ISOLATION AND CHARACTERIZATION OF NATURAL PRODUCTS FROM SELECTED *RHUS SPECIES*

UNIVERSITY *of the*
WESTERN CAPE

MKHUSELI KOKI

MTECH CHEMISTRY, CAPE PENINSULA UNIVERSITY OF TECHNOLOGY

A thesis submitted in partial fulfilment of the requirements for the degree of

Doctor of Philosophy

JUNE 2020

Department of Chemistry

Faculty of Natural Sciences

University of the Western Cape

Supervisor: Prof. T.W Mabusela

ABSTRACT

Searsia is the more recent name for the genus (*Rhus*) that contains over 250 individual species of flowering plants in the family *Anacardiaceae*. Research conducted on *Searsia* extracts to date indicates a promising potential for this plant group to provide renewable bioproducts with the following reported desirable bioactivities; antimicrobial, antifungal, antiviral, antimalarial, antioxidant, antifibrogenic, anti-inflammatory, antimutagenic, antithrombin, antitumorigenic, cytotoxic, hypoglycaemic, and leukopenic (Rayne and Mazza, 2007, Salimi *et al*., 2015).

Searsia glauca, Searsia lucida and *Searsia laevigata* were selected for this study. The aim of this study was to isolate, elucidate and evaluate the biological activity of natural products occurring in the plants selected.

From the three *Searsia* species seven known terpenes were isolated and characterized using chromatographic techniques and spectroscopic techniques: Moronic acid (**C1 & C5**), 21*β*-hydroxylolean-12-en-3-one (**C2**), Lupeol (**C11a),** β-Amyrin (**C11b & C10**), α-amyrin (**C11c**) and a mixture β-Amyrin (**C12a**) and α-amyrin (**C12b**) of fatty acid ester. Six known flavonoids were isolated myricetin-3-*O*-*β*-galactopyranoside (**C3**), Rutin (**C4**), quercetin (**C6**), Apigenin (**C7**), Amentoflavone (C**8**), quercetin-3-*O*-*β*-glucoside (**C9**).

The *in vitro* anti-diabetic activity of the extracts was investigated on selected carbohydrate digestive enzymes. The enzyme inhibition effect was conducted at 2.0 mg/ml for both carbohydrate digestive enzymes. The α-glucosidase inhibition effect percentage (%) results from various crude extracts *S. glauca* (Hexane, 59,13; DCM, 85.22 ; EtOAc, 62.50 ; Butanol, 55.76), *S. Lucida (*Hexane, 91.85; DCM, 49.28; EtOAc, 93.54; Butanol, 67.74) and *S. Laevigata* (Hexane,73.92; DCM,68.28 ; EtOAc,79.99 ; Butanol, 90.10) and α-amylase inhibition effect percentage results are as follows *S. glauca* (Hexane, 41.24 ; DCM,50.08 ; EtOAc, 39.85; Butanol, 47.71), *S. lucida* (Hexane,24.95 ; DCM,33.47 ; EtOAc,50.16 ;

Butanol,60.88) *and S. laevigata* (Hexane, 41.81; DCM,40.99 ; EtOAc,52.31 ; Butanol,59.72. Results exhibited from extracts inhibition effects indicate that extracts from these plants are possible sources of compounds with anti-diabetic activity.

The isolated compounds were screened at 125.0 µg/ml and other compounds did not show any activity against both digestive enzymes at that concentration. The pure compounds that showed activity at 125.0 µg/ml were further screened to determine IC_{50} values on both digestive enzymes. The α-glucosidase and α-amylase results from **Amentoflavone (C8) (**IC_{50} 5.57 ±1.12 ug/ml and 19.84 ± 1.33 ug/ml**)** and **Moronic acid (C1) (**IC_{50} 10.62 ± 0.89 and 20.08 ± 0.56).

The *in vitro* inhibition of oxidative stress by hexane, dichloromethane, ethyl acetate, methanol and butanol extracts were investigated as total antioxidant capacity using (FRAP, ORAC, TEAC). The results exhibited that methanol extract, ethyl acetate and butanol extracts of *S. lucida* showed high FRAP values of 1038.39 ± 80.41 µM AAE/g, 137.24 ± 16.54 µM AAE/g and 680.01 ± 96.34 µM AAE/g and while hexane and dichloromethane extracts revealed less FRAP values of 12.99 ± 1.82 µM AAE/g and 40.69 ± 2.11 µM AAE/g. The FRAP values obtained from methanol, ethyl acetate and butanol extracts of *S. laevigata* showed significant activity i.e; 411.58 ± 79.73, 86.28 ± 9.16 and 977.88 ± 71.24 respectively. It was observed that butanol, ethyl acetate and dichloromethane extracts from *S. glauca* showed high FRAP values i.e; 1008.67 ± 41.65 µM AAE/g, 557.09 ± 39.41 µM AAE/g and 431.62 ± 39.34 µM AAE/g respectively.

Ethyl acetate and butanol extracts of *S. glauca* exhibited the high ORAC values of 4574.93 ± 109.12 and 5653.36 ± 328.66 µM TE/g respectively. The results showed that ethyl acetate, methanol and butanol extracts exhibited the highest ORAC values of 4010.56 ± 73.52, 5793.45 ± 27.30, and 4198.42 ± 166.53 respectively. Hexane and dichloromethane extracts showed the lowest ORAC values of 1013.65 ± 12.26 and 1048.15 ± 35.35 respectively.

Methanolic extract of *S. lucida* exhibited the high TAEC value of 1512.08 ± 0.06 (µM TE/g) and butanol extract of *S. glauca* indicated the high TEAC activity of 1438.63 ± 13.53 (µM TE/g). Ethyl acetate of *S. glauca* showed the higher TEAC values of 1095.42 ± 28.42 (µM TE/g). Hexane and dichloromethane extracts of *S. lucida* showed the less TEAC values 20.26 ± 4.42 and 52.20 ± 2.82 (µM TE/g) respectively.

Toxicity assay was conducted in brine shrimp lethality test. The hexane and dichloromethane extracts exhibited less activity with an LC50 greater than a highest tested dosage (1000 µg/mL), while *S. glauca, S. lucida, and S. laevigata* ethyl acetate extracts showed activity values of 966.74, 905.22, 855.79 µg/mL and butanol extracts showed an activity of 621.24, 676.68, 571.71 µg/mL respectively.

DECLARATION

I, Mkhuseli Koki hereby declares that this research is a result of my own investigation, where use was made of outside material, proper acknowledgements have been made in the text. This thesis has not been submitted for any degree or examination in any university.

Signed……Date……..Mkhuseli Koki

Signed……Date……

Professor WT Mabusela

ACKNOWLEDGEMENT

I express my sincere gratitude to my supervisor, Professor Wilfred T Mabusela for his supervision, guidance, and encouragement throughout my studies.

I am grateful to Prof Ahmed Mohammed for the support and guidance.

I am thankful to my family: Nozakufa / Vavandin (mom), Vusi, Musa, Nkululeko, and Asithandile your steadfast support and enduring love.

I thank Medical Research Council (MRC-SA) for the financial support.

I am grateful to FORENSIC SCIENCE LABORATORY (SAPS) for allowing me to use their instrumentation during the course of this study.

Special thanks goes to Masande Yalo and Mthandazo Rondo your contribution is appreciated.

To Ndikho, Masixole, Sinazo, Nangamso, Sazi, Zandile and Dr Henry Mwangi it was a great experience working in the lab with you guys, thanks for everything.

DEDICATION

This work is dedicated to my late sister ***Vuyokazi Koki***

LIST OF ABBREVATIONS

AOC-Antioxidant capacity

BuOH- Butanol

COSY - Correlated Spectroscopy

DEPT-Distortionless Enhancement by Polarization Transfer

DCM-Dichloromethane

DMSO-Dimethylsulfoxide

DPPH-2, 2-diphenyl-1-picrylhydrazine

ESI-MS-Electrospray ionization trap mass spectroscopy

ES-MS- Electrospray ionization mass spectroscopy

EtOAc-Ethyl acetate

ET-Electron transfer

FRAP-Ferric reducing antioxidant power

GC-Gas Chromatography

HMBC-Heteronuclear multiple bond connectivity

HMQC-Heteronuclear multiple quantum correlation

HREI (MS) - High resolution electron impact Mass Spectroscopy

HSQC-Hetero-nuclear Single Quantum Coherence

HPLC-high Performance liquid chromatography

HSV- Herpes Simplex Virus

LC_{50} -Concentration that gives 50% inhibition

IR-Infra Red

MeOH- Methanol

MS- mass Spectroscopy

NMR-Nuclear Magnetic Resonance

NOESY-Nuclear Overhauser Enhancement Spectroscopy

ORAC- Oxygen radical absorption capacity

TEAC-Trolox equivalent antioxidant capacity

TLC-Thin Layer Chromatography

TMS-Tetramethylsilane

TMSO-trimethylsilyl methanesulfonate

TE-Trolox equivalent

1D- One dimensional

2D-Two Dimensional

HDL- high-density lipoprotein

LDL-low-density lipoprotein

LIST OF FIGURES

Chapter One: General introduction

LIST OF TABLES

TABLE OF CONTENT

CHAPTER ONE: GENERAL INTRODUCTION

1.1 Background

Medicinal plants, since time immemorial, have been used in virtually all cultures as a source of medicine. They have been used as valuable medicines in India, China, Egypt and Greece and a number of modern drugs have been developed from them. Currently, the World Health Organization estimates that approximately 80% of the world's inhabitants rely on traditional medicine for their primary health care; especially for developing countries where conventional medicines are largely unaffordable. Secondary metabolites are naturally occurring plant-derived substances with minimal or no industrial processing. However, given the current scientific advancement, application of herbal medicines based on long-term empirical and traditional uses is no longer sufficient. This hence calls for the need of up-to-date scientific information on herbal medicines to assure their quality, safety and efficacy. Effects are suspected to be due to their chemical constituents; hence chemical analysis facilitates identification of such compounds. Analysis of secondary metabolites in plants is a challenging task due to their chemical diversity, low abundance and high variability even within the same species (Steinman & Ganzera, 2011). In fact, medicinal plants comprise of constituents that belong to different compound classes with diverse chemical and physical properties (Zhou *et al*., 2009). Medicinal plants are chemically complex and diverse. Their botanical compounds elicit a wide range of biological and pharmacological properties (Patwardhan & Gautam, 2005). Searching for active agents against diseases among natural products is a more effective drug screening platform than random screening.

Natural products continue to be a major source of pharmaceuticals and for the discovery of new molecular structures. Various plant products such as alkaloids, terpenes, sterols, flavonoids, lignans, and saponins continue to be drug targets (Steinmann & Ganzera, 2011).

These phytochemicals are non-nutritive plant chemicals that have protective properties. Natural products, either as pure compounds, or standardized plant extracts, provide unlimited opportunities for new drug leads due to their unmatched availability and chemical diversity (Maregesi, 2008). Plants produce them to protect themselves but recent research shows that they can also protect humans against both communicable and non-communicable diseases. The multi-purpose nature of herbal medicine, from an ethnopharmacological point of view, may be associated with the broad diversity of their natural product composition and hence it is becoming imperative that they are tested against an ever-increasing range of bioassays.

1.2 Oxidative stress and human health

Oxidative stress is an imbalance between production of oxidants (free radicals) and antioxidant such that the production of oxidants overcomes the antioxidant defense system. Free radical species mediate damage to protein, lipids, mitochondria, and DNA and may activate the cell cycle; overwhelm endogenous antioxidant defenses in the brain; and contribute to neuronal damage (Lovell et al., 2007; Montine et al., 2002). Obesity and type 2 diabetes are associated with an increase in oxidative stress. Therefore, it would be important to consider antioxidants supplements in the management of diabetes and obesity (Abdali et al., 2015).

Free radicals are highly reactive molecules that can be classified as reactive oxygen species (ROS) or nitrogen reactive species (NOS). ROS includes superoxide anion, hydrogen peroxide, hydroxyl radical, singlet molecular oxygen and organic peroxide radicals. Under stress, the body produces more ROS, such as superoxide anion and hydroxyl radical, which are highly reactive and potentially damaging transient chemical species.

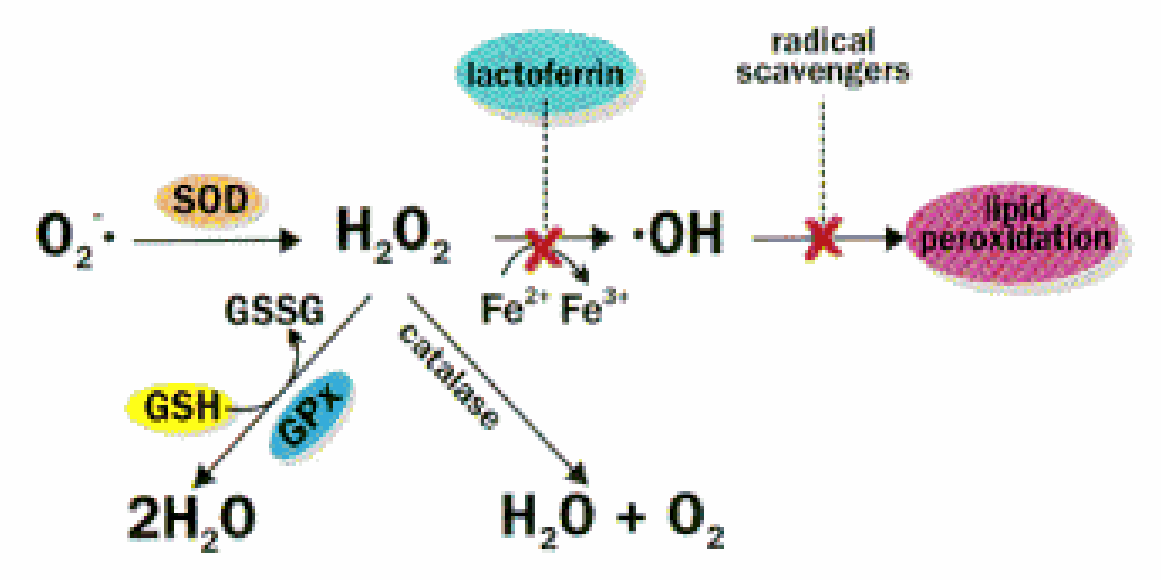


Figure 1. 1: Destructive effects of free radical are neutralized by endogenous antioxidant defence system in producing harmless molecules.

The antioxidant defense system is made up of enzyme (Catalase, superoxide dismutase, glutathione, peroxidases, and glutathione reductase) and non-enzymatic antioxidants (Rebamang A. Mosa, 2014). Free radical formation in diabetes by non-enzymatic glycation of proteins, glucose oxidation and increased lipid peroxidation leads to damage of enzymes, cellular machinery and also increased insulin resistance due to oxidative stress (Maritim et al., 2003).

Oxidative stress has been implicated in various pathophysiological disorders and diseases such as inflammation, hyperlipidemia, cancer, obesity, atherosclerosis, diabetes, and neurodegenerative diseases (Shibata and Kobayashi, 2008; Chang *et al.*, 2010; Shalaby and Hamouda, 2013). Oxidative stress has been reported to contribute in molecular and cellular tissue damage mechanism in a wide spectrum of human diseases (Valko et al., 2007). Evidence have been reported that proves the contribution of oxidative stress in the pathogenesis of both type 1 and 2 diabetes. (Maritim et al., 2008).

Development of new and naturally derived antioxidants for food and health applications, with safety profile is a major goal in sustainable bio-products. Previous studies reported that extracts from the stems, roots, bark, leaves, fruits, and seeds of many plants have antioxidant potential (Kotebagilu *et al*., 2014). Natural antioxidants represent a potentially side-effect-free alternative to synthetic antioxidants (Krishnaiah *et al*., 2011). A study conducted by Asgarpanah and Saati, 2014 revealed that the *Searsia* species possess pharmacological properties such as antibacterial, antifungal, antioxidant and hypoglycemic activities.

These diabetic complications are likely to be mediated by oxidative stress. The control of postprandial hyperglycaemia, hyperlipidemia and reduction of oxidative stress is important in preventing diabetes-associated complications (Santos *et al*., 2012).

1.3 Obesity

Obesity is a common chronic metabolic disorder of lipids and carbohydrate which is characterized by excessive deposition of fats in adipose tissue and other internal organs. Little *et al*, 2007, Ordovas *et al*., 2008, Mohamed *et al*., 2014 reported that physiological, genetical, behavioral and environmental factors such as excessive intake of high caloric food, dietary lifestyle, all contribute to the development of excessive weight and obesity. Obesity has reached epidemic levels globally with about 300 million adults being clinically obese (WHO, 2009). It has also been projected that if not controlled, the number will reach 1.12 billion by 2030 (Kelly *et al.*, 2008). Thus, obesity is a global concern and the major contributor to increased prevalence of various pathophysiological disorders such as hyperlipidemia, diabetes mellitus, certain types of cancer, inflammation, cardiovascular diseases and other atherothrombotic related diseases (Gurevich- Panigrahi *et al*., 2009; Yun, 2010; Roberts *et al*., 2010).

A common type of pathogenesis in many obesity-associated diseases is oxidative stress (Marseglia et al., 2015). Oxidative stress has been reported as one of the mechanisms implicated in vascular complications of diabetes and in pancreatic β-cell failure in diabetes, and obese

people without diabetes also display increased level of systematic oxidative stress (Matsuda and Shimomura, 2013).

1.4 Diabetes

Diabetes is rapidly emerging as a global health problem that threatens to reach epidemic levels by 2030 (Shaw *et al*., 2010). It is another serious chronic metabolic disorder characterized by chronic hyperglycaemia. Hyperglycaemia results from abnormal metabolism of carbohydrate, lipids and protein. *Diabetes mellitus* is characterized by hyperglycemia resulting from defects in insulin secretion, action or both (Mamun-or-Rashid, 2014). The condition is commonly classified into type 1 and type 2. Type 1 is usually referred to as insulin-dependent since it results from failure of the pancreatic cells to secrete insulin and its complications are managed by injection of exogenous insulin. Contrary to Type I diabetes, where there is insufficient insulin production, in Type II diabetes the pancreas produces normal or greater than normal quantities of insulin.

The management of the disease is an important step for its control which involves lowering the postprandial increase in blood glucose levels by inhibiting the enzymes, α-amylase, and α-glucosidase, responsible for hydrolysis of carbohydrates to simple sugars such as glucose (Zia-Ul-Haq *et al*., 2011). Currently insulin and oral anti-diabetic chemical agents (glucosidase inhibitor, biguanides, insulin sensitizer and sulfonylureas) are used in clinical practice as therapies for *Diabetes mellitus* (Zhang *et al*., 2014). Many of these have limitations and side effects such as liver and kidney failure, hypoglycaemia, diarrhoea and lactic acidosis which are difficult to tolerate (Wang *et al*., 2007).

The hyperglycaemia and hyperlipidemia commonly observed in diabetes are considered the main contributors to the development of micro and macro vascular complications of diabetes (Ortiz-Andrade *et al.,* 2007; Gutierrez, 2013). Natural products may be feasible alternative

remedies for the treatment of diabetes or be complementary to currently used treatments (Coman *et al*., 2012).

1.4.1 Enzyme inhibition

Diabetes type 2 is the most common type of diabetes, it is a metabolic disorder of multiple aetiologies characterized by carbohydrate, lipid and protein metabolic disorders that include defects in insulin secretion, with a major contribution of insulin resistance (Albert and Zimmet, 1998). Diabetes has been treated by decreasing post prandial hyperglycemia (Kumar et al., 2012). This has been achieved by retarding and reducing the digestion and absorption of glucose through the inhibition of carbohydrate hydrolyzing enzymes such as α-glucosidase and α-amylase in the digestive tract (Tundis et al., 2010). Inhibition of alpha-glucosidase retard the digestion of the carbohydrates, resulting to a reduction in the rate of glucose absorption. Acarbose is being used as alpha glucosidase inhibitor but it has side effects such as bloating, flatulence, and diarrhea (Chakrabarati and Rajagopalan, 2002). Medicinal plants have been used worldwide for the therapy of Diabetes type 2 (*Diabetes mellitus*).

1.4.2 Drugs used for the treatment of diabetes

The choice of specific antihyperglycemic agents is prognosticated on the basis of their capacity to reduce glucose, extraglycemic effects that may reduce long-term complications, safety profiles, tolerability, and affordability (Nathan *et al*., 2009). The available anti-diabetic interventions (Kemp, 2012; Patel *et al.*, 2012) as summarized in Table 1.1.

Table 1. 1 Glucose-lowering interventions in patients with type 2 diabetes (Kemp, 2012, Golightly, *et al*., 2012, Nathan *et al*., 2009).

Drug class	**Advantages**	**Disadvantages**
Sulfonylurea	Rapidly effective	Hypoglycemia, weight gain
Metformin	Weight neutral	GI side effects, contraindicated with renal insufficiency
Thiazoladinediones	More durable effect on glycemic control	Weight gain & fluid retention, with peripheral edema & two-fold increased risk for congestive heart failure
α-Glucosidase inhibitors	Weight neutral	Frequent GI side effects, three times per day dosing, expensive
DPP-4 inhibitors	Weight neutral	Long-term safety not established
GLP-1 agonist	Weight loss	Frequent GI side effects, expensive, 2 injections daily,
Insulin	Rapidly effective, no dose limit, improved lipid profile	Weight gain, hypoglycaemia, analogues are expensive

Table 1. 2: Plant flavonoids with hypoglycemic activity (R.M Perez et al., 1998)

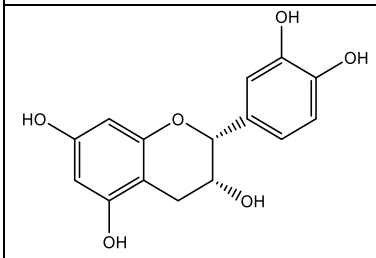
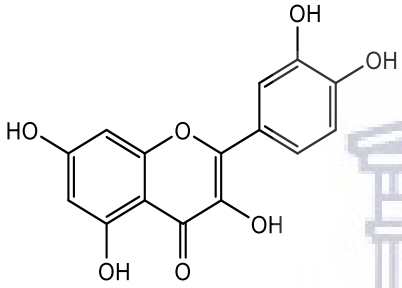
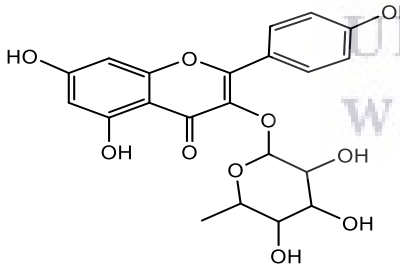
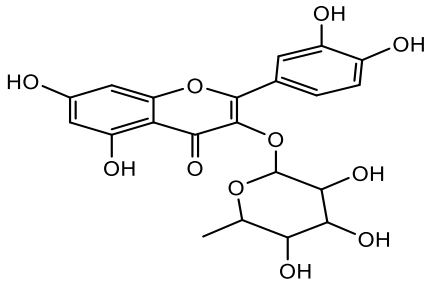
Hypoglycemic agent	Source	Plant part	Properties
 (-)-Epicathechin	*Pterocarpus marsupium* Roxb. (*Leguminosae*)	Bark	Extracts of *P. marsupium* have been reported to be useful as hypoglycemic agents in the treatment of DM. It has been reported that the active hypoglycemic principal of the bark is (-)-Epicathechin (Sheehan and Zemaitis 1983). It also has been reported that the hypoglycemic effect of this compound is attributed to regeneration of β cells in the pancreatic islets of alloxan diabetic rats (Hii and Howell 1984, Chakravarthy et al., 1981, Kolbet et al., 1982).
 Quercetin	*Baubinia Purpurea L. (Leguminosae)*	Leaf	The effects of some f1avonoids has been reported to possess antidiabetic activities on insulin release and $^{45}Ca^{2}$ handling, have been studied in isolated rat islets of Langerhans. Insulin release was enhanced by approximately 44 - 47% when islets were exposed to quercetin, naringenin and chrysin. The quercetin inhibited $^{45}Ca^{2+}$ efflux in the presence and absence of extracellular Ca^{2+}. These findings suggestl that stimulatory compounds such as quercetin may, at least in partl, exert their effects on insulin release via changes in Ca^{2+} metabolism (Hii and Howell 1985).
 Kaempferol-3-O-rhamnoside  Quercetin-3-O-rhamnoside	*Zizipbus rugosa* Lam.(Ramnaceae)	Leaf	The f1avonoids kaempferol-3-0-rhamnoside, quercetin3-0-rhamnoside and myricetin-3-0-rhamnoside isolated from Z, *rugosa* produced hypoglycemia activity in rabbits (Khosa et aI., 1983).

Table 1.2: Plant flavonoids with hypoglycemic activity (continued)

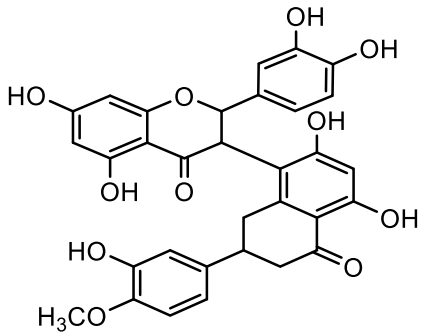
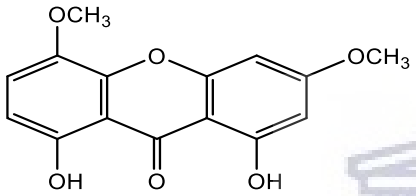
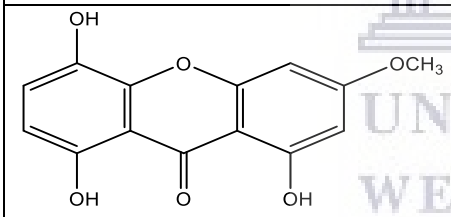
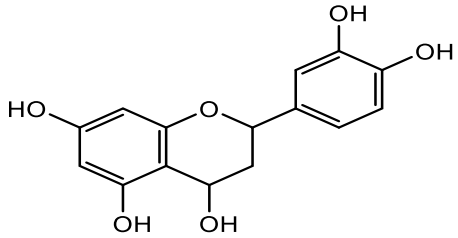
Hypoglycemic agent	***Source***	**Plant part**	**Properties**
 Kolaflavanone	*Garcinia kola* Hook F. (Gutiferae)	Leaf	Blood glucose level in normal and alloxan-treated diabetic mice were measured after oral administration of various doses of the Kolaflavanone. It was concluded that this compound produced a significant hypoglycemic effect in normal and alloxan-induced diabetes mice (Coterill et al., 1976).
 Swerchirin	*Swertia chirayita* Roxb.(Gentianaceae)	Leaf	Xanthone was isolated from the hexane fraction of S.*chirayita* and identified as 1,8-dihydroxy-3,5-Dimethoxyxanthone (Swerchirin). It has been reported to possess a very significant blood sugar lowering effect in fasted, fed, glucose loaded, and tolbutamide pretreated albino rat models (Mukherjee and Mukherjee, 1987).
 Bellidifolin	*Swertia japonica* Makino (Gentianacea e)	Leaf	Fractionation led to the isolation of known xanthones bellidifolin, methylbellidifolin, swertianin and methylswertianin of S. *japonica;* and their identification was based on spectroscopic methods. Bellidifolin and methylbellidifolin showed a potent and dose-dependent hypoglycemic activity in streptozotocin (STZ) induced diabetic rats after both i.p. and p.o. administration but methylswertianin and swertianin did not show any activity (Basnet et al. 1994).
 Leucocyanidin	*Ficus bengalensis* L. (Moraceae)	Leaf	The leaf of F. *bengalensis* is used in traditional medicine for the management of diabetes mellitus. Leucocyanidin has shown to have a hypoglycemic effect in fasted normal rats, it has shown to check hyperglycemia induced by the effects of alloxan (Brahmachari et al., 1964).

Table 1. 3: Steroids and triterpenoids

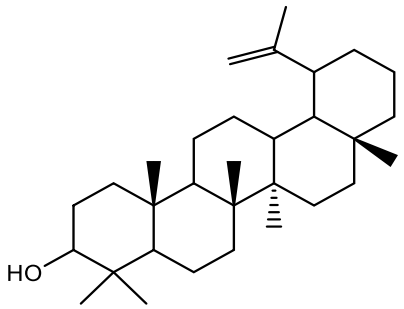
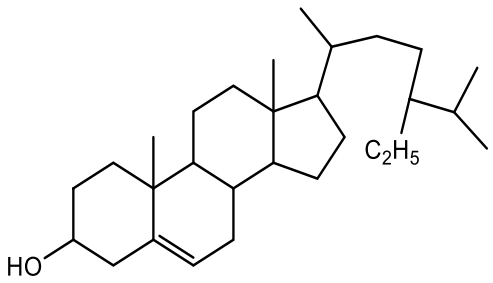
Hypoglycemic agent	Source	Plant part	Properties
 Fagasterol	*Phyllanthus emblica* H. B. K. (Euphorbiaceae)	Leaf	Hypoglycemic effects were seen in normal mice. A reduced blood glucose level was also observed when fagasterol was injected to alloxan-induced hyperglycemic mice (Cuellar et al., 1980).
Gymnemic acid	*Gymnema sylvestre* R. Br. (Asclepiadaceae)	Leaf	A mixture of triterpenoid saponins extracted from the leaves of *Gymnema sylvestre* suppressed the sweet taste sensation in man, but also inhibited the glucose absorption in the rat small intestine, resulting to a reduction in plasma glucose in the oral glucose tolerance test (Shigemasa, 1992). Serum enzymes and histological observations suggested reduced tissue damage in diabetic animals. Glycogenesis and protein anabolism were improved and the activity of insulin-dependent enzymes such as hexokinase and glycogen synthase was increased (Shanmugasundaram et aI., 1983).
 β-sitosterol	*Coffea arabica* L. (Rubiaceae)	Green beans	The β-sitosterol exhibited significant hypoglycemic activity in normal and hyperglycemic mich (Sampaio et aI., 1979).

Table 1.3: Steroids and triterpenoids (continued)

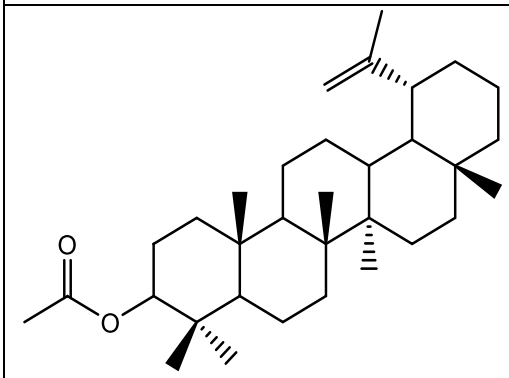
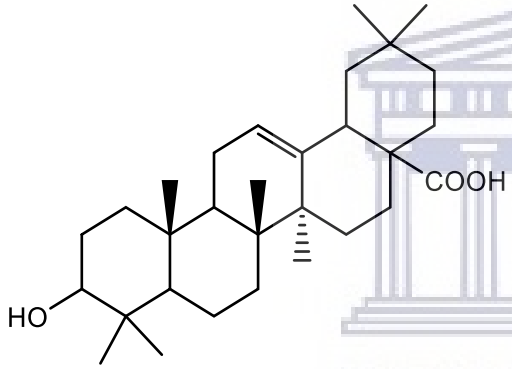
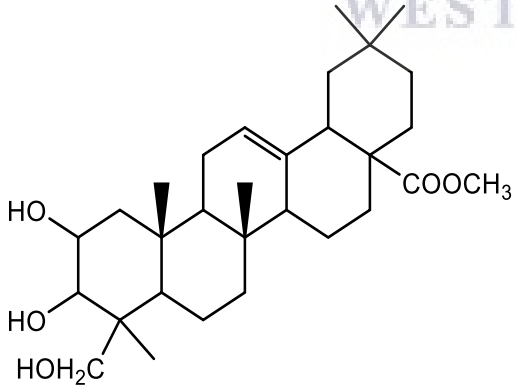
Hypoglycemic agent	Source	Plant part	Properties
 Lupeol acetate	*Phoenix dactylifera* L. (Palmaceae)	Leaf	Lupeol acetate exhibited hypoglycemic activity in rabbits (Harborne and Baxter, 1993).
 Oleanolic acid	*Momordica cochinchinensis* Sprengel (Cucurbitaceae) *Comus officinalis* Sieb.(Cornaceae)	Seeds	Oleanolic acid and ursolic acid were isolated together from C. *officinalis*, they decreased the amount of water consumption and urine volume in diabetic rats (Re-za-ul-jalil et aI., 1986).
 Sapogenol	*Bumelia sartorum* L. (Sapotaceae)	Root bark	Sapogenol elicited a hypoglycemic effect in normal and alloxan induced hyperglycemic mice (Nobrega et aI.,1985).

Table 1.3: Steroids and triterpenoids (continued)

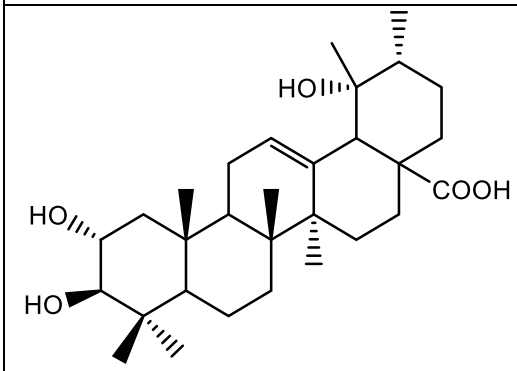
Hypoglycemic agent	Source	Plant part	Properties
 Tormentic acid	*Poterium ancistroides* Desf. (Rosaceae)	Leaf	The hypoglycemic activity of tormentic acid was determined in norrnoglycemic (Villar et aI.,1986), Hyperglycemic and streptozotocin diabetic rats. This principle reduced the fasting plasma glucose level with a corresponding increase in circulating insulin levels. It improved the glucose tolerance test by increasing insulin secretory response to glucose. Tormentic acid did not change the insulin and glucose levels in streptozotocin induced diabetic rats. Effects have been compared with that of glibenclamide. These findings suggest that tormentic acid, like glibenclamide, may act by increasing insulin secretion from islets of Langerhans (Ivorra et aI., 1988.
Glycoside	*Xanthium strumarium* L. (Compositae)	Leaf	A glycoside from *Xanthium strumarium* had hypoglycemic activity at a dose of 1- 5 mg/kg i.v. in laboratory animals (Ogzewalla et al., 1974).

Table 1.3: Steroids and triterpenoids (continued)

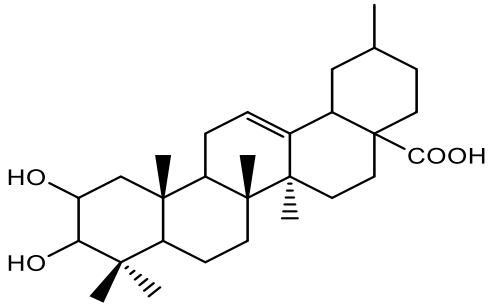
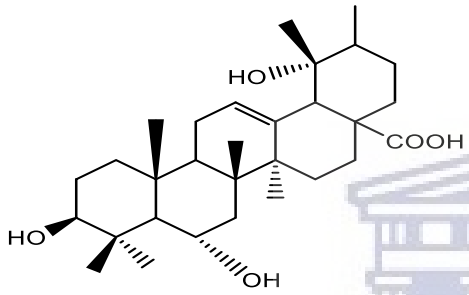
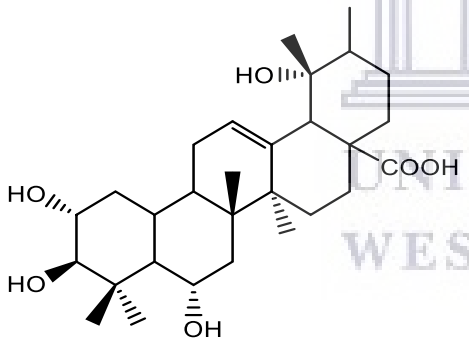
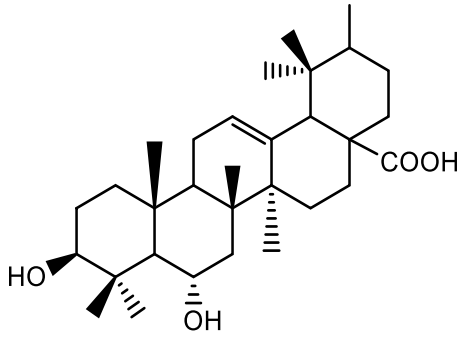
Hypoglycemic agent	Source	Plant part	Properties
   Polyhydroxylated triterpenoids	*Eriobotrya japonica* Linde (Rosaceae)	Leaf	The effect of the polyhydroxylated triterpenoids isolated from *Eriobotrya japonica* by MeOH extraction were studied in genetically diabetic mice and norrnoglycemic rats. They exhibited a marked inhibition of glycosuria and reduced blood glucose levels in normogycemic rats (Tommasi et aI., 1991).
 Ursolic acid	*Comus officinalis* Sieb, (Cornaceae)	Seeds	Ursolic acid produced hypoglycemic activity in rats with diabetes induced by STZ (Yamahara et al., 1981).

Table 1.3: Steroids and triterpenoids (continued)

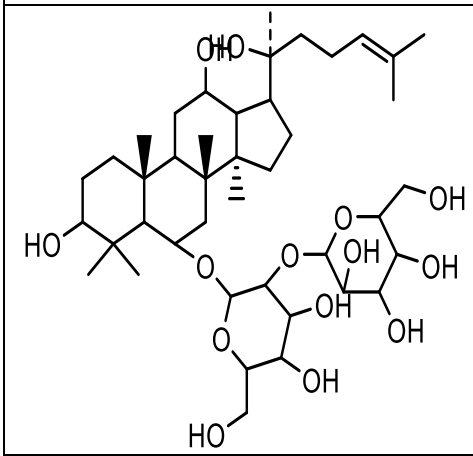
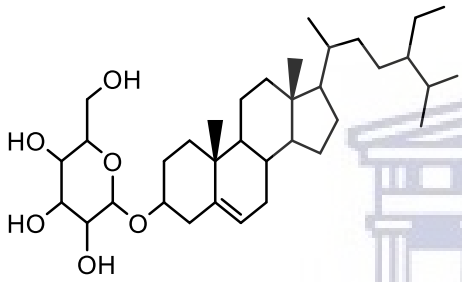
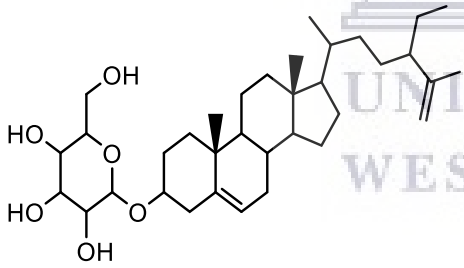
Hypoglycemic agent	Source	Plant part	Properties
	*Panax Ginseng* Meyer (Araliaceae)	Leaf	Ginseng saponins had a hypoglycemic action in rats with streptozotocin-induced diabetes.
 Sitosterol D-glucoside  5,25- Stigmastadienol-glucoside	*Momordica charantia* L. (Cucurbitaceae)	Fruit	A range of compounds have been isolated from *M. charantia* (karela) fruit, seeds and vines: saponins (sitosterol and stigmastadienol glucosides), proteins (p-insulin), steroidal glycosides (momordicines and momordicosides) and pyrimidine nucleoside (vicine) (Raman and Lau, 1996). Sitosterol and stigmastadienol glucosides when administered to normal rabbits produced a gradual but significant fall in blood sugar. Pancreatectomy was found to reduce but not abolish the hypoglycemic effect of charantin (mixture of sitosterol and stigmastadienol glucosides) (Lotlikar and Rajarama, 1966).

Table 1. 4: Alkaloids and other nitrogen compounds (R.M Perez G. et al., 1998)

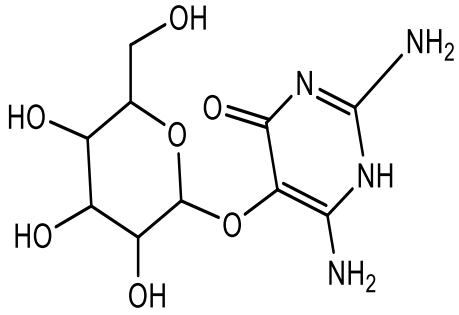
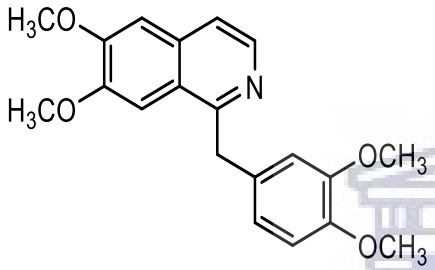
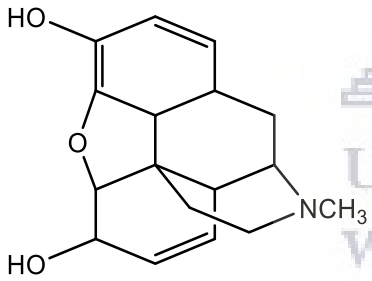
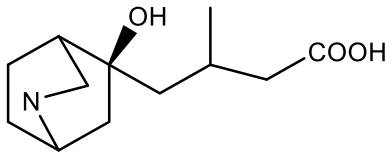
Hypoglycemic agent	Source	Plant part	Properties
 Vicine	*Momordica charantia* Linn (Cucurbitaceae)	Fruit	The pyrimidine nucleoside vicine has been isolated from the seeds of kare1a. Administration caused a hypoglycemic response in normal fasting albino rats. Note: *M. charantia* shows toxicity in animals to the liver and reproductive system (Raman and Lau, 1996).
 Papaverine  Morphine	*Papaver somnirerum* L. (Papaveraceae)	Fruit	The hypoglycemic effect of morphine *(40* mg) injected intrathecally was studied and compared with the effects of other hypoglycemic agents. The hypoglycemic effects of both morphine and insulin appear to be attributed largely to an increased glucose uptake by muscle (White et aI., 1993). The papaverine is an phosphodiesterase inhibitor which increases intracellular cAMP by preventing its breakdown (Hill et al., 1987).
 Dioscoretine	*Dioscorea dumetorum* Pax (Dioscoreaceae)	Tubers	Dioscoretine, the hypoglycemic principle of D. *dumetorum*, was isolated by bioassay-guided fractionation of the methanolic extract of the tuber of D. *dumetorum* when administered intra peritoneally to normal and alloxan diabetic rabbits. Dioscoretine produces hypoglycemic effects at a dose of 20 mg/kg (lwu et aI., 1990).

Table 1. 5: Some medicinal plants with antioxidant activity used in hyperlipidemia (Mahmoud Bahmani et al., 2015).

Scientific name of the plant	**Therapeutic effect**
Amirkabiria odorastissima Mozaffarian	Reduces blood lipids in hypercholesterolemic rabbit model
Rheum ribes L	Stimulating liver and gall bladder, regulating the blood cholesterol. In clinical studies, consumption of 27 g of *Rheum ribes* L for four weeks caused the decrease in cholesterol and LDL.
Red yeast Rice	Stimulating blood cycle and digestion by thought-provoking secretion and increase HDL and reduce triglycerides and LDL
Pistacia atlantica	Feeding 20 % of *Pistacia atlantica* for 3 weeks led to reduction in cholesterol-LDL
Juglans regia	Anti-lipid effects of walnuts in humans has been demonstrated
Cynara Scolymus	Stimulating the liver and gall bladder and bile secretion and is prescribed to reduce cholesterol levels. Improves digestion problems due to lack of bile secretion and increased bile secretion to help digest fats. Inhibit cholesterol synthesis in the liver cells and protects the liver from chemical toxins damage.
Ginseng	Daily intake of 150 mg per kg body weight of ginseng extract for 6 weeks in rats reduced 40% cholesterol

Olea europaea	Olive oil consumption increased HDL, decreased LDL and cholesterol, reducing triglycerides and LDL-cholesterol ratio
Citrus limetta	Studies show that rats fed lemon peel have concluded the lower level liver and plasma cholesterol, which suggested this decrease is related to the flavonoids
Cyamopsis tetragonoloba (Guargum)	Reduce intestinal absorption of fat in food and fecal steroid excretion and bile production increased by using this plant
Trigonella foenum graeam L.	Consumption of a warm extraction of the plant for 2 months caused the decrease in serum triglycerides
Allium Sativum L.	Consumption of garlic and garlic coated tablets caused the decrease in cholesterol and LDL. Garlic caused the decrease in formation of atherosclerosis

1.5 Natural products as antioxidants

Alternative medicine is increasing by leaps and bounds as indicated by the increase in demand of natural health products (Sahib *et al*., 2012). As per modern theory of free radical biology and medicine, reactive oxygen species are involved in a number of disorders. The negative implications of the free radicals can be blocked by antioxidant substances which scavenge the free radicals and detoxify the organism (Maestri et al., 2006). Due to the toxicological concerns of synthetic antioxidants, interests for identifying phenolic components from the plants to reduce or retard lipid oxidation in lipid-based food products have increased. The majority of these natural antioxidants come from fruits, vegetables, spices, grains and herbs.

Oxidative stress plays an important role in the development and progression of Alzheimer's disease (AD) pathology (V. Chauhan and A. Chauhan, 2006). Altered glutathione metabolism in association with increased oxidative stress has been implicated in the pathogenesis of AD (Reid and Jahoor, 2001). The Ginkgo biloba extract EGb 761 has been reported to exert neuroprotective effects in several animal models, and to improve or maintain cognitive function in AD patients.

Parkinson's disease (PD) is a progressive neurodegenerative disease in the elderly, and no cure or disease-modifying therapies. Oxidative stress has been implicated in the process of neurodegeneration in PD pathogenesis. Reported evidence suggest that mitochondrial dysfunction and oxidative stress have a key role in the dopaminergic neurodegeneration of Parkinson's disease (Jin et al., 2014). Mitochondrial dysfunction due to oxidative stress, mitochondrial DNA deletions, altered mitochondrial morphology and the interaction of pathogenic proteins with mitochondria all result in dopaminergic neurodegeneration. Therefore, therapeutic strategies targeting mitochondrial dysfunction and oxidative stress may provide a great promise for a cure for PD. Suppressing mitochondrial reactive oxygen species

(ROS) generation with specific antioxidants can potential reduce complications arising from PD.

Diabetes cardiomyopathy (DCM) is a common and severe complication of diabetes and results in high mortality (Yan et al., 2017). Oxidative stress is a key mechanism by which diabetes induces DCM. The improvement of the antioxidative mechanisms and the suppression of the oxidative stress are considered as key targets in the treatment of DCM. Supplementation of aged garlic extract in the diet reduced oxidative stress and improved endothelial dysfunction in humans (Dillon et al., 2002, Weiss et al., 2006).

As conventional drugs fail to give desirable long-term results, more interest has been generated towards the use of medicinal plants to prevent development of obesity. Several plant species and natural products have been examined for their potential antiobesity effect both *in vitro* and *in vivo*. There is increasing evidence that plants and their products can exert antiobesity effects through various mechanisms such as antilipase, or anti adipogenesis effect, or suppression of appetite. The potential of natural products for treating obesity continues to be under exploration. This may present an excellent alternative strategy for developing future effective, and safe anti-obesity drugs (Mohamed *et al*., 2014, Birari & Bhutani, 2007). Natural products, including crude extracts and isolated pure natural compounds can induce body weight reduction and prevent diet-induced obesity (Mohamed *et al*., 2014).

1.6 Brine shrimp Toxicity

It is important that medicinal plants cytotoxicity and microbial properties are well investigated. The toxicity bioassay was conducted using the brine shrimp lethality method where the nauplii of Artemia Salina were used in the analysis (Pelka *et al*., 2000; Chan-Bacab *et al*., 2003). The (LD_{50}) Lethality of substances to brine shrimp nauplii has been linked to the potential of such compounds to kill cancer cells (antitumor activity), possibly pesticidal and antimicrobial activity (Mc Laughlin *et al*., 1991). This was achieved by looking at the ratio between dead

larvae (no motility) and living larvae (high motility) in comparison to a control without any toxic substances which was used in estimating the toxicity of the test solutions.

1.7 Rationale of the study

Species from the South African wild genus *Searsia* have been traditionally used for medicinal purposes for years and even cultivated for the same purpose. Diabetes mellitus is one of the major health problems in Africa. A number of synthetic medicines are available for the management of the disease never the less drugs of natural origin have attracted a great interest. The anti-diabetic activity demonstrated by the compounds isolated from *S. mysorensis* and α-glucosidase activity exhibited by the compounds isolated from *S. javanica* have motivated the choice of the three selected *Searsia* species.

A comprehensive investigation of the plant extracts exhibiting alpha glucosidase, α-amylase inhibitory activities will be of great importance to health practitioners. Since poorer societies depend mainly on traditional medicine for primary health care, analysis of these medicines need to be vigorously examined to understand their mode of action, efficacy and safety.

1.8 The aim of the study

A chemical study on three *Searsia* species (*Searsia glauca, Searsia lucida; and Searsia laevigata*), to isolate the chemical constituents, and perform the biological assays such as enzyme inhibition and antioxidative stress on the extracts.

1.8.1 Specific objectives

i) To prepare crude extracts using various organic solvents and isolate pure compounds from the selected plants using chromatographic techniques.

ii) To characterize and elucidate the pure isolates using selected spectroscopic techniques

iii) To determine antioxidant capacity, anti-diabetic activity on extracts and selected compounds and conduct toxicity using brine shrimp lethality assay on the crude extracts.

CHAPTER TWO: LITERATURE REVIEW

2.1 *Searsia* family

The special focus of this research is the *Searsia genus*. *Searsia* is the more recent name for the genus (*Rhus*) that contains over 250 individual species of flowering plants in the family *Anacardiaceae*. A number of the species grown in southern Africa, belonging to the *Rhus* genus, have been placed in *Searsia*. *Searsia* was named after Paul B. Sears (1891–1990) who was head of the Yale School of Botany, USA. The *Anacardiaceae* family includes plants that produce gums, resins or latex from which antimicrobial compounds may be isolated. In this study we are going to use *Searsia* instead of *Rhus*.

2.1.1 Searsia genus

These plants are found in temperate and tropical regions worldwide. In general, *Searsia* species can grow in non-agriculturally viable regions without necessarily competing with food production in terms of the land use and have been used by indigenous cultures for medicinal and other purposes (Van Wyk *et al*., 2004). These are plants having trifoliate leaves with small flowers that eventually produce fruits which are in the category of drupes.

Research conducted on *Searsia* extracts to date indicates a promising potential for this plant group to provide renewable bioproducts with the following reported desirable bioactivities; antimicrobial, antifungal, antiviral, antimalarial, antioxidant, antifibrogenic, anti-inflammatory, antimutagenic, antithrombin, antitumorigenic, cytotoxic, hypoglycaemic, and leukopenic (Rayne and Mazza, 2007, Salimi *et al*., 2015). Some compounds from the *Searsia* species show anti-HIV-1 activities (Wang *et al.*, 2008), antioxidant properties, anti-diabetic activities and these plants can serve as sources for the development of novel anti-HIV agents (Rayne and Mazza, 2007; Saleh Ibrahim Alqasoumi et al., 2016). Previous studies reported antibacterial and antifungal activity of extracts prepared from air dried branches of *S. glabra* (McCutcheon *et al*., 1992, 1994). Furthermore, the bioactive components can be extracted from the plant material using environmentally benign solvents that allow for both food and industrial

end-uses. However, not all of the species known to date have been fully profiled for potential bioactive components and biological activities.

2.2 Botanical information on *Searsia* species

2.2.1 *Background Information on Searsia glauca*

Searsia glauca is a small tree or bush that has bluish leaflets and up to 4.0 m high that is commonly found along the coastline and the adjacent interior from Velddrif in the South Western Cape to near Kentani, Transkei (Eastern Cape) (Bothalia *et al*., 1994).

Figure 2. 1: *Searsia glauca*

2.2.2 Background Information on Searsia Lucida

Searsia lucida, previously known as *Searsia lucida (isiXhosa: intlokotshane ebomvu; umchane; amapozi)*. The tree seldom reaches a height of more than 2 meters and has attractive shiny leaves. It produces small, creamy-white flowers, which bear fruits 4 mm in diameter, which are initially green and turn shiny brown as they mature. The fruits are eaten by birds. It is found in scrub or forest areas from sea level to 2000 meters above.

Figure 2. 2: *Searsia lucida (Intlokoshane)*

This small tree has a distribution along the West Coast of South Africa from Saldanha Bay around the Cape and up the East Coast almost until the Mozambique border. Its distribution area also encompasses the whole of Kwazulu-Natal and stretches in an arm past Swaziland, right up the Lowveld areas of Mpumalanga and into Limpopo Province, almost to the Zimbabwe border.

2.2.3 Background Information on Searsia Laevigata

Searsia laevigata (or Dune Currant Rhus) is a small, bushy, evergreen tree that occurs in fynbos and coastal shrub in South Africa. It looks very similar to its close relative *Searsia glauca* but has larger leaflets.

Figure 2. 3: *Searsia laevigata*

2.3 Ethnomedicinal uses of *Searsia species*

Some species of the *genus Searsia* are used in traditional medicine either as antimicrobial concoctions or for their cytotoxic properties, while others display insecticidal activities against aphids (Saxena et al., 1994). *Searsia* species such as *S. coriaria, S. chirindensis, and S. verniciflua* crude extracts have been reported to exhibit significant *In vitro* and *In vivo* hypoglycemic activities (Giancarlo et al., 2006; Jung et al., 2006; Ojewole et al., 2007). *Searsia chinensis* is a plant that has been used by traditional healers in Asia; it has been used in the treatment of cold fever, malaria, and diarrhea. *Searsia glabra* has been used by native people from North America in the treatment of bacterial disease such as syphilis, gonorrhea, dysentery and gangrene (Erichsen-Brown, 1989). The leaves and fruits of *S. coriaria* have been reported to possess defensive and beneficial effects on a wide set of diseases, including, but not limited to, diabetes mellitus, cancer, stroke, oral-diseases, inflammation, diarrhea, and dysentery. (Abu-Reidah et al., 2014). The leaves of *Searsia semialata* have been used as a folk medicine

for the treatment of diarrhea, spermatorrhea and malaria. Some of the *Searsia* species that have been traditionally used for medicinal purposes are listed in (Table 2.1).

Table 2. 1 traditional medicinal use of *Searsia genus*

***Searsia* species**	**Medicinal indication**	**Reference**
S. toxicodendron	For fever, swollen glands, and other diseases	Donald, 2008
S. verniciflua	For gastritis stomach cancer and arteriosclerosis	Choi et al., 2014
S. semialata	For treating diarrhea & dysentery	Gaire & Subedi, 2011
S. Chinensis	For HIV, Cold fever, malaria	Wang et al., 2008
Searsia. javanica	Antidiarrhoeal activities	Vareinshang and Yadav, 2004
Searsia pentheri	For epilepsy	Svenningsen et al., 2006
Searsia chirindensis	Used to strengthen the body, Stimulate circulation and for Rheumatism	Pujol, 1990
Searsia dentata	For epilepsy	Pedersen et al., 2010.
Searsia longipes	For asthma and malaria infection	Olorunnisola, et al., 2017

2.4 Chemistry of *Searsia Genus*

The chemistry of the *Searsia* genus is complex with occurrence of different secondary metabolites identified as terpenoids, and flavonoids. Examination of *Searsia pyroides Burch* (*Anacardiaceae*) led to the identification of rhuschalcone and traces of biflavonoids which were not identified (Masesane *et al*., 2000). Essential oils as natural products have a great potential to be an alternative raw material in food, perfume and pharmaceutical industries (Webber *et al.*, 1999). Some of these oils have exhibited antibacterial, antifungal or antioxidant activity (Gundidza, 1993; Lee *et al*., 2003). In the latter study, essential oil was prepared from fresh leaves of *S. lancea* and the chemical profile was analyzed by GC-MS (Subhash and Mathela, 2014). The essential oil of the aerial parts of *Searsia cotinus*, which is dominated by monoterpenes, was reported to exhibit significant antioxidant activity by inhibiting ß-carotene

bleaching, ferric reducing antioxidant power and by scavenging DPPH free radical (Subhash and Mathela, 2014). A number of aldehydes, fatty acids, long chain alcohols, terpenes, terpenoids and waxes of commercial or bioactive potential in essential oils and non-polar extracts have been reported from selected *Searsia* species (Sierra Rayne, 2011). *S. coriaria* plant is known as an abundant source of tannins (condensed and hydrolysable), phenolic acids, anthocyanins, gallic acid derivatives, flavonoid glycosides, and organic acids (Abu-Reidah *et al*., 2014). Selected compounds isolated from *Searsia* species are reported in table 2.2 along with their biological activities where such information is available, some structures of biologically active isolates are presented in figures 2 and 3.

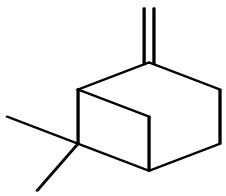
2.4.1 Chemical constituents isolated and detected from Searsia species

Phytochemical examination of several *Searsia* species led to the isolation and identification of many biological active compounds. GC and GC-MS analysis of the volatile oil from the aerial parts of *S. cotinus* from Kumaon Himalaya exhibited the major and minor chemical constituents. Selected constituents are from these classes are listed in table 2.1.2. Major monoterpenes were constituted of β-pinene (30.6%), camphene (13.6%), limonene (12.4%), α-pinene (5.2%), and *p*-cymene (4.6%). Oxygenated monoterpenes were composed of 1.8-cineole (1.8%), α-terpenol (0.3%), linalool (0.2%), terpin-4-ol (2.8%). Major sesquiterpene hydrocarbons were composed of β-caryophyllene (4.4%), germacrene D (2.0%) and bicyclogermacrene (12.6%). Oxygenated sesquiterpene constituted of relatively less amount with the minor presence of *epi*-α-cardinol (1.0%) and β-eudesmol (1.0%). The following minor constituents were identified γ-gurjurene, *epi*-cubebol, γ-cadinene, germacren D-4-ol, spathulenol, caryophyllene oxide, cubebol, hummulene epoxide, α-hummulene,α-cadinol, α-terpinene, α-thujene, α-terpineol, bornyl acetate, δ-elemene, α-copaene (Subhash and Mathela, 2014). The composition of the essential oil was dominated by monoterpene hydrocarbons

(65.9%), followed by the sesquiterpene hydrocarbons (20.6%). Oxygenated monoterpenes (5.8%) and oxygenated sesquiterpenes (4.7%) (Subhash and Mathela, 2014).

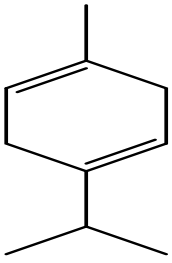
GC-MS analysis of *S. coriaria* fruit from three different geographic origin led to the identification of a number volatile constituents categorized in ten different classes such as alcohols, aromatics, esters, aldehydes, hydrocarbons, ketons, monoterpene hydrocarbons, oxide and sesquiterpene hydrocarbons, these constituents are indicated from figure 3.1 to figure 3.9 (Morshedloo et al., 2018). Phytochemical screening of *S. coriaria* using HPLC-DAD/QTOF-MS led to the detection and characterization of a number of phenolic compounds (Abu-Reidah et al., 2015). These phenolic compounds are listed in table 2.2 (from 93 – 162).

Figure 3. 1: Monoterpenes hydrocarbons

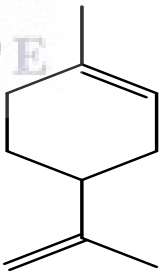


β-Pinene

β-myrcene

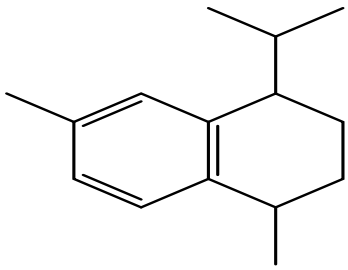


γ-Terpinene

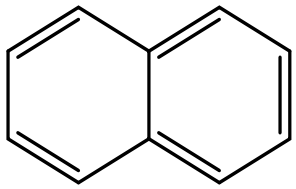


Cimonene

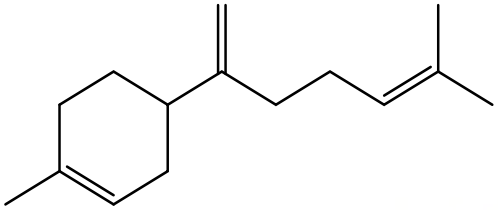
Figure 3. 2: Sesquiterpene



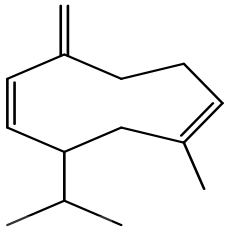
Calamanene



Naphthalene

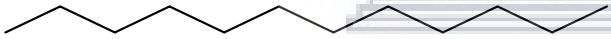


β-Bisabolene



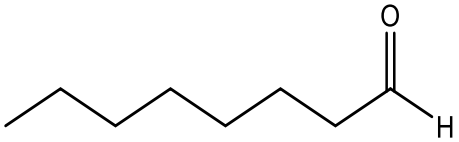
Germacrene D

Figure 3. 3: Hydrocarbons

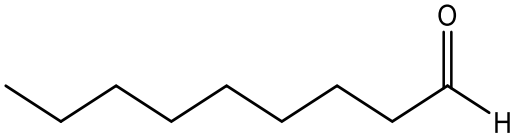


Dodecane

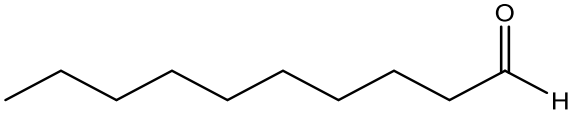
Figure 3. 4: Aldehydes



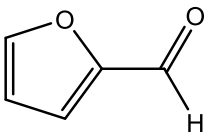
Octanal



nonanal

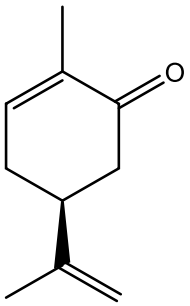


Decanal

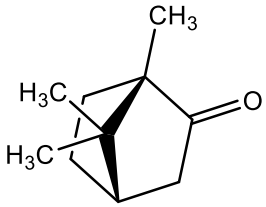


Furfural

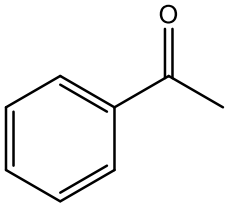
Figure 3. 5: Ketons



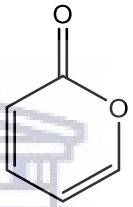
Carvone



Camphor

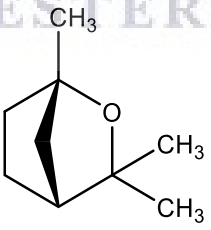


Acetophenone



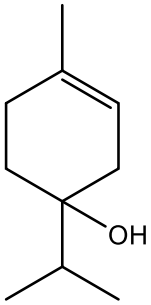
Pyranone

Figure 3. 6: Oxides

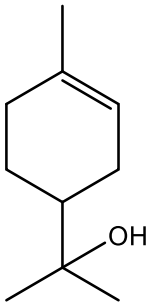


Cineole

Figure 3. 7: Alcohols

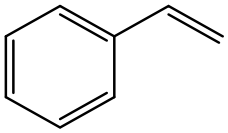


4-Terpineol

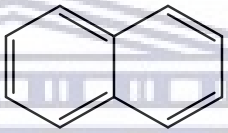


α-Terpineol

Figure 3. 8: Aromatics

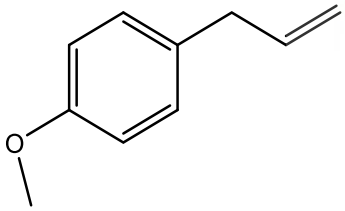


Styrene

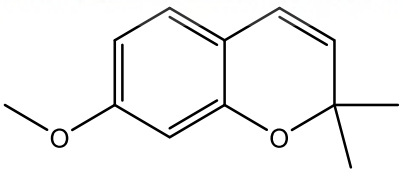


Naphthalene

Figure 3. 9: Ethers

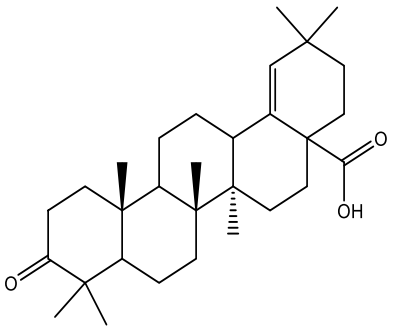


Estragole

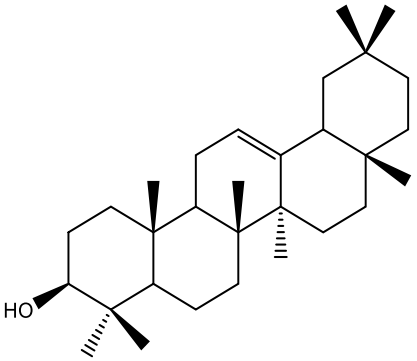


Prococene I

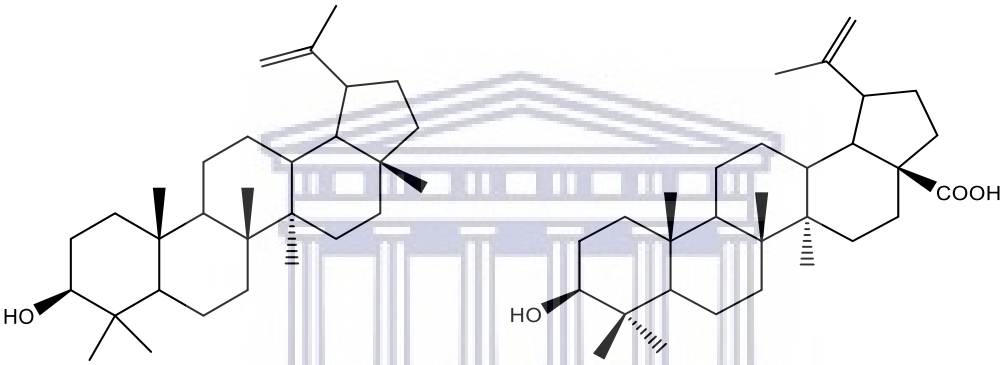
Figure 3. 10: Structures of some reported pentacyclic and tetracyclic terpenoids with biological activities.



Moronic acid

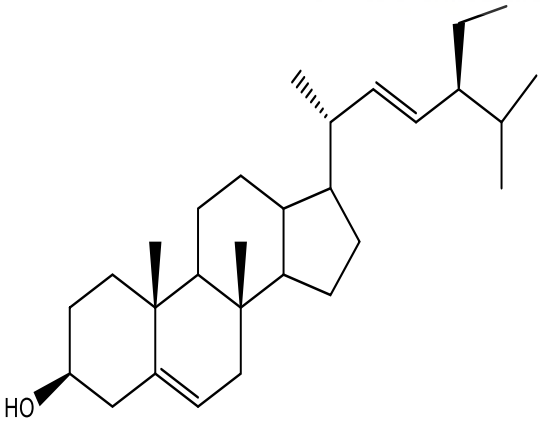


β-Amyrin

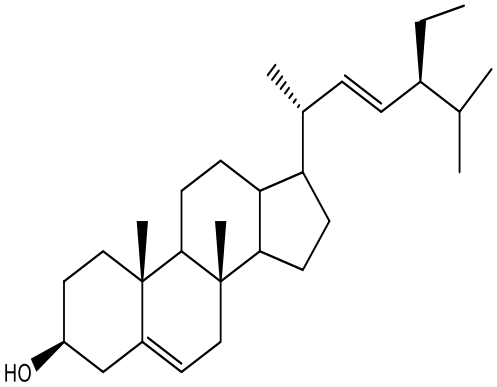


Lupeol

Betulinic acid



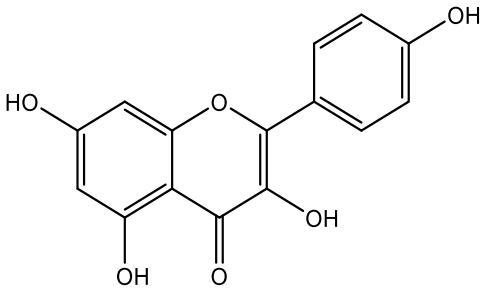
β-Sitosterol



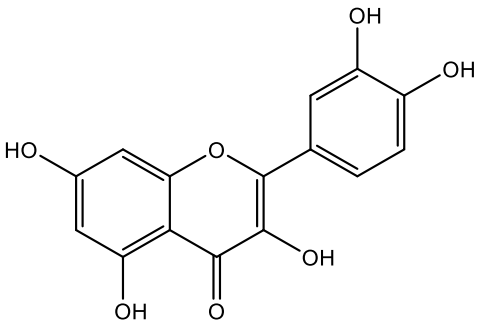
Stigmasterol

Figure 4. 1: Flavonoids; Structures of some reported compounds with biological activities in the *Searsia genus*

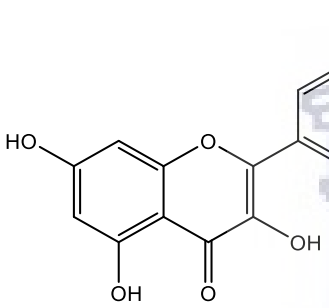
Figure 4.1. 1: Flavonol



Kaempferol

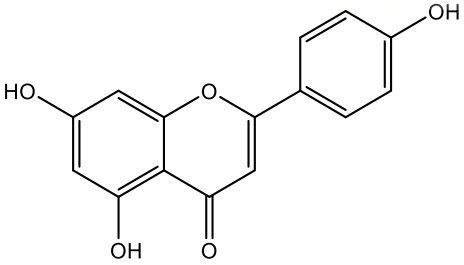


Quercetin

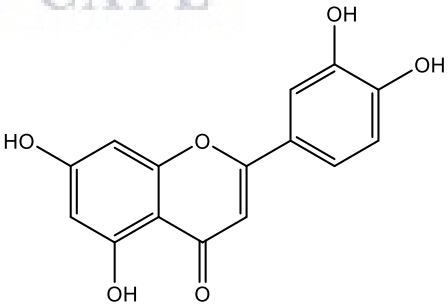


Myricetin

Figure 4.1. 2: Flavone

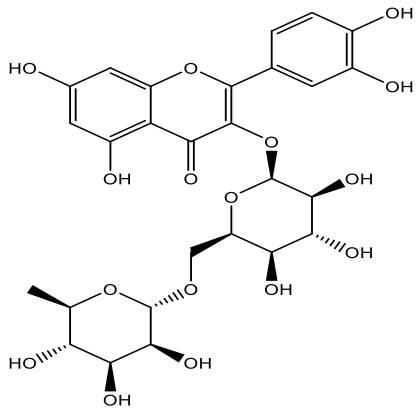


Apigenin

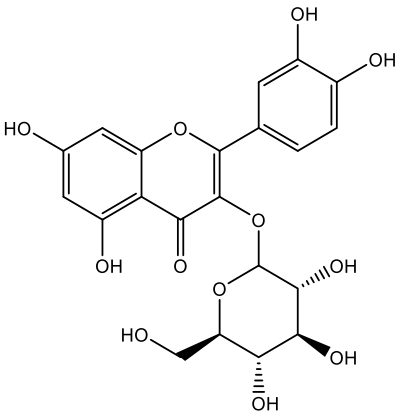


Luteolin

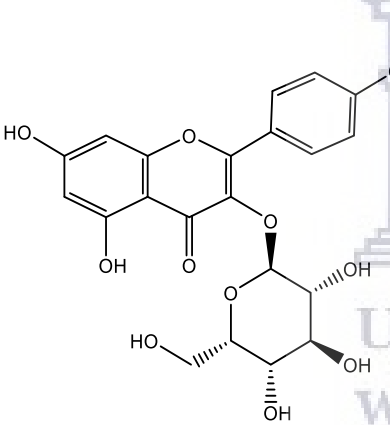
Figure 4.1. 3: Flavonol glycosides



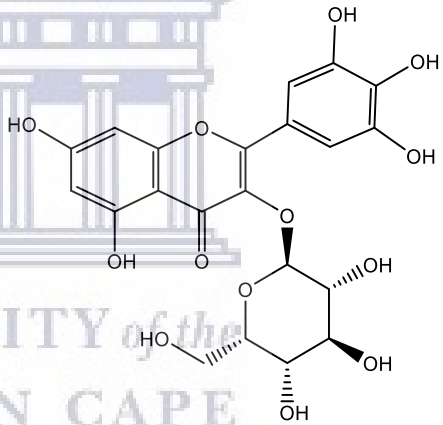
Rutin



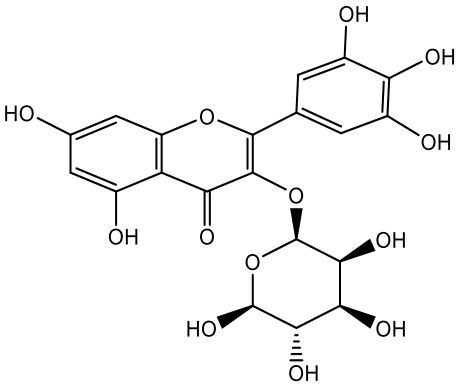
quercetin 3-O-β-D-glucopyranoside



Kaempferol-3- O-glucoside

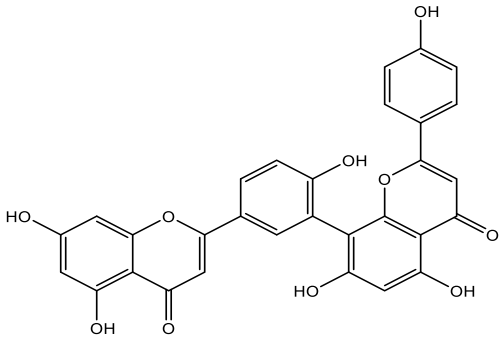


Myricetin- 3- O-glucoside

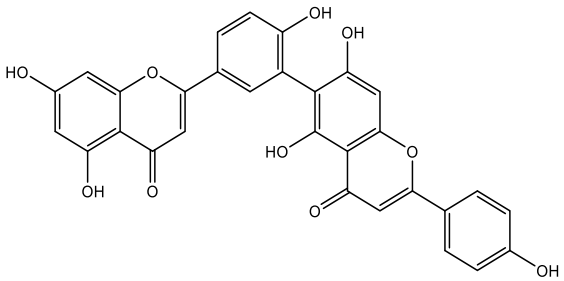


Myricetin-3-O-rhamnoside

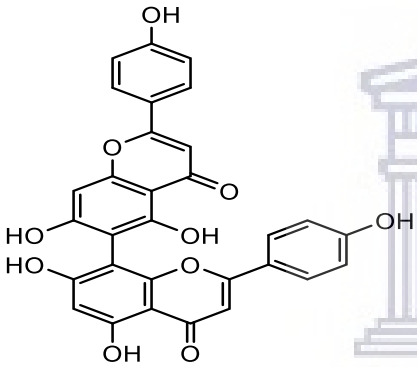
Figure 4.1. 4: Biflavonoids



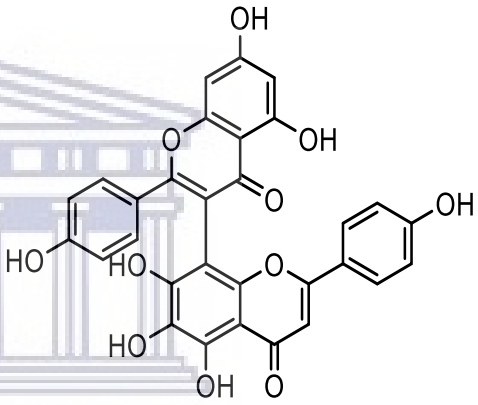
Amentoflavone



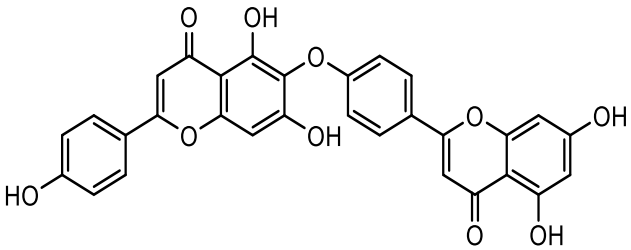
Robustaflavone



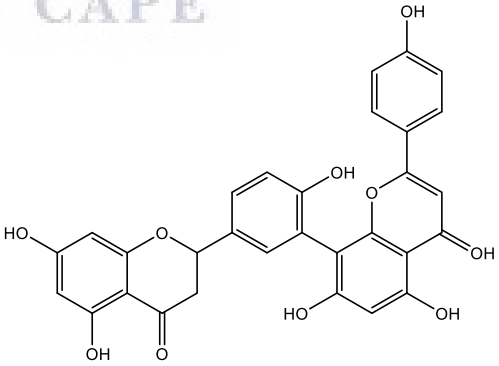
Agathisflavone



Sumaflavone



Hinkiflavone



2.3-dihydroamentoflavone

Table 2. 2 Selected compounds isolated and detected from *Searsia* species and their biological activities.

s/n	**Compound**	**Plant source**	**Biological activity**	**Reference**
1	Moronic acid	*S. Chinensis, S. Javanica*	Anti-HIV-1 activity	Gu *et al*., 2007; Soler *et al*., 1996; Pengsuparp e*t al.*,1994; Kashiwada *et al*., 1998, Kurokawa *et al*., 1998, Lee et al., 2005
2	4',5,7-trihydroxyflavanone	*S. javanica*		Lee *et al*., 2005
3	Betulin	*S. Chinensis*		Gu *et al*., 2007
4	Betulonic acid	*S. javanica*	Selective growth inhibitor of human melanoma, and malignant, triggers apoptosis in chemoselective cells	Kurokawa *et al*., 1998, Lee *et al*., 2005,
5	Betulinic acid	*S. javanica*		Lee *et al*., 2005
6	Lantabetulinic acid	*S. javanica*		Lee *et al*., 2005
7	3β-hydroxyolean-18-en-28-oic acid	*S. javanica*		Lee *et al*., 2005
8	3-oxo-6β-hydroxyolean-18-en-28-oic acid	*S. javanica*		Lee *et al*., 2005
9	methyl gallate	*S. glabra*	antimicrobial activity	Saxen, 1994

10	4-methoxy-3,5-dihydroxybenzoic acid	*S. glabra*	antimicrobial activity	Saxen, 1994
11	gallic acid	*S. glabra*	antimicrobial activity	Saxen, 1994
12	Semimoronic acid	*S. javanica*		Lee *et al*., 2005
13	3-O-methyl semimoronic acid	*S. javanica*		Lee *et al*., 2005
14	3-oxoolean-18-en-28-oic acid	*S. javanica*		Lee *et al*., 2005
15	Oleanolic acid	*S. javanica*		Lee *et al*., 2005
16	7-*O*-methylnaringenim	*S. retinorrhea*	Antimicrobial activity	Ahmed *et al*., 2001
17	7.3'-*O*-dimethylquercetin	*S. retinorrhea*	antimalarial activity	Rayne and Mazza, 2007
18	7-*O*-methylapigenin	*S. retinorrhea*	antimalarial activity	Rayne and Mazza, 2007
19	7-*O*-methylluteolin	*S. retinorrhea*	antimalarial activity	Rayne and Mazza, 2007
20	Robustaflavanone	*S. succedanea*		Kosar *et al*., 2006
21	Amentoflavone	*S. succedanea; S. retinorrhea, S. pyroides*	antimalarial activity, Anti-HSV-I and Anti-HSV-2	Kosar *et al*., 2006 ; Ahmed *et al*, 2001; Svenningsen *et al*., 2006; Lin *et al*., 1991
22	Agathisflavanone	*S. succedanea, S. pyroides*	Inhibition of growth of leukemia cell, affinity for GABA	Kosar *et al*., 2006; Svenningsen *et al*., 2006;

			A/benzodiazepine receptor	
23	volkensiflavanone,	*S. succedanea*		Kosar *et al*., 2006
24	succedaneaflavanone	*S. succedanea*		Kosar *et al*., 2006
25	Rhuschromone	*S. natalensis*	Antimicrobial activity	Mwangi *et al*., 2013
26	2',4'-dihydroxychalcone-(4-*O*-5''')-4'',2''',4'''-trihydroxychalcone	*S. natalensis*		Mwangi *et al*., 2013
27	Garbazol	*S. verniciflua*	Antimutagenic	Lee *et al*., 2004
28	Sulfuretin	*S. verniciflua*	Antimutagenic	Lee *et al*., 2004, Park et al., 2004
29	Fisten	*S. verniciflua*	Antimutagenic	Lee *et al*., 2004, Park et al., 2004
30	Fustin	*S. verniciflua*	Antimutagenic	Lee *et al*., 2004, Park et al., 2004
31	Mollisacasidin	*S. verniciflua*	Antimutagenic	Lee *et al*., 2004, Park et al., 2004
32	Butein	*S. verniciflua*	Antifibrogenic	Lee *et al*., 2003
33	Chrysoeriol-7-O-β-D-glucopyranoside	*S. parviflora*		Shrestha *et al.*, 2012
34	Luteolin-7-*O*-β-D-glucopyranoside	*S. parviflora*		Shrestha *et al.*, 2012
35	Quercetin-3-*O*-β-D-glucopyranoside	*S. parviflora*	Diuretic, antioxidant, antifungal activity	Shrestha *et al.*, 2012

36	Quercetin-3-*O*-α-L-rhamnopyranoside	*S. parviflora*		Shrestha *et al.*, 2012
37	Lantanolic acid	*S. javanica*		Lee *et al*., 2005
38	3-oxotirucalla-7,24-dien-21-oic acid	*S. javanica*		Lee *et al*., 2005
39	Dipterocapol	*S. javanica*		Lee *et al*., 2005
40	3β-hydroxy-22,23,24,25,26,27-hexanordammaran-20-one	*S. javanica*		Lee *et al*., 2005
41	β-sitosterol	*S. javanica*		Lee *et al*., 2005
42	Stigmastane-4-3-one	*S. javanica*		Lee *et al*., 2005
43	Stigmast-4-ene-3,6-dione	*S. javanica*		Lee *et al*., 2005
44	Stigmast-7-en-3-ol	*S. javanica*		Lee *et al*., 2005
45	Pinoresinol	*S. javanica*	Has a potential to be used as natural and safe food preservative.	Lee *et al*., 2005, Zhou et al., 2017
46	4-oxopino-resinol	*S. javanica*		Lee *et al*., 2005
47	trans-3,4',7-trihydroxyflavanone	*S. javanica*		Lee *et al*., 2005
48	Methyl ferulate	*S. javanica*		Lee *et al*., 2005
49	β-Amyrin	*S. natalensis*	antifungal activity against *A.rabiei*	Saleh Ibrahim Alqasoumi *et al*., 2016; Jabeen *et al.*, 2011
50	β-sitosterol-glucose	*S. natalensis*		Saleh Ibrahim Alqasoumi *et al*., 2016

51	Diosmetin	*S. natalensis*	Antimicrobial, anti-inflammatory, & Chemopreventive	Saleh Ibrahim Alqasoumi *et al*., 2016, Pinzon et al., 2011
52	Diosmin	*S. natalensis*	Deactivation of NF-kB targets; Suppression of monocyte chemoattractant protein-1 (MCP), tumor necrosis factor (TNF-α), and interleukins (IL-1β and 6)	Saleh Ibrahim Alqasoumi *et al*., 2016; Gotha *et al*., 2016; Jain *et al*., 2014
53	Hesperidin	*S. natalensis*	Inducer for the production of naringinase	Saleh Ibrahim Alqasoumi *et al*., 2016; Fukumoto *et al*.,1979
54	Rutin	*S. natalensis*	antidiabetic activity by inhibiting inflammatory cytokines	Saleh Ibrahim Alqasoumi *et al*., 2016, Niture *et al*., 2014
55	Quercetin	*S. tripartita*	has potential anticancer properties, antiproliferative & antioxidant	Mohammed, 2015; Lamson and Brignall, 2000

56	Myricetin	*S. tripartita*		Mohammed , 2015
57	Kampferol-3-O-α-L-rhamnopyranoside	*S. tripartita*		Mohammed, 2015
58	Kampferol-7-O-α-L-rhamnopyranoside	*S. tripartita*		Mohammed, 2015
59	β- sitosteryl-3-O-β-glucopyranoside	*S. tripartita*		Mohammed, 2015
60	Gallocatechin	*S. tripartita*		Mohammed, 2015
61	2-(3,4-dihydroxyphenyl)-hydroxy-4H-chromen-4-one	*S. Mysorensis*	Antidiabetic activity at 150 mg/ml	Ganji *et al*., 2017
62	5,6,7-trihydroxy-2-phenyl-4H-chromen-4one	*S. Mysorensis*	Antidiabetic activity at 150 mg/ml	Ganji *et al*., 2017
63	2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxy-4H-chromen-4-one	*S. Mysorensis*		Ganji *et al*., 2017
64	2-(3,4-dihydroxyphenyl)-3,7-dihydroxy-4H-chromen-4-one	*S. Mysorensis*		Ganji *et al*., 2017
65	Apigenin	*S. typhina*	Activates ERK1/2, Attenuates the production of pro-inflammatory cytokines	Qiu *et al.*, 2016; Gotha et al., 2016
66	Kampferol	*S. typhina*	AMPK activation; Reduce the fasting blood glucose, and	Qiu *et al.*, 2016; abo-Salem, 2014; Zang *et al*, 2015

			improved insulin resistance	
67	Daidzein	*S. typhina*		Qiu *et al.*, 2016
68	Orobol	*S. typhina*		Qiu *et al.*, 2016
69	3',5,5',7-tetrahydroxyflavanone	*S. typhina*		Qiu *et al.*, 2016
70	Naringenin	*S. typhina*	Activation of AMPK and suppression of NF-kB pathways; Increases the glucose tolerance and insulin sensitivity	Qiu *et al.*, 2016 ; Tsai et al., 2011; Choi et al., 1991
71	(-)-catechin acid	*S. typhina*		Qiu *et al.*, 2016
72	Quercetin-3-O-α-L-(3"-O-galloy)-rhamnoside	*S. typhina*		Qiu *et al.*, 2016
73	2-hydroxybenzoic acid	*S. typhina*		Qiu *et al.*, 2016
74	4-hydroxybenzaldehyde	*S. typhina*		Qiu *et al.*, 2016
75	Vanillin	*S. typhina*		Qiu *et al.*, 2016
76	Methyl 3,4-dihydroxybenzoate	*S. typhina*		Qiu *et al.*, 2016
77	3,5-dihydroxybenzamide	*S. typhina*		Qiu *et al.*, 2016
78	Tyrosol	*S. typhina*		Qiu *et al.*, 2016
79	Caffeic acid	*S. typhina*		Qiu *et al.*, 2016
80	3-(2,4,6-trihydoxyphenyl)-1-(4-hydroxyphenyl)-propan-1-one	*S. typhina*		Qiu *et al.*, 2016
81	Phlorizin	*S. typhina*		Qiu *et al.*, 2016

82	Friedelin	*S. typhina*	Qiu *et al.*, 2016
83	4,4-dimethyl-heptanedioic acid	*S. typhina*	Qiu *et al.*, 2016
84	Anthranilic acid	*S. typhina*	Qiu *et al.*, 2016
85	Lumichrome	*S. typhina*	Qiu *et al.*, 2016
86	scoparone	*S. typhina*	Qiu *et al.*, 2016
87	Uracil	*S. typhina*	Qiu *et al.*, 2016
88	Phlorizin	*S. javanica*	Cho *et al*., 2013
89	Scopoletin	*S. javanica*	Cho *et al*., 2013
90	5-methylresorcinol	*S. javanica*	Cho *et al*., 2013
91	3-hydroxy-5-methylphenol 1-O-b-D-(6'-galloy)glucopyranoside	*S. javanica*	Cho *et al*., 2013
92	3,4,5-trihydroxy-acephenone 4-D-B-D-glucopyranoside	*S. javanica*	Cho *et al*., 2013
93	Quinic acid	*S. coriaria*	Abu-Reidah et al., 2015.
94	Malic acid	*S. coriaria*	Abu-Reidah et al., 2015.
95	Malic acid hexoside	*S. coriaria*	Ley et al., 2006
96	Oxydisuccinic acid	*S. coriaria*	Abu-Reidah et al., 2015.
97	Galloylhexose	*S. coriaria*	Frohlich et al., 2002
98	Caftaric acid	*S. coriaria*	Abu-Reidah et al., 2015.
99	Levoglucosan gallate	*S. coriaria*	Abu-Reidah et al., 2015.
100	O-galloylnorbergenin	*S. coriaria*	Abu-Reidah et al., 2015.

101	Digalloyl-hexoside	*S. coriaria*	Abu-Reidah et al., 2015.
102	Protocatechuic acid hexoside	*S. coriaria*	Abu-Reidah et al., 2015.
103	Gallic acid dihexose	*S. coriaria*	Abu-Reidah et al., 2015.
104	Protocatechoic acid	*S. coriaria*	Shabana et al., 2011
105	Galloylshikimic acid I	*S. coriaria*	Abu-Reidah et al., 2015.
106	Syringic acid hexoside	*S. coriaria*	Abu-Reidah et al., 2015.
107	Galloylquinic acid I	*S. coriaria*	Abu-Reidah et al., 2015.
108	Coumaryl-hexoside	*S. coriaria*	Abu-Reidah et al., 2015.
109	Trigalloyllevoglucosan I	*S. coriaria*	Chen & Bergmeier, 2011
110	Tri-galloyl-hexoside I	*S. coriaria*	Regazzoni et al., 2013
111	Penstemide	*S. coriaria*	Rodriguez-Perez et al., 2013
112	Isorhamnetin hexoside II	*S. coriaria*	Abu-Reidah et al., 2013
113	Apigenin glucoside I	*S. coriaria*	Shabana et al., 2011

114	Kaempferol-hexose malic acid I	*S. coriaria*	Perestrelo et al., 2012
115	Hydroxymethoxyphenyl-O-(Ogalloyl)-hexose	*S. coriaria*	Abu-Reidah et al., 2015.
116	Apigenin-7-O-(6-Ogalloyl)-β-D-glucopyranoside	*S. coriaria*	Tian et al., 2010
117	7-O-Methyldelphinidin-3-O-(2 galloyl)-galactoside I	*S. coriaria*	Kirby et al., 2013
118	Methyl-dihydroquercetin hexoside	*S. coriaria*	Abu-Reidah et al., 2013
119	Myricetin galloylhexoside	*S. coriaria*	Abu-Reidah et al., 2013
120	Myricetin-hexose malic acid I	*S. coriaria*	Abu-Reidah et al., 2013
121	Eriodictyol xyloyl-deoxyhexose	*S. coriaria*	Abu-Reidah et al., 2013
122	Tetra-O-galloylhexoside	*S. coriaria*	Regazzoni et al., 2013
123	Di-O-galloyl-3,4 - (S)-hexahydroxydiphenoyl Protoquercitol I	*S. coriaria*	Nishimura, Nonaka, & Nishioka, 1984
124	O-Galloyl-arbutin	*S. coriaria*	Shi & Zou , 1992
125	Umbelliferone	*S. coriaria*	
126	Trigalloyllevoglucosan VI	*S. coriaria*	Chen & Bergmeier, 2011

127	Tetra-O-galloyl-scyllo-quercitol	*S. coriaria*	Nishimura et al., 1984
128	Glycitein 7-O - glucoside	*S. coriaria*	Abu-Reidah et al., 2013
129	Ampelopsin glucoside	*S. coriaria*	Yeom et al., 2003
130	Myricetin-3-O-glucuronide	*S. coriaria*	Regazzoni et al., 2013
131	Trigallic acid	*S. coriaria*	Nishimura et al., 1983
132	Apiin I	*S. coriaria*	Abu-Reidah et al., 2013
133	Mingjinianuronide B	*S. coriaria*	Tan & Zuo, 1994
134	Apigenin neohesperidoside I	*S. coriaria*	Matsuda, 1966
135	Spicoside E	*S. coriaria*	Albach et al., 2005
136	Myricetin-rhamnose malic acid	*S. coriaria*	Abu-Reidah et al., 2013
137	Dihydroxybenzoic acetate-digallate I	*S. coriaria*	Hahn & Fekete, 1954
138	Kaempferol rutinoside I	*S. coriaria*	Ding et al., 2009

139	Methyl digallate	*S. coriaria*	Shabana et al., 2011
140	Kaempferol 3-glucuronide	*S. coriaria*	Al Sayed et al., 2010
141	Quercetin arabinoside	*S. coriaria*	Buziashvili et al., 1970
142	Myricitrin O-gallate	*S. coriaria*	Moharram et al., 2006
143	Genistein-hexose malic acid	*S. coriaria*	Abu-Reidah et al., 2013
144	Galloyl-valoneic acid bilactone	*S. coriaria*	Sanz et al., 2010
145	Hexagalloyl-hexoside	*S. coriaria*	Regazzoni et al., 2013
146	Camellianin A	*S. coriaria*	Abu-Reidah et al., 2013
147	Isorhamentin hexosemalic acid	*S. coriaria*	Abu-Reidah et al., 2013
148	Kaempferol rhamnose malic acid	*S. coriaria*	Abu-Reidah et al., 2013
149	Homoprotocatechuic acid	*S. coriaria*	Abu-Reidah et al., 2013
150	Quercetin dimer	*S. coriaria*	Abu-Reidah et al., 2013
151	Ascorbyl monomyristate	*S. coriaria*	Abu-Reidah et al., 2013

152	Dihydroxypalmitic acid	*S. coriaria*	Abu-Reidah et al., 2013
153	Hexadecadienoic acid	*S. coriaria*	Abu-Reidah et al., 2013
154	Deacetylforskolin	*S. coriaria*	Zhang et al., 2009
155	Rhamnetin I	*S. coriaria*	Abu-Reidah et al., 2013
156	Vapiprost	*S. coriaria*	Abu-Reidah et al., 2013
157	Sespondelo	*S. coriaria*	Abu-Reidah et al., 2013
158	Linoleic acid amide	*S. coriaria*	Abu-Reidah et al., 2013
159	Moroctic acid	*S. coriaria*	Abu-Reidah et al., 2013
160	Linoleylhydroxamate I	*S. coriaria*	Abu-Reidah et al., 2013
161	Isovitexin	*S. coriaria*	Abu-Reidah et al., 2013
162	Homoprotocatechuic Acid	*S. coriaria*	Abu-Reidah et al., 2013

2.5 Biological activity of *Searsia* extracts

Plant species from *Searsia* genus in the Anacardiaceae family such as *Searsia lancea*, *Searsia incisa* (Van der Merwe *et al*, 2001; McGaw *et al*., 2008), *S. javanica* (Vareinshang and Yadav, 2004), *S. natalensis* (John *et al.*, 1995), *S gueinzii* (Elgorashi *et al*., 2003), *S. leptodictya diels* (Sebothoma, 2010), *S. rogersii Schonland* (Samie *et al*., 2010), and *S. pendulina* (Coates-Palgrave, 2002) are used by traditional medicine practitioners in South Africa and other African countries for treating intestinal disorders, including diarrhea. *Searsia* extracts have been shown

to exhibit a wide range of biological activities in relation to antioxidant, antidiabetic, antimicrobial and antiviral activities.

Searsia species traditionally used in South Africa to treat microbial infections and gastrointestinal disorders were selected for *in vitro* examination of biological activities and cytotoxicity. *Searsia* extracts are most acknowledged for their antibacterial activities, although not much information is available on their antifungal and antiviral activities. Extracts from *S. chinensis* showed that extracts from this plant have promising potential as antiviral, antidiarrheal, anticancer, antidiabetic and hepatoprotective (Djakpo and Yao, 2010).

2.5.1 Antioxidants from the Searsia genus

Previous studies conducted on *Searsia* extracts have investigated antioxidant activity, and there is a potential for commercial development of the products from a number of species (Rayne and Mazza, 2007). Villa-Ruano *et al*., 2013 reported that *R. chondroloma* showed high levels of flavonoids and tannins as well as terpenes and steroids. The antioxidant activity of *R. chondroloma* ethanolic extract is probably linked to the high flavonoid and phenolic content whereas the anti-lipase effects could be associated with the latter compounds but possibly with other metabolites such as steroids. The essential oil from *R. lancea* exhibited antioxidant activity by showing a mean zone of colour of 19.2 mm, which is almost the same as that noted from ascorbic acid used as positive control in this study (20 mm) (Gundidza *et al*., 2008).

2.5.2 Antidiabetic activity of Searsia genus

The fruits could improve the life of type 2 diabetic patients by exerting mild antihyperglycemic and potent antioxidant properties. *Searsia coriaria* is more often recommended for the blood lipids adjustment in diabetic patients. The hypoglycemic efficacy of the plant extracts has been investigated through inhibiting the α-amylase enzyme. Anwer *et al.,* 2013 suggested that the methanol extract of *S. coriaria* can largely delay the onset of hyperinsulinemia and glucose intolerance, and it can also improve insulin sensitivity in rats.

Swathi *et al*., 2005 investigated the anti-diabetic effect of *Searsia mysorensis* against streptozotocin induced diabetes in Wistar rats, using hydroethanolic root extracts of *Searsia*

mysorensis. The findings exhibited significant anti-diabetic activity, which provide some scientific evidence for its traditional claims (Swathi *et al*., 2005).

2.5.3 Other biological activities of the Searsia genus

Phytochemical investigation conducted from *Searsia* chinensis led to the isolation of compounds with anti-HIV-1 activity (Gu *et al*., 2007, Wang *et al*., 2008). Among the isolated compounds 5-hydroxy-3-(propan-2-ylidene)-7-(3, 7, 11, 15-tetramethylhexadeca2, 6, 10, 11-tetraenyl)-2(3H)-benzofuranone and 5-hydroxy-7-(3, 7, 11, 15-tetramethylhexadeca-2, 6, 10, 11-tetraenyl)-2(3H)-benzofuranone exhibited significant results in suppressing HIV-1 replication (Gu *et al*., 2007). Moronic acid, 3-oxo-6b-hydroxyolean-12-en-28-oic acid and 3-oxo-6b-hydroxyolean-18-en-28-oic acid have been reported to possess anti-HIV activity (Pengsuparp *et al*., 1994, Soler *et al*., 1996, Kashiwada *et al*., 1998). Antimutagenic flavonoids such as Garbanzol, Sulfuretin, Fisten, Fustin and Mollisacasidin (Table 2.2) were reported from the ethyl acetate extract of *S. verniciflura* (Lee *et al*., 2004, Park *et al*., 2004). When these compounds were put to the Ames test, Sulfuretin exhibited a potential to effectively prevent the metabolic activation, or scavenge the electrophilic intermediates, capable of causing mutation (Sierra and Mazza, 2007). A chemical investigation conducted on *S. natalensis* led to the isolation of rhuschromone which showed significant activity against *S. aureus* ATCC 25923 (Mwangi *et al*., 2013). Monavari *et al*., 2007 reported that *S. coriacoria*, amongst twenty-five species investigated, showed significant activity against HSV-1 and adenovirus type 5 at non-toxic concentrations.

CHAPTER THREE: EXPERIMENTAL PRECEDURE

3. Methodology

3.1 General Experimental Procedure

3.2 Reagents and solvents

Hexane, Dichloromethane, Ethyl acetate, Methanol and Butanol were of analytical grade (supplied by Merck, South Africa). Acetonitrile, Dimethyl sulfoxide (DMSO), formic Acid, and methanol were of HPLC grade (supplied by fisher, South Africa).

3.3 Chromatography

3.3.1 Thin Layer Chromatography (TLC)

Pre-coated plates of silica gel 60 F254 (Merck, Germany) were used for TLC profiling. TLC spots were viewed under UV at 254nm and 366nm using UV lamp followed by spraying with vanillin/sulphuric acid reagent and heating (Wagner, 1984). TLC development was chosen from the following TLC systems: (hexane: ethyl acetate, (9:1); (7:3), DCM-methanol, (95:5), (90:10).

3.3.2 Column chromatography (CC)

Glass columns (20-25 mm diameter) wet packed with silica gel 60 (0.040-0.063mm) (230-400 Mesh ASTM, Merck) were used for column chromatography.

3.3.3 High Pressure Liquid Chromatography (HPLC)

3.3.3.1 Analytical System:

System: waters HPLC System with MS Quattro micro API detector

Column: Waters Symmetry, C18, 3.5 micron, 4.6 x 100 mm

Preparative System: Waters PLC 2020

Mobile Phase: Acetonitrile/Methanol: H_20 solvent gradient with 0.1% formic acid. Sample purification was performed using Waters preparative HPLC ESI-MS equipped with MS Quattro micro API detector automated fraction collector, injector, and quaternary pump.

***3.3.3.2 Preparative HPLC-MS parameters*:**

The chromatographic separation of secondary metabolites was carried out using reverse phase C-18 (RP C-18) column. The mobile phase consisted of solvent A: water-formic acid (99.9 %: 0.1%) and solvent B: Acetonitrile/methanol. The HPLC binary pump with a flow rate of 20 ml/min was programmed to run the mobile phase over ten minutes.

3.3.3.3 Electrospray ionization mass spectroscopy (ESI-MS)

High resolution mass spectroscopy (HRMS) analysis was performed on waters Quattro micro API with electrospray ionization (ESI) interface working in the positive mode.

3.4 Spectroscopy

3.4.1 Nuclear magnetic resonance (NMR) spectroscopy

NMR spectra were recorded at 25^0 C, using deuterated methanol (CD_3OD), DMSO (CD_3SOCD_3), Pyridine (C_5D_5N) and chloroform ($CDCl_3$) as solvents, on a Bruker Avance 400 MHz NMR spectrometer. Chemical shifts of ^{13}C and ^{1}H were measured relative to tetramethyl silane as internal reference.

3.4.2 Mass spectroscopy (MS)

Mass spectroscopy (MS) analysis was performed on waters Quattro micro API with electrospray ionization (ESI) interface working in the positive and or negative mode.

3.4.3 Infrared (IR) spectroscopy

Attenuated total internal reflectance FTIR measurements were performed using Spectrum 100 (Perkin Elmer Corporation). Spectra recording were achieved using the interface "Spectrum". Samples were dissolved using Dichloromethane (DCM).

3.5 Plant material

3.5.1 Collection and identification of plant material

The aerial parts of *Searsia glauca*, S*earsia lucida* and *Searsia laevigata* were collected in February 2013 from the Cape Nature Reserve at UWC in Cape Town, South Africa. Species were identified and confirmed by Mr Fransz Wertz from University of Western Cape Herbarium, Samples were dried and powdered. Powdered samples were stored in dark until further use.

3.6 Extraction and fraction of plants

3.6.1. Extraction and isolation of compounds from S. glauca

Leaves, flowers of *Searsia glauca* (300.05g) were air dried at room temperature, blended and extracted (2.5L x 2) with 80% methanol for 48 hours. Methanol extract was evaporated at 40^0C using rotary vapour. Methanol extract was partitioned with hexane (15.42g), dichloromethane (10.14g), ethyl acetate (8.25g) and butanol (6.12g) respectively. The extracts were loaded on the column chromatography with silica gel and eluted with hexane: EtOAc (100:0; 95:05, 90:10; 80:20; 70:30; 60:40; 5050; 40:60; 20:80; 0:100). The collected fractions (1-40) were concentrated using rotary vapour, and profiled using TLC. All the fractions with similar profiles were combined and labelled alphabetical (A-E) as shown in Table 3.1.

Table 3. 1: Main fractions obtained after fractionation of Hexane extract of *S. glauca*

Fraction number	Combine fractions	Elution mixture
1-4	A	(9:1)
6-11	B	(7:3)
12-18	C	(9.5: 0.5)
19-27	D	(9:1)
28-40	E	(9:1)

3.6.1.1 Isolation of compound C1, C2, &C10 Column chromatography of main fraction B

Fraction B (1.27g) was reloaded column chromatography silica gel (0.063-0.2 mm (70-230 mesh ASTM) and eluted with (Hex: EtOAc) (9:1), & (7:3). 100 mL of each fraction was collected, and evaporated using rotary vapour. Fractions with similar profiles were combined as indicated on Table 3.2.

Table 3. 2: Sub fractions from Fraction B

Fraction	Weight	Designated code
4-7	340 mg	A1
8-14	273 mg	A2
15-18	110 mg	A3
19-23	80 mg	A4

The chromatography of sub fraction A1 (340.23g) on silica gel (hex/EtOAc, 7:3) resulted on the isolation of pure compound C1 (82 mg) and C10 (11 mg). The compound was crystalized by using methanol.

3.6.1.2 Isolation of compound C2, C3 and C10 from dichloromethane and ethyl acetate extracts of S. glauca

The TLC profiles of dichloromethane and ethyl acetate were similar, hence they were combined and loaded on column chromatography with silica gel. The collected fractions (**1-40**) were concentrated and evaporated using rotary vapour. The fractions with similar profiles were combined and coded alphabetical (**F-J**).

Table 3. 3: Main fractions obtained from the combined extracts of DCM/EtOAc of *S. glauca*

Fraction number	**Combined fractions**	**Elution mixture**
1-3	F	9:1
4-6	G	9:1
7-14	H	8:2
15-29	I	7:3
30-40	J	(1:1)

The main fraction H (350 mg) was further chromatography on preparative HPLC/MS waters system and afforded compounds C2 (09 mg), C10 (11.0mg) and C3 (12 mg).

3.6.1.3 Isolation of compound C4 from butanol extract

The main fractions from butanol extract were profiled and those with similar profiles were combined and the total mass of the combined fractions was 250 mg. The combined fraction further purified in a prep-HPLC-MS and afforded compound C4 (12.90 mg).

3.6.2 Extraction and fractionation of *S. Lucida*

Leaves, flowers of *Searsia Lucida* (280.12g) were air dried at room temperature, blended and extracted (2.5L x 2) with 80% methanol for 48 hours. Methanol extract was evaporated at 40^0C using rotary vapour. Methanol extract was partitioned with hexane (16.42g), dichloromethane (12.14g), ethyl acetate (10.25g) and butanol (6.12g) respectively. The crude extracts were each subjected to column chromatography (CC) for the isolation of pure compounds. Crude extracts were eluted with Hexane: ethyl acetate; dichloromethane: ethyl acetate (100: 0; 80:20; 60:40; 50:50; 20:80; 0:100) and washed with 100 %. Fractions with the similar profile after TLC profiles were combined and further purified on (CC) or Preparative HPLC-MS.

3.6.2.1 Isolation of the pure compounds from Searsia lucida

3.6.2.2. Isolation of compound C5 (moronic acid) from hexane extract

Main fraction FH6 (250.36 mg) was loaded on the silica column and eluted with hexane/ethyl acetate (8:2). After profiling the fractions with TLC plate a pure compound C9 (40.52 mg) was obtained.

3.6.2.3 Isolation of Compound C6 (Quercetin) column chromatography

During fractionation dichloromethane and ethyl extracts were combined due to their similar TLC profiles. Main fraction FDE-24 (150.12 mg) was chromatographed on silica gel and eluted with mobile phase of dcm/ethyl acetate (7:3) and afforded pure quercetin (15.81 mg).

3.6.2.4 Isolation of compound C7 and C8 column chromatography

Main fraction FDE-34 (300.56 mg) was chromatographed on silica gel eluted with dcm/ethyl acetate (1:1). Subfractions from 12-17 precipitated and yellow porous powder was observed. The yellow powder was further rinsed with methanol. FDE-34 afforded two compounds C8 and C9 compound of 6.12 mg and 110.21 mg respectively.

3.6.3.1 Extraction and fractionation of Searsia Laevigata

Leaves, flowers of *Searsia Laevigata* (200.10g) were air dried at room temperature, blended and extracted (2.5L x 2) with 80% methanol for 48 hours. Methanol extract was evaporated at 40^0C using rotary vapour. Methanol extract was partitioned with hexane (10.54g), dichloromethane (7.22 g), ethyl acetate (5.18 g) and butanol (4.12 g) respectively. All the crude extracts were profiled using TLC. Crude extracts were subjected to column chromatography (CC) respectively and eluted with Hexane: ethyl acetate; DCM; and EtOAc: MeoH depending on the polarity of the extract.

3.6.3.2 Isolation of compounds C11a, C11b and C11c (mixture) from S. laevigata

Fraction A from a DCM extract was subjected to column chromatography over silica gel. The eluting mobile phase was (8:2) hexane: EtOAc. The sub-fractions with similar profiles were combined and re-chromatography over flash chromatography silica gel, eluting with 100% DCM and the TLC showed one spot. The amount of the mixture-1 was 22.23 mg.

3.6.3.3 Isolation of an inseparable mixture of α-amyrin fatty acid ester C12a and β-amyrin fatty acid ester C12b from S. laevigata

Fraction B from DCM extract was subjected over flash chromatography on silica gel, eluted with 100 % DCM and afforded mixture-2 (5.32 mg).

3.6.3.4 Isolation of compound C9 from S. laevigata (quercetin-3-O-β-glucoside)

Fraction A from butanol extract was subjected over chromatography silica gel and eluted with EtOAc/MeOH (8:2). After five repeated runs over the silica gel sub-fractions with similar profiles were further purified on preparative HPLC-MS, and afforded compound C9 (8.03 mg).

3.7 General experimental procedure for biological assays

3.7.1. Reagents

Standards (purity > 99.0%) for antioxidant, trolox (6-Hydroxyl-2, 5,7,8-tetramethylchroman-2-carboxylic acid, Saarchem Cat nr.: 238831), and other reagents including ABTS (2,2'-Azino-bis (3-ethylbenzo thiazoline-6-sulfonic acid) Diammonium salt (Sigma Cat nr.: A1888), Potassium-peroxodisulphate (Merck Cat nr.: 105091), Ethanol (Saarchem Cat nr 2233540LP), fluorescein sodium salt (Sigma Cat nr F6377), AAPH (2, 2'-Azobis (2-methylpropionamidine) dihydrochloride (Aldrich Cat nr.: 440914), Sodium dihydrogen orthophosphate-1-hydrated ($Na_2HPO_4.2H_2O$, Merck Cat nr.: 5822880EM), PCA (Perchloric acid , Saarchem Cat nr.: 494612), TPTZ (2,4,6-tri[2-pyridyl]-s-triazine, Iron (III) chloride hexahydrate, copper sulphate, were purchased from Sigma-Aldrich, Inc. (St. Louis, MO, USA). L-Ascorbic acid was procured from Sigma Aldrich, South Africa. Antioxidant assvylujb89yhays including FRAP, TEAC, were measured using Multiskan spectrum plate reader, whilst automated ORAC assay was examined by Floroskan spectrum plate reader.

3.8 Antioxidant

3.8.1 Ferric-ion reducing antioxidant power (FRAP) assay

FRAP is established on the rapid reduction in Ferric-tripyridyltriazine (Fe^{3+}-TPTZ) by antioxidant present in the samples forming ferrous-tripydyltriazine (Fe^{2+}-TPTZ), a blue-coloured product (Benzie & Strain, 1996). A standard curve was prepared as per method described by Benzie & Strain, 1996. L-Ascorbic acid (Sigma Aldrich, South Africa) was used as a standard with concentrations varying between 0 and 1000 µM. The results were expressed as µM L-Ascorbic acid equivalents per milligram dry weight (µM AAE/g) of the test samples.

3.8.1.1 Preparation of reagents

Preparation of the FRAP reagent in a 50 mL conical flask was composed of 30 mL Acetate buffer 300 mM pH 3.6 (1.627 g Sodium acetate + 16 mL Glacial acetic acid and made up to 1L with distilled water) + 3 mL TPTZ 10 mM solution (0.0093g TPTZ and 3 mL of 40 mM HCl in 15 mL flask) + 3 mL $FeCl_3$ solution (0.054 g $FeCl_3.6H_2O$ and 10 mL distilled water) and 6.6 mL of distilled water. A control solution was prepared by dissolving 0.00352 g of Ascorbic acid in a 50 mL and diluted to the mark with distilled water.

3.8.1.2 Sample analysis

Preparation of standard series six (6) Eppendorf tubes marked from A-F. Added the amount of standard stock solution and diluents to each tube as described in the Table 3.2. Diluted the stock solution as per Table 3.2 to make a series of standards. Dilutions were made to the extracts that were highly concentrated and dilution factors were noted and considered in calculations of the affected extracts. In a 96-well clear microplate, 10 µL of the stock solution of the extracts (Hexane, DCM, ethyl acetate, Methanol, and Butanol of three plants were mixed with 300 µL FRAP reagent. The plate was read at a wavelength of 593 nm in a Multiskan Spectrum plate reader.

Table 3. 4: Ascorbic standard wells

Tube	**Ascorbic acid stock solution µL**	**Distilled water µL**	**Standard concentration µM**	**Well number**
A	0	1000	0	A1-A3
B	50	950	50	A4-A6
C	100	900	100	A7-A9
D	200	800	200	A10-A12
E	500	500	500	B1-3
F	1000	0	1000	B4-6

3.9.1 Trolox equivalent absorbance capacity (TEAC) assay

The total antioxidant activity of the extracts was determined by previously described methods (Pellegrini, et al., 1999; Re, et al., 1999).

3.9.2.1 Preparation of reagents

ABTS (2, 2'-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) Diammonium salt 7 mM solution was prepared by dissolving 0.0192 g with 5 mL distilled water. Potassium-peroxodisulphate 140 mM solution was prepared by dissolving 0.1892 g with 5 mL distilled water. The ABTS mix solution was prepared by adding 88 µl $K_2S_2O_8$ solution to 5 mL ABTS solution. The ABTS mix solution was allowed stay in the dark for 24 hours at room temperature before use. Trolox (6-hydrox-2, 5, 7, 8-tetramethylchroman-2-carboxylic acid) 1.0 mM was prepared by dissolving 0.0125 g of Trolox in 50 mL of ethanol and used as the standard with concentrations ranging between 0 and 500 µM. The stock control (Trolox) 200 µM was prepared by dissolving 0.0025 g of Trolox in 50 mL ethanol. The ABTS mix solution was diluted with ethanol to read a start-up absorbance of approximately 2.0 (± 0.1). The test extracts were prepared by dissolving 1 mg of an extract in 1mL of methanol and allowed to react with 300 µL ABTS solution in the dark at room temperature for 30 min.

3.9.2.2 Sample analysis

Standard series were prepared in tubes marked A-F, an amount of standard stock solution was diluted as per Table 3.2. The absorbance was measured at 734 nm at 25 °C in the plate reader. The results were expressed as µM Trolox equivalents per milligram dry weight (µM TE/g) of the test samples.

Table 3. 5: Preparation of standard series

Tube	Trolox standard µl	Ethanol µl	Trolox conc. µM	Well number
A	0	1000	0	A1-A3
B	50	950	50	A4-A6
C	100	900	100	A7-A9
D	150	850	150	A10-A12
E	250	750	250	B1-3
F	500	500	500	B4-6

3.10. Automated oxygen radicals absorbance capacity (ORAC) assay

ORAC was determined according to the previously described method (Prior, et al., 2003) with some modifications (Cao et al, 1997; 1998). Fluorescein was used as the fluorescent probe. The loss of fluorescence of fluorescein was an indication of the extent of its oxidation through reaction with the peroxyl or the hydroxyl radical. The protective effect of an antioxidant was measured by assessing the fluorescence area under the curve plot relative to that of a blank in which no antioxidant was present. The ORAC value is calculated by dividing the area under the sample curve by the area under the Trolox curve with both areas being corrected by subtracting the area under the blank curve.

3.10.1 Preparation of reagents

Phosphate buffer: 75mM, pH 7.4 was prepared by dissolving 1.035g of sodium di-hydrogen orthophosphate-1-hydrate ($NaH_2PO_4.H_2O$) with 100mL of distilled water. Second solution was prepared by dissolving 1.335g of di-sodium hydrogen orthophosphate dehydrate ($NaH_2PO_4.2H_2O$) with 100mL of distilled water. Mixed 18 ml of the first solution with 82 ml of the second solution. Fluorescein sodium salt was prepared by dissolving 0.0225g of

$C_{20}H_{10}Na_2O_5$ in 50 mL Phosphate buffer. Peroxyl radical: AAPH (2.2'-Azobis (2-methylpropionamidine) dihydrochloride 25 mg/mL was prepared by dissolving 150 mg into a 15 ml screw cap tube. PCA (70% Perchloric acid) 0.5 M was prepared by diluting 15 ml of 70 % perchloric acid with 195 ml of distilled water into a 250 ml bottle. Trolox standard of 500 µM was prepared by dissolving 0.00625g 6-Hydroxy-2, 5, 7, 8-tetra-methylchroman-2-carboxylic acid with 50 ml phosphate buffer. Trolox control of 250 µM stock solution was prepared by dissolving 0.00312g of 6-Hydroxy-2, 5, 7, 8-tetra-methylchroman-2-carboxylic acid with 50 ml phosphate buffer.

3.10.2 Sample analysis

The Trolox standard series were prepared in tubes marked A-F, an amount of standard stock solution and was diluted as per Table 3.3. The fluorescein solution and sample were added in the wells of an illuminated 96 well plate, 12 µL of each of our sample (in stock solution of 1 mg/mL) was combined with 138 µL of a fluorescein working solution followed by addition of 50 µL of 150 mg of AAPH prepared in-situ in 6 mL Phosphate buffer. Absorbance was measured with Fluoroskan spectrum plate reader with the excitation wavelength set at 485 nm and the emission wavelength at 530 nm. Extracts without perfect curve were further diluted and the dilution factors were used in the calculations of ORAC values. Results were expressed as micromoles of Trolox (TE) equivalents per milligram of sample (µM TE/g). Trolox was used as a control in the experiment.

Table 3. 6: Trolox standard series

Tube	**Standard concentration µM**	**Trolox stock solution µL**	**Phosphate Buffer µL**	**Well number**
A	0	0	750	A1-A3
B	83	125	625	A4-A6
C	167	250	500	A7-A9
D	250	375	375	A10-A12
E	333	500	250	B1-3

F	417	625	125	B4-6

3.11 Brine shrimp lethality assay

The method was done following Solis protocol with some modification, (Solis *et al*., 1993). Brine shrimp eggs (*Artemia salina*) were obtained and hatched by placing 1 g of these eggs in 1L of artificial sea water prepared using 40.23 gm of sea salt in a large conical flask. A constant supply of oxygen was carried was carried out throughout the hatching period. Incubation of the Brine shrimp eggs was kept for 48 hours in continuous illuminated tank. The larvae (nauplii) were attracted to one side of the vessel towards the light source. Extracts were prepared by dissolving them in dimethylsulfoxide (DMSO) and diluted with artificial sea water so that final concentration did not exceed 0.05 %. Different stock solutions of the test extracts were prepared at 1000 µl and from this; 100 µl and 10 µl of the solutions were prepared. Each dosage was tested in triplicate. After 48 hours ten (10) nauplii were placed in each of a series of the test tubes containing the test extracts at different concentrations. After 24 hours surviving nauplii were inspected using magnifying glass and counted to determine the percentage mortality. A control experiment was prepared in the same manner as that of the extracts but the test extract was excluded. Regression equations of concentration versus percentage mortality were used to estimate LC50 from Microsoft Excel Plus 2013 (Persoone et al., (1979).

3.12 In *vitro* enzyme inhibition

3.12.1 Reagents

α-glucosidase (*Saccharomyces cerevisiae*), α-amylase (procaine pancreas) and 3, 5, di-nitro salicylic acid (DNS), P-nitro-phenyl-α-D-glucopyranoside (p-NPG), sodium carbonate ($Na_2 CO_3$), Soluble starch, sodium dihydrogen phosphate, di-sodium hydrogen phosphate purchased from Sigma-Aldrich, South Africa.

3.12.2 In-vitro Assay: α-amylase inhibitory activity

α-amylase inhibitory activity of extracts was carried out according to the standard method with minor modification (Ademuluyi & Oboh, 2013). In a 96-well plate, reaction mixture containing 50 µl phosphate buffer (100 mM, pH = 6.8), 10 µl α–amylase (2 U/ml), and 20 µl of plant extracts at 2.0 mg/ml and was preincubated at 37°C for 20 min. Then, the 20 µl of 1% soluble starch (100 mM phosphate buffer pH 6.8) was added as a substrate and incubated further at 37°C for 30 min; 100 µl of the DNS colour reagent was then added and boiled for 10 min. The absorbance of the resulting mixture was measured at 540 nm using Multiplate Reader (Multiska thermo scientific, version 1.00.40). Acarbose was used as a control. The extracts or isolated compounds were measured in triplicates. The results for both α-amylase and α-glucosidase were expressed as percentage inhibition, which was calculated using the formula,

Inhibitory activity (%) = $(1 - As/Ac) \times 100$

Where,

As is the absorbance in the presence of test substance and Ac is the absorbance of control.

3.12.3. In vitro assay: α-glucosidase inhibitory activity

α-glucosidase inhibitory activity of extract was carried out according to the standard method with minor modification (Shai et al., 2011). In a 96-well plate, reaction mixture containing 50 µl phosphate buffer (100 mM, pH = 6. 8), 10 µl α-glucosidase (1 U/ml), and 20 µl of extract at 2.0 mg/ml was pre-incubated at 37°C for 15 min. Then, 20 µl P-NPG (5 mM) was added as a substrate and incubated further at 37°C for 20 min. The reaction was stopped by adding 50 µl Na_2CO_3 (0.1 M). The absorbance of the released p-nitrophenol was measured at 405 nm using Multiplate Reader. Quercetin was used as a control. Each extract was tested in triplicates.

CHAPTER FOUR: RESULTS AND DISCUSSION

4. SECTION A: PHYTOCHEMISTRY

4.1. Chemical constituents isolated from Searsia glauca

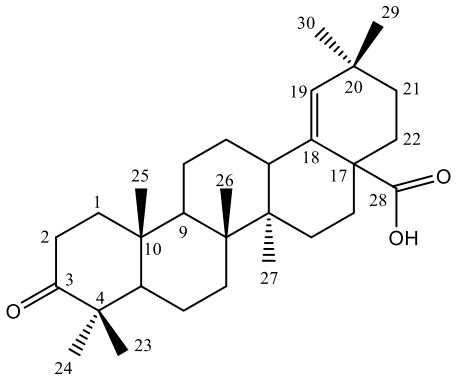


Figure 5. 1: moronic acid (C1)

4.1.1 Characterization of compound (C1)

Compound C1 was isolated as white needle crystals. The chemical structures of compound C1 showed the pseudo molecular ion of m/z 455.21 ESI-MS $[M+H]^+$. The ^1H NMR spectrum of compound C1 showed seven methyl groups resonating at 0.81 (3H, s), 0.96 (3H, s), 0.99 (3H, s), 1.02 (3H, s), 1.03 (3H, s), 1.04 (3H, s), and 1.09 (3H, s). The proton signals from 1.24 – 2.54 ppm were an indication of saturated cyclic and or acyclic methylene and methine protons and accounts for twenty four (24) protons. An olefinic proton was observed at 5.19 (1H, s, H-19) ppm and correlated with 133.3 (C-19) in the HSQC.

The HMBC correlation between proton H_δ 2.19 (2H, t) and 181.3 confirmed that the carboxyl group is allocated at C-28.

The ^{13}C NMR and DEPT 135 spectra exhibited a keto-carbonyl carbon at δ_C 218.3 (C-3), seven primary carbons, ten secondary carbons, three tertiary carbons, seven quaternary carbons, an olefinic carbon at δ_C 136.6 (C-19) and a carboxylic group at δ_C 181.3, (C-28).

The chemical structure of compound C1 was determined by comparing (^1H and ^{13}C) spectral data with that of moronic acid, reported on the literature (Majumder *et al*., 1979; Gonalez *et*

al., 1983; Ahsan *et al*., 1995). Compound C1 was previously isolated from *S. Javanica (*Kurokawa, *et al.,* 1998). Moronic acid has been reported as a major anti-HSV compound from *S. javanica*, and it exhibited novel anti-HSV activity that was different from that of ACV (Kurokawa *et al*., 1999).

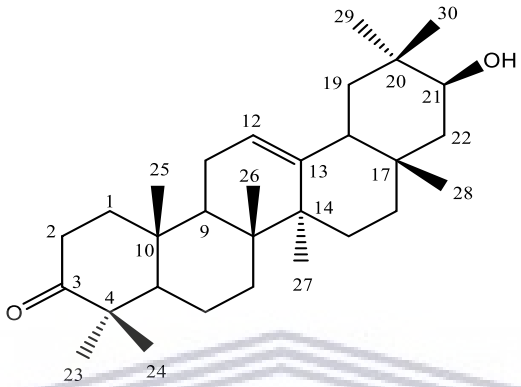


Figure 5. 2 : 21-β-hydroxylolean-12-en-3-one (C2)

4.1.2 Characterization of 21β-hydoxylolean-12-en-3-one (C2)

Compound **C2** was isolated as a white amorphous powder. The HRESI-MS exhibited a pseudo molecular peak at m/z 441.39 $[M+H]^+$ corresponding to $C_{30}H_{48}O_2$. The ^{1}HNMR spectrum showed characteristic signals of eight methyl groups, a proton attached to an oxygenated carbon atom (δ_H 3.22, dd, J = 11.2, 5.3 Hz), and an olefinic proton at (δ_H 5.33, t, J = 7.08 Hz). The ^{13}C NMR spectrum showed eight primary carbons, nine secondary carbons, three tertiary carbons, an oxygenated carbon (δ_C 78.9), seven quaternary carbons, an olefinic carbon at (δ_C 122.4, C-12), a carbonyl group at (δ_C 217.7). From the ^{1}H NMR and ^{13}C NMR spectra compound C2 was proposed to be a 12-oleanene type triterpene with a secondary hydroxyl group and keto group.

In the HMBC spectrum correlations between the hydroxymethine signal and two methyl carbons signals at δ 28.0 C-29 and δ 15.3 C-30, methylene signal at δ 45.2 C-22, and a quaternary carbons signal at δ 36.7 C-20 exhibited that the secondary hydroxyl group is at C-21 .Further HMBC spectrum, showed a correlation between proton methyl peaks at δ 0.98 H-

29 and 0.78 H-30 exhibited a long range correlations with the methine carbon signal at δ 78.9 (C-21), a methylene signal at δ 45.8 (C-19), and the quaternary carbon at δ 36.7 (C-20). The methyl proton signal at δ_H 0.98 (H-29) indicated correlation with the methyl signal at δ 15.3 (C-30), and the methyl signal at δ_H 0.78 (H-30) indicated correlation with the methyl signal at δ 28.0 (C-29).The configuration at C-21 was substantiated by the coupling constant of H-21, which exhibited axial-axial and axial-equatorial coupling values (3.22 dd, J = 11.2, 5.3 Hz) with C-22 protons, indicating that the hydroxyl group must be equatorial (β-oriented). Confirmation of the keto group at C-3 and allocation of C-23 and C-24 gem-dimethyl group signals was justified by the long range coupling between the carbonyl signal at δ 217.7 and both methyl signals at δ_H 0.96 and 0.86 (H-23 and H-24) respectively. The assignment of the ^{13}C NMR spectrum is shown in Table 4.1. After comparing the entire experimental spectroscopic data with previously reported data, compound C2 was identified as 21*β*-hydroxylolean-12-en-3-one and this compound has been isolated from *Hippocratea excelsa* (Mena-Rejon, *et al*., 2007).

Table 4. 1: ^{1}H (400 MHZ, m J Hz) and ^{13}C (100 MHz) NMR spectral data of C1, and C2 in $CDCl_3$

No	**C1 (moronic acid)**		**C2 (**21β-hydroxylolean-12-en-3-one)	
	^{13}C	**^{1}H**	**^{13}C**	**^{1}H**
1	39.8	1.98 (m)	39.9	1.90 (m)
2	33.8	2.40 (m)	34.2	2.41 (m)
3	218.3		217.8	
4	47.3		47.6	
5	55.3	1.39(m)	55.3	1.33 (m)
6	19.6	1.38 (m)	19.6	1.51 (m)
7	33.9	2.49 (m)	33.8	2.37 (m)
8	40.6		41.3	
9	50.5	1.39 (m)	50.6	1.34 (m)
10	36.8		37.1	
11	21.4	1.33 (m)	20.1	1.46 (m)
12	26.0	1.28 (m)	122.4	5.33 t, J = 7.08 Hz
13	41.6	2.27(m)	143.6	
14	42.6		41.7	
15	29.3	1.27 (m)	29.7	1.27 (m)
16	33.7	1.48 (m)	29.6	1.36 (m)
17	47.9		36.7	
18	136.6		46.8	0.78
19	132.4	5.19 (s)	45.8	1.19 (m)
20	32.1		36.7	
21	33.4	2.22 (t)	78.9	3.22 dd, J = 11,2; 5.3 Hz)
22	33.3	2.19 (t)	45.2	1.19 (m)
23	20.9	1.05 (3H, s)	23.6	0.96 3H, (s)
24	26.8	1.09 (3H, s)	26.4	0.86 (3H, s)
25	15.8	1.04 (3H,s)	15.4	0.79 (3H, s)
26	16.7	0.97 (3H,s)	16.8	0.84 (3H, s)
27	14.8	0.81 (3H, s)	25.8	1.10 (3H, s)
28	181.3		27.8	0.99 (3H, s)
29	29.1	0.99 (3H, s)	28.0	0.98 (3H, s)
30	30.4	1.02 (3H, s)	15.3	0.79 (3H, s)
C=O				
CH_3				

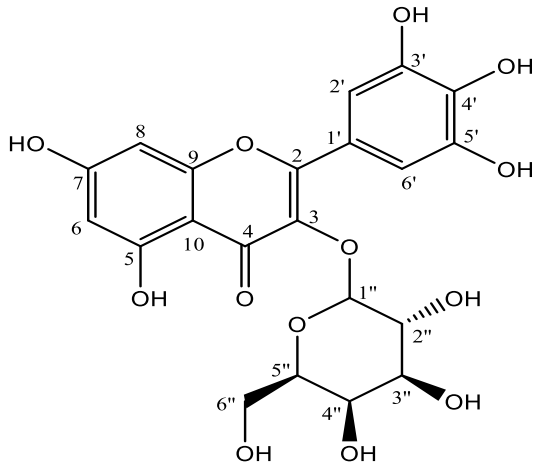


Figure 5. 3 : myricetin-3-*O*-*β*-galactopyranoside (C3)

4.1.3 Characterization of myricetin-3-O-β-galactopyranoside (C3)

The butanol extract afforded one compound C3 and its formula was suggested to be $C_{12}H_{20}O_{13}$, by the NMR data and ESI-MS m/z 574.10 $[M+H]^+$. The ^{1}HNMR spectrum of compound C3 exhibited two meta-coupled aromatic protons at 6.2 (d, J =2.08 Hz) and 6.39 (d, J = 1.92 Hz). These are the characteristics of flavonoids with oxygen substituents at positions 5 and 7. The ^{1}H NMR spectrum exhibited a proton signal at 7.38 ppm which confirms symmetry at positions (s, H-2' and H-6').

The ^{13}C NMR spectrum compound C3 showed carbon signals at δ_C 156.9 (C-2), 134.5 (C-3), 161.6 (C-5), 98.4 (C-6), 164.6 (C-7), 93.2 (C-8), 157.2 (C-9), 105.7 (C-10), 120.2 (C-1'), 108.5 (C-2' and C-6'), 136.7 (C-4'), 144.9 (C-3' and C-5'), and a carbonyl carbon signal at δ_C 178.0 (C-4; C=O). The high intensity signals at δ_C 108.5 and 144.9, exhibited the presence of two pairs of equivalent carbons i.e., C-2' with C-6', and C-3' with C-5', respectively. The sugar carbon atoms were observed to resonate at δ_C 60.5 (C-6"), 68.6 (C-4"), 71.8 (C-2"), 73.7 (C-3"), 75.8 (C-5"), and the anomeric carbon was observed at δ_C 104.1 (C-1"). The sugar moiety was identified as galactopyranose with chemical shifts for H-1" and C-1" at (δ_H 5.21, J = 7.8 Hz, δ_C 104.1). The coupling constant of 7.8 Hz on the anomeric proton confirmed the β-orientation of sugar moiety.

The glycosyl linkage at C-3 was confirmed by the HMBC long range correlation between anomeric proton (δ_H 5.45) and carbon C-3 (δ_C 134.5). Correlations between H-8/H-6, C-9, C-6 and C-10 confirmed the presence of a benzopyran ring system. H-2' showed a long range correlation to C-2, which confirmed the substitution of the B-ring to C-2. HMBC spectrum also showed a correlation between H-1" and C-3. This proved that the sugar moiety was connected to C-3 of the flavonol. The configuration of the anomeric proton was proposed to be β-form based on larger coupling constant (H-1", J = 7.8 Hz). The proposed structure for this compound C3 was in agreement with the literature data previously reported (Gürbüz *et al*., 2015).

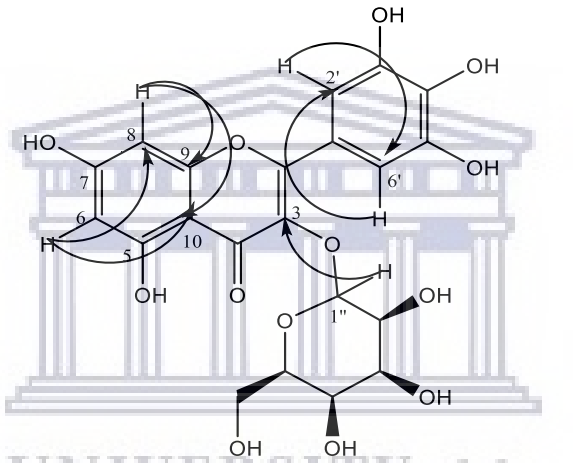


Figure 5. 4: Key HMBC correlations in compound myricetin-3-*O*-*β*-galactopyranoside (C3)

Flavonoids and their glycosides derivatives are reported to possess biological activity such as antioxidants, anti-inflammatory and anticancer (Antunes-Ricardo *et al*., 2014, Veitch and Grayer, 2011). Fractionation of the methanol extract resulted in the isolation of myricetin-3-*O*-*β*-galactopyranoside which has been reported to contribute for the antinociceptive effect of *Davilla elliptica* extract, a secondary metabolite significantly more potent than diclofenac, used as reference drug (Camposa *et al*., 2013).

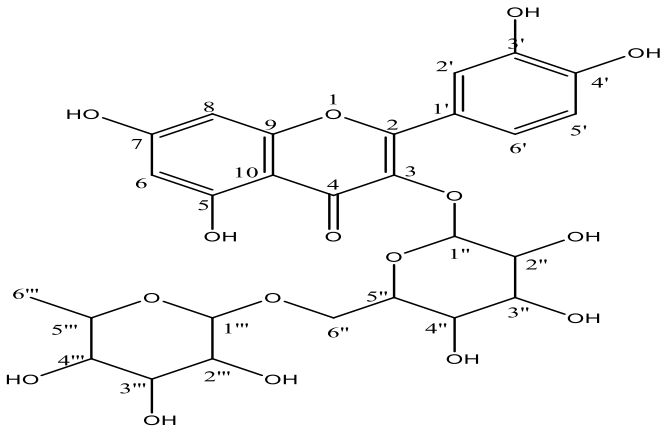


Figure 5. 5 : Rutin (C4)

4.1.4 Characterization of Rutin (C4)

Compound C4 was obtained as a yellow solid material, ESI-MS showed a pseudo molecular ions at m/z 632.23 [M+Na^+] suggesting an elemental composition of $C_{27}H_{30}O_{16}$. In the ^{1}H NMR spectrum of compound C4, two meta-coupled aromatic protons were observed at δ_H 6.23 (d, J = 2.04 Hz) and 6.42 (d, J = 2.04 Hz) assigned to H-6 and H-8 protons of 5, 7 dihydroxylated ring A from a flavonoid skeleton. The ^{1}H NMR spectrum exhibited three signals corresponding to an ABX aromatic spin system at δ_H 6.89 (d, J = 8.48 Hz), 7.65 (dd, J = 8.4, 2.12 Hz) and 7.69 (d, J = 2.12 Hz) suggesting a 3', 4'substitued B-ring of a flavonoid derivative. The H NMR spectrum also supported the presence of rhamnose and glucose moieties with the rhamnose anomeric proton signal at δ_H 4.54 and glucose H-1'' peak at δ_H 5.09. The presence of L-rhamnose unit was confirmed by a doublet of methyl group at high field δ_H 1.15 ppm (3H, J = 6.16 Hz). The rest of the proton peaks were observed between 3.30 ppm and 3.55 ppm.

In the ^{13}C NMR spectrum of compound C4**,** twelve aromatic carbons were observed at δ_C 93.5 (C-8), 98.6 (C-6), 104.2 (C-10), 114.7 (C-2'), 116.3 (C-5'), 121.7 (C-6'), 122.2 (C-1'), 144.4 (C-3'), 148.4 (C-4'), 158.0 (C-9), 161.5 (C-5), and 164.6 (C-7) and are attributed to ring A and B of the parental structure. Two quaternary carbons at δ_C 134.3 (C-3), and 157.1 (C-2), and carbonyl carbon at δ_C 178.0 (C-4, C=O) were observed and are attributed to ring C of the flavonoid moiety. From the ^{13}C NMR spectrum two anomeric carbons at δ_C 103.6 (C-1'') and

δ_C 101.6 (C-1''') were observed suggesting that a flavonoid skeleton is attached to two sugar moieties. Signals attributed to the glucose moiety were observed to resonate at δ_C 67.1 (C-6''), 69.9 (C-4''), 74.2 (C-2''), 75.7 (C-5''), 76.7 (C-3''). While those which indicated L-rhamnose moiety were observed at 17.0 (C-6'''), 68.3 (C-4'''), 70.6 (C-2'''), 70.8 (C-3'''), 72.0 (C-5''').

The DEPT spectrum of compound C3 exhibited the presence of one methyl carbon at δ_C 17.0 ppm of rhamnose, one methylene carbon at δ_C 67.1 ppm, 15 methine carbons and 10 quaternary carbons.

In the HMBC spectrum correlations were observed between H-6 at δ_H 6.23 with C-5 at δ_C 161.5, C-7 at δ_C 164.6, C-8 at δ_C 93.5, and C-10 at δ_C 104.2, H-8 at δ_H 6.42 connected with C-7 at δ_C 164.6 and C-10 at δ_C 104.2, H-1'' at δ_H 5.09 correlated with C-3 at δ_C 134.3, H-1''' at δ_H 4.54 correlated with C-6'' of the glucose at δ_C 67.1 ppm confirming that the glycosylation of the glucose unit by the rhamnose occurred on C-6'' - hydroxyl. Confirmation of the position of attachment of the sugar moiety was obtained from the HMBC spectrum of C4 in which key ^{3}J correlations was observed between the anomeric proton signal of glucose at δ_C 5.09 ppm and C-3 resonance of the aglycone at δ_C 134.3 ppm indicating that C-3 is the site of glucosilation. The chemical structure of compound C4 was proposed to be Rutin by comparison with the previously reported data (Quispe et al., 2013). This compound was reported from *R. natalensis* by Saleh Ibrahim Alqasoumi et al., 2016.

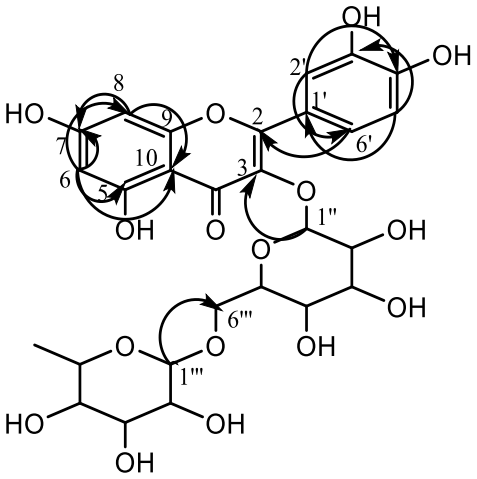


Figure 5. 6: Key HMBC correlations of Rutin (C4)

Rutin is reported to have antimicrobial, antifungal, antithrombotic, antioxidant, anticarcinogenic, cytoprotective, hepatoprotective, vasoprotective, smooth muscle relaxing, anti-allergic activities and tissue protein glycation inhibiting activities (Kamalakkannan & Prince, 2006, Srinivasan, 2005, Yidizoglu-Ari et al., 1991. Rutin reported to possess significant antidiabetic activity by inhibiting inflammatory cytokines, and improved the antioxidant and plasma lipid profiles in high fat and streptozotocin-induced type 2 diabetic model (Niture *et al*., 2014).

Table 4. 2: ^{1}H (400 MHZ, m J Hz) and ^{13}C (100 MHz) NMR spectral data of C3 and C4 in $CDOD_3$

Position	Compound C3		Compound C4	
	^{13}C	^{1}H	^{13}C	^{1}H
2	157.2		157.1	
3	134.5		134.3	
4	177.9		178.0	
5	161.6		161.5	
6	98.4	6.2 (d, J =2.08 Hz)	98.6	6.23 (d, J = 2.04 Hz)
7	164.6		164.6	
8	93.2	6.39 (d, J = 1.92 Hz)	93.5	6.42 (d, J = 2.04 Hz)
9	156.9		158.0	
10	105.7		104.2	
1’	120.2		121.7	
2’	108.5	7.38 (s)	114.7	6.89 (d, J = 8.5 Hz)
3’	144.9		144.3	
4’	136.7		148.4	
5’	144.9		116.3	7.69 (d, J = 2.1 Hz)
6’	108.5	7.38 (s)	122.2	7.65 (dd, J = 8.4 ,2.1Hz)
galactopyranoside			**glucose**	
1’’	104.1	5.21 (d, J = 7.8 Hz)	103.6	5.09 (d, J = 7.6 Hz)
2’’	71.2	3.82	74.2	3.52
3’’	73.2		76.7	3.44
4’’	68.6	3.87 (d, J = 3.36 Hz)	69.9	3.29
5’’	75.8	3.49	75.7	
6’’	60.5		67.1	
			rhamnose	
1’’’			101.0	4.54 (d , J = 1.2 Hz)
2’’’			70.6	
3’’’			70.8	3.55 (d, J = 2.3 Hz)
4’’’			72.5	3.30
5’’’			68.3	3.46 (d, J = 2.4 Hz)
6’’’			17.0	1.15 (d, J = 6.2 Hz)

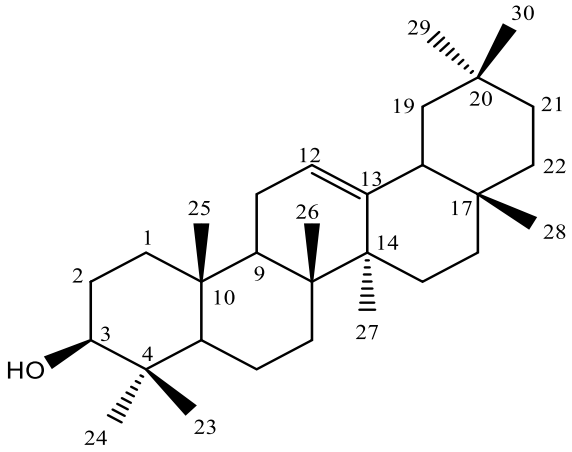


Figure 5. 7: *β*-amyrin (C10)

4.1.5 Characterization of β-amyrin (C10)

The carbon multiplicities of compound C10 (Table 4.3) were confirmed by ^{13}C NMR spectrum and HSQC correlations. Compound C10 exhibited major carbon signals, eight primary carbons at δ_C 27.99 (C-23), 15.02 (C-24), 15.36 (C-25), 16.93 (C-26), 23.49 (C-27), 18.27 (C-28), 29.65 (C-29), 22.98 (C-30), seven quaternary carbons, three tertiary carbons and a secondary hydroxyl bearing carbon 78.98 ppm at C-3. It also exhibited an olefinic carbon atom at $\delta_{C\text{-}12}$ 122.41. The ^{13}C NMR spectrum showed the chemical shifts of olefinic carbons C-12 at $\delta_{C\text{-}12}$ 122.41 and C-13 at $\delta_{C\text{-}13}$ 143.63 ppm which confirmed the presence of *β*-amyrin. The ESI-MS spectrum indicated a pseudomolecular ion of m/z 426.69. The NMR data of C10 (see table 4.3) has been compared with previously reported data of *β*-amyrin (Zhang *et al*., 2012).

Table 4. 3: ^{1}H (400 MHZ, m J Hz) and ^{13}C (100 MHz) NMR spectral data of C10 in $CDCl_3$

Position	**C10**		**Lit (**Vázquez et al., 2012)
	δ^{13} C	**δ^{1}H**	**δ^{13} C**
1	38.97		38.7
2	27.68		27.2
3	78.98	3.24 (dd, J = 5.3, 11.2 Hz)	79.3
4	39.01		38.5
5	55.33	0.76 (d, J = 11.7 Hz)	55.1
6	19.57		18.6
7	32.19		32.4
8	39.12		39.8
9	47.77		47.6
10	36.78		36.9
11	22.98		23.6
12	122.41	5.33 (t, J = 3.5 Hz)	121.7
13	143.63		145.2
14	41.16		41.7
15	26.44		26.2
16	25.80		26.1
17	32.38		32.6
18	47.45		47.2
19	46.52		46.8
20	30.68		31.0
21	34.16		34.7
22	37.12	1.77	37.1
23	27.99	0.84	28.0
24	15.02	0.96	15.4
25	15.36	0.79	15.4
26	16.93	0.89	16.8
27	23.49	0.95	25.9
28	28.5	0.99	28.4
29	32.6	0.93	33.8
30	21.46	1.06	23.7

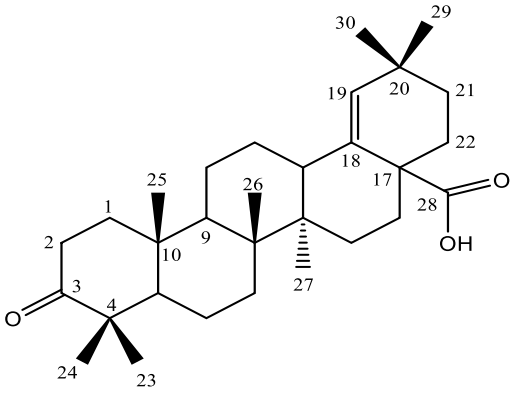


Figure 5. 8 : moronic acid (C5)

4.1.6 Characterization of moronic acid (C5)

Compound C5 was obtained as a white amorphous solid. The molecular formula of compound C5 was determined to be $C_{30}H_{46}O_3$, based on the ^{1}H and ^{13}C-NMR data. The ^{1}H-NMR spectrum showed characteristic signals of seven methyl groups (0.80 (3H, s), 0.96 (3H, s), 0.99 (3H, s), 1.02 (3H, s), 1.03 (3H, s), 104 (3H, s), and 1.09 (3H, s), an olefinic proton at (δ_H 5.18, s). The signals in the ^{13}C NMR spectrum of compound C6 exhibited a carbonyl carbon at δ_C 218.3, C-3 (C=O), a double bond at δ_C (133.2 C-19, 136.6 C-18) and carboxylic group at δ_C 182.3 (C-28). The rest of assignments were compared with the literature data (Junko, *et al*., 2001, David Salinas et al., 2001). Moronic acid was isolated previously from R. *Chinensis*, Gu *et al*., 2007, R. *javanica*, Kurokawa *et al*., 1998).

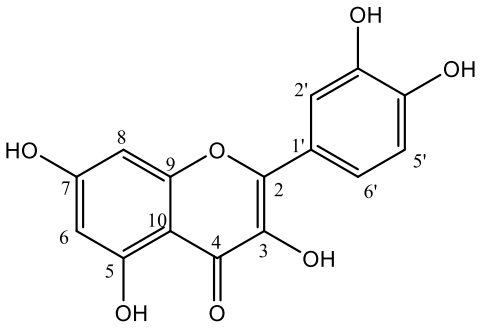


Figure 5. 9: quercetin (C6)

4.1.7 Characterization of quercetin (C6)

Compound C6 was obtained as yellow powder. The HRES-MS showed a pseudo molecular peak at m/z 303.2 $[M+H]^+$ confirming the molecular formula $C_{15}H_{10}O_7$. The ^1H NMR spectrum showed the characteristic 5-OH signal at 12.18, and a pair of *meta*-coupled doublets signals of an AB spin-system at 6.08 (d, J = 2.04 Hz, H-6) and 6.29 (d, J = 2.12 Hz, H-8) in agreement with a 5, 7-disubstituted ring A. An ABC spin-system suggesting a flavonol with 3', 4'-disubstituted ring B was supported by the three aromatic signals at 6.79 (1H, d, J = 8.48 Hz, H-5'), 7.54 (1H, dd, J = 2.16 and 8.48 Hz, H-6') and 7.63 (1H, d, J = 2.16 Hz, H-2'). The ^{13}C NMR spectrum of C7 showed 15 signals, with the characteristic C-3 signal of a flavonol at δ 135.8 and carbonyl carbon at 175.9 (C-4), the rest of chemical shifts of carbon nucleus are indicated in Table 4.4.

The HMBC correlations between H-6 to C-5, C-7, C-8, C-10, H-8 to C-6, C-7, C-9, C-10, H-2' to C-6', C-4', H-6' to C-2', H-5', C-1', and C-3' were observed confirming the quercetin aglycone structure. The ^{13}C NMR data of C7 was compared with the published ^{13}C NMR data of quercetin (Mohammed, 2015). This compound has been isolated from *R. tripartita* (Mohammed, 2015).

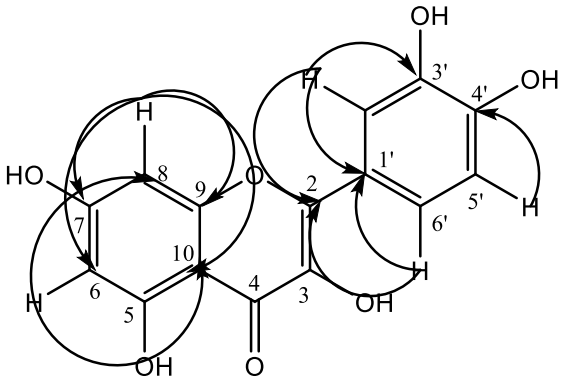


Figure 5. 10 : Key HMBC correlations in quercetin (C6)

Quercetin is known for its anti-inflammatory, antihypertensive, vasodilator effects, antiobesity, antihypercholesterolemic and antiatherosclerotic activities (David, *et al.,* 2016). Quercetin has been reported to show antibacterial effects against almost all strains of bacteria, particularly affecting gastrointestinal, respiratory, urinary, and dermal system. Viruses which respond to flavonoids are adenovirus, herpes simplex virus, Japanese encephalitis virus, and respiratory syncytial virus (Johari J, *et al*., 2012, Ramos FA, *et al*., 2006, Cushinie TP, *et al*., 2005). Quercetin is reported to inhibit the uptake of glucose from the blood, blocks the fat cell production, and enhances fat cell necrosis (Strobel P, et al., 2005**,** Yang JY, et al., 2008). Quercetin has potential anticancer properties which include antiproliferative, growth factor suppression, and antioxidant (Lamson and Brignall, 2000).

Table 4. 4: ^{1}H (400 MHz: m, J Hz) and ^{13}C (100 MHz) NMR spectral data of isolated compounds C6 in CD_3OD

POSITION	**Quercetin (C6)**	
	^{13}C	**^{1}H**
2	147.4	
3	135.8	
4	175.9	
5	161.1	
6	97.8	6.08 (d, J = 2.04 Hz, H-6)
7	164.2	
8	92.9	6.29 (d, J = 2.12 Hz, H-8)
9	156.8	
10	103.1	
1’	122.7	
2’	114.8	7.63 (1H, d, J = 2.16 Hz, H-2’)
3’	144.8	
4’	146.6	
5’	114.6	6.79 (1H, d, J = 8.48 Hz, H-5’)
6’	120.2	7.54 (1H, dd, J = 2.16 & 8.48 Hz, H-6’)
5-OH		12.18, brs

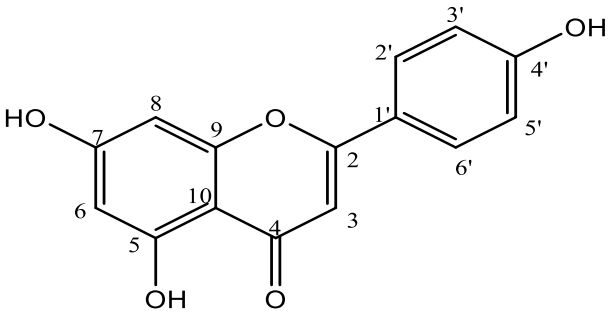


Figure 5. 11 : apigenin (C7)

4.1.8 Characterization of apigenin (C7)

Compound **C7** was obtained as yellow amorphous powder. The EI-MS indicated a pseudo molecular ion of m/z 269.9 $[M+H]^+$, this matched with the molecular formula $C_{15}H_{10}O_5$. The ^1H NMR spectrum of compound **C7** indicated an apigenin skeleton which was suggested by the presence of a hydroxyl at δ_H 12.6 (s, 5-OH). Two doublets observed at δ_H 6.1 (H-6, d, J = 1.72 Hz) and 6.2 (H-8, d, J = 1.8 Hz) which were an indication of 5, 7 disubstituted A ring. An AA'BB' spin-system was exhibited by two doublets at δ_H 7.6 (d, J = 8.84 Hz) and δ_H 7.32 (d, J = 8.56 Hz) which were assigned to H-2', 6' and H-3', 5' of the flavone chromophore, respectively.

The ^{13}C NMR spectrum of compound **C7** exhibited the presence of δ_C [155.2, 99.8, 165.9, and 94.4] for the A- ring, δ_C [162.0, 104.5, 183.4, 109.5, and 161.7] for the C-ring, and δ_C [121.1, 129.1, 116.4 and 154.5] for the B-ring. The structure of compound **C7** was proposed in accordance with the data of apigenin found in the literature (Yang *et al*., 2007, Liu *et al*., 2012, Alwahsh *et al*., 2015).

Apigenin is abundant in various fruits, vegetables, and medicinal plants, such as parsley, onion, orange, paper mulberry, *Veronica linariifolia*, and *Rhizoma Polygoni Cuspidati* and is reported to be useful as pharmaceutical agents (Miean *et al*., 2001). In addition to its anti-inflammatory and antioxidation activities, apigenin has been used as a dietary supplement because of its anticancer properties (Fotsis *et al*., 1997). Apigenin has been reported to exhibit anticancer

activity in numerous human cancer cells, such as prostate cancer, colon carcinoma, and breast cancer, with low cytotoxicity and no mutagenic activity (Lin *et al*., 2012).

Table 4. 5: ^{1}H (400 MHz: m, J Hz) and ^{13}C (100 MHz) NMR spectral data of isolated compound C7 Apigenin) in DMSO.

	C7		**Lit** *(*Owen *et al.*, 2003*)*
Position	**^{13}C**	**^{1}H**	
2	162.0		165.30
3	104.5		103.78
4	183.4		183.07
5	165.9		166.46
6	99.8	6.1 (d, J = 1.72 Hz)	99.71
7	155.2		159.6
8	94.4	6.2 (d, J = 1.8 Hz)	93.99
9	154.5		157.33
10	109.5		103.6
1’	121.1		121.17
2’	129.1	7.6 (d, J = 8.84 Hz)	128.48
3’	116.4	7.2 (d, J = 8.60 Hz)	115.97
4’	161.7		162.9
5’	116.4	7.2 (d, J = 8.60 Hz)	115.97
6’	129.1	7.6 (d, J = 8.84 Hz)	128.48
5-OH		12.6 (1H, s)	

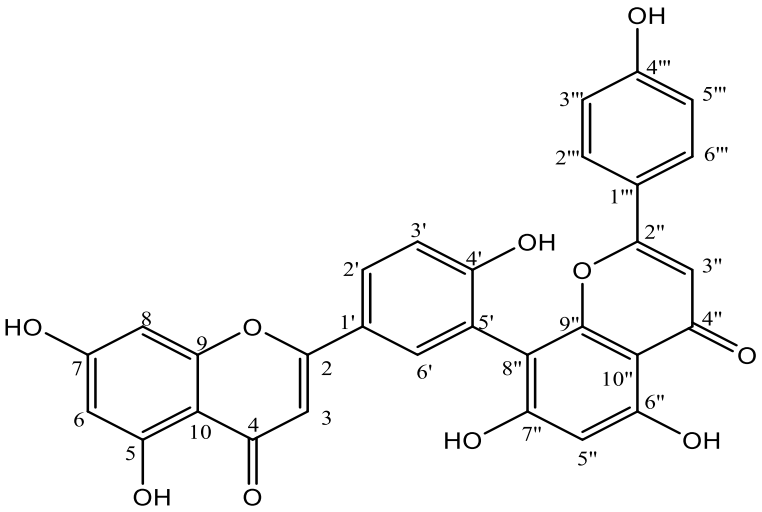


Figure 5. 12 : amentoflavone (C8)

4.1.9 Characterization of amentoflavone (C8)

The elemental composition of compound C8 was found to be $C_{30}H_{18}O_{10}$ as determined from ESI-MS, m/z 538.43 $[M+H]^+$ and 1D and 2D NMR data. The ^{1}H-NMR spectrum of compound C8 exhibited two singlets at δ 13.88 (s, H-5'') and 13.37 (s, H-5), of OH-5 of flavone indicating the presence of two flavonoids linked together as biflavonoid. The two doublets at δ 6.65 (d, J = 2.04 Hz, H-6) and at δ 6.54 (d, J = 2.04 Hz, H-8) were due to a pair of meta-coupling protons of an AB spin-system. An AA'XX' spin-system was exhibited by two doublets at δ 7.66 (d, J = 8.84 Hz, 2H) and δ 7.32 (d, J = 8.56 Hz, 2H) which were assigned to H-2''', 6''' and H-3''', 5''' of the flavanone moiety, respectively.

The HMBC spectrum exhibited correlations from H-6 (δ 6.53) to C-5, C-7, C-8 and C-10, H-8 (6.63) to C-6, C-7, C-9, and C-10 accounting for the tetra-substituted aromatic A-ring. The correlation from a proton signal 5'-H (δ 7.32) to C1 and C3, and the proton signal 2'-H (δ 7.88) showed correlations to C-4', C-6', and C-8'' established the B-ring, and correlations from 3-H to C-2, C-4, and C-1 were observed. Further HMBC spectrum analysis exhibited correlations 6''-H to C-5'', and C-8''. Additional HMBC examination showed correlations from 5'''-H to C-1''', and C-3'''. Further HMBC correlations from 2''-H to C-2, C-1, C-8'' and 6''-H to C-

5'', C-7'' and C-8'' indicated that two flavonol units were linked at C-3 and C-8'' positions. The interflavonoid linkage between two apigenin units corresponding to flavone series (3'– 8'' linkage) was confirmed by the HMBC correlation between H-2' and C- 8''. The ^{13}C-NMR spectra of C8 showed two conjugated ketones at δ183.80 and δ 183.28. The ^{13}C NMR spectrum of compound C8 was compared with those of an amentoflavone isolated from *Podocarpus nakaii* (Yeh *et al.,* 2012). This compound was isolated from *S. succedanea, S. retinorrhea, S. pyroides* (Ahmed *et al*., 2001, Kosar *et al*., 2006, Svenningsen *et al.,* 2006).

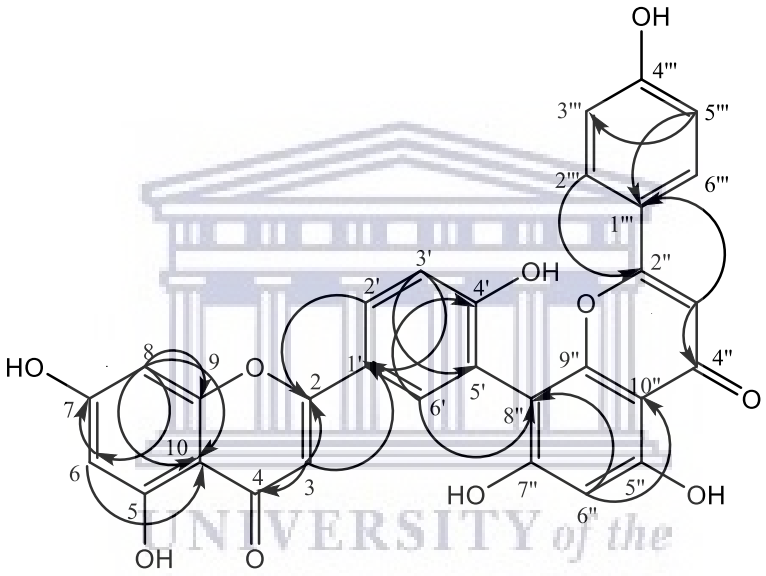


Figure 5. 13 : Selected HMBC correlations in compound C8

Bioflavonoids belonging in this group of flavonoids are known for a variety of biological activities of high importance such as anti-inflammatory (Kim *et al*., 2008, Zhou *et al*., 2011), anticancer (Silva et al., 1995, Li *et al*., 2014), antibacterial (Kaikabo and Eloff, 2011), antiviral (Coulerie *et al*., 2013) activity.

Table 4. 6: ^{1}H (400 MHz: m, J Hz) and ^{13}C (100 MHz) NMR spectral data of isolated compounds C8 (Amentoflavone) in Pyridine

Position	**^{13}C**		**^{1}H**	
	Unity I	**Unity II**	**Unity I**	**Unity II**
2	166.1	162.7		
3	102.4	102.4		
4	183.2	183.5		
5	163.02	162.7		
6	100.3	99.8	6.65 (d, J = 2.04 Hz)	6.89 (d, J = 8.8 Hz)
7	165.6	165.4		
8	94.6	103.9	6.54 (d, J = 2.04 Hz)	
9	155.7	154.5		
10	109.4	109.4		
1’	121.4	120.5		
2’	129.1	129.1		7.88 (d, J = 8.8 Hz)
3’	116.8	116.2	7.14 (d, J = 8.8 Hz)	7.32 (d, J = 8.56 Hz)
4’	161.2	159.1		
5’	120.1	116.2		7.32 (d, J = 8.65 Hz)
6’	132.4	129.1	7.65 (d, J = 8.56 Hz)	7.88 (d, J = 8.8 Hz)

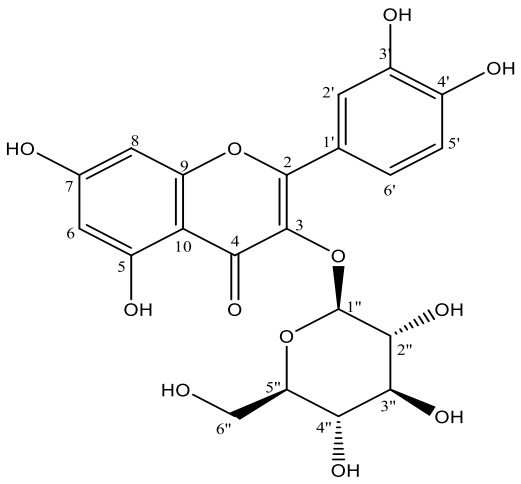


Figure 5. 14 : quercetin-3-*O*-*β*-glucoside (C9)

4.1.10 Characterization of quercetin-3-O-β-glucoside (C9)

Compound C9 was isolated as a yellow solid material. In the HRESI-MS $[M+H]^+$ spectrum a molecular ion signal at m/z 486.37 was observed $[M+Na^+]^+$, suggesting the elemental composition of $C_{21}H_{19}O_{12}$. In the ^1H NMR spectrum two meta-coupling aromatic proton signals were observed at δ_H 6.42 (H-8, d, J = 2.04 Hz) and 6.23 (H-6, d, J = 2.04 Hz), indicating a 5, 7 disubstituted A ring. The AMX spin-system suggesting a flavonol with 3', 4'-disubstituted ring B was confirmed by the three aromatic signals at 6.89 (1H, d, J = 8.52 Hz, H-5'), 7.86 (1H, dd, J = 2.16 and 8.48 Hz, H-6') and 7.63 (1H, d, J = 2.16 Hz, H-2'). The ^{13}C NMR spectrum exhibited 21 carbon signals. The linkage of sugar moiety to quercetin was supported by HMBC correlation between H-1'' (5.17) and C-3 at δ_C 134.36. The anomeric proton at δ_H 5.17 (d) had a coupling constant of 7.76 Hz confirming the *β*-orientation of sugar moiety.

From the HMBC data, correlations (Figure 4.9.5) between H-6 (6.23) to C5, C8, C10; H-8 (6.42) to C4, C6, C7, C9, and C10; H-2' (7.86) to C1', C3' and C4'; H-5' (6.89) to C3', C4', and C6'; H-6' (7.59) to C2, C2' and C4' were observed. The results presented here were compared with the literature data from Zhang et al, 2014, compound C9 was proposed to be quercetin 3-*O*- *β*-glucoside.

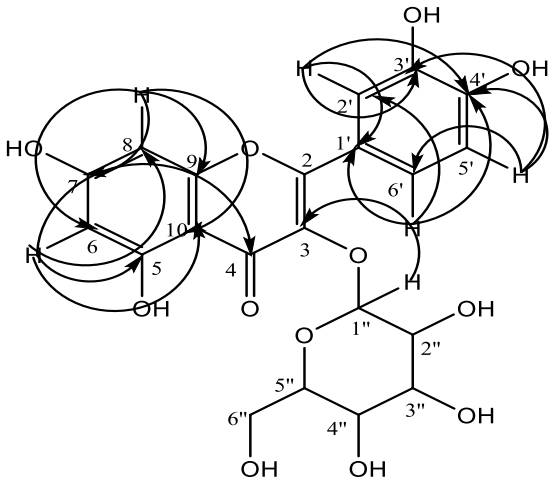


Figure 5. 15 : Key HMBC correlations of quercetin 3-*O*-β-glucoside (C9).

Quercetin-3-O-*β*-glucoside showed high antioxidant activity that makes it potent for defense against oxidative stress and free radical scavenging activities in plants (Razavi et al., 2009). Quercetin and its glycosilated derivatives has been reportedly shown to have a capacity to inhibit several key enzymes e.g. phospholipase A_2 and C, tyrosin protein kinases, lipoxygenase, cyclooxygenase, cyclic nucleotide phosphodiesterase and cytochrome P_{450} systems (Razavi et al., 2009). Quercetin3-*O*-*β*-glucoside has been reported to exhibit antihistamine, anti-inflammatory, and anticarcinogenic properties and may help to lower symptoms such as fatigue, depression, anxiety, coronary heart disease and cancer (Wolffram et al., 2002, Yamamoto et al., 1999).

Table 4. 7: ^{1}H (400 MHz: m, J Hz) and ^{13}C (100 MHz) NMR spectral data of isolated compounds C9 (quercetin-3-O-*β*-glucoside) in CD_3OD

Compound C9			
Atom	**^{13}C**	**^{1}H**	**δ_{lit} (**Zhang et al.,2014)
2	157.05		156.79
3	134.36		133.99
4	178.14		177.97
5	161.61		161.71
6	98.53	6.23 (d, J = 2.04 Hz)	99.16
7	164.74		164.66
8	93.34	6.42 (d, J = 2.04 Hz)	93.98
9	157.40		156.79
10	104.22		104.39
1’	121.46		121.59
2’	116.39	7.86 (d, J = 2.16 Hz)	115.67
3’	144.42		145.30
4’	148.56		148.95
5’	114.69	6.89 (d, J = 8.52 Hz)	116.44
6’	121.53	7.62 (dd, J = 2.16/8.52 Hz)	122.46
1’’	103.99	5.17 (d, J = 7.76 Hz)	102.33
2’’	71.77	3.58 (1H, m)	71.70
3’’	73.68	3.84 (1H, m)	73.69
4’’	68.60	3.87 (1H, m)	68.41
5’’	75.77	3.49 (1H, m)	76.32
6’’	60.52	3.65 1H, (m)	60.62

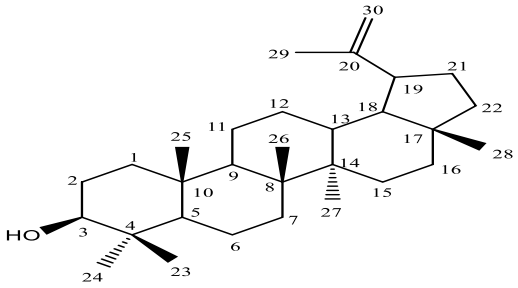


Figure 5. 16 : Lupeol (C11a)

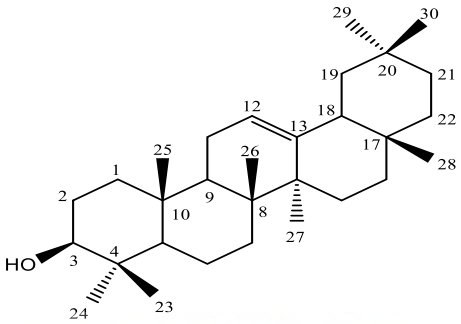


Figure 5. 17 : *β*-amyrin (C11b)

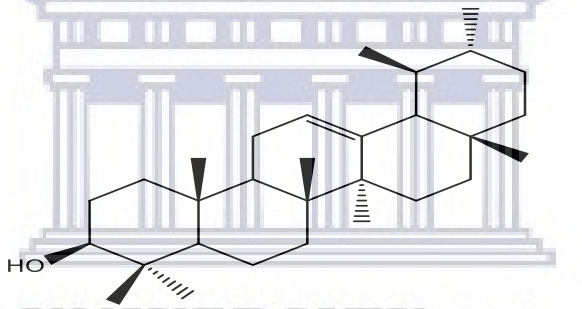


Figure 5. 18 : *α*-amyrin (C11c)

4.1.11 Characterization of an inseparable mixture of compound Lupeol (C11a), α-amyrin (C11c) and (β-Amyrin (C11b)

An inseparable mixture of three isomeric triterpenoids (C11a, C11b and C11c) was isolated from dichloromethane extract. It showed a pseudo-molecular ion peak at m/z 426.31 $[M+H]^+$ in the HRESI-MS spectrum which is in agreement with $C_{30}H_{50}O$. The sp2 carbons at δ_C 109.8 and δ_C 150.9 (Table 4.8) were an indication of a lupane type triterpene, pentacyclic terpenoids. The deshielded signal at δ_C 79.0 was attributed to C-3 with a hydroxyl group attached to it. The HSQC correlation of compound (**C11a)** showed 2 terminal olefinic protons at δ_H 4.56 and at δ_H 4.68 ppm attached on the same carbon resonance at 109.8 ppm that confirmed the terminal

methylene structure. In the HSQC spectrum, one methine carbon at δ_C 79.01 ppm coupled with a proton at 3.17 ppm (H-3). Compound (**C11a)** was established to be Lupeol.

In the ^{1}H NMR spectrum of the mixture eight methyl signals were observed. From the ^{13}C NMR spectrum of the mixture the following signals were observed for oxygenated carbon (δ_C 79.03), an olefinic and quaternary carbons at δ_C 121.5 (C-12) and at δ_C 145.8 (C-13) respectively. HSQC correlation indicated an olefinic proton at δ_H 5.18 (H-12) attached on δ_C 121.5 ppm and δ_H 3.22 attached at δ_C 79.03. In accordance with previously reported data (Dias *et al*., 2011). ^{1}H NMR and ^{13}C NMR of the mixture indicated the presence of *β*-Amyrin (**C11b**)**.**

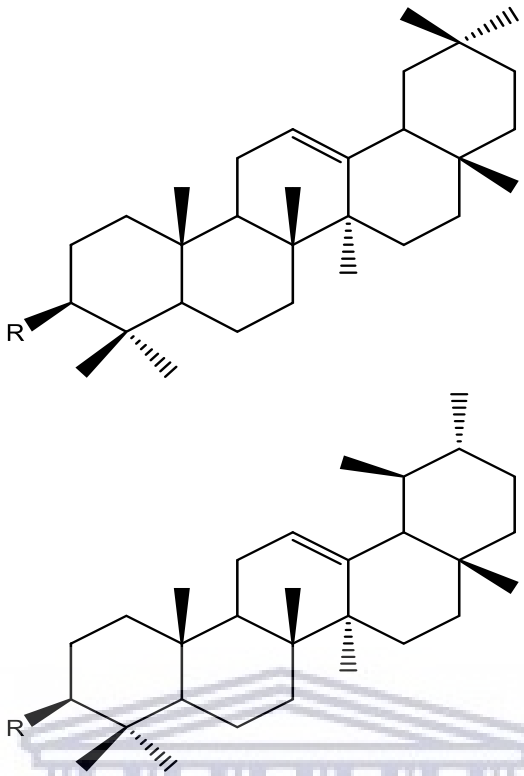
The ^{13}C NMR spectrum of the mixture showed a quaternary and methine carbon signals at δ_{C-13} 139.6, and δ_{C-12} 124.4 respectively. HSQC correlations indicated an olefinic proton 5.12 attached on δ_C 124.4 ppm, and proton signal δ_H 3.21 attached at δ_C 79.06 ppm C-3 and there was a carbon signal at δ_C 59.1 which corresponds to C-18 of α-amyrin moiety. The downfield shift of one of the methyl group at δ_H 1.32, compared with 3 hydroxyurs-12-ene (Galgon et al., 1999) assisted in assigning one of the methyl groups to C-19. After comparing the experimental data of the mixture **(C11c)** with the reported data from Vázquez *et al.,* 2012 we confirmed the presence of the α-amyrin in the mixture.

α and *β*-Amyrin have been previously reported to have anti-microbial, anti-inflammatory and analegisic properties (Vázquez *et al.*, 2012; Recio *et al*., 1995; Madeiros *et al*., 2007; Okoye *et al*., 2014; Otuki *et al*., 2005; Soldi *et al.*, 2008). *β*-amyrin exhibited antifungal activity against *A.rabiei* with an MIC value of 0.0156 mg/mL (Jabeen *et al.*, 2011). Lupeol has been reported to induce differentiation and inhibits the cell growth of mouse melanoma and human leukemia cells (Aratanechemuge et al., 2004; Hata et al., 2002). The morphological observations of leukemia nuclei and the gel electrophoresis analysis of DNA extract from leukemia cells treated

with Lupeol and its derivatives exhibited that Lupeol induces apopotosis in these cells (Wal et al., 2011).

Table 4. 8: ^{1}H NMR (400 MHz) and ^{13}C NMR (100MHz) data of the mixture-1 (C11a, C11b and C11c) in $CDCl_3$, δ in ppm, J in Hz

Position	α-amyrin (C11c)		β-amyrin (C11b)		Lupeol (C11a)	
	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C
1		38.8		38.7		38.6
2		28.7		27.2		27.5
3	3.22 (dd, J = 5.2; 10.8 Hz)	79.06	3.21 (dd, J= 4.8; 10.5 Hz)	79.03	3.17(d, J= 5.1 Hz)	79.0
4		38.7		38.5		39.8
5	0. 76	55.3	0.72	55.3	0.69	55.1
6		18.4		18.6		19.0
7		32.2		32.4		34.3
8		40.7		39.8		41.7
9		47.7		47.6		50.7
10		36.6		36.9		37.2
11		23.3		23.6		21.2
12	5.13 (t, J = 3.6 Hz)	124.4	5.19 (t, J= 3.5 Hz)	121.7		25.3
13		139.6		145.2		38.6
14		42.1		41.7		42.8
15		27.2		26.2		27.2
16		26.6		26.1		35.9
17		33.7		32.6		43.0
18	1.31	59.1	1.54	47.8	2.39	48.3
19		39.6	1.92	47.3		47.7
20		39.6		31.0		150.9
21		31.2		34.7		30.1
22	1.85	41.5		37.1		40.8
23	0.83	28.1	0.77	28.0	0.80	28.7
24	0.76	15.6	0.90	15.5	0.77	15.7
25	0.73	15.6	0.73	15.4	0.84	16.2
26	0.83	16.9	0.93	16.1	1.04	16.1
27	1.01	23.2	1.19	25.9	0.96	14.5
28	0.94	28.1	1.07	28.4	0.80	18.1
29	0.79	17.9	0.87	33.8	4.69 s 4.56 s	109.3
30	0.86	19.4	0.80	23.7	1.70	19.8



C12a & C12b R = Long chain fatty acid ester

Figure 5. 19 : α-amyrin and *β*-amyrin fatty acid ester

4.1.12 Characterization of inseparable mixture of α-amyrin fatty acid ester (C12a) and *β*-amyrin fatty acid ester (C12b)

The ^{1}H NMR spectrum of an inseparable mixture showed olefinic protons at 5.18 (t, J = 3.6 Hz) ppm attached to $\delta_{C\text{-}12}$ 121.6 and at 5.12 (t, J = 3.5 Hz) attached to $\delta_{C\text{-}12}$ 124.3. Further analysis of the mixture exhibited a broad signal at 1.25 ppm attributed to the hydrocarbon chain, a deshielded proton signal at $\delta_{H\text{-}3}$ 4.5 (1H, dd J = 6.2; 10.6 Hz), a proton signal at $\delta_{H\text{-}2'}$ 2.25 attached to C-2' methylene of the carbonyl group, and a triplet at $\delta_{H\text{-}n}$ (0.87 3H, t, J = 7.5 Hz) attributed to the terminal methyl group of the hydrocarbon.

The ^{13}C NMR spectrum of an inseparable mixture showed the methine ($\delta_{C\text{-}12}$ 121.6, 124.3) and quaternary ($\delta_{C\text{-}13}$ 145.2, 139.) carbons signals corresponding to that of *β*-amyrin and α-amyrin. Further analysis of the ^{13}C NMR spectrum indicated that the mixture is esterified with a fatty acid. This was substantiated by the presence of an added methyl signal (δ_C 14.13) attributed to

the terminal Sp^3 carbon (CH_3) of the hydrocarbon chain, many methylenes (δ_C 29.18-31.93) and acyl group (δ_C 173.69). An oxymethine resonated at a higher chemical shift (δ_C 80.58) relative to the chemical shift of *β*-amyrin and α-amyrin that normally resonates at 79.0 ppm. Comparing the data of the mixture with previously reported data **C12a** and **C12b** were proposed to be α-amyrin fatty acid ester and β-amyrin fatty acid ester respectively (Consolacio Y. Ragasa et al., 2013)**.** Due to an amount of the mixture we could not do transesterification to establish the length and the nature of the hydrocarbon chain of the natural ester.

Table 4. 9: ^{1}H NMR (400 MHz) and ^{13}C NMR (100MHz) data of the mixture-2 (C12a and C12b) in $CDCl_3$, δ in ppm, J in Hz

Position	C12a α-amyrin fatty acid ester		C12b β-amyrin fatty acid ester	
	^{13}C	^{1}H	^{13}C	^{1}H
1	38.4		38.2	
2	28.1		27.9	
3	80.6	4.50 (dd, J = 6.2; 10.6 Hz)	80.6	4.50 (dd, J = 6.2; 10.6 Hz)
4	37.8		37.1	
5	55.1	0.86	55.5	0.83
6	18.2		17.5	
7	32.5		32.4	
8	40.0		39.8	
9	47.6		47.5	
10	36.8		36.7	
11	23.3		23.5	
12	124.2	5.12 (t, J = 3.5 Hz)	121.6	5.18 (t, J = 3.6 Hz)
13	139.6		145.2	
14	42.0		41.7	
15	27.2		26.1	
16	27.1		26.6	
17	32.8		32.4	
18	59.1	1.29	47.5	
19	39.8		47.2	
20	39.7		31.0	
21	31.2		34.7	
22	41.2		36.7	
23	28.0	0.87	28.7	0.86
24	15.7	0.97	15.7	0.97
25	15.5	0.96	16.8	0.98
26	16.9	0.99	16.7	0.96
27	23.2	0.87	25.2	0.97
28	28.1	0.83	27.8	0.79
29	17.8	0.79	33.2	0.87
30	21.4	0.91	23.5	0.87
1’	173.6		173.6	
2’	34.8	2.29 (t, J = 7.5 Hz)	34.8	2.29 (t, J= 7.5 Hz)
3’$(CH_2)n$	29.1-31.9	1.25	29.1-31.9	1.25
(CH_3)	14.13	0.87(t, 7.5Hz)	14.13	0.87 (t, J = 7.5Hz

4.2 Physico-chemical properties of isolated compounds

4.2.1 Physico-chemical data of isolated compounds from S. glauca

4.2.1.1 Moronic acid (C1)

Molecular formula $C_{30}H_{46}O_3$ MW 454.32

Description: needle white crystals

Mass spectrum: ESI- MS m/z (ES+) 455.21 $[M+H]^+$

NMR spectrum: ^1H and ^{13}C see Table 4.4

4.2.1.2 21-β- hydroxylolean-12-en-3-one (C2)

Molecular formula $C_{30}H_{48}O_2$ MW 440.70

Description: white amorphous powder

Mass spectrum: ESI-MS m/z (ES+) 441.39 $[M+H]^+$

NMR spectrum: ^1H and ^{13}C see Table 4.4

4.2.1.3 Myricetin 3-O-β-galactopyranoside (C3)

Molecular formula: $C_{25}H_{26}O_{14}$ MW 573.12

Description: yellow solid material

Mass spectrum: ESI-MS m/z (ES+) 574.10 $[M+H]^+$

NMR spectrum: ^1H and ^{13}C see Table 4.5

4.2.1.4 Rutin (C4)

Molecular formula $C_{27}H_{30}O_{16}$ MW 610.15

Description: yellow solid material

Mass spectrum: ESI-MS m/z (ES+) 632.28 $[M+Na]^+$

NMR spectrum: ^1H and ^{13}C see Table 4.5

4.3 Physico-chemical data of isolated compounds from *S. lucida*

4.3.1.1 Quercetin (C6)

Molecular formula: $C_{15}H_{10}O_7$ MW 302.26

Description : yellow powder

Mass spectrum : ESI-MS m/z 303.2 $[M+H]^+$

NMR spectrum: ^1H and ^{13}C NMR see Table 4.4

4.3.1.2 Apigenin (C7)

Molecular formula: $C_{15}H_{10}O_5$ MW 270.05

Description : yellow powder

Mass spectrum : ESI-MS m/z (ES+) 269.9 $[M+H]^+$

NMR spectrum: ^1H and ^{13}C NMR see Table 4.5

4.3.1.3 Amentoflavone (C8)

Molecular formula: $C_{30}H_{18}O_{10}$ MW 538.46

Description: yellow powder

Mass spectrum: ESI-MS m/z (ES+) 538.12 $[M+H]^+$

NMR spectrum: ^1H and ^{13}C NMR see Table 4.6

4.4 Physico-chemical data of isolated compounds from *S. laevigata*

4.4.1 Quercetin-3-O-ß-glucoside (C9)

Molecular formula: $C_{21}H_{19}O_{12}$ MW 463.31

Description: yellow solid material

Mass spectrum: ESI-MS (ES+) m/z 486.37 $[M+Na^+]$

NMR spectrum: ^1H and ^{13}C NMR data see Table 4.7

4.4.1.2 Lupeol (C11a)

Molecular formula: $C_{30}H_{48}O$ MW 426.78

Description: white powder

Mass spectrum: ESI-MS (ES+) m/z 424.69 $[M+H]^+$

NMR spectrum: ^{1}H and ^{13}C NMR data see Table 4.8

4.4.1.3 β-Amyrin (C11b)

Molecular formula: $C_{30}H_{50}O$ MW 426.7

Description: white powder

Mass spectrum: ESI-MS (ES+) m/z 426.69 $[M+H]^+$

NMR spectrum: ^{1}H and ^{13}C NMR data see Table 4.8

4.4.1.4 α-amyrin (C11c)

Molecular formula: $C_{30}H_{50}O$ MW 426.7

Description: white powder

Mass spectrum: ESI-MS (ES+) m/z 426.69 $[M+H]^+$

NMR spectrum: H and C NMR data see Table 4.8

CHAPTER FOUR: BIOLOGICAL ASSAYS

4.5 Section B: biological evaluations

4.5.1 General experimental procedure

4.5.1.1 Reagents and solvents

All the reagents and solvents used are stated in section 3.7.1.

4.5.2 Antioxidant

All experimental procedure for the various antioxidant capacity assays (FRAP, ORAC, and TEAC), were followed as stated in chapter three section 3.8 to 3.10.2.

4.5.2.1 Evaluating ORAC activity of Rhus extracts of glauca, lucida and laevigata species

The oxygen radical absorbance (ORAC) assay has emerged as a robust method to measure the peroxyl radical absorbing capacity (with AAPH) of antioxidants and serum or other biological fluids. The ORAC assay is derived on free radical damage to a fluorescent probe, most commonly fluorescein, caused by an oxidizing reagent resulting in a loss of fluorescent intensity over time (Ou et al., 2001). The amount of the oxidant present in the sample test is correlated with the resultant damage. Conversely inhibition of oxidative damage to the fluorescent probe can be coordinated with the antioxidant capacity of a compound acting as a free radical scavenger (Brescia, 2012). The results were reported as the ORAC values which refers to the net protection area under the quenching curve of β-PE (fluorescein) in the presence of an antioxidant. As indicated from Table 4.10 to Table 4.12 the extracts from three *Rhus species* which were prepared by sequential extraction with hexane, dichloromethane, ethyl acetate, methanol and butanol and their ORAC values were measured. Calibration solutions of Trolox standard solutions were tested to establish a standard curve. Samples were analyzed in triplicate. The area under the curve (AUC) was determined for each extract by integrating the relative fluorescene curve. The regression equation between net AUC and Trolox concentration was established and ORAC values were reported as µM TE/g of plant extract using the standard curve determined previously.

The results presented in Table 4.10 showed that ethyl acetate and butanol extracts of *S. glauca* exhibited the high ORAC values of 4574.93 ± 109.12 and 5653.36 ± 328.66 µM TE/g respectively. The results in Table 5.4 showed that ethyl acetate, methanol and butanol exhibited the highest ORAC values of 4010.56 ± 73.52, 5793.45 ± 27.30, and 4198.42 ± 166.53 respectively. Hexane and dichloromethane extracts showed the lowest ORAC values of 1013.65 ± 12.26 and 1048.15 ± 35.35 respectively.

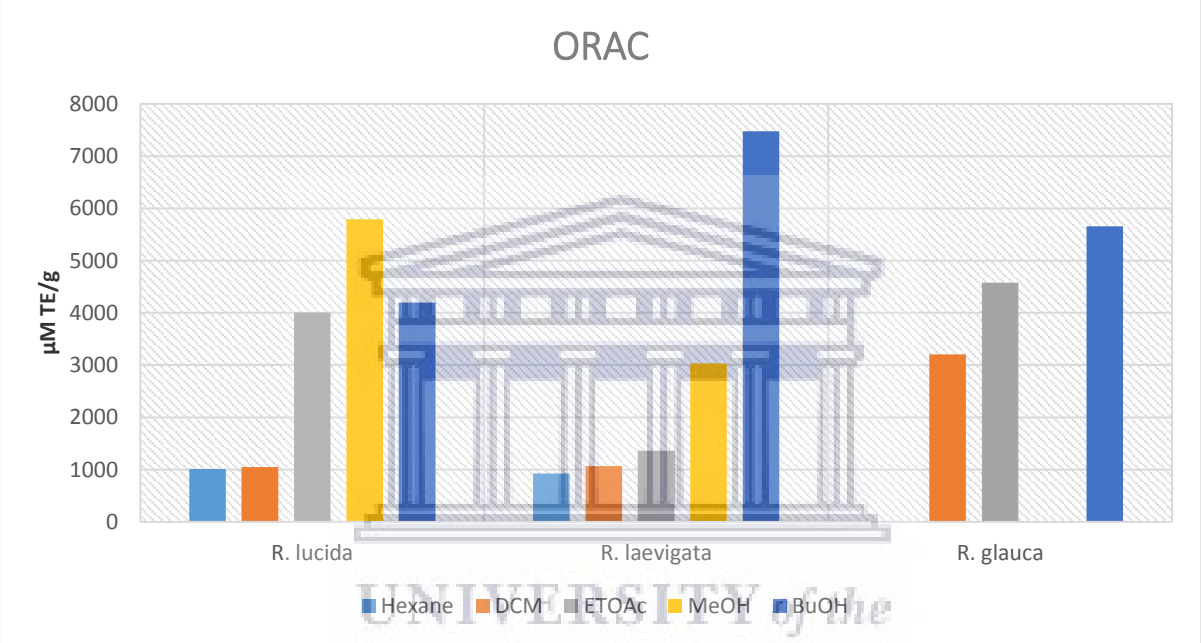


Figure 6. 1: Bar chart of ORAC values of extracts from *R. lucida, R. laevigata and R. glauca*

Table 4. 10: Oxygen radical absorbance capacity (ORAC) of *S. glauca* extracts

Sample	**µM TE/g**
dichloromethane	3207.09 ± 79.34
Ethyl acetate	4574.93 ± 109.12
Butanol	5653.36 ± 328.66

Data presented as mean ± SD (standard deviation)

Table 4. 11: ORAC activity of *S. lucida* extracts

Sample	**µM TE/g**
Hexane	1013.65 ± 12.26
Dichloromethane	1048.15 ± 35.35
Ethyl acetate	4010.56 ± 73.52
Methanol	5793.45 ± 27.30
Butanol	4198.42 ± 166.53

Data presented as mean ± SD (standard deviation)

Table 4. 12: ORAC activity of *S. laevigata* extracts

Sample	**µM TE/g**
Hexane	924.25 ± 17.77
Dichloromethane	1067.17 ± 38.95
Ethyl acetate	1363.86 ± 72.80
Methanol	3033.18 ± 222.16
Butanol	7475.11 ± 73.23

Data presented as mean ± SD (standard deviation)

4.5.2.2 Evaluating FRAP activity of the *S. glauca, S. lucida, and S. laevigata* extracts.

The FRAP assay uses an oxidation/reduction reaction to measure the ability of a sample to reduce Fe^{3+} to Fe^{2+}. The method is derived from the reduction of a ferric tripyridyltriazine (Fe^{3+}-TPTZ) colourless complex to Fe^{2+} ferrous form, which has an intense blue colour formed by the action of electron donating antioxidant at low pH. The reaction is monitored by measuring the change in absorption at 593nm. The change in absorbance, therefore, is directly related to the reducing power of the electron donating antioxidants present in the reaction mixture. An antioxidant donates electrons as a reductant in an oxidation/reductions, so it is assumed that the FRAP assay is a method for evaluating antioxidant capacity. However, it does not directly determine the antioxidant capacity of a potential antioxidant. The FRAP values were generated by comparing the absorbance change in the test mixture with those obtained from increasing concentrations of Fe^{3+} and expressed as µM AAE/g of the sample.

The FRAP activity of the extracts from *S. glauca, S. lucida and S. laevigata* is presented in Table 4.13. The results presented in Table 4.13 exhibited that methanol extract, ethyl acetate and butanol extracts of *S. lucida* exhibited high FRAP values of 1038.39 ± 80.41 µM AAE/g, 137.24 ± 16.54 µM AAE/g and 680.01 ± 96.34 µM AAE/g and while hexane and dichloromethane extracts revealed low FRAP values of 12.99 ± 1.82 µM AAE/g and 40.69 ± 2.11 µM AAE/g. The FRAP values obtained from methanol, ethyl acetate and butanol extracts of *S. laevigata* showed significant activity i.e; 411.58 ± 79.73, 86.28 ± 9.16 and 977.88 ± 71.24 respectively. It was observed that butanol, ethyl acetate and dichloromethane extracts from *S. glauca* showed high FRAP values i.e; 1008.67 ± 41.65 µM AAE/g, 557.09 ± 39.41 µM AAE/g and 431.62 ± 39.34 µM AAE/g respectively. The good antioxidant activity from these results can be attributed to more phenols and flavonoids normally found in these extracts. The low FRAP values showed from hexane extracts from *R. lucida* and *R. laevigata* can be due to the absence of phenols and flavonoids.

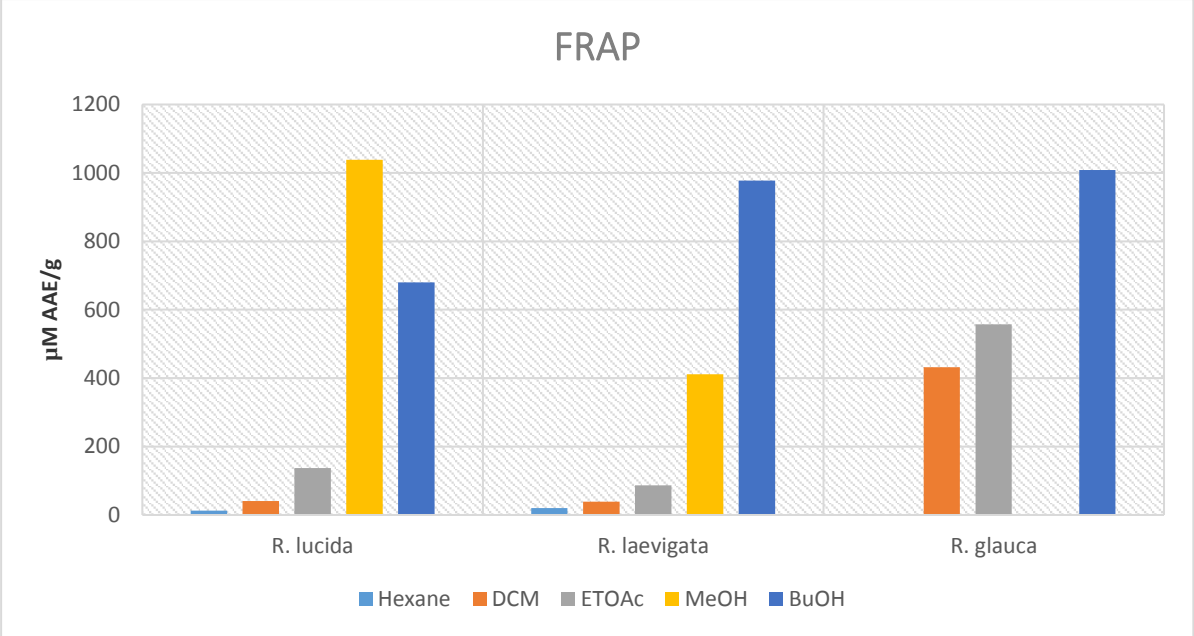


Figure 6. 2 : Bar chart of FRAP values of extracts from *S. lucida, S. laevigata and S. glauca*

Table 4. 13: Ferric ion reducing (FRAP) and trolox equivalent antioxidant capacities (TEAC) of *S. lucida, S. Laevigata and S. glauca extracts*

Sample	FRAP (µM AAE/g)	TEAC (µM TE/g)
	S. lucida	
Hexane	12.99 ± 1.82	20.26 ± 4.42
Dichloromethane	40.69 ± 2.11	52.20 ± 2.82
Ethyl acetate	137.24 ± 16.54	543.24 ± 9.34
Methanol	1038.39 ± 80.41	1512.08 ± 0.06
Butanol	680.01 ± 96.34	1075.2 ± 114.89
	S. laevigata	
Hexane	20.52 ± 4.27	0.00
Dichloromethane	39.34 ± 6.94	35.77 ± 4.01
Ethyl acetate	86.28 ± 9.16	150.50 ± 12.68
Methanol	411.58 ± 79.73	715.66 ± 6.76
Butanol	977.88 ± 71.24	1104.67 ± 24.61
	S. glauca	
Dichloromethane	431.62 ± 39.34	900.44 ± 49.14
Ethyl acetate	557.09 ± 39.41	1095.42 ± 28.42
Butanol	1008.67 ± 41.65	1438.63 ± 13.53

Data presented as mean ± SD (standard deviation)

4.5.2.3 Evaluating ABTS/ TEAC activity of the *S.glauca, S. lucida, and S. laevigata* extracts.

The antioxidant power of the extracts was examined by using ABTS radical decolorisation assay, which measures the relative antioxidant ability to scavenge the radical $ABTS^+$ (2.2'-azinobis (3-ethylbenzothiazoline-6-sulfonic acid)), a blue-green chromophore with a maximum absorption at 734 nm that decreases in its intensity in the presence of antioxidant. Antioxidant can neutralize the radical cation $ABTS^+$, generated from ABTS, by either direct reduction via electron donation, and the balance of these two mechanism is determined by antioxidant

structure and pH of the medium (Prior et al., 2005). The TEAC values of various extracts of *S. lucida*, *S. laevigata* and *S. glauca* are shown in Table 4.13. Methanolic extract of *S. lucida* exhibited the highest TAEC value of 1512.08 ± 0.06 (µM TE/g) and butanol extract of *S. glauca* indicated the highest TEAC activity of 1438.63 ± 13.53 (µM TE/g). Ethyl acetate of *S. glauca* showed the highest TEAC values of 1095.42 ± 28.42 (µM TE/g). Hexane and dichloromethane extracts of *S. lucida* showed the low TEAC values 20.26 ± 4.42 and 52.20 ± 2.82 (µM TE/g) respectively. Generally, extracts with high content of phenolics exhibited high radical scavenging and antioxidant activity (Lu and Foo, 2001; Miliauskas et al., 2004; Murthy et al., 2002; Madhavi et al., 1996).

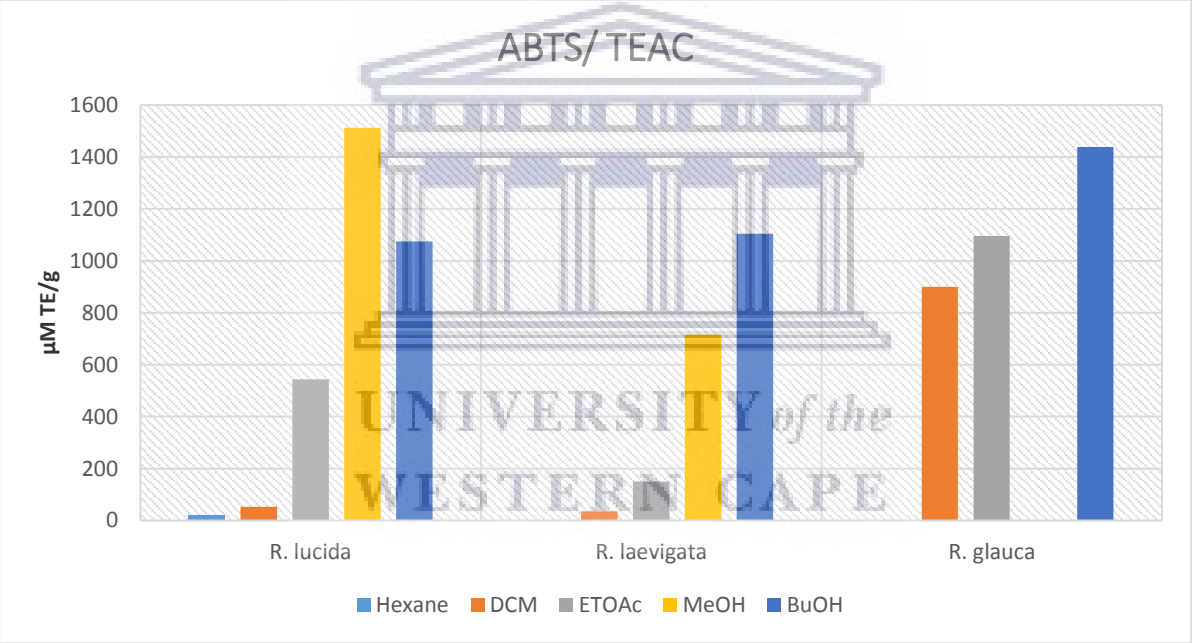


Figure 6. 3 : Bar chart of ABTS/TEAC values of extracts from *S. lucida*, *S. laevigata* and *S. glauca*.

4.5.3 Toxicity

4.5.3.1 Brine Shrimp lethality test

Brine shrimp assay is considered as an important tool for preliminary screening of toxicity and it has been used for the detection of plant extracts toxicity (McLaughlin et al., 1998), toxicity of heavy metals (Martinez et al., 1999), pesticides (Barahona and Sanchez-Fortun, 1999) and toxicity testing of dental materials (Pelka et al., 2000), toxicity of nanoparticles (Maurer-Jones et al., 2013), as well as screening of marine natural products (Carballo et al., 2002).The brine shrimp lethality assay was performed in all extracts of *S. glauca*, *S. lucida* and *S. laevigata*. The brine shrimp lethality assay of hexane, dichloromethane, EtOAc, and BuOH extracts was conducted at 10, 100 and 1000 µg/ml concentration. Triplicate of test tubes were each filled with ten nauplii at different concentrations of plant extracts (10, 100, and 1000 µg/ml). Control was prepared by adding 10 nauplii in three test tubes with simulated sea water, and no mortality rate was observed. The number of surviving nauplii counted after 24 hours were used to calculate percentage mortality. The results shown in Table 4.14 indicated that ethyl acetate and butanol extracts were regarded as active due to their high percentage mortality.

Table 4. 14: Number of dead nauplii after 24 hours

Sample	**Vials at 1000 µg/ml**			**Vials at 100 µg/ml**			**Vials at 10 µg/ml**			**Control**
	1	**2**	**3**	**1**	**2**	**3**	**1**	**2**	**3**	
							R. glauca			
Hexane	1	1	1	1	1	1	0	0	0	0
DCM	2	2	2	1	1	1	0	0	1	0
EtOAc	5	4	6	1	2	2	1	1	0	0
Butanol	7	7	8	2	3	1	1	1	1	0
							R. lucida			
Hexane	1	1	0	1	1	0	1	0	1	0
DCM	2	2	1	1	1	1	1	1	1	0
EtOAc	6	5	5	2	3	2	1	1	1	0
Butanol	7	6	8	2	3	3	1	1	2	0
							R. laevigata			
Hexane	1	2	1	1	1	1	0	0	0	0
DCM	2	2	1	2	1	1	1	0	0	0
EtOAc	5	6	6	1	2	2	0	1	1	0
Butanol	7	8	7	3	3	2	1	2	2	0

Table 4. 15: Brine shrimp lethality assay for three extracts (*S. glauca, S, lucida and S. laevigata)* percentage mortality after 24 hours.

Sample	**1000 µg/ml**	**100 µg/ml**	**10 µg/ml**	**LC50**	**Remarks**
	S. glauca				
Hexane	10	10	0	>1000	Inactive
DCM	20	10	3.3	>1000	Inactive
EtOAc	50	13.3	6.6	996.74	Active
Butanol	73.3	20	10	621.24	Active
S. lucida					
Hexane	6.7	3.3	3.3	>1000	Inactive
DCM	16.7	10	10	>1000	Inactive
EtOAc	53.3	23.3	10	905.22	Active
Butanol	70.0	26.7	13.3	676.68	Active
S. laevigata					
Hexane	13.3	10	0	>1000	Inactive
DCM	16.7	13.3	3.3	>1000	Inactive
EtOAc	56.7	16.7	6.7	855.79	Active
Butanol	73.3	26.7	16.7	571.1	Active

Values are mean of three replicates

4.5.4 Enzyme inhibition

4.5.4.1 In vitro activity on carbohydrate digestive enzymes

The extracts of *Searsia* species were screened to determine their inhibition activity to enzymes relevant to the management of diabetes. A therapeutic approached used in managing non-insulin dependent hyperglycaemia is through inhibition of glucosidase and amylase that control the breakdown and absorption of glucose and its precursors in the small intestine (Deol et al., 2016). The extracts were tested at 2.0 mg/ml. The results for both enzymes are shown in Table 4.16. The extracts with 25-50 % inhibitory effect on the α-glucosidase (low potent), extracts with 50-75 % (moderate potency), 75 -100 % (high potency) (Ahmad et al., 2008). The enzyme inhibition from *S. glauca* extracts were between 41.2 to 50.08 % for α-amylase and between 55.76 to 85 % for α-glucosidase; *S. lucida* extracts were between 24.95 to 60.88 % for α-amylase and between 49.28 to 93.54 % for α-glucosidase; *S. laevigata* extracts showed an enzyme inhibition between 40.99 to 59.72 % for α-amylase and for α-glucosidase between 68.28 to 90.10 %.

Table 4. 16: Inhibitory activity (%) of *Searsia* extracts on some carbohydrate digestive enzymes

	α-amylase			**α- glucosidase**		
	S. glauca	***S. lucida***	***S. laevigata***	***S. glauca***	***S. lucida***	***S. laevigata***
Hexane	41.2 ± 1.23	24.95 ± 2.12	41.81 ± 1.04	59.13±1.02	91.85±1.30	73.92±2.30
DCM	50.08 ±1.06	33.47 ± 1.21	40.99 ± 2.12	85.22±2.07	49.28±1.19	68.28±1.12
EtOAc	39.85 ± 1.41	50.16 ± 1.71	52.31 ± 1.01	62.50±1.05	93.54±2.01	79.99±1.23
Butanol	47.71 ± 2.54	60.88±2.21	59.72 ± 2.14	55.76±1.45	67.74±1.27	90.10±2.06

Data expressed as mean ± SD.

The isolated compounds from the three plants were screened against α-glucosidase and α-amylase at 125.0 µg/ml to determine their inhibitory activity. The compounds that showed activity during screening were tested further to determine their IC_{50} values from various concentrations from (125.0; 62.5; 31.25; 15.63; 7.82; 3.91; 1.95 µg/ml). It was only compound **C1** and **C8** that showed activity at 125.0 µg/ml, and were tested against the two digestive enzymes to examine their IC_{50} values. Compound **C1** and **C8** showed more potent inhibition values for α-glucosidase and α-amylase (IC_{50} 10.57 ± 2.02; 20.08 ± 0.98) and (IC_{50} 5.57 ± 1.17; 19.84 ± 1.03) respectively. Compound **C1** showed high potency on α-glucosidase compared with the positive control (Quercetin) with an IC_{50} value of 105.41 ± 2.30 µg/ml and **C8** showed the highest potency with an IC_{50} value of 5.57 µg/ml.

Table 4. 17: IC_{50} values of tested compounds on α-glucosidase and α-amylase

	IC_{50} (µg/ml)	
Compound	**α-glucosidase**	**α-amylase**
Amentoflavone (C8)	5.57 ± 1.17	19.84 ± 1.03
Moronic acid (C1)	10.57 ± 2.02	20.08 ± 0.98
Quercetin	105.41 ± 2.30	-
Acarbose	-	10.25 ± 1.23

5. CONCLUSION

This thesis explores the phytochemistry of *S. glauca, S. lucida and S. laevigata* and the biological activities of extracts from the selected *Searsia* species. The phytochemistry of the aerial parts of these plants has not been investigated before.

After the preparation of the crude extracts using different solvents such as Hexane, DCM, Ethyl acetate, Butanol and Methanol, eleven (11) pure compounds were isolated.

The pure compounds were structural characterized and elucidated using chromatographic and spectroscopic techniques. The pure compounds isolated from *Searsia glauca* were identified as, Moronic acid (**C1**), 21-β-hydroxylolean-12-en-3-one (**C2**), β-amyrin (**C10**) and two flavonoids, Myricetin 3-O-β-galactopyranoside (**C3**), and Rutin (**C4**).

The phytochemical investigation of the extracts from the aerial parts of *Searsia lucida* led to the isolation of four known compounds; one triterpene, Moronic acid **(C5),** and three flavonoids quercetin (**C6**), Apigenin (**C7**), and Amentoflavone (**C8**).

The phytochemical investigation of *Searsia laevigata* led to the isolation of six known compounds namely, quercetin-3-O-*β*-glucoside (**C9**), and a mixture of Lupeol (**C11a**), β-amyrin (**C11b**) and α-amyrin (**C11c**) and a mixture of β-amyrin fatty acid ester (**C12b**) and α-amyrin fatty acid ester (**C12a**).

The crude extracts from the plants were screened for FRAP, TEAC and ORAC activities. *S. lucida* showed FRAP values for Hexane 12.99 ± 1.82, DCM 40.69 ± 2.11, EtOAc 137.24 ± 16.54, Butanol 680.01 ± 96.34, and MeOH 1038.39 ± 80.41; TEAC values were (Hexane 20.26 ± 4.42, DCM 52.20 ± 2.82, EtOAc 543.24 ± 9.34, Butanol 1075.2 ± 114.89, and MeOH 1512.08 ± 0.06); ORAC values were (Hexane 1013.65 ± 12.26, DCM 1048.15 ± 35.35, EtOAc 4010.56 ± 73.52, and Butanol 4198.42 ± 166.53, MeOH 5793.45 ± 27.30). *S. laevigata* showed FRA values (Hexane 20.52 ± 4.27 , DCM 39.34 ± 6.94 , EtOAc 86.28 ± 9.16 , Butanol 977.88 ±

71.24, and MeOH 411.58 ± 79.73; TEAC values were (Hexane 0.00 , DCM 35.77 ± 4.01 , EtOAc 150.50 ± 12.68 , Butanol 1104.67 ± 24.61, MeOH 715.66 ± 6.76); ORAC values were (Hexane 924.25 ± 17.77 , DCM 1067.17 ± 38.95 , EtOAc 1363.86 ± 72.80 , Butanol 7475.11 ± 73.23, MeOH 3033.18 ± 222.16); *S. glauca* : FRAP values (DCM 431.62 ± 39.34, EtOAc 557.09 ± 39.41, Butanol 1008.67 ± 41.65), TEAC values were (DCM 900.44 ± 49.14, EtOAc 1095.42 ± 28.42, Butanol 1438.63 ± 13.53); ORAC values were (DCM 3207.09 ± 79.34 , EtOAc 4574.93 ± 109.12, Butanol 5653.36 ± 328.66). Extracts shown from ethyl acetate, methanol and butanol extracts showed that these extracts have a large amount of phenolics. Some of the secondary metabolites isolated from these extracts are reported to exhibit high radical scavenging, antiviral activity and antioxidant activity. The antioxidant activity of the methanol, and butanol extracts of the aerial parts of these selected *Searsia* species showed that these plants have a potential to be used as sources of antioxidant agents due to the compounds isolated from these extracts. The isolated compounds and mixtures, each with their own biological activity could play a key role in the bioactivity of the extracts and the medicinal value of the plant extracts administered as traditional medicine.

The brine shrimp lethality assay results from extracts showed the following LC_{50} for all the selected plants; *S. glauca* (Hexane > 1000, DCM >1000, EtOAc 996.74, Butanol 621.24); *S. lucida* (Hexane >1000, DCM >1000, EtOAc 905.22, Butanol 676.68); *S. laevigata* (Hexane >1000, DCM >1000, EtOAc 855.79, Butanol 571.1). The extracts ethyl acetate and butanol extracts showed the highest percentage mortality while hexane and dichloromethane extracts exhibited less percentage mortality.

Enzyme inhibition of the extracts from these *Searsia species* plants exhibited strong inhibition activity of more than 90 % against α-glucosidase and some indicated more than 50 % against α-amylase. The enzyme inhibition from *S. glauca* extracts were between 41.2 to 50.08 % for α-amylase and between 55.76 to 85 % for α-glucosidase; *S. lucida* extracts were between 24.95

to 60.88 % for α-amylase and between 49.28 to 93.54 % for α-glucosidase; *S. laevigata* extracts showed an enzyme inhibition between 40.99 to 59.72 % for α-amylase and for α-glucosidase between 68.28 to 90.10 %. Selected compounds isolated from these plants showed good IC-50. The enzyme inhibitory effects indicated by the plants extracts and compounds isolated afford these plants an opportunity to be used as agents for antidiabetic management.

This research has contributed to the knowledge relative to the photochemistry and biological activities of the *Searsia* species.

6. REFERENCES

Abd El-Salam I. Mohammed. (2015) Phytoconstituents and the study of antioxidant, antimalarial and antimicrobial activities of *Rhus tripartita* growing in Egypt. *Journal of Pharmacognosy and Phytochemistry*. 4(2): pp 276-281.

Ademiluyi AO, Oboh G. (2013). Soybean phenolic-rich extracts inhibit key-enzymes linked to type 2 diabetes (a-amylase and a-glucosidase) and hypertension (angiotensin I converting enzyme) in-vitro. Exp. Toxicol. Pathology. 65: pp 305 -309.

Ahmad Gholamhoseinian, Hossein Fallan, Fariba Sharifi-far, Mansour Mirtajaddini. (2008). The inhibitory effects of some Iranian plants extracts on the alpha glucosidase. *Iranian Journal of Basic Medical Science:* 11(1), pp 1-9.

Ahmed MS, Galal AM, Ross SA, Ferreira D, Elsohly MA, Ibrahim AS, Mossa JS, El- Ahn YJ, Lee CO, Kweo JH, ahn JW, Park JH. (1998). Growth-inhibitory effects of Galla Rhois-derived tannins on intestinal bacteria. Journal of applied Microbiology. 84: pp 439-443.

Ahn, J.H. Liu, Q. Lee, C. Ahn, M. Yoo, H. Hwang, B.Y. Lee, M.K. (2012). A new pancreatic lipase inhibitor from *Broussonetia kanzinoki*. *Bioorganic and Medicinal Chemistry Letters* 22, pp 2760-2763.

Al Sayed E., Martiskainen, O., Sinkkonen, J, Pihiaja K, Ayoub, N., Singab, A.E., et al. (2010). Chemical composition and bioactivity of *Pleiogynium timorense* (*Anacardiaceae*). *Natural Product Communications*. 5: pp 545-550.

Albach, D.C, Grager R.J, Kite G.C, and Jensen S.R. (2005). Veronica: Acylated flavone glycosides as chemosystematic markers. *Biochemical Systematics and Ecology*. 33: pp 1167-1177.

Alberti K.G.M.M. and Zimmet P.Z. (1998). Definnition, diagonosis, and classification of diabetes mellitus and its complications. Part 1: diagonosis and classification of diabetes mellitus. Provisional report of a WHO consultation. *Diabetes Med*. 15: pp 539-553.

Anwer T, Sharma M, Khan G, Iqbal M, Ali M S, Alam M S, Safhi M M and Gupta N. (2013). *Rhus coriaria* ameliorates insulin resistance in non-insulin-dependent diabetes mellitus (NIDDM) rats. *Acta Pol. Pharm - Drug Research*, **70**: pp 861-867.

Aratanechemuge Y, Hibasami H, Sanpin K, Katsuzaki H, Imai K, Komiya T. (2004). Inductionof apopotosis by Lupeol isolated from mokumen (*Gossampinus malabaria L. Merr*.) in human promyelotic leukemia HL-60 cells. *Oncology. Rep*. 11: pp 289-292.

Asgarpanah J, and Saati S, (2014). An overview on phytochemical and pharmacological properties of Rhus coriaria L., *Research Journal of Pharmacognosy*, 1(3), pp 47-54.

Babak Baharvand-Ahmadi, Mahmoud Rafiean-Kopaei, Mohammad M, Zarshenas and Mahmoud Bahmani, (2015). Contrasting actions of various antioxidants on hyperlipidemia: A review and new concepts, *Der Pharmacia lettre*, 7(12), pp 81-88.

Barahona M.V and S. Sanchez-Fortun. (1999). Toxicity of carbamates to the brine shrimp Artemia salina and the effect of atropine, BW28c51, iso-OMPA and 2-PAM on carbaryl toxicity. *Environmental. Pollution*. 104: pp 469-476.

Basnet, P., Kadota, S., Shimizu, M., Namba, T. (1994). Bellifolin: A potent hypoglycemic agent in streptozotocin (STZ)-induced diabetic rats from Swertia japonica. *Planta Med*. 60: pp 507- 511.

Benzie, I.F.F and Strain, J.J. (1996). The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": The FRAP Assay. *Analytical Biochemistry*, 238, pp. 70-76.

Bhakta Prasad Gaire and Lalita Subedi. (2011). Medicinal Plant Diversity and their Pharmacological Aspects of Nepal Himalayas. *Pharmacognosy Journal*. 3(25): pp 6-17.

Birari Rahul. B & Bhutani Kamlesh K, (2007), Pancreatic lipase inhibitors from natural sources: unexplored potential, *Drug Discovery Today*, 12(19/20), pp 879-889.

Bothalia. (1994). Names of the South Africa species of *Rhus* (*Anacardiaceae*) and their etymology. 24(1): pp 67-76.

Brahmachari, H. D., Augusti, K. T. (1964). Isolation of orally effective hypoglycemic compounds from Ficus bengalensis. *Industrial Journal of Physiological*. *Pharmacal*. 8: pp 60 – 64.

Brescia Peter J., (2012). Determination of antioxidant potential using an oxygen radical absorbance capacity (ORAC) assay with Synergy H4. Application Note. *BioTek Instruments*, Inc. Winooski, VT.

Cao, G., and Prior, R. (1999). Measurement of Oxygen Radical Absorbance Capacity in Biological samples. Oxidants and Antioxidants. *Methods Enzymology*, 299: pp 50-62.

Cao, G, and Prior, R.L. (1998). Comparison of analytical methods for assessing total antioxidant capacity of human serum. *Clinical Chemistry*, 44: pp 1309-1315.

Cao Shugeng, Rebecca Clare Guza, James S. Miller, Rabodo Andriantsiferana, Vincent E. Rasamison, and David G. I. Kingson. (2004). Cytotoxic triterpenoids from Acridocarpus vivy from the Madagascar rain Forest. *Journal of Natural Products*. 67(6): pp 986-989.

Carballo, J.L., Hernandez-Inda, Z.L., Perez, P., Garcia-Gravalos, M.D., (2002)., A comparison between two brine shrimp assays to detect in vitro cytotoxicity in marine natural products. *BMC Biotechnology*. 2, 17.

Chang, C. Zhang, L. Chen, R. Wu, C. Huang, H. Roy, M.C. Huang, J. Wu, Y. Kuo, Y. (2010). Quiquelignan A-H, eight new lignoids from the rattam palm Calmus quiquesetinervious and their antiradical, anti-inflammatory and antiplatelet aggregation activities. *Bioorganic & Medicinal Chemistry* 18, pp 518-525.

Chakravarthy, B. K., Cupta, S., Gambhir, S. S. and Gode, K. D. (1981). Pancreatic Beta-cell regeneration in rats by (-)-Epicatechin. *Lancet.* .pp 759 - 273.

Charkrabarati Ranjan, and Rajagopalan Ramanujam. (2002). Diabetes and insulin resistance associated disorders: Disease and the therapy. *Current Science*. 83(12): pp 1533-1538.

Chen JC, HO TY, Chang YS, Wu SL, Li CC, Hsiang CY. (2009). Identification of Escherichia coli enterotoxin inhibitors from traditional medicinal herbs by in silico, in vitro, and in vivo analyses. *Journal of Ethnopharmacology*.121: pp 372-378.

Chen, X., & Bergmeier, S. (2011). Compositions of glucose transport inhibitors as antitumor agents. PCT Int. Appl., WO 2011119866 A1 20110929.

Chitemerere, T. and Mukankanyama, S. (2011). *In vitro* antibacterial activity of selected medicinal plants from Zimbabwe. *The African Journal of Plant Science and Biotechnology* 5(1): pp 1-7.

Coman Cristina, Olivia Dumitrita Rugina, Carmen Socaciu, (2012), Plants and natural compounds with antidiabetic action, *Not Bot Horti Agrobo*, 40(1),pp 314-325.

Consolacio Y. Ragasa, Kimberly B. Cornelio. (2013). Triterpenes from *euphorbia hirta* and their cytotoxicity. *Chinese Journal of natural medicines*.11 (5): pp 0528-0533.

Coterill, P., Scheinmannan, E, Stenhouse, I. **(1976).** Kolaflavanone a new biflavanone from *Garcinia kola.* J. *chem. Soc. Perkin Trans* 6: pp 532 - 539.

Cutillo F., Abrosca B. D, Greca M. D., Fiaorentino A., & Zarelli A,J. (2003). Agricultural Food chemistry. 51, pp 6165 - 6172.

Cristina Quispe, Ezequiel Viveros-Valdez, Jose A. Yarleque, Marco R, Juan C, Paniagua, Guillermo Schmeda-Hirschmann, (2013), High speed centrifugal countercurrent chromatography (HSCC) isolation and identification by LC-MS analysis of the polar phenolics from Vasconcellea qurecifolia. *Journal Chil. Chemistry. Society*, (58):3

Cuellar, A., Estevez, P. (1980). A phytochemical study of Cuban plants. *Rev. Cubana Farm.* 14: pp 63 - 68.

Cushnie TP, Lamb AJ. (2005). Antimicrobial activity of flavonoids. *International Journal of Antimicrobial Agents.* 26: pp 343–356.

Da-Ren Qiu, D-Cheng Wang, Sheng-Xiang Yang, Ya-Mei Zhang, Dong-Sheng Wei, Ming-Zhe Zhang, Ji-Zhu Sun, Jie Cong, Jie Guo, Shu-Liang He, Jia-Chun Qin, (2016), Chemical constituents from the fruits of *Rhus typhina L* and their chemotaxonomic significance. *Biochemical systematic and Ecology* 69. pp 261-265.

Daniyal Abdali, Sue E. Samson, Ashok Kumar Grover. (2015). How effective are antioxidant supplements in obesity and diabetes. 24: pp 201-215.

David M. Nathan, John B. Buse, Mayer B. Davison, Ele Ferrannin, Rury R. Holman, Robert Sherwin, Bernard Zinman, (2009). Medical management of hyperglycemia in type 2 diabetes: A consensus algorithm for the initiation and adjustment of therapy. *Diabetes Care*, 32(1): pp 193-203.

Deol Permal, Hewawasam Erandi, karakoulaskis Aris, Claudie J. David, Nelson Robert, Simpson S. Bradley, Smith M. Nicholas, And Semple J. Susan. (2016). *In vitro* inhibitory activities of selected Australian medicinal plant extracts against protein glycation, angiotensin converting enzyme (ACE) and digestive enzymes linked to type II diabetes**.** *BMC Complementary and alternative Medicine*. 16(435): pp 1-11.

Dhaliya Salam, A, Surya A.S, Dawn V Tomy, Dr. Betty Carla, Dr. Arun Kumar, Dr. C. Sunil, (2013), A riview of hypelipidemia and medicinal plants, *Hetero group of Journal*,2(4), pp 219-237.

Dillon S.A, Lowe G.M, Billington D, and Rahman K. (2002). Dietary supplementation with aged garlic extract reduces plasma and urine concentrations of 8-iso-prostaglandin F (2 alpha) in smoking men and women. *The Journal of Nutrition*, 132(2): pp 168-171.

Ding Y, Nguyen H.T, Choi E.M, Bae K, and Kim Y.H. (2009). *Rhusonoside A*, a new megastigmane glucoside from *Rhus sylvestris*, increases the function of esteoblastic MC3T3-E1 Cells. Planta Medica. 75: pp 158-162.

Eisenbrand G., Pool-Zobel B., Baker V., Balls M., Baauboer B.J, Boobis A., Carere A., Kevekordes S., Lhuguenot J.-C., Pieters R., Keiner J., (2002). Methods of *in vitro* toxicology. *Food and chemical toxicology* 40: pp 193-236.

Erichsen-Brown, C (1989). Medicinal and Other Uses of North American Plants: A Historical Survey with Special Reference to the Eastern Indian Tribes. New York, NY, USA: Dover Publications.

Fatma Hadruch, Zouhaier Boullagui, Han Junkyu, Hiroko Isoda, and Sami Sayadi. (2015). The α-glucosidase and α-amylase enzyme inhibitory of hydroxtryrosol and oleuropein .*Journal of Oleu Science* 64. (8), pp 835-843.

Feraly FS. (2001). A weakly antimalarial biflavanone from Rhus retinorrhoea. Phytochemistry 58: pp 599-602.

Fotsis, T.; Pepper, M.S.; Aktas, E.; Breit, S.; Rasku, S.; Adlercreutz, H.; Wahala, K.; Montesano, R.; Schweigerer, L. (1997). Flavonoids, dietary-derived inhibitors of cell proliferation and *in vitro* angiogenesis. *Cancer Res*. 57: pp 2916-2921.

Fiamegos, Y.C. Kastritis, P.L. Exarchou, V. Han, H. Bonvin, A.M.J.J. et al. (2011). Antimicrobial and efflux pump inhibitory activity of caffeoylquinic acids from *Artemisia absinthium* against Gram-positive pathogenic bacteria. *PLoS ONE* 6(4): e18127 doi:10.1371/journal.pone.0018127.

Floris Alexander van de Laar, (2008), Alpha-glucosidase inhibitors in the early treatment of type 2 diabetes, *Vascular Health and Risk Management*. 4(6), pp 1189-1195.

Fukumoto J, Okado S (1973). Naringinase production by fermentation. Japanese Patent 7306554.

Ganji Renuka rani, Singara Charya Ma, M. Viswanadham, Murali Krishna Thupurani, (2017), Antidiabetic activity of the compounds isolated from *Rhus Mysorensis* plant extract. *Journal of Biotechnology and Biochemistry*. 3(3): pp 37-42.

Galgon, T, Hoke, D, Drager, B. (1999). Identification and quantification of betulinic acid. *Phytochemical analysis*. 10(4): pp 184-194**.**

Gelfand M, Drummond RB, Mavi S, Ndemera B: The traditional medical practitioner in Zimbabwe: his principles of practice and pharmacopoeia. 1985, Gweru: Mambo Press.

Gonzalo J. Mena-Rejon, Aida R. Perez-Espadas, Rosa E. Moo-Puc, Roberto Cedillo-Rivera, I.L. Bazzocchi, I.A. Jimenez-Diaz, and Leovigildo Quijano, (2007), Antigiardial activity of triterpenoids from root bark of *Hippocratea excelsa*. *Journal of Natural Products*, 70: pp 863-865.

Giancarlo S, Rosa LM, Nadjafi F, Francesco M, (2006), Hyperglycemic activity of two species extracts: *Rhus coriaria L* and *Bunium persicum boiss*, Natural product Research, 20(9), pp 882-886.

Gundidza M, Gweru N, Mmbengwa V, Ramalivhana N.J, Magwa Z. and Samie A. (2008). Phytoconstituents and biological activities of essential Oil from *Rhus lancea* L. F. *African Journal of Biotechnology*.7 (16) pp 2787-2789.

Gurevich-Panigrahi, T. Panigrahi, S. Wiechec, E. Los, M. (2009). Obesity: pathophysiology and clinical management. *Current Medicinal Chemistry* 16, pp 506-521.

Gutierrez, R.M.P. (2013). Evaluation of the hypoglycemic and hypolipidemic effects of

triterpenoids from *Prosthechea michuacana* in STZ-induced type 2 diabetes in mice. *Pharmacologia*, DOI: 10.5567/pharmacologia. pp 170-179.

Guvenalp, Z., Omur, D. L. (2005). Flavonol Glycosides from *Asperula arvensis* L. *Turk. Journal Chem.* 29: pp 163-169.

Guvenalp, Z., Nurcan, K., Kazaz, C., Yusuf, K., Omur, D. L. (2006). Chemical Constituents of *Galium tortumense*. *Turk. Journal of Chemistry* .30: pp 515-523.

Hata K, Hori K, Takahash S. (2002). Differentiation and apopotosis-inducing activities by pentacyclic triterpenes on a mouse melanoma cell line. *Journal of Natural Products* 65: pp 645-648.

Han-Seok Choi, Hye Sook Seo, Soon Re Kim, Youn Kyung Choi, Bo-Hyoung Jang, Yong-Cheol Shin And Seong-Gyu Ko. (2014). Anti-inflammatory and anti-proliferative effects of *Rhus verniciflua* Stokes in RAW264.7 cells. Molecular Medicine Reports. 9: pp 311-315.

Haixu Zhou, Jiali Ren, Zhonghai Li. (2017). Antibacterial activity and mechanism of pinoresinol from Cinnamomum Camphora leaves against food-related bacteria. *Food Control*. 79: pp 192-199.

Harborne, J. B. and Baxter, H. (1993). Phytochemical dictionary a handbook of bioactive compounds from plants. ed. Burgess Science Press Basingstoke. London 12 - 567.

Henry M. Mwangi, Wilfred T. Mabusela, Berhanu M. Abegaz and Onani O. Martin. (2012). Antimicrobial activities of a novel biflavonoid and other constituents from *Rhus natalensis*, Journal *of Medicinal Plants Research*, 7(10): pp 619-623.

Hill, R. S., Oberwetter, J. M., Boyd, A. E. (1987). Increase in cAMP levels in b-cell line potentiates insulin secretion without altering cytosolic free-calcium concentration. *Diabetes* 36: pp 440 - 446.

Hii, C S. *T*., Howell, S. L. (1984). Effects of (-)-Epicatechin on rat islets of Langerhans. *Diabetes*, 33: pp 291- 296.

Hii, C S. T. and Howell, S. L. (1985). Effects of flavonoids on insulin secretion and $^{45}Ca^{2+}$ handling in rat islets of Langerhans. *Journal of Endocrinol.* 107: pp 1- 8.

Ibrahim M. Abu-Reidah, Rana M. Jamous and Mohammed S. Ali-Shtayeh, (2014), Phytochemistry, Pharmacological Properties and Industrial Applications of *Rhus coriaria L*. Jordan *Journal of Biological Science*. 7(4): pp 233-244.

Ivorra, M. D., Paya, M., Villar, A. (1988). Hypoglycemic and insuline release effects of tormentic acid: a new hypoglycemic natural product. *Planta Med*. pp 282 - 286.

Iwu, M., Okunji, CO., Akah, P. T, Corley, D. (1990). Dioscoretine the hypoglycemic principle of *Dioscarea dumetorum. Planta Med.* 56: 119 -120.

Ji Hye Kim, Yong Cheol Shin, and Seong-Gyu Ko, (2014). Integrating Traditional Medicine into Modern Inflammatory Diseases Care: Multitargeting by *Rhus verniciflua Stokes. Mediators of inflammation*. pp 1-17.

Johari J, Kianmehr A, Mustafa MR, Abubakar S, Zandi K. (2012). Antiviral activity of baicalein and quercetin against the Japanese encephalitis virus. *International Journal Molecular Science*. 13:16785–16795.

Junk Ito, Fang-Rong Chang, Hui-Kang Wang, Yong Kun Park, Masaharu Ikegaki, Nicole Kilgore, and Kuo-Hsiung Lee, (2001). Anti-Aids agents.48. Anti-HIV activity of Moronic Acid Derivatives and the New Melliferone-Related Triterpenoid Isolated from Brazilian Propolis. *Journal of Natural Products*.64: pp 1278- 1281.

Kamalakkannan N & Prince P S, (2006). Antihyperglycemic and antioxidant effect of rutin, a polyphenolic flavonoid, in streptozotocin-induced diabetic Wistar rats, *Basic Clinical Pharmacological Toxicology*. (98), pp 97.

Kelly, T. Yang, W. Chen, C. Reynolds, K. He, J. (2008). Global burden of obesity in 2005 and projections for 2030. *International Journal of Obesity* 32, pp 1431-37.

Kemp, D. M. (2012). Type 2 Diabetes: Disease Overview. In "New Therapeutic Strategies for Type 2 Diabetes: Small Molecule Approaches", *The Royal Society of Chemistry*, pp 1-14.

Khosa, R. L., Pandey, V. B. and Singh, J. P. (1983). Experimental studies on *Zizypbus rugosa* (lam) bark. *Indian Drugs 20:* pp 241- 243, 1983.

Kosar M, Bozan, T. F & Baser K. (2006). antioxidant *(Rhus- Corairai L)* extract, Food Chem. 103, pp 956-959.

Kotebagilu Namratha Pai, Vanitha Reddy Palvai, and Asna Urooj, (2014), Protective Effect of Selected Medicinal Plants against Hydrogen Peroxide Induced Oxidative Damage on Biological Substrates, *International Journal of Medicinal Chemistry*, pp 1-7.

Kolb, H., Kiesel, H., Greulich, B., Vander Bosh,]. (1982). Lack of antidiabetic effect of (-) Epicatechin. *Lancet* 1: pp 1303-1304.

Krishnaiah D, R. Sarbatly R, and Nithyanandam R, (2011). A review of the antioxidant potential of medicinal plant species, *Food and Bioproducts Processing*, 89(3), pp 217–233.

Kumar Sunil, Smita Narwal, Vipin Kumar, and Om Prakash, (2011) α-glucosidase inhibitors from plants: A natural approach to treat diabetes, Jan-Jun, 5(9), pp 19-29.

Kumar B, Gupta SK, Nag TC, Srivastava S, Saxena R. **(2012).** Green tea prevents hyperglycemia induced retinal oxidative stress and inflammation in streptozotocin-induced diabetic rats. *Ophthalmic Res*. 47:103-108.

Lamson DW, Brignall MS. (2000). Antioxidants and cancer, part 3: Quercetin. Alternative Med Rev. 5: pp 196–208.

Larry K. Golightly, Caitlin C. Drayna and Michael T. McDermott, (2012), Comparative clinical pharmacokinetics of dipeptidyl peptidase-4 inhibitors. *Clinical Pharmacokinetics*. 51(8): pp 501-514.

Lee SH, Nan JX, Zhao YZ, Woo SW, Park EJ, Kang TH, Seo GS, Kim YC, Sohn DH. (2003). The chalcone butein from *Rhus verniciflua* shows antifibrogenic activity. *Plana Med*. 69: pp 990-994.

LEE, J.C., (2004). "Extract from *Rhus verniciflua* Stokes Is Capable of Inhibiting the Growth of Human Lymphoma Cells," *Food and Chemical Toxicology*, 42(9): pp. 1383 -1388.

Ley, J., Kindel, G., Krammer, G., Widder, S., Pickenhagen, W., Rotzoll, N., et al. (2006). Use of Malic acid glucosides as flavoring substances. PCT Int. Appl., WO 2006003107 A1 20060112.

Lin, C.C., Chuang, Y.J., Yu, C.C., Yang, J.S., Lu, C.C., Chiang, J.H., Lin, J.P., Tang, N.Y., Huang, A.C., Chung, J.G, (2012). Apigenin induces apoptosis through mitochondrial dysfunction in U-2 OS human osteosarcoma cells and inhibits osteosarcoma xenograft tumor growth *in vivo*. *Journal of Agricultural Food Chemistry 60*:11395-11402.

Liu, J., Chen, L., Cai, S., et al 2012. Semisynthesis of apigenin and acacetin-7-O-β-D-glycosides from naringin and their cytotoxic activities. *Carbohydrates. Res*., 357: pp 41- 46.

Liu, Q. Ahn, J.H. Kim, S.B. Hwang, B.Y. Lee, M.K. (2012). New phenolic compounds with anti-adipogenic activity from the aerial parts of *Pulsatilla koreana*. *Planta Medica* 78, pp 1783-1786.

Little, T. Horwitiz, M. Feinle-Bisset, C. (2007). Modulation by high-fat diets of gastrointestinal function and hormone associated with the regulation of energy intake:

implications for the pathophysiology of obesity. *American Journal of Clinical Nutrition* 86, pp 531-541.

Lotlikar, M. M. and Rajarama, R. (1996). Pharmacology of a hypoglycaemic principle isolated from the fruits of *Momordica charantia Linn. Indian Journal of Pharmacy*. 28: pp 129 - 133.

Lovell M.A, Markesbery W.R, (2007). Oxidative damage in mild cognitive impairment and early Alzheimer's disease. *J. Neurosci. Res*. 85(14): pp 3036-3040.

Lu, Y., Foo, Y.L. (2001). Antioxidant activities of polyphenols from sage (*Salvia officinalis*). *Food Chemistry* 75, pp 197–202.

Lunesa C. Pinzon, Mylene M. Uy, Kung Hong Sze, Mingfu Wang and Ivan Keung Chu. (2011). Isolation and characterization of antimicrobial, anti-inflammatory and chemopreventive flavones from *Premna odorata* Blanco. *Journal of medicinal Plants Research.* 5(13). pp 2729-235.

Lucia Marseglia, Sara Manti, Gabriella D' Angelo, Antonio Nicoreta, Eleonora Parisi, Gabriella Di Rosa, Eloisa Gitto and Teresa Arrigo. (2015). Oxidative stress in obesity: A critical component in human diseases. *International. Journal of Molecular Science*. 16: pp 378-400. doi: 10.3390/ijms16010378.

Shugeng Cao, Rebeca Clare Guza, James S. Miller, Rabodo Andriantsiferana, Vincent E. Rasamison, and David G.I Kingston, (2004), Cytotoxic triterpenoids from *Acridocarpus vivy* from the Madagascar rain forest. *Journal of Natural Products*. 67: pp 986-989.

Madeiros R, Otuki MF, Avellar MC, Calixto JB. (2007). Mechanisms underlying the inhibitory actions of the pentacyclic triterpene-amyrin in the mouse skin inflammation induced by phorbol ester 12-O-tetradecanoylphorbol-13-cetate. *Eur. Journal of Pharmacology*. 55 (9): pp 227-235.

Madhavi, D.L., Singhal, R.S., Kulkarni, P.R. (1996). Technological aspects of food antioxidants, in food antioxidants: Madhavi, D.L., Deshpande, S.S., Salunkhe, D.K. (Eds.). Technological, Toxicological, and Health Perspectives. Marcel, Dekker, New York, pp 159-265.

Masahiko Kurokawa, Purusotam Basnet, Mizue Ohsugi, Toyoham Hozumi, Shigetoshi Kadota, Tsuneo Namba, Takashi Kawana, and Kimiyasu Shiraki. (1999).Anti-Herpes Simplex Virus Activity of Moronic Acid Purified from *Rhus javanica* In Vitro and In Vivo. *Journal of Pharmacology and Experimental Therapeutics*. 289 (1) 72-78.

Marcus V. Bahia, Juceni P. David, Jorge M. David, (2010). Occurrence of biflavones in leaves of Caesalpinia pyramidalis specimens. 33(6), pp 1297- 1300.

Maroyi A. (2011). Ethnobotanical study of medicinal plants used by people in Nhema communal area, Zimbabwe. J Ethnopharmacol.136: pp 347-354.

Masahiko Kurowaka, Purusotam Basnet, Mizue Ohsugi, Toyoharu Hozumi, Shigetoshi Kadota, Tuneo Namba, Takashi Kawana, and Kimiyasu Shiraki, (1998). Anti-Herpes Simplex Virus activity of moronic acid purified from Rhus javanica In vitro and In vivo. *The Journal of Pharmacology and Experimental Therapeutics.* 289(1): pp 72-78.

Manuel Jesus Chan-Baacab, Elfride Balanza, Eric Deharo, Victoria Munoz, Rafael Duran Garcia, Luis Manuel Pena-Rodriguez. (2003), Variation of leishmanicidal activity in four populations of Urechites andrieuxii. *Journal of Ethnopharmacology* 86: pp 243-247

Mamun-or-Rashid ANM, MD Shamim Hossain, Naim Hassan, Biplab Kumar Dash, MD.Ashrafuzzaman Sapon, Monokesh Kumer Sen, (2014). A review on medicinal plants with antidiabetic activity, *Journal of Pharmacognosy and Phytochemistry*, 3(4), pp 149-159.

Maragesi M S., Pieters L., Ngasapa D., Apers S., Vingerboets R., Cos P., Berghed A V. & Vlietick, A. J. (2008). Screening of some Tanzanian medicinal plants from Bunda distract for antibacterial, antifungal and antiviral activities, *Journal Ethnopharm*.119: pp 58-66.

Marrelli, M.; Loizzo, M.R.; Nicoletti, M.; Menichini, F.; Conforti, F. **(2013)**, Inhibition of key enzymes linked to obesity by preparations from Mediterranean dietary plants: Effects on α-amylase and pancreatic lipase activities. *Plant Foods Hum. Nutr*. 68, pp 340–346.

Martinez, M. Ramo J.D, Torreblanca A., Diaz-Mayans, (1999). Effect of cadmium exposure on zinc levels in the brine shrimp *Atermia parthenogenetica*. Aquaculture, 172: pp 315-325. **Mates JM, Perez-Gomez C, and Nunez de CastroI**. (1999), Antioxidant enzymes and human diseases. *Clinical Biochemistry*. 32, pp 595-603.

Maurer-Jones, M.A., Love, S.A., Meierhofer, S., Marquis, B.J., Liu, Z., Haynes, C.L., (2013). Toxicity of nanoparticles to brine shrimp: An introduction to nanotoxicity and interdisciplinary science. *Journal of Chemistry Education*. 90: pp 475-478.

Maritim A.C, Sanders R.A, Watkins J.B, (2003). Diabetes, oxidative stress, and antioxidants: a review, *J. Biochem. Mol. Toxicol.* **17(1): pp 24-28.**

Matsuda H., (1966). Constituents of the leaves of Rhus and some species of related genera in japan. *Chemical & Pharmaceutical Bulletin*. 14: pp 877-882.

McLaughlin, J.L., Rogers L.L and Anderson J.E. (1998). The use of biological assays to evaluate botanicals. *Drug inform. Journal*, 32: ppm 513-524.

Mclaughlin J.L, (1991), Crown-Gall Tumours in Potato Discs and Brine Shrimp Lethality: Two Simple Bioassays for higher plant screening and fractionation. In: Methods in plant Biochemistry: Assays for Bioactivity, Hostettmann, K. (Ed) Academic Press, London, ISBN: 0-124610161, pp 1-31.

Meyer B.N., Ferrigni, N.R., Putnam, J.E., Jacobsen, L.B., Nichols, D.E., and McLaughlin, J.L., (1982). Brine shrimp: a convenient general bioassay for active plant constituents. *Planta Medica,* 45: 31-34.

Mohamed Gamal. A, Sabrih R.M. Ibrahim, Ehab S. Elkhayat, Riham Salah El Dine, (2014), Natural anti-obesity Agents. *Bulletin of Faculty of Pharmacy* 52, pp 269-284.

Mohammed Reza Morshedloo, Filippo Maggi, Hossein Tavakoli Neko, Morteza Soleinmani Aghdam. (2018). Sumac (Rhus coriaria L.) Fruit: Essential oil variability in Iranian populations. *Industrial Crop & Products* 111: pp 1 – 7.

Morihiro Matsuda, lichiro Shimomura, (2013). Increased oxidative stress in obesity: implications for metabolic syndrome, diabetes, hypertension, dyslipidemia, atherosclerosis, and cancer. *Obesity Research & Clinical Practice*. 7 e330-e341.

Montine T.J, Neely M.D, Quinn J.F. (2002). Lipid peroxidation in aging brain and Alzheimer's disease. *Free Radical Biol. Med*. 33(5): pp 620-626.

Mikael E. Pedersen, Roger A. Baldwin, Jerome Miquet, Gary I. Stafford, Johannes van staden, Claude G. Wasterlain, and Anna K. Jager. (2010). Anticonvulsant effects of *Searsia dentata* (Anacardiaceae) leaf extract in rats. *Phytotherapy Research*. 24: pp 924-927.

Mohammadi S, Kouhsari Montasser S and Feshani Monavar A. 2010. Antidiabetic properties of the ethanolic extract of *Rhus coriaria* fruits in rats. *DARU, Journal of Pharmaceutical Science.,* 18: 270-275.

Monavari H R, Hamkar R, Norooz-Babaei Z, Adibi L, Noroozi M, Ziaei A. (2007). Antiviral effect assay of twenty five species of various medicinal plants families in Iran, 1(2): pp 49-59.

Mohamed Ali A. Alwahsh, Melati Khairuddean and Wong Keng Chong, (2015), Chemical constituents and antioxidant activity of *Teucrium barbeyanum* Aschers. Rec. Nat. Prod. 9: pp 159-163.

Miean, K.H.; Mohamed, S. (2001). Flavonoid (myricetin, quercetin, kaempferol, luteolin, and apigenin) content of edible tropical plants. *J. Agric. Food Chem.*49: pp 3106–3112.

Miliauskas, G., Venskutonis, P.R., Van Beek, T.A. (2004). Screening of radical scavenging activity of some medicinal and aromatic plant extracts. *Food Chemistry* 85, pp 231–237.

Mukherjee, B. and Mukherjee, S. K. (1987). Blood sugar lowering activity of *Swertia chirata* extract. *International* Journal of *Crude drugs Res.* 25: pp 97-102.

Murthy, K.N.C., Singh, R.P., Jayaprakasha, G.K. (2002). Antioxidant activity of grape (*Vitis vinifera*) pomace extracts. *Journal of Agricultural and Food Chemistry* 50, pp 5909 - 5914.

Naquvi Kamran J, Javed Ahamad Showkat R. Mir, Mohd. Ali, Mohd. Shuaib, (2011). Review on role of natural Alpha-Glucosidase inhibitors for management of diabetes mellitus. *International Journal of Biomedical Research*. (6), pp 374-380.

Nishumura H., Nonaka G, and Nishioka I. (1984). Tannis and related compounds. Two new ellagitannis containing a proto-quercitol core from Quercus stenophylla Makino. *Chemical & Pharmaceutical Bulletin*, 32: pp 1750-1753.

Niture NT, Ansari AA, and Naik SR. (2014). Anti-hyperglycemic activity of rutin in streptozotocin-induced diabetic rats: an effect mediated through cytokines, antioxidants and lipid biomarkers. *India Journal of Experimental Biology*. (52): pp 720-727.

Nobrega, R., Almeida, J., Barbosa, J. M., Ramnath, S. N. (1985). Chemistry and pharmacology of an ethanol extract of Bumelia sartorum. *Journal of Ethnopharmacology* 14: pp 173 -185.

Okoye NN, Ajaghaku DL, Okeke HN, ILodigwe EE, Nworu CS, Okoye FBC. (2014). beta-Amyrin and Alpha-amyrin acetate isolated from the stem of *Alstonia boonei* display profound anti-inflammatory activity. *Pharmacological Biol*ogy. 52(11): pp 1478-1486.

Olubukula S. Olorunnisla, Adewale Adetutu, Abiodum O. Owoade, Babatunde T. Adesina, Peter Adegbola, (2017), Toxicity evaluation and protective effects of *Rhus longipes* Engl. leaf extract in paracetamol induced oxidative stress in wister rats. *The Journal of Phytopharmacology:* 6 (2): pp 73-77.

Otuki C, Ferreira J, Lima F, Meyre-Silva C, Malheiros A, Muller L, Cani G, Santos A, Odilon Djakpo and Weirong Yao. (2010). *Rhus Chinensis* and *Galla Chinensis-Folklore* to modern evidence: A review. *Phytochemistry Research* 24: pp 1739-1747.

Ogzewalla, P.O., Wayne, C. D., Schell, EM. (1974). Isolation and characterization of a hypoglycemic agent from *Xanthium strumarium. Journal of Pharmaceutical Science*. 63: pp 1166 -1167.**Oshima R, Kumanotani J, (1984),** Structural studies of plant gum from sap of the lac tree, *Rhus vernicifera.* Carb Res. 127: pp 43-57.

Ordovas JM, Shen J., (2008) Gene-environment interactions and susceptibility to metabolic syndrome and other chronic diseases. *Journal of Periodontology*: 799, pp 1508-13.

Ortiz-Andrade, R. R, García-Jiménez, S. Castillo-España, P. Ramírez-Avilla, G.Villalobos-Milona, R. Estrada-Soto, S. (2007). Alpha glucosidase inhibitory activity of the methanolic extract from *Tournefortia hartwegiana*: an anti-hyperglycemic agent. *Journal of Ethnopharmacology* 109(1): 48-53.

Ou, B., Hamsch-Woodill, and Prior, R. (2001). Development and validation of an improved oxygen radical absorbance capacity assay using Flourescein as the Fluorescent probe. *Journal of Agric. Food chemistry* 49: pp 4619-4626.

Patel, D. K., Kumar, R., Laloo, D., and Hemalatha, S. (2012). Natural medicines from plant source used for therapy of diabetes mellitus: An overview of its pharmacological aspects. *Asian Pacific Journal of Tropical Disease* 2, 239-250.

Patwardhan, B., & Gautam, M. (2005). Botanical immunodrugs: Scope and opportunities. *Drug Discovery Today*, 10(7), pp 495-502.

Park KY, Jung GO, Lee KT, Choi J, Choi MY, Kim GT, Jung HJ, Park HJ, (2004), Antimutagenic of *Rhus verniciflua*. *Journal of Ethnopharmacology*. 90: pp 73-79.

Parasuraman. (2016). Overviews of Biological Importance of Quercetin: A Bioactive Flavonoid. *Pharmacognosy Reviews*. 10(20): pp 84- 89.

Pen-Ho Yeh, Yun-Dar Shieh, Li-Chun Hsu, Li-Ming Yang Kuo, Jhih-Hu Lin, Chia-Ching Liaw and Yao-Haur Kuo. (2012). Naturally occurring cytotoxic [3'-8'']-Biflavonoids from *Podocarpus nakaii*. *Journal of Traditional and Complementary Medicine*. 2 (3): pp. 220-226.

Park, C.H. Chung, B.Y. Lee, S.S. Bai, H.W. Cho, J.Y. Jo, C. Kim, T.H. (2013). Radiolytic transformation of rotenone with potential anti-adipogenic activity. *Bioorganic and Medicinal Chemistry Letters* 23: pp 1099-1103.

Pelka, M., Danzl, C., Distler, W., Petschelt, A. (2000). A new screening test for toxicity testing of dental materials. *Journal of Dentistry* 28, pp 341–345.

Pellegrini, N., Re, R., Yang, M., Rice-Evans, C.A. (1999). Screening of dietary carotenoid-rich fruit extracts for antioxidant activities applying ABTS radical cation decolorisation assay. *Methods in Enzymology*, 299: 379-389.

Pengsuparp T, Cai LN, Fong HHS *et al*., (1994). Pentacyclic triterpenes derived from Maprouena Africana are potent inhibitors of HIV-1 reverse transcriptase. *Journal of Natural Products* 57: pp 415-522

Perihan Gürbüz, Lütfiye Ömür Demirezer, Zühal Güvenalp Ayse Kuruüzüm-Uz and Cavit Kazaz. (2015). Isolation and Structure Elucidation of Uncommon Secondary metabolites from *Cistus salviifolius* L. *Rec. Natural Products*. 9(2) pp 175-183.

Perez G. R.M, M.A Zavala S, S Perez G, C. Perez G. (1998). Antidiabetic effect of compounds isolated from plants. *Phytomedicine*. 5(1): pp 55-57.

Perestrelo R, Lu Y, Santos S.A. O., Silvestre A. J.D., Neto C.P., Camara J.S. et al., (2012). Phenolic profile of Sercial and Tinta Negra *Vitis Vinifera L*. grape skins by HPLC-DAD-ESI-MS: Novel phenolic compounds in *Vitis Vinifera L*. grape. *Food Chemistry*. 135: pp 94-104.

Persoone G, Sorgeloos P, Roels O, Jaspers E, Editors. The brine shrimp *Artemia*. Proceedings of the international symposium on the brine shrimp *Artemia Salina:* 1979 August 20-23; Texas, USA. Belgium: Universal Press: 1980.

Prior R.L, Hoang H, Gu L, Wu X, Hamsch-Woodill M, Huang D, OU B, Jacob B, (2003). Assays for hydrophilic and lipophilic antioxidant capacity (Oxygen radical absorbance capacity (ORAC (RL)) of plasma and other biological and food samples. *Journal of Agricultural food chemistry*. 21:51(11) pp 3273-3279.

Prior R.L., Wu X., and Schaich K., (2005). Standardized methods for the determination of antioxidant capacity and phenolics in foods and dietary supplements. *Journal of Agricultural Food Chemistry*. 53: pp 4290-4302.

Poujade C, Evers M *et al*., (1996). Beulinic acid derivatives: A new class of specific inhibitors of human immune-deficiency virus type 1 entry. *Journal of medicinal chemistry* 39: pp 1069-1083.

Pujol, 1990. NaturAfrica: The Herbalists handbook. Jean Pujol natural Healers Foundation, Durban.

Raman, A., and Lau, C., (1996). Anti-diabetic properties and phytochemistry of *Momordica charantia L. (*Cucurbitaceae*). Phytomedicine* 2: pp 349 - 362.

Ramos FA, Takaishi Y, Shirotori M, Kawaguchi Y, Tsuchiya K, Shibata H, *et al*. (2006). Antibacterial and antioxidant activities of quercetin oxidation products from yellow onion (*Allium cepa*) skin. *Journal of Agricultural Food Chemistry*. 54: 3551–3557.

Razavi SM, ZAhri S, Zarrini G, Nazemiyeh, and Mohammadi S. (2009). Biological activity of quercetin-3-O-glucoside, a known plant flavonoid. *Russian Journal of Bioorganic Chemistry*. 35(3): pp 376-378.

Ramirez-Espinosa JJ, Poali, Rios MY, Lopez-Martinez S, Lopez-Vallejo F, Medina-Franco JL, Poali P, et al. (2011). Antidiabetic activity of some pentacyclic acid triterpenoids, role of PTP-1B: in vitro, in silico, and in vivo approaches. *Europian Medicinal Chemistry* (46), pp 2243-4451.

Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., Rice-Evans, C. (1999). Antioxidant activity applying an improved ABTS radical cation assay. *Free radical Biology and Medicine*, 26: pp 1231-1237.

Recio MC, Giner RM, Manez S, Rios JL. (1995). Structural requirements for the anti-inflammatory activity of natural triterpenoids. *Planta Med*. 61(2): pp 181-185.

Regazzoni L, Arlandini E, Garzon D, Santagati N.A, Beratta G, Maffei F. R. (2013). A rapid profiling of gallotannins and flavonoids of aqueous extract of *Rhus coriaria L*. by flow injection analysis with high resolution mass spectroscopy assisted with a database searching. *Journal of Pharmaceutical & Biomedical analysis*. 72: pp 202-207

Reza-Ul-Jalil, J., Hasan, C. M. (1986). Hypoglycemic activities of the glycosides of Momordica cochinchinensis. *Journal of Bangladesh Academic Science*. 10: pp 25 – 30.

Rhee MH, Park HJ, Cho JY. Salicornia. (2009). herbaceae: botanical, chemical and pharmacological review of halophyte marsh plant. *J Med Plants Res.* 3(8): pp 548-555.

Rios MY, Salinas D, Villarreal ML. (2000). Cytotoxic Activity of moronic acid and identification of the new triterpene 3.4-seco-Olean-18-ene-3.28-dioic acid from *Phoradendron reichenbachianum***.** *Planta med*. 67: pp 443-446.

Risa, J., Risa, A, Adsersen, A, Gauguin, B., Stafford, G.I., Van Starden, J., Jager, A.K. (2004), Screening of plants used in Southern Africa for epilepsy and convulsions in the GABAA-benzodiazepine receptors assay. *Journal of Ethnopharmacology* 93, pp 177-182.

Roberts, D.L. Dive, C. Renehan, A.G. (2010). Biological mechanisms linking obesity and cancer risk: new perspective. *Annual Reviews of Medicine* 61, pp 301-316.

Roberts, D.L. Dive, C. Renehan, A.G. (2010). Biological mechanisms linking obesity and cancer risk: new perspective. *Annual Reviews of Medicine* 61, pp 301-316.

Rui-Rui Wang, Qiong Gu, Yun-Hua Wang, Xue-Mei Zhang, Liu-Meng Yang, Jun Zhou, Ji-Jun Chen, Yong-Tang Zhen. (2008). Anti-HIV-1 activities of compounds isolated from the medicinal plant *Rhus chinensis. Journal of Ethnopharmacology*. 117. pp 249–256

R.W. Owen R. Haubner W. Mier A. Giacosa W.E. Hull B. Spiegelhalder H. Bartsch. (2003). Isolation, structure elucidation and antioxidant potential of the major phenolic and flavonoid compounds in brined olive drupes. *Food and Chemical Toxicology* 41: pp 703–717.

Sabina Shrestha, Dae-Young Lee, Ji-Hae Park, Jin-Gyeong Cho, Woo-Duck Seo, Hee Cheol Kang, Yong-Jin Jeon, Seung-Woo Yeon, Myun-Ho Bang, Nam-In Baek, (2012). Flavonoid Glycosides from the fruit of Rhus parviflora and Inhibition of cyclin dependent kinases by hyperin. *Journal of Korean Society*.55: pp 689-693.

Sahib Najla Gooda, Nazamid Saari, Amin Ismail, Alfi Khatib, Fawzi Mahomoodally, and Azizah Abdul Hamid, (2012), Plants' Metabolites as Potential Antiobesity Agents, *The Ethnopharmacology,* 2(3), pp 165-172.

Sampaio, E. M., Furtado, E A. S., Furtado, J.S., Cavalacante, M. N. M. and Riedel, O. O. (1979). Hypoglycemic producing activities of raw coffee beans (*Coffea arabica). Rev. Medicine Univ. Fed. Doceara* 19: pp 49 - 54.

Sancheti, S. Sancheti, S. Lee S Lee, J. Seo, S. (2011). Screening of Korean medicinal plant

extracts for α-glucosidase inhibitory activities. *Iranian Journal of Pharmaceutical Research* 10(2): pp 261-264.

Santos, F.A. Frota, J.T. Arruda, B.R. de Melo, T.S. da Silva, A.A. Brito, G.A. Chaves, M.H.Rao, V.S. (2012). Antihyperglycemic and hypolipidemic effects of α-amyrin, a triterpenoid mixture from *Protium heptaphyllum in mice*. *Lipids in Health and Disease*, 11(98), pp 1-8.

Saleh Ibrahim Alqsoumi, Omer Ahmed Basudan, Prawez Alam and Maged Saad Abdel-Kader, (2016). Antioxidant study of flavonoids derivatives from the aerial parts of Rhus natalensis growing in Saudi Arabia. Pak. *Journal of Pharmaceutical. Science*. 29 (1): pp 97-103.

Sebothoma Costar, (2009), Isolation and characterization of antibacterial compounds from *Rhus Leptodictya,* Univesity of Limpopo, South Africa.

Slanc, P. Doljak, B. Kreft, S. Lunder, M. Janeš, D. Štrukelj, B. (2009). Screening of selected food and medicinal plant extracts for pancreatic lipase inhibition. *Phytotherapy Research* 23, pp 874-877.

Saleem, M. Nazir, M. Ali, M.S. Hussain, H. Lee, Y.S. Raiz, N. Jabbar, A. (2010). Antimicrobial natural products: an update on future antibiotic drug candidates. *Natural Products Reports* 27: 238-254.

Shabana M M, El Sayed A M, Yousif M F, El Sayed A M and Sleem A A. (2011). Bioactive constituents from Harpephyllum caffrum Bernh. and *Rhus coriaria L*. *Pharmacognosy Magazine*, 7: pp 298-306.

Shalaby, M.A. and Hamouda, A.B. (2013). Antiobesity, antioxidant and antidiabetic activities of red Ginseng plant extract in obese diabetic rats. *Journal of Intercultural Ethnopharmacology,* 2(3), pp. 165-172.

Shaw, J.E. Sicree, R.A. Zimmet, P.Z. (2010). Global estimates of the prevalence of diabetes

for 2010 and 2030. *Diabetes Research Clinical Practice* 87: pp 4-14.

Shanmugasundaram, K. R., Panneerselv, c., Samudram, P., Shanmugasundaram E. R. B. (1983). Enzyme changes and glucose utilisation in diabetic rabbits: the effect of *Gymnema sylvestre RBr*. *Journal of Ethnopharmacology*. 7: pp 205 - 34.

Shai LJ, Magano SR, Lebelo SL, Mogale AM. (2011). Inhibitory effects of five medicinal plants on rat alpha-glucosidase: Comparison with their effects on yeast alpha-glucosidase. J Med Plant Res. 5: pp 2863–2867.

Sheehan, E. W. and Zemaitis M. A. (1983), A constituent of *Pterocarpus marsupium,* (-)- Epicatechin, as a potential antidiabetic agent. *Journal Natural Products.* 46: pp 232 – 234.

Shi Q, and Zuo C., (1992). Chemical compounds of the leaves of *Pistacia chinensis*, Bge. Zhongguo Zhongyao Zazhi 17: pp 422-423.

Shibata, N. and Kobayashi, M. (2008). The role for oxidative stress in neurodegenerative diseases. *Brain Nerve*, 60(2), pp 157-70.

Shigemasa, H. (1992). Partial purification of crude gymnemic acids by affinity chromatography and effects of purified fractions on the oral glucose tolerance test in rats. *Yonago Igaku Zasshi*. 43: pp 350 - 364

Srinivasan K, (2005), Partial protective effect of rutin on multiple low dose streptozotocin, *Indian Journal of Pharmacology*., (37), 327.

Sierra Rayne, G. Mazza, (2007), Biological activities of extracts from Sumac (*Rhus spp*): A review, *Plant Foods Human Nutrition*. 62, pp 165-175.

Sierra Rayne, 2011, Chemical profiles of essential oils and non-polar extractables Sumac **(***Rhus spp***).** Nature Precedings: doi:1038/npre.2011.5926.1: Posted in 26 April 2011.

Steinmann, D., & Ganzera, M. (2011). Recent advances on HPLC/MS in medicinal plant analysis. *Journal of Pharmaceutical and Biomedical Analysis*, 55(4), pp 744-757. **Soler F,**

Strobel P, Allard C, Perez-Acle T, Calderon R, Aldunate R, Leighton F. (2005). Myricetin, quercetin and catechin-gallate inhibit glucose uptake in isolated rat adipocytes. *Biochemistry Journal*. 386(Pt 3): pp 471–478.

Subhash C. Joshi and C.S Mathela, (2014). Essential oil composition of *Rhus cotinus* and its antioxidant activity. Journal of Natural Product Resource. 4(3): pp 39-43.

Sun Na-Nong, Tsung-Yen Wu, and Chi-Fai Chau. (2016), Natural Dietary and Herbal Products in Anti-Obesity Treatment, *Molecules* 2016, 21(1351), pp 1-15.

Svenningsen, A. B., Madsen, K. D., Liljefors, T.; Stafford, G. I., Staden, J. v., Jager, A. K. J. (2006). Biflavones from Rhus species with affinity for GABAA/benzodiazepine receptor. *Ethnopharmacology*. 103: 276-280.

Swathi H, T Shekshavali, IJ Kuppast, MC Ravi, Priyanka R, (2005), A Review on *Rhus Mysorensis, The Pharma Innovation Journal*,4(8): pp 94-96.

Tan G.S, and Zuo X., (1994). Chemical constituents of *Hylotelephium mingjinianum* (S.H.FU) H. Ohba, Yaoxue Xuebao, 29: pp 519-525.

Tian Y., Sun L.M., Liu X.Q., Li B., Wang Q., & Dong J.X. (2010). Anti-HBV active flavone glucosides from Euphorbia humifusa Wild. *Fitoterapia* 81: pp 799-802.

Tommasi, N., Simone, E, Cirino, G., Pizza, C. (1991). Hypoglycemic effects of sesquiterpene glycosides and polyhydroxylated triterpenoids of *Eriobotrya Japonica*. *Planta Med*. 57: pp 414 - 416.

Tsong-Huei Lee, Jong-Liang Chiou, Ching-Kuo Lee and Yue-Hsiung Kuo, (2005). Separation and determination of chemical constituents in the roots of *Rhus javanica L.var. roxburghiana, Journal of the Chinese Chemical Society*, 52: pp 833-841.

Tucci, S.A. Boyland, E.J. Halford, J.S.G. (2010). The role of lipid and carbohydrate digestive enzyme inhibitors in the management of obesity: a review of current and emerging therapeutic agents. *Diabetes, Metabolic Syndrome and Obesity: Targets and Therapy* 3, pp 125-143.

Tundis R, Loizzo M. R, Menichini F. (2010). Natural products as α-amylase and α-glucosidase inhibitors and their hypoglycemic potential in the treatment of Diabetes: An Update. *Mini-Review in Medicinal Chemistry*. 10(4): pp 315-331.

USDA (2007) Germplasm Resources Information Network. Beltsville, MD, USA: United States Department of Agriculture, Agricultural Research Service. http://www.arsgrin.gov/npgs/aboutgrin.html.

Vázquez LH, Palazon J, Navarro-Ocana A. (2012). The pentacyclic triterpenes, α, β-amyrins: A review of sources and biological activities, Phytochemicals- A Gobal Perspective of their role in nutrition and health, Rao V (Ed.), ISBN: 9 78-953-51-0296-0, InTech,

Villar, A., Paya, M., Hortiguela, M. D., Cortes, M. (1986). Tormentic acid, a new hypoglycemic agent from Poterium ancistroides. *Planta Med*. 52: pp 43 - 45.

Von Breitenbach, Dr. F 1974, Southern Cape Forests and Trees, the Government Printer, Pretoria. Coates Palgrave, Meg 2002, Keith Coates Palgrave Trees of Southern Africa, Struik, Cape Town. Other information sources are listed on the References page.

Wang. T, Shankar. K, Ronis M.J, Mehendale, H.M, (2007), Mechanisms and Outcomes of drug and Toxicant-Induced Liver Toxicity in Diabetes. *Crit. Rev. Toxicol*., 37, pp 413-459.

Wang RR, Gu Q, Wang YH et al., (2008). Anti-HIV-1 activities of compounds isolated from medicinal plant *Rhus chinensis. Journal of Ethnopharmacology* 117: pp 249-256.

Weiss N, Ide N, Abahji T, Nill L, Keller C, and Hoffman U. (2006). Aged garlic extract improves homocysteine-induced endothelial dysfunction in macro and microcirculation. *The Journal of Nutrition* 136(3): pp 750s – 754s.

White, C., Ward, C., Dombrowski, D. S., Dunlow, L. D., Brase, D. A., Dewey, W. 1. (1993). Effect of intrathecal morphine on the fate of glucose. Comparison with effects of insuline and xanthan gum in mice. *Biochemistry. Pharmacology*. 45: pp 459 - 464.

Wiseman SA, Balentine DA, Frei B. Antioxidants in tea. *Critical Review of Food Science*. 1997; 37: pp 705-718.

Wolffram S, Block, Ader P., (2002). Quercetin-3-glucoside is transported by the glucose carrier SGT1 across the brush border membrane of the rat small intestine. *Journal of Nutrition.* 132 (4): pp 630-635.

W.H.O (2009). Obesity and overweight. <http://www.who.int/dietphysicalactivity/

Yamahara, J., Mibu, H., Sawada, T., Fujimore, H., Takinos, S., Yoshikawa, M., Kitagawa, L. (1981). Biologically active principles of crude drugs. Antidiabetic principles of *Corni fructus* in experimental diabetes induced by streptozotocin. *Yakugaku Zasshi*. 101: 86 - 90.

Yamamoto N, Moon JH, Tsushida T, Nagao A, Terao J. (1999). Inhibitory effect of quercetin metabolites and their derivatives on copper ion-induced lipid peroxidation in human low-density lipoprotein. *Arch. Biochem. Biophys*. 372(2): pp 347-354.

Yang H, Zheng S, Meijer L et al., (2005). Screening the active constituents of Chinese Medicinal herbes as potent inhibitors of Cdc25 tyrosine phosphatase, an activator of mitosis-inducing p34cdc2 kinase. J Zhejiang Univ. Sci. 6B: pp 656-663.

Yang JY, Della-Fera MA, Rayalam S, Ambati S, Hartzell DL, Park HJ, et al. (2008). Enhanced inhibition of adipogenesis and induction of apoptosis in 3T3-L1 adipocytes with combinations of resveratrol and quercetin. *Life Science*. 82: pp 1032–1039.

Yang, A.M., Lu, R.H., Shi, Y.P., 2007. Study on flavonoids from *Lagotis ramalana* Batalin. *China. Pharmacology Journal*, 19: pp 1459-1461.

Yeom S. H, Kim M.K, Kim H.J, Shim J.G, Lee J.H, and Lee M.W. (2003). Phenolic compounds from Seeds of *Astragalus Sinicus* and their oxidative activities, Saengyak Hakhoechi, 34: pp 344-351.

Yidizogglu-Ari N, Altan V, Altinkurt O & Ozturk Y, (1991), Pharmalogical effects of rutin, Phototherapeutic Res, 5, (19).

Yuliana ND, Jahangir M, Korthout H, Choi Y.H, Kim H.K , and Verpoorte R, (2011) "Comprehensive review on herbal medicine for energy intake suppression," *Obesity Reviews*, 12(7),pp 499 - 514.

Yun JW (2010). Possible anti-obesity therapeutics from nature – A review. *Phytochemistry* 71, pp 1625-1641.

Yunes R, Calixto J. (2005). Antinociceptive properties of a mixture of α-amyrin and β-amyrin triterpenes: evidence for participation of protein kinase C and protein kinase A pathway. *Journal of Pharmacology Expo. Therapeutics* 31 (1): pp 310-318.

Zahra Kalhori, (2015), Antioxidant effect of aqueous extract of Sumac (*Rhus coriaria L*) in the alloxan-induced diabetic rats, *Indian journal Physiol. Pharmacology*. 59(1), pp 87-93

Zahra salami, Azade Eskandary, Reza Headar, Vahid Nejati, Mojataba Moradi and Zaitoun S, Al-Ghazawi A, Al-Qudah A., (2007), Bee pollination and fruit set of Sumac (Rhus coriaria, Anacardiaceae) as a native herbal plant grown under semiarid Mediterranean conditions in Jordan. *Adv Hortic Sci*.: 21(3): pp 183–187.

Zhang X, Wu C, Wu H, Sheng L, Su Y, et al. (2013), Anti-Hyperlipidemic Effects and Potential Mechanisms of Action of the Caffeoylquinic Acid-Rich Pandanus tectorius Fruit Extract in Hamsters Fed a High Fat-Diet. *PLoS ONE* 8(4), pp 1-12.

Zhang Y, Wang D, Yang L, Zhang J. (2014). Purification and characterization of flavonoids from the leaves of *Zanthoxylum bungeanum* and correlation between their structure and antioxidant activity. PLoS ONE 9(8):e105725. doi:10.1371/journal.pone.0105725.pp 1-11.

Zhou, J., Qi, L., Li, P. (2009). Herbal medicine analysis by liquid chromatography/time-of-flight mass spectroscopy. *Journal of Chromatography*, 1216(44), pp 82-7594.

Zia-Ul-Haq, M., Cavar, S., Qayum, M., Imran, I., and de Feo, V. (2011). Compositional studies: antioxidant and antidiabetic activities of Capparis decidua (Forsk.) Edgew. *International. Journal of Molecular Science.* 12, pp 8846–8861.

ANNEXURE ONE: NMR SPECTRUM OF COMPOUNDS ISOLATED FROM *RHUS LUCIDA*

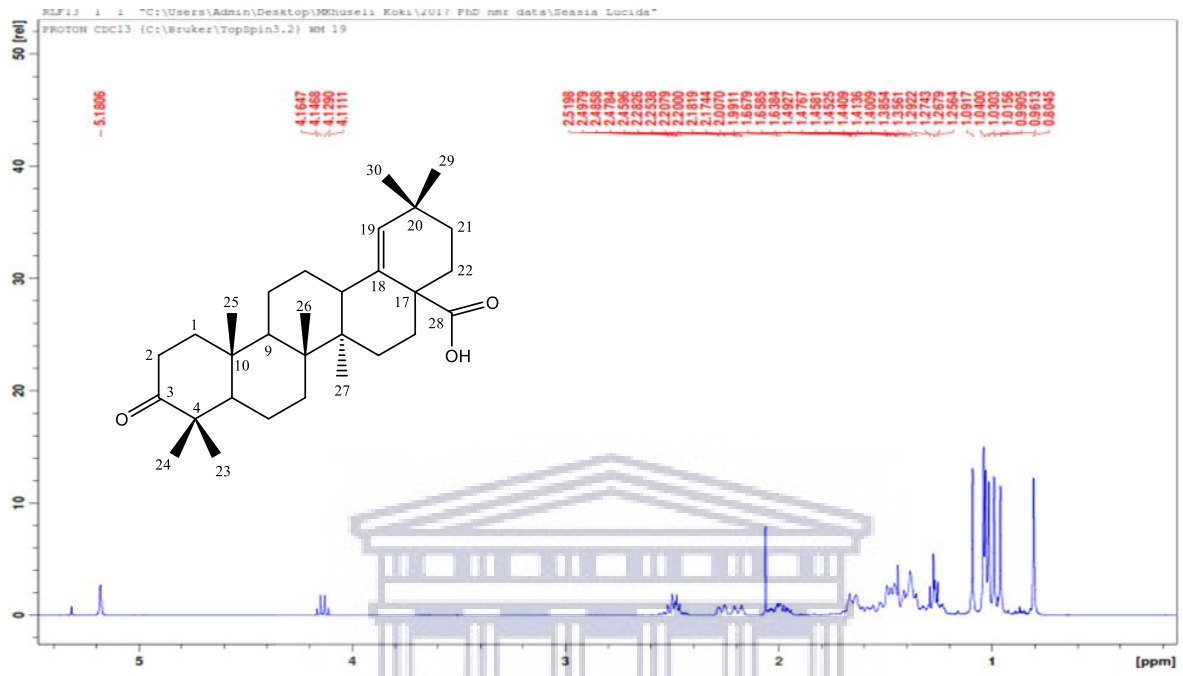


Figure 7. 1: The ^{1}H NMR moronic acid (C5) in $CDCl_3$

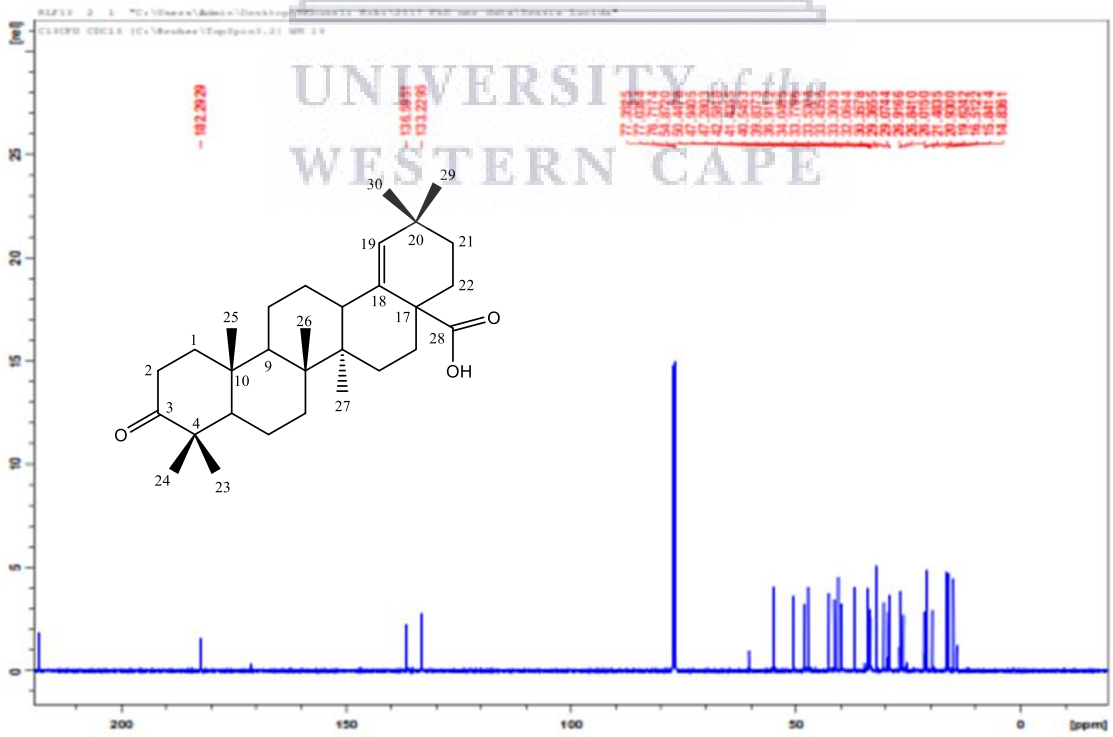


Figure 7. 2 : The ^{13}C NMR of moronic acid (C5) in $CDCl_3$

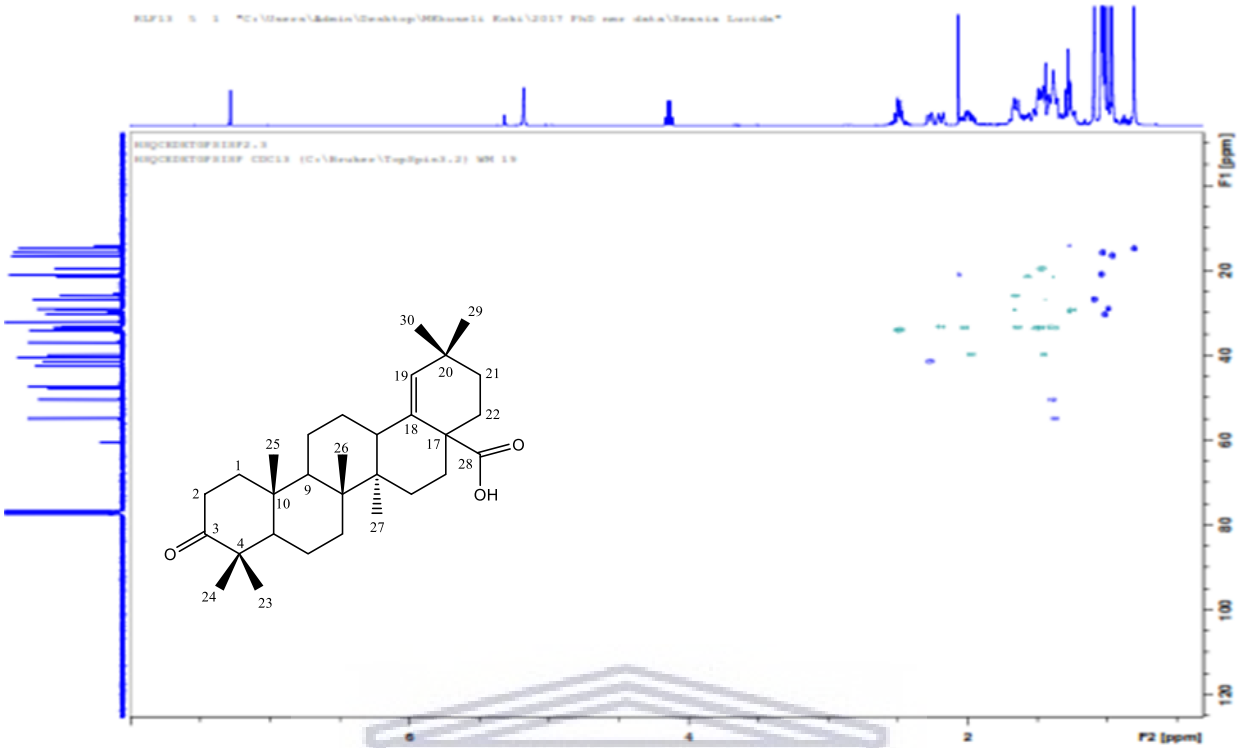


Figure 7. 3: The HSQC of moronic acid (C5) in $CDCl_3$

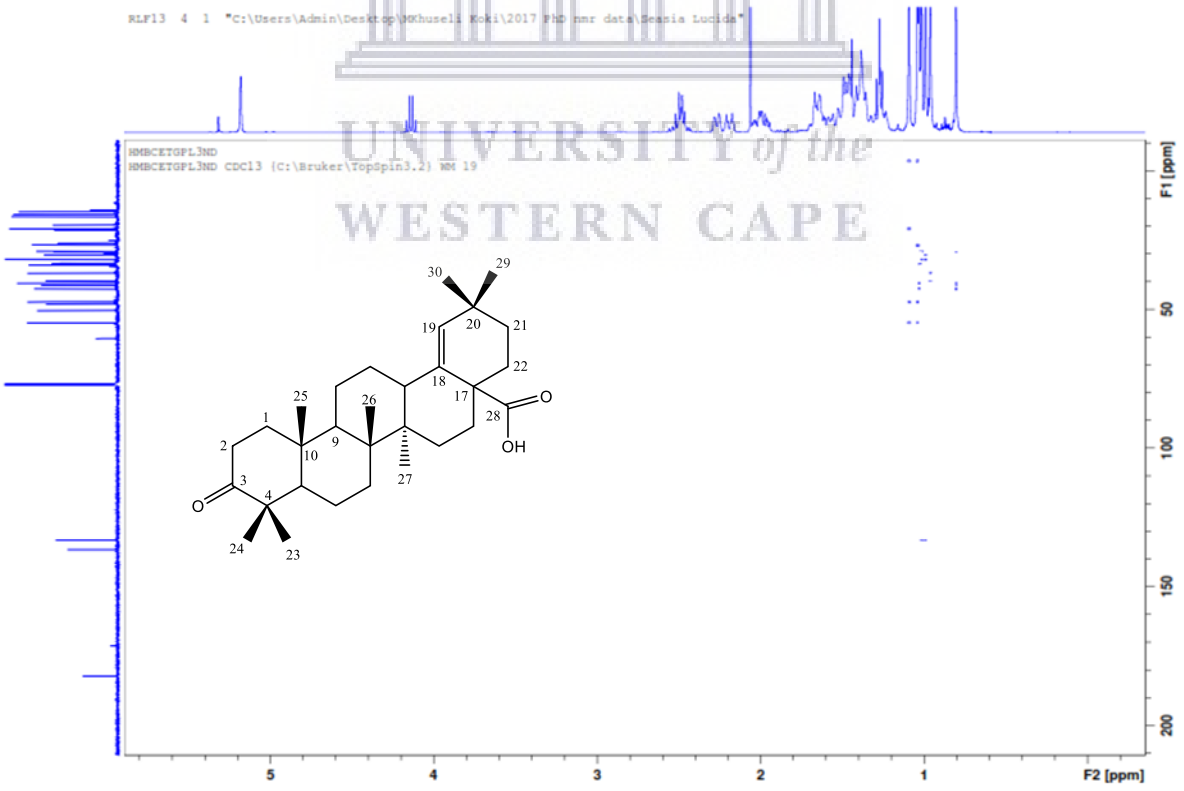


Figure 7. 4 : The HMBC of moronic acid (C5) in $CDCl_3$

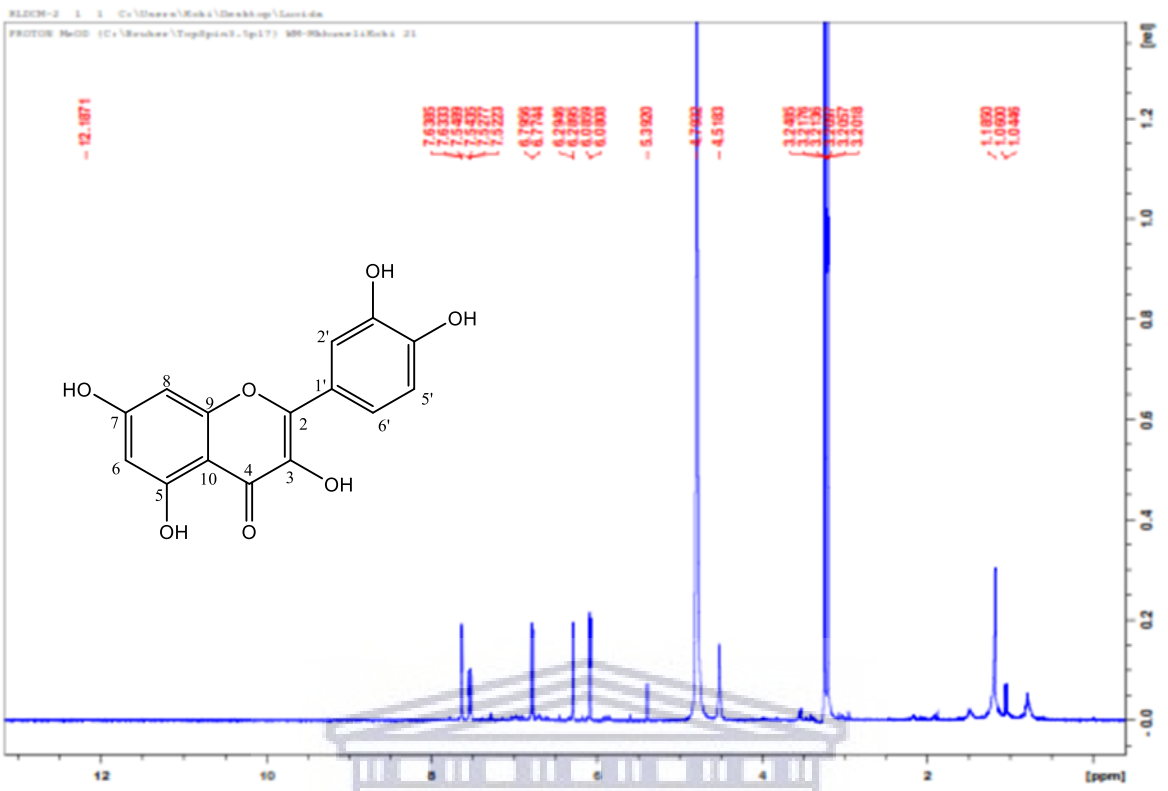


Figure 7. 5 : The ^{1}H NMR of quercetin (C6) in CD_3OD

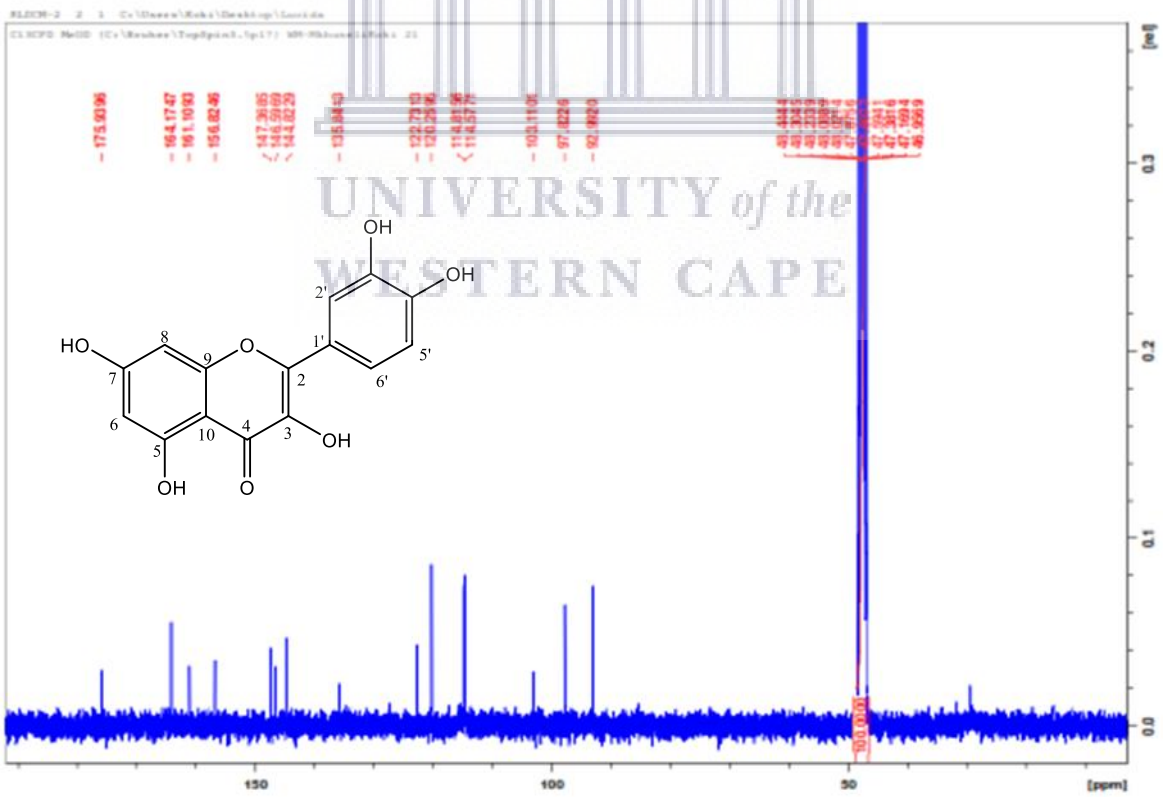


Figure 7. 6 : The ^{13}C NMR of quercetin C6 in CD_3OD

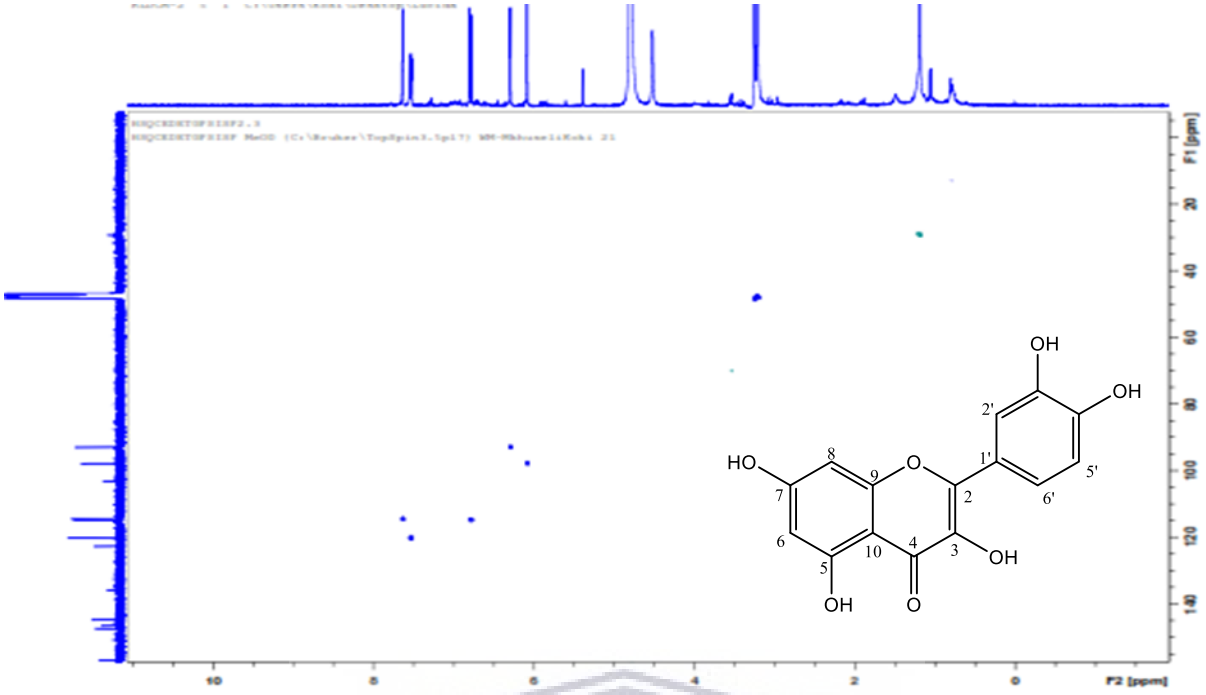


Figure 7. 7 : The HSQC of quercetin (C6) in CD_3OD

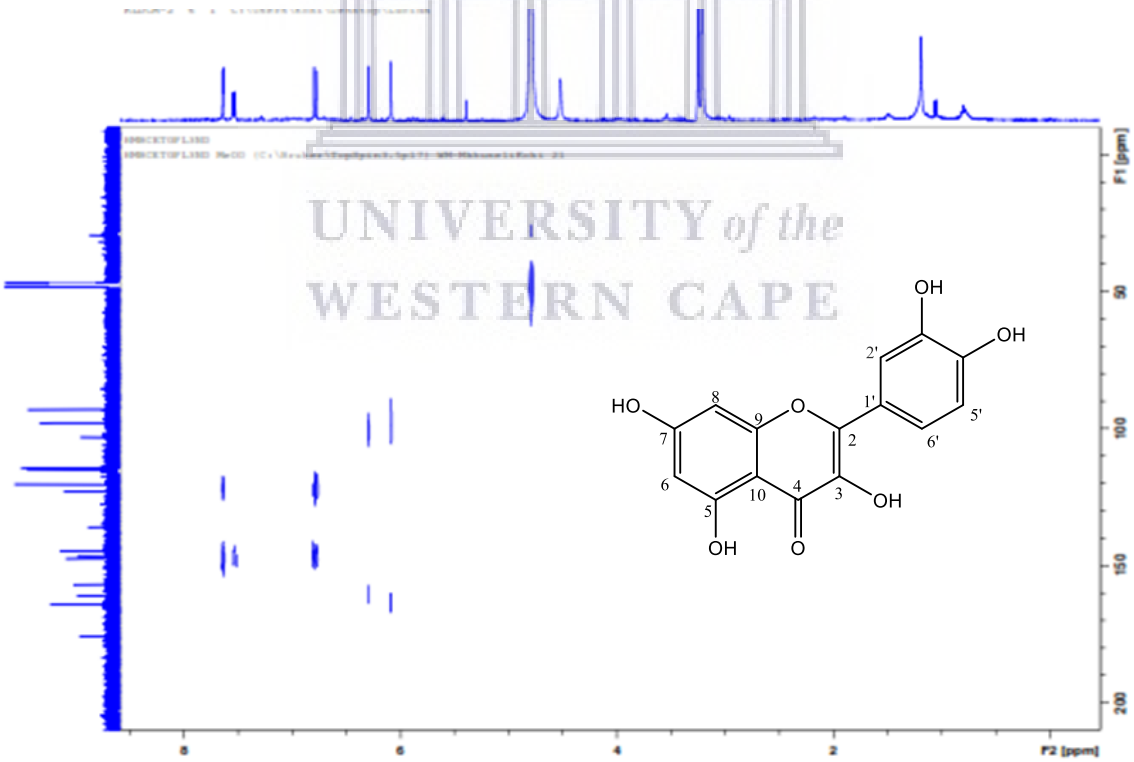


Figure 7. 8 : The HMBC of quercetin (C6) in CD_3OD

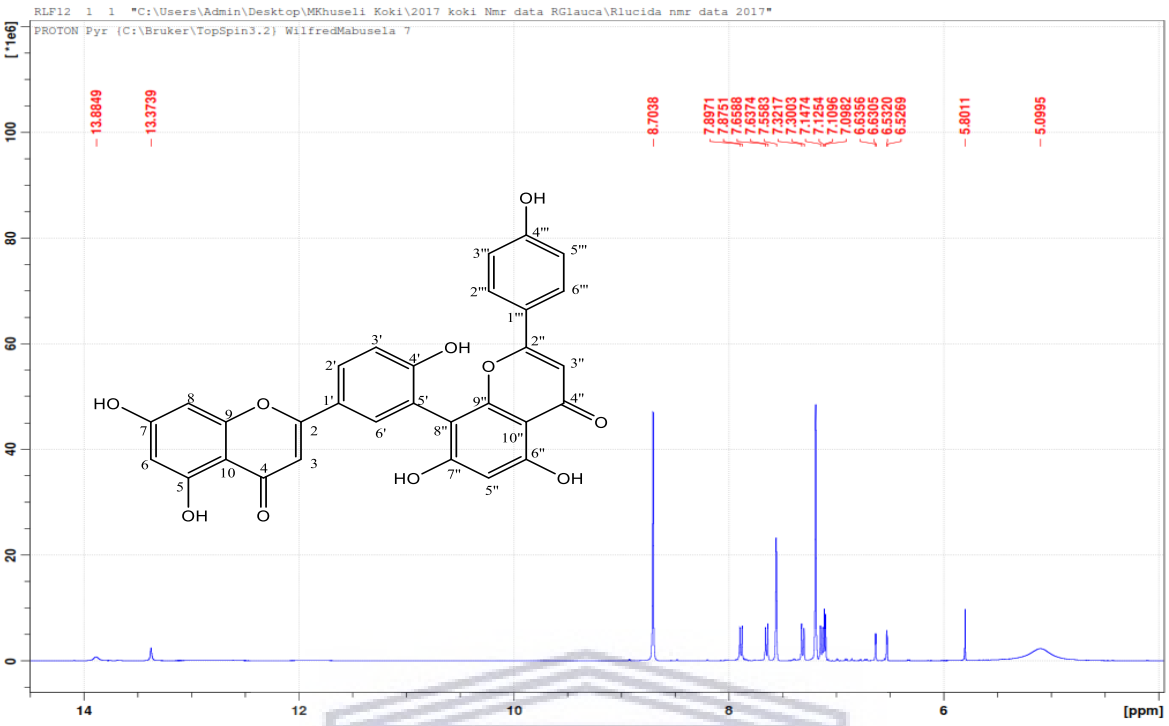


Figure 7. 9 : The ^{1}H NMR of amentoflavone (C8) in Pyridine-d_5

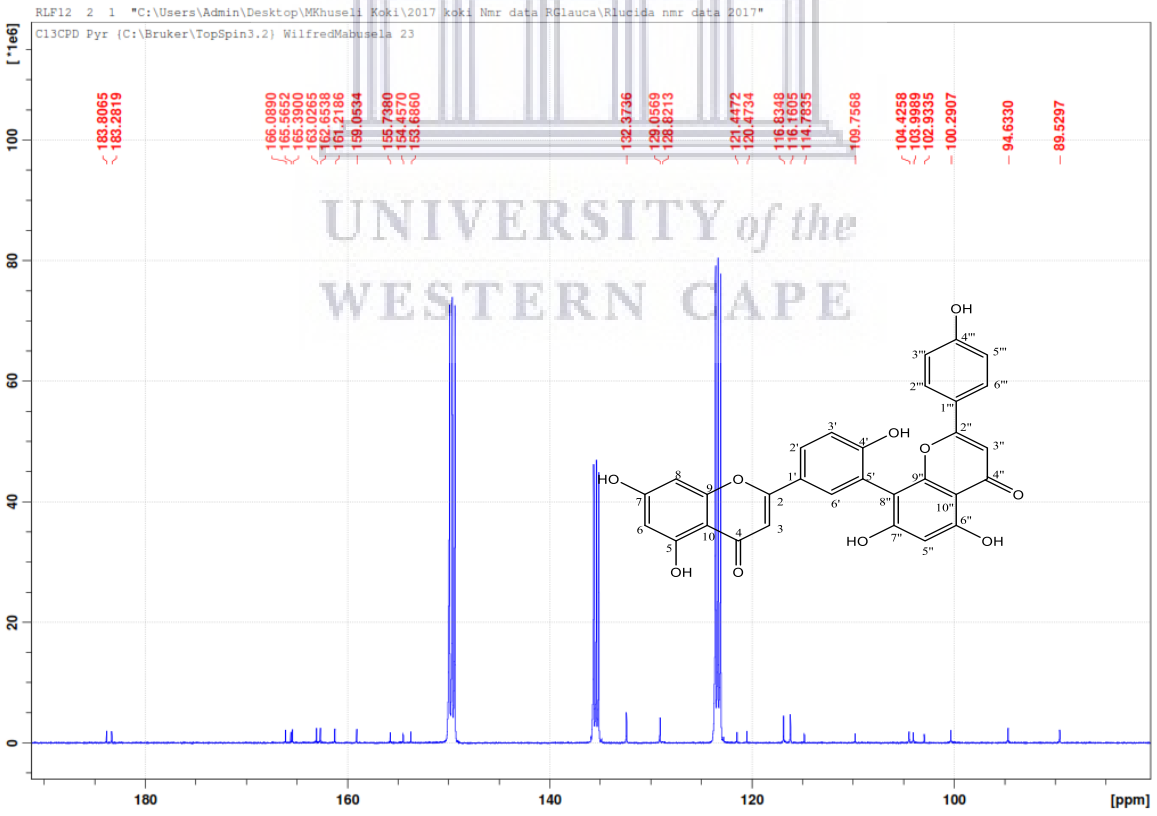


Figure 7. 10 : The ^{13}C NMR of amentoflavone (C8) in Pyridine-d_5

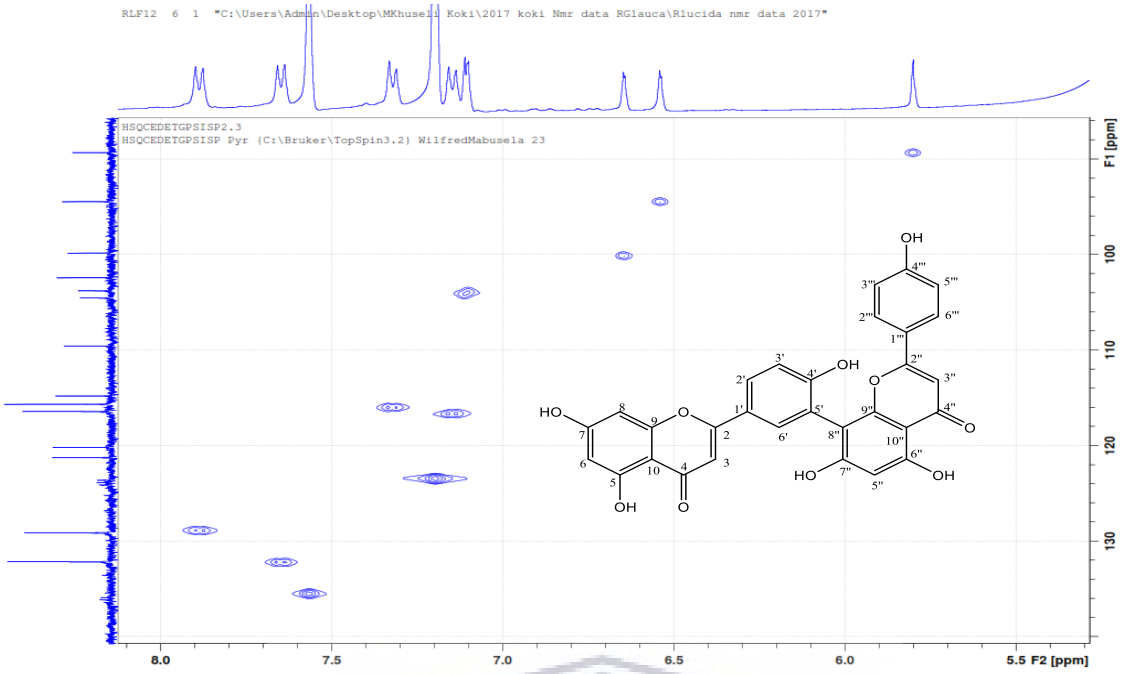


Figure 7. 11: The HSQC of amentoflavone C8 in Pyridine-d_5

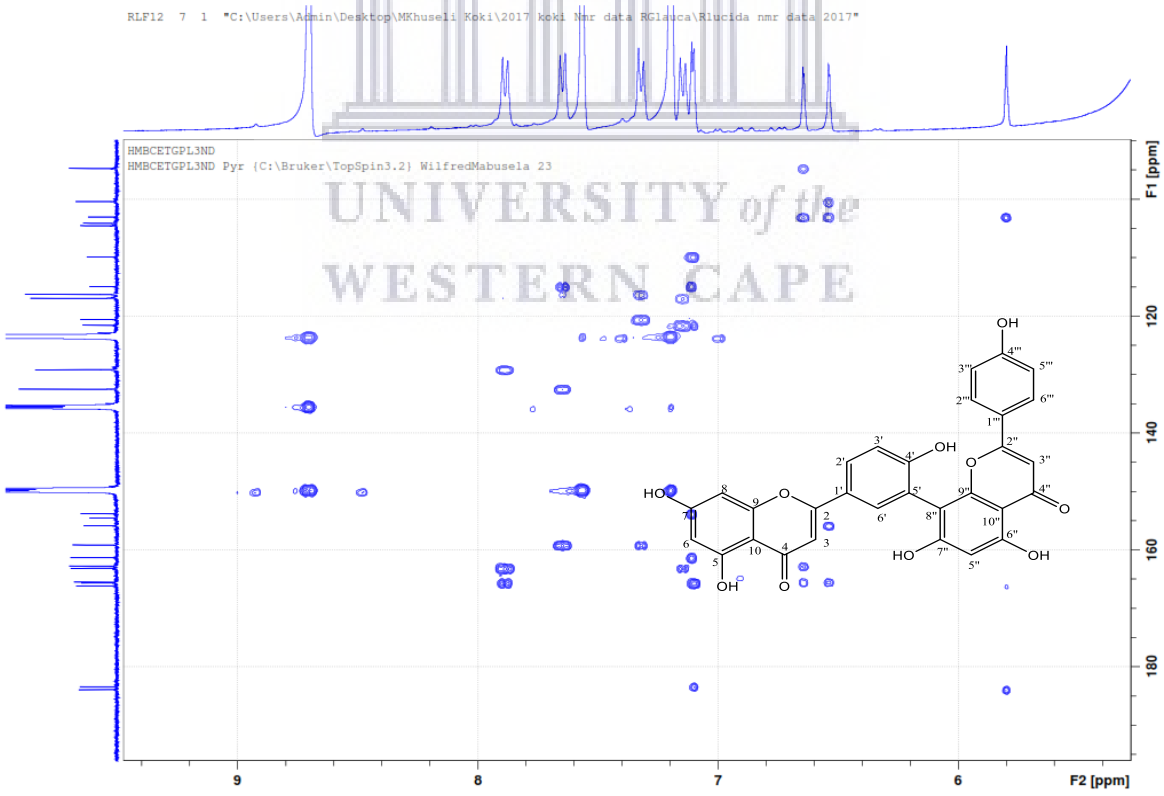


Figure 7. 12 : The HMBC of amentoflavone (C8) in Pyridine-d_5

ANNEXURE TWO: NMR SPECTRUM OF COMPOUNDS ISOLATED FROM *RHUS GLAUCA*

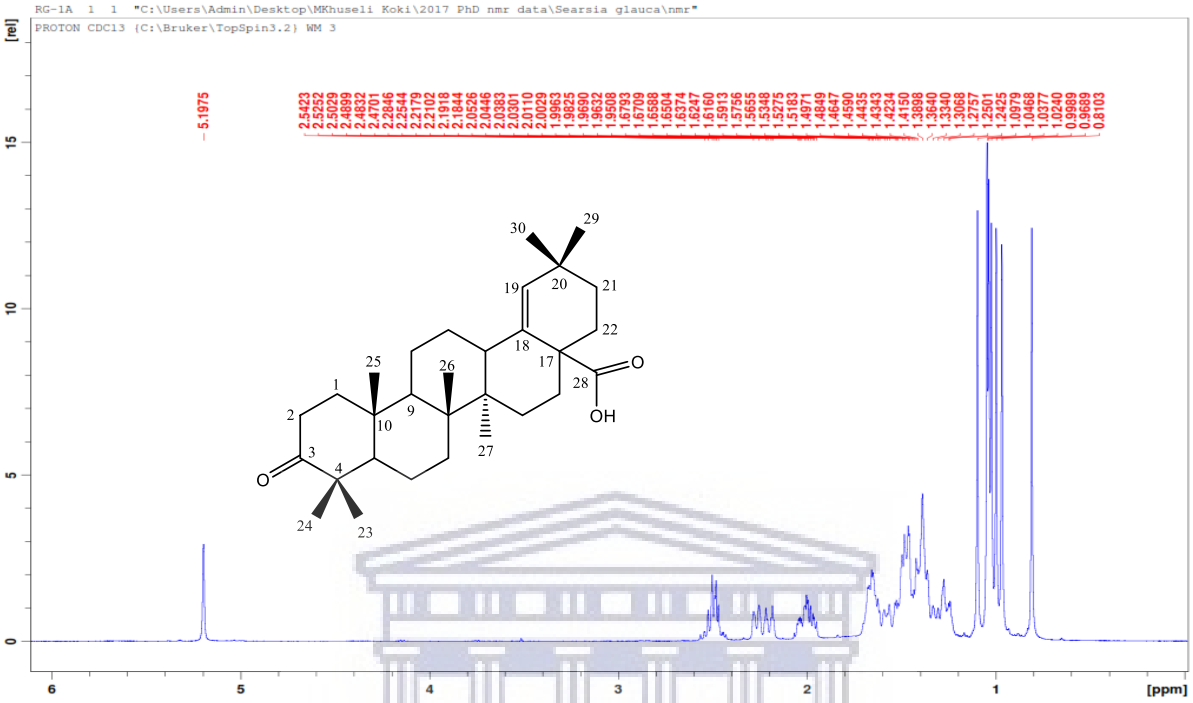


Figure 7. 13 : The ^{1}H NMR of moronic acid (C1) in $CDCl_3$

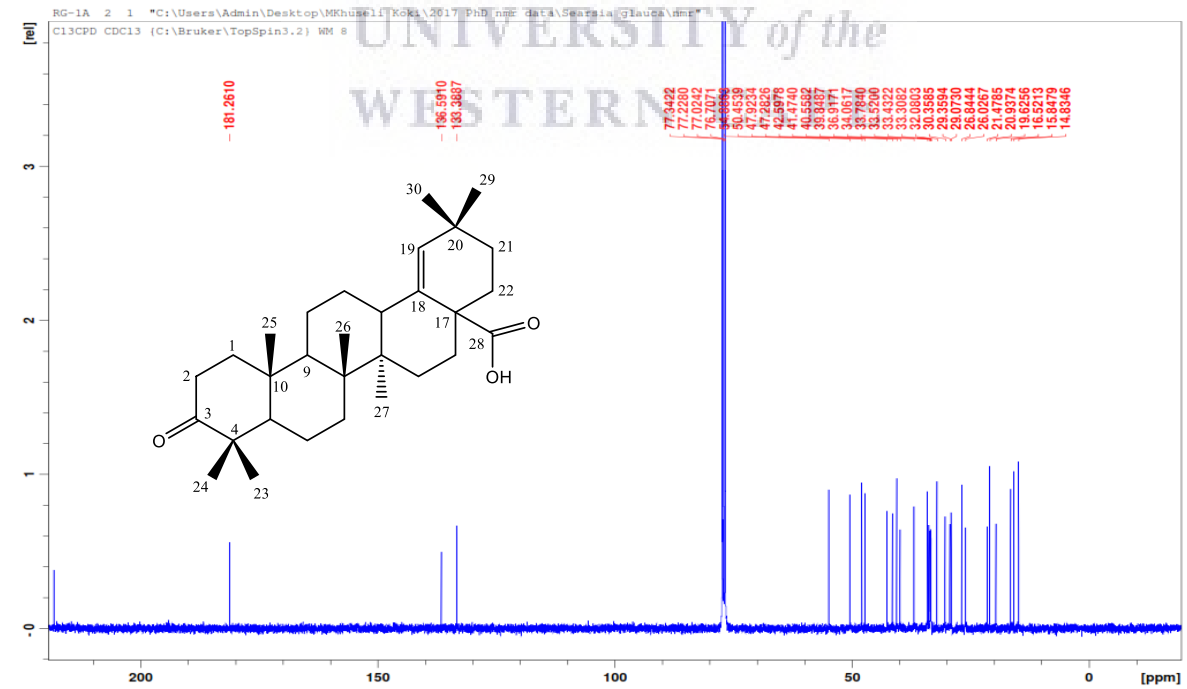


Figure 7. 14 : The ^{13}C NMR of moronic acid (C1) in $CDCl_3$

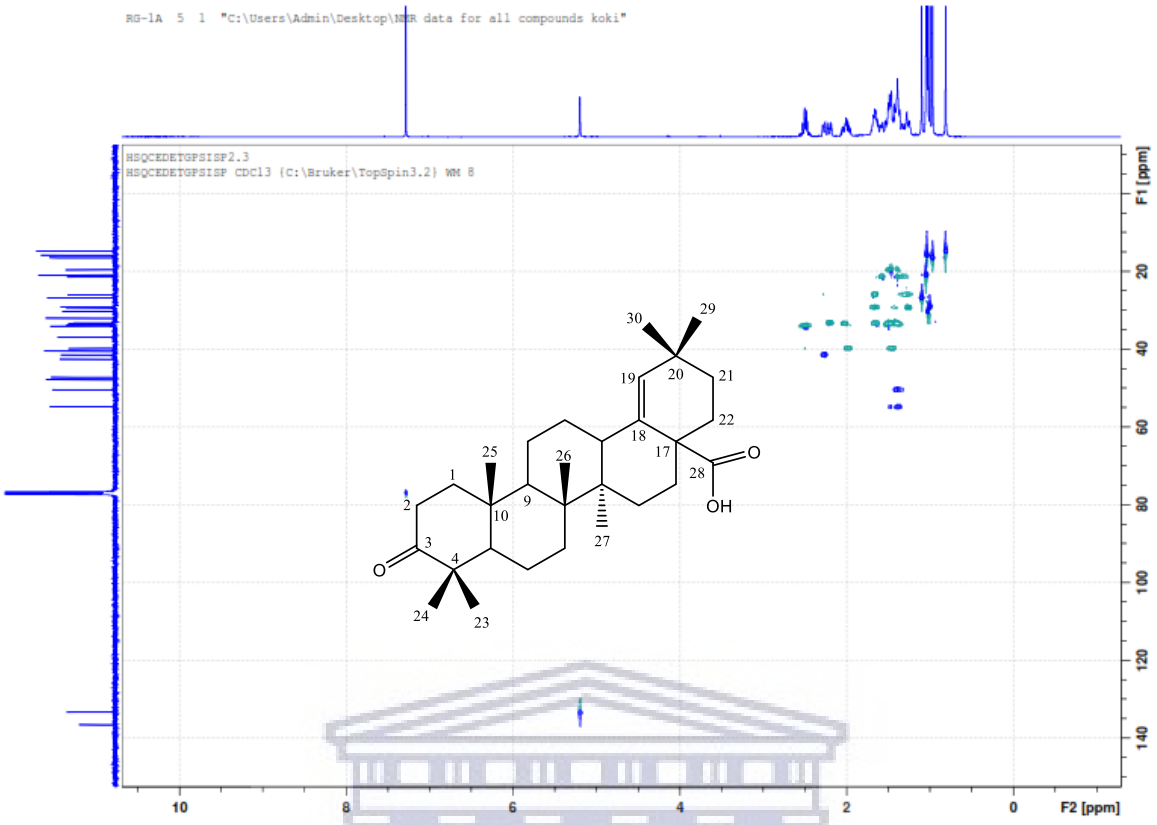


Figure 7. 15 : The HSQC of moronic acid (C1) in $CDCl_3$

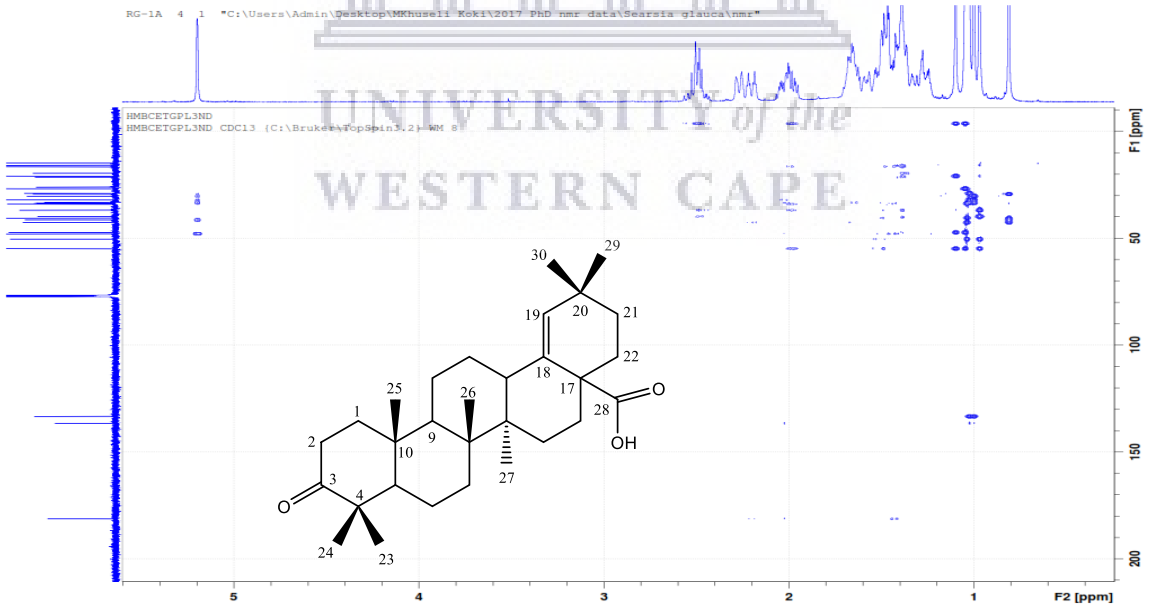


Figure 7. 16 : The HMBC of moronic acid (C1) in $CDCl_3$

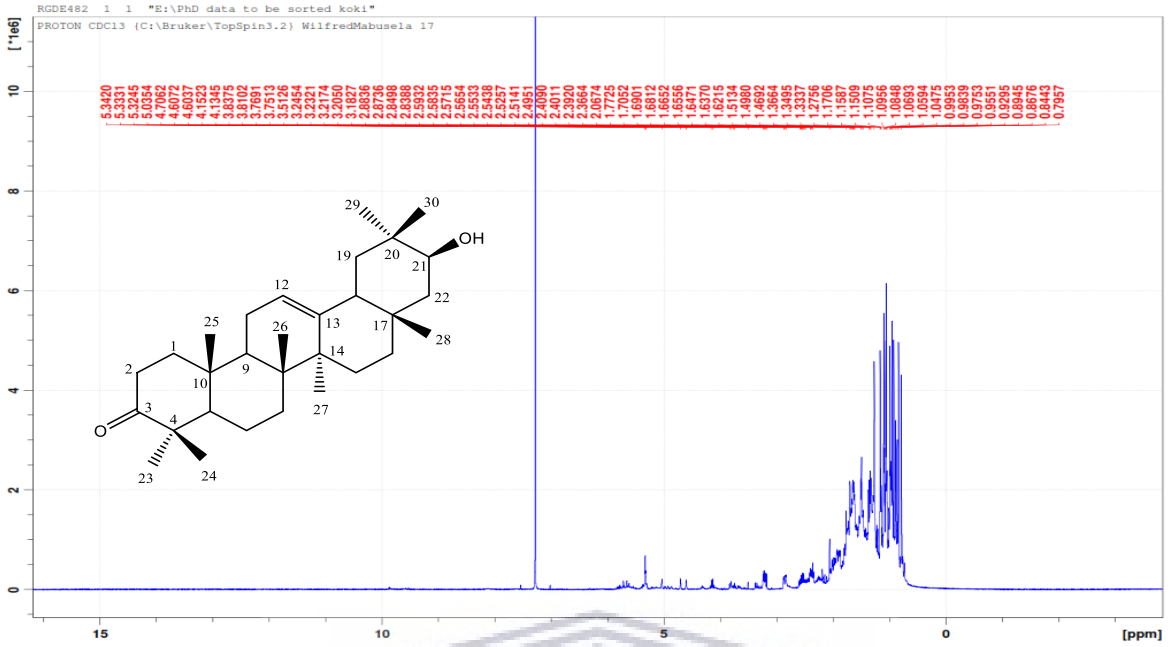


Figure 7. 17: The ^{1}H NMR spectrum of 21-β-hydroxylolean-12-en-3-one (C2) in $CDCl_3$

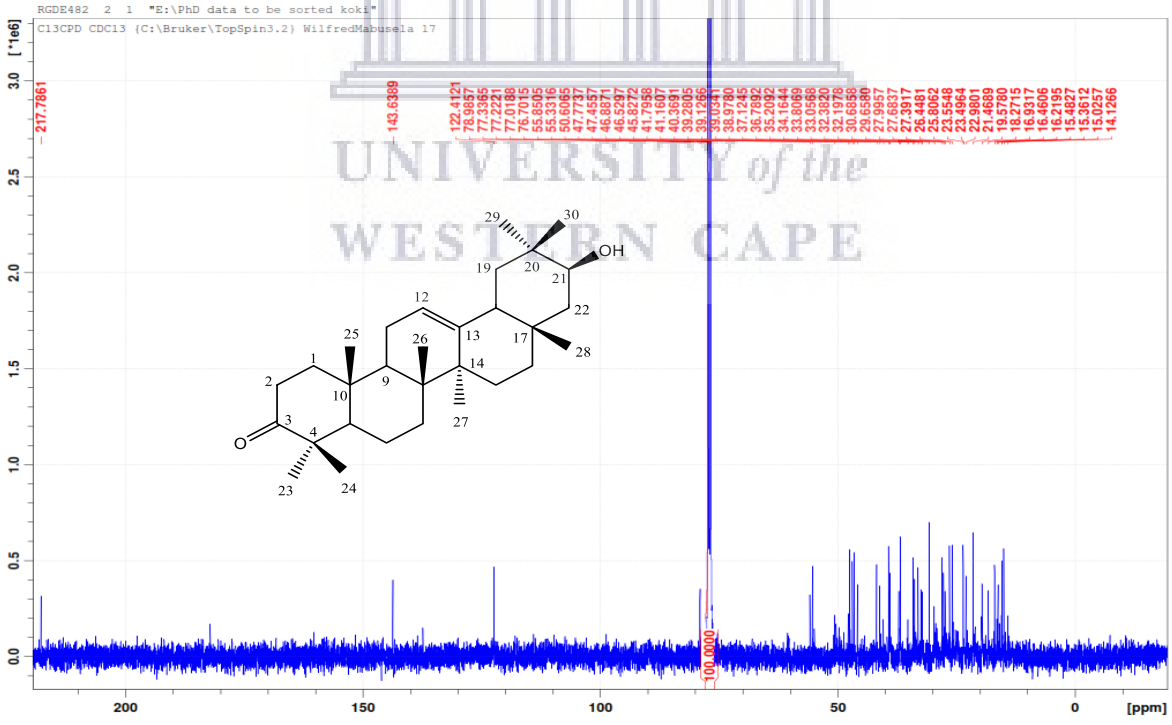


Figure 7. 18 : The ^{13}C NMR spectrum of 21-β-hydroxylolean-12-en-3-one (C2) in $CDCl_3$

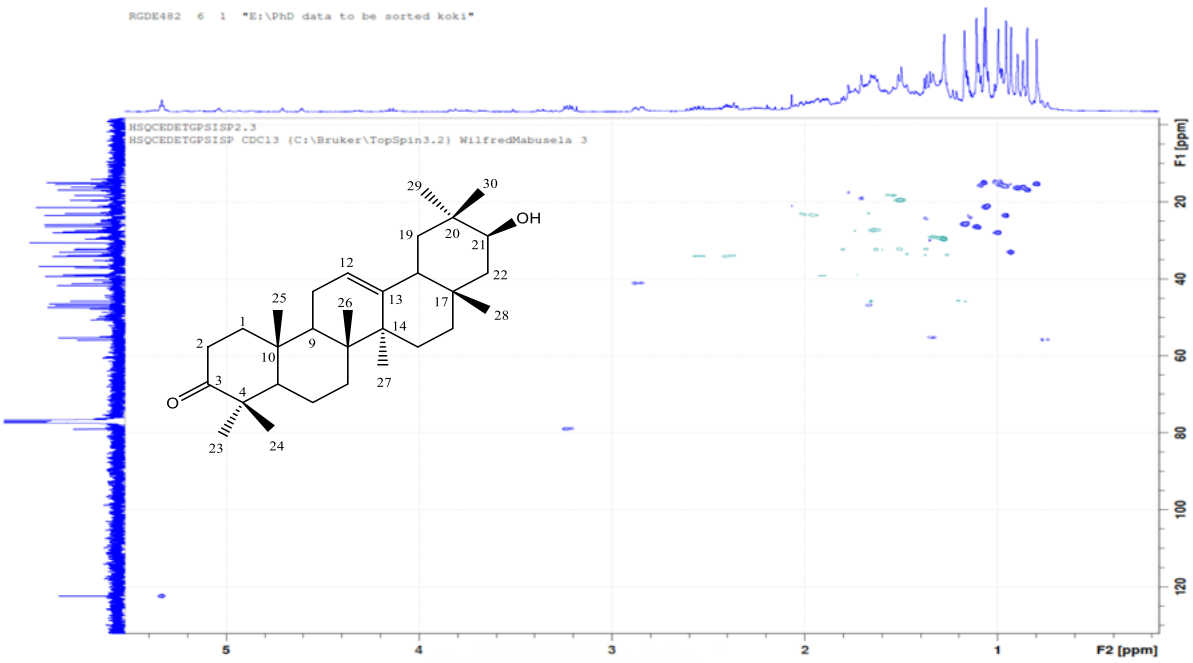


Figure 7. 19 : The HSQC spectrum of 21-β-hydroxylolean-12-en-3-one (C2) in $CDCl_3$

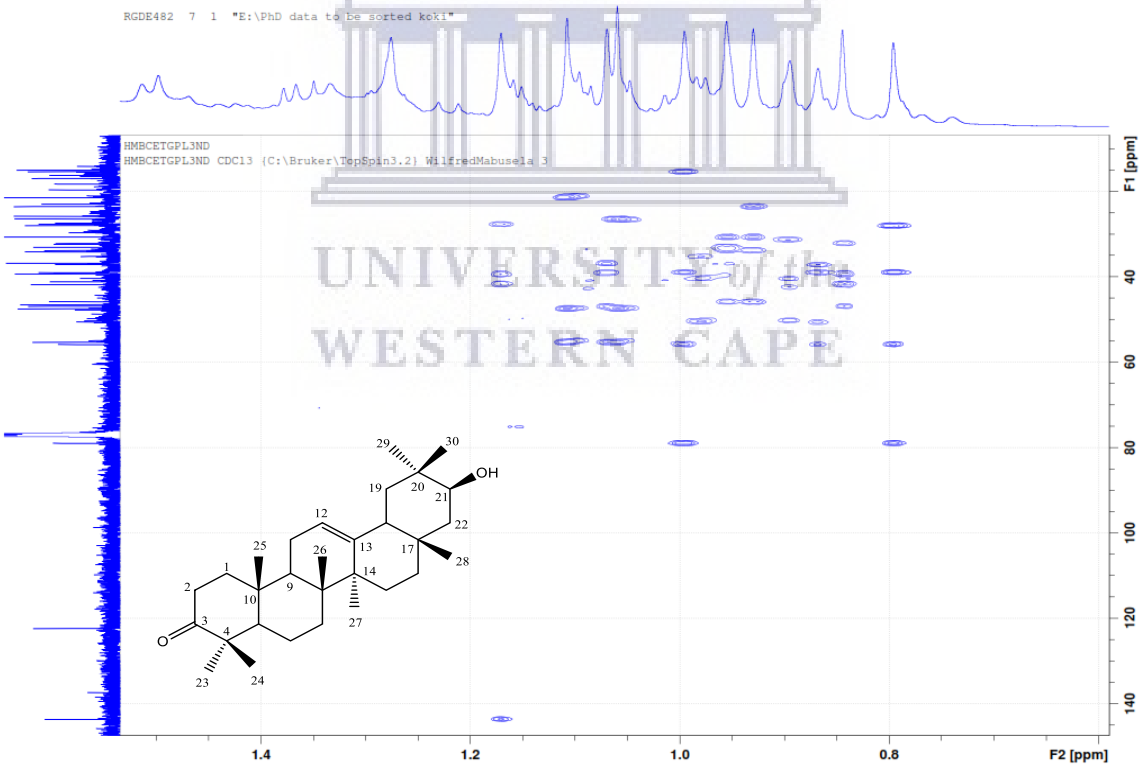


Figure 7. 20 : The HMBC of 21-β- hydroxylolean-12-en-3-one (C2) in $CDCl_3$

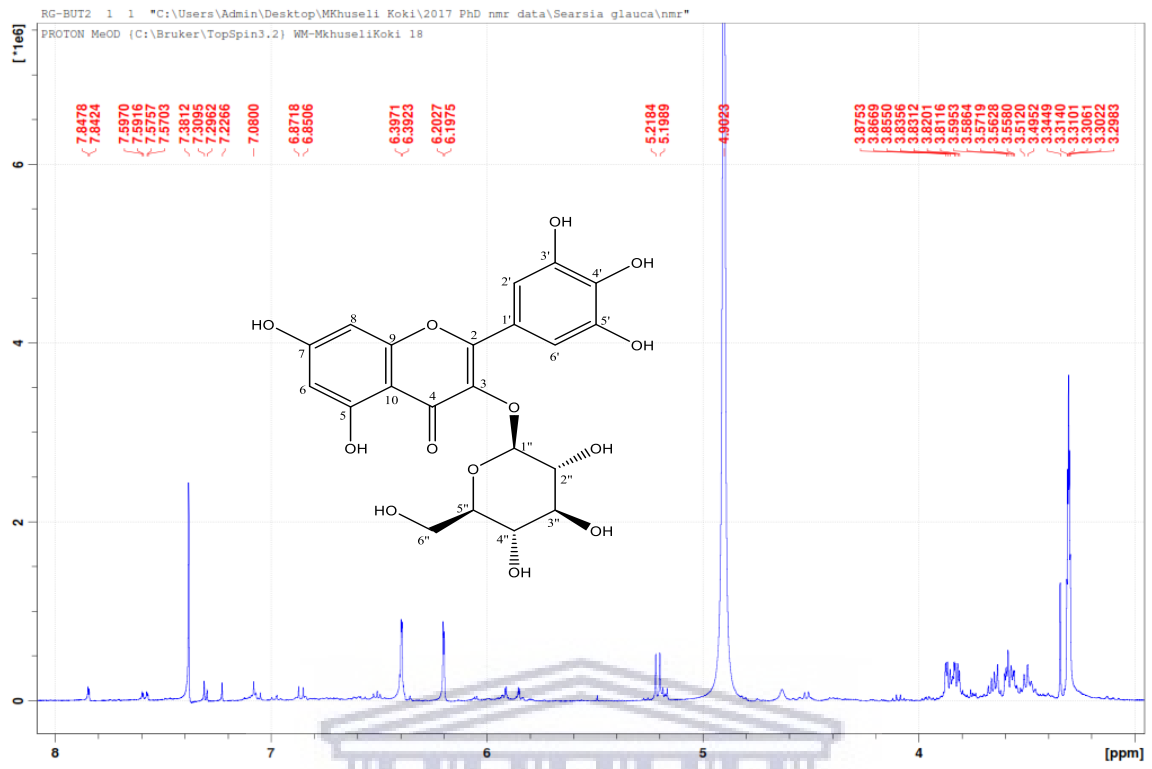


Figure 7. 21 : The ^{1}H-NMR of myricetin 3-*O*-*β*-galactopyranoside (C3) in CD_3OD

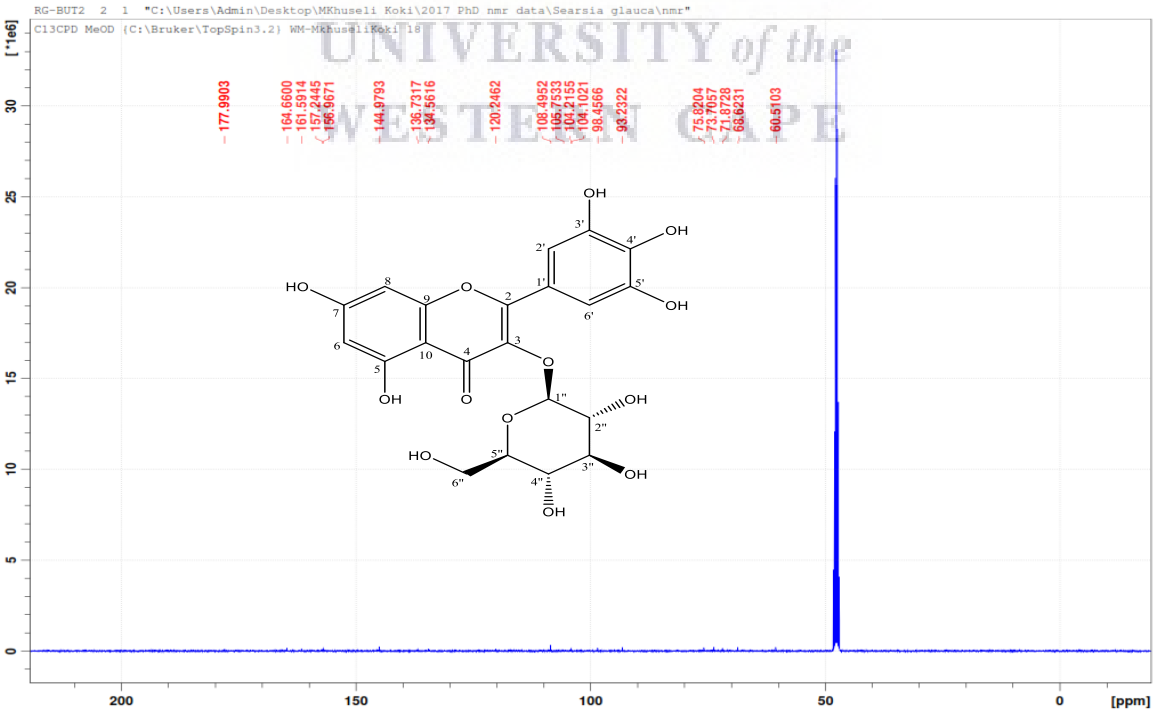


Figure 7. 22 : The ^{13}C-NMR of myricetin 3-*O*-*β*-galactopyranoside (C3) in CD_3OD

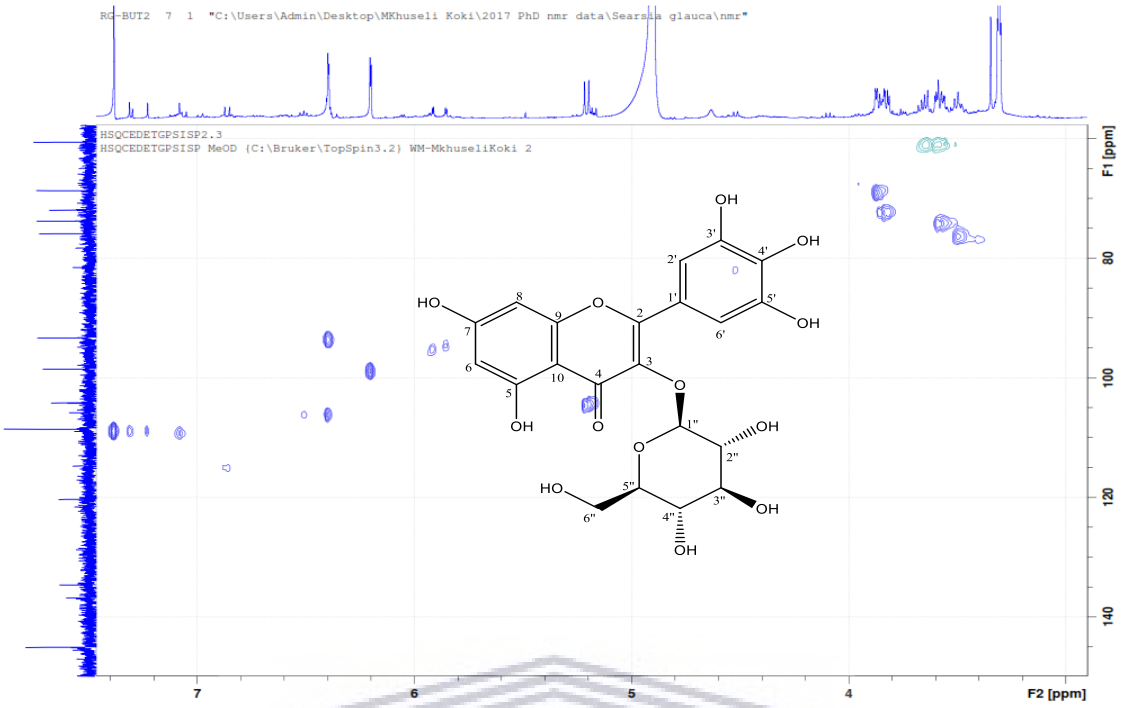


Figure 7. 23 : The HSQC of myricetin 3-*O*-*β*-galactopyranoside (C3) in CD_3OD

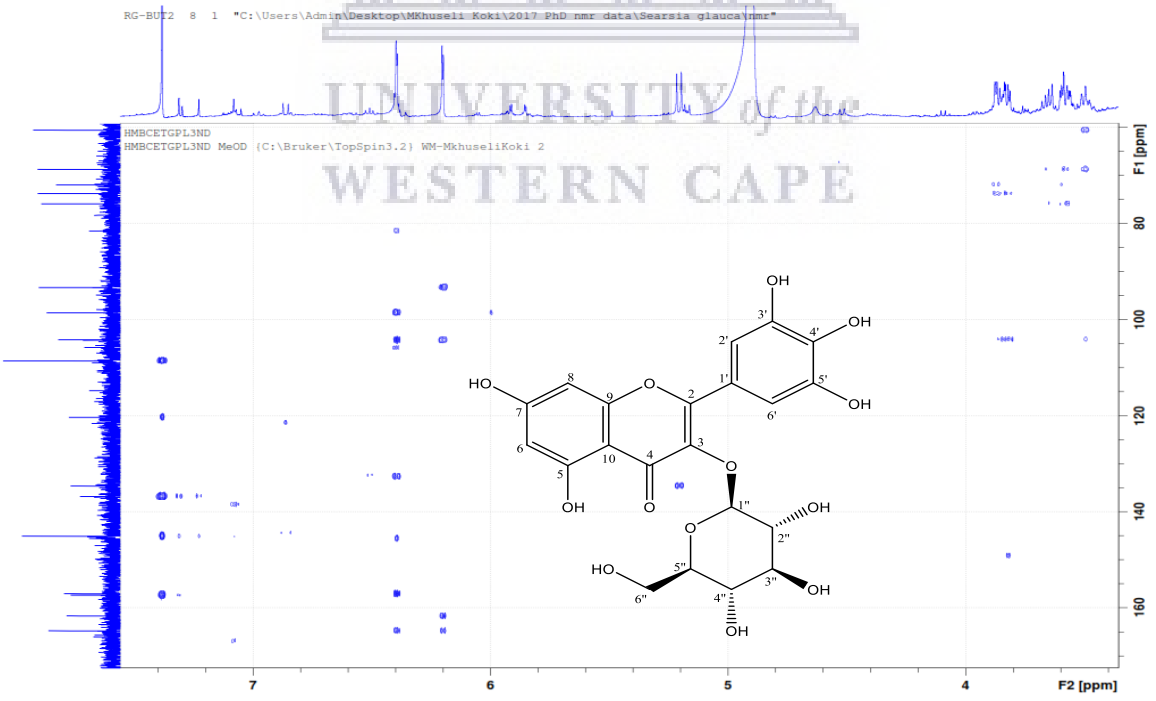


Figure 7. 24 : The HMBC of myricetin 3-*O*-*β*-galactopyranoside (C3) in CD_3OD

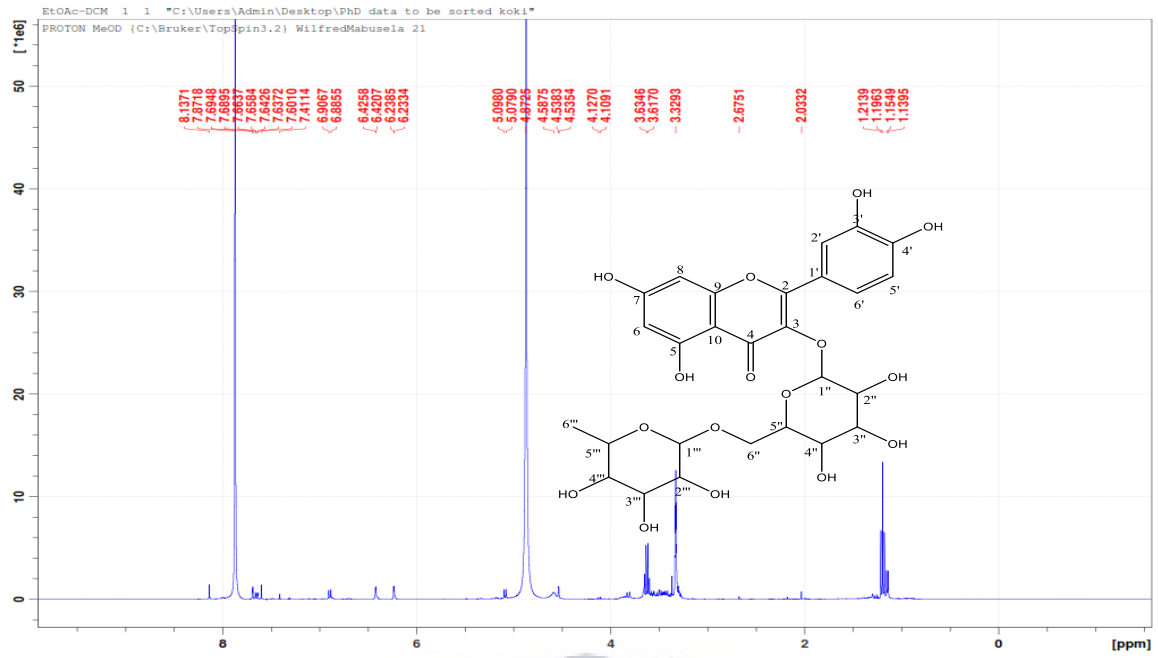


Figure 7. 25 : The ^{1}H NMR of Rutin (C4) in CD_3OD

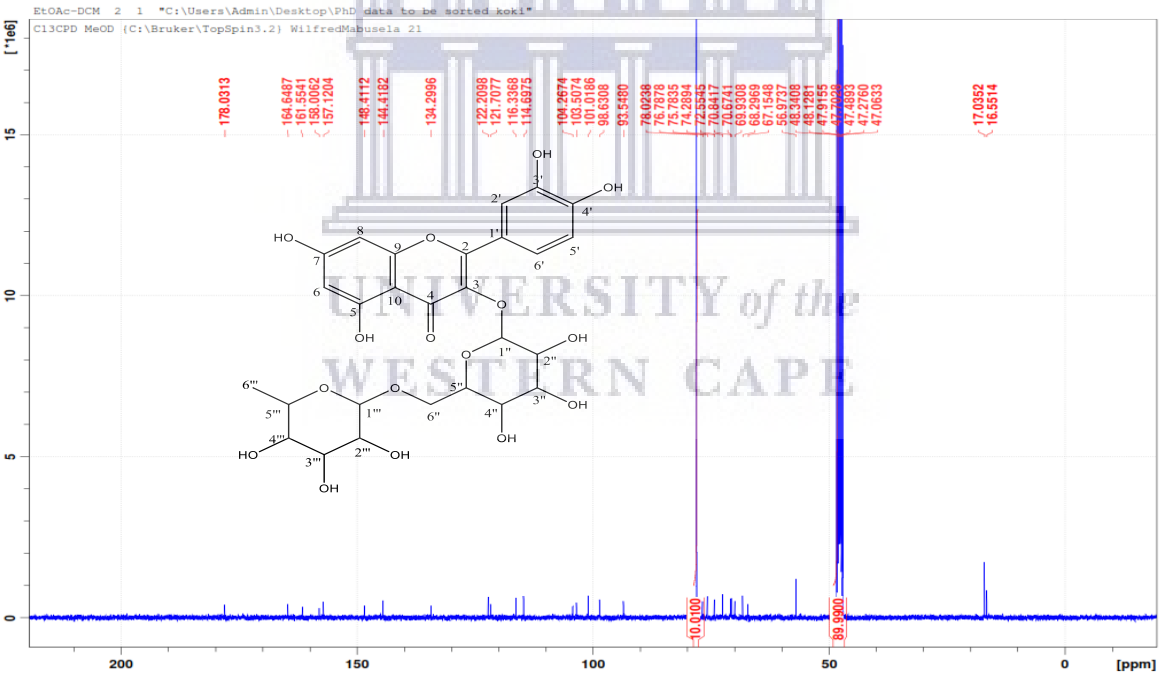


Figure 7. 26 : The ^{13}C NMR of Rutin (C4) in CD_3OD

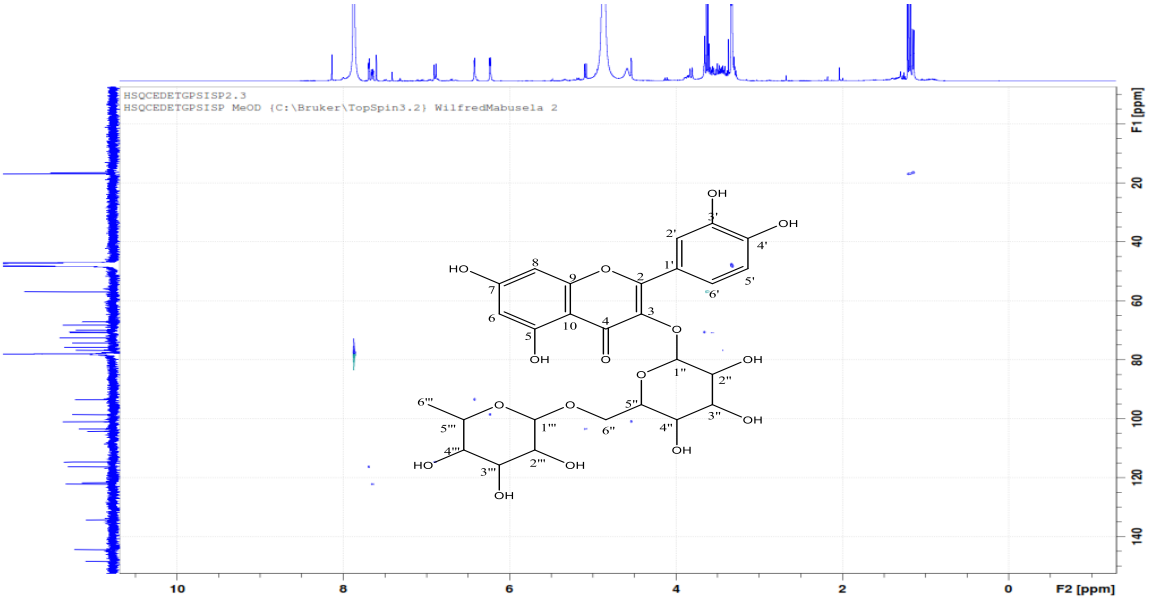


Figure 7. 27 : The HSQC of Rutin (C4) in CD_3OD

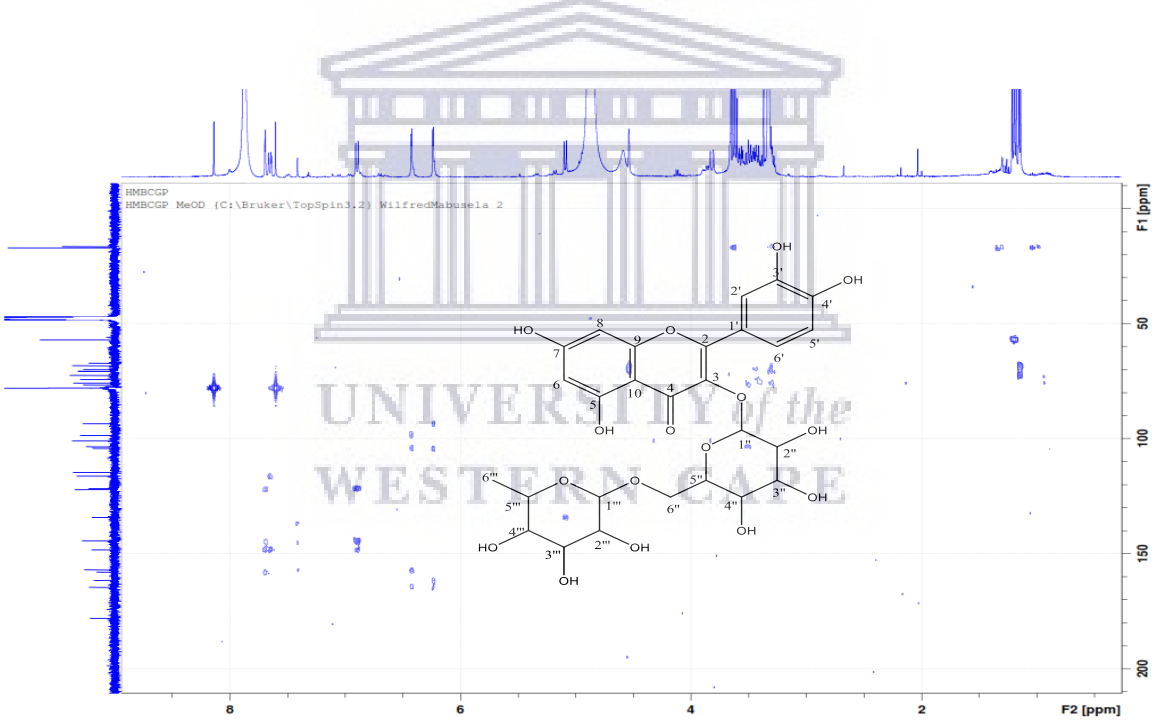


Figure 7. 28 : The HMBC of Rutin (C4) in CD_3OD

ANNEXURE THREE: NMR spectrum of compounds isolated from *R. laevigata*

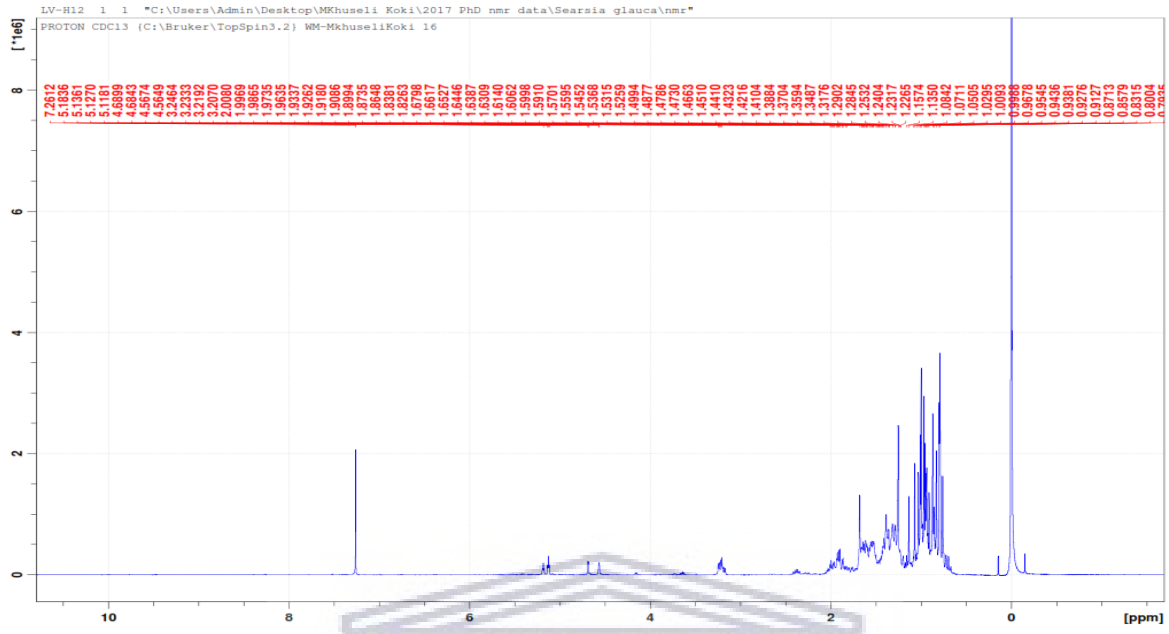


Figure 7. 29: The ^{1}HNMR of α-amyrin (C11a), β-Amyrin (C11b) and Lupeol (C11c) in $CDCl_3$

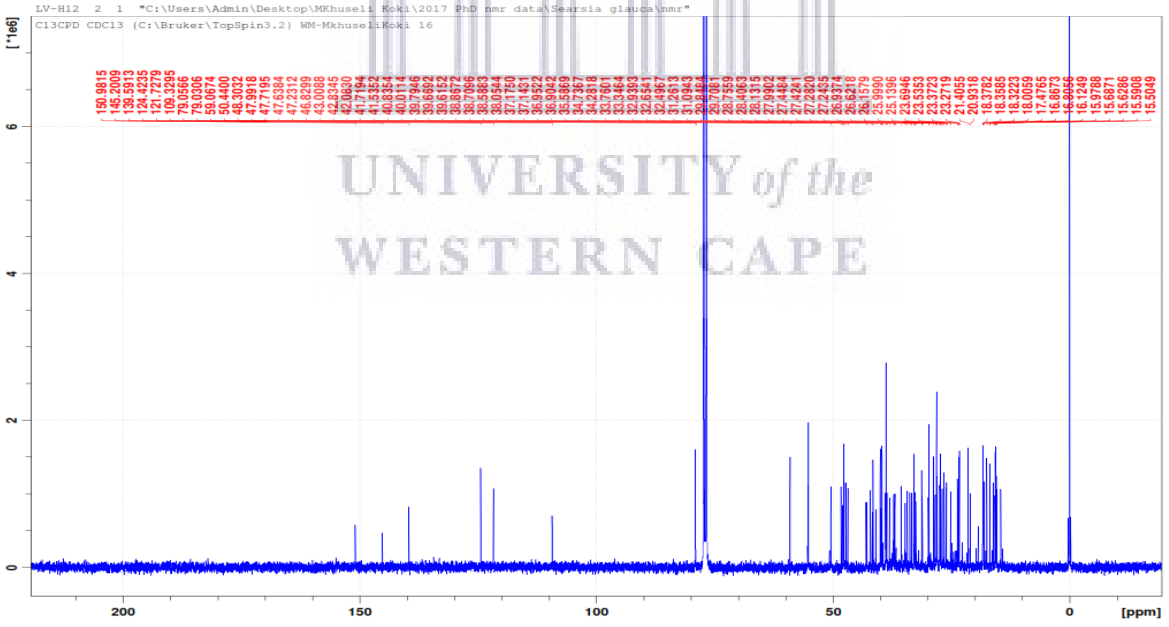


Figure 7. 30: The ^{13}CNMR of α-amyrin (C11c), and β-Amyrin (C11b) and Lupeol (C11a) in $CDCl_3$

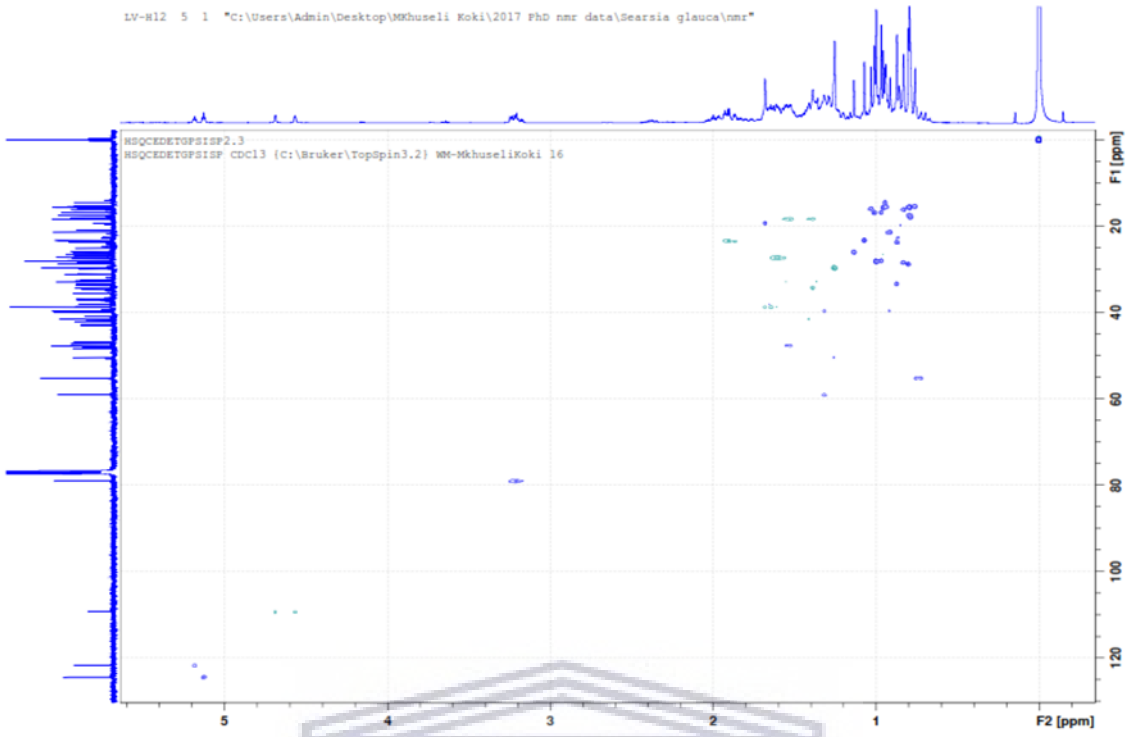


Figure 7. 31 : The HSQC of α-amyrin (C11c), and β-Amyrin (C11b) and Lupeol (C11a) in $CDCl_3$

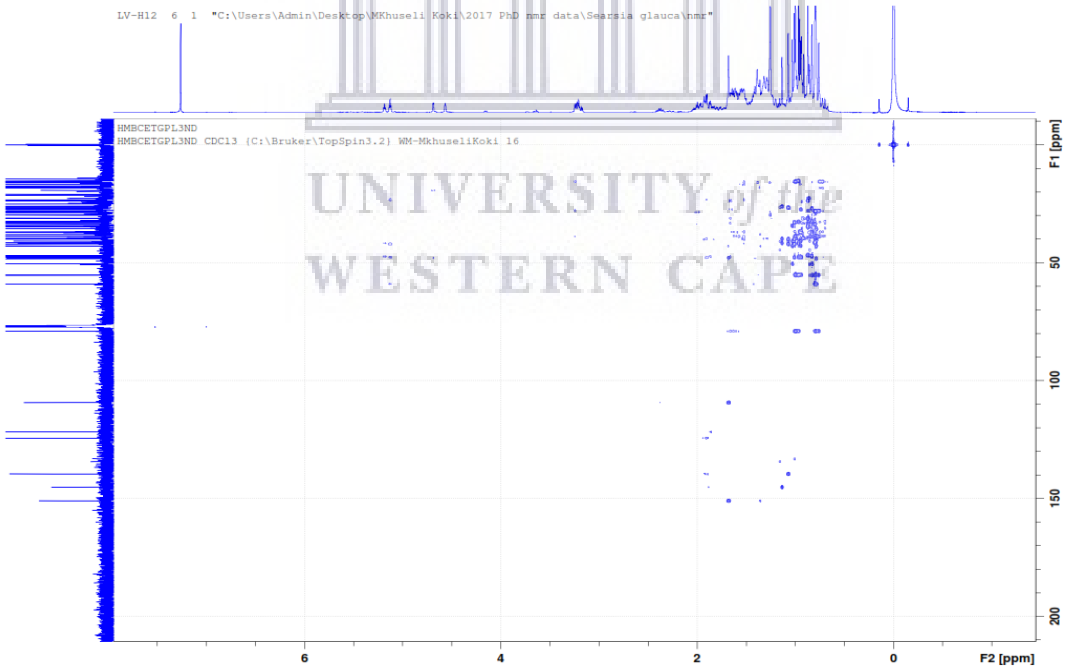


Figure 7. 32: The HMBC of α-amyrin (C11c), and β-Amyrin (C11b) and (Lupeol) (C11a) in $CDCl_3$

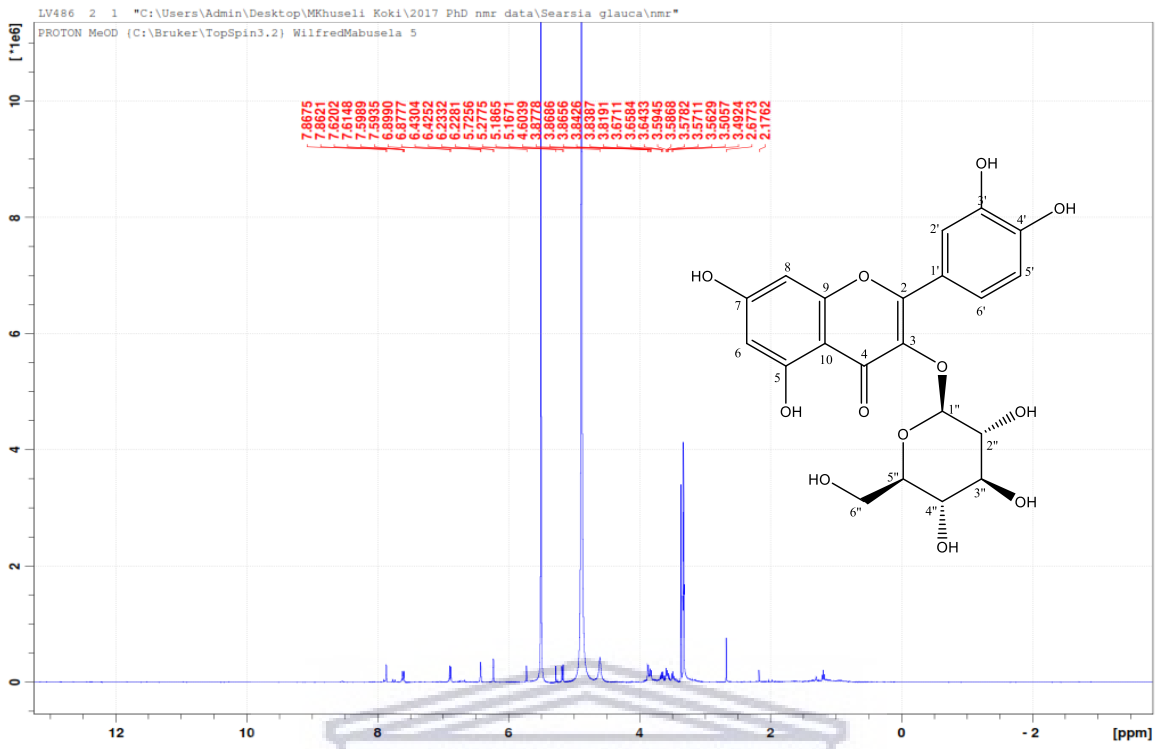


Figure 7. 33 : The ^{1}H NMR of Quercetin-3-*O*-*β*-glucoside (C9) in CD_3OD

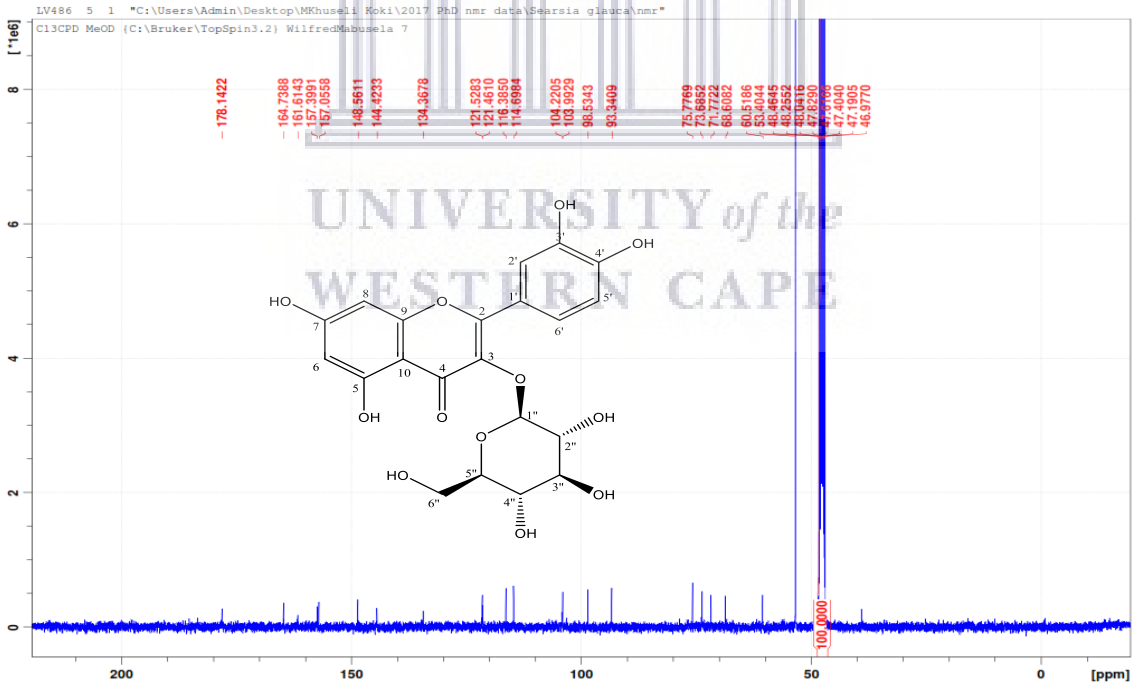


Figure 7. 34 : The ^{13}C NMR of Quercetin-3-*O*-*β*-glucoside (C9) in CD_3OD

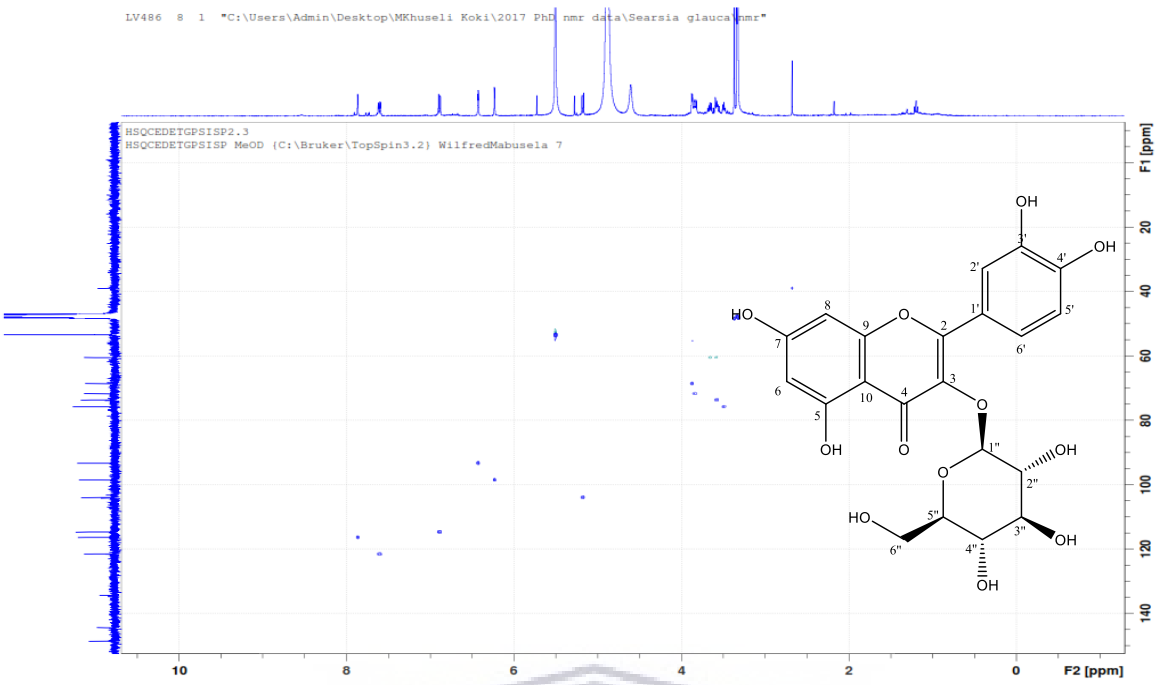


Figure 7. 35 : The HSQC of Quercetin-3-*O*-*β*-glucoside (C9) in CD_3OD

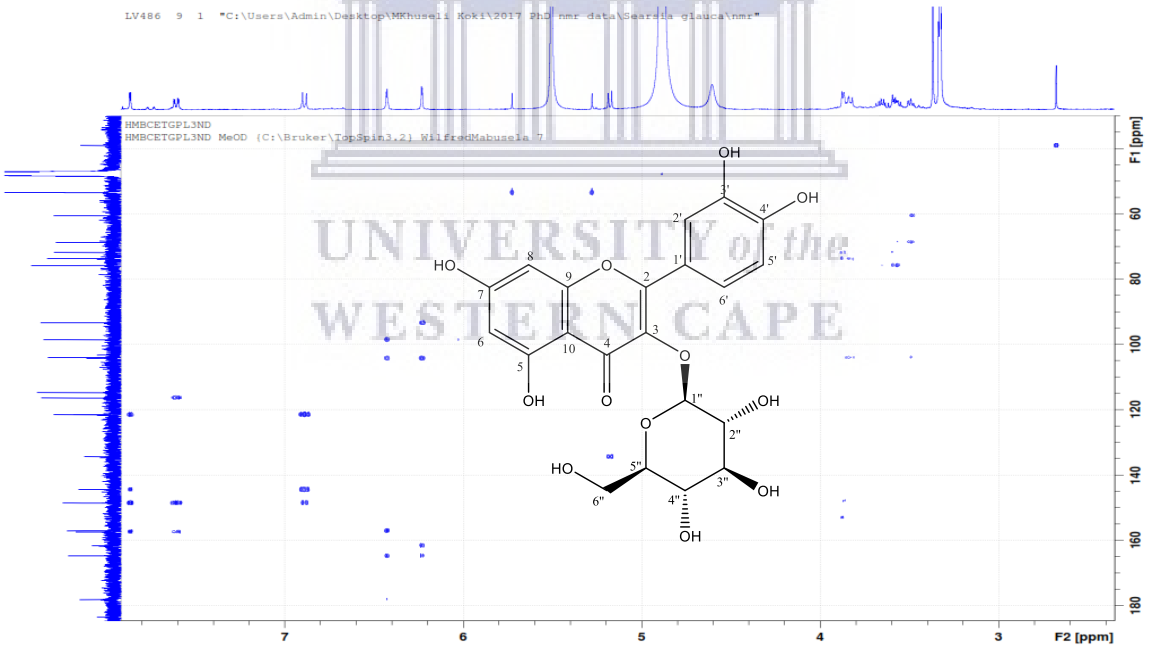


Figure 7. 36 : The HMBC of Quercetin-3-*O*-*β*-glucoside (C9) in CD_3OD

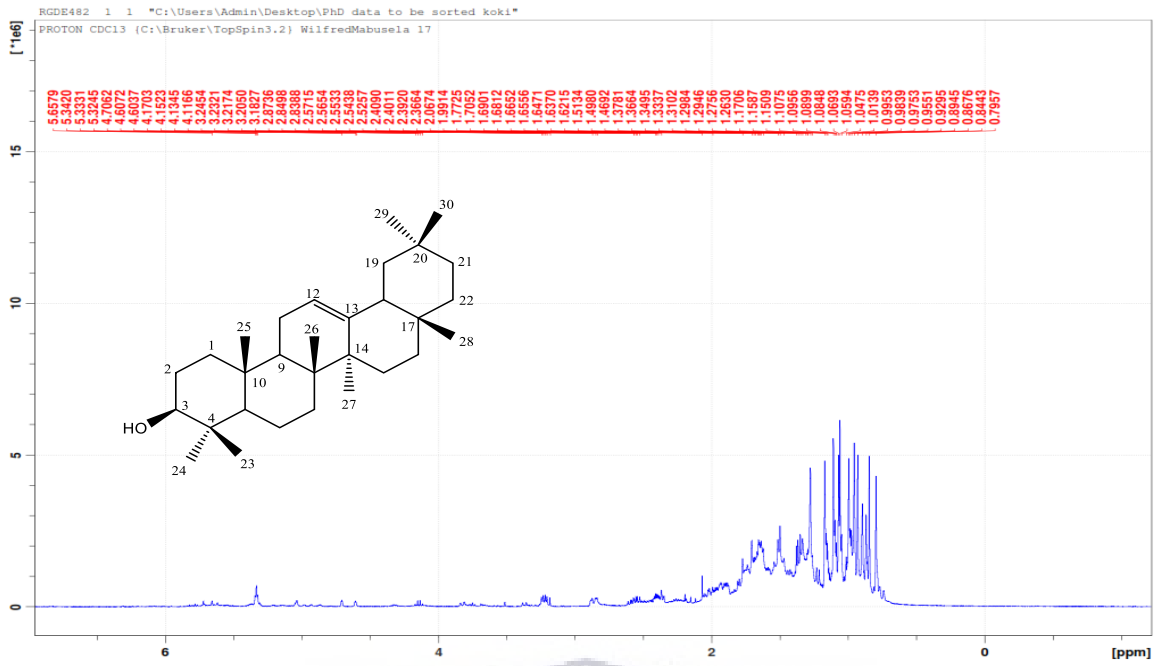


Figure 7. 37 : The ^{1}H NMR spectrum of β-amyrin (C10) in $CDCl_3$

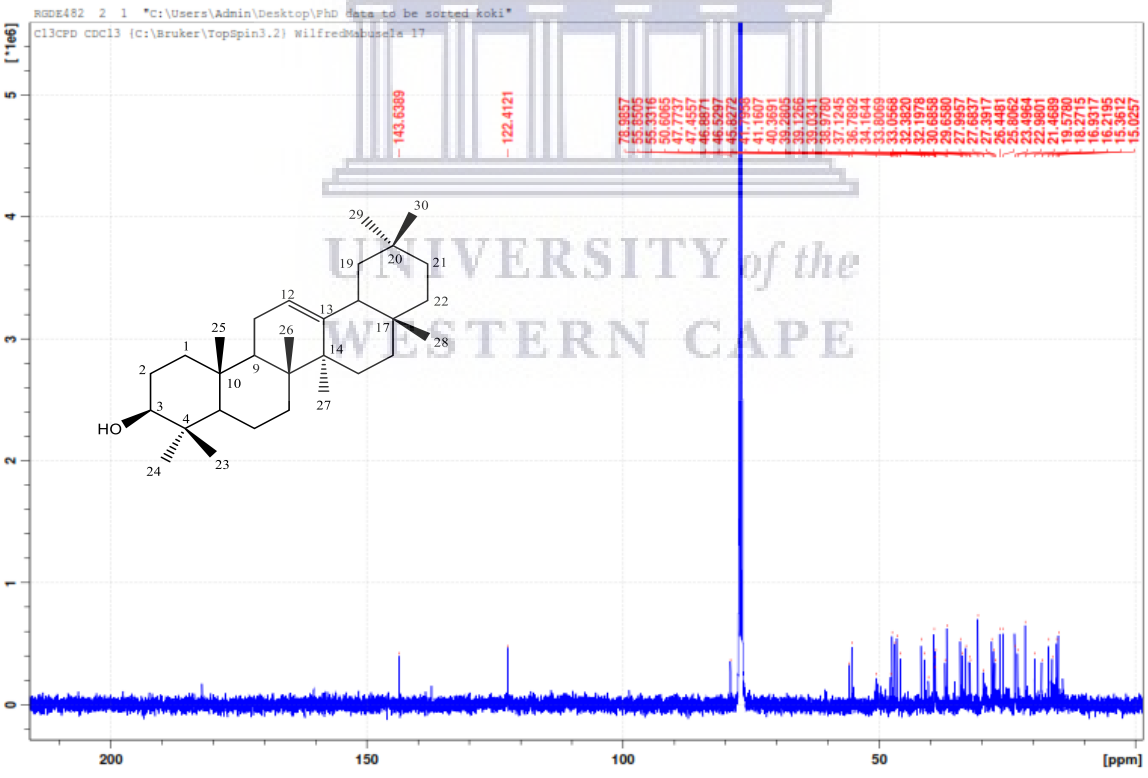


Figure 7. 38 : The ^{13}C NMR spectrum of β-amyrin (C10) in $CDCl_3$

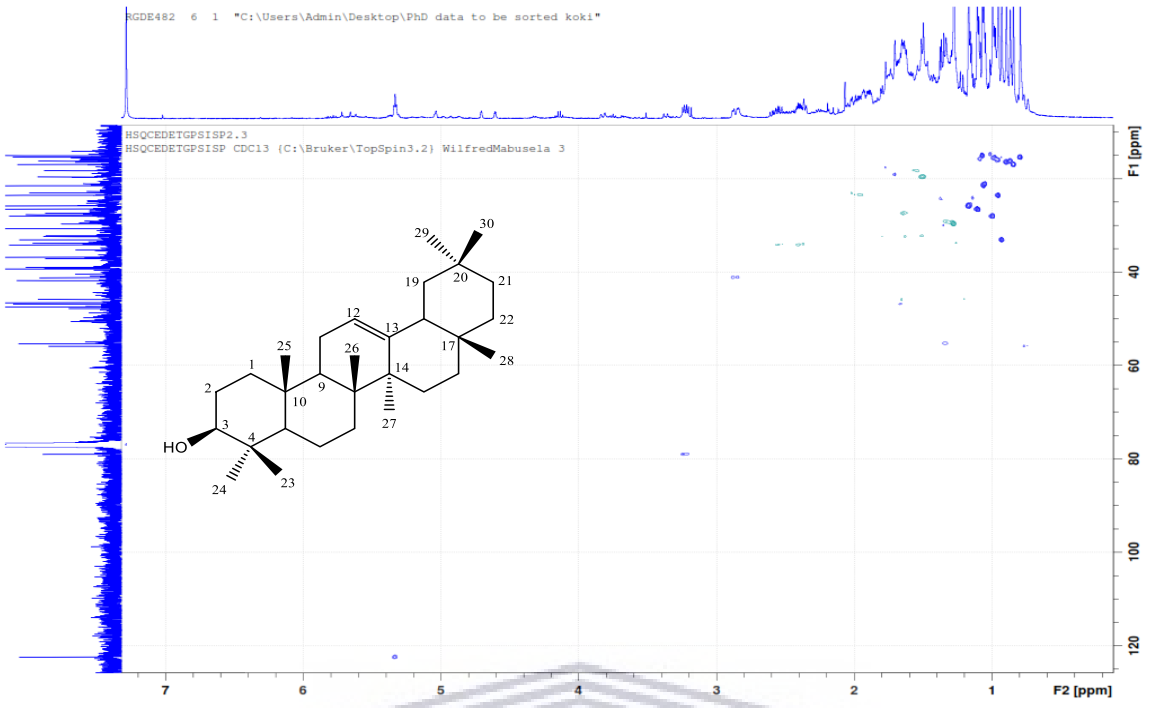


Figure 7. 39 : The HSQC spectrum of *β*-amyrin (C10) in $CDCl_3$

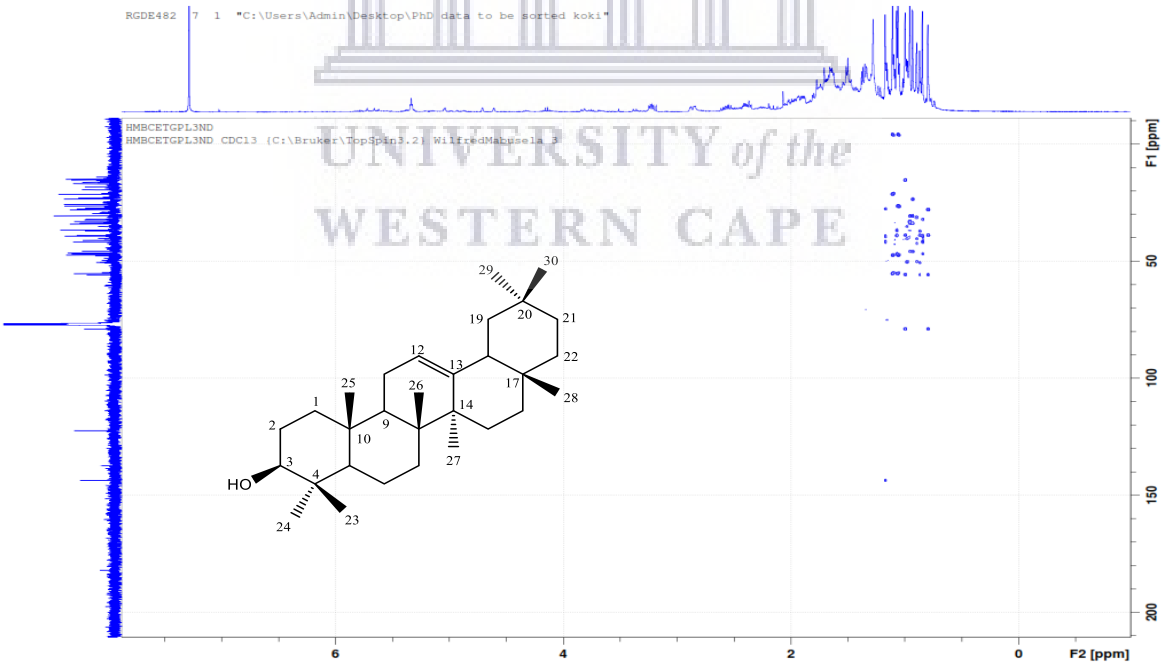


Figure 7. 40 : The HMBC spectrum of *β*-amyrin (C10) in $CDCl_3$