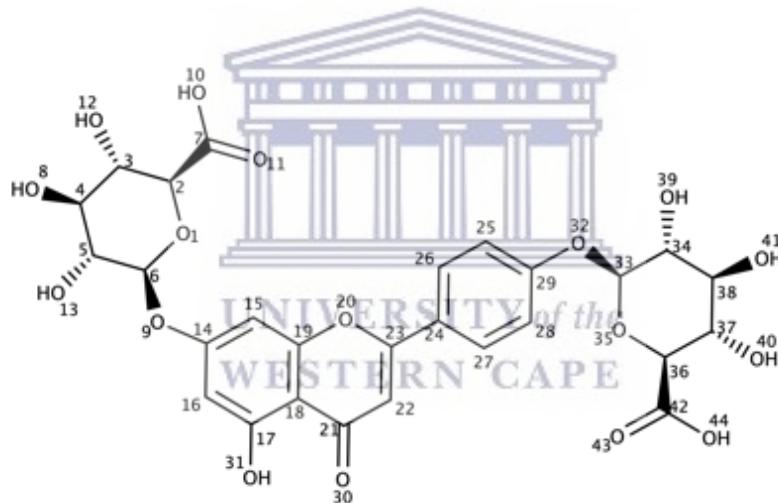
Scheme 1:5 steps to Hernolactone	7
Scheme 2: Syringaldehyde to Trimethoprim	8
Scheme 3: from 3,4,5-trimethoxybenzaldehyde to trimethoprim both methods	8
Scheme 4: Isolation of natural products from <i>Uromycladium tepperianum</i> MacAlpine Fungus	
.....	39



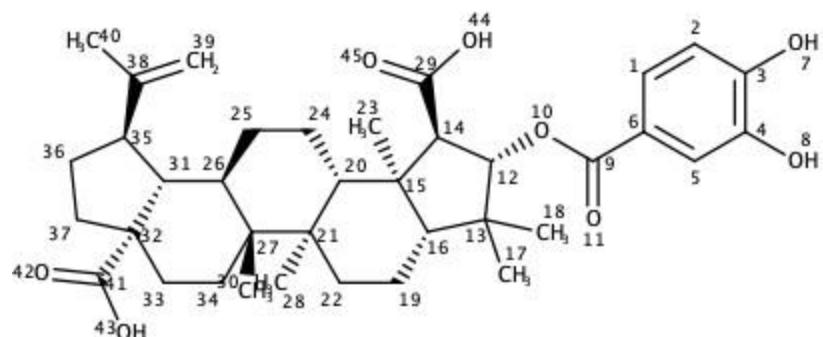
List of Isolated compounds



compound 37: 7-O-Methylquercetin-3-O-galactoside-6''-rhamanoside



compound 38: 4'-O-GlcA-7-O-GlcA Apigenin



compound 39: 3-O-protocatechuoyl ceanothic acid

Chapter 1

1.1 Introduction: Secondary Metabolites From the Epiphytic fungus *Uromykladium Tepperianum*

Uromykladium Tepperianum MacAlpine is a rust fungus that produces large and irregular sized galls on the leaves and stems of the plant (Wood, 2012). It is native to Australasia and has affected over 100 species of Acacia related genera, including Paraserianthes in Australia, South-East Asia, the South Pacific and New Zealand (Doungsa-ard *et al.*, 2015) The rust fungus was introduced in South Africa purposefully to control the spread of *Acacia saligna* bushes (Besaans, 2015). There is no literature on the natural product composition of this fungus. *U. Tepperianum* MacAlpine fungus consists of single-celled teliospores produced on pedicels where the ridged teliospores are produced on the surface of the galls as a brown powder and are approximately 0.02mm in diameter (Wood, no date). The wind is responsible for the spreading of the Teliospores (Newcombe, 2006). The brown powder consisting of teliospores can easily be brushed off when fresh and then infect the tree, penetrating the epidermal cells of the young phyllodes (leaves) as well as stems and flower buds (Ratio, 2017). The fungus then surrounds the plant tissue and induces the formation of the galls (Wood, 2012). The galls are quickly germinated during spring, which is the season where plants and flowers grow and reproduce the most (Aime *et al.*, 2017). The teliospores are formed between 10°C to 20°C when there is free water available on the plant's surface, which can be due to a light rain or overnight dew especially during the spring season (Wood en Morris, 2007). Since the fungus is spread rapidly by wind there is no need for artificial redistribution of the fungus (Wood, no date). New galls often develop in February to March since the fungus generally infects the plant the previous rainy season, thus new galls can be produced any time from then until late spring (Wood, 2012).

The fungus utilizes the plant's nutrients (Wood, 2012) to nourish itself, thus causing limitation of growth and seed production of that specific plant, due to the heavy galls on the plants leaves and stems (Davis en Agrios, 1970). The heavy gall makes the plant unable to cope with drought on drier months and other environmental stresses leading to the death of the plant. Once the plant has died, the fungus also dies since it lives inside the plant as a parasite (Wood, no date). Intense fires and clearing operations may lead to the extinction of the rust fungus, but later on

naturally reinvade the area, thus minimization and avoiding the clearance and burning of the areas affected would help to keep the fungus alive (Wood, 2012)



1.2 Problem statement

Among a wide range of plants, a few are classified as medicinal plants, which are widely used for the treatment of, or drug fabrication to combat or cure diseases. By utilizing modern biotechnical extraction processes, concentrated extracts of medicinal plants may be prepared for human use. In recent times, research towards the discovery of drugs and other treatments for currently incurable diseases and drug-resistant pathogens has intensified. However, the development of such new products often faces challenges which may include causation of side effects, or not being compatible with all physiological systems among others. Thus, exploring the natural product composition of the *Uromycladium Tepperianum* MacAlpine endophytic fungus, may offer opportunities for the lead discovery of new drugs as has been achieved with some fungal species. To our knowledge, no such research on the *Uromycladium tepperianum* MacAlpine endophytic fungus has been pursued to date. The host plant of the fungus, *Acacia saligna*, has been studied for its phytochemical composition, and has been found to contain compound classes such as terpenoids and phenolics. It may thus be expected that some of these compounds may be assimilated into the fungus, while the fungus may also be able to produce its own natural products for its survival.

1.2.1 Hypothesis

Some fungal species have been shown to produce secondary metabolites which possess diverse biological activities, and which have been exploited for health benefits among humankind. These chemical structures may be used in medicine as well as agricultural products, and thus exploring the natural product composition of *Uromycladium tepperianum* MacAlpine fungus, may open opportunities for the development of new medicines, drugs, or leads for new drugs. The research work is thus based on the hypothesis that *Uromycladium tepperianum* MacAlpine fungus contains secondary metabolites, some of which may display potential for development into therapeutic drugs.

1.3 Aims and Objectives

General aims and objectives

The objective of the investigation is to discover and understand the chemical composition of the *Uromycladium tepperianum* MacAlpine fungus due to the lack of background knowledge on the fungus. The investigation aims to conduct a systematic solvent extraction of the *Uromycladium tepperianum* fungus and determine its nature and composition. Preparation of both organic and aqueous solvent based maceration and crude extraction from the *Uromycladium tepperianum* fungus; isolation followed by structural elucidation of secondary metabolites.

Specific objectives

1. Use organic and aqueous solvents to perform maceration and crude extractions from the *Uromycladium tepperianum* MacAlpine fungus.
2. Carry out phytochemical screening of the *Uromycladium Tepperianum MacAlpine* extracts (Hexane [*n*-hex], Ethyl Acetate [EtOAc], Dichloromethane [DCM], Butanol [BuOH]) for various classes of compounds.
3. Carry out fractionization of organic extracts, followed by the isolation of pure compounds subsequently using various chromatographic techniques.
4. Carry out compound characterization with a combination of various spectroscopic techniques, reagent tests and hydrolysis.

Chapter 2

Literature review

2.1 Introduction to fungi

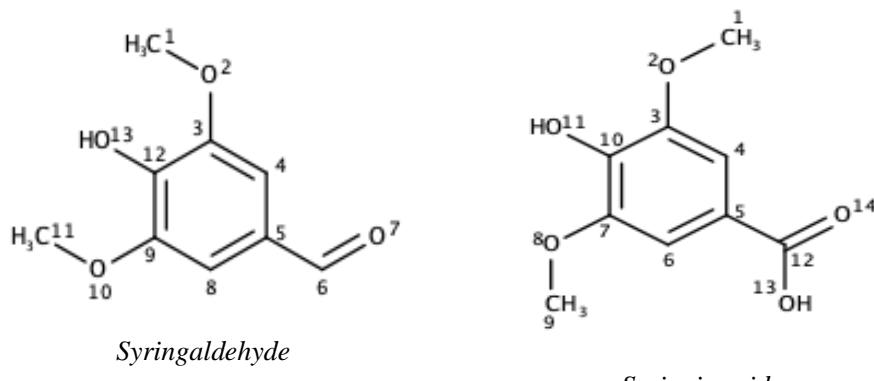
Fungi originally classified as plants, are now categorized as a separate kingdom, and considered to be among the most important organisms on earth. When combined with certain bacteria, fungi are responsible for the conversion of dead material into helpful material for soil (Mohmand *et al.*, 2011). Fungi are natural decomposers, parasites, or pathogens, were majority are beneficial partners in symbiosis with animals, plants, and algae (Reid en Webster, 1973).

The fungi kingdom consists of mushrooms, rusts, smuts, truffles, morels, moulds, and yeasts and each of these known fungi belongs to one of three groups: Eumycota, Oomycota, and Myxomycota (Gurnani *et al.*, 2014).

Rusts are diseases prone to grow on plants, consequently, acquiring nutrients from the plant itself and reproducing on its host (Tattar, 1989). Several species of rusts have evolved in a manner that they solely grow, infect, colonize and reproduce in specific plant species (Kolmer, Ordonez en Groth, 2009).

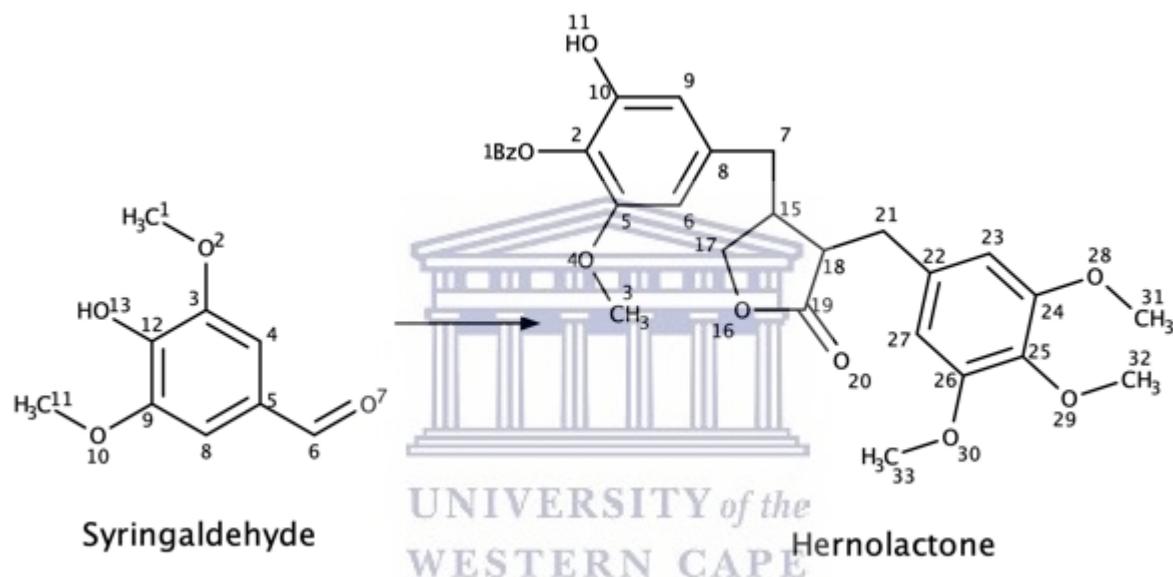
Smuts are cellular fungi characterized by their sizable amount of teliospores. Smuts represent the second-largest fungi cluster after rusts (Pandey, 2018).

Truffles are fruity bodies, part of the genus Tuber. These are widely used as food and medicine, wealthy in bioactive compounds such as terpenoids, flavonoids, polysaccharides, and others which supply its anti-inflammatory, anti-mutagenic, anti-cancer, and inhibition of antioxidant properties. Some of these compounds are (Reyna en Garcia-Barreda, 2014):

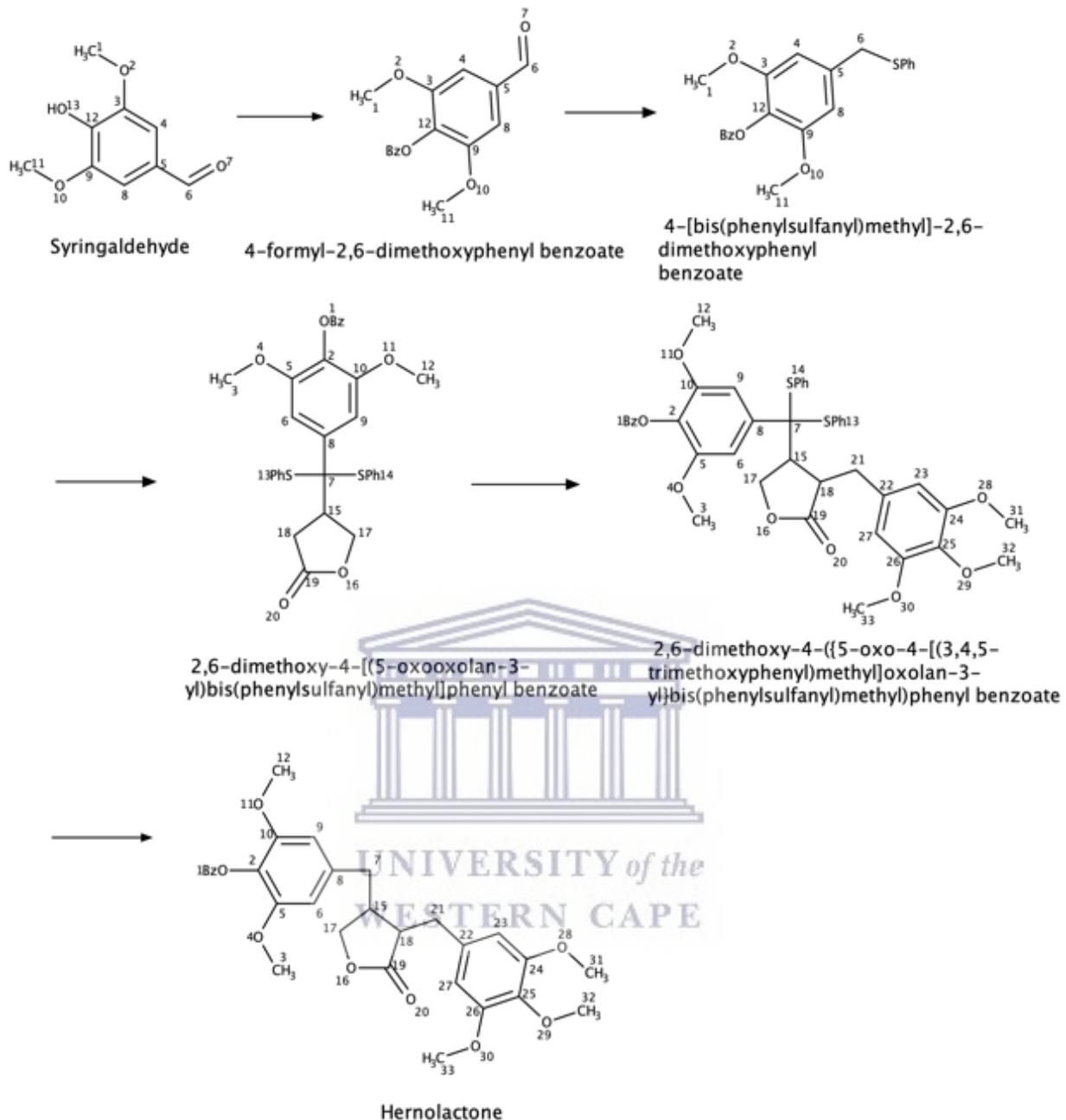


Syringaldehyde, a valuable pharmaceutical starting material, is produced when lignin oxidation takes place in vanillin with molecular oxygen (Mathew *et al.*, 2018). Alcoholic hydrolysis and heating, degrade the lignin and generates aldehydes. Oxidation and the association with lignin and aldehydes produce phenolic acids, hence syringaldehyde is able to convert to syringic acid and other compounds (Aging, 2012).

Hernolactone a lignan which inhibits the activity of cyclooxygenase-2, reducing inflammation in the human body, may also be naturally isolated from the seeds of *H. Ovigera*, or is chemically synthesized with syringaldehyde as its direct precursor (Vi *et al.*, 1987).

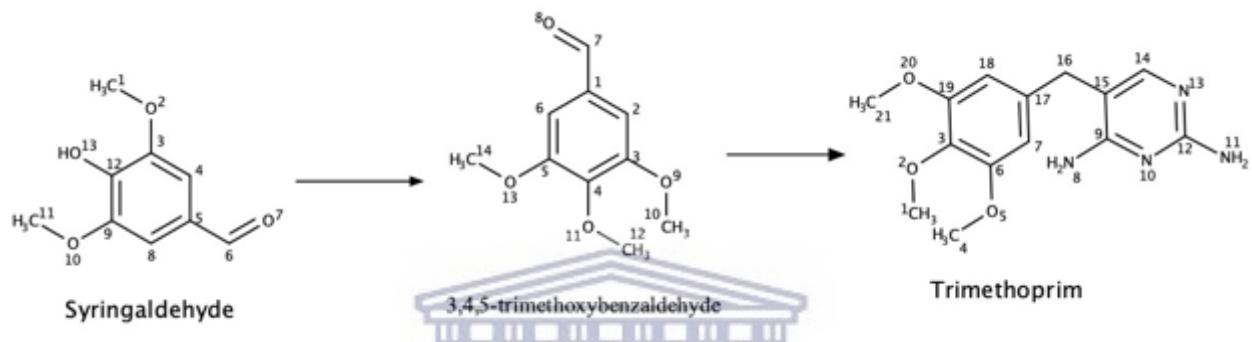


To produce hernolactone, syringaldehyde reacts with benzyl chloride under the presence of anhydrous potassium carbonate submerged in dimethylformamide, to form benzyl syringaldehyde (Chen, 2000), followed by the condensation with butanolide which occurs by Michael addition in *n*-butyllithium which followed thereafter the benzyl syringaldehyde reaction with thiophenol, converting it to its corresponding phenyldithioacetal in the presence of boron trifluoride-etherate, and finally hernolactone is obtained(Arimoto, Yamaguchi en Nishibe, 1995).



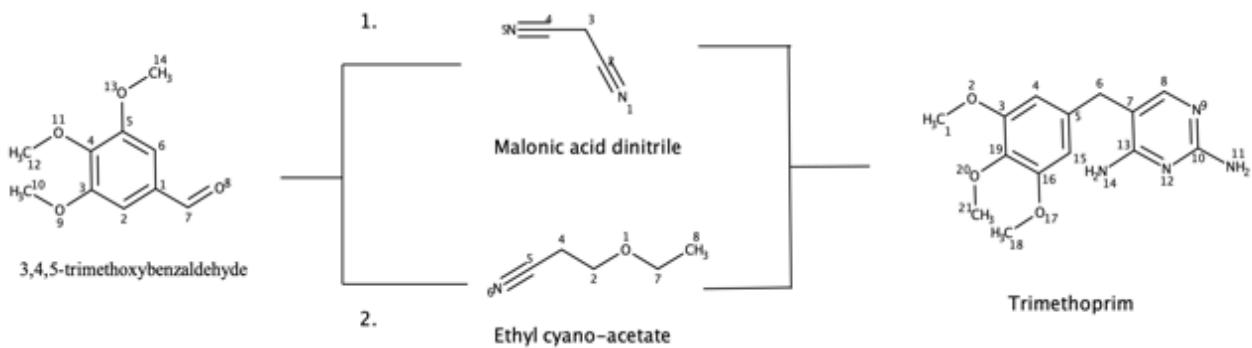
Scheme 1: 5 steps to Hernolactone

Additionally, syringaldehyde is the main precursor of 3,4,5-trimethoxybenzaldehyde (Mathew *et al.*, 2018). By utilizing a copper [1]- catalyzed exchange of bromine by methoxide in DMF and separating the syringaldehyde from 3,5-dibromo-4-hydroxybenzaldehyde and 5-bromovanillin, followed by stirring the mixture containing syringaldehyde, acetone treated with dimethylsulfate, disodium carbonate and potassium hydroxide in water, 3,4,5-trimethoxybenzaldehyde is then synthesized (Kolb, 1993). 3,4,5-trimethoxybenzaldehyde is an intermediate in the synthesis of pharmaceutical drugs such as, Cintriamide, Bactrim (Biseptol), Trimethoquinol, trimethoprim and others (Sriprasanthi, 2012).



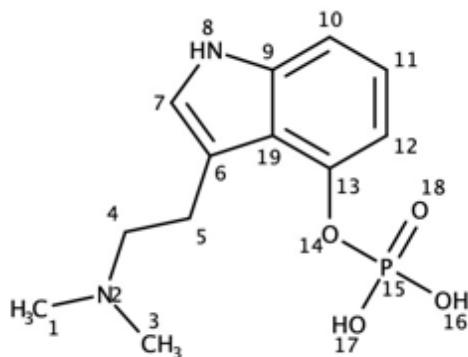
Scheme 2: Syringaldehyde to Trimethoprim

Trimethoprim synthesis with 3,4,5-trimethoxybenzaldehyde as a main precursor occurs by condensation of 3,4,5-trimethoxybenzaldehyde with malonic acid dinitrile in a Knoevenagel reaction, or Knoevenagel condensation of 3,4,5-trimethoxybenzaldehyde with ethyl cyanoacetate reaction (R.S.Vardanyan en V.J.Hruby, 2006).



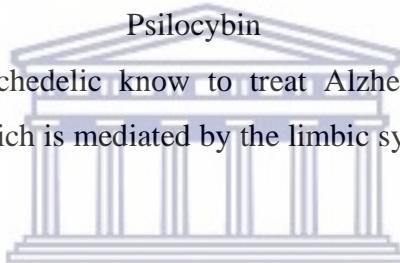
Scheme 3: from 3,4,5-trimethoxybenzaldehyde to trimethoprim both methods

Mushrooms are fleshy spore-bearing fungi and are subdivided into, edible and non-edible mushrooms. Some mushrooms contain hallucinogenic properties such as the *Psilocybe semilanceata* mushroom that contain the compound Psilocybin which is responsible for the hallucinogenic properties within the mushroom (Wieczorek *et al.*, 2015):

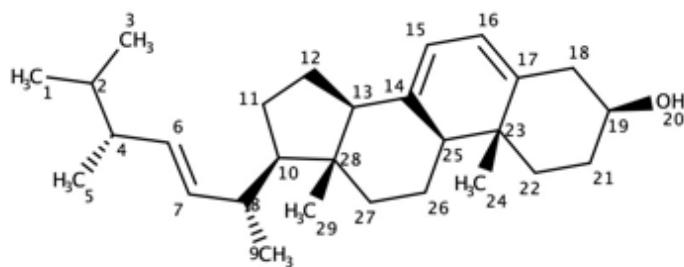


Psilocybin

Psilocybin is an alkaloid psychedelic known to treat Alzheimers disease, dementia, and depression by micro-dosing which is mediated by the limbic system (Vann Jones en O'Kelly, 2020).



Morels possess bioactive compounds that permits it to possess antimicrobial, anti-fungal anti-oxidative, anti-inflammatory, anti-tumor, and immunostimulatory properties (Bulam, 2019). Ergosterol is a neutral lipid and the main component of the fungal membranes that treats severe human fungal infections (Alcazar-Fuoli en Mellado, 2012).

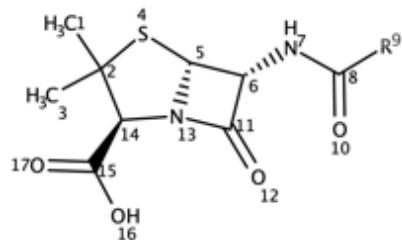


Ergosterol

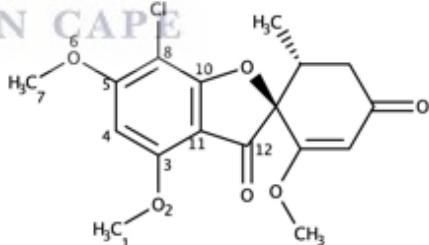
The pathway for the biosynthesis of ergosterol requires 20 enzymes and the synthesis of squalene from mevalonate (Alcazar-Fuoli en Mellado, 2012). Squalene is a cholesterol and triglyceride level lowering compound which is currently being utilized in a variety of cancer therapies (Reddy en Couvreur, 2009).

Yeasts are single-celled fungi. Yeasts such as *Saccharomyces*, are used in bread baking and alcohol production due to its abundance in vitamin B (Joseph en Bachhawat, 2014). Other yeasts such as *Candida albicans* are often present in the human body without causing disease unlike malignant yeasts, such as cryptococcosis, which affect the lungs, meninges, and the coverings of the brain and spinal cord of the human body (Hernday *et al.*, 2010).

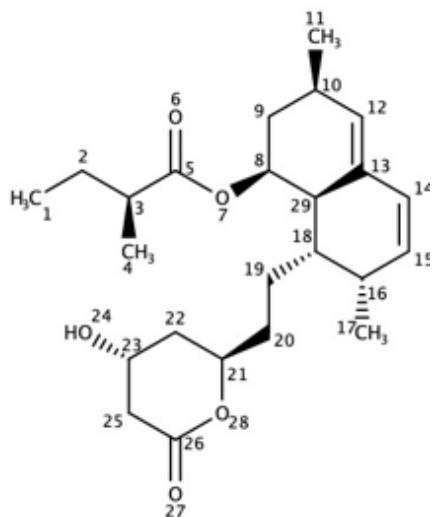
Moulds are long filamentous fungi commonly referred to as Hyphae .The *Aspergillus* and *Penicillium* fungi species are considered to be of human or animal disease importance (Houbraken, de Vries en Samson, 2014). Found as Saprophytes, both these fungi species are ubiquitous, unlike certain species viewed as immune-compromisers (Pitt, 1994). The *Aspergillaceae* fungal species produces secondary metabolites known to be pharmaceuticals, some of them being, penicillin which is an antibiotic found in *Penicillium rubens*, mycophenolic acid which is an immunosuppressant found in *Penicillium brevicompactum*, Griseofulvin which is an antifungal found in *Penicillium griseofulvum* and lovastatin known as the cholesterol lowering agent found in *Aspergillus terreus* and Streptomycin which is an antibiotic found in *actinobacterium Streptomyces griseus* (Holt, 1975) (Marinelli en Marcone, 2011) :



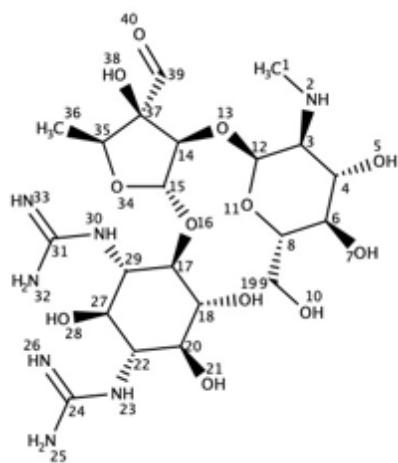
Penicillin



Griseofulvin



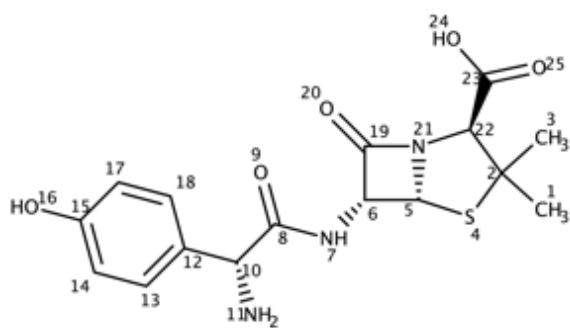
Lovastatin



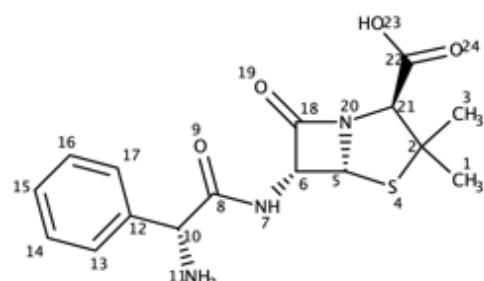
Streptomycin



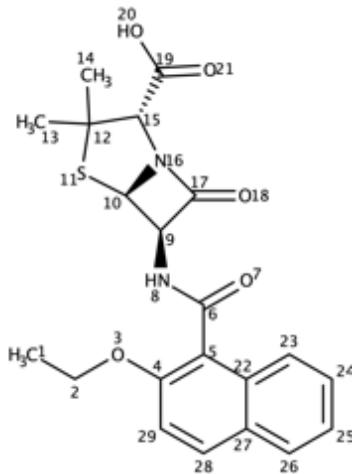
A number of medications are now derived or contain penicillin, some of them being,



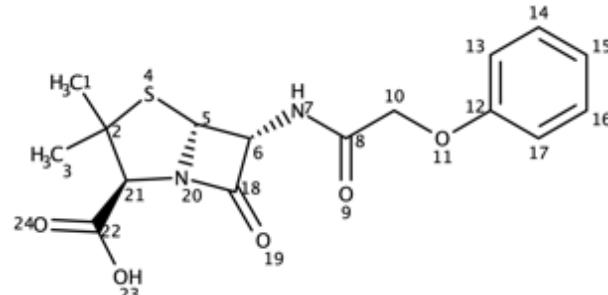
Amoxicillin,



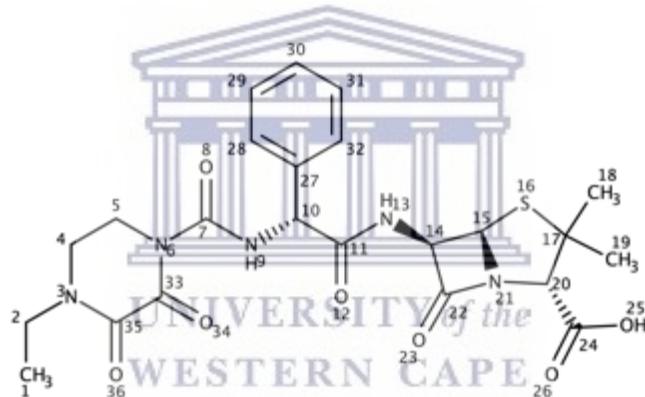
Ampicillin,



Nafcillin,

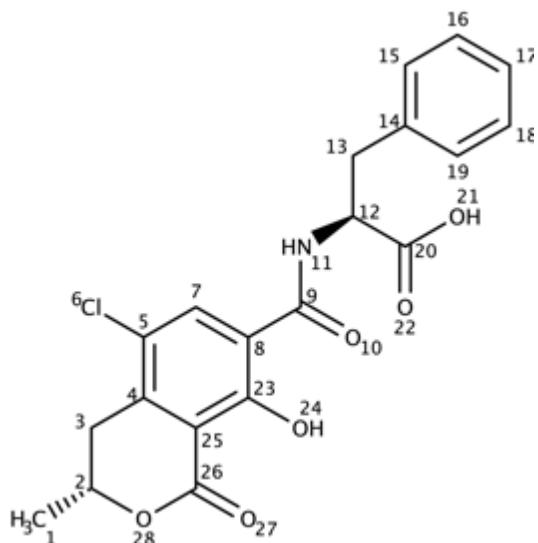


Penicillin V,

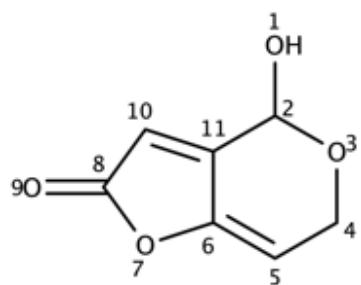


Piperacillin

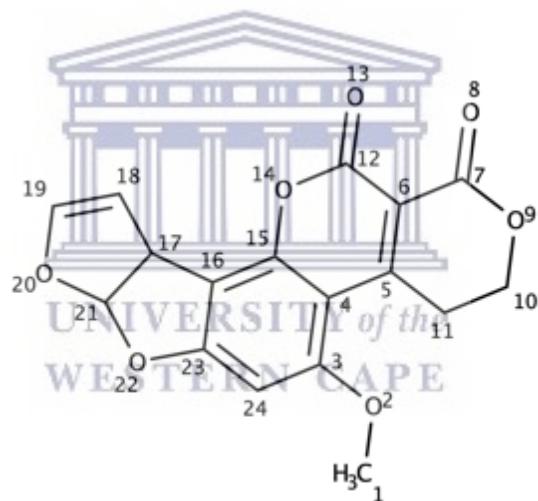
Moreover, not all secondary metabolites found in these fungi species are safe for human and animal consumption or used in medicinal uses (Frisvad, 2014). Mycotoxins are considered to be any toxic compounds, or secondary metabolites, produced by a fungus, mostly grown in food. Some of these mycotoxins found in the *Aspergillus* and *Penicillium* fungi species are (Haschek en Voss, 2013):



Ochratoxin A



Patulin



Aflatoxin

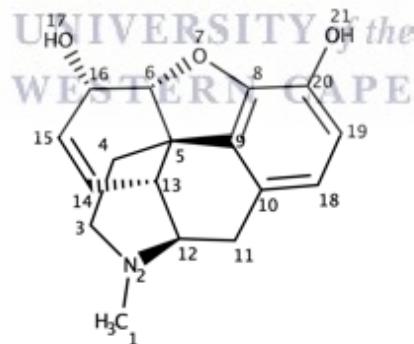
2.1.1 Natural Products and potential drug development

2.1.1.a) Plants

Natural products are any naturally occurring substances generally taken as a secondary metabolite (Gurnani *et al.*, 2014). Over 300,000 secondary metabolites exist, and their primary function is to increase the likelihood of survival of an organism by attracting or repelling other organisms (Barrios-González, 2018).

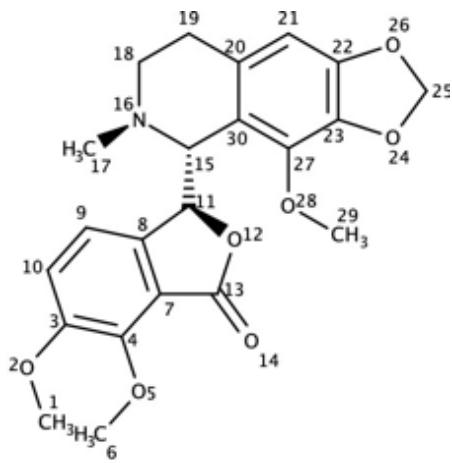
Natural products, specifically compounds and products isolated from plants, have been utilized to treat human ailments, in the forms of extracts, decoction, oils, powders, and mixtures for centuries (Mpofu *et al.*, 2014). They contain an extensive range of unknown and unexplored compounds where several are nearly impossible to emulate, thus natural products will remain a potential source of drug discovery and development in the future (Müller *et al.*, 2000).

In 1803 Wilhelm Sertürner isolated a crystalline substance from *opium* and named it morphine (Huxtable en Schwarz, 2001). With formula $C_{17}H_{19}NO_3$ and IUPAC name (4R,4aR,7S,7aR,12bS)-3-methyl-2,4,4a,7,7a,13-hexahydro-1H-4,12-methanobenzofuro[3,2-e]isoquinoline-7,9-diol morphine is used as a pain reliever (Holstege, 2005).



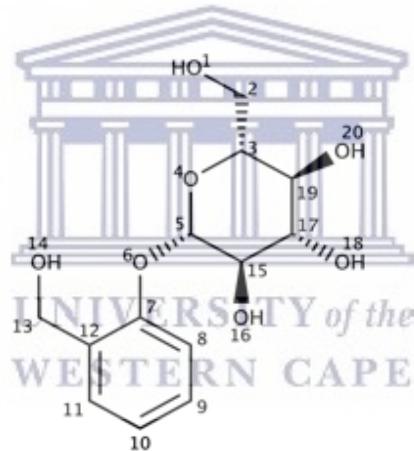
Morphine

Narcotine also known as noscapine, like morphine isolated from the *Papaveraceae* plant species or as otherwise known as *opium*, possesses painkilling, cough-suppressing, anti-cancer and various other properties (Hao, Gu en Xiao, 2015).

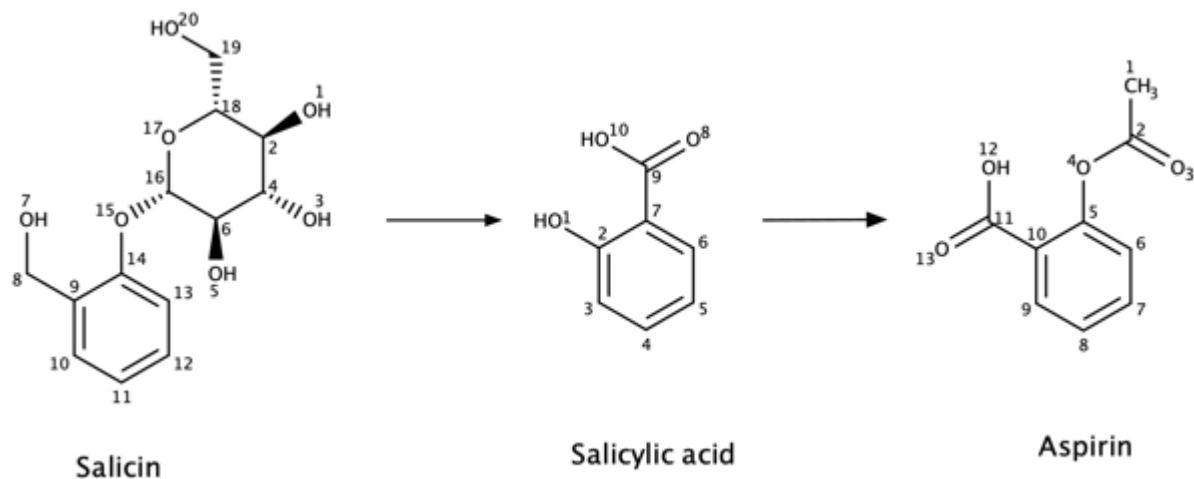


Narcotine

A few years later, in 1899, Bayer introduced aspirin which was the first semi-synthetic pure drug based on a naturally occurring compound named Salicin, obtained from the bark of the plant species *Salix* (Yunes *et al.*, 2005).



Salicin

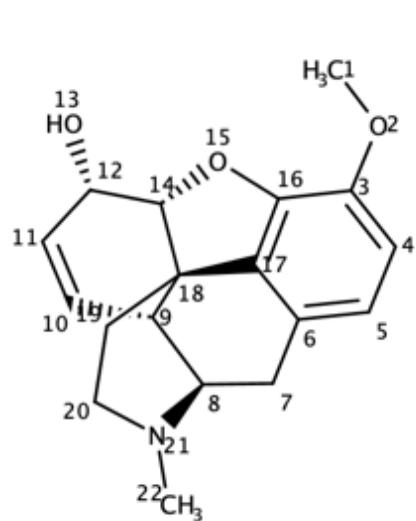


Salicin

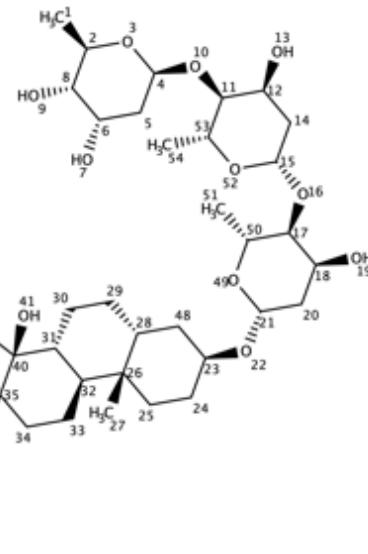
Salicylic acid

Aspirin

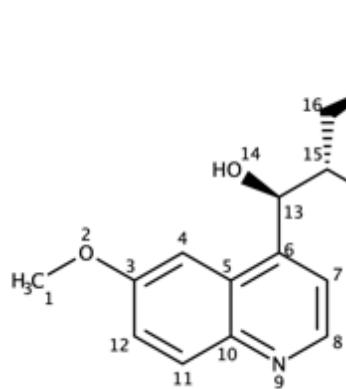
Subsequently, isolation of other drugs followed by the successful isolation of morphine and aspirin. Some of the early isolated drugs post morphine and aspirin are: (Veeresham, 2012):



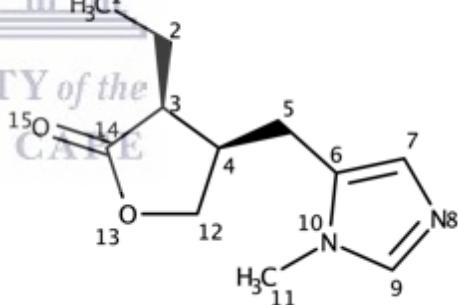
Codeine



Digitoxin



Quinine



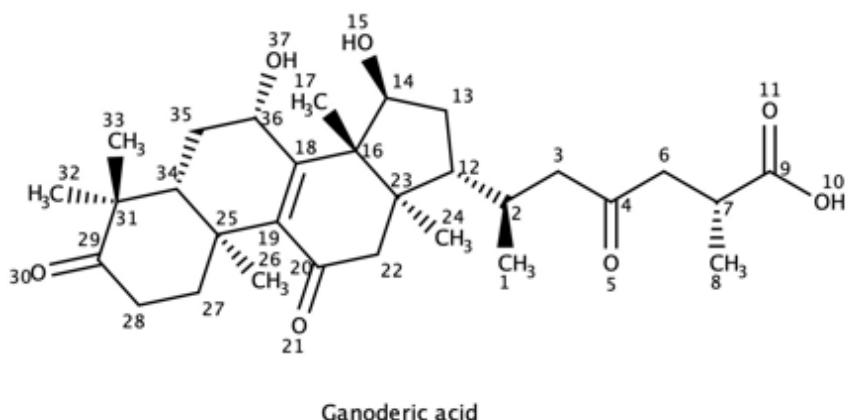
Pilocarpine

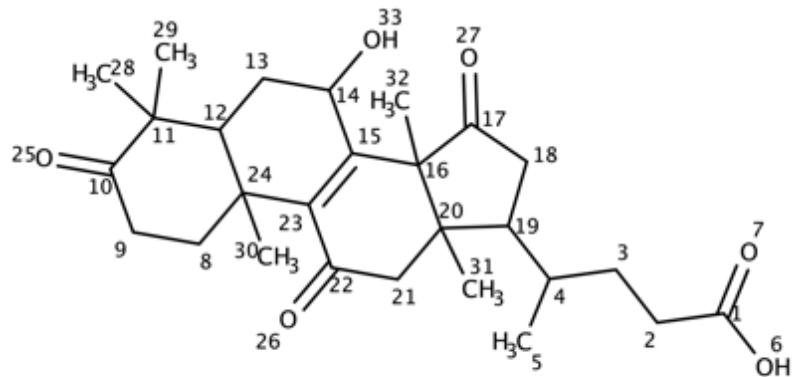
2.1.1.b) Fungi

The introduction of sulphonamide antibiotics and penicillin revolutionized the drug discovery process by using natural products as a source, decreasing the fatality rates associated with bacterial infections (Groman, 2014). More than 80% of drug substances involved in drug discovery in that era were reported to be natural products, or they were inspired by natural product structures (Krause en Tobin, 2013).

Anti-cancer drugs have had natural products as their most comprehensive and single source of material since their approval in the Western countries and Japan, where 85 compounds representing 48,6% out of 100%, were natural products or compounds derived from natural products (Krause en Tobin, 2013). The study of fungi metabolites has shown that many compounds found in fungi are sources or have been proven to be antibiotics, antifungal, immunosuppressive and cholesterol-lowering agents (Kumar *et al.*, 2017).

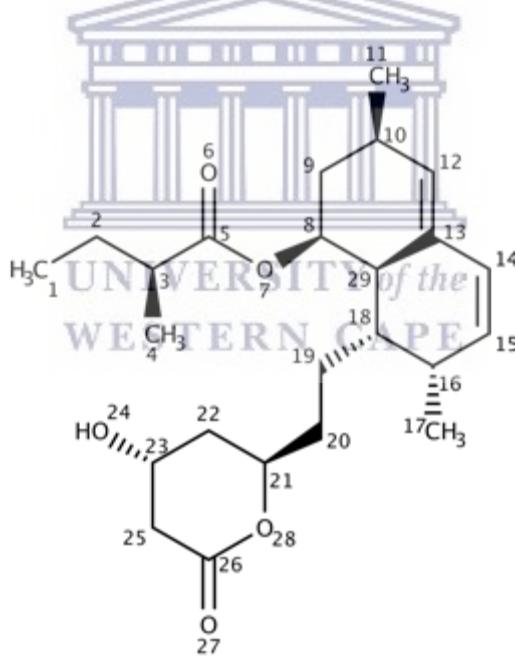
Fungi have been extensively used as medicine due to their curative properties. Lingzhi is the rarest and most precious medicinal fruit body of the *Ganoderma* fungus in Chinese culture (Geng *et al.*, 2020). This fungus is known to prevent and mitigate a variety of clinical conditions such as amnesia, chronic bronchitis, neurasthenia, coronary heart disease, stroke, asthma, insomnia, a female endocrine disorder, cancer, arthritis, chronic hepatitis, thrombosis, and many other diseases (Moore, 2001). Some of the compounds responsible for the prevention of these diseases are: Ganoderic acid A, B and F and Lucidenic acid A, B, C, N and F with the following backbones (Haubrich, 2018).





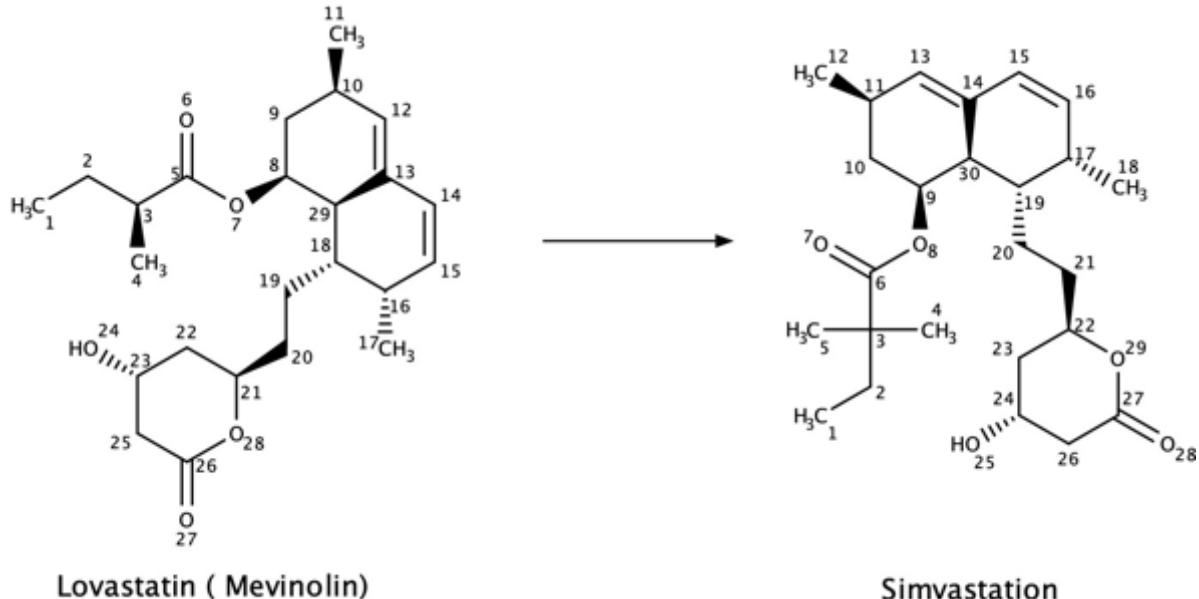
Lucidenic acid

In the western side of the world, the use of fungi increased due to their interest in fungi metabolic products in medicine after the success of penicillin (Jakubczyk en Dussart, 2020). Other successful stories of drugs developed with fungi as a source, is the cholesterol-lowering agent Mevinolin or otherwise known as Lovastatin. (Alberts *et al.*, 1980).



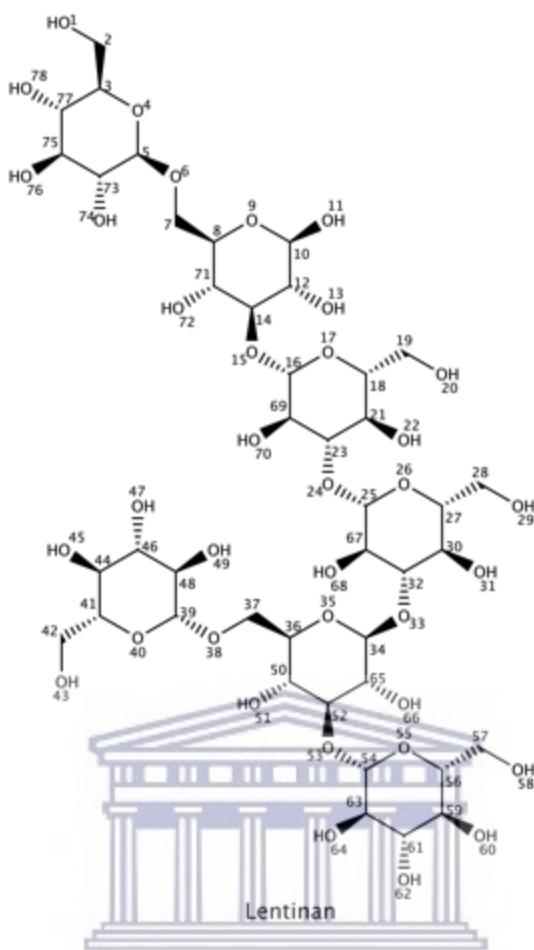
Mevinolin (Lovastatin)

Mevinolin was isolated from *Aspergillus terreus* and *asperlicin*, where the structure manipulation of this compound has led to the discovery of benzodiazepines, which are used to treat severe anxiety or insomnia, an example would be Simvastatin (Guina en Merrill, 2018).

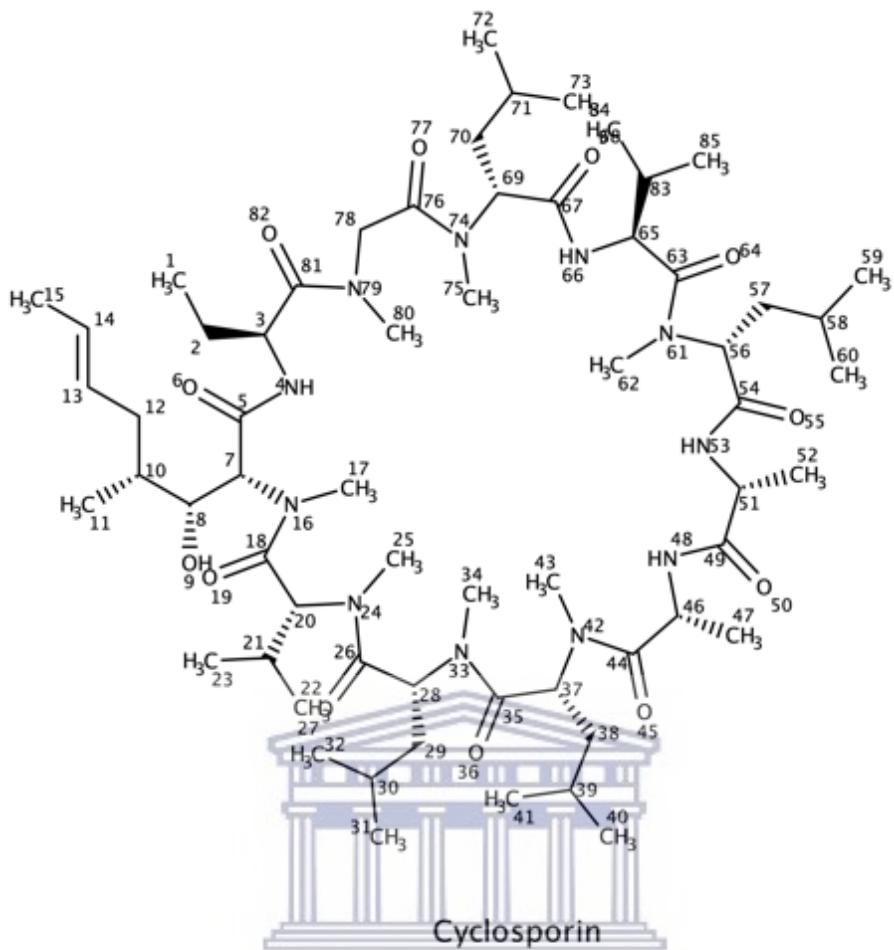


The *Cryptosporiopsis quercina* and endophytic fungus isolated from *Tripterygium wilfordii* have shown antifungal activity against some fungal pathogens in humans such as *Candida albicans* and *Trycophyton mentagrophyte* (Strobel *et al.*, 1999).

Aspergillus parasiticus is an endophytic fungus of the coastal redwood which produces sequoiatones A and B, which show selective inhibition of human tumour cells, specifically, breast cancer cell lines (Stierle, Stierle en Bugni, 1999). Amrubicin hydrochloride was isolated from *Streptomyces peucetius*. This species is related to *Doxorubicin* which is used to treat soft tissue, acute leukaemia, lung cancer, bone sarcomas, thyroid cancer, and both Hodgkins and non-Hodgkins lymphomas (López-González *et al.*, 2013). More in cancer research, lentinan, a polysaccharide isolated from the *L. edodes* mushroom has been used to treat stomach and various other cancers types (Yang, Zhou en Zhang, 2019).

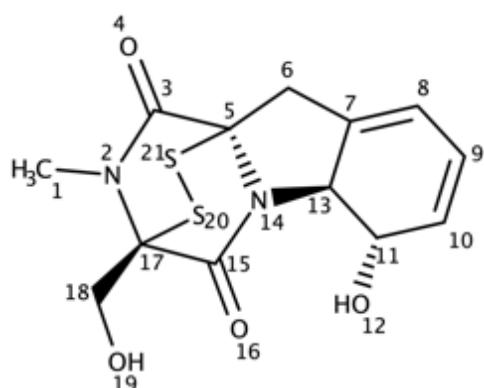


Natural products that were first discovered as antibiotics have recently shown to possess other activities in mammals, for example, cyclosporin (Herbrecht, Lid en Bergerat, 1990). Cyclosporin is capable of suppressing the recipient's immune response to avoid organ rejection during organ transplant (n, Eunsung Mouradian, 2008). This fungi is currently widely used and known as an immunosuppressant, and has played an essential role in improving the rate of successful transplant operations (Tabassum Khan, 2017).



UNIVERSITY of the WESTERN CAPE

Gliotoxin is a mycotoxin found in marine fungi that contain sulphur, it belongs to a naturally occurring 2,5-diketopiperazines and is also utilized to regulate the immune system and manage the postoperative recovery of transplant patients (Kumari en Srividhya, 2020).



Gliotoxin

2.1.2 Mushrooms

Mushrooms possess tonic, medical properties and attributes, generally contain higher protein contents compared to most vegetables and wild plants due to the high amounts of essential amino acids, making them the best sources of vitamins and minerals for animals and humans (Valverde, Hernández-pérez en Paredes-lópez, 2017)(Temesgen, 2018). They possess eight important amino acids, polyunsaturated fatty acids, and small amounts of saturated fatty acids (Kakon, Choudhury en Saha, 2012).

Mushroom fungi play key roles in forest ecosystems due to their unique ability to break down organic matter such as leaves, wood, and various others (Onifade *et al.*, 2019).. Wild mushrooms contain extremely high amounts of vitamin D2 and small amounts of vitamin B-complex and vitamin C (Cardwell *et al.*, 2018).



2.1.3 Mushrooms and health benefits

From a nutritional perspective, since mushrooms contain high levels of protein and fiber the cultivation of mushrooms has been considered as an alternative to protein supply in countries with high rates of malnutrition (Figueiredo en Régis, 2017).

Certain mushrooms are known to contain medicinal properties, which have been widely used and studied as possible sources of treatment or drug development for diseases such as cardiovascular , cancer, viruses, bacteria, parasites, and many others (Hyde *et al.*, 2019).

Polysaccharide-proteins extracted from medicinal mushrooms may enhance innate immune responses, resulting in antitumor activities in animals and humans (Lull, Wickers en Savelkoul, 2005).

Certain species of mushrooms are utilized as a source of healing, prevent the spread of tumor cells and AIDS (Wang, Wang en Ng, 2007), treat colds, stomach aches, headaches, hepatitis B, reduce fatigue, cholesterol levels, blood levels and strengthening of the immune system (Jong en Birmingham, 1992).

Methanolic extracts of six wild mushrooms, showed significant antimicrobial activity against *B. subtilis*, *S. aureus*, *E. coli*, *P. aeruginosa*, and *Candida Albicans* (Venturini *et al.*, 2008). These six mushrooms are: *Lycoperdon perlatum*, *Cantharellus cibarius*, *Romaria formosa*, *Marasmius oreades*, *Pleurotus pulmonarius* and *Chelonistele vermicularis* (Ahmed *et al.*, 2015).

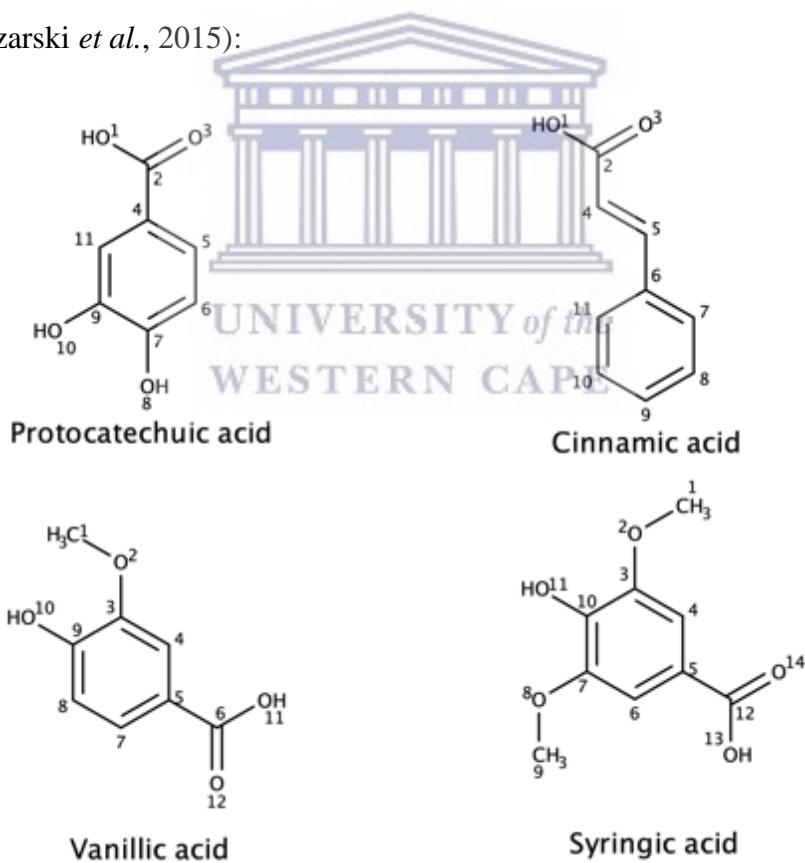
In addition mushrooms are good sources of zinc, which benefits normal fetal growth during pregnancy (Mohmand *et al.*, 2011) . Certain species have also shown potential *in vitro* for lowering the level of oestrogen and testosterone while others demonstrate anti-inflammatory effects (Thornton, 2002).

2.1.4 Kinds of mushrooms

Mushrooms are divided into, edible and non-edible mushrooms. Edible mushrooms possess desirable taste and aroma and are naturally of white colour and non-edible mushrooms are naturally coloured mushrooms with the exception of certain white mushrooms. Some of these mushrooms possess compounds with properties which are useful in medicine and in human health (Fernandes et al., 2014). Some of these mushrooms are:

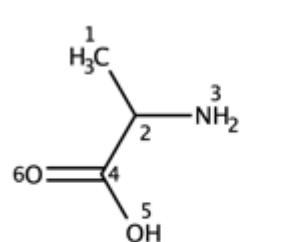
Macrolepiota

The parasol mushroom, *macrolepiota* is part of the *Agaricaceae* family. This mushroom is an edible mushroom, a basidiomycete fungus that contains bioactive compounds which are responsible for its antioxidant properties and anticancer activities against the human lung cancer cell line. Some of the compounds responsible for this antioxidant properties are the following: (Kozarski et al., 2015):

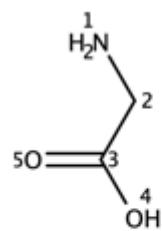


Auricularia

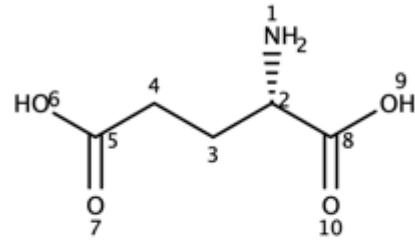
Auricularia is part of the *Auriculariaceae* family. This fungus is also an edible mushroom and it resembles an ear possessing a gelatinous texture. This fungus contains small amounts of essential amino acids such as the following and others: (Kadnikova *et al.*, 2015)



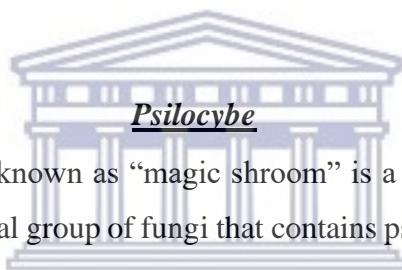
Alanine



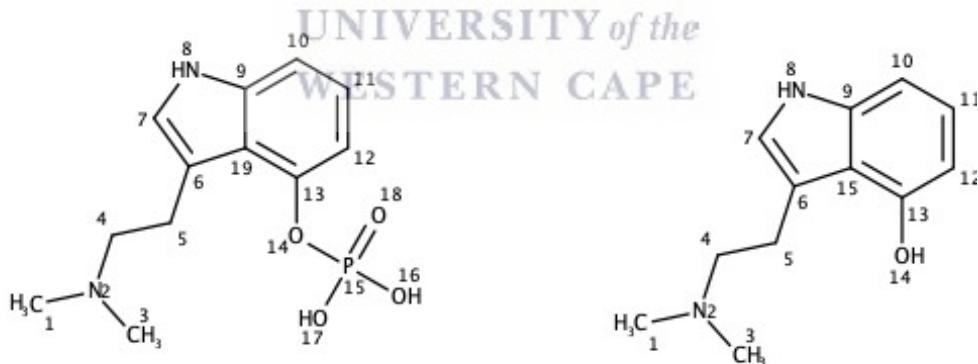
Glycine



Glutamic acid



Psilocybe mushrooms are also known as “magic shroom” is a poisonous mushroom, and are polyphyletic which is an informal group of fungi that contains psilocybin and psilocin (Seneca, 2007).



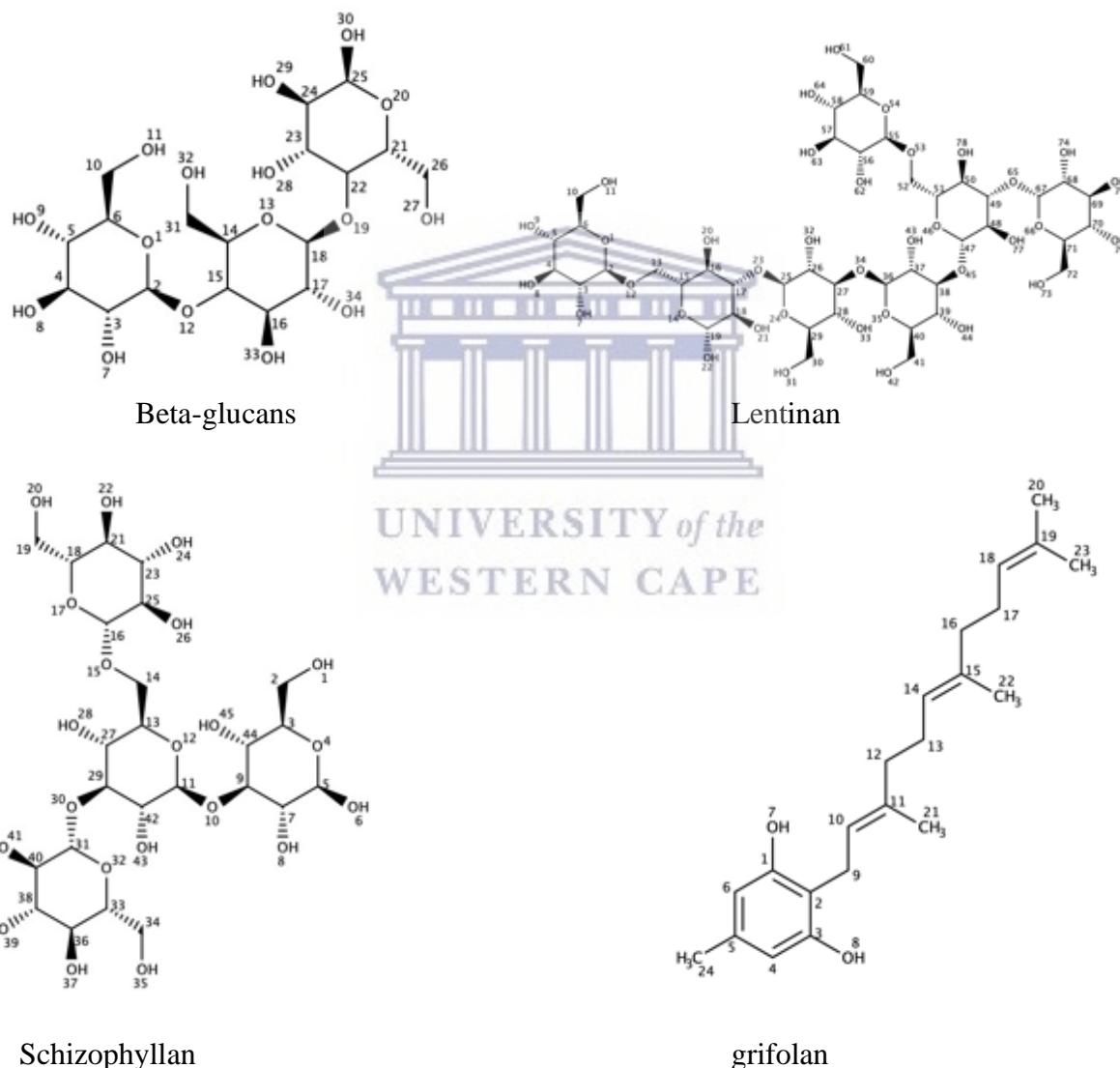
Psilocybin

Psilocin

These compounds are responsible for creating the feeling of disorientation, lethargy, giddiness, euphoria, joy, depression, and even paranoia when given in high doses (Clarke, 2007) however, when taken in micro doses, psilocybin and psilocin are used to treat mental disorders (Singh, 2017).

2.1.5 Mushrooms in cancer research

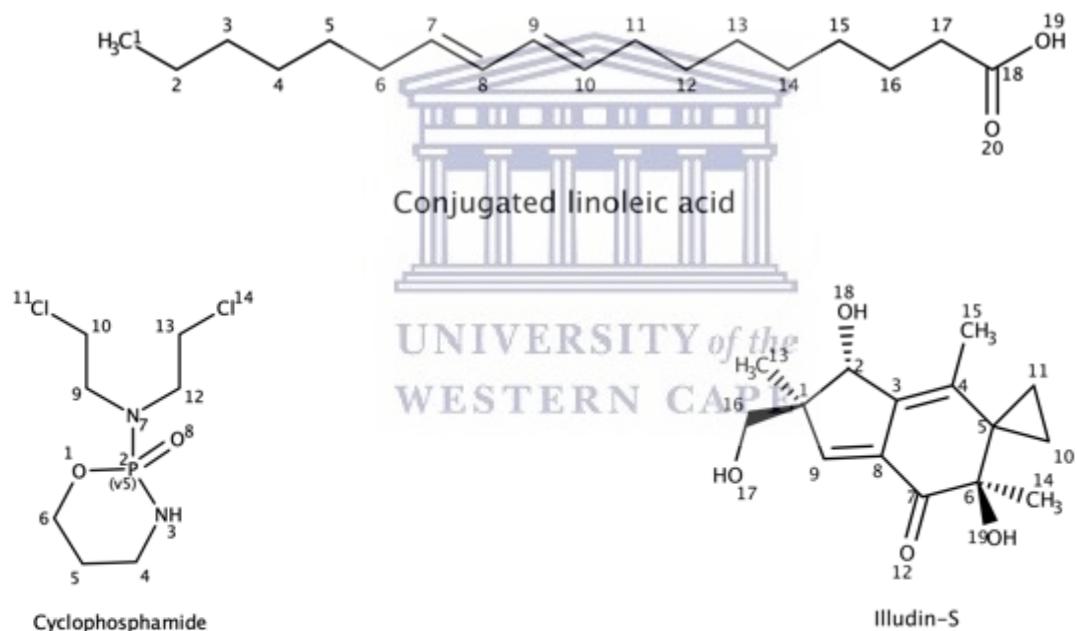
Mushrooms contain bioactive compounds and it is considered as a source of drug and nutraceutical developments, making them a functional food source (Üstün, Bulam en Pekşen, 2018). Many pharmaceutical substances that contain unique and potent properties were isolated from mushrooms (Kimura, 2013). Five mushrooms, *Lentinus edodes*, *Schizophyllum commune*, *Grifola frondosa*, *Sclerotinia*, *Pleurotus rimosus* (Chatterjee en Patel, 2016) are known to possess antitumor activities due to their SSG constituents (C.F.R. Ferreira *et al.*, 2012):



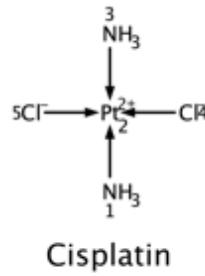
Mushrooms that contain anti-cancerous compounds, play an important role as inducers of reactive oxygen species, inhibitors of mitotic kinase, angiogenesis, topoisomerase, and anti-mitotic, leading to apoptosis and finally checking cancer proliferation (Patel en Goyal, 2012).

The ethyl acetate, methanol, and aqueous extracts of *Pleurotus rimosus* produces compounds which inhibit Dalton's Lymphoma Ascites cell line induced solid tumor and EAC cell line induced ascites tumor in mice and the antitumor effects are higher in ethyl acetate extracts compared to other extracts (Ajith en Janardhanan, 2007). In the methanol and aqueous extracts, a significant amount of antitumor properties helps inhibit tumor development (Khan *et al.*, 2020).

The *Agaricus bisporus* mushroom, aids on the prevention and combats breast, colorectal, ocular, and prostate cancer (Wang *et al.*, 2021). Some of the compounds responsible for the medicinal properties of the *Agaricus bisporus* are; conjugated linoleic acid (CLA), Cyclophosphamide (CP), Illudin-s and various others (Chen *et al.*, 2006).

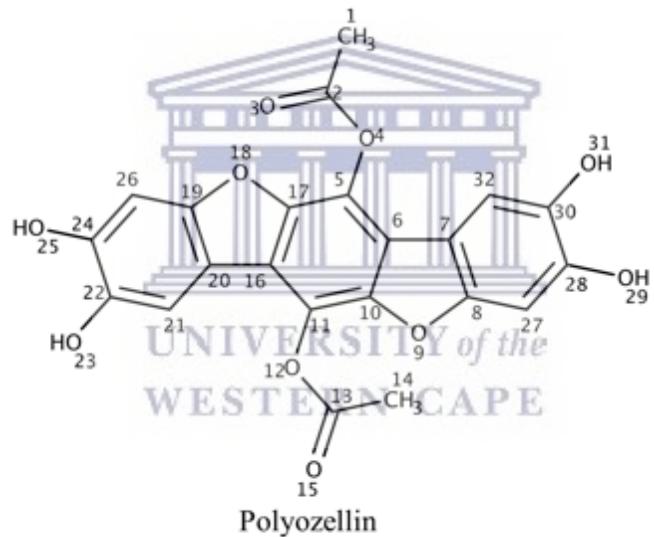


The *Maitake (D fraction)* mushroom, aids to combat breast cancer. The compounds responsible for these properties are (Alonso *et al.*, 2018): Cisplatin, sulphated polysaccharide (S-GAP-P), A novel polysaccharide-peptide GFPPS1b (Shi *et al.*, 2007).



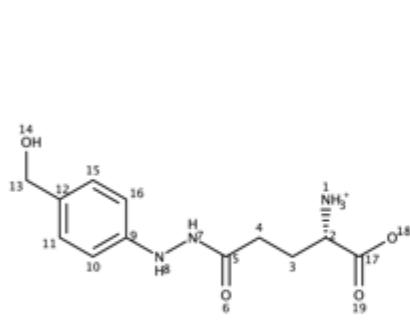
Pleurotus ostreatus, combats breast cancer. The compounds responsible are the water-soluble polysaccharide (POPS-1) (Radzki *et al.*, 2016).

Polyozellus multiplex, known to combat hepatocellular carcinoma, colon cancer, leukemia, and gastric carcinoma, and the compound responsible is Polyozellin (Chen, Lee en Kirschner, no date).

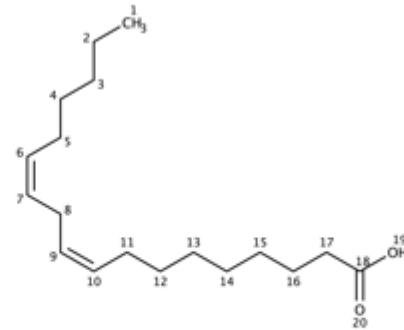


Pleurotus eryngii, is known to combat hepatocellular carcinoma, and the protein responsible is lectin (Wang, Gao en Ng, 2000).

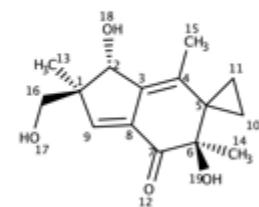
Agaricus blazei (Murill), is known to combat myeloma, hepatic cancer, leukemia, stomach and lung cancer, and the compounds and proteins responsible are, Agaritine, Agaricus bisporus lectin (ABL) Agaricus polytricha proteins (APP), Linoleic Acid and Illudin-s (Rodríguez P *et al.*, 2017).



Agaritine

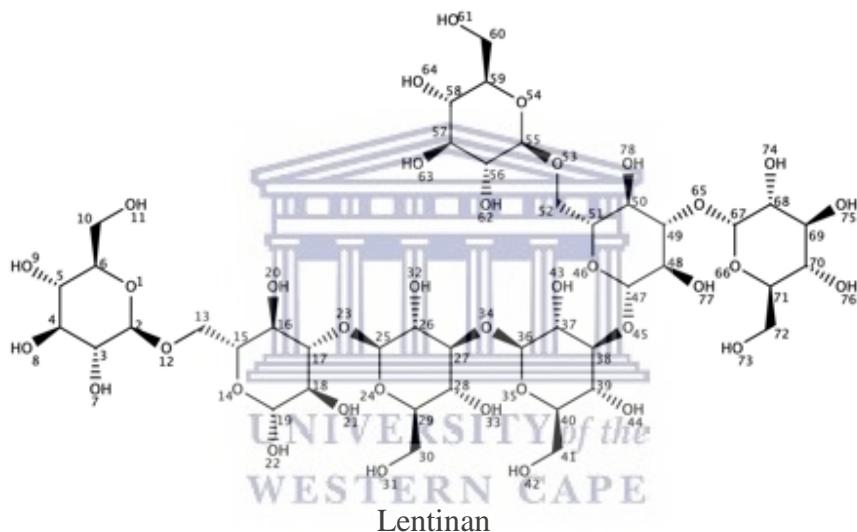


Linoleic acid



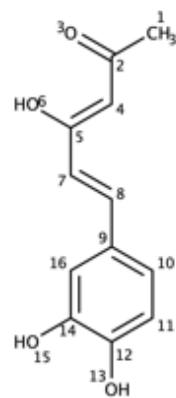
Illudin-s

Lentinula edodes is known to combat lung, cervical/ovarian, gastric and skin cancer. The compound responsible is lentinan (Zhang *et al.*, 2019).



UNIVERSITY of
WESTERN CAPE
Lentinan

Phellinus linteus it is known to combat colon cancer and the compound responsible is Hispolon (Sarfraz *et al.*, 2020).



Hispolon



UNIVERSITY *of the*
WESTERN CAPE

2.1.6 Rust fungi

Rust fungi are parasites that grow on plants by obtaining their nutrients, reproduce and complete their life cycle on plants (Voegele en Mendgen, 2011). Rust fungi cause diseases in economically important plant species and certain species of this fungus are highly specific on what plant to infect, colonize and reproduce (Kolmer, Ordóñez en Groth, 2009).

Rust fungi possess a complex life cycle called the macrocyclic cycle and it undergoes five distinctive spore stages on two unrelated hosts (Ma *et al.*, 2020).

Aecial colonies reflect the pycnial clusters, and are formed by the growth of the rust species that infect leaves on the lower leaf surface as dikaryotic hyphae (Chen *et al.*, 2014). Dikaryotic aeciospores can travel long distances and are produced in chains and quantity within pustules (Leonard en Szabo, 2005). Unlike aecial colonies, urediniospores are produced singly on stalks (Shattock *et al.*, 2003). When the nutrients from the plant start to decline the uredinia converts to telia and produces increasing numbers of teliospores which are thickly walled and resistant to cold or drying as they begin to darken (Mendgen, 1984).

The *Uromycladium tepperianum* MacAlpine, the fungi under research, is a rust fungus that produces large, irregular-sized galls on the leaves and stems of the plant (Ratio, 2017). It was introduced in South Africa for the control and spread of the acacia saligna bushes (Control *et al.*, 2010). The *Uromycladium tepperianum* MacAlpine fungus causes the limitation in the growth and seed production of the acacia tree due to them feeding on the plants' nutrients and then forming heavy large galls around the tree (Wood en Morris, 2007).

Currently, no discoveries of bioactive compounds have been made on rust fungi, hence the investigation on the *Uromycladium tepperianum* MacAlpine fungus was conducted due to the limitation of the growth and seed production this fungus carries out on the acacia tree species.

2.1.7 Application of LC-MS and MS in plant extract analysis.

Chromatographic analysis, aids on the optimization of samples and complex plant extract mixtures containing numerous metabolites in which the chemical composition of these may differ between extracts (Keskes *et al.*, 2017). The detection of all secondary metabolites found in a plant may not be permitted when different types of chromatography detectors are utilized. Some of these chromatographic techniques are, RI (retention index), electrochemical light scattering, UV (Ultraviolet-visible spectroscopy) and various others, unlike MS (mass spectrometry) (Keskes *et al.*, 2017). MS is considered to be the most sensitive method of molecular analysis due to its power of mass separation and since all natural compounds possess a molecular weight, MS is one of the most reliable chromatographic techniques (Wolfender en Hostettmann, 1995).

LC-MS like MS is a suitable interface for secondary metabolite analysis, its interface possesses the capability of detecting weak chromophoric compounds specifically when extracts contain numerous metabolites with low yields (Wolfender en Hostettmann, 1995).

With the aid of databases these chromatographic techniques are utilized to separate and characterize complex compound mixtures and isomers. In the field of organic chemistry, the database Global natural products social molecular network is one of the most reliable natural product databases due to it being a mass spectrometry ecosystem based database, possessing open access, to annotated fragmentation mass spectrometry data, aiding on the identification of data and compound characterization.

Chapter 3

Materials and methods

3.1 Reagents and General methods

3.1.1 Chemicals and reagents

Solvents: dichloromethane (DCM), *n*-hexane (*n*-hex), ethyl acetate (EtOAc), butanol (BuOH), methanol (MeOH), Chloroform (CHCl₃), Acetone, vanillin, standard solution, phenol, sulphuric acid, dragendorff reagent, and ninhydrin reagent.

3.1.2 General experimental methods

The employed experimental methods in the study included, NMR Spectroscopy, Mass Spectrometry, column chromatography, Liquid Chromatography - Mass Spectroscopy, Gas Chromatography- Mass Spectroscopy, ninhydrin and Dragendorff reagents, hydrolysis, phenol sulphuric acid test and UV-lamp.

3.1.3 Evaporation of the solvents

Solvent evaporation was performed on a Buchi Rotavapor R-114. The water bath temperature was maintained at 50 °C for plurality of evaporation, however, kept at 60 °C when evaporating BuOH.

3.2 Spectroscopy

3.2.1 Nuclear magnetic resonance (NMR) spectroscopy

NMR Spectroscopic analysis was conducted in 1 and 2 dimensions and recorded at 25 °C on a 400 MHz Bruker advanced IIIHD Nanobay Spectrometer, using a 5mm BBO probe, deuterated chloroform CDCl₃ as the solvent solution. The coupling constants and chemical shifts were expressed as (J, Hz) and δ ppm respectively.

3.3 Chromatography

3.3.1 Column Chromatography (CC)

Silica gel 60 (0.040 - 0.063 mm) 230 - 400 mesh particle size (Merck) were packed in glass columns (10 - 30 mm diameter) for column chromatography.

3.3.2 Thin layer Chromatography (TLC)

Thin layer chromatography was carried out on the pre-coated silica gel of 0.2 mm layer thickness 60 F_{254} plates (Merck). TLC spot visualization was carried out under UV light at 254 nm and 366 nm, followed by the spraying of the TLC plate with vanillin spray reagent, prepared by dissolving 15 g of vanillin in 250 ml ethanol and 2.5 ml concentrated sulphuric acid. After the TLC plates were sprayed heating of the plates revealed the spots of colour.

3.4 Mass spectrometry (MS)

3.4.1 Gas Chromatography Mass Spectrometry (GC-MS)

GC-MS spectra were recorded by an Agilent technology gas chromatography coupled to 5977E MSQ model mass spectrometer at an isothermal temperature of 250 °C , equipped with a flame ionisation detector (FID) on a DB 225 capillary column. Helium was the carrier gas used. A temperature of 225 °C was programmed once the samples of 10 μ l were injected into the GC analyser on a flow rate of 1.4 ml/min.

3.4.1 Liquid Chromatography-Mass Spectrometry (LC-MS)

LC-MS analysis was performed at the University of Stellenbosch. LC-MS spectra were recorded by Waters Synapt G2, using modes ESI probe injecting into a stream of water and into a stream of acetonitrile, data was collected in ESI positive and negative modes with cone voltage 15 V.

3.5 *Uromycladium tepperianum* MacAlpine preparation

3.5.1 Plant Material

The *Uromycladium tepperianum* plant material provided by the Chemistry department and Professor Mabusela weighed 42,4 g, harvested in Khayelitsha suburb of Cape Town, South Africa GPS coordinates (34°01'05.4"S 18°36'26.1"E). A second and third collection was required to conduct the experiment due to small product yields. The second collection of the plant material weighed 772.49 g, and the final collection weighed 5 kg (5000 g).



Figure 1: Uromycladium tepperianum MacAlpine fungus and material in powder form
<http://hdl.handle.net/20.500.11939/1000>

3.5.2 Extract preparation

Maceration and crude extraction of the material were the two methods of preparation conducted. Part of the freshly ground material was macerated using 80% methanol and 20% water over a period of 24 hours (process was repeated five times), followed by evaporation of the methanol and freeze-drying of the residue. The surplus ground material was utilized to perform crude extraction by the addition of *n*-Hex, DCM, EtOAc and BuOH (singularly, leaving each solvent submersed in the plant material over a period of 24 hours) followed by filtration of the material and evaporation of the solvents utilizing the Buchi Rotavapor R-114, allowing the samples to dry into powder form. After acquiring the desired amounts of extract, the extracts were then singularly placed in a column, where fractions were collected, purified, isolated, and characterized utilizing chromatographic techniques, NMR, TLC, GC-MS and LC-MS.

3.5.3 Maceration Method (the first collection of material used 42,4g and the second batch used 300g)

Post maceration (80% MeOH and 20% water), filtration, and solvent evaporation (Buchi rotavapor), the sample was freeze dried, followed by solvent-solvent extractions (the process was repeated three times). The solvent-solvent extractions were conducted by four different solvents, *n*-hex , DCM, EtOAc, and BuOH. The dissolution of the dried samples was conducted by distilled water and transferred into a separating funnel were *n*-Hex, EtOAc, DCM, and BuOH were singularly added, followed by shaking of the funnel (three times). The aqueous and organic layers were left to separate over a period of four hours, followed by the separation of the organic layer.

3.5.4 Crude extraction method (second collection of material used 472,49 g)

The ground material was submersed in either: *n*-hex, DCM, EtOAc, or BuOH singularly, and left immersed overnight. The process was repeated 3-4 times followed by evaporation of the solvent with aid of a Buchi Rotavapor. After dry, the samples were weighed and screened using TLC.

3.5.5 Tannin containing compounds and its removal by acetone precipitation

The aqueous extract, left post solvent-solvent extraction and the separation of the organic layer was divided into two.

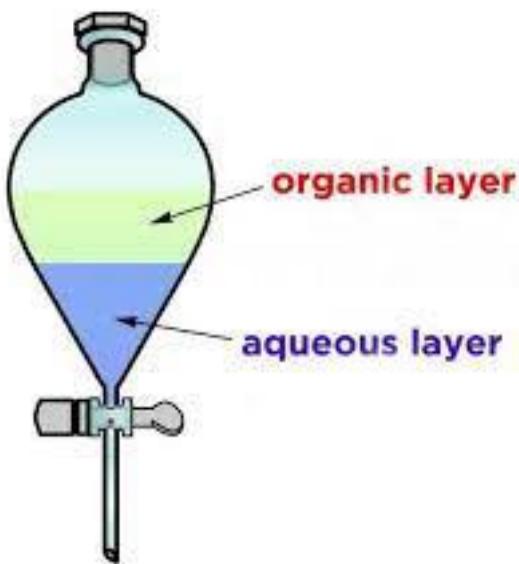


Figure 2: solvent-solvent extraction, layer separation

11,15 g (first experiment), and 6,23 g (second experiment) were utilized to conduct this experiment. 40 % water (80 ml) was added to a flask for material dissolution, followed by the gradual addition of 60 % acetone (120 ml), while stirring. The solution was left overnight to stir. The stirred solution was then placed in a centrifuge at 2300 speed for 20 minutes. The precipitate separated from the aqueous tannin-containing solution, was decanted and dissolved in deionized water, followed by the evaporation of the remaining acetone and freeze-drying of the material. Subsequently, the sample was prepared using hydrolysis methodology, for LC-MS and NMR characterization.

3.5.6 Hydrolysis

The proportions of the polysaccharides and other carbohydrates were determined by firstly, conducting hydrolysis of the crude material, where 100 mg of the sample and 10 ml of 2 M trifluoroacetic acid was added into a test tube and heated in a hot water bath at 100 °C for 24 hours, followed by LC-MS and NMR characterization (Van Wychen en Laurens, 2015).

3.5.7 Reagents test

Dragendorff reagent was utilized to detect the presence of alkaloids in the sample, by revealing a bright orange spot on TLC after submerging the plate spotted with the crude extract into the orange solution/reagent.

Ninhydrin reagent was used to detect the presence of amino acid in the sample, by revealing a bright purple spot on TLC after submerging the plate spotted with the crude extract into the solution/reagent.

Phenol/sulfuric acid test was conducted in order to detect the presence of carbohydrates in the sample post hydrolysis. A colour change to orange indicated the presence of carbohydrates in the sample (Ismail, 2017).

3.6 Isolation and purification of compounds

3.6.1 Fractionation of the hexane extract

The *n*-hex extract acquired from the second collection was merged with the first collection *n*-hex extract, and it was utilized to conduct this experiment due to the possession of higher yields. The *n*-hex extracts (2,34 g + 0,845 g+ 0,125g = 3,31g) was placed in a glass column for fractionization, by adsorption on silica gel by gravity elution . The following *n*-hex elution were utilized: 2 L of 100% *n*-hex for initial elution, and 2 L mixtures of hexane and EtOAc in the following ratios (*n*-hex 9,5: EtOAc 0,5), (*n*-hex 9: EtOAc 1), (*n*-hex 8: EtOAc 2) , (*n*-hex 7: EtOAc 3), (*n*-hex 6: EtOAc 4), (*n*-hex 5: EtOAc 5), (*n*-hex 4: EtOAc 6), (*n*-hex 3: EtOAc 7), (*n*-hex 2: EtOAc 8), (*n*-hex 1: EtOAc 9) and 100 % EtOAc. The collected fractions were analysed by TLC using 10 ml of *n*-hex and EtOAc mixtures as tank solution in the following ratios: (*n*-hex 9: EtOAc 1), (*n*-hex 8: EtOAc 2), (*n*-hex 7: EtOAc 3), (*n*-hex 6: EtOAc 4). Fractions with similar retention factors were merged. Fractions 35 to 55 were combined and sent to NMR for characterization leading to the isolation of compounds 1 and 2. Fractions 16-26 were combined for bearing the same retention factor. Subsequently purification of the fractions utilizing a silica gel column was conducted using MeOH as the eluting solvent. Fractions 4, 5 and 6 were obtained, combined according to retention factor, sent to NMR for characterization, thus leading to compounds 3 and 4 isolation.

3.6.2 Fractionation of the EtOAc extract

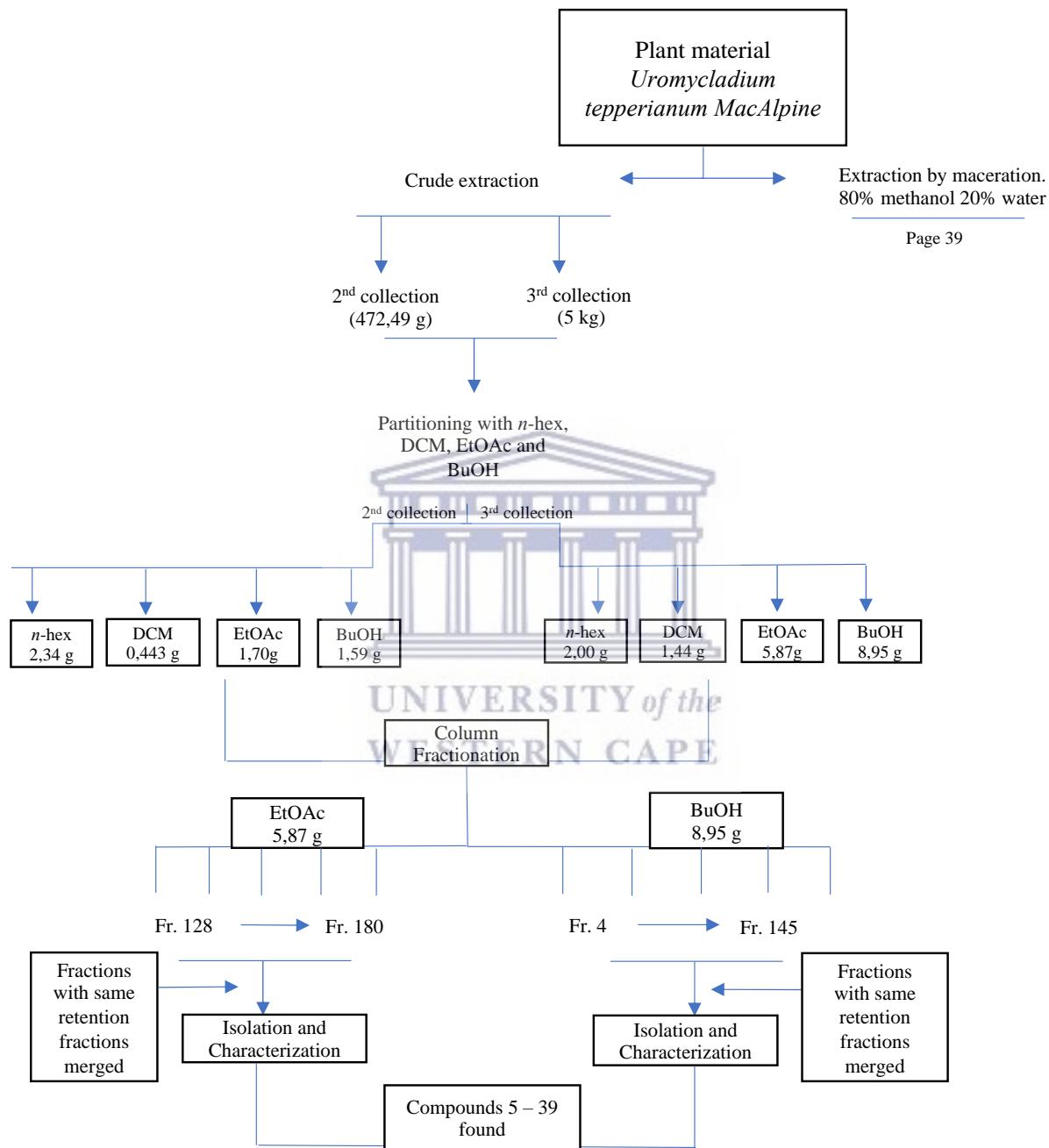
The EtOAc extract acquired from the third collection was utilized to conduct this experiment due to the possession of higher yield. The EtOAc extract (5,87 g) was placed in a glass column for fractionization, and adsorbed on silica gel by gravity elution. EtOAc elution were utilized: 2 L of 100 % DCM for initial elution , and 2 L of mixtures of DCM and EtOAc in the following ratios (9:1), (8:2), (7:3) , (6:4), (5:5), (4:6), (3:7), (2:8), (1:9) and 100 % EtOAc. The collected fractions were analysed by TLC using *n*-hex and EtOAc mixtures as tank solutions in the following ratios: (9:1), (8:2), (7:3), (5:5). Fractions with similar retention factors were merged together such as 128-140 , 181-190 , 201-207, 208-215, and 161-180. The first four merged fractions (128-140 , 181-190 , 201-207, 208-215) were prepared and sent to LC-MS for further

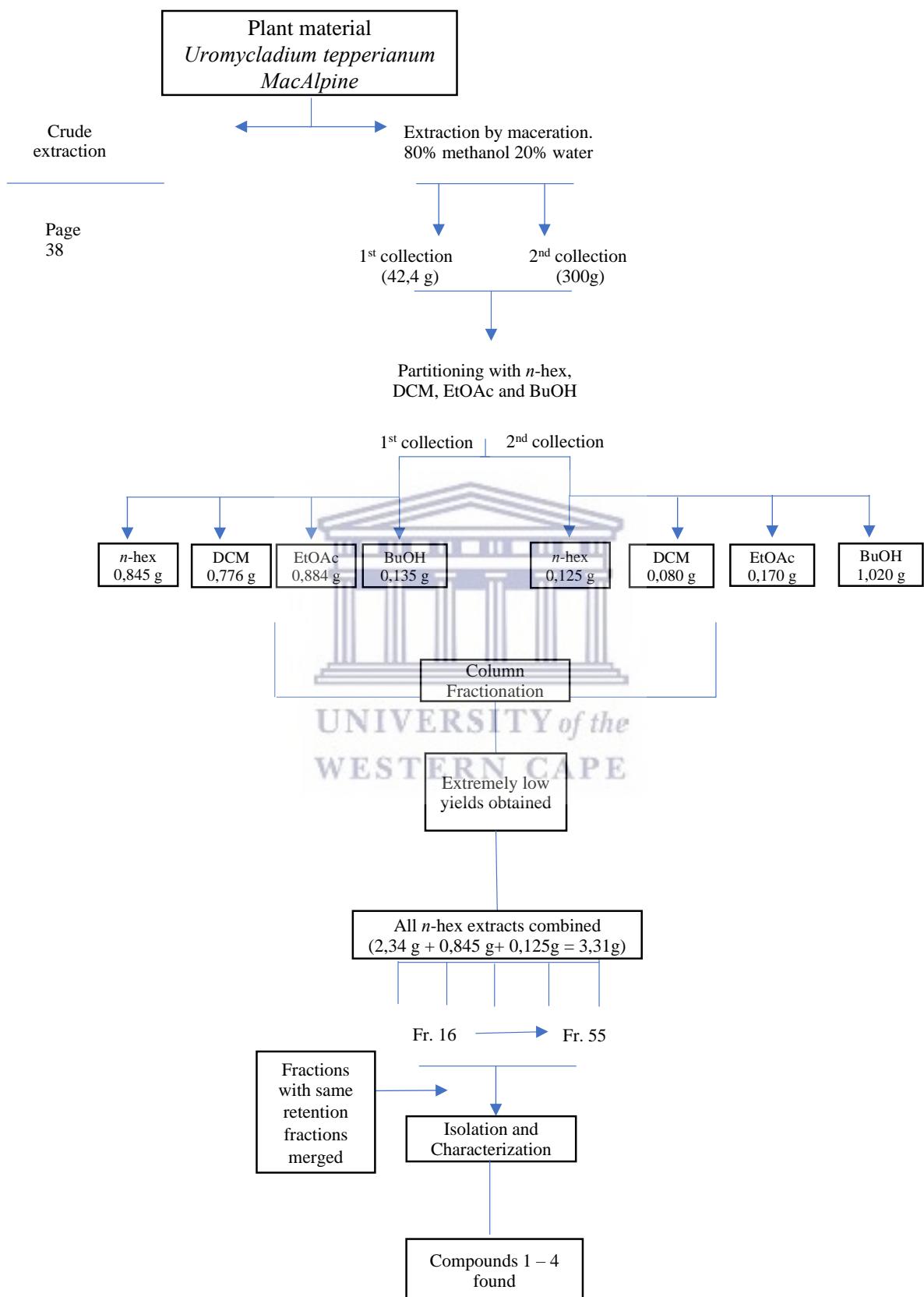
analysis followed by database characterization. Fractions 161-180 were merged and loaded onto a silica gel column for further separation, with elution mixtures of 2 L of 100 % DCM for initial elution , and 2 L of mixtures of DCM and EtOAc in ratios of (9:1), (8:2), (7:3) , (6:4), (5:5), (4:6), (3:7), (2:8), (1:9) and 100 % EtOAc, obtaining fractions 43-51 which were merged due to possessing the same retention factor, followed by LC-MS analysis at the University of Stellenbosch.

3.6.3 Fractionation of the BuOH extract

The BuOH extract acquired from the third collection was utilized to conduct this experiment due to the possession of higher yield. The BuOH extract (8,95 g) was placed in a glass column for fractionization, adsorbed on silica gel by gravity elution. DCM elution was utilized: 2 L of 100 % DCM for initial elution, and 2 L of mixtures of DCM and EtOAc in the following ratios (9:1), (8:2), (7:3) , (6:4), (5:5), (4:6), (3:7), (2:8), (1:9) and 100 % EtOAc. The collected fractions were analysed by TLC using chloroform and methanol mixtures as tank solutions in the following ratios: (9,5:0,5), (9:1).Fractions with similar retention factors were merged, such as fractions 103-145 and 96-102. The second set of fractions merged (96-102) were prepared and sent to LC-MS for further analysis. Fractions 103-145 were merged and loaded onto a silica gel column for further separation with elution mixtures of; 2 L of 100 % DCM for initial elution, and 2 L of mixtures of DCM and EtOAc in the following ratios (9:1), (8:2), (7:3) , (6:4), (5:5), (4:6), (3:7), (2:8), (1:9) and 100 % EtOAc, obtaining fractions 4-7, and 91-110 which were merged due to bearing the same retention factor and sent to LC-MS for analysis at the University of Stellenbosch.

Scheme 4: Isolation of natural products from *Uromycladium tepperianum* MacAlpine Fungus





Chapter 4

4.1 Results and Discussion

Subsequently to both methods of extraction, all samples underwent dry column chromatography. The results recorded are to be found in **Table 1**.

Table 1: Collections and Extract quantities

Solvents	1 st collection material weight: 42,4 g	2 nd collection material weight:300g	2 nd collection Crude material weight: 472,49g	3 rd collection material weight:5kg
Yield post maceration	4% yield 1,70 g post freeze dry	4% yield 13,27 g post freeze dry	N/A	10% yield 401,52 g post freeze dry
Hexane	0,845 g	0,125g	2,34 g	2,00 g
EtOAc	0,884 g	0,17g	1,70 g	5,87 g
DCM	0,776 g	0,08g	0,443 g	1,44 g
BuOH	0,135 g	1,02g	1,587 g	8,95 g

UNIVERSITY of the

WESTERN CAPE

The primary collection, the plant material was a mature, dry gall, with brown and powdery texture. Two extra collections were required to perform this experiment due to small product yields post maceration and crude extractions. The second collection weighed 772,49 g and it was divided into two parts, 300 g was utilized to conduct maceration and 472,49 g was utilized to conduct crude extraction. Moreover, the plant material was mature, but not as dry, brown, yellow, and powdery within. The last collection weighed 5 kg. Unlike the first collection the galls were not brown and dry, instead, within the gall, white and light green colours where observed, resembling an apple or a pear, it also possessed a damp and tough texture. It is suspected that due to climate, season and environmental conditions the phytochemical characteristics of the material were different for every collection, and this can be observed below on the TLC plates.

Lane representation

Lane 1: 1st collection, Lane 2: 2nd collection, Lane 3: 3rd collection

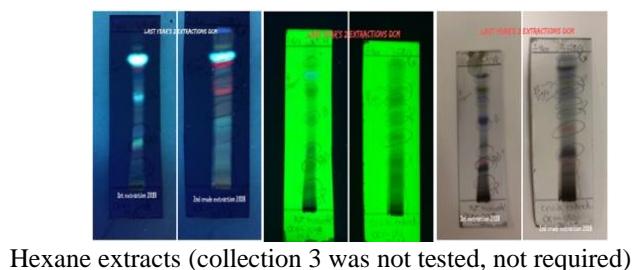
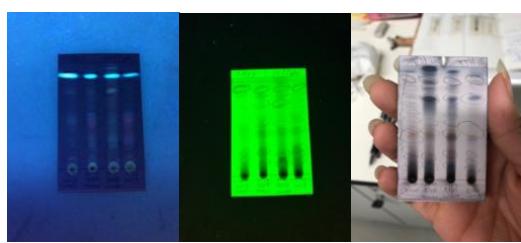
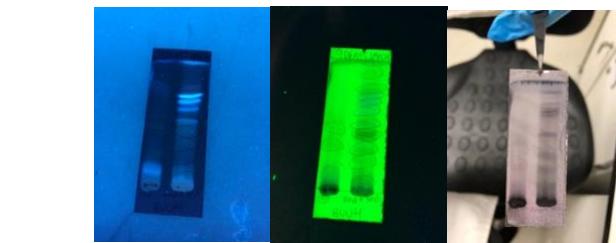
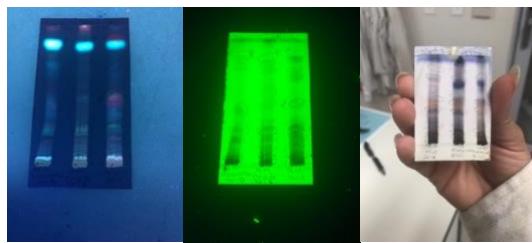
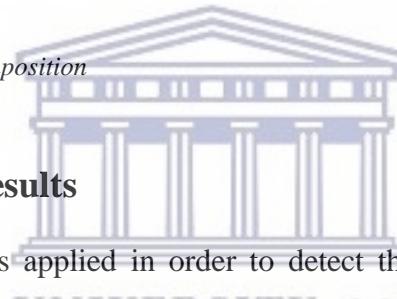


Figure 3: Difference in chemical composition



4.1.1 Reagent tests and results

The **Dragendorff reagent** was applied in order to detect the presence of alkaloids in the sample. Orange spots were observed post spraying the TLC plate with the reagent, displaying a positive result for the presence of alkaloids in the sample.

The **ninhydrin reagent** was applied in order to detect the presence of amino acid in the sample, no purple spots were seen post spraying the TLC plate with the reagent, displaying a negative or inconclusive result for the presence of amino acids in the sample.

The **phenol; sulfuric acid** test was conducted in order to detect the presence of carbohydrates in the sample. A colour change to orange indicated the presence of carbohydrates in the sample.



Figure 4: Presence of carbohydrates

4.1.1 Structure elucidation of Spinasterol (compound 1) and Schottenol (compound 2) found in the hexane extract

Flake like compounds (compound 1 and 2) were obtained from fractions 35-55 directly from the main hexane extract column, followed by NMR characterization. No further purification was needed. The compounds were isolated as a mixture due to their isomeric characteristic to one another. Spinasterol was the most abundant compound out of the two, making Schottenol the least abundant compound, thus the focus was on Spinasterol when analysing the NMR and GC-MS spectra. From the NMR data using ^1H -NMR, ^{13}C -NMR, COSY, and HMBC spectra the compounds and structures were identified and the data showed typical steroidal characteristics. The compounds in the mixture differ on the double bond positions since Spinasterol possesses a double bond at position 22 and Schottenol only contains one double bond at position 7 such as Spinasterol, and no other double bonds. The proton NMR spectral data displayed a signal at (δ_c 21) δ -0,962ppm (3H, d), J =6,8 Hz Me, a signal at (δ_c 22) δ 5,09 ppm (H, dd) 8, 8 Hz & 15,2 Hz, a signal at (δ_c 23) δ 4,96 (H, brs) 8,4Hz & 15,2Hz, another signal at (δ_c 26) δ 0,78 (3H, d) J =6,4 Hz Me, another signal at (δ_c 27) 0,73 (3H,s), (δ_c 29) 0,75 (3H,t) J =7,2Hz Me, where found. These are the main structural characteristics of Spinasterol. ^{13}C NMR spectrum has 29 carbons for the Spinasterol structure. From this spectrum, two double bonds were found present as well as a secondary alcohol. A DEPT spectrum showed 3 quaternary carbon atoms and, 6 methyl resonances. See appendix for NMR Spectra of compounds 1, 2,3,4 and 5 and GCMS spectra for compounds 6,7 and 8.

Table 2: NMR interpretation of compound 1

Position/ position on the structure.	¹³ C-shift (ppm)	¹ H-shift			HMBC
		(integration, multiplicity),	(ppm)	Most important J coupling	
1/C8	139,6 (C)	-	-	-	H-9, H-14
2/C22	138,1 (CH)	5,09 (H,dd) (mixture)	8,8Hz & 15,2Hz	H-20, H ₃ – 21, H-23	
3/C23	129,5 (CH)	4,96 (H, brs)	8,4Hz & 15,2Hz	H-20, H-22, H-24, H- 25, H ₂ – 28 _a	
4/C7	117,5 (CH)	5,09 (3H, dd)	-	H-a, H-14	
5/C3	71,1 (CH)	3,53 (H, m)	-	H-1b, H-2b, H-4	
6/C17	55,9 (CH)	1,187 (H, m)	-	H ₂ – 12, H-15a, H ₂ – 16, H ₃ – 21, H-22	
7/C14	55,1 (CH)	1,74 (H, m)	-	H-12b, H-15b, H ₃ – 18	
8/C24	51,2 (CH)	1,47 (H, m)	-	H-22, H-23, H-25, H-28, H ₃ – 26, H ₃ – 26, H ₃ – 27, H ₃ – 29	
9/C9	49,5 (CH)	1,58 (H, m)	-	H-7, H ₂ – 12, H – 14, H ₃ – 19	
10/C13	43,3 (C)	-	-	H ₂ – 11, H12a, H – 15b, H ₂ – 16, H ₃ – 18	
11/C20	40,8 (CH)	1,95 (H, m)	-	H – 21, H – 22, H – 23	
12/C5	40,3 (CH)	1,33 (H, m)	-	H-1b, H ₂ – 4, H – 7, H ₃ – 19	
13/C12	39,6 (CH ₂)	1,14 & 1,93 (H, m)	-	H-9, H ₃ – 18	
14/C4	38,0 (CH ₂)	1,65 & 1,20 (H, m)	-	H-5, H-2b	
15/C1	37,2 (CH ₂)	1,755 & 1,01 (H, m)	-	H ₂ – 9, H ₃ – 19	
16/C10	34,2 (C)	-	-	H-1b, H-4b, H-2b, H ₃ – 19	
17/C25	31,9 (CH)	1,47 (H, m)	-	H-23, H ₃ – 26, H ₃ – 27, H – 28 _a	
18/C2	31,5 (CH ₂)	1,73 & 1,31 (H, m)	-	H-1b, H ₂ – 4	
19/C6	29,7 (CH ₂)	1,198 & 1,69 (H, m)	-	H-7	
20/C16	28,5(CH ₂)	1,68 & 1,201 (H, m)	-	H-12b, H-15a, H-17	
21/C28	25,4 (CH ₂)	1,34(H, m)	-	H-23, H ₃ – 29	
22/C15	23,0 (CH ₂)	1,43 & 1,32(H, m)	-	H ₂ – 16, H – 17	
23/C11	21,6 (CH ₂)	1,51 & 1,40(H, m)	-	H-9, H ₂ – 12	
24/C21	21,4 (CH ₃)	0,962 (3H, d)	J=6,8Hz Me	H-17, H-20, H-22	
25/C26	21,1 (CH ₃)	0,78 (3H, d)	J=6,4Hz Me	H ₃ – 27	
26/C27	19,0 (CH ₃)	0,76 (3H, d)	J=6,0Hz Me	H ₃ – 26	
27/C19	13,0 (CH ₃)	0,73 (3H, s)	-	H-1a	
28/C29	12,2 (CH ₃)	0,75 (3H, t)	J=7,2Hz Me	H ₂ – 28	
29/C18	12,0 (CH ₃)	0,48 (3H, s)	-	H-12a, H-14, H-17	

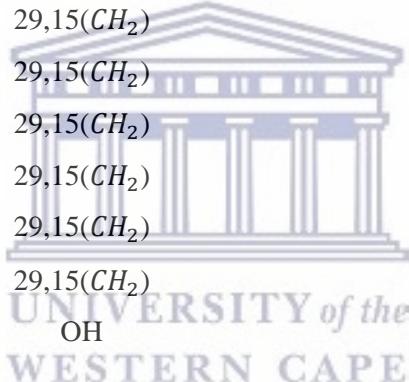
Research on phytoestrogens found in the *Pueraria* roots was conducted by Gook-che and associates (Daejeon, 2005). The roots were found to be mainly used as a rejuvenating folk medicine. Investigations on the toxicity of the Spinasterol in several cancer cell lines was conducted, and it was found that the *Pueraria* roots possess anti-tumor, anticancer activities and antiproliferative effects (Shattock, 2003).

4.1.2 Structure elucidation of Palmitic Acid (compound 3) and linoleic Acid (compound 4) found in the hexane extract.

Hex 5 fraction (compound 3 and 4) was obtained from fractions 16-26 taken from the main hexane extract column. Compounds with the same retention factor were combined followed by evaporation. The sample was further purified in order to isolate the desired compounds. To perform the isocratic elution, a smaller column was utilized and run using 100 % MeOH (methanol), where fractions 4, 5 and 6 were obtained, and screened with a solvent system of 80 % Hexane and 20 % EtOAc in the TLC plate tank. Moreover NMR characterization post isolation was conducted. The compounds were isolated as a mixture. In the mixture there were two compounds found, palmitic acid and linoleic acid. Palmitic acid was the most abundant compound out of the two, hence, the focus was on the palmitic acid when analysing the NMR Spectra and GC-MS spectra. From the ^1H -NMR, ^{13}C -NMR, DEPT, COSY, and HMBC spectra the compounds were identified to be of typical terpenes. The peaks identified as the "typical" fatty acid peaks are 2 triplets, a singlet, and a multiplet. at (δc 2) δ -1.40ppm (2H,t), (δc 16) δ -0,91ppm (3H,t), (δc 3) δ -2.32ppm (2H,s), (δc 15) δ -1.39ppm (2H, M). Spectra 9, 10,11,12 and 13 for NMR and spectra 14, 15 and 16 for GC-MS.

Table 3: NMR Interpretation of compound 3

Position/ position on the structure.	¹³ C-shift (multiplicity), (ppm)	¹ H-shift (integration, multiplicity), (ppm)
1	178,39 (C)	-
2/C16	130,24 (CH_3)	0.91 (3H,t)
3/C15	33,76(CH_2)	1.39 (2H,m)
4/C14	31,90(CH_2)	1.31 (2H,m)
5/C13	31,54(CH_2)	1.31(2H,m)
6/C12	27,22(CH_2)	1.31(2H,m)
7/C11	29,15(CH_2)	1.31(2H,m)
8/C10	29,15(CH_2)	1.31(2H,m)
9/C9	29,15(CH_2)	1.31(2H,m)
10/C8	29,15(CH_2)	1.31(2H,m)
11/C7	29,15(CH_2)	1.31(2H,m)
12/C6	29,15(CH_2)	1.31(2H,m)
13/C5	29,15(CH_2)	1.31(2H,m)
14/C4	29,15(CH_2)	1.31(2H,m)
15/C2	29,15(CH_2)	1.40 (2H,t)
16/C3	29,15(CH_2)	2.32 (2H,s)
17	OH	9.78



Palmitic acid was found in marine red algae, *Amphiroa zonata*. It was found to contain selective cytotoxicity to human leukemic cells by inducing apoptosis. It was also found that palmitic acid does not affect DNA topoisomerase II, which then suggests that palmitic acid may be a lead for anti-cancer drugs (Jeon *et al.*, 2005).

4.1.3 Compounds found in EtOAc and BuOH extracts

Post fractionization of the columns, eight fractions were chosen. Within these eight, five fractions were collected from the column eluent with EtOAc, and three were collected from the column eluted with BuOH. The fractions were then prepared, and submitted for LC-MS analysis. The below fractions were selected according to the number of compounds viewed on TLC. These possessed low value yields, after the attempt on the isolation of compounds. View **Table 4**.

Table 4: EtOAc and BuOH fractions sent to LC-MS

Fraction name	Extract	Weight
C13-C14 (91-110)	BuOH	5,5mg
K9 (208-215)	EtOAc	5,6mg
C17 (96-102)	BuOH	8,5mg
K29 (128-140)	EtOAc	9,2mg
K32 (181-190)	EtOAc	9,9mg
K33 (201-207)	EtOAc	7,4mg
LV3	EtOAc	0,6mg
C13-C14 (4-7)	BuOH	0,03mg

A set of LC-MS data was received from the University of Stellenbosch post analysis of the eight given samples. Compounds were characterized utilizing the following data bases: Global natural products social molecular network, FoodDB, STOFF (environment), KNAPSAcK (Natural product), PubChem (biomolecules), PlantCyc (plant), ChBI (Biomolecules), NANPDB (Natural product), UNPD (Natural Product)

Table 5: LC-MS of EtOAc and BuOH extracts

Compounds Found			
Compound number	Average Rt (min)	Average Mz	Structure Rank
5	3.289	121.02876	Benzoic Acid
6	4.621	225.11133	Tuberonic acid
7	4.621	243.12206	Pandangolide 1a
8	5.632	267.02768	Coumesterol
9	4.338	269.04468	Apigenin
10	4.977	281.08237	5,7-Dimethoxyflavone
11	5.082	285.03842	Kaempferol
12	4.977	293.08362	Dehydrocycloguanandin
13	4.389	299.05392	Chrysoeriol
14	4.674	313.07132	Cirsimaritin
15	6.561	313.12134	Cribrostatin 5
16	4.992	341.10123	4',5,6,7-Tetramethoxyflavone
17	4.988	353.10263	(+)-Sesamin
18	4.988	371.11349	Sesamolinol
19	4.108	403.1041	Chalconaringenin 2'-xyloside
20	4.07	419.17023	Eupaformosanin
21	5.029	431.13525	Medicocarpin
			Schizandrin;(+)-Schizandrin;Schisandrol A;Wuweizichun A
22	3.378	431.20508	Jatamanvaltrate G;(-)-Jatamanvaltrate G
23	3.697	441.215	Platanic acid
24	10.819	457.32974	Varixanthone
25	2.918	467.16714	Kushenol H
26	3.543	471.2012	Phelligradin F;(-)-Phelligradin F
27	5.609	477.11978	Diversolide G
28	4.108	483.16541	(-)-(7" ^R ,8" ^S)-4",5,7-trihydroxy-3',5'-dimethoxy-4',8"-oxyflavonolignan-7",9"-diol
29	5.497	495.12512	Ninandrographolide;Andropanoside
30	6.775	495.26041	Poricoic acid H;(+)-Poricoic acid H
31	10.166	499.3389	Myrianthic Acid
32	7.19	503.33783	Icariside II;Baohuoside 1;3-[(6-Deoxy-alpha-L-mannopyranosyl)oxy]-5,7-dihydroxy-2-(4-methoxyphenyl)-8-(3-methyl-2-butenyl)-4H-1-benzopyran-4-one
33	4.116	513.17542	Volkensiflavone
34	4.337	539.09674	Rubriflorin A
35	4.812	555.21954	Baccatin III;(-)-Baccatin III
36	5.236	585.2337	

After obtaining the LC-MS data sheet, the raw files provided by Mr Taylor from the University of Stellenbosch were placed in the Global natural products social molecular network database and the following compounds were found.

Table 6: Global Natural Products Social Molecular Network database compounds

Compound number	Average Mz	Peak	Structure rank	Spectra number
37	621.109	269.0	4'-O-GlcA-7-OGlcA Apigenin	17
38	769.22	300.2	7-O-Methylquercetin-3-O-galactoside-6''-rhamnoside	18
39	621.341	109.0	3-O-protocatechuoyl ceanothic acid	19

Insufficient literature on 4'-O-GlcA-7-OGlcA apigenin and 7-O-methylquercetin-3-O-galactoside-6''-rhamnoside was provided on the database and various others besides the certitude of them being natural products.

4.1.4 Literature review on compounds obtained through LC-MS

3-O-protocatechuoyl ceanothic acid

3-O-protocatechuoyl ceanothic acid, is found in plants and foods such as olives. This compound possesses antioxidant, anti-inflammatory, anti-apoptotic, antihyperglycemic activities (Semaming *et al.*, 2015).

Benzoic Acid

Benzoic Acid is also known as phenylformic acid. It is an aromatic carboxylic acid naturally present in plants and animal tissues. This may be found in some berries(cranberries, strawberries), prunes, apples, yogurt, cayenne pepper, and other foods. Benzoic Acid prevents

infections caused by bacteria hence acting as an antibacterial and antifungal. Benzoic acid can treat skin irritations and inflammation caused by insect bites, burns, or eczema, they also possess antioxidant properties, are used as flavouring agents in food, used in the production of cosmetics, hygiene and other pharmaceutical products. They are active ingredients in pesticide products and biocidal products in veterinary hygiene .(Khotimchenko, Vaskovsky en Titlyanova, 2002).

Tuberonic Acid

Tuberonic acid is an oxo monocarboxylic acid, and it can be found in jasmonic acid in potatoes. They act as antimicrobials, possess insecticidal functions and they regulate the defence mechanisms (del Olmo, Calzada en Nuñez, 2017).

Pandangolide 1a

Pandangolide 1a has been isolated from the Cladosporium fungus which was extracted from the red sea sponge *Niphates rowi*, and it is said to possess anti-cancer and anti-inflammatory activities (Wakuta *et al.*, 2010).

Coumesterol

Coumesterol is a phytochemical organic compound, first identified in *ladino clover* and *alfalfa*. It can also be found in soybeans, brussels sprouts, spinach, and many other different legumes. Coumesterol mimics the bioactivity of estrogen, it has antioxidant properties, and it's an anti-inflammatory agent. Coumesterol also has the ability to decrease bone resorption and promote mineralization of the bone. Daily injections of coumesterol have shown to reduce bone loss in rats. It also decreases ovarian weight and increases apoptotic cell death in ovaries of adult rats exposed during lactation (Hill, 2006).

Apigenin

Apigenin is found in a variety of plants such as, fruits, vegetables, and Chinese herbs. It possesses anti-inflammatory, antioxidant, antibacterial, and antiviral functions and it is also used as a blood pressure reducer. Apigenin also triggers cell apoptosis and cell autophagy, induces cell cycle arrest, suppresses the cell migration and invasion in the organs and it helps stimulate immune responses being a great candidate for an anti-cancer agent (Moon *et al.*, 2009).

5,7-Dimethoxyflavone

5,7-Dimethoxyflavone, is found in *Kaempferia parviflora*. The *Kaempferia parviflora* has been found to possess various types of activities such as; cellular metabolism-regulating, anti-cancer, cardioprotective, neuroprotective, antiallergic, anti-inflammatory, due to the 5,7-dimethoxyflavone which is the specific compound responsible for its anti-inflammatory activities (Yan *et al.*, 2017).

Kaempferol

Kaempferol is a polyphenol flavonoid found in a variety of plants such as, kale, beans, spinach, and broccoli. It reduces the risk of chronic diseases such as cancer by augmenting the human body's antioxidant defences against free radicals. It also inhibits apoptosis, angiogenesis, inflammation, and metastasis (Chen *et al.*, 2018).

Dehydrocycloguanandin

Dehydrocycloguanandin was isolated from the mangrove plant and it is known to have antinociceptive, anti-inflammatory, and antipyretic activities (Chen en Chen, 2013).

Chrysoeriol

Chrysoeriol is an anti-inflammatory and diabetic control activity containing flavonoid found in the methanolic extract of the *Cardiospermum halicacabum* and *Gladularia selloi*. This compound also shows activities of plasma reduction in glucose, hemoglobin, and glycosylated hemoglobin, while rising the plasma insulin sensitivity (Shilpi *et al.*, 2012).

Cirsimaritin

Cirsimaritin is a strong antioxidant, antimutagen, antibacterial compound found in *Baccharis trimera less* also known as Carqueja, a folk medicine commonly used in Brazil to prevent cancer and increase antimutagens and antitumor promoting activities, it is also found in *Rosmarinus officinalis L.* and other medicinal plants (Krishnan *et al.*, 2020).

Cribostatin 5

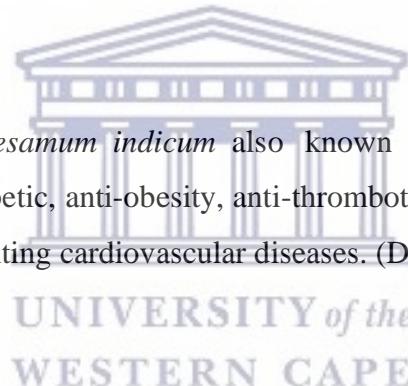
Cribostatin 5 is one of the five Cribostratin compounds found and isolated from the marine sponge *Cribrochalina* species in the Republic of the Maldives. Compounds, Cribostratins 3, 4, and 5 provide cancer cell line inhibitory activities, antibacterial and antifungal activities. Cribostratins 1-5 are also sources for strong antibiotics production due to their antibiotic activities. (Nakasugi en Komai, 1998)

4',5,6,7-Tetramethoxyflavone

4',5,6,7-Tetramethoxyflavone, isolated from the peels of the *Citrus aurantium* and *Citrus reticulata blanco* cv. *Ponkan*, possesses antimutagenic, anticarcinogenic, and antitumor activity (Pettit *et al.*, 2000) which show antiproliferative activities against cancer cells (Chen, Montanari en Widmer, 1997).

(+)-Sesamin

Sesamin, isolated from the *Sesamum indicum* also known as sesame seeds/ sesame oil, possesses antioxidant, anti-diabetic, anti-obesity, anti-thrombotic, anti-hypertensive, and anti-inflammatory properties preventing cardiovascular diseases. (Du en Chen, 2010).



Sesamolinol

Sesamolinol like Sesamin isolated from the *Sesamum indicum*, possesses antioxidant, and anti-inflammatory properties. (Dalibalta, Majdalawieh en Manjikian, 2020).

Chalconaringenin 2'-xyloside

Chalconaringenin 2'-xyloside was isolated from the ethanol extract of the Uzbek medicinal plant *Helichrysum maracandicum*, it contains anticarcinogenic properties and antiproliferative activity (Taylor, no date).

Eupaformosanin

Eupaformosanin, isolated from *Eupatorium formosanum* (Lee *et al.*, 1977), is an antitumor, antileukemic, and antisarcoma agent with the purpose of affecting the nucleic acid and the protein of Ehrlich Ascites carcinoma cells(Yagura *et al.*, 2008). Eupaformosanin also acts as

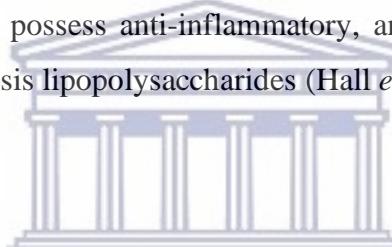
an antineoplastic agent in the cells metabolism inhibiting deoxyribonucleic acid synthesis (Lee *et al.*, 1977).

Medicocarpin

Medicocarpin is a flavonoid derivative from the *Ononis Sicula Guss* species mainly found in Egypt and from the *Glycyrrhiza pallidiflora* hairy root species. It is utilized in skin care products due to its anti-aging, anticancer and antioxidant properties (Oliveira en Rodrigues, 2018).

(+)-Schisandrin; Schisandrol A; Wuweizichun A

Schisandrol A has been isolated from the *Schisandra Chinensis* bail fruit (Kwon *et al.*, 2018). Schisandrin or otherwise called Schisandrol A is the main active ingredient in the fruit, and it has been studied and found to possess anti-inflammatory, and antioxidant effects, it has a protective effect on induced sepsis lipopolysaccharides (Hall *et al.*, 1980).



Jatamanvaltrate G

Jatamanvaltrate G was isolated from the *Valeriana jatamansi* plant species and it is considered an iridoid compound (Ma *et al.*, 2021). Iridoids from the *Valeriana jatamansi* contain anti-inflammatory and anti-proliferative properties Jatamanvaltrate G being one of them. These are also known to inhibit the proliferation of human glioma stem cell lines (Yu *et al.*, 2008).

Platanic Acid

Platanic Acid, isolated from the *Syzygium claviflorum* leaves, and found to contain anti-HIV activity (Kahnt *et al.*, 2018) . It is an inhibitor of HIV replication in the H9 lymphocyte cells in the human body and an anti-AIDS agent(Liu *et al.*, 2021).

Varixanthone

Varixanthone, isolated from the *Emericella variecolor* marine Fungus and this compound shows antimicrobial activity (From en Clavzflqrum, 1994).

Kushenol H

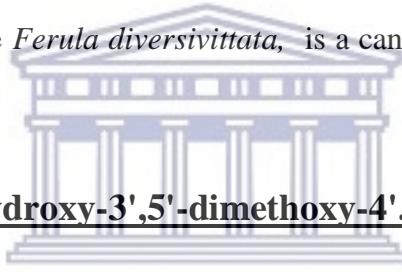
Kushenol H, isolated from the dried roots named Kushen ("Kujinn") of the *Sophorae flavescens* plant species, original to Japan, have been utilized as Chinese and Japanese traditional medicine for centuries. The Kushenols O - A are known flavonoids known to constitute antibacterial and antiandrogen activities (Malmstrøm *et al.*, 2002).

Phelligradin F;(-)-Phelligradin F

Phelligradin F, isolated from the *Inonotus xeranticus* mushroom species is a new hispidin derivative. This compound was found to have antioxidant activities (Kuroyanagi *et al.*, 1999).

Diversolide G

Diversolide G isolated from the *Ferula diversivittata*, is a cancer chemo-preventive agent in humans. (Lee *et al.*, 2006).



(-)-(7''R,8''S)-4'',5,7-trihydroxy-3',5'-dimethoxy-4',8''-oxyflavonolignan-7'',9''-diol

(-)-(7''R,8''S)-4'',5,7-trihydroxy-3',5'-dimethoxy-4',8''-oxyflavonolignan-7'',9''-diol is a flavonolignan found in the *Calamus quiquesetinervius* plant/vegetable native of Northern Taiwan. This phenolic compound was found to have cardiovascular protective activities (Iranshahi *et al.*, 2010).

Ninandrographolide;Andropanoside

Andropanoside or otherwise known as Ninandrographolide is a compound found in the Andrographis paniculata species and these were produced by glucosylation at the C19-hydroxyl. This compound is known to be an anti-inflammatory, anti-cancer, immunomodulatory, antimalarial, antidiabetic, fever combating, laryngitis, gastric infections reduction agent, and many more(Chang *et al.*, 2010).

Poricoic acid H;(+)-Poricoic acid H

Poricoic acid H has been isolated from the *Poria cocos* herb species which originated from China and is used as a prescribed diuretic and as a sedative. Poricoic acid H was found to inhibit

cytotoxicity to all cancer cell lines and other tumor-promoting effects in conjunction with the other poricoic acids (Sun *et al.*, 2019).

Myrianthic Acid

Myrianthic Acid is a terpenoid isolated from the *Vaccinium emarginatum* plant species and it is found to have cytotoxic and anti-inflammatory activities (Ukiya *et al.*, 2002).

Icariside II;Baohuoside 1;3-[(6-Deoxy-alpha-L-mannopyranosyl)oxy]-5,7-dihydroxy-2-(4-methoxyphenyl)-8-(3-methyl-2-butenyl)-4H-1-benzopyran-4-one

Icariside II is an icariin metabolite that derives from the *herba Epimedii*. This compound is known to induce apoptosis in the A431 human epidermoid carcinoma cells (Tu *et al.*, 2020).

Volkensiflavone

Volkensiflavone was isolated from both *Garcinia multiflora* and *Rhus succedanea* plant species. This compound is known to have antiviral activities against respiratory viruses such as influenza A and B, parainfluenza type 3, measles, respiratory syncytial and adenovirus type 5, and herpes viruses(Wu *et al.*, 2013).

Rubriflorin A

Rubriflorin A has been isolated from the stems of the *Schisandra rubriflora* and it is a dibenzocyclooctene lignan. This compound is known to possess anti-tumor and anti-cancer activities (Lin *et al.*, 1998).

Baccatin III;(-)-Baccatin III

Baccatin III was isolated from the heartwood of *Taxus baccata L.* and it's considered to be a taxoid. Anti-inflammatory and antinociceptive activities are medicinal characteristics of this compound. This was tested in vivo (Li *et al.*, 2004).

Chapter 5

5.1 Conclusion

The assessment of the phytochemistry and the identification of several of the compounds present in the *Uromycladium tepperianum* MacAlpine fungus was important, due to the lack of knowledge and, literature reports on this fungus. Since rust fungi are mostly known to be diseases which affect plants, this investigation may be deemed as a first of its kind and may now be documented.

Moreover, the investigation conducted on the *Uromycladium Tepperianum* MacAlpine fungus was found to provide unexpected results since, apart from certain yeasts and moulds, mushrooms are the only types of fungi widely reported on the contribution towards medicinal treatments, and drug developments, unlike rust fungi, which are considered to be plant diseases with no medicinal uses. A detailed investigation on the phytochemistry of this fungus was conducted, leading to the isolation of 39 simple and complex compound structures. Compounds 1, 2, 3 and 4 were characterized by utilizing NMR GC-MS and MS (Spinasterol, Schottenol, Palmitic acid and Linoleic acid), compounds 37, 38 and 39 (4'-O-GlcA-7-O GlcA apigenin , 7-O-Methylquercetin-3-O-galactoside-6''-rhamnoside and 3-O-protocatechuoyl ceanothic acid) were characterized by utilizing LC-MS and the Global natural products social molecular network database. Lastly compounds 5 to 36 were characterized by utilizing LC-Ms with the aid of various other databases.

The majority of the bioactive compounds found in this fungus possessed isomeric characteristics with extremely low yields, making isolation and characterization in NMR exceedingly difficult. Since NMR requires at least 5mg of sample to confirm the organic purity and characterization of the product, LC-MS and various data bases [Global natural products social molecular network, FoodDB, STOFF (environment), KNAPSAcK (Natural product), PubChem (biomolecules), PlantCyc (plant), ChBI (Biomolecules), NANPDB (Natural product), UNPD (Natural Product), Elemental composition, Pubchem, Chemspider Science direct, Synapt G2 qTOF, MZmine, Chem Calc, and Science finders Scholar] were utilized to isolate and characterize the remaining compounds.

3.5.5 Future recommendations

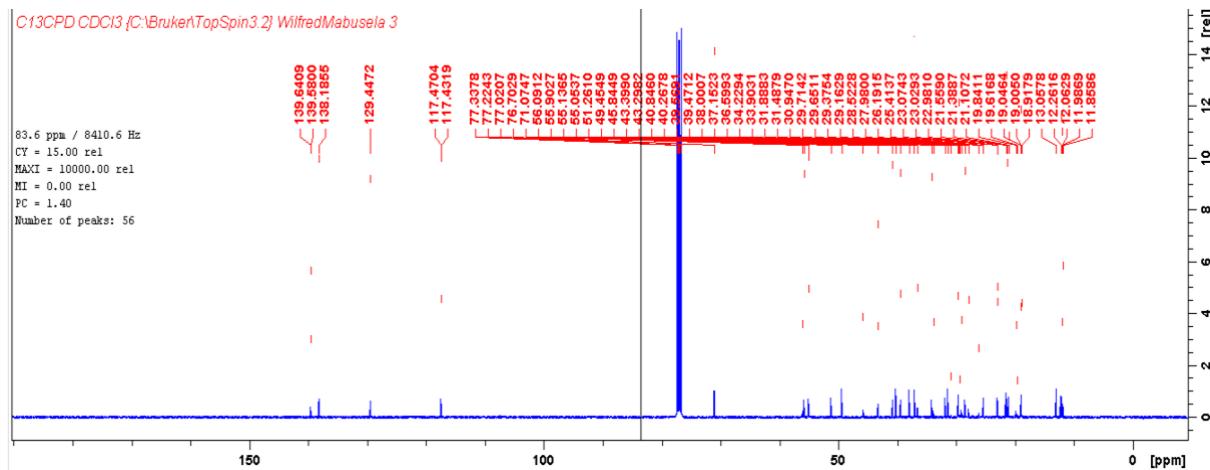
A sizeable amount of material is required (of at least 20 kgs) in the future for further investigation or confirmation of compound structures, as to be able to obtain higher amounts of the organic constituents present in the fungus for better separation, isolation, purification and characterization.

Further research is necessary to investigate and compare the phytochemistry and chemical composition of this fungus when collected in different seasons and environments on all four extracts (*n*-hex, EtOAc, DCM and BuOH) using HPLC as a method for seasonal profiling of the compounds present in the fungus.

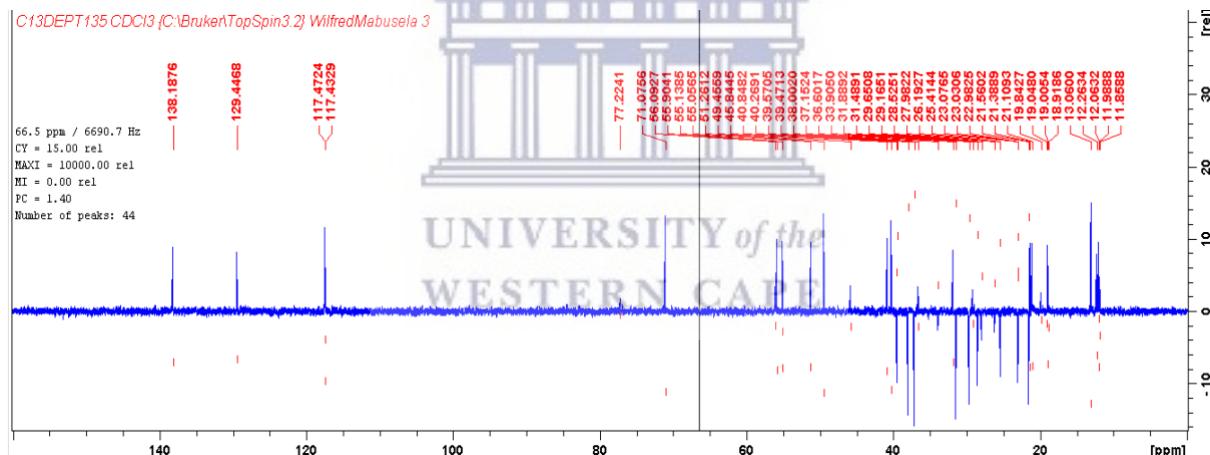


Appendix

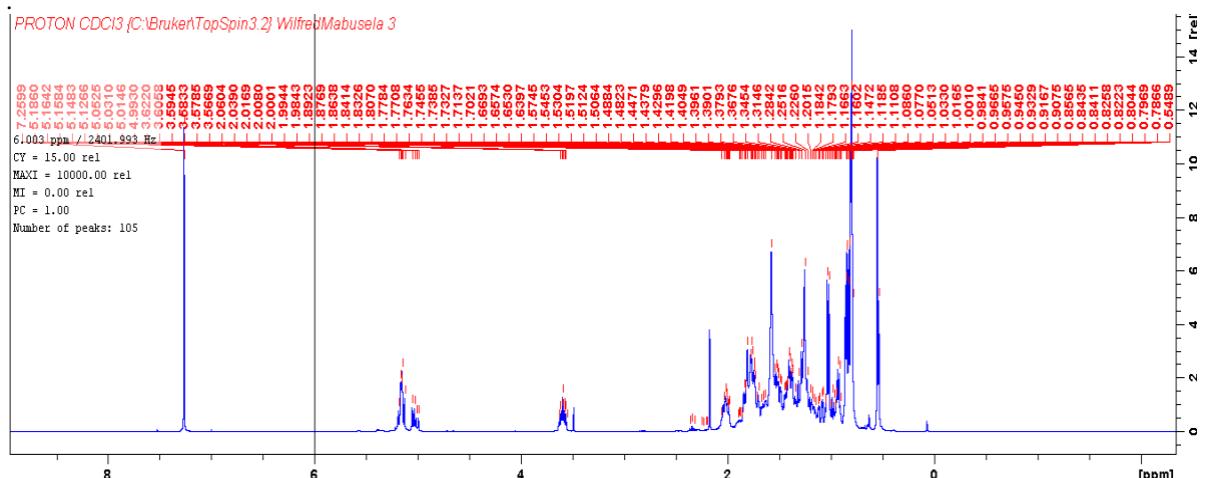
NMR Spectra (compound 1 and 2)



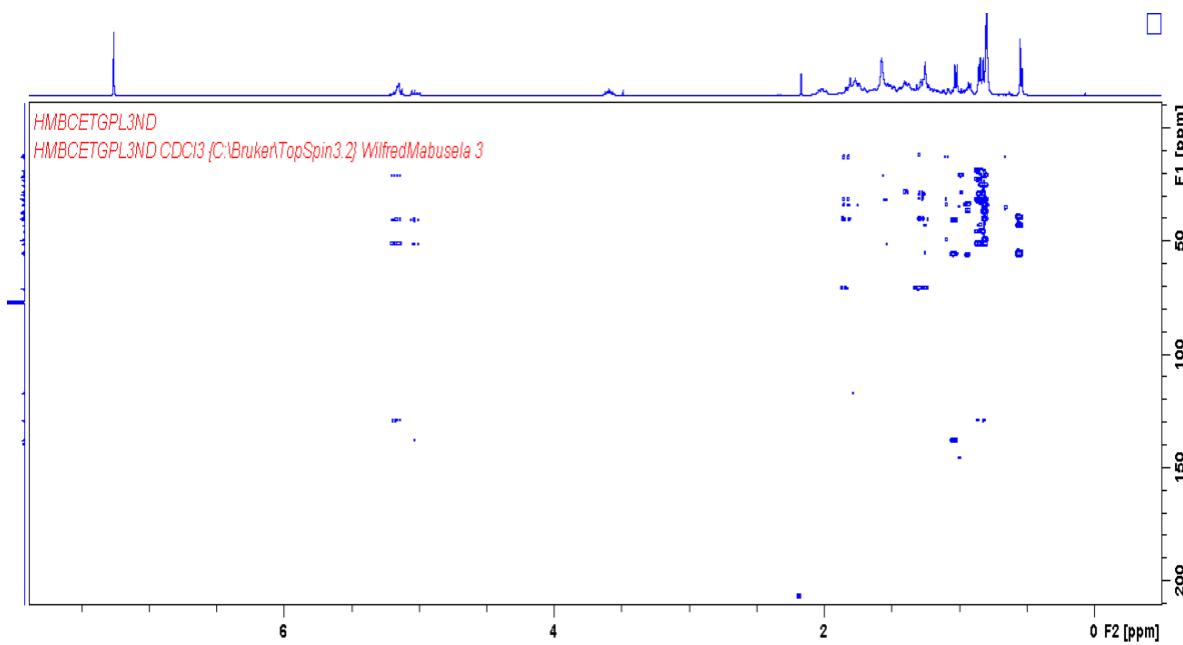
spectra 1:Spectra C13 compound 1 and 2.



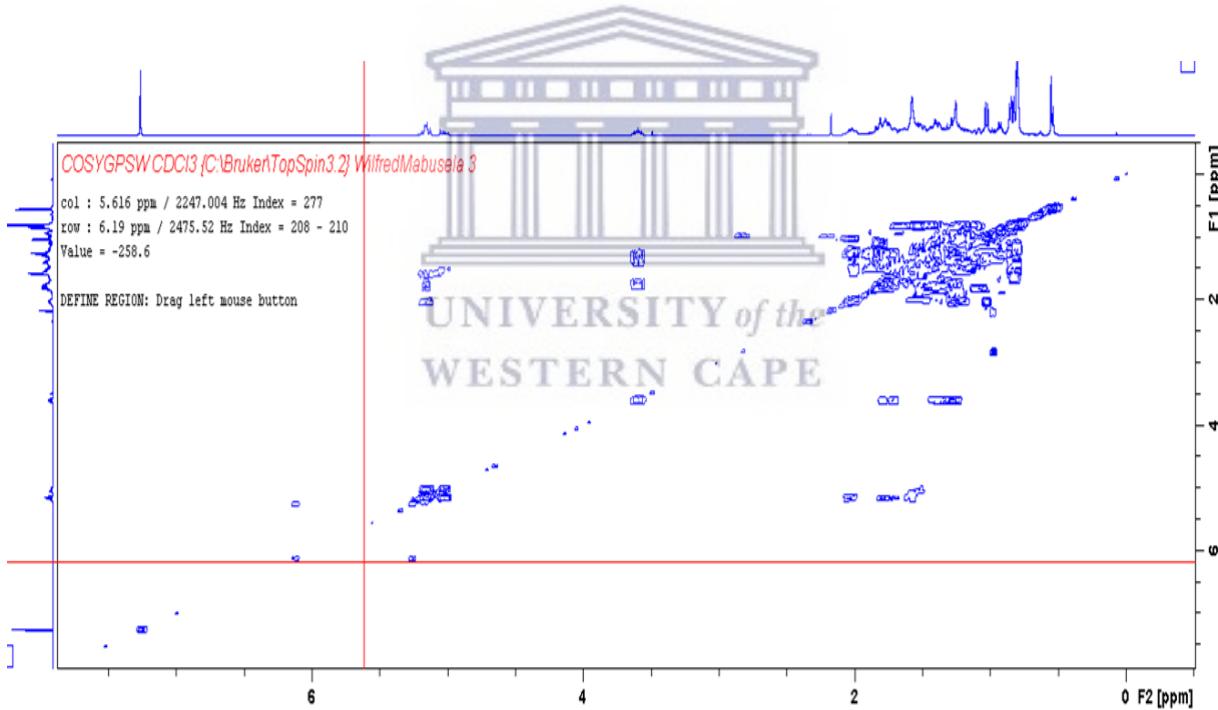
spectra 2:Spectra DEPT compound 1 and 2



spectra 3:Spectra Proton compound 1 and 2.



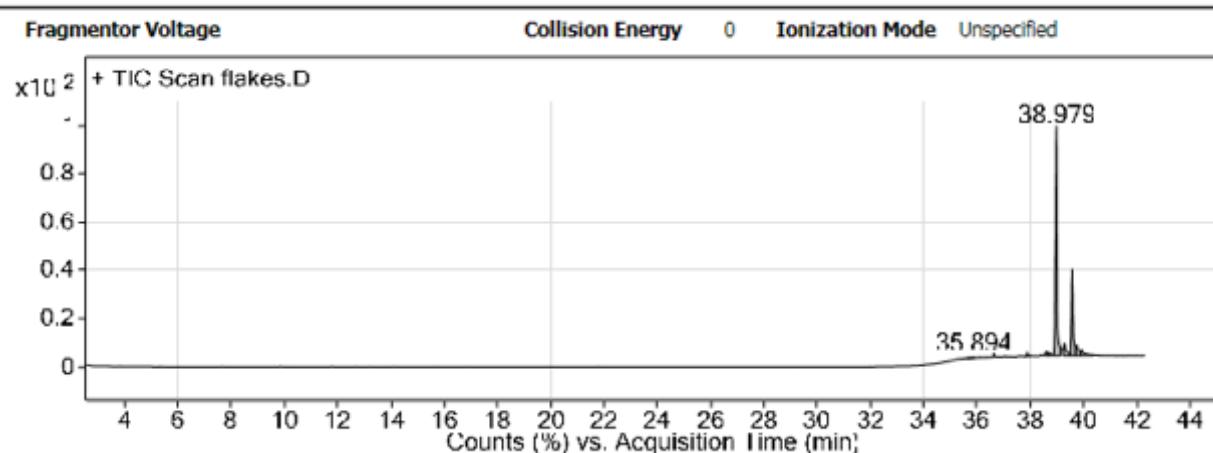
spectra 4:Spectra HMBC compound 1 and 2.



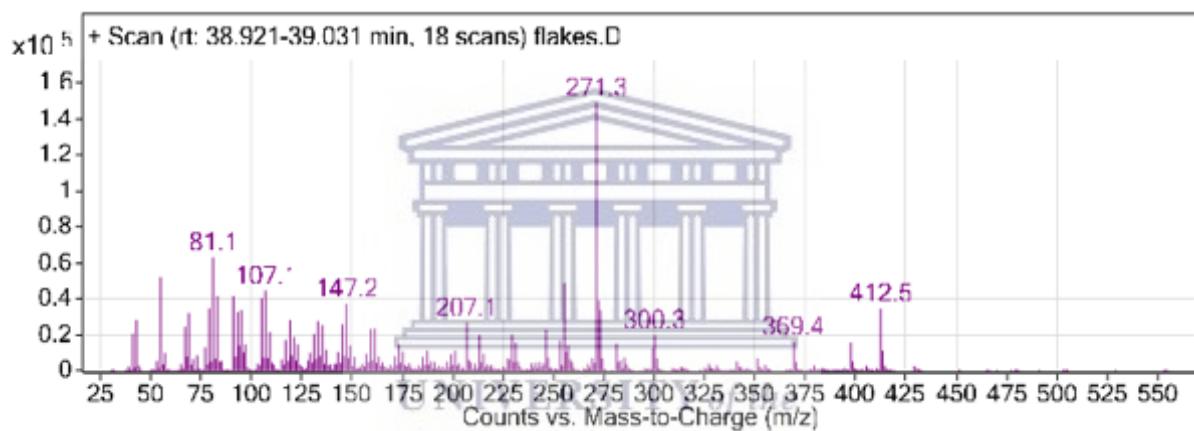
spectra 5:Spectra Cosy compound 1 and 2.

GC-MS Spectra of compound 1 and 2

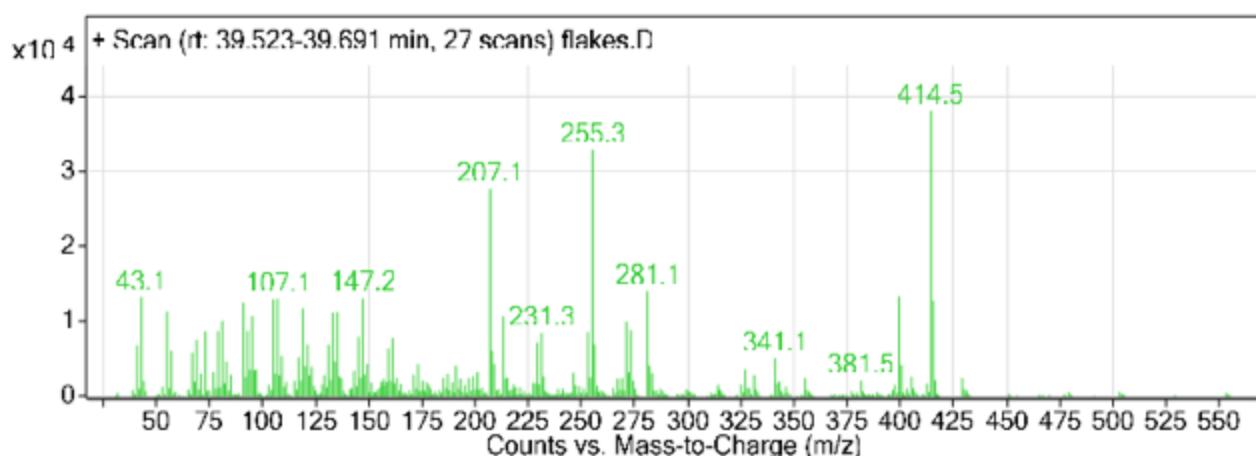
User Chromatograms



spectra 6: GC-MS Spectra visualization of both compound 1 and 2. (mixture)

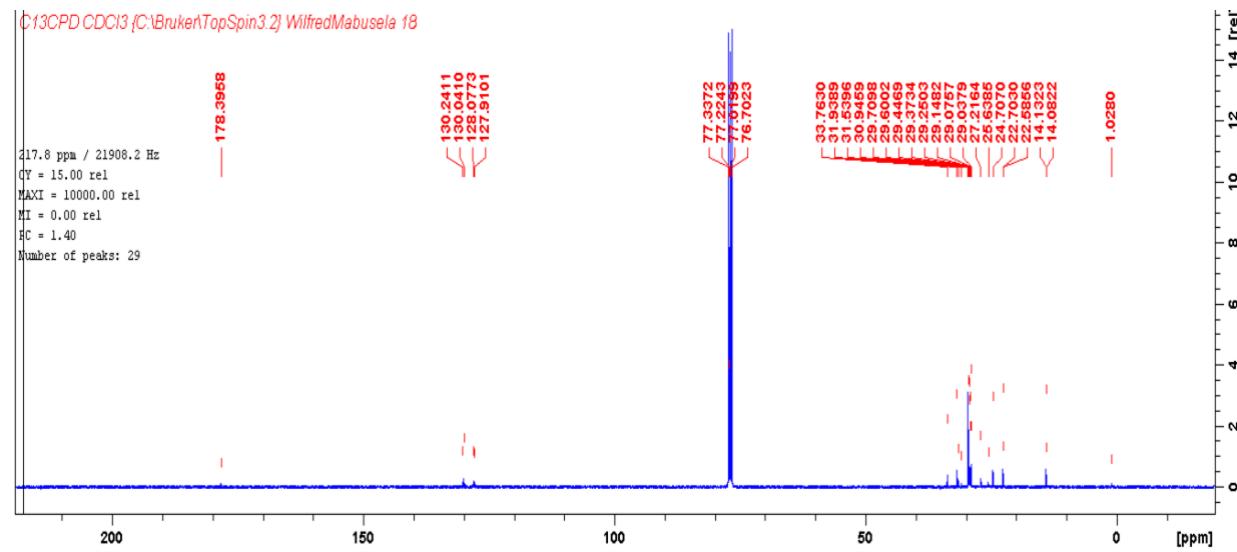


spectra 7: GC-MS Spectra molar mass of Spinasterol compound 1

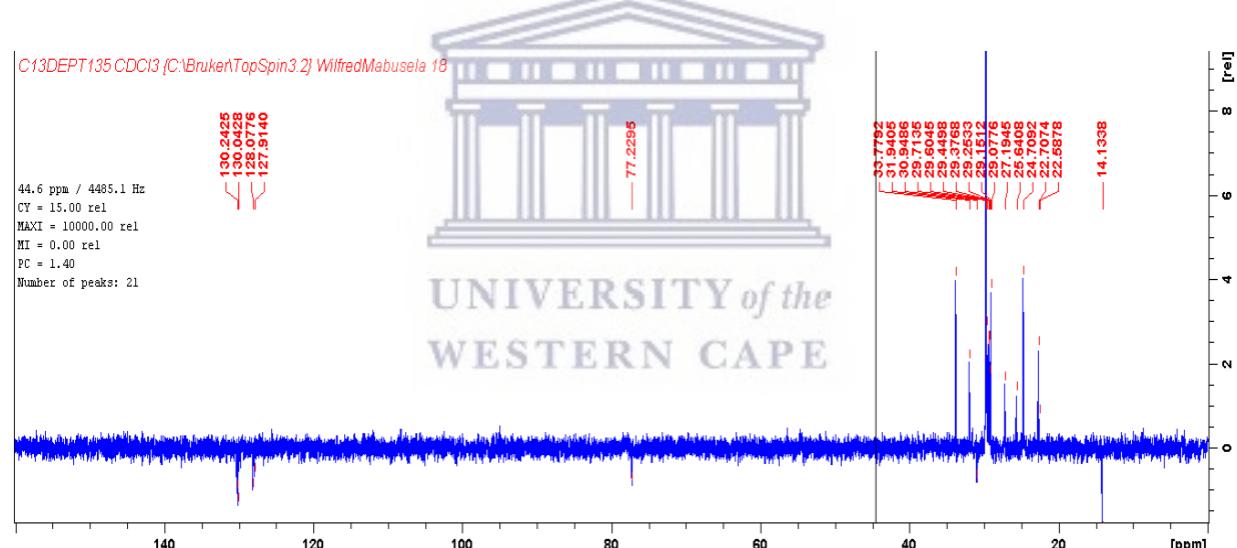


spectra 8: GC-MS Spectra molar mass of Schottenol compound 2.

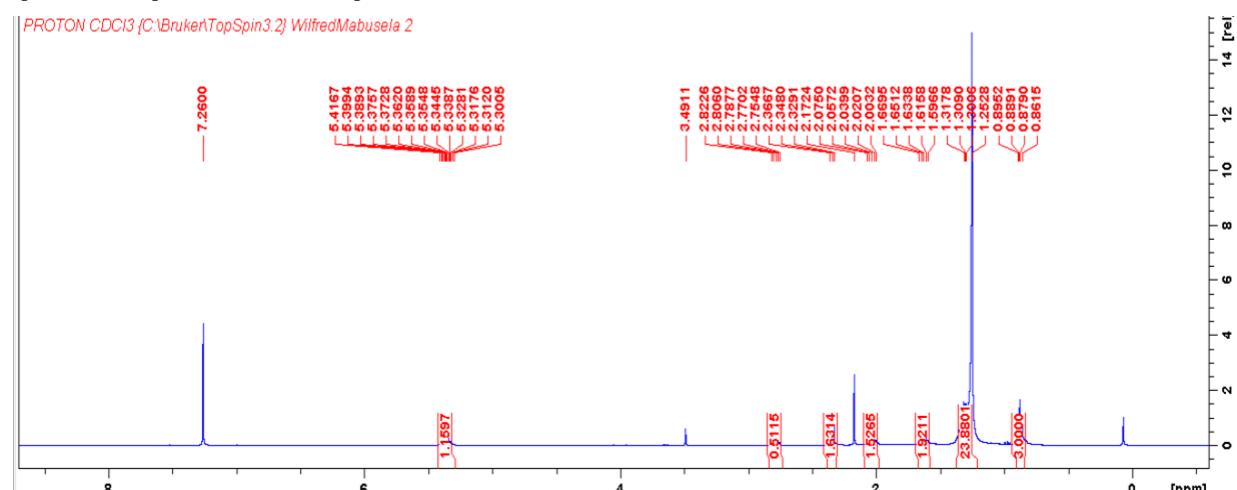
NMR Spectra compound 3 and 4



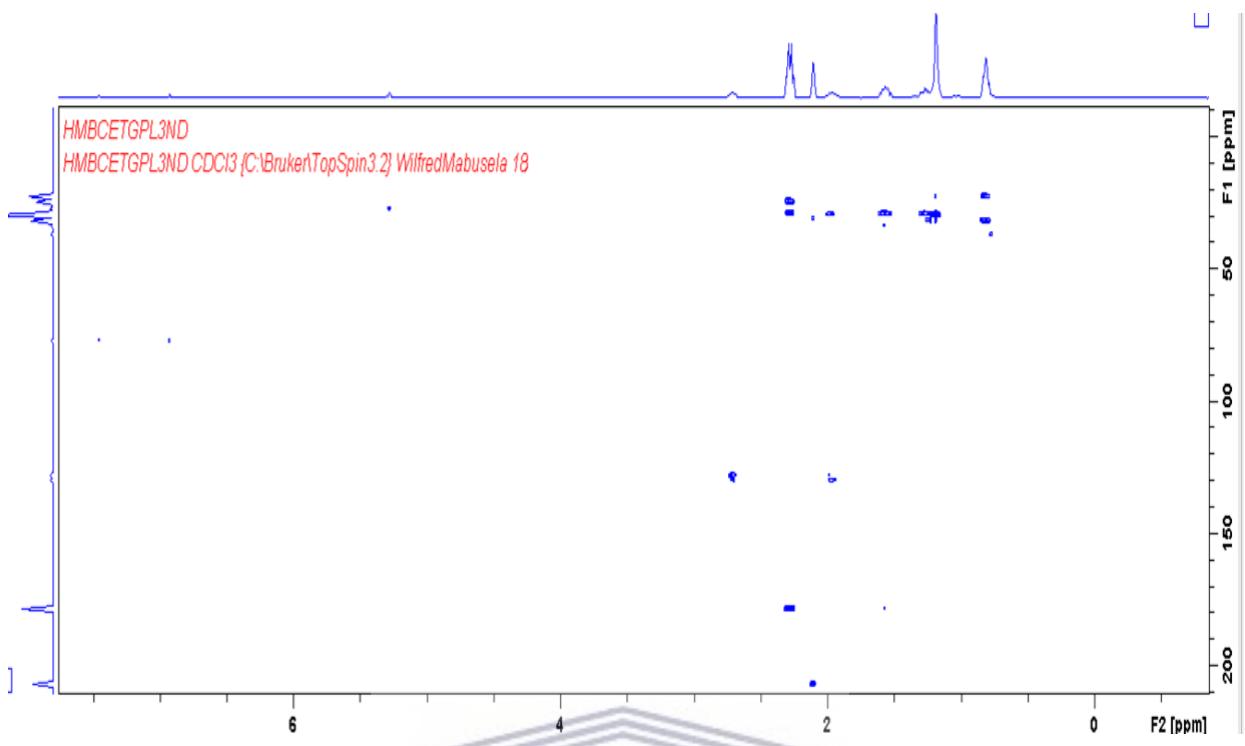
spectra 9:Spectra C13 compounds 3 and 4



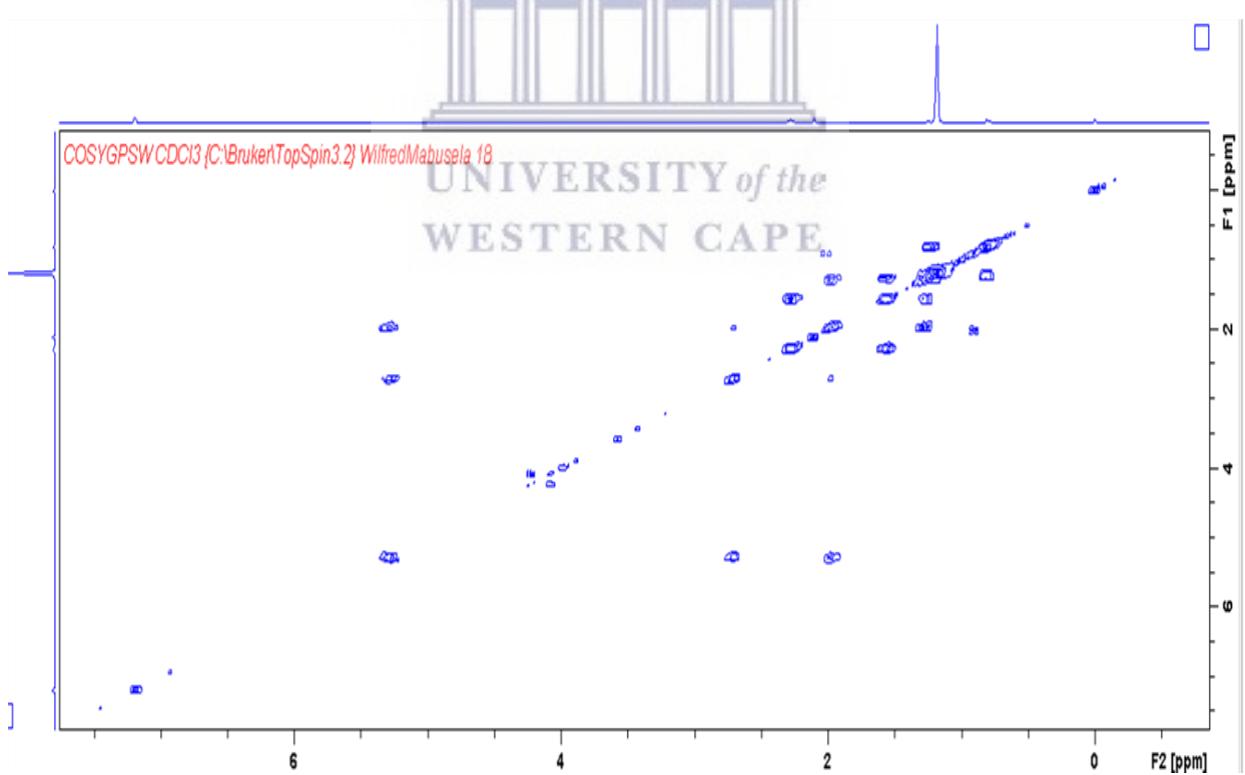
spectra 10:Spectra DEPT compounds 3 and 4



spectra 11:Spectra Proton compounds 3 and 4



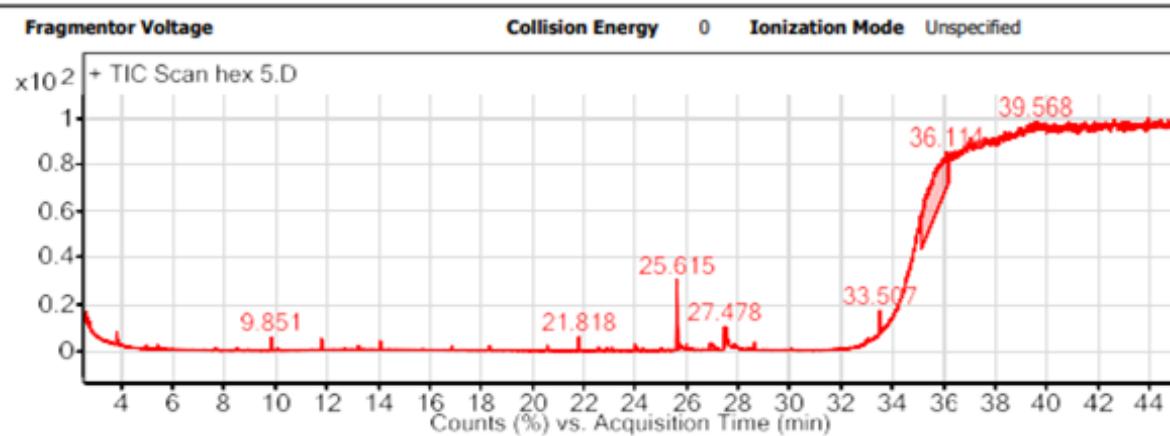
spectra 12:Spectra HMBC compounds 3 and 4



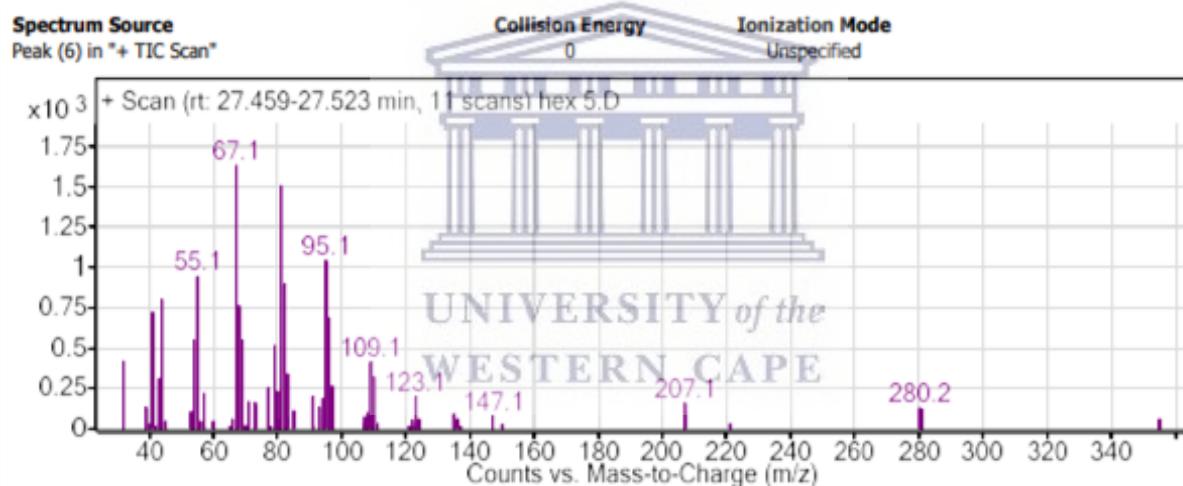
spectra 13:Spectra COSY compounds 3 and 4

GC-MS spectra compounds 3 and 4

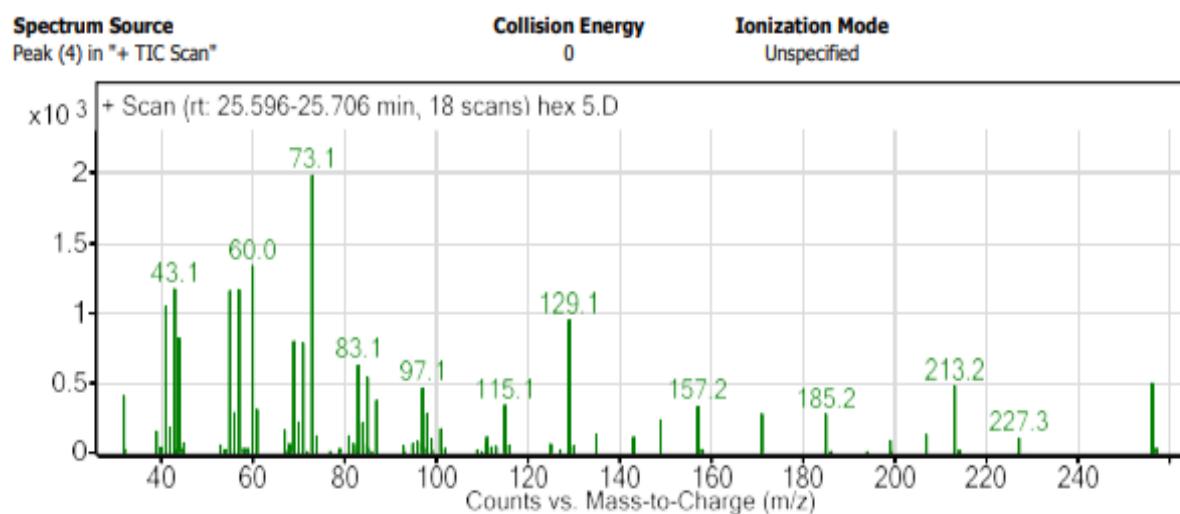
User Chromatograms



spectra 14: GC-MS Spectra visualization of compounds 3 and 4 mixture.

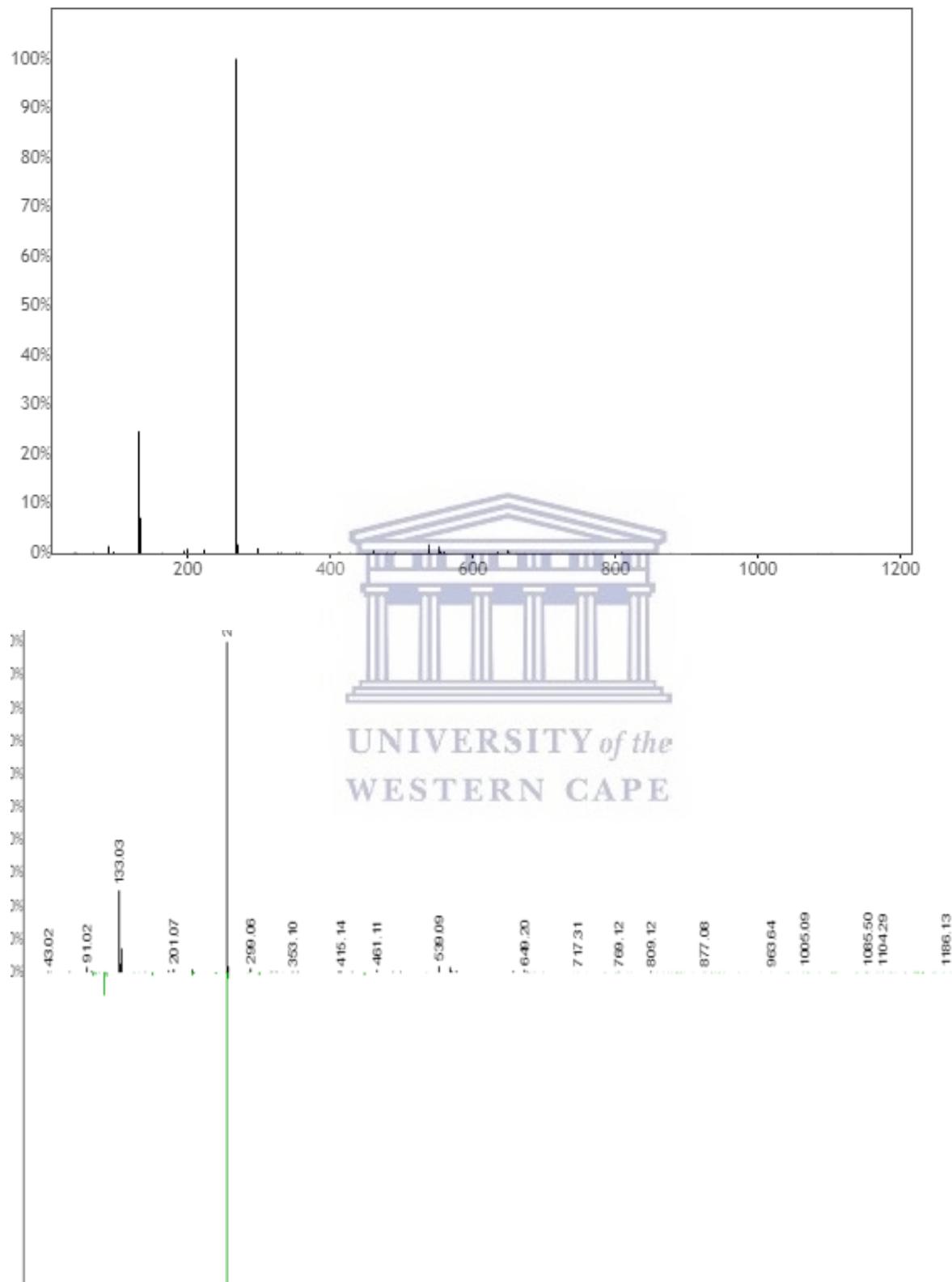


spectra 15: GC-MS Spectra molar mass of linoleic Acid compound 4.

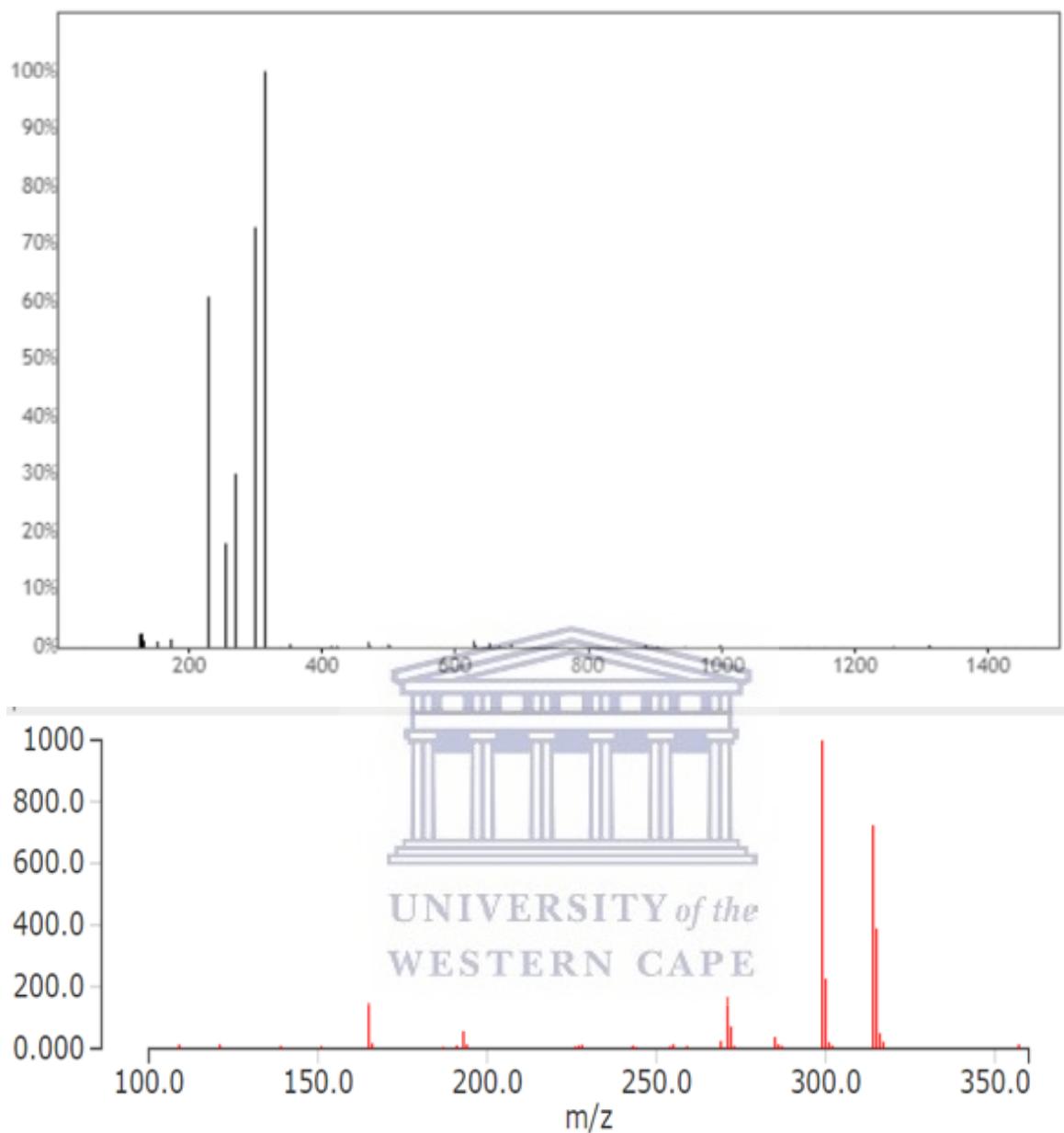


spectra 16: GC-MS Spectra molar mass of Palmitic acid compound 3.

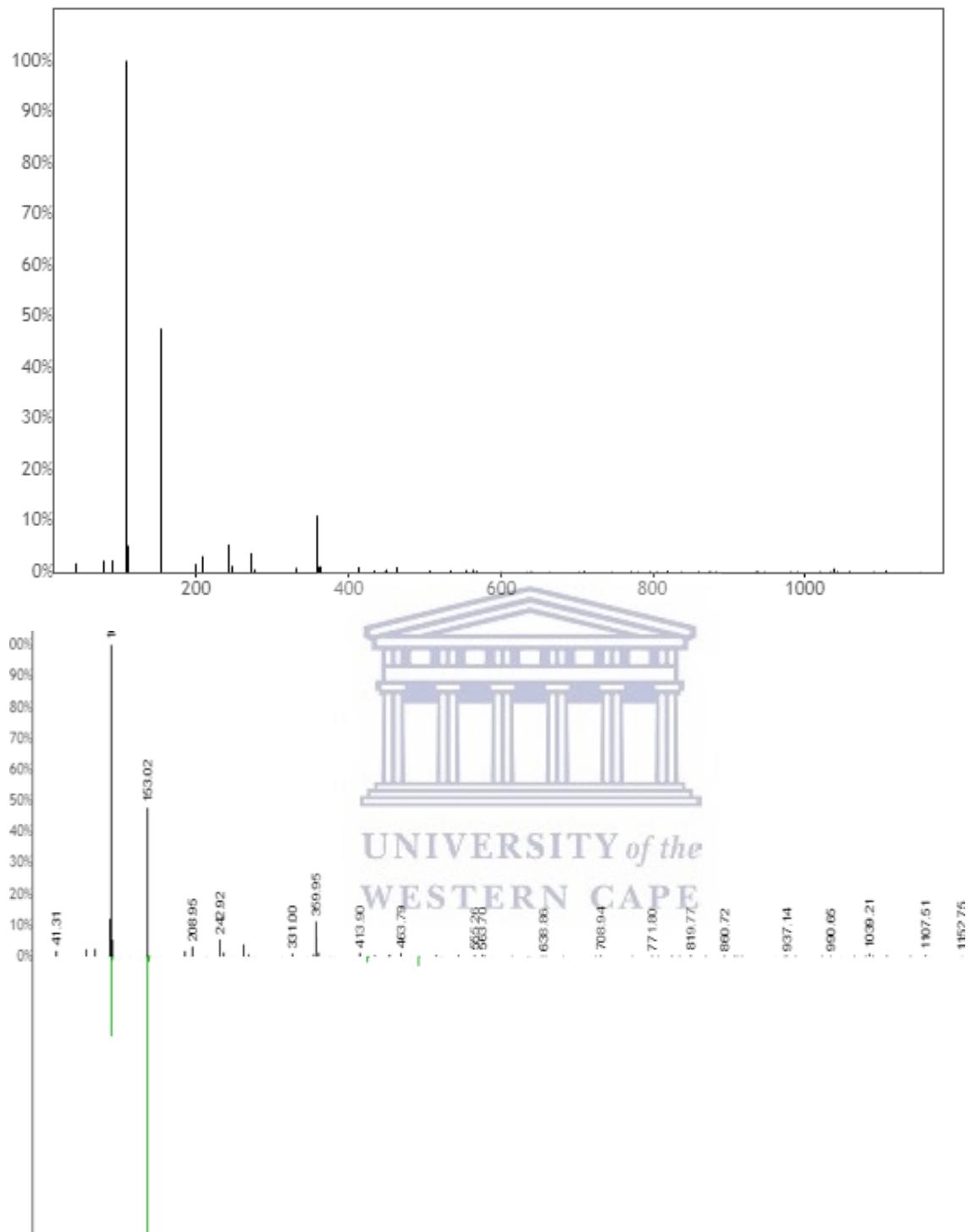
Global natural products social molecular network database spectra



spectra 17: compound 37 peaks



spectra 18: compound 38 peak



spectra 19: compound 39 spectra.

References

- Aging, C. (2012) “Chemical Aging”, *Enological Chemistry*, bll 375–388. doi: 10.1016/b978-0-12-388438-1.00021-2.
- Ahmed, D. *et al.* (2015) “Antimicrobial activities of methanolic extract of Carissa opaca roots and its fractions and compounds isolated from the most active ethyl acetate fraction”, *Asian Pacific Journal of Tropical Biomedicine*, 5(7), bll 541–545. doi: 10.1016/j.apjtb.2015.05.006.
- Aime, M. C. *et al.* (2017) “Phylogenetics and Phylogenomics of Rust Fungi”, *Advances in Genetics*, 100, bll 267–307. doi: 10.1016/bs.adgen.2017.09.011.
- Ajith, T. A. en Janardhanan, K. K. (2007) “Recent Advances in Indian Herbal Drug Research
Guest Editor: Thomas Paul Asir Devasagayam

Indian Medicinal Mushrooms as a Source of Antioxidant and Antitumor Agents”, *Journal of Clinical Biochemistry and Nutrition*, 40(3), bll 157–162. Available at: https://www.jstage.jst.go.jp/article/jcbn/40/3/40_3_157/_article.
- Alberts, A. W. *et al.* (1980) “Mevinolin: A highly potent competitive inhibitor of hydroxymethylglutaryl-coenzyme A reductase and a cholesterol-lowering agent”, *Proceedings of the National Academy of Sciences of the United States of America*, 77(7 II), bll 3957–3961. doi: 10.1073/pnas.77.7.3957.
- Alcazar-Fuoli, L. en Mellado, E. (2012) “Ergosterol biosynthesis in *Aspergillus fumigatus*: Its relevance as an antifungal target and role in antifungal drug resistance”, *Frontiers in Microbiology*, 3(JAN), bll 1–6. doi: 10.3389/fmicb.2012.00439.
- Alonso, E. N. *et al.* (2018) “Antitumoral and antimetastatic activity of Maitake D-Fraction in triple-negative breast cancer cells”, *Oncotarget*, 9(34), bll 23396–23412. doi: 10.18632/oncotarget.25174.
- Arimoto, M., Yamaguchi, H. en Nishibe, S. (1995) *Structure elucidation and synthesis of the lignans from the seeds of Hernandia ovigera L., Studies in Natural Products Chemistry*. Elsevier Masson SAS. doi: 10.1016/S1572-5995(96)80034-2.
- Barrios-González, J. (2018) “Secondary Metabolites Production”, *Current Developments in Biotechnology and Bioengineering*, bll 257–283. doi: 10.1016/b978-0-444-63990-5.00013-x.

Besaans, L. (2015) “ARC - PPRI FACT SHEETS ON INVASIVE ALIEN PLANTS AND THEIR CONTROL IN SOUTH AFRICA Compiled by : Lin Sztab & Lesley Henderson (2015) © ARC Plant Protection Research Institute”, *Arc-Lnr*, (iv), bl 2015.

Bulam, S. (2019) “True Morel (*Morchella spp.*) Mushroom in Turkey”, (January).

C.F.R. Ferreira, I. *et al.* (2012) “Compounds from Wild Mushrooms with Antitumor Potential”, *Anti-Cancer Agents in Medicinal Chemistry*, 10(5), bl 424–436. doi: 10.2174/1871520611009050424.

Cardwell, G. *et al.* (2018) “A review of mushrooms as a potential source of dietary vitamin D”, *Nutrients*, 10(10), bl 1–11. doi: 10.3390/nu10101498.

Chang, C. L. *et al.* (2010) “Cardiovascular protective flavonolignans and flavonoids from *Calamus quiquesetinervius*”, *Phytochemistry*, 71(2–3), bl 271–279. doi: 10.1016/j.phytochem.2009.09.025.

Chatterjee, B. en Patel, T. (2016) “Edible mushroom - A nutritious food improving human health”, *Int. J. Clin. and Biomed. Res.*, 2(1), bl 34–37. Available at: file:///Users/carolinavegaoliveros/Library/Application Support/Mendeley Desktop/Downloaded/Chatterjee, Patel - 2016 - Edible mushroom - A nutritious food improving human health.pdf%0Afile:///Users/carolinavegaoliveros/Library/Application%0ASupport/Mendele.

Chen, A. N. and L.-S. (2000) “NII-Electronic Library Service”, *Chemical Pharmaceutical Bulletin*, 3(1).

Chen, A. Y. en Chen, Y. C. (2013) “A review of the dietary flavonoid, kaempferol on human health and cancer chemoprevention”, *Food Chemistry*, 138(4), bl 2099–2107. doi: 10.1016/j.foodchem.2012.11.139.

Chen, D. *et al.* (2018) “Kaempferia parviflora and its methoxyflavones: Chemistry and biological activities”, *Evidence-based Complementary and Alternative Medicine*, 2018. doi: 10.1155/2018/4057456.

Chen, J., Montanari, A. M. en Widmer, W. W. (1997) “Two New Polymethoxylated Flavones, a Class of Compounds with Potential Anticancer Activity, Isolated from Cold Pressed Dancy Tangerine Peel Oil Solids”, *Journal of Agricultural and Food Chemistry*, 45(2), bl 364–368. doi: 10.1021/jf960110i.

Chen, S. et al. (2006) “Anti-aromatase activity of phytochemicals in white button mushrooms (*Agaricus bisporus*)”, *Cancer Research*, 66(24), bll 12026–12034. doi: 10.1158/0008-5472.CAN-06-2206.

Chen, S., Lee, I. en Kirschner, R. (no date) “Medicinal Mushrooms for Cancer: Science and Practice”, bll 81–114.

Chen, W. et al. (2014) “10.1111/mpp.12116”, *Molecular Plant Pathology*, 15(5), bll 433–446. doi: 10.1111/mpp.12116.

Clarke, Z. (2007) “Psilocybin”, (1), bll 1–4.

Control, P. et al. (2010) “Acacia saligna (Port Jackson willow) -Management and Control”, *Plant Ecology*, (September), bll 1–3.

Daejeon, B. (2005) “Antitum or activity of spinasterol isolated from Pueraria roots”, 37(2), bll 111–120.

Dalibalta, S., Majdalawieh, A. F. en Manjikian, H. (2020) “Health benefits of sesamin on cardiovascular disease and its associated risk factors”, *Saudi Pharmaceutical Journal*, 28(10), bll 1276–1289. doi: 10.1016/j.jps.2020.08.018.

Davis, D. en Agrios, G. N. (1970) “Plant Pathology”, *Mycologia*, 62(1), bl 207. doi: 10.2307/3757727.

Doungsa-ard, C. et al. (2015) “Uromycladium falcatarium sp. nov., the cause of gall rust on Paraserianthes falcatoria in south-east Asia”, *Australasian Plant Pathology*, 44(1), bll 25–30. doi: 10.1007/s13313-014-0301-z.

Du, Q. en Chen, H. (2010) “The methoxyflavones in Citrus reticulata Blanco cv. ponkan and their antiproliferative activity against cancer cells”, *Food Chemistry*, 119(2), bll 567–572. doi: 10.1016/j.foodchem.2009.06.059.

Figueiredo, L. en Régis, W. C. B. (2017) “Medicinal mushrooms in adjuvant cancer therapies: an approach to anticancer effects and presumed mechanisms of action”, *Nutrire*, 42(1), bll 1–10. doi: 10.1186/s41110-017-0050-1.

Frisvad, J. C. (2014) *Penicillium: Penicillium/Penicillia in Food Production*. Second Edi, *Encyclopedia of Food Microbiology: Second Edition*. Second Edi. Elsevier. doi: 10.1016/B978-0-12-384730-0.00249-4.

From, A. P. en Clavzflqrum, S. (1994) "ANTI-HIV ACTIVITY OF STRUCTURALLY RELATED TRITERPENOIDS En ", 57(2), bll 243–247.

Geng, X. et al. (2020) *Preventive and therapeutic effect of Ganoderma lucidum on kidney injuries and diseases*. 1st ed, *Advances in Pharmacology*. 1st ed. Elsevier Inc. doi: 10.1016/bs.apha.2019.10.003.

Groman, R. P. (2014) *Miscellaneous antibiotics*. Second Edi, *Small Animal Critical Care Medicine, Second Edition*. Second Edi. Elsevier Inc. doi: 10.1016/B978-1-4557-0306-7.00181-1.

Guina, J. en Merrill, B. (2018) "Benzodiazepines I: Upping the Care on Downers: The Evidence of Risks, Benefits and Alternatives", *Journal of Clinical Medicine*, 7(2), bl 17. doi: 10.3390/jcm7020017.

Gurnani, N. et al. (2014) "Natural Products : Source of Potential Drugs Natural Products Lab , School of Studies in Chemistry & Bio-Chemistry ", 6(6), bll 171–186. doi: 10.5829/idosi.ajbas.2014.6.6.21983.

Hall, I. H. et al. (1980) "Antitumor agents XLI: Effects of eupaformosanin on nucleic acid, protein, and anaerobic and aerobic glycolytic metabolism of ehrlich ascites cells", *Journal of Pharmaceutical Sciences*, 69(3), bll 294–297. doi: 10.1002/jps.2600690313.

Hao, D. C., Gu, X.-J. en Xiao, P. G. (2015) *Phytochemical and biological research of Papaver pharmaceutical resources, Medicinal Plants*. doi: 10.1016/b978-0-08-100085-4.00006-2.

Haschek, W. M. en Voss, K. A. (2013) *Mycotoxins*. Third Edit, *Haschek and Rousseaux's Handbook of Toxicologic Pathology*. Third Edit. Elsevier. doi: 10.1016/B978-0-12-415759-0.00039-X.

Haubrich, B. A. (2018) "Microbial sterolomics as a chemical biology tool", *Molecules*, 23(11), bll 1–37. doi: 10.3390/molecules23112768.

Herbrecht, R., Lid, K. L. en Bergerat, J. P. (1990) "Interactions of cyclosporine with antimicrobial agents", *Reviews of Infectious Diseases*, 12(2), bl 371. doi: 10.1093/clinids/12.2.371.

Hernday, A. D. et al. (2010) *Genetics and molecular biology in Candida albicans*. 2nd ed, *Methods in Enzymology*. 2nd ed. Elsevier Inc. doi: 10.1016/S0076-6879(10)70031-8.

- Hill, R. A. (2006) “Marine natural products”, bll 123–137. doi: 10.1039/b515100g.
- Holstege, C. P. (2005) “Opium”, *Encyclopedia of Toxicology*, bll 299–300. doi: 10.1016/B0-12-369400-0/00710-9.
- Holt, R. J. (1975) “New Antifungal Drugs”, *Drugs*, 9(6), bll 401–405. doi: 10.2165/00003495-197509060-00001.
- Houbraken, J., de Vries, R. P. en Samson, R. A. (2014) *Modern taxonomy of biotechnologically important aspergillus and penicillium species*. 1st ed, *Advances in Applied Microbiology*. 1st ed. Copyright © 2014 Elsevier Inc. All rights reserved. doi: 10.1016/B978-0-12-800262-9.00004-4.
- Huxtable, R. J. en Schwarz, S. K. (2001) “The isolation of morphine--first principles in science and ethics.”, *Molecular interventions*, 1(4), bll 189–191.
- Hyde, K. D. et al. (2019) “The amazing potential of fungi: 50 ways we can exploit fungi industrially”, *Fungal Diversity*, 97(1), bll 1–136. doi: 10.1007/s13225-019-00430-9.
- Iranshahi, M. et al. (2010) “Cancer chemopreventive activity of diversin from Ferula diversivittata in vitro and in vivo”, *Phytomedicine*, 17(3–4), bll 269–273. doi: 10.1016/j.phymed.2009.05.020.
- Ismail, B. P. (2017) “Food Analysis Laboratory Manual-Ash Content Determination”, *Food Analysis Laboratory Manual*, bll 117–119. doi: 10.1007/978-3-319-44127-6.
- Jakubczyk, D. en Dussart, F. (2020) “Selected fungal natural products with antimicrobial properties”, *Molecules*, 25(4), bll 1–18. doi: 10.3390/molecules25040911.
- Jeon, G. C. et al. (2005) “Antitumor activity of spinasterol isolated from Pueraria roots”, *Experimental and Molecular Medicine*, 37(2), bll 111–120. doi: 10.1038/emm.2005.15.
- Jong, S. C. en Birmingham, J. M. (1992) “Medicinal Benefits of the Mushroom Ganoderma”, *Advances in Applied Microbiology*, 37(C), bll 101–134. doi: 10.1016/S0065-2164(08)70253-3.
- Joseph, R. en Bachhawat, A. K. (2014) *Yeasts: Production and Commercial Uses*. Second Edi, *Encyclopedia of Food Microbiology: Second Edition*. Second Edi. Elsevier. doi: 10.1016/B978-0-12-384730-0.00361-X.
- Kadnikova, I. A. et al. (2015) “Chemical Composition and Nutritional Value of the

Mushroom Auricularia auricula-judae”, *Journal of Food Nutrition and Research*, 3(8), bll 478–482. doi: 10.12691/jfnr-3-8-1.

Kahnt, M. et al. (2018) “Platanic acid: A new scaffold for the synthesis of cytotoxic agents”, *European Journal of Medicinal Chemistry*, 143, bll 259–265. doi: 10.1016/j.ejmech.2017.11.046.

Kakon, A., Choudhury, M. B. K. en Saha, S. (2012) “Mushroom is an Ideal Food Supplement”, *Journal of Dhaka National Medical College & Hospital*, 18(1), bll 58–62. doi: 10.3329/jdnmch.v18i1.12243.

Keskes, H. et al. (2017) “LC-MS-MS and GC-MS analyses of biologically active extracts and fractions from tunisian juniperus phoenice leaves”, *Pharmaceutical Biology*, 55(1), bll 88–95. doi: 10.1080/13880209.2016.1230139.

Khan, T. et al. (2020) “Anticancer plants: A review of the active phytochemicals, applications in animal models, and regulatory aspects”, *Biomolecules*, 10(1). doi: 10.3390/biom10010047.

Khotimchenko, S. V., Vaskovsky, V. E. en Titlyanova, T. V. (2002) “Fatty acids of marine algae from the pacific coast of North California”, *Botanica Marina*, 45(1), bll 17–22. doi: 10.1515/BOT.2002.003.

Kimura, T. (2013) “Natural products and biological activity of the pharmacologically active cauliflower mushroom Sparassis crispa”, *BioMed Research International*, 2013. doi: 10.1155/2013/982317.

Kolb, M. (1993) “Synthetic communications: An international journal for rapid communication of synthetic organic chemistry”, *Synthetic Communications*, 23(1), bl vii. doi: 10.1080/00397919308020392.

Kolmer, J. A., Ordonez, M. E. en Groth, J. V (2009) “The Rust Fungi”, *Encyclopedia of Life Sciences*, (January). doi: 10.1002/9780470015902.a0021264.

Kozarski, M. et al. (2015) “Antioxidants of edible mushrooms”, *Molecules*, 20(10), bll 19489–19525. doi: 10.3390/molecules201019489.

Krause, J. en Tobin, G. (2013) “Using Old Solutions to New Problems - Natural Drug Discovery in the 21st Century”, *Using Old Solutions to New Problems - Natural Drug Discovery in the 21st Century*, bll 3–36. doi: 10.5772/46069.

Krishnan, B. et al. (2020) “Chrysoeriol ameliorates hyperglycemia by regulating the carbohydrate metabolic enzymes in streptozotocin-induced diabetic rats”, *Food Science and Human Wellness*, 9(4), bll 346–354. doi: 10.1016/j.fshw.2020.05.014.

Kumar, A. et al. (2017) *Secondary metabolism and antimicrobial metabolites of penicillium, New and Future Developments in Microbial Biotechnology and Bioengineering: Penicillium System Properties and Applications*. Elsevier B.V. doi: 10.1016/B978-0-444-63501-3.00003-X.

Kumari, N. en Srividhya, S. (2020) “Secondary metabolites and lytic tool box of trichoderma and their role in plant health”, *Molecular Aspects of Plant Beneficial Microbes in Agriculture*, bll 305–320.

Kuroyanagi, M. et al. (1999) “Antibacterial and antiandrogen flavonoids from *Sophora flavescens*”, *Journal of Natural Products*, 62(12), bll 1595–1599. doi: 10.1021/np990051d.

Kwon, D. H. et al. (2018) “Schisandrin A suppresses lipopolysaccharide-induced inflammation and oxidative stress in RAW 264.7 macrophages by suppressing the NF-κB, MAPKs and PI3K/Akt pathways and activating Nrf2/HO-1 signaling”, *International Journal of Molecular Medicine*, 41(1), bll 264–274. doi: 10.3892/ijmm.2017.3209.

Lee, I. et al. (2006) “Hispidin Derivatives from the Mushroom *Inonotus xeranticus* and Their Antioxidant Activity”, bll 299–301.

Lee, K. H. et al. (1977) “Structure and stereochemistry of eupaformosanin, a new antileukemic and antisarcoma germacraneolide from *Eupatorium formosanum*”, *Phytochemistry*, 16(7), bll 1068–1070. doi: 10.1016/S0031-9422(00)86734-5.

Leonard, K. J. en Szabo, L. J. (2005) “Stem rust of small grains and grasses caused by *Puccinia graminis*”, *Molecular Plant Pathology*, 6(2), bll 99–111. doi: 10.1111/j.1364-3703.2005.00273.x.

Li, L. et al. (2004) “Rubriflorin A and B , Two Novel Partially Saturated Dibenzocyclooctene Lignans from *Schisandra rubriflora* 19 species , mostly growing in the southwestern part of the country on mountains 1500 m”, 87, bll 3–7.

Lin, Y. et al. (1998) “Antiviral Activities of Biflavonoids”, bll 120–125.

Liu, H. et al. (2021) “Iridoids from *Valeriana jatamansi* with anti-inflammatory and antiproliferative properties”, *Phytochemistry*, 184(January), bl 112681. doi:

10.1016/j.phytochem.2021.112681.

López-González, A. et al. (2013) “The role of anthracyclines in small cell lung cancer”, *Annals of Translational Medicine*, 1(1), bll 4–9. doi: 10.3978/j.issn.2305-5839.2013.01.05.

Lull, C., Wicher, H. J. en Savelkoul, H. F. J. (2005) “Antiinflammatory and immunomodulating properties of fungal metabolites”, *Mediators of Inflammation*, 2005(2), bll 63–80. doi: 10.1155/MI.2005.63.

Ma, X. et al. (2020) “Identification of Berberis spp. as Alternate Hosts for Puccinia achnatheri-sibirici Under Controlled Conditions and Morphologic Observations of Sexual Stage Development of the Rust Fungus”, *Frontiers in Microbiology*, 11(June), bll 1–14. doi: 10.3389/fmicb.2020.01278.

Ma, Y. et al. (2021) “A narrative review of botanical characteristics, phytochemistry and pharmacology of Valeriana jatamansi jones”, *Longhua Chinese Medicine*, 4(12), bll 5–5. doi: 10.21037/lcm-20-54.

Malmstrøm, J. et al. (2002) “Bioactive Metabolites from a Marine-Derived Strain of the Fungus *Emericella variecolor*”, 82, bll 364–367.

Marinelli, F. en Marcone, G. L. (2011) *Microbial Secondary Metabolites*. Second Edi, *Comprehensive Biotechnology, Second Edition*. Second Edi. Elsevier B.V. doi: 10.1016/B978-0-08-088504-9.00539-0.

Mathew, A. K. et al. (2018) *Lignocellulosic biorefinery wastes, or resources?*, *Waste Biorefinery: Potential and Perspectives*. Elsevier B.V. doi: 10.1016/B978-0-444-63992-9.00009-4.

Mendgen, K. (1984) “Development and Physiology of Teliospores”, *The Cereal Rusts*, bll 375–398. doi: 10.1016/b978-0-12-148401-9.50018-2.

Mohmand, A. Q. K. et al. (2011) “Medical importance of fungi with special emphasis on mushrooms”, *ISRA Medical Journal*, 3(1), bll 1–44.

Moon, H. J. et al. (2009) “Lactational coumestrol exposure increases ovarian apoptosis in adult rats”, *Archives of Toxicology*, 83(6), bll 601–608. doi: 10.1007/s00204-008-0400-0.

Moore, D. (2001) “Slayers, Saviors, Servants and Sex”, *Slayers, Saviors, Servants and Sex*, (April). doi: 10.1007/978-1-4613-0135-6.

Mpofu, S. et al. (2014) “Interactive efficacies of Elephantorrhiza elephantina and Pentanisia prunelloides extracts and isolated compounds against gastrointestinal bacteria”, *South African Journal of Botany*, 94, bll 224–230. doi: 10.1016/j.sajb.2014.07.002.

Müller, H. et al. (2000) “Natural products in drug discovery.”, *Ernst Schering Research Foundation workshop*, (32), bll 205–216. doi: 10.1007/978-3-662-04042-3_7.

n, Eunsung Mouradian, M. M. (2008) “基因的改变NIH Public Access”, *Bone*, 23(1), bll 1–7.

Nakasugi, T. en Komai, K. (1998) “Antimutagens in the Brazilian Folk Medicinal Plant Carqueja (Baccharis trimera Less.)”, *Journal of Agricultural and Food Chemistry*, 46(7), bll 2560–2564. doi: 10.1021/jf9711045.

Newcombe, G. (2006) “Rust Diseases”, *Encyclopedia of Entomology*, (December), bll 1928–1928. doi: 10.1007/0-306-48380-7_3730.

Oliveira, B. P. P. en Rodrigues, F. (2018) *Plant Extracts in Skin Care Products, Plant Extracts in Skin Care Products*. doi: 10.3390/books978-3-03897-161-0.

del Olmo, A., Calzada, J. en Nuñez, M. (2017) “Benzoic acid and its derivatives as naturally occurring compounds in foods and as additives: Uses, exposure, and controversy”, *Critical Reviews in Food Science and Nutrition*, 57(14), bll 3084–3103. doi: 10.1080/10408398.2015.1087964.

Onifade, E. O. et al. (2019) “The medical benefits of Mushrooms in Public Health”, 2(2), bll 1–3.

Pandey, A. K. (2018) “SMUT FUNGI : AN OVERVIEW”, (April).

Patel, S. en Goyal, A. (2012) “Recent developments in mushrooms as anti-cancer therapeutics: a review”, *3 Biotech*, 2(1), bll 1–15. doi: 10.1007/s13205-011-0036-2.

Pettit, G. R. et al. (2000) “Antineoplastic agents 430. Isolation and structure of cibrostatins 3, 4, and 5 from the Republic of Maldives Cribrochalina species”, *Journal of Natural Products*, 63(6), bll 793–798. doi: 10.1021/np990618q.

Pitt, J. I. (1994) “The Current role of aspergillus and penicillium in human and animal health”, *Medical Mycology*, 32(S1), bll 17–32. doi: 10.1080/02681219480000701.

R.S.Vardanyan en V.J.Hruba (2006) “Sulfonamide drugs and trimethoprim”, *Synthesis of Essential Drugs*, bll 499–523.

Radzki, W. et al. (2016) “Effect of processing on the content and biological activity of polysaccharides from Pleurotus ostreatus mushroom”, *LWT - Food Science and Technology*, 66, bll 27–33. doi: 10.1016/j.lwt.2015.10.016.

Ratio, S. I. (2017) “基于多元素和稳定同位素的特基拉酒原产地鉴定 张建1, 2 , 马义虔1, 2 , 田玲3 *, 邵飞龙1, 2 , 卢垣宇1, 2 , 陈兴林1, 2 1..”, (January), bll 2–5.

Reddy, L. H. en Couvreur, P. (2009) “Squalene: A natural triterpene for use in disease management and therapy”, *Advanced Drug Delivery Reviews*, 61(15), bll 1412–1426. doi: 10.1016/j.addr.2009.09.005.

Reid, D. A. en Webster, J. (1973) “Introduction to Fungi”, *Kew Bulletin*, 28(1), bl 166. doi: 10.2307/4117096.

Reyna, S. en Garcia-Barreda, S. (2014) “Black truffle cultivation: A global reality”, *Forest Systems*, 23(2), bll 317–328. doi: 10.5424/fs/2014232-04771.

Rodríguez P, L. et al. (2017) “A review of paraneoplastic syndromes in gastrointestinal tumors”, *Rev. colomb. gastroenterol.*, 32(3), bll 230–244. doi: 10.22516/25007440.155.

Sarfraz, A. et al. (2020) “Hispolon: A natural polyphenol and emerging cancer killer by multiple cellular signaling pathways”, *Environmental Research*, 190(July). doi: 10.1016/j.envres.2020.110017.

Semaming, Y. et al. (2015) “Pharmacological properties of protocatechuic acid and its potential roles as complementary medicine”, *Evidence-based Complementary and Alternative Medicine*, 2015. doi: 10.1155/2015/593902.

Seneca (2007) “Alkaloid Chemistry”, *Alkaloids - Secrets of Life*, (Table 19), bll 61–139. doi: 10.1016/b978-044452736-3/50004-0.

Shattock (2003) “Further Reading”, *Annals of Applied Biology*, bll 165–169.

Shi, B. J. et al. (2007) “Anticancer activities of a chemically sulfated polysaccharide obtained from Grifola frondosa and its combination with 5-Fluorouracil against human gastric carcinoma cells”, *Carbohydrate Polymers*, 68(4), bll 687–692. doi:

10.1016/j.carbpol.2006.08.003.

Shilpi, J. A. *et al.* (2012) “Antinociceptive, anti-inflammatory, and antipyretic activity of mangrove plants: A mini review”, *Advances in Pharmacological Sciences*, 2012. doi: 10.1155/2012/576086.

Singh, D. R. (2017) “A Review on Different Benefits of Mushroom”, *IOSR Journal of Pharmacy and Biological Sciences*, 12(01), bll 107–111. doi: 10.9790/3008-120102107111.

Sriprasanthi, R. B. (2012) “PEER-REVIEW ARTICLE”, (May 2014).

Stierle, A. A., Stierle, D. B. en Bugni, T. (1999) “Sequoiatones A and B: Novel antitumor metabolites isolated from a redwood endophyte”, *Journal of Organic Chemistry*, 64(15), bll 5479–5484. doi: 10.1021/jo9902771.

Strobel, G. A. *et al.* (1999) “Cryptocandin, a potent antimycotic from the endophytic fungus Cryptosporiopsis cf. quercina”, *Microbiology*, 145(8), bll 1919–1926. doi: 10.1099/13500872-145-8-1919.

Sun, W. *et al.* (2019) “The genome of the medicinal plant Andrographis paniculata provides insight into the biosynthesis of the bioactive diterpenoid neoandrographolide”, *Plant Journal*, 97(5), bll 841–857. doi: 10.1111/tpj.14162.

Tabassum Khan, N. (2017) “Cyclosporin A Production from Tolipocladium inflatum”, *General Medicine: Open Access*, 05(04), bll 4–6. doi: 10.4172/2327-5146.1000294.

TATTAR, T. A. (1989) “Rust Diseases”, *Diseases of Shade Trees*, bll 168–188. doi: 10.1016/b978-0-12-684351-4.50017-x.

Taylor, P. (no date) “Agricultural and Biological Chemistry”, (November 2014), bll 8–10. doi: 10.1080/00021369.1985.10867272.

Temesgen, T. (2018) “Application of Mushroom as Food and Medicine”, *Advances in Biotechnology & Microbiology*, 11(4). doi: 10.19080/aibm.2018.11.555817.

Thornton, M. J. (2002) “The biological actions of estrogens on skin”, *Experimental Dermatology*, 11(6), bll 487–502. doi: 10.1034/j.1600-0625.2002.110601.x.

Tu, P. *et al.* (2020) “Cytotoxic and Anti-inflammatory Terpenoids from the Whole Plant of Vaccinium emarginatum”, bll 7–16.

Ukiya, M. *et al.* (2002) “Inhibition of Tumor-Promoting Effects by Poricoic Acids G and H

and Other Lanostane-Type Triterpenes and Cytotoxic Activity of Poricoic Acids A and G from *Poria cocos*”, 4(28), bll 462–465.

Üstün, N. S., Bulam, S. en Pekşen, A. (2018) “The use of mushrooms and their extracts and compounds in functional foods and nutraceuticals”, *Türkmen, A.(ed.)*, (June). Available at: https://www.researchgate.net/profile/Sanem_Bulam/publication/328939374_The_Use_of_Mushrooms_and_Their_Extracts_and_Compounds_in_Functional_Foods_and_Nutraceuticals/links/5bec3d16299bf1124fd1e43f/The-Use-of-Mushrooms-and-Their-Extracts-and-Compounds-in-Fun.

Valverde, M. E., Hernández-pérez, T. en Paredes-lópez, O. (2017) “Inside Front Cover (Editorial Board)”, *Phytochemistry Letters*, 20(Table 1), bl IFC. Available at: <http://linkinghub.elsevier.com/retrieve/pii/S1874390017303634>.

Vann Jones, S. A. en O’Kelly, A. (2020) “Psychedelics as a Treatment for Alzheimer’s Disease Dementia”, *Frontiers in Synaptic Neuroscience*, 12(August), bll 10–15. doi: 10.3389/fnsyn.2020.00034.

Veeresham, C. (2012) “Natural products derived from plants as a source of drugs”, *Journal of Advanced Pharmaceutical Technology and Research*, 3(4), bll 200–201. doi: 10.4103/2231-4040.104709.

Venturini, M. E. et al. (2008) “Antimicrobial activity of extracts of edible wild and cultivated mushrooms against foodborne bacterial strains”, *Journal of Food Protection*, 71(8), bll 1701–1706. doi: 10.4315/0362-028X-71.8.1701.

Vi, L. L. et al. (1987) “TABLE I . 13C-NMR Data for Hernolactone (ppm)”, 35, bll 4162–4168.

Voegele, R. T. en Mendgen, K. W. (2011) “Nutrient uptake in rust fungi: How sweet is parasitic life?”, *Euphytica*, 179(1), bll 41–55. doi: 10.1007/s10681-011-0358-5.

Wakuta, S. et al. (2010) “Identification of a β-glucosidase hydrolyzing tuberonic acid glucoside in rice (*Oryza sativa L.*)”, *Phytochemistry*, 71(11–12), bll 1280–1288. doi: 10.1016/j.phytochem.2010.04.025.

Wang, H., Gao, J. en Ng, T. B. (2000) “A new lectin with highly potent antihepatoma and antisarcoma activities from the oyster mushroom *Pleurotus ostreatus*”, *Biochemical and Biophysical Research Communications*, 275(3), bll 810–816. doi: 10.1006/bbrc.2000.3373.

- Wang, J., Wang, H. X. en Ng, T. B. (2007) “A peptide with HIV-1 reverse transcriptase inhibitory activity from the medicinal mushroom *Russula paludosa*”, *Peptides*, 28(3), bll 560–565. doi: 10.1016/j.peptides.2006.10.004.
- Wang, X. *et al.* (2021) “White button mushroom (*Agaricus bisporus*) disrupts androgen receptor signaling in human prostate cancer cells and patient-derived xenograft”, *Journal of Nutritional Biochemistry*, 89, bl 108580. doi: 10.1016/j.jnutbio.2020.108580.
- Wieczorek, P. P. *et al.* (2015) *Bioactive alkaloids of hallucinogenic mushrooms, Studies in Natural Products Chemistry*. doi: 10.1016/B978-0-444-63462-7.00005-1.
- Wolfender, J.-L. en Hostettmann, K. (1995) “Applications of Liquid Chromatography-Mass Spectrometry to the Investigation of Medicinal Plants”, *Phytochemistry of Medicinal Plants*, bll 189–215. doi: 10.1007/978-1-4899-1778-2_9.
- Wood, A. (no date) “The Acacia Gall Rust Fungus , *Uromycladium tepperianum* A fungal pathogen of Port Jackson (*Acacia saligna*) in South Africa”.
- Wood, A. R. (2012) “*Uromycladium tepperianum* (a gall-forming rust fungus) causes a sustained epidemic on the weed *Acacia saligna* in South Africa”, *Australasian Plant Pathology*, 41(3), bll 255–261. doi: 10.1007/s13313-012-0126-6.
- Wood, A. R. en Morris, M. J. (2007) “Impact of the gall-forming rust fungus *Uromycladium tepperianum* on the invasive tree *Acacia saligna* in South Africa: 15 years of monitoring”, *Biological Control*, 41(1), bll 68–77. doi: 10.1016/j.biocontrol.2006.12.018.
- Wu, J. *et al.* (2013) “Icariside II induces apoptosis via inhibition of the EGFR pathways in A431 human epidermoid carcinoma cells”, *Molecular Medicine Reports*, 8(2), bll 597–602. doi: 10.3892/mmr.2013.1557.
- Van Wychen, S. en Laurens, L. M. L. (2015) “Determination of Total Carbohydrates in Algal Biomass - Laboratory Analytical Procedure (LAP)”, *Laboratory Analytical Procedure (LAP)*, (December). Available at: www.nrel.gov/publications.
- Yagura, T. *et al.* (2008) “Anticarcinogenic compounds in the Uzbek medicinal plant, *Helichrysum maracandicum*”, *Journal of Natural Medicines*, 62(2), bll 174–178. doi: 10.1007/s11418-007-0223-y.
- Yan, X. *et al.* (2017) “Apigenin in cancer therapy: Anti-cancer effects and mechanisms of action”, *Cell and Bioscience*, 7(1), bll 1–16. doi: 10.1186/s13578-017-0179-x.

Yang, D., Zhou, Z. en Zhang, L. (2019) *An overview of fungal glycan-based therapeutics*. 1st ed, *Progress in Molecular Biology and Translational Science*. 1st ed. Elsevier Inc. doi: 10.1016/bs.pmbts.2019.02.001.

Yu, L. et al. (2008) “Anti-inflammatory effects of schisandrin isolated from the fruit of Schisandra chinensis Baill”, 591, bll 293–299. doi: 10.1016/j.ejphar.2008.06.074.

Yunes, R. A. et al. (2005) “The use of natural products as sources of new analgesic drugs”, *Studies in Natural Products Chemistry*, 30(C), bll 191–212. doi: 10.1016/S1572-5995(05)80033-X.

Zhang, M. et al. (2019) *Mushroom polysaccharide lentinan for treating different types of cancers: A review of 12 years clinical studies in China*. 1st ed, *Progress in Molecular Biology and Translational Science*. 1st ed. Elsevier Inc. doi: 10.1016/bs.pmbts.2019.02.013.

