ANTIMICROBIAL ACTIVITIES OF EXTRACTS AND ISOLATES FROM *Senna didymobotrya* FLOWERS.

BY:

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DECLARATION

This thesis has never been presented for examination in Maseno University or in any other University. This is my original work and all sources of information have been duly supported by the relevant references.

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DEDICATION

This work is dedicated to my elder brother Nelson. Your encouragements are highly appreciated.

ABSTRACT

The emerging trends in pathogenic microorganisms becoming resistant to available antibiotics coupled with the evolution of new strains of pathogens causing diseases, is a major concern to the global health community and this requires urgent remedy. Prolonged treatment with conventional antimicrobial drugs has led to their toxicities among patients and therefore, for effective treatment of diseases caused by pathogenic microbes, search for new pharmaceuticals or some potential sources of novel drugs are necessary. Medicinal plants commonly used by various communities could be potential sources for bioprospection to help in the fight against this menace. This study focused on evaluating the antimicrobial activity of *Senna didymobotrya* flowers (Caesalpiniaceae), a plant widely used in Kenyan folklore medicine to manage bacterial and fungal infections. In parts of Kisumu, the flowers of the plant are used to manage diarrhoea and itching of the skin. Although the flowers are traditionally used by herbalists to manage microbial infections, very little work has been done to evaluate the efficacy of the extracts against pathogenic microbes. In addition, compounds responsible for the antimicrobial activity in the flowers have not been isolated and characterized. The objectives of this study were to evaluate the extracts and isolated compounds from flowers for antifungal and antibacterial activities. The dry and pulverized flowers of the plant were sequentially extracted with *n*-hexane, ethyl acetate and methanol. The methanol extract significantly ($P \leq 0.05$) inhibited *M. gypseum (*MIC 3.9µg/ml), *T. mentagrophyte* (MIC 7.8µg/ml), *K. pneumoniae* and *C. albicans* (MIC value 31.3 µg/ml each). This was followed by ethylacetate extract which gave moderate results against *M. gypseum (*MIC 15.6µg/ml), *S. faecalis, B. anthracais, C. albicans* and *T. mentagrophyte* (MIC value 62.5 µg/ml each). *n*-Hexane extract afforded MIC values ranging between 125 µg/ml to over 250 µg/ml. Fractionation of the various extracts led to the isolation of twelve compounds whose structures were determined using physical and spectroscopic methods as well as comparison with literature data. The compounds were: chrysophanol (**7**), physcion (**9**), emodin (**10**), quercetin (**253**), *β*-sitosterol (**302**), stigmasterol (**303**), lupeol (**323**), betulinic acid (**324**), oleanolic acid (**335**), 4', 5-dihydroxystilbene-3-*O*-glucoside (**336**), 5-hydroxyflavone (**337**) and 7-hydroxyflavone (**338**). All the isolated compounds were screened for antimicrobial activities.The most active metabolite was emodin which gave a MIC in the range 15.6-250 µg/ml against all the microorganisms tested except *E. coli* (MIC >250 µg/ml). 7-Hydroxyflavone also afforded good results by displaying MIC values in the range 31.3-250 µg/ml against all microorganisms that were tested. The least active metabolites were *β*-sitosterol and stigmasterol which were observed to show mild activities against *S. aureus* (MIC 250 µg/ml). Activities of the three extracts and isolated compounds against the tested pathogenic microbes were significantly ($P \leq 0.05$) lower compared to the activities exhibited by kanamycin and fluconazole which were used as standards against bacteria and fungi, respectively. This study has authenticated the traditional use of *S.didymobotrya* flowers in the management of bacterial and fungal infections. It has also identified the individual compounds responsible for the activities. Compounds that exhibited high antimicrobial activities such as emodin, and 7-hydroxyflavone may be developed into formulations to be used as antimicrobials.

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ACRONYMS AND ABBREVIATIONS

CC	Column Chromatography
$CDCl_3$	Deuterated Chloroform
^{13}C NMR	Carbon -13 Nuclear Magnetic Resonance
COSY	Correlation Spectroscopy
DEPT	Distortionless Enhancement by Polarization Transfer
DMSO	Dimethyl Sulphoxide
ESIMS	Electrospray Ionization Mass Spectroscopy
HMBC	Heteronuclear Multiple Bond Coherence
^{1}H NMR	Proton Nuclear Magnetic Resonance
Hz	Hertz
IR	Infrared
MIC	Minimum Inhibitory Concentration
MS	Mass Spectroscopy
TMS	Trimethylsilane
TLC	Thin Layer Chromatography
UV	Ultraviolet
s	Singlet
d	Doublet
dd	Doublet doublet
t	Triplet
br s	Broad singlet
m	Multiplet
J	Coupling constant
m/z	Mass to charge ratio

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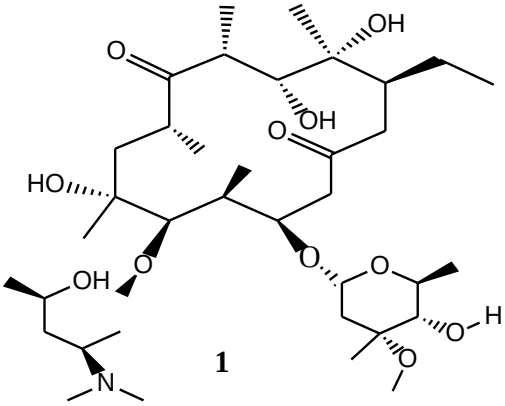
CHAPTER ONE

INTRODUCTION

1.1 Background

Pathogenic diseases are known to be one of the leading causes of high mortality rate worldwide (WHO, 1999; 2000; Bandow *et al*., 2003; Parekh and Chanda, 2007) with over 15 million deaths annually (Morens *et al*., 2004). Diseases caused by pathogenic microorganisms cause major health problems particularly in developing countries due to emergence of microbe strains that are resistant to conventional drugs (Komolafe, 2003). The problem of pathogenic microbes' resistance to antibiotics is due to misuse of drugs and emergence of genetically versatile microbes (Olila *et al*., 2002). Despite the high mortality rate caused by pathogenic diseases, effective and safe drugs that can be used to manage the diseases are still lacking. Some of the conventional antimicrobial drugs currently in clinical applications to manage bacterial infections include erythromycin (**1**), amoxicillin (**2**), cefuroxime axetil (**3**) among others, while fluconazole (**4**), nystatin (**5**) and griseofulvin (**6**) are some of the conventional drugs used to manage the fungal infections. All of them have been found to have limited applications and side effects. For instance, compound (**1**), an antibiotic used to treat Gram-positive bacteriasuch as *Neisseria, Brucella, Campylobacter, Mycoplasma* among others are no longer effective against the microbes (Washington II and Wilson, 1985). The side effects associated with the antibiotic include skin blistering, chills, dizziness, fast heartbeats and skin rash (Jelic and Antoloric, 2016). Compound (**2)** is used against pneumonia, skin and urinary tract infections among other indications. Its limited applications to *Staphylococcus aureus* and *Helicobacter pylori* has been documented (Picoli *et al*., 2014). The side effects associated with the drug are nausea, rash, risk of yeast infection and diarrhea which cause discomfort to patients (Kar, 1997). Compound (**3**) is a semisynthetic cephalosporin which is

used to treat a wide variety of diseases caused by both Gram-positive and Gram-negative bacteria. The pathogens, *Escherichia coli, Haemophilus influenzae, Klebsiella pneumoniae, Haemophilus parainfluenzae, Moraxella catarrhalis,* and *Neisseria gonorrhoeae* have developed resistance to the drug (Brogden, *et al*., 1979). Its side effects include diarrhea, fever, chills, body aches, flu, chest pain and nausea (Leder and Carson, 1997). On the other hand, the fungal drug fluconazole (**4**) is used for preventing and treating a variety of fungal and yeast infections (Albertson *et al.*, 1996). The yeast *Candida albicans* has developed resistance to this drug (Albertson *et al*., 1996). The drug has also elicited side effects among patients including headache, dizziness, diarrhea, stomach and abdominal pains and heartburn (Novelli and Holzel, 1999). Nystatin (**5**) is another antifungal remedy used against fungal diseases of the gut and there have been reports of fungal resistance to the antibiotic (Scheibler *et al*., 2017). The common side effects associated with the drug are diarrhea, nausea, contact dermatitis, skin irritation and hypersensitivity (Scheibler *et al.*, 2017). The unsuccessful treatment of dermatophytes using griseofulvin (**6**) is common (Finkelstein *et al*., 1996). The side effects associated with the drug are similar to those of compound **5**. Though the conventional drugs are effective in controlling microbial infections, their limited capabilities together with their adverse side effects make them unsuitable for use as antibiotics. This has necessitated the need to further bio prospect natural products with an aim of finding a lasting solution to the problem of drug resistance and side effects.



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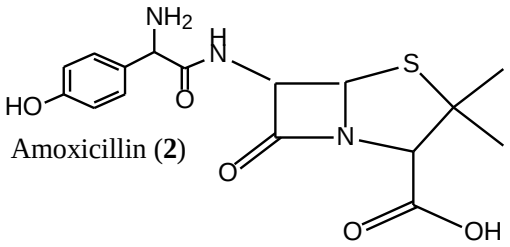
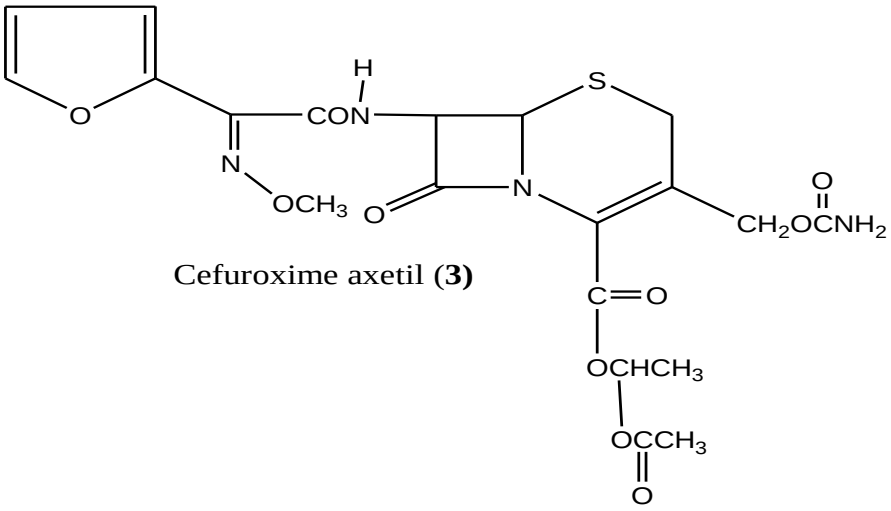
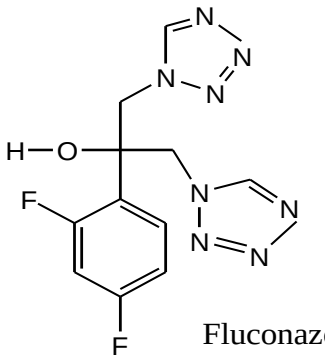


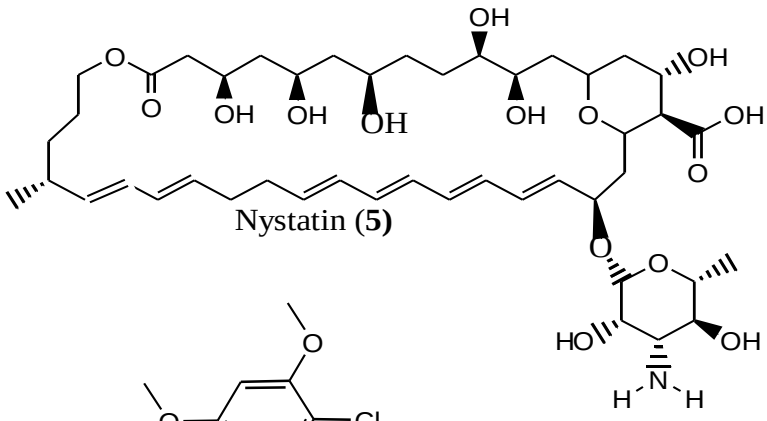
Amoxicillin (**2**)



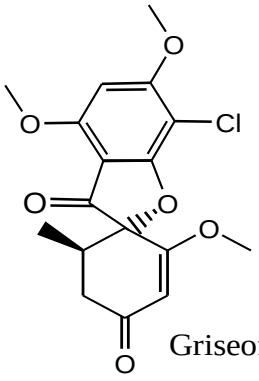
Cefuroxime axetil (**3)**



Fluconazole (**4**)



Nystatin (**5)**



Griseofulvin (**6**)

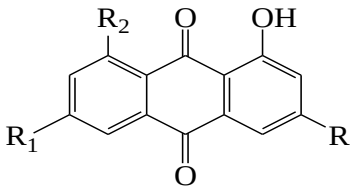
Herbal medicine contributes immensely to primary health care needs of the world population and this is attributed to exorbitant costs or inaccessibility of conventional drugs (Chawla *et al*., 2013). In many indigenous African communities medicinal plants play significant role as readily available alternatives to conventional drugs. In sub-Saharan Africa approximately 80 % of rural population still rely on herbal medicine for primary healthcare and veterinary use (Doughari, 2006). Thus, the issue of population health and socio-economic development is crucial in Africa where infection cases are prevalent (Bloom and Canning, 2008).

In Kenya, herbal medicine is widely practiced and this is evidenced by documented ethnobotanical surveys (Kareru *et al*., 2007; Njoroge and Bussmann, 2006). The limited capability, side effects, high costs and inaccessibility of the antibiotics has led to increased dependence on herbal medicine. Thus, herbal medicine is perceived to be an efficient system deserving consideration from cultural point of view (Kareru *et al*., 2007). Although medicinal plants are used as remedy for microbial infections because of their low costs and ease of availability, in many cases their application has not been subjected to thorough scientific evaluation to validate their use in traditional medicine.

Senna didymobotrya Fresen is a well-known shrub acclimatized in East and Central Africa (Cherono and Akoo, 2000). It is widely used by herbalists as laxative, purgative and treatment of skin related disorders (Nyamwamu *et al.*, 2015). Anthraquinones are considered as chemotaxonomic markers of the genus *Senna* and are believed to be responsible for both the laxative, purgative and antimicrobial activities (Nyamwamu *et al.*, 2015). In Kenya, the plant parts find numerous applications as antimicrobials. The leaves, stem and roots are used to manage microbial infections (Njoroge and Bussmann, 2006). In Kisumu, the flowers of the plant are used to manage diarrhoea and itching of the skin (P. Atieno, "personal communication," January 4th,

2014). Methanolic extract of *Senna didymobotrya* flowers showed antimicrobial activities against *S. aureus* and *E. coli* (Jeruto *et al*., 2018). However, the active components were not isolated and characterized. In addition, the study did not evaluate the antimicrobial activity of the extract against most pathogens used in this study. Extracts from other parts of the plant including leaf, seed, root and stem barks as well as anthraquinones associated with these parts have also showed antimicrobial activities (Anil and Rajmuhon, 2010; Jose *et al*., 2010; Kitonde *et al*., 2014; Korir *et al*., 2012; Chekwujekwu *et al*., 2006). The leaves, pods, stem and root barks have been phytochemically investigated for phytopharmaceutical products and the major phytochemical constituents associated with the parts have been reported to be anthraquinones and flavonoids (Mining *et al*., 2014; Muiru *et al*., 2019). Different parts of *Senna didymobotrya* have been found to have different compounds whereas certain compounds are commonly found in all the other parts of the plant that have been studied. For instance, chrysophanol (**7**), aloe-emodin (**8**), physcion (**9**), emodin (**10**) and rhein (**11)** are compounds which have been isolated from the leaves (Alemayehu *et al*., 1989). Further search for anthraquinones from the pods led to isolation of emodin (**10**) and other compounds (Alemayehu *et al*., 1993) which were not isolated from the leaves. Elsewhere, phytochemical study using the plant stem bark extract resulted in isolation of chrysophanol (**7**), aloe-emodin (**8**), physcion (**9)** together with two other compounds (Legesse, 1998) which were neither isolated from leaves nor from the pods. Chemical evaluation of the root extract of the same plant led to isolation of similar anthraquinones from the leaves and two others (Alemayehu *et al*., 2015) which had not been isolated from the other parts.The phytochemical constituents associated with flowers of *Senna didymobotrya* include terpenoids, flavonoids, phenols, steroids and alkaloids (Jeruto *et al*., 2016). However, the study did not involve isolationof compounds. So far, there are no reports of isolated compounds from *Senna didymobotrya* flowers, hence, it is not

known whether the flowers have similar compounds or different from the ones already isolated from the other parts of the plant.Compounds **7-11** isolated from different parts of *S. didymobotrya* including the roots, stem, leaves and pods have all been found to have antimicrobial activities (Chekwujekwu *et al*., 2006). This suggests that flowers of the plant could also be having phytoconstituents with antimicrobial activities. Therefore, the present work evaluated the extracts and phytoconstituents of flowers against pathogenic microbes.



7 R = CH_3, R_1 = H, R_2 = OH

8 R = CH_2OH, R_1 = H, R_2 = OH

9 R = CH_3, R_1 = OCH_3, R_2 = OH

10 R = CH_3, R_1 = OH, R_2 = OH

11 R = CO_2H, R_1 = H, R_2 = OH

1.2 Statement of the Problem

Diseases caused by pathogenic microbes are major health impediments globally due to emergence of microbe strains that are resistant to conventional drugs. Also side effects associated with available conventional drugs have urged the need for bioprospecting natural products that are effective and safe to overcome the paradox of drug resistance and side effects. Different parts of *Senna didymobotrya* including stem and root barks, leaves, pods and seeds as well as flowers are widely used ethnobotanically as a remedy for various ailments including microbial infections. For instance, root and stem barks, pods and seeds as well as leaf decoctions have been observed to possess activities such as antibacterial and antifungal. Although the plant flower decoction is used by herbalist as a remedy for microbial related diseases, evaluation of the extract against pathogenic microbes is limited. Phytochemical reports on *S. didymobotrya* parts have afforded an array of secondary metabolites, some of which are antimicrobials. However, there are no reports on isolation of secondary metabolites from the flowers of the plant and their biological activities. In

fact, it is not known whether the flowers contain antimicrobial metabolites which have been observed in other part

1.3 Objectives

General objective

To evaluate extracts and isolates of *S. didymobotrya* flowers for their antimicrobial activities.

Specific objectives

1. To determine the antibacterial and antifungal activities of extracts of *S. didymobotrya* flowers.
2. To isolate and characterize metabolites from flowers of the plant.
3. To determine the antibacterial and antifungal activities of isolates from flowers.

1.4 Null hypothesis

1. Extracts from the flowers of *Senna didymobotrya* do not exhibit antimicrobial activities against the test microorganisms.
2. Isolates from flower extracts do not exhibit antimicrobial activities.

1.5 Research Question

1. Have the compounds from the flowers of *Senna didymobotrya* been isolated and characterized?

1.6 Justification

Humanity has suffered loss of human labor especially in the rural areas due to increasing rate of microbial infections. This is due to the limited capabilities of conventional antibiotics caused by

microbial resistance and their side effects. Therefore, there is urgent need to further bio prospect natural products with an aim of finding compounds which can act as templates in synthesis of effective and safe drugs against microbialin fections, hence reduced death rate and improved work productivity.

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CHAPTER TWO

LITERATURE REVIEW

Pharmaceutical companies have made tremendous efforts to manufacture an array of new antibiotics during the last three decades to combat infectious diseases. Pathogenic resistance to antibiotics is a worrying trend that the World Health Organization (WHO) warns of a 'post-antibiotic era' (WHO, 2009). For instance, 5% of *Escherichia coli* isolated from hospitals in the Netherlands are resistant to fluoroquinolones (Goettsch *et al*., 2000). In an attempt to manage and control microbial infections, WHO recommends for sustainable investment in new medicines (WHO, 2014). Palombo (2009) also recommended the continued search for novel active principles from medicinal plants to counter the problem of microbial resistance. The problem is a global concern as the number of people infected in hospitals with new bacterial strains, which are multi-drug resistant has increased leading to high mortalities. There are reports of high incidences of resistant microorganisms in clinics all over the world (Montelli and Levy, 1991; Cohen, 1992) and the problem continues to grow with the outlook for the use of antimicrobial drugs as the lead therapeutic agents in the future remaining bleak. Therefore, there is need to counter this situation by development of research to introduce new drugs, either through synthesis or bioprospecting natural resources.

Plants have been used in nearly all cultures with varying degree of success as a source of medicine (Kinghorn *et al*., 2011). They have been identified as promising avenues for bioactive compounds (Khan *et al*., 2015). The widespread applications of plants in traditional medicine particularly in developing countries is attributed to their availability andconvinience as a source of primary health care for the rural folk. In fact, approximately 80% of Africansdepend on traditional medicine and out of this about 85% use plant extracts in their healthcare systems (Farnsworth, 1985). Farnsworth (1985) also observed that about 20% of patients seeking medication in hospitals first consult

herbalists. Use of medicinal plants as a remedy for microbial infections is considerably a cheaper option, however, in many cases their application has not been subjected to thorough scientific evaluation to validate their use in traditional medicine (WHO 2002-2005).

2.1 Herbal Medicine Practice in Kenya.

Traditional medicine is a concept that is well known in developing countries including Kenya due to its social, economic and cultural significance. World Health Organization considers herbalism as the art of knowledge, skills and practices which are based on the theories, beliefs and experiences local to different cultures (WHO 2002-2005). In Kenya, management of pathogenic diseases has been done by the use of medicinal plants (Kokwaro, 2009). The plants constitute great economic wealth and strategic potential to the Kenyan population. However, realization of full exploitation of the potentially useful plants is yet to be achieved due to poor documentation and lack of proper scientific validation of the herbal drugs, the safety and efficacy (Kigen *et al*., 2013). The country healthcare system is struggling to cope with demand, high costs and adverse effects of conventional drugs as well as drug resistant microbes and all these promote the significance of traditional medicine application in Kenya (Olila *et al*., 2002). Herbal medicine is the most popular form of traditional medicine which contribute immensely to healthcare particularly in resource limited settings (Calixto, 2000; Okumu *et al*., 2017). Kenya has vast resources of medicinal plants and knowledge supporting their use (Okumu *et al*., 2017; Kigen *et al*., 2013). Traditional medicine practice in Kenya has a rich and diverse culture which brings together different ethnic groups each with a unique and inherent knowledge of medicinal plants (Njoroge and Bussman, 2007). It is estimated that about 1,200 plant species are used to offset the healthcare needs of communities and animals in Kenya (Njoroge and Bussman, 2007). The use of traditional medicine in Kenya is widespread particularly in rural areas with the ratio of traditional medicine practitioners to patients

being approximately 1:378 in the rural and about 1: 833 in the urban areas (Orwa *et al*., 2007; Nyika, 2007). Tropical diseases are on the rise in Kenya and this is causing strain on the available resources (Maundu *et al*, 2006). Maundu *et al*, (2006) also observed that majority of the population who are unable to afford modern health care visit traditional medicine practitioners whenever they fall ill. The National Museums of Kenya and the Institute of Primate Research have teamed up to tap the potential of traditional medicine practitioners with an aim to develop and commercialize products arising from the practice of traditional medicine (Maundu *et al.,* 2006).

2.2 Microbial Infections in Kenya.

2.2.1 Bacterial Infections

Bacterial infection is a proliferation of a harmful strain of bacteria on or inside the body. Persistent bacterial infections pose significant public health problems (Kovacs *et al*., 1997). Resistance to drugs used to treat bacterial infections causes prolonged hospitalization and sometimes death of the infected person (Holmberg *et al*., 1987). This leads to high economic burden due to increased cost of treatment and loss of skilled personnel, hence need for continued search for new compounds which exhibit activity against bacterial infections.

2.2.2 Fungal Infections

Kenya has a high rate of tuberculosis (TB) and HIV infection burden. These two diseases predispose patients to the development of opportunistic fungal infections (Saag, 1997). Nearly 7% of the Kenyan population suffer from serious fungal infections each year (Guto *et al*., 2016). This causes reduced work productivity hence economic losses. The antifungal agents currently available for managing fungal infections are expensive, have side effects and are ineffective leading to increase in occurrence of resistant strains of pathogenic microbes (Frontling, 1987). For

instance, the fungal drug used in treatment of cetaceous and systematic fungal disease cause liver problem (Frontling *et al.,* 1987). This calls for further research to identify new compounds which exhibit activity against fungal infections.

2.3 Fabaceae Family.

The family Fabaceae also known as Leguminosae represents the largest family of flowering plants (Hegnauer and Grayer-Barkmeyer, 1993). It is compost of over 20000 species spread in 650 genera (Schrire *et al*., 2005). Fabaceae family is traditionally divided into three subfamilies namely; Caesalpinioideae, Mimosoideae, and Faboideae (or Papilionoideae) of which each has been considered a separate plant family in the past (Schrire *et al*., 2005). Schrire *et al*., (2005) also observed that classifications based on molecular analyses now separate Caesalpinioideae into several lineages and recognize the tribe Cercideae as a separate and more basal group in the family. Phytochemicals associated with the family include flavonoids, anthraquinones, alkaloids and terpenoids (Wojciechowski, *et al*., 2004). The family is economically exploited as a source of medicine, ornaments, dyes, timber, fodder, tannins, resin, essential oils, flavor, pesticides and food (Kokwaro, 2009).

2.4 The Sub-family Caesalpinioideae.

The Caesalpinioideae subfamily (classified as a family, Caesalpiniaceae, by some authorities) is a heterogeneous group of plants consisting of approximately 160 genera and some 2,000 species (Hou *et al.,* 1996). This sub-family is the most basal lineage among the legumes and the one from which the other two subfamilies, Mimosoideae, and Faboideae evolved (Pettigrew and Watson, 1977). Caesalpinioideae legumes are found throughout the world but are primarily woody plants in the tropics. Their moderate secondary invasion of temperate regions is mostly by herbaceous (non woody) evolutionary derivatives (Pettigrew and Watson, 1977). The leaves are usually

compound, flowers vary in symmetric form, from radial to bilateral to irregular (Hou *et al*., 1996). Many of the Caesalpiniaceae species are prized ornamentally as well as finding applications in folklore medicine (Monkheang *et al*., 2011). The plants produce many kinds of chemical substances such as anthraquinones, alkaloids, flavonoids, tannins, and the free amino acids (Kubmarawa*et al*., 2007).

2.5 The Genus Senna

This is a genus of flowering plants within the subfamily Caesalpinioideae tribe. It is native throughout the tropics, with a small number of species in temperate regions (Randell and Barlow, 1998). About 50 species of *Senna* are in cultivation (Randell and Barlow, 1998). The genus is composed of herbs, shrubs, and trees (Huxley *et al*., 1992). The leaves are pinnate with opposite paired leaflets. The inflorescences are racemes at the ends of branches or emerging from the leaf axils. The flower has five sepals and five usually yellow petals. There are ten straight stamens. The stamens may be different sizes, and some are staminodes. The fruit is a legume pod containing several seeds (Huxley *et al*., 1992).

2.5.1 Ethnobotany of *Senna* Species

Ethnobotanical studies have shown that *Senna* species have various medicinal uses (Ogunkule *et al*., 2006). Members of the genus *Senna* have been used as laxatives and purgatives in different countries (Kokwaro, 2009). The leaves, flowers, pods, stem and root barks of these plants have been extensively used to treat diseases including malaria, skin disease, gonorrhea, anthelmintics, syphilis and stomachache (Kokwaro, 2009). Different parts of *Senna didymobotrya* find numerous applications as antimicrobials. The leaves, stem and roots are used to manage microbial infections in Kenya (Njoroge and Bussmann, 2006). In Kisumu, the flowers of *Senna didymobotrya* are used to manage diarrhoea and skin diseases (P. Atieno, "personal communication," January 4th, 2014).

Some of the traditional uses of Senna species, as documented by (Kokwaro, 2009) are tabulated in Table 1.0.

Table 1.0: Ethnobotany of *Senna* Species

Plant name	Plant part used	Purpose
S. didymobotrya	Root and stem barks, leaves, and pods	Antimalarial, laxative, purgative, gonorrhea, cattle skin diseases, backaches, against ringworm, appetizer, antidote
S. petersiana	Root, leaf,	Gonorrhea, hematuria, sterility, purgative, stomachache, anthelmintic, skin disease, coughs, syphilis
S. occidentalis	Leaf, root	Abdominal pain, snake-bite, against round worm, oedema, fevers, malaria, antidote, stomachache, kidney troubles, pain-killer
S. singueana	Root, bark, leaf	Gonorrhea, heartburn, purgative, stomach troubles,
S. sophera	Seed, root	Laxative, skin disease, acute bronchitis
S. italic	Leaves, roots	Laxative, gonorrhea
S. septemtrionalis	Leaves, fruits	Promote menstruation, purgative
S. alexandrina	Root, bark, leaves	Purgative
S. obtusifolia	Upper parts of the plant	Stomach trouble, quicken birth, antibiotic
S. bicapsularis	Roots	Stomach complaints in children

2.5.2 Antimicrobial Activities of Genus *Senna*

Some species from this genus have been found to have antimicrobial activities as shown in Table 2.0. *Senna didymobotrya* is widely used in traditional medicine as laxative, purgative and treatment of skin related disorders (Nyamwamu *et al.*, 2015). In Kenya, the plant parts find numerous applications as antimicrobials (Kokwaro, 2009). The leaf, seed, root and stem bark extracts as well

as anthraquinones associated with these parts of *S. didymobotrya* showed antimicrobial activities (Anil and Rajmuhon, 2010; Jose *et al*., 2010; Kitonde *et al*., 2014; Korir *et al*., 2012; Chekwujekwu *et al*., 2006). *Senna didymobotrya* flower extract has showed antimicrobial activities against *S. aureus* and *E. coli* (Jeruto *et al*., 2018). However, the active components were not isolated and characterized. In addition, the study did not evaluate the antimicrobial activity of the extract against most pathogens used in this study.

Table 2.0 Antimicrobial Activities of some Senna Species

Species	**Part or compound responsible for the antimicrobial activity**	**Reference**
S. didymobotrya	Leaf, flowers, root and stem barks and seeds extracts, anthraquinones	Anil and Rajmuhon, 2010; Jose *et al*., 2010; Kitonde *et al*., 2014; Korir *et al*., 2012; Jeruto *et al*., 2018; Chekwujekwu *et al*., 2006.
S. alata	leaf extract	Palanichamy *et al*., 1990; Timothy *et al*., 2012
S bakeriana	Leaf extract	Cunha *et al*., 2017
S glauca	seed extract	Deepak *et al*., 2013
S. occidentalis	Root bark extract, anthraquinones	Chukwujekwu *et al*., 2006
S. surattensis	Flower extract	Sumathy *et a*l., 2014
S. auriculata	Leaf and flower extract	Subhadradevi *et al*., 2011
S. obtusifolia	Leaf extract	Doughari *et al*., 2008
S. fistula	Flower extract	Bhalodia *et al*., 2011

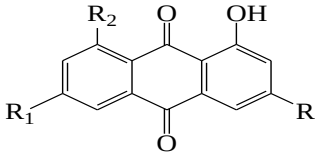
2.6 Phytochemistry of Senna Species

Phytochemical studies on the genus *Senna* has led to the isolation and characterization of different classes of secondary metabolites with anthraquinones being the most abundant.

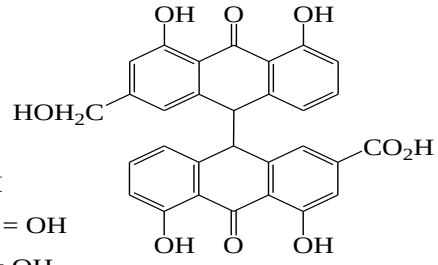
Alkaloids, flavonoids, xanthones, tannins and terpenoids have also been reported to occur in this genus (El-Sayyad and Ross, 1983).

2.6.1 Anthraquinone Compounds of the Genus *Senna*

Anthraquinones are secondary metabolites which are taxonomic markers of the genus *Senna* and their existence is almost evidenced in all parts of these plants. These compounds with the anthracene skeleton have been documented as dihydroxy, trihydroxy, pentahydroxy and hexahydroxy derivatives (Mining *et al.*, 2014; Muiru *et al.*, 2019). Also a number of dimeric anthraquinones have been isolated (Kinjo *et al.*, 1994) and all of them have only C-C linkage in between the two units. No bianthraquinone has been reported with a C-O-C interanthraquinoid linkage. Phytochemical analysis of leaf and root extracts of *Senna absus* for anthraquinone derivatives led to the isolation of chrysophanol (**7**) and aloe-emodin (**8**) (Krishna Rao *et al*., 1979). Compounds **7** and **8,** along with physcion (**9**), emodin (**10**), rhein (**11**), aloe-emodin-8-*O*-*β*-D-glucoside (**12**), emodin-8-*O*-*β*-D-glucoside (**13**), rhein-8-*O*-β-D-glucoside (**14**), sennidin C (**15**) and sennidin (**16**) have been reported from culture suspension of *S. acutifolia* (Nazif *et al*., 2000).



7 R = CH_3, R_1 = H, R_2 = OH
8 R = CH_2OH, R_1 = H, R_2 = OH
9 R = CH_3, R_1 = OCH_3, R_2 = OH
10 R = CH_3, R_1 = OH, R_2 = OH
11 R = CO_2H, R_1 = H, R_2 = OH
12 R = CH_2OH, R_1 = H, R_2 = Oglu
13 R = CH_3, R_1 = OH, R_2 = Oglu
14 R = CO_2H, R_1 = H, R_2 = Oglu



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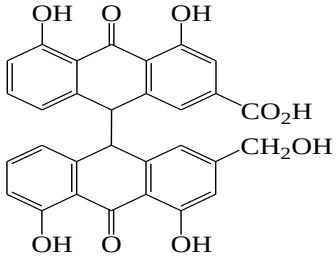
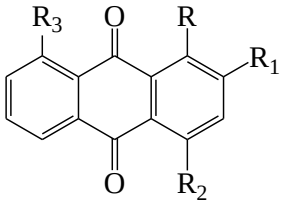
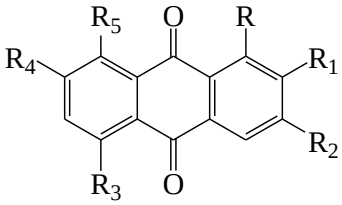


16

Senna alata is a plant widely distributed in the tropics mainly in western and eastern Africa and India. Fractionation of the various plant parts have resulted in the isolation of compounds **7-11**

together with isochrysophanol (**17**), 1,4-dihydroxy-2-hydroxymethylanthraquinone (**18**), 2-hydroxymethylanthraquinone (**19**), 1,3,8-trihydroxy-2-methylanthraquinone (**20**), 1,5,7-trihydroxy-3- hydroxymethylanthraquinone (alatinone) (**21**) and 2-formyl-1,3,8-trihydroxyanthraquinone) (alatonal) (**22**) (Hemlata and Kalidhar,1993; Hofilena *et al*., 2000; Panichayupakaranant and Intaraksa, 2003).





17 R = R_3 = OH, R_1 = CH_3, R_2 = H

18 R = R_2 = OH, R_1 = CH_2OH, R_3 = H

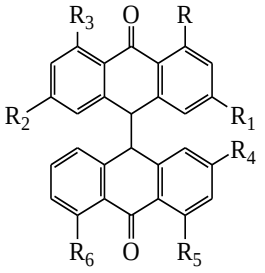
19 R = R_2 = R_3 = H, R_1 = CH_2OH

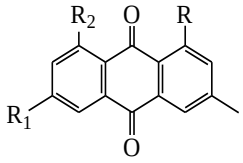
20 R = R_2 = R_5= OH, R_1 = CH_3, R_3 = R_4 = H

21 R = R_3 = R_4 =OH, R_2 = CH_2OH, R_1 = R_5=H

22 R = R_2 = R_5 = OH, R_1 = CHO, R_3 = R_4 =H

On the other hand, chemical analysis of *C. angustifolia* leaves revealed the presence of the usual anthraquinones **7-11** along with aloe-emodin dianthraone (**23**), emodin-8-*O*-sophoroside (**24**), aloe-emodin dianthrone diglucoside (**25**), sennoside A (**26**), sennoside B (**27**), sennoside C (**28**) and sennoside D (**29**) (Dave and Ledwani, 2012). From the pods, callus culture and seedlings of the plant, Friedrich and Baier (1973) reported the isolation of all compounds reported from the leaves in addition to chrysophanein (**30**) and physcionin (**31**).





23 $R_1 = R_4 = CH_2OH$, $R = R_3 = R_6 = OH$, $R_2 = H$

24 $R = R_1 = OH$, R_2 = Oglu (2, 1)-glu

25 $R_1 = R_4 = CH_2OH$, $R_6 = R_3$ = Oglu, $R = OH$, $R_2 = H$

30 R = Oglu, $R_1 = H$, $R_2 = OH$

26 $R = R_5 = OH$, $R_1 = R_4 = CO_2H$, $R_2 = H$, $R_3 = R_6$ = Oglu

31 $R = OH$, $R_1 = OMe$, R_2 = Oglu

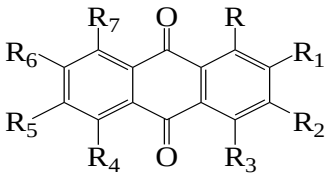
27 $R = R_6$ = Oglu, $R_1 = H$, $R_2 = R_4 = CO_2H$, $R_3 = R_5 = OH$

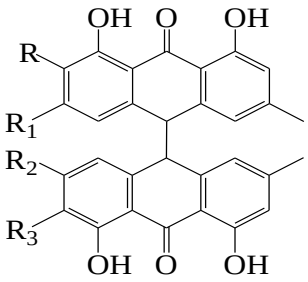
28 $R = R_5 = OH$, $R_1 = CH_2OH$, $R_4 = CO_2H$, $R_3 = R_6$ = Oglu, $R_2 = H$

29 $R = R_6$ = Oglu, $R_1 = H$, $R_4 = CO_2H$, $R_3 = R_5 = OH$, $R_2 = CH_2OH$

Senna didymobotrya is a widely used medicinal plant in its native range. Anthraquinone derivatives **7-11** together with fallacinol (**32**), parietenic acid (**33**) and torosachrysone (**34**) have been reported from the leaves of the plant (Alemayehu *et al*., 1989). Two years later Monache *et al* (1991) reported the isolation of 7-acetyl chrysophanol (**35**) and chrysophanol-physcion-10,10'-bianthrone (**36**) from culture suspension of the same plant. Further search for anthraquinones from the plant pods led to the isolation of compound **10** together with chrysophanol-10,10`-bianthrone (**37**), chrysophanol-isophyscion-10,10'-bianthrone (**38**) and physcion-10,10'-bianthrone (**39**) together with 10-hydroxyl-10-10'-(physcion-7'-yl)-chrysophanolanthrone (**40**), 9-(physcion-7'-yl)-5, 10-dihydroxy-2-methyl-1,4-anthraquinone (**41**) and knipholone (**42**) (Alemayehu, 1996). Alemayehu *et al.*, (1993), using callus culture documented the isolation of an array of anthraquinone derivatives including compound **34**, emodin-6, 8-dimethyl ether (**43**), chrysophanol-8-methyl ether (**44**), emodin-1, 6-dimethyl ether (**45**), nataloe-emodin-8-methyl ether (**46**), questin (**47**) and germychrysone (**48**) . Elsewhere, phytochemical study using the plant stem bark extract resulted in the extraction of compounds **7-9** together with 2-methoxystypandrone

(**49**), 10-(chrysophanol-7‘-yl)-10-hydroxychrysophanol-9-anthrone (**50**) (Legesse, 1998). Chemical evaluation of the root extract of the same plant led to the isolation of seven anthraquinone derivatives **7, 9, 34, 36, 49** together with obtusifolin (**51**) and 1,6-*O*-dimethylemodin (**52**) (Mining *et al*., 2014). One year later, fractionation of *Senna didymobotrya* CH_2Cl_2/CH_3OH (1:1) root extract afforded a phenylanthraquinone (**53**). This marked the first report of the compound from the genus Senna **(**Alemayehu *et al*., 2015). From literature, phytochemical analysis of *Senna didymobotrya* has led to isolation of different compounds from different parts. Some compounds are commonly found in all the parts that have been studied. It is not known if flower extract also has similar or different compounds compared to the ones already isolated from the other parts since so far there are no phytochemical work on the plant flowers. Compounds **7-11** isolated from different parts of *S. didymobotrya* including the roots, stem, leaves and pods have all been found to have antimicrobial activities (Chekwujekwu *et al*., 2006). This suggests that flowers of the plant could also be a potential source of antimicrobial compounds. Therefore, the present work evaluated the extracts and phytoconstituents of flowers against pathogenic microbes.





32 R=R_7 =OH, R_2 = CH_2OH, R_1= R_3=R_4=R_6=H, R_5 = OMe

33 R=R_7 =OH, R_2 = CO_2H, R_1= R_3=R_4=R_6=H, R_5 = OMe

35 R =R_7= OH, R_1 = R_3 = R_4 = R_5=H, R_6 =Ac

36 R = R_1 = H, R_2 = OMe, R_3 =H

37 R = R_1 = R_2= R_3= H

38 R = R_1 = R_2=H, R_3 = OMe

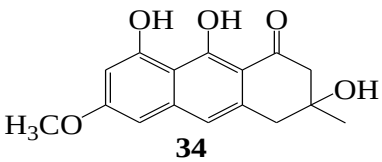
39 R=R_3=H, R_1=R_2=OMe

43 R=OH, R_1=R_3= H, R_5=Me, R_6=H, R_2 =Me, R_7=OMe

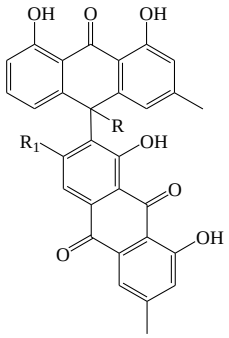
44 R= OH, R_2=Me, R_1=R_3=R_4=R_5=R_6 =H, R_7= OMe

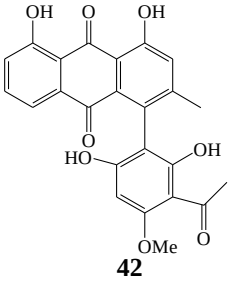
45 R=R_5=OMe, R_1=R_3=R_4=R_6=H, R_2= Me

46 R=R_5=OH, R_2= Me, R_1=R_3=R_4=R_6=H, R_7=OMe



34





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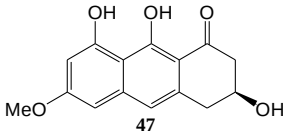

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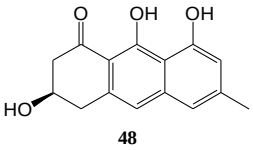
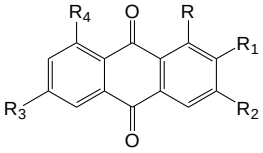


48

40 R = OH, R_1 = OMe

41 R = H, R_1 = OMe

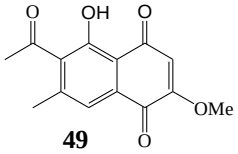
50 R = OH, R_1= H



51 R = OMe, R_1 = R_4 = OH, R_2 = Me, R_3 = H

52 R = R_3 = OMe, R_1 = H, R_2 = Me, R_4 = OH

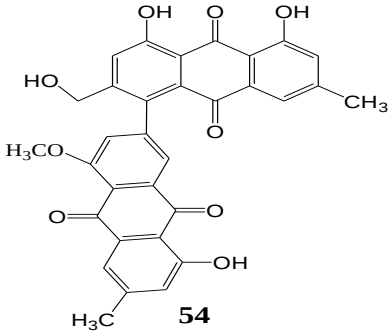
53 R = R_2 = R_3 = R_4 = H, R_2 = ph



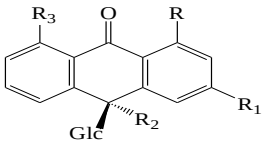
49

Chemical studies done on the leaves, pods, callus and seedlings of *S. angustifolia* resulted in the isolation and identification of a bianthraquinone 5, 7'-biphyscion (floribundone-1) (**54**) along with several known anthraquinone derivatives (Kinjo *et al*., 1994). Similar work on *S. auriculata* parts by Lohar *et al* (1981) afforded 1,5,8-trihydroxy-6-methoxy-2-methylanthraquinone-3-*O*-β-D-glalactopyranosyl(1→4)-*O*-β-D-mannopyaranoside (**55**), 3-hydroxy-6,8-dimethoxy-2-methyl anthraqinone-1-*O*-β-D-galactoside (**56**) and rubiadin (**57**), along with compounds **7-9**. From the flower extract of *S. biflora* compounds **7** and **9** have been reported (Hemlata and Kalidhar, 1995). Phytochemical evaluation of *S. fistula* wood and leaf extracts gave rhein glucoside (**58**) together with known compounds **7, 9, 11, 26** and **27** (Dutta and De, 1998). Rani and Kalidhar (1998) isolated fistulic acid (**59**), fistulin (**60**), fistulin rhamnoside (**61**), 1,8-dihydroxy-6-methoxy-3-methyl anthraquinone (**62**), 3-formyl-1-hydroxy-8-methoxyanthraquinone (**63**), aloin (**64**), and barbaloin (**65**) along **7, 8, 10, 11, 26, 27, 28, 29, 30** and **60** from the plant pods, fruit pulp, seeds, stem bark, flowers and pods. Studies on *S. garrettiana* heartwood afforded cassialoin (10-hydroxy-

10-C-D-glucosylchrysophanol-9-anthrone) (**66**) and the common anthraquinone derivatives (Hata *et al*., 1978).

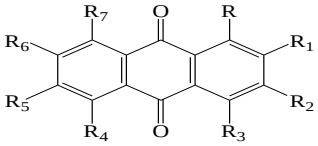


54



65 R = H, R_1 =CH_2OH, R_2 = H, R_3 = OH

66 R = R_3 = OH, R_1 = Me, R_2 = H



55 R = R_4 = R_7 = OH, R_1 = Me, R_2 =O-gal (1–6)-man, R_3 = R_6 = H, R_5 = OMe

56 R = O-gal, R_1 = Me, R_2 = OH, R_3 = R_4 = R_6 = H, R_5= R_7 = OMe

57 R = R_2 = OH, R_1 = Me, R_3 = R_4 = R_5 = R_6 R_4 = H= R_7 = H

58 R = OH, R_1 = R_3 = R_4 = R_5 = R_6 = H, R_2 = CO_2H, R_7 = O-glc

59 R = R_3 =R_7 = OH, R_1 = Me, R_2 = CO_2H, R_4= H, R_5 = R_6 = OMe

60 R = R_3 = R_7 =OH, R_1 = Me, R_2 = R_4 = H, R_5 = R_6 = OMe

61 R = O-rha, R_1 = Me, R_2= R_4= H, R_3 = R_7 = OH, R_5 = R_6 = H

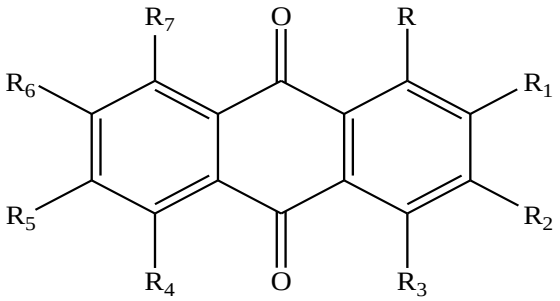
62 R = R_7 = OH, R_1 = R_3 = R_4= R_6 = H, R_2 = Me, R_5 = OMe

63 R = OH, R_2 = CHO, R_1 = R_3 = R_4 = R_5 = R_6 = H, R_7 = OMe

64 R = R_2 = OH, R_1 = Me, R_3 = R_4 = R_5 = R_6 = R_7 = H

Chemical analysis of *S. glauca* stem bark and leaves extracts led to the isolation of 8-hydroxy-6-methoxy-3-methylanthraquinone-1-*O*-α-L-rhamnopyranosyl-(1→6)-β-D glucopyranoside (**67**) and compounds **7, 9** , **10** and **62 (**Hemlata and Kalidhar, 1994). Similarly, phytochemical evaluation of *S. grandis* pods afforded 1,3,4-trihydroxy-6,7,8-trimethoxy-2-methyl anthraquinone-3-*O*-β-D-glucopyranoside (**68**) while from its seeds 1,2,4,8,-tetrahydroxy-6-methoxy-3-methylanthraquinone-2-*O*-β-Dglucopyranoside (**69**), 3-hydroxy-6,8-dimethoxy-2-methylanthraquinone-3-*O*-β-D-glucopyranoside (**70**) and 1,3-dihydroxy-6,7,8-trimethoxy-2-methylanthraquinone-3-*O*-β-D-glucopyranoside (**71**) have been reported (Rani and Kalidhar , 1998). Phytochemical investigation of *S. greggii* root bark extract by Gonza'lez *et al* (1992) led to

the isolation and characterization of anthraquinone derivatives namely, 5-hydroxy-1,4,6,7-tetramethoxy-2-methylanthraquinone (**72**), 1,5,7-trihydroxy-4,6-dimethoxy-2-methylanthraquinone (**73**), 5,6-dihydroxy-1,4,7-trimethoxy-2-methylanthraquinone (**74**), 1-hydroxy-4,7-dimethoxy-5,6-methylenedioxy-2-methylanthraquinone (**75**), 5,7-dihydroxy-1,4,6-trimethoxy-2-methylantraquinone (**76**), 4,5-dihydroxy-1,6,7-trimethoxy-2 methylanthraquinone (**77**), and 5,6-dihydroxy-4,7-dimethoxy-2-methylanthraquinone (**78**). Singh and Singh (1986) reported the isolation of an anthraquinone metabolite characterized as 4,4′-bis(1,3,8-trihydroxy-2-methyl-6-methoxy anthraquinone) (**79**) from the seeds of *C. hirsuta*.



67 R = O-glc (1-6)-rha, $R_1 = R_3 = R_4 = R_6 = H$, R_5=OMe, R_2 = Me, R_7 =OH

68 R = $R_2 = R_3$ = OH, R_1 = Me, R_2 = Oglc, R_4 = H, $R_5 = R_6 = R_7$ = OMe

69 R = $R_1 = R_3 = R_7$ = OH, R_1 = Oglc, R_2 = Me, $R_4 = R_6$ = H, R_5 = OMe

70 R = $R_3 = R_4 = R_6$ = H, R_2 = O-glc, R_2 = OMe, $R_5 = R_7$ = OMe

71 R = R_2 = OH, R_1 = Me, R_2 = O-glc, $R_3 = R_4$ = H, $R_5 = R_6 = R_7$ =OMe

72 R = $R_3 = R_5 = R_6$ = OMe, R_1 = Me, $R_2 = R_7$= H, R_4 = OH

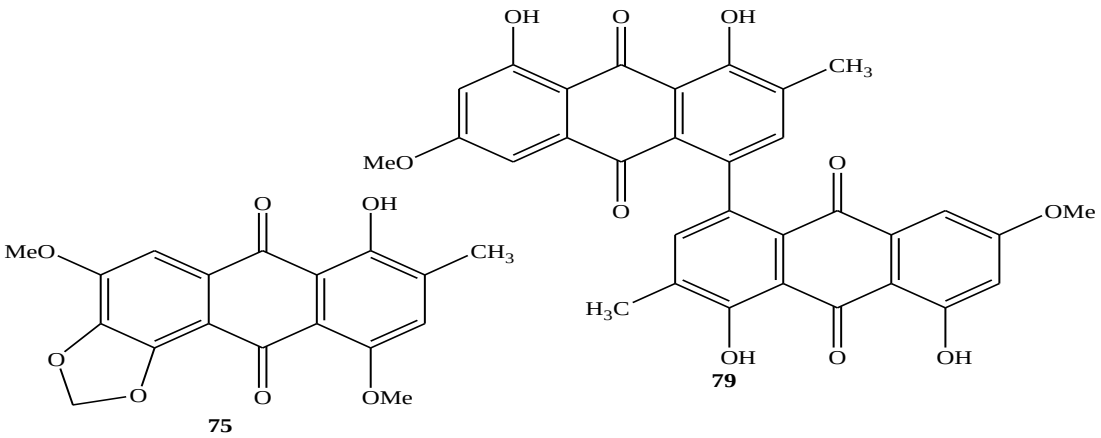
73 R = $R_4 = R_6$ =OH, R_1 = Me, $R_2 = R_7$= H, $R_3 = R_5$ = OMe

74 R = $R_3 = R_6$ = OMe, R_1 = Me, $R_2 = R_7$ =H, $R_4 = R_5$ = OH

76 R = $R_3 = R_5$ = OMe, R_1 = Me, $R_2 = R_7$ = H, $R_4 = R_6$ = OH

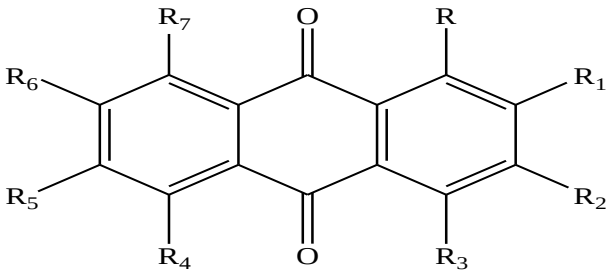
77 R = $R_5 = R_6$ = OMe, R_1 = Me, $R_2 = R_7$ = H, $R_3 = R_4$ = OH

78 R = $R_2 = R_7$ = H, R_1 = Me, $R_3 = R_6$ = OMe, $R_4 = R_6$ =OH



75 **79**

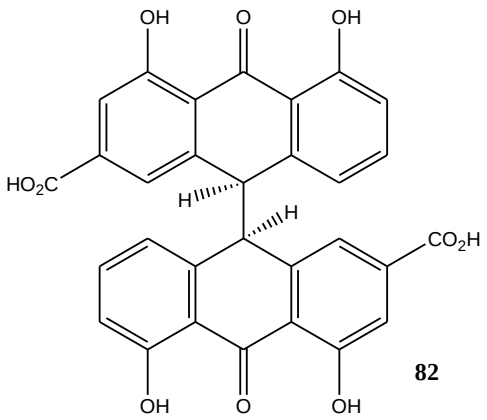
The species *S. italica* is widely used as a herb in its native habitat. Common anthraquinones reported from the plant includes compound **7, 8** and **10** along with emodin rhamnoside (**80**) and physcion glucosylrhamnoside (**81**) (Rastogi and Mehrotra, 1995).



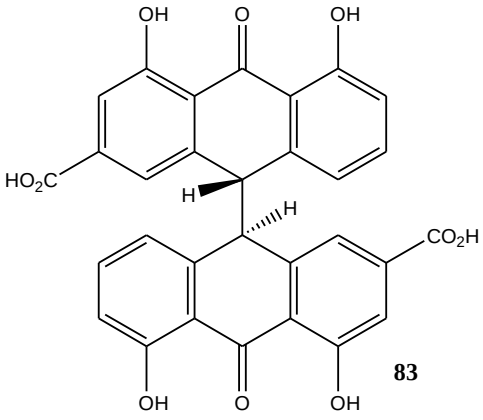
80 R = O-rha, R_2 = Me, R_1 = R_3 = R_4 = R_6 = H, R_5= R_7 = OH

81 R = O-glc (1➤6)-rha, R_1 = R_3 = R_4 = R_6 = H, R_5 = OMe, R_7 = OH

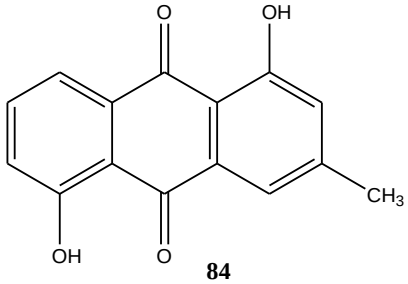
Further chemical analysis of the plant leaves also afforded the common anthraquinones **7, 8** and **11**, sennidins A (**82**) and B (**83**), along with 1,5-dihydroxy-3-methyl anthraquinone (**84**) (Kazmi *et al*., 1994).



82



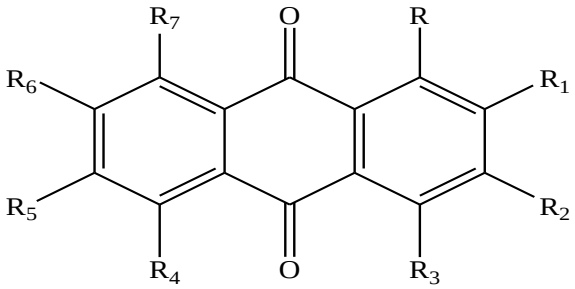
83



84

In a separate study, Tiwari and Singh (1979) phytochemically evaluated *S. javanica* root bark extract resulting in the isolation of emodin-8-rhamnoside (**85**), 5-hydroxyemodin-8-rhamnoside (**86**), 1,3-dihydroxy-5,6,7-trimethoxy-2-methylanthtraquinone (**87**), 1,4-dihydroxy-8-methoxy-2-methylanthraquinone-3-*O*-β-D-glucopyranoside (**88**), 1,8-dihydroxy-6,7-dihydroxy-2-methyl anthraquinone (**89**). From the leaves and seed extracts of the same plant was reported compounds **7-11**, physcion 1-*O*-*β*-glucoside (**90**), 1,5-dihydroxy-4,7-dimethoxy-2-methylanthraquinone rhamnopyranoside (**91**) and 1,3,6,7,8-pentahydroxy-4-methoxy-2-methylanthraquinone (**92**) (Chaudhuri and Chalwa, 1987). On the other hand chemical analysis of the plant stem bark extract yielded 1,3-dihydroxy-6,8-dimethoxy-2-methyl-anthraquinone (**93**), 1,3,5,8-tetrahydroxy-6-methoxy-2-methylanthraquinone (**94**), 1,3,4,6-tetrahydroxy-5,8-dimethoxy-2-methylanthraquinone (**95**), 1,4-dihydroxy-6,7,8-trimethoxy-2-methylanthraquinone (**96**), 1-hydroxy-3,6,7,8-tetramethoxy-2-methylanthraquinone (**97**) and 4,4'-bis(1,5-dihydroxy-7-

hydroxymethyl-2-methyl-3-methoxy) anthraquinone (**98**) (Singh and Singh, 1988; 1999 and 2008).



85 R = R_5 = OH, R_2 = Me, R_1 = R_3 = R_4 =R_6 = H, R_7 = O-rha

86 R = R_4 =R_5 = OH, R_1 = R_3 = R_6 =H, R_2 = Me, R_7 = O-rha

87 R = R_2 = OH, R_1 = Me, R_4= R_5 = R_6 = OMe, R_3 = R_7 =H

88 R = R_3 = OH, R_1 = Me, R_2=O-glc, R_4 = R_5= R_6 = H, R_7= OMe

89 R = R_5 = R_6 = R_7 =OH, R_1 = Me, R_2 = Me, R_3 = R_4 = H

90 R = O-glc, R_1 = H, R_2 = Me, R_3 = R_4 = R_6 = H, R_5 = OMe, R_7 = OH

91 R = R_4 = OH, R_2 = R_5 = H, R_1 = Me, R_3 = R_6 = OMe, R_7 = O-rha

92 R = R_2 = R_5 = R_6 = R_7 = OH, R_1 = Me, R_3 = OMe, R_4=H

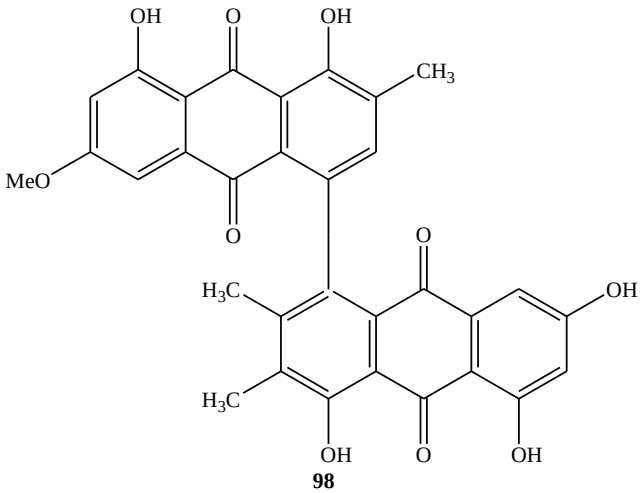
93 R = R_2 = OH, R_1 = Me, R_3 = R_4 = R_6 = H, R_5 =R_7 = OMe

94 R = R_2 =R_4 = R_7 = OH, R_1 = Me, R_3 = R_4 = R_6 =H, R_5 = OMe

95 R = R_2 = R_3=R_5 = OH, R_1 = Me, R_3= R_6 = H, R_4 = R_7 = OMe

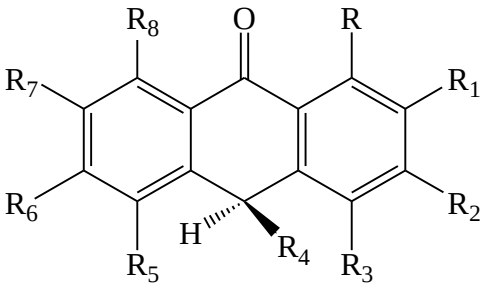
96 R =R_3= OH, R_1 = Me, R_2= R_4 = H, R_4 = R_5= R_6 = R_7= OMe

97 R = OH, R_2 = R_5 = R_6 = R_7 = OMe, R_1 = Me, R_4 = H



98

From *S. kleinii* aerial parts and roots have been documented the isolation of kleinioxanthrones-1 (**99**), 2 (**100**), 3 (**101**), 4 (**102**) (Anu and Rao, 2001; Ibid, 2002).



99 R = R_8 = OH, R_1 =R_3 = R_5 =R_7 = H, R_4 = OCO $(CH_2)_8CH_3$, R_6 = OMe

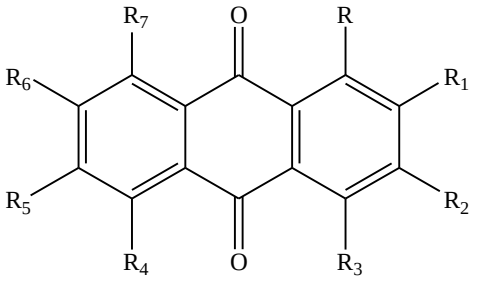
100 R = R_8 = OH, R_1 =R_3 = R_5 = R_6 = R_7 = H, R_4 = OCO $(CH_2)_8CH_3$

102 R = R_8 = OH, R_1 =R_3 = R_5 = R_6 =R_7 = H, R_4 = OCO $(CH_2)_8CH_3$

102 R = R_8 = OMe, R_1 =R_6 = R_7 = OH, R_2 = Me, R_4 = OCO $(CH_2)_8CH_3$

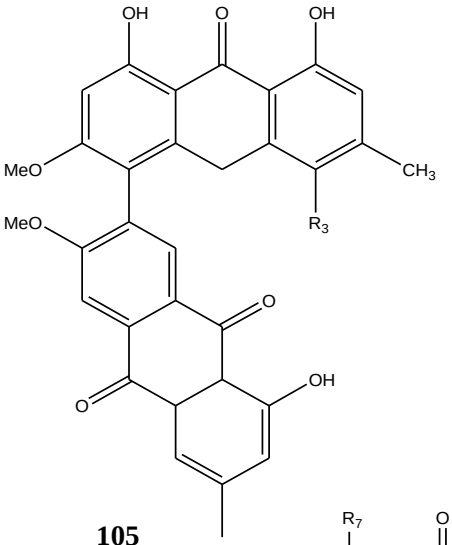
The species S. *laevigata* Wild *(*syn. *S. floribunda* Ca*v)* has been reported to contain purgative metabolites along with some new anthraquinones. Chemical analysis of the plant root, seeds and pods extract yielded common anthraquinones **7-11** and physcion-8-*O*-β-D-galactoside (**103**), 1,8-dihydroxy-6-methoxy-3-methyl-anthraquinone (**62**), 1-hydroxy-6-methoxy-3-methylanthraquinone-8-*O*- β-D-galactosyl (1→4)-*O*-β-D-galactopyranoside (**104**) (Tiwari and Singh, 1979; Singh *et al*., 1980). On the other hand, from the leaves of the same plant, 5, 7′-biphyscion (**54)** and 5, 7′-physcion-physcionanthrone (floribundone 2) (**105**) together with compounds **7** and **10** have been reported (Alemayehu *et al*, 1988). The species *S. marginata (*syn. *S. roxburghii*) commonly known as "Red Cassia" is acclimatized in India where it finds wide application in herbal medicine. From the plant seeds have been documented the isolation of 1,3-dihydroxy-2-methylanthraquinone-8-*O*-α-L-arabinopyranoside (**106**), 1,3-dihydroxy-6-8-dimethoxy-2-isoprenylanthraquinone (**107**), physcion-8-*O*-α-L-xylopyranoside (**108**), emodin-8-*O*-α-L-arabinopyranoside (**109**) and 1,3-dihydroxy-6-8-dimethoxyanthraquinone (**110**) and 4,4'-bis(1,3-dihydroxy-6,8-dimethoxy-2-methylanthraquinone) (**111**) besides 1,3,5,8-tetrahydroxy-2-methyl-anthraquinone (**112**) **7** and **9** (Duggal and Misra, 1982; Singh and Singh, 1987). Previous research on the plant flowers, leaves and heartwood by Ashok and Sharma (1985) afforded

roxburghinol (**113**) along with compounds **7, 9** and **11**. Phytochemical studies undertaken on *S. mimosoides* aerial parts gave 1, 8-dihydroxy-6-methoxy-2-methyl anthraquinone (**114**) and emodic acid (**115**) together with known compounds **62,7**, **9** and **10** (Mukherjee *et al*., 1987). Phytochemical exploration undertaken on the species *S. multijuga* seeds and roots targeting anthraquinones gave an array of the secondary metabolites. From the seeds, Singh (1981) documented the isolation of 1,3,8-trihydroxy-2-methylanthraquinone (**20**), 1,3-dihydroxy-6,8-dimethoxy-2-methyl anthraquinone (**116**), 3-hydroxy-6,8-dimethoxy-2-methylanthraquinone-1-O-β-D(+) glucopyranoside (**117**) and 3-hydroxy 6,8-dimethoxy-2-methylanthraquinone 1-*O*-rhamnopyranosyl (1→6) glucopyranoside (rutinoside) (**118**). A further search for the secondary metabolites in the roots produced 1,3-dihydroxy-2-methyl anthraquinone (**119**), 1,3-dihydroxy 6,8-dimethoxy-2-methyl anthraquinone (**120**), 1,3,8-trihydroxy-6-methoxy-2-methylanthraquinone (**121**), 1,8-dihydroxy-2-methylanthraquinone-3-*O*-rutinoside (**122**), 1-hydroxy-6,8-dimethoxy-2-methylanthraquinone-3-*O*- rutinoside (**123**) and 1,8-dihydroxy-6-methoxy-2-methylanthraquinone-3-*O*- rutinoside (**124**) (Obodozie *et al*., 2004; Ayo *et al*., 2007). Elsewhere, search for anthraquinones in *S. nigricans* whole plant resulted in the isolation of **10**, 4-hydroxyanthraquinone-2-carboxylic acid (**125**) and citreorosein (**126**), along with emodic acid (**115**) (Ayo *et al*., 2009). On the other hand, chemical evaluation of *S. nomame* aerial parts by Kitanaka and Takido (1985) reported the isolation ofphyscion-9-anthrone (**127**), emodin-9-anthrone (**128**), and physcion 10,10`-bianthrone (**39**) along with **7, 9, 10**. However, a similar study done on *S. obtusa* roots yielded 1, 3-dihydroxy-6-methoxy-7-methylanthraquinone (**129**) and 1, 3-dihydroxy-3, 7-diformylanthraquinone (**130**) (Sekar *et al*., 1999).



103 R = OH, R_1 =R_3 = R_4 = R_6 = H, R_2 = Me, R_5 = OMe, R_7 = Ogal

104 R = OH, R_1 =R_3 = R_4 = R_6 = H, R_2 = Me, R_5 = OMe, R_7 = Ogal(1➔4)-gal



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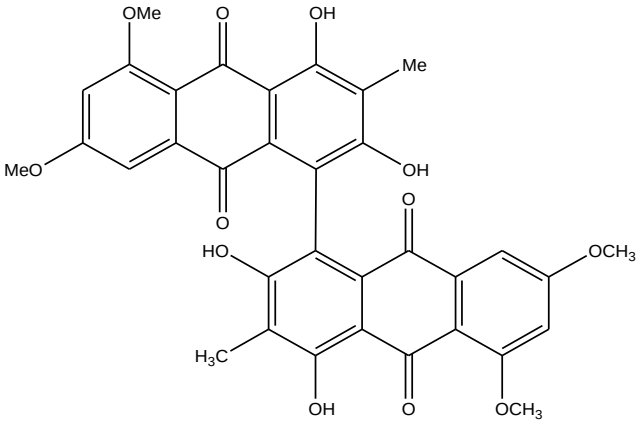

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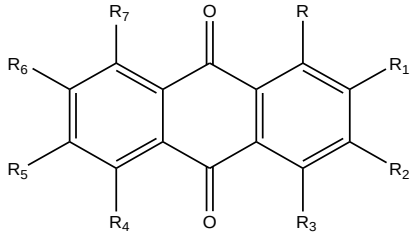


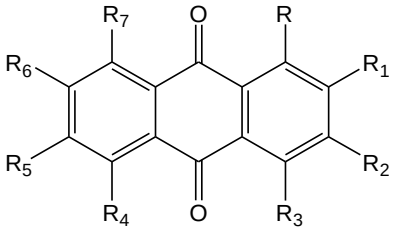
106 R = R_2 = OH, R_1 = Me, R_3 = R_4 = R_5 = R_6 = H, R_7 = O-ara

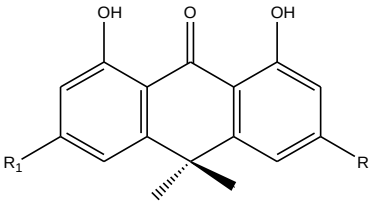
107 R = R_2 = OH, R_1 = CH_2-CH=C $(CH_3)_2$, R_3 = R_4 = R_5 = R_6 = H, R_5 = R_7 = OMe

108 R = OH, R_1 = R_3 = R_4 = R_6 = H, R_2 = Me, R_5 = OMe, R_7 = O-xyl

109 R = R_5 = OH, R_1 = R_3 = R_4 = R_5 = R_6 = H, R_2 = Me, R_7 = O-ara

110 R = R_2 = OH, R_1 = R_3 = R_4 = R_6 = H, R_5 = R_7 = OMe





127 R=OMe, R_1=Me
128 R=Me, R_1=OH

112 R =R_2=R_4= R_7 = OH, R_1 = Me, R_2 = R_3 = R_4 =R_5 = R_6 = H

113 R = R_7 = OH, R_1 =Me, R_2=R_3 = R_4 = R_6 = H, R_5 = OMe

114 R = R_7 =OH, R_1 =R_2= R_3 = R_4 = R_6 = H, R_5=OMe,

115 R = R_5 = R_7 =OH, R_1 = R_3 = R_4 = R_6 = H, R_2 = CO_2H

116 R = R_2 = OH, R_3 = R_4 = H, R_5=R_7 = OMe, R_1 = Me

117 R= O-glu, R_2 = OH, R_1 = Me, R_3 = R_4=H, R_5= R_7 = OMe

118 R_2 = OH, R_1 = Me, R = O-rha (1➤6)-glc, R_3 = R_4 = R_6 = H, R_5 =R_7= OMe

119 R=R_2= OH, R_1 = Me, R_3 = R_4 = R_5 =R_6= R_7=H

120 R = R_2 = OH, R_1 = Me, R_3 = R_4 = H, R_5 =R_7= OMe

121 R = R_2 = R_7 = OH, R_1 =Me, R_3 = R_4 = R_6 = H, R_5 = OMe

122 R = R_7 = OH, R_2=O-rut, R_3 = R_4 = H, R_5 = OMe, R_1 = Me

123 R = OH, R_1 =Me, R_2=O-rut, R_3 = R_4 = H, R_5 =R_7=OMe

124 R=R_7=OH, R_5=OMe, R_1=Me, R_1=R_2=R_3=R_4=H

125 R = R_2 = R_4 = R_6 = R_5 = R_6 = R_7 = H, R_1 = CO_2H, R_3 = OH

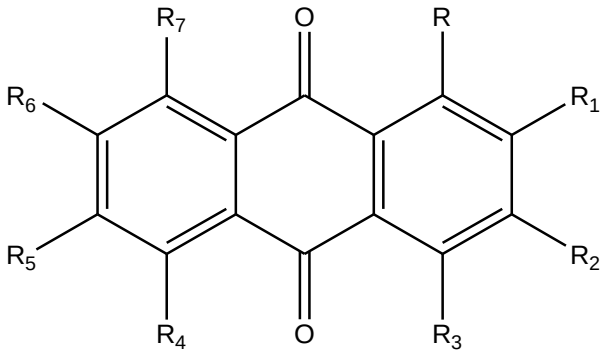
126 R = R_5 = R_7 = OH, R_1 = R_3 = R_4 = R_6 = H, R_2 = CH_2OH

129 R=R_2=OH, R_5=OMe, R_6=Me, R_1=R_3=R_4=R_7=H

130 R = R_2 = OH, R_1 = R_3 = R_4 = R_5 = R_7 = H, R_2=R_6 = CHO

The species S. *obtusifolia* has been widely studied and a wide range of anthraquinones documented from the plant various parts. Phytochemical analysis of seeds by Li *et al* (2004) resulted in isolation of **8**, 1-methylaurantio-obtusin-2-*O*-*β*-*D*-glucopyranoside (**131**), **10**, 1,2-dihydroxyanthraquinone (**132**), obtusin (**133**), chrysoobtusin (**134**), aurantioobtusin (**135**), gluco-obtusin (**136**), glucoaurantioobtusin (**137**), gluco-chryso-obtusin (**138**), 1-desmethylaurantio-obtusin (**139**), 1-desmethylaurantio-obtusin-2-*O*-*β*-*D*-glucopyranoside (**140**), 1-desmethylchryso-obtusin (**141**), 1-

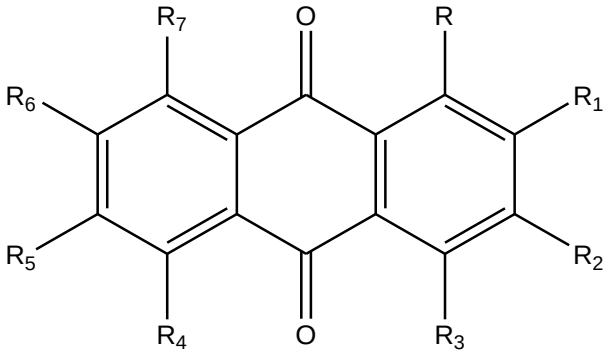
desmethyl-obtusin(**142**), aurantio-obtusin-6-*O*-*β*-*D*-glucopyranoside (**143**), alaternin-1-*O*-*β*-*D*-glucopyranoside (**144**), chrysoobtusin-2-*O*-*β*-*D*-glucopyranoside (**145**), Chrysophanol-8-*O*-β-glucoside (**146**), obtusifolin-8-*O*-β-glucoside (**147**), 8-*O*-methyl-chrysophanol (**148**), emodin-1-*O*-*β*-gentio-bioside (**149**),chrysophanol-1-*O*-*β*-gentiobioside (**150**), physcion-8-*O*-*β*-gentiobioside (**151**), physcion-8-*O*-*β*-glucoside (**152**), chrysophanol-1-*O*-*β*-*D*-glucopyranosyl-(1→3)-*β*-*D*-glucopyranosyl-(1→6)-β-D-glucopyranoside (**153**) and 1,3-dihydroxy-8-methylanthraquinone (**154**). The leaves as well as the root bark extracts have been evaluated for anthraquinones resulting in isolation of **7-10**together with, 1-hydroxy-7-methoxy-3-methylanthraquinone (**155**), 8-*O*-methylphyscion (**156**), 1-*O*-methylchrysophanol (**157**) and 1,2,8-trihydroxy-6,7-dimethoxyanthraquinone (**158**), islandicin (**159**), helminthosporin (**160**) and xanthorin (**161**) (Yun Choi *et al*., 1990; Guo *et al*., 1998; Yang *et al*., 2006; Ying Tang *et al*., 2008; Sob *et al*., 2008; Wu *et al*., 2011).



131 R=R_2=Me, R_1=R_5=R_7=OH, R_6=OMe, R_3=R_4=H
132 R = R_1 = OH, R_2 = R_3 = R_4 = R_5 = R_7 = H
133 R = R_6 = OH, R_1 = R_2 = R_7 = OMe, R_5 = Me, R_3 = R_4 = H
134 R = R_1 = R_2 = R_7 = OMe, R_5 = Me, R_3 = R_4 = H
135 R = R_2 = R_6 = OH, R_1 = R_7 = OMe, R_5 = Me, R_3 = R_4 = H
136 R = OMe, R_1 = O-glc, R_2 = Me, R_7 = OH, R_3 = R_4 = R_5 = H
137 R = R_6 = OH, R_1 = R_7 = OMe, R_2= O-glc, R_5 = Me, R_3 = R_4 = R_5 = H
138 R = R_5 = R_6 = R_7 = OMe, R_1 = O-glc, R_2 = Me, R_3 = R_4 = R_5 = H
139 R = R_1 = R_5 = R_6 = R_7 = OH, R_2 = Me, R_3 = R_4 = R_5 = H
140 R = R_5 = R_7 = OH, R_1 = O-glc, R_6 = OMe, R_2 = R_3 = R_4 = H
141 R = OH, R_1 = R_3 = R_4 = H, R_2 = Me, R_5 = R_6 = R_7 = OMe
142 R = R_2 = R_7 = OH, R_2 = Me, R_5 = R_6 = OMe, R_3 = R_4 = H
143 R = R_6 = R_7 = OMe, R_1 = R_7 = OH, R_2 = Me, R_3 = R_4 = H, R_5 = O-glc
144 R = R_5 = R_6 = R_7 = OMe, R_1 = O-glc, R_2 = Me, R_2 = R_3 = R_4 = H
145 R = OH, R_1 =O-glu, R_3 = R_4 = R_6 = H, R_2 = Me, R_7 = OMe
146 R = OH, R_1 = R_3 = R_4 =R_5= R_6 =H, R_2 = Me, R_7 = O-glu
147 R=OMe, R_1= R_4=OH, R_2=Me, R_3= R_5=R_6=H, R_7=O-glu
148 R=OH, R_1=R_3=R_4=R_5=R_6=H, R_2=Me, R_7=OMe
149 R = O-gentibiose, R_1= R_3= R_4 = R_6 =H, R_2 = Me, R_5 = R_7 = OH
150 R = O-gentibiose, R_1= R_3= R_4 = R_5=R_6 =H, R_2 = Me, R_7 = OH
151 R = OH, R_1= R_3= R_4 = R_6 =H, R_2 = Me, R_5 = OMe, R_7 = O-gentbiose
152 R = OH, R_1= R_2 = R_3= R_4 = R_6 =H, Me, R_5 = OMe, R_7 = O-glucoside
153 R = O-glu, R_1= R_3= R_4 =R_5= R_6 =H, R_2 = Me, R_7 = OH
154 R=R_2=OH, R_1=R_3=R_4=R_5=R_6=H, R_7=Me
155 R=OH, R_1=R_3=R_4=R_5=R_7=H, R_6=OMe, R_2=Me
156 R=OH, R_1=R_3=R_4= R_6=H, R_5=OMe, R_2=Me, R_7=OMe
157 R=OMe, R_1=R_3=R_4=R_5=R_6=H, R_2=Me, R_7=OH
158 R=R_1=R_7=OH, R_5=R_6=OMe, R_2 = R_3= R_4=H
159 R=R_3=R_7=OH, R_1= R_4= R_5=R_6=H, R_5=OMe, R_2=Me
160 R=R_4=R_7=OH, R_1= R_3= R_5=R_6=H, R_2=Me
161 R=R_4=R_7=OH, R_1= R_3= R_6=H, R_5=OMe, R_2=Me

Senna occidentalis is another species in the genus Senna that has been widely studied for anthraquinone metabolites. From the plant leaves and seed extracts have been reported the isolation and identification of common anthraquinones namely: chysophanol (**7**), emodin (**10**), physcion (**9**), rhein (**11**), Islandicin (**159**), questin (**47**), chrysophanol-10,10'-bianthrone (**37**), germichrysone (**48**) along with 1, 8-dihydroxy-2-methylanthraquinone (**162**) and 1,4,5-trihydroxy-7-methoxy-3-methylanthraquinone (**163**) (Rastogi and Mehrotra, 1990) while from the roots emodin (**10**), chrysophanol (**7**), physcion (**9**), islandicin (**159**), rhein (**11**), aloe-emodin (**8**), chrysophanol-10, 10'-bianthrone (**37**) and germichrysone (**48**) have been reported (Chukwujekwu *et al*., 2006). Further search for the anthraquinone constituents in flowers and callus culture of the plant afforded 7-methylphyscion (**164**) and 7-methyltorosachrysone (**165**) as well as emodin (**10**), physcion (**9**) and its glucoside (**146**) (Yadav *et al*., 2010). From the species *S. podocarpa* parts including leaves, pods as well as callus culture have been documented the isolation of rhein (**11**) its glucoside (**14**), emodin (**10**), chrysophanol (**7**), rhein-*8-O-B-D*-glucoside (**14**), sennoside A (**26**) and sennoside B (**27**) (Rai and Obayemi , 1973; Elujoba *et al*., 1994). Elsewhere phytochemical investigation of *S. pudibunda* root bark extract afforded chrysophanol dimethyl ether (**166**) together with **7** and **9** (Messana *et al*, 1991) while a similar scientific research undertaken using *S. pumila* whole plant by Sharma *et al* (2012) gave **7, 166**, and **9**. Phytochemical investigation of stem bark extract of *S. racemosa* yielded racemochrysone (**167**), **7 and 9**. (Mena-Rejona *et al*., 2002; Moo-Pucc *et al*., 2007). Search for anthraquinone derivatives in *S. renigera* parts namely leaves, stem bark as well as seeds culminated in the isolation and identification of purgative ananthraquinones **7, 9, 11** along with 1-hydroxy-3,8-dimethoxy-2-methylanthraquinone (**168**), 1,5,6-trihydroxy-3-methylanthraquinone-8-*O*-β-D-glucoside (**169**), 1,8-dihydroxy-3,5,7-trimethoxy-2-methylanthraquinone (**170**), 1,5,8-trihydroxy-6,7-dimethoxy-2-

methylanthraquinone-3-*O*-α-L-rhamnopyranosides (**171**) and 1-hydroxy-8-methoxy-2-methylanthraquinone (**172**) (Ledwani and Singh, 2005).



162 R = R_7 = OH, R_1 = Me, R_2 = R_3 = R_4 = R_5 = R_6 = H

163 R = R_3 = R_4 = OH, R_2 = Me, R_1 = R_5 = R_7 = H, R_6 = OMe

164 R = R_7 = OH, R_1 = R_3 = R_4 = H, R_2 = R_6 = Me, R_5 = OMe

166 R = R_7 = OMe, R_1 = R_3 = R_4= R_5 = R_6 = H, R_2 = Me

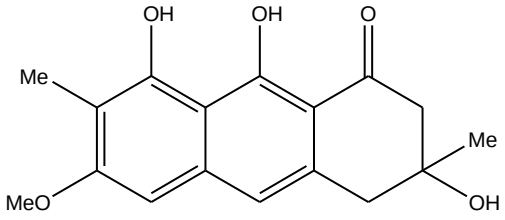
168 R = OH, R_1 = Me, R_2= R_7 =OMe, R_3 = R_4 = R_5 = R_6 = H

169 R = R_4 = R_5 = OH, R_1 = R_3 = R_6 = H, R_7 = O-glu, R_2 = Me

170 R = R_7 = OH, R_1 = Me, R_2 = R_4 = R_6 = OMe, R_3 = R_5 = H

171 R = R_4=R_7 = OH, R_5 = R_6 = OMe, R_1= Me, R_3 = H, R_2 = O-rha

172 R = OH, R_1 = Me, R_2 = R_3 = R_4 = R_6 = H, R_7 = OMe



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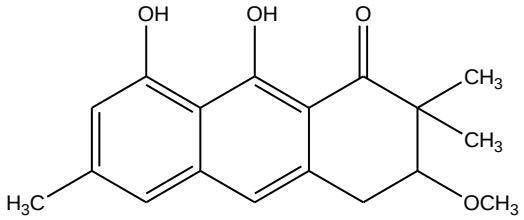


167

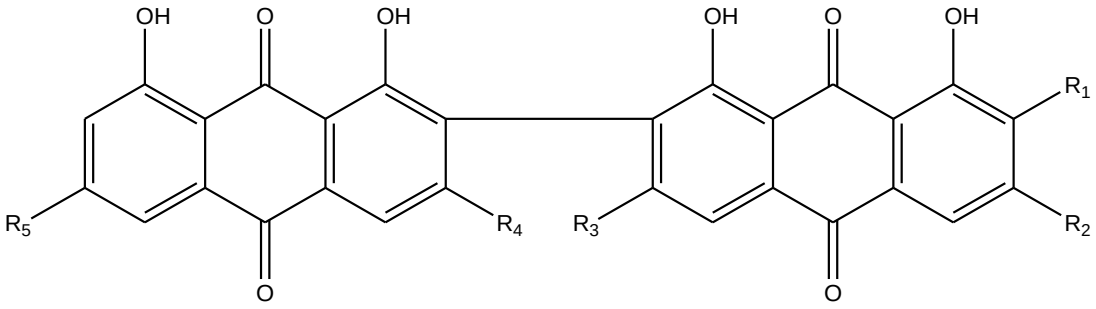
The common anthraquinones **7, 8, 10, 11** have been reported from *S. reticulata* leaves and flowers (Messmar *et al*., 1968). *Senna siamea* parts have been phytochemically evaluated for anthraquinone derivatives. A compound coded cassiamin A (**173**) together with. **7, 9, 11** and sennoside B (**27**) have been reported from the leaves of the plant (Rastogi and Mehrotra 1995). Chemical evaluation of the plant heartwood resulted in the isolation of 4,4'-bis(1,3-dihydroxy-

6,8-dimethoxy-2-methylanthraquinone) (**111**), cassiamin A (**173**) and 1,1′-bis (4,5-dihydroxy-2-methyl anthraquinone) (**174**) together **7, 10** have been reported (Singh *et al*., 1992; Ingkaninan *et al*., 2000; Kaur *et al*., 2006). A similar study targeting the stem and root barks of the plant by Ledwani and Singh (2006) resulted in the isolation and characterization of **7, 9, 10,** cassiamin A (**173**),B (**175**,) and C (**176**), siameanin (**177**), siameadin (**178**), 1,1′,3,8,8′-pentahydroxy-3′,6-dimethyl[2,2′-bianthracene]-9,9′,10,10′-tetrone (**179**), 7-chloro-1,1′,6,8,8′-pentahydroxy-3,3′-dimethyl[2,2′-bianthracene]-9,9′,10,10′-tetrone (**180**). Anthraquinones characterized as 1-hydroxy-6, 8-dimethoxy-2-methylanthraquinone-3-*O*-rutinoside (**123**) and 1, 5, 8-trimethoxy-2-methylanthraquinone-3-*O*- β-D-galactopyranoside (**181**) have also been reported from root extracts of the plant (Koyama *et al*., 2001).

The other species in the genus Senna that has attracted wide attention due to it medicinal value is *S. singueana*. From its root extract has been documented the isolation and identification of torosachrysone (**34**), germichrysone (**48**), singueanol-I (**182**), singueanol-II (**183**), 7-methylphyscion (**164**), and cassiamin A (**173**) (Endo and Naoki, 1980; Mutasa *et al.*, 1990). Rastogi and Mehrotra (1995) phytochemically evaluated *Senna sophera* leaves leading to the isolation of sennoside (**174**) while from flower extract **7** was isolated. Further chemical search using the plant root bark led to the isolation of 1,8-dihydroxy-2-methylanthraquinone 3-neohesperidoside (**184**), 1,8-dihydroxy-3,6-dimethoxy-2-methyl-7-vinylanthraquinone (**185**) and 1,3-dihydroxy-5,7,8-trimethoxy-2-methylanthraquinone (**186**) together with compounds **7, 8** (Dass *et al*., 1984; Joshi *et al*., 1985). Similarly, a phytochemical investigation of the plant heartwood afforded 1,2,7-trihydroxy-6,8-dimethoxy-3-methylanthraquinone (**187**), 1,2,6-trihydroxy-7,8-dimethoxy-3-methylanthraquinone (**188**) and sopheranin (**189**) in addition to compounds **7, 9,10** (Dass *et al*, 1984). Besides the species *Senna sophera*, another species which

has been documented to possess medicinal value is *Senna spectabilis*. Chemical analysis of both plant leaves and flower buds resulted in the isolation of common purgative anthraquinones **7, 9** along with 1,3,8-trihydroxy-2-methylanthraquinone (**20**).

In another study, Ashok and Sharma, (1988) documented the existence of 1,8-dihydroxy-6-methoxy-3-methyl-anthraquinone (**62**) from the plant flowers. From *Senna tomentosa* whole plant sengulone (**190**), **54** together with **10** have been reported (Rastogi and Mehrotra, 1995).



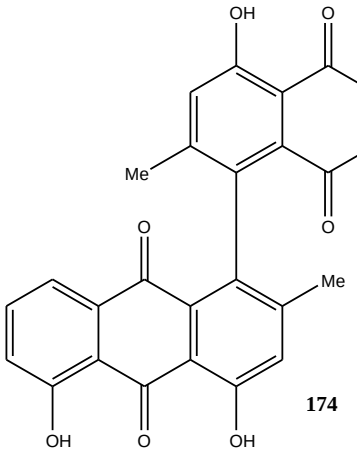
173 $R_1 = R_5 = H$, $R_2 = OH$, $R_3 = R_4 = Me$

175 $R_1 = H$, $R_2 = R_5 = OH$, $R_3 = R_4 = Me$

176 $R_1 = R_2 = R_5 = H$, $R_3 = R_4 = Me$

179 $R_1 = R_5 = H$, $R_2 = R_4 = Me$, $R_3 = OH$

180 $R_1 = Cl$, $R_2 = OH$, $R_3 = R_4 = Me$, $R_5 = H$



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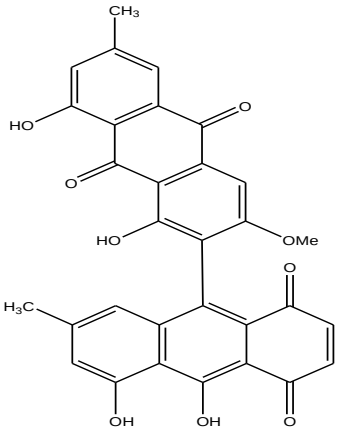

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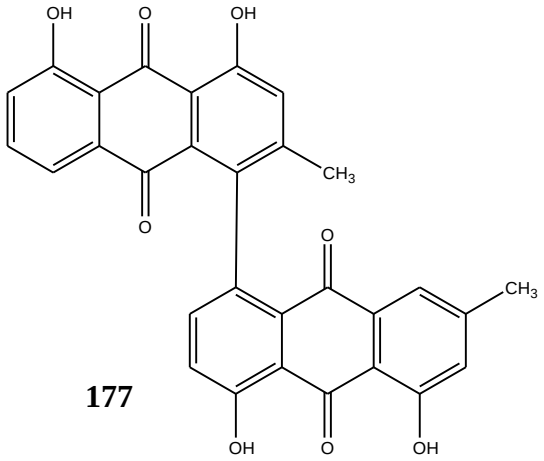

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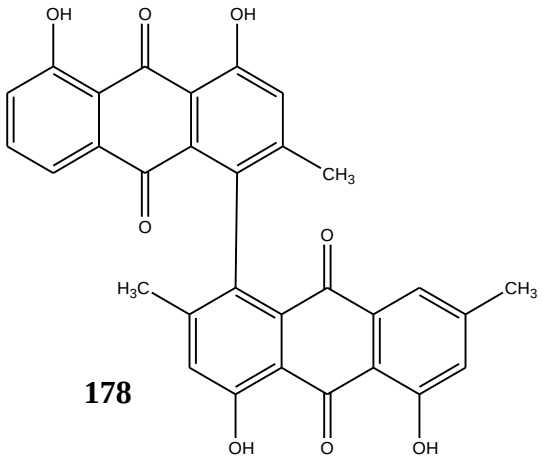

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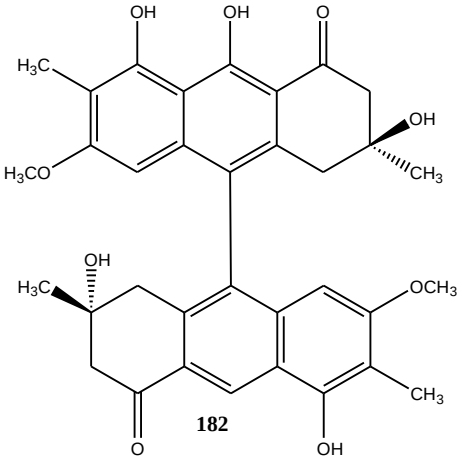

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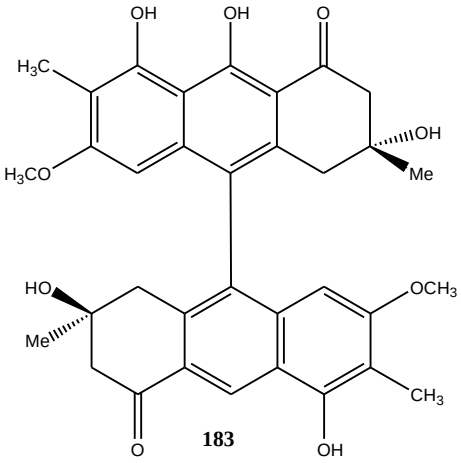

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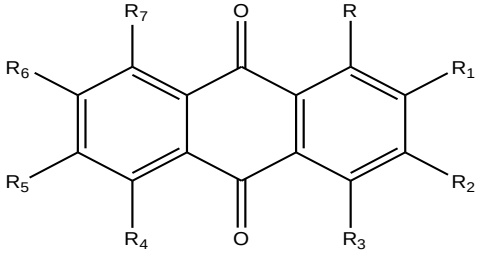


181 R= R_4= R_7=OMe, R_1=Me, R_2= *O*gal, R_3=R_5=R_6

184 R = R_7 = OH, R_1 = Me, R_2 = *O*glu (1-2) rha, R_2 = R_4 = R_6 = H

185 R = R_7 = OH, R_1 = Me, R_2 = R_5 = OMe, R_3= R_4 = H, R_6 = CH= CH_2

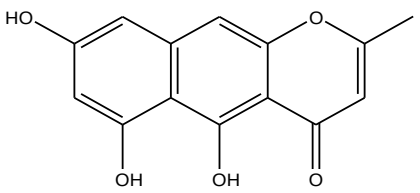
186 R = R_2 = OH, R_1 = Me, R_3 = R_5 = H, R_4= R_6 = R_7 =OMe,

187 R = R_1 = R_6 = OH, R_2 = Me, R_3 = R_4 = H, R_6 = R_7 = OMe

188 R = R_2 = R_5 = OH, R_2 = Me, R_3 = R_4 = H, R_5 = R_7 = OMe

189 R = R_2 = R_5 = R_7 = OH, R_1 = Me, R_3 = R_4 = H, R_6 = CH=CH_2

Similarly, phytochemical evaluation *Senna tora* seeds by Upadhaya and Singh (1986) led to the isolation of several anthraquinone metabolites including chrysoobtusin (**134**), aurantio-obtusin (**135**), obtusin (**133**), chryso-obtusin-2-*O*-β-D-glucoside (**191**), **7, 9, 10, 11, 51**, obtusifolin-2-*O*-β-D-glucoside (**192**), 1-[(β-D-glucopyranosyl-(1→3)-*O*-β-D-glucopyranosyl-(1→6)-*O*-β-D-glucopyranosyl)oxy]-8-hydroxy-3-methyl-9,10-anthraquinone (**193**), 1-[(β-D-glucopyranosyl-(1→6)-*O*-β-D-glucopyranosyl-(1→3)-*O*-β-D-glucopyranosyl-(1→6)-*O*-β-D-glucopyranosyl)oxy]-8-hydroxy-3-methyl-9,10-anthraquinone (**194**), 2-(β-D-glucopyranosyloxy)-8-hydroxy-3-methyl-1-methoxy-9,10-anthraquinone (**195**), alaternin-2-*O*-β-D-glucopyranoside (**196**), alaternin (**197**), 8-hydroxy-3-methylanthraquinone-1-β-gentiobioside (**198**), rubrofusarin (**199**) and its 6-β-gentiobioside (**200**) , nor- rubrofusarin (**201**), torachrysone (**202,**) (Sui-Ming *et al*., 1989., Lee *et al*., 1998; Choi *et al*., 1998; Maity and Dinda, 2003). From the plant leaves, root and stem bark extracts 1,3,8-trhydroxy-2-methylanthraquinone (**20**), 1,3,5, 8-tetrahydroxy-6,7-dimethoxy-2-methylanthraquinone (**203**), 1-hydroxy-5-methoxy-2-methylanthraquinone (**204**) and its glycoside (**205**), 5-methoxy-2-methylanthraquinone-1-*O*-α-L-rhamnoside (**206**) together with compounds **7-11** has been reported (Maity and Dinda, 2003; Rai and Kumari, 2006).



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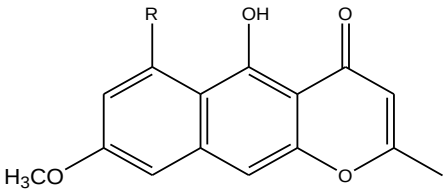
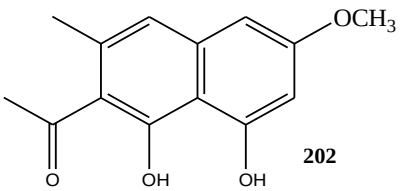


199 R=OH
200 R=Oglu (1➤ 6) glu



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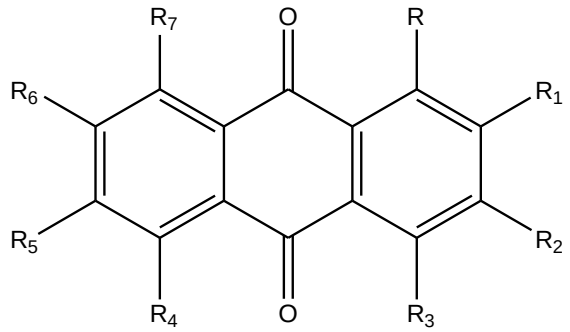


191 R = R_2 = R_7 = OMe, R_1 = Oglu, R_2 = Me, R_3 = R_4 =R_5= H

192 R = OMe, R_1 = Oglu, R_2 = Me, R_3 = R_4 = R_5 = R_6 =R_7= H, R_4 =OH

193 R = Oglu (1-3) glu (1-6) glu, R_1 = R_3 = R_5 = R_6 = H, R_2 = Me, R_7 = OH

194 R = Oglu (1-6) glu (1-3) glu (1-6) glu, R_1 = R_3 = R_4 = R_5 = R_6 = H, R_2 = Me, R_7 = OH

195 R = OMe, R_1 = Oglu, R_2 = Me, R_3 = R_4 = R_5 = R_6 = H, R_7 = OH

196 R = R_5 = R_7 =OH, R_1 = Oglu, R_2 = Me, R_3 = R_4 = R_6 = H

197 R = R_1 = R_5 = R_7 = OH, R_2 = Me, R_3 = R_4 = R_6 = H

198 R = Oglu (1-6) glu, R_1 = R_3 = R_4 = R_5 = R_6 = H, R_2 = Me, R_7 = OH

203 R = R_2 = R_4 = R_7 = OH, R_1 = Me, R_3 = H, R_5 = R_6 = OMe

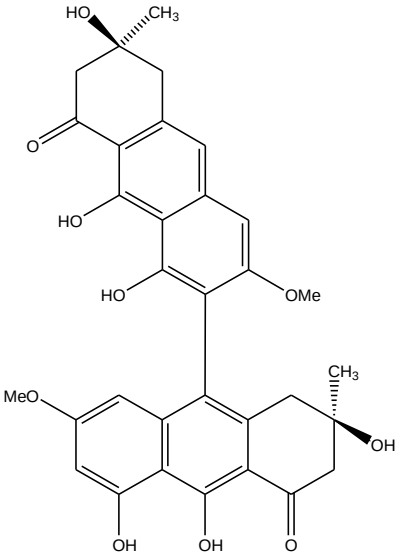
204 R = OH, R_1 = Me, R_2 = R_3 = R_5 = R_6 =R_7 = H, R_4 = OMe

205 R = Oglu, R_1 = Me, R_2 = R_3 = R_5 = R_6 = R_7 = H, R_4 = OMe

206 R = Orha, R_1 = Me, R_2 = R_3 = R_5 = R_6 = R_7 = H, R_4= OMe

Chemical analysis of *S. torosa* seeds by Kitanaka and Takido, 1982 led to the isolation of phlegmacin (**207**), anhydrophlegmacin-9,10-quinone (**208**), germichrysone (**48**), germitorosone (**209**) and methylgermitorosone (**210**). The compounds namely, torosachrysone (**34**), physcion-9-anthrone (**127**), physcion-10,10′-bianthrone (**39**), anhydrophlegmacin B2 [2-(6′-methoxy-3′-methyl-3′,8′,9′-trihydroxy-1′-oxo-1′,2′,3′,4′-tetrahydroanthracene-10′-yl)-1,8-dihydroxy-3-methoxy-6-methyl-9-oxo-9,10-dihydroanthracene] (**211**), torosanin [2-(6′-methoxy-3′-methyl-3′, 8′,9′-trihydroxy-1′-oxo-1′,2′,3′,4′-tetrahydroanthracene-5′-yl)-1,8-dihydroxy-3-methoxy-6-methyl-9-oxo-9,10-dihydroanthracene (**212**), torosachrysone-8-β-D-gentiobioside (**213**), physcion-8-*O-β-D*-gentiobioside (**151**) as well as, **9, 11** and xanthorin (**161**) have been reported from plant leaves (Takido, 1982; Kitanaka and Takido, 1982; Kitanaka and Takido, 1984;

Kitanaka and Takido, 1990; Kitanaka and Takido, 1994; Kitanaka and Takido, 1995). However, a similar study conducted using flowers of the same plant by Kitanaka and Takido (1994) yielded torosaol-III (**214**), Physcion-5,7'-physcionanthrone (**215**), 5,7'-biphyscion (**54**), torosanin-9,10-quinone (**216**), 5,7-dihydroxy-chromone (**217**), norengenin (**218**), chrysoeriol (**219**). On the other hand, evaluation of root and leaves extracts afforded torosaols I (**220**) and II (**221**) as well as torososide A (**222**) (Kitanaka and Takido, 1995).



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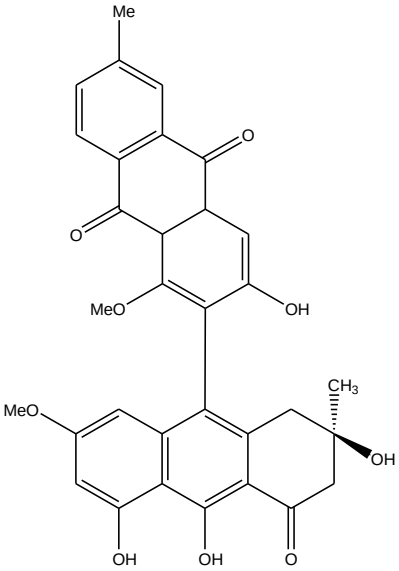

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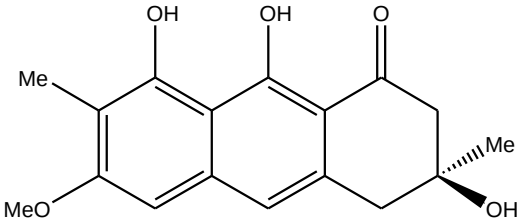

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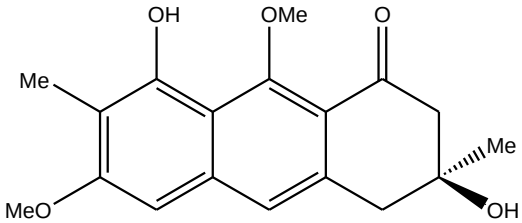

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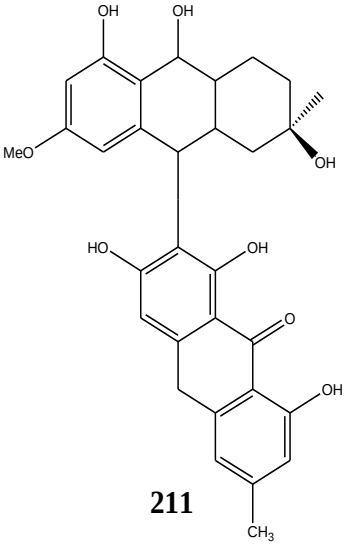

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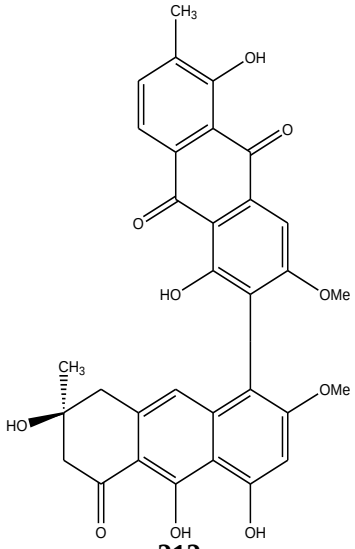

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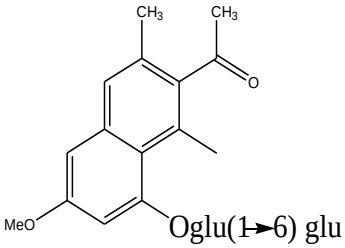
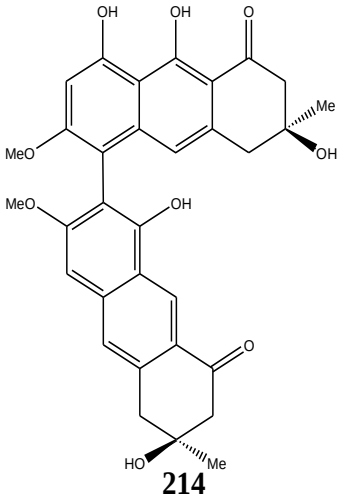


Oglu(1→6) glu

213



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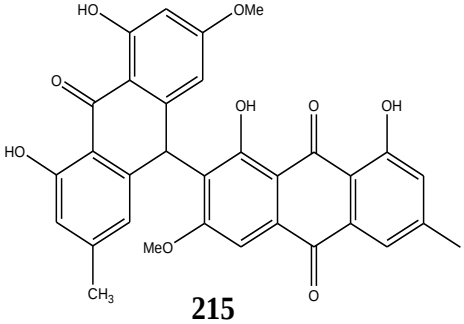

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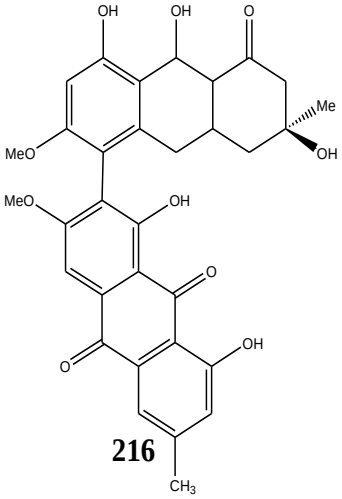

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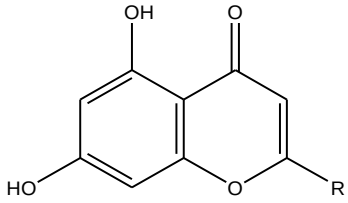
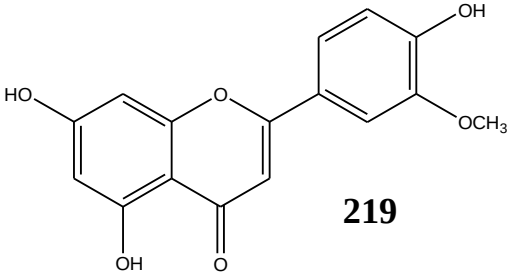


217 R = H

218 R = Me



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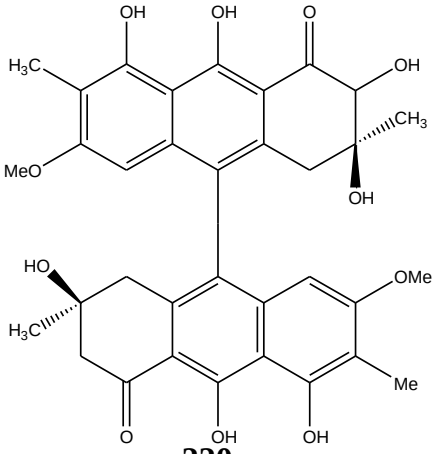

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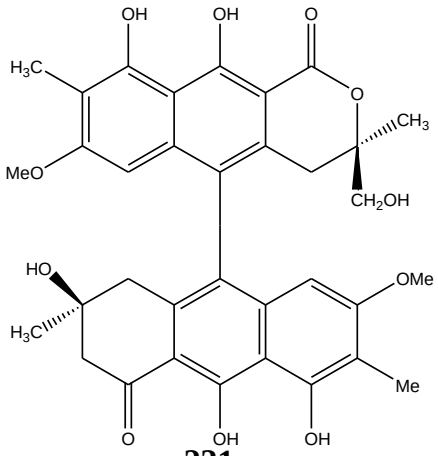

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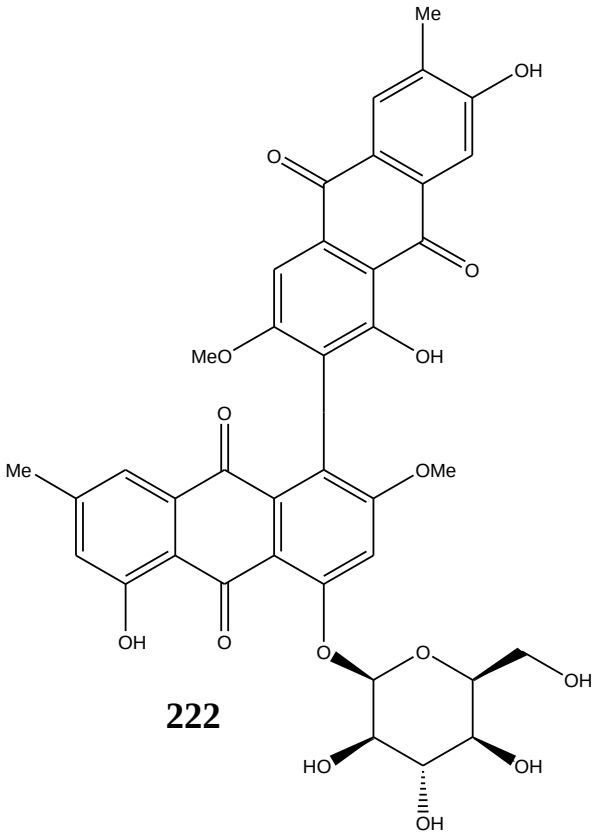
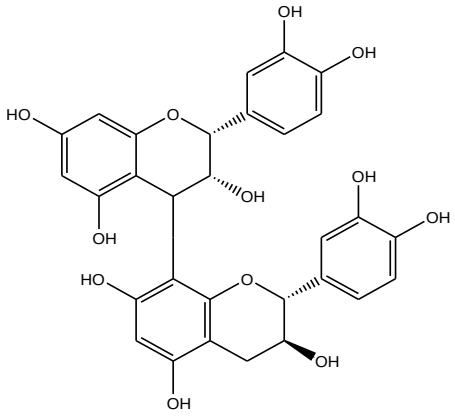


222

2.6.2 Phenolic Compounds Isolated From the Genus Senna.

Vaishnav *et al*., (1993), Vaishnav and Gupta (1996) reported the isolation of rhamnetin 3-*O*-gentiobioside (**223**) from the roots of *Cassia fistula* while from the stem bark extract of the plant the isolation of two flavonol glycosides identified as 5, 7, 3', 4'-tetrahydroxy-6, 8-

dimethoxyflavonol-3-O-α-arabinopyranoside (**224**) and 5,7,4'-trihydroxy-6, 8, 3'-trimethoxyflavonol-3-O-α-L-rhamnosyl (1—>2)-O-β-D-glucopyranoside (**225**) together with a xanthone glycoside characterized as 1, 8-dihydroxy-3, 7-dimethoxyxanthone-4-O-α-L-rhamnosyl (1—>2)-O-β-D-glucopyranoside (**226**) (Vaishnav and Gupta (1996). From the plant fruits Sartorelli *et al*., (2009) isolated an isoflavone biochanin A (**227**) from dichloromethane extracts. A phytochemical research on the fruit pulp by Nadkarni (2009) documented the isolation of a dimeric proanthocyanidin CFI (**228**) along with (–) epiafzelechin (**229**), (+) catechin (**230**), kaempferol (**231**) and dihydrokaempferol (**232**). The isolation of bioactive flavone glycoside namely; 5, 3', 4'-trihydroxy- 6-methoxyflavonol-7-O-α-Lrhamnopyranosyl-(1 → 2)-O- β - D-galactopyranoside (**233**) has also been reported from defatted seeds of the plant (Nadkarni, 2009). Metabolites (+)-epiafzelechin (**234**) and (+)-epicatechin (**235)** with the usual 2S configuration have been reported in the pods along with common flavan-3-ol (**236**).



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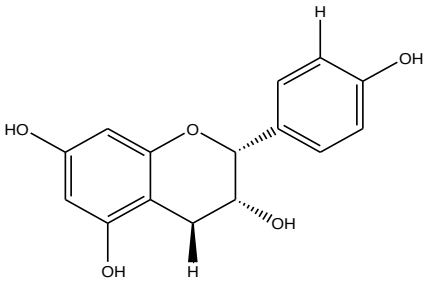

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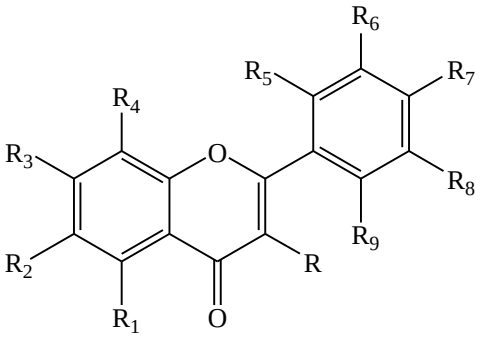


223 R = Ogent, $R_1 = R_6 = R_7 = OH$, $R_2 = R_4 = R_5 = R_8 = R_9 = H$, R_3=OMe

224 R = Ora, R_1=$R_3 = R_6 = R_7 = OH$, $R_2 = R_4 = OMe$, $R_5 = R_8 = R_9 = H$

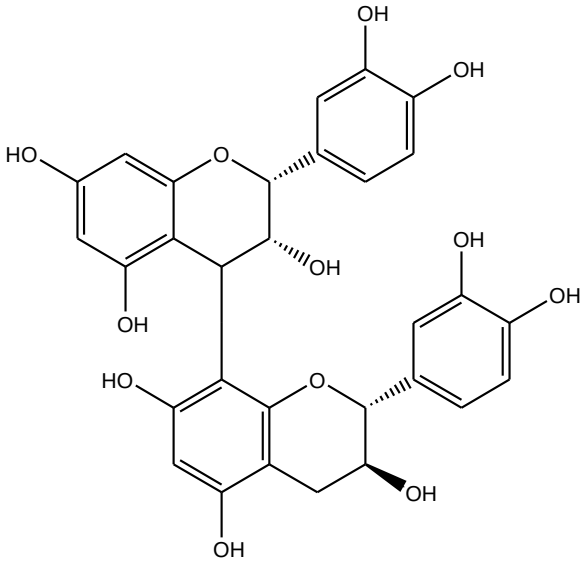
225 R = Ora (1-2) glu, $R_1 = R_3 = R_7 = OH$, $R_2 = R_4 = R_6 = OMe$, $R_5 = R_8 = R_9 = H$

227 $R = R_2 = R_4 = R_7 = OH$, $R_2 = R_4 = R_5 = R_6 = R_8 = R_9 = H$, $R_1 = R_4 = R_3 = OH$, $R_7 = OMe$

231 $R_2 = R_4 = R_5 = R_6 = R_8 = R_9 = H$, $R=R_1 = R_3 = R_7 = OH$

232 $R_5 = R_6 = R_8 = R_9 = H$, $R= R_1 = R_2= R_3= R_4= R_7=OH$

233 $R_1 = R_6 = R_7 = OH$, R_2= OMe, R_3= O-rha (2-1) gal, $R_3= R_4=R_5 = R_8 = R_9 = H$



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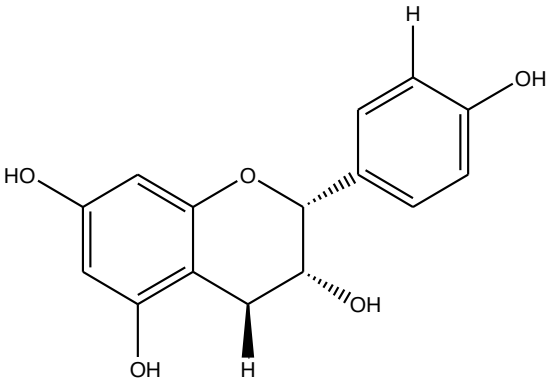

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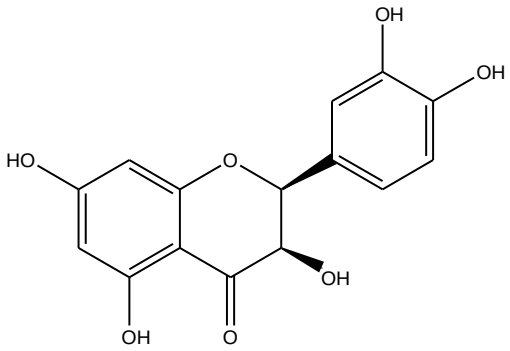

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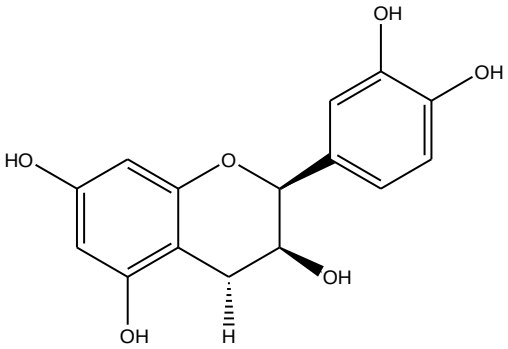

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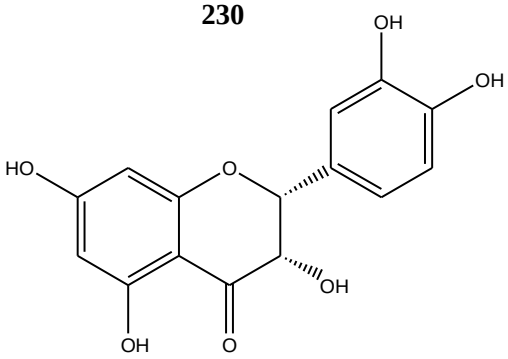

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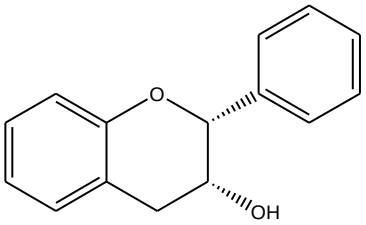
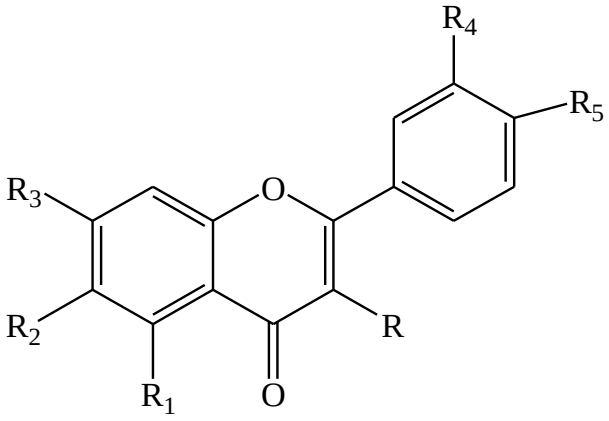


236

In a bioassay-guided chemical analysis of *S.nigricans* leaf extract, a flavonoid, luteolin (**237**) has been isolated (Georges*et al*., 2008). Similarly, chemical evaluation of *S. angustifolia* leaf methanol, ethanol and ethyl acetate extracts by HPLC-MS revealed the presence of three bioactive flavonoid compounds characterized as quercimeritrin (**238**), scutellarein (**239**), and rutin (**240**) (Ahmed *et al*., 2016). From flowers of *Senna tora* has been reported the isolation of kaempferol (**231**) and leucopelargonidin (**241**) (Asba and Meeta, 2017). Yadav *et al*., (2010) reported the isolation of apigenin (**242**) and quercetin-3-O-rhamnoside (**243**) from *Senna occidentalis*. Further study by Yadav and Satnami (2011) on the plant seeds led to isolation and characterization of three flavonoid derivaties namely, 3', 4'-dihydroxyflavone 5 -O-β-D-xylopyranosyl-7-O-β-L-rhamnopyranosyl-(1—>3)-O - α - L-arabinopyranoside (**244**), 5, 3', 4'-trihydroxyflavonol 3-O - α-L-rhamnopyranosyl- 7 - O - β - D- glucopyranosyl-(1—>3)-O - β - D-xylopyranoside (**245**) and 7, 3, 3', 4' - tetrahydroxy- 6 methoxyflavonol -5 - O - β -L-arabinopyranosyl (1—>4) - O - α-L -

rhamopyranosyl- (1—>3) - O-β - D-galactopyranoside (**246**). Three C-glycosidic flavonoids coded cassia occidentalins A (**247**), B (**248**) and C (**249**) were isolated from aerial parts of the plant (Hatano *et al*., 1999).



237 R = R_2 = H, R_1 = R_3 = R_4 = R_5 = OH

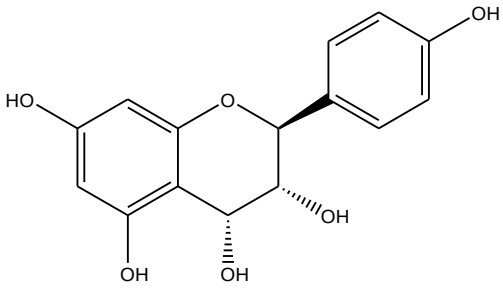
238 R = R_1 = R_4 = R_5 = OH, R_3 = Oglu, R_2 = H

239 R = R_4 = H, R_1 = R_2 = R_3 = R_5 = OH, R_2 = H

240 R = Oglu (1-6) rha, R_1 = R_3 = R_4 = R_5 = OH, R_2 = H

242 R = R_1 = R_2 = R_4 = H, R_1 = R_3 = R_5 = OH

243 R = Orha, R_1 = R_3 = R_4 = R_8 = OH, R_2 = H



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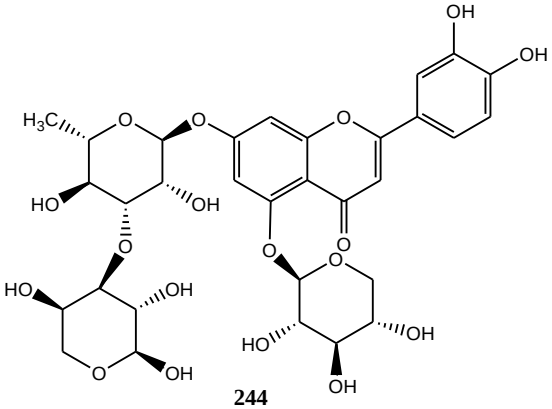

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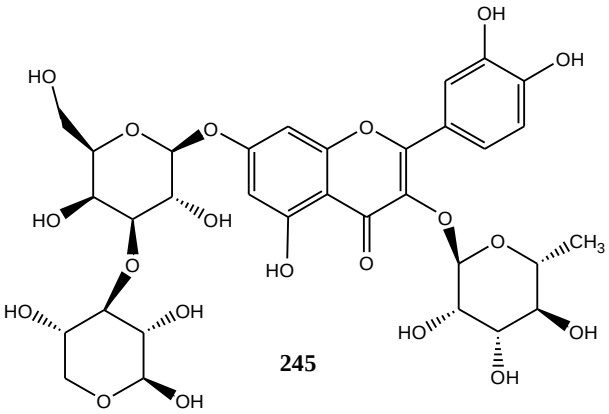

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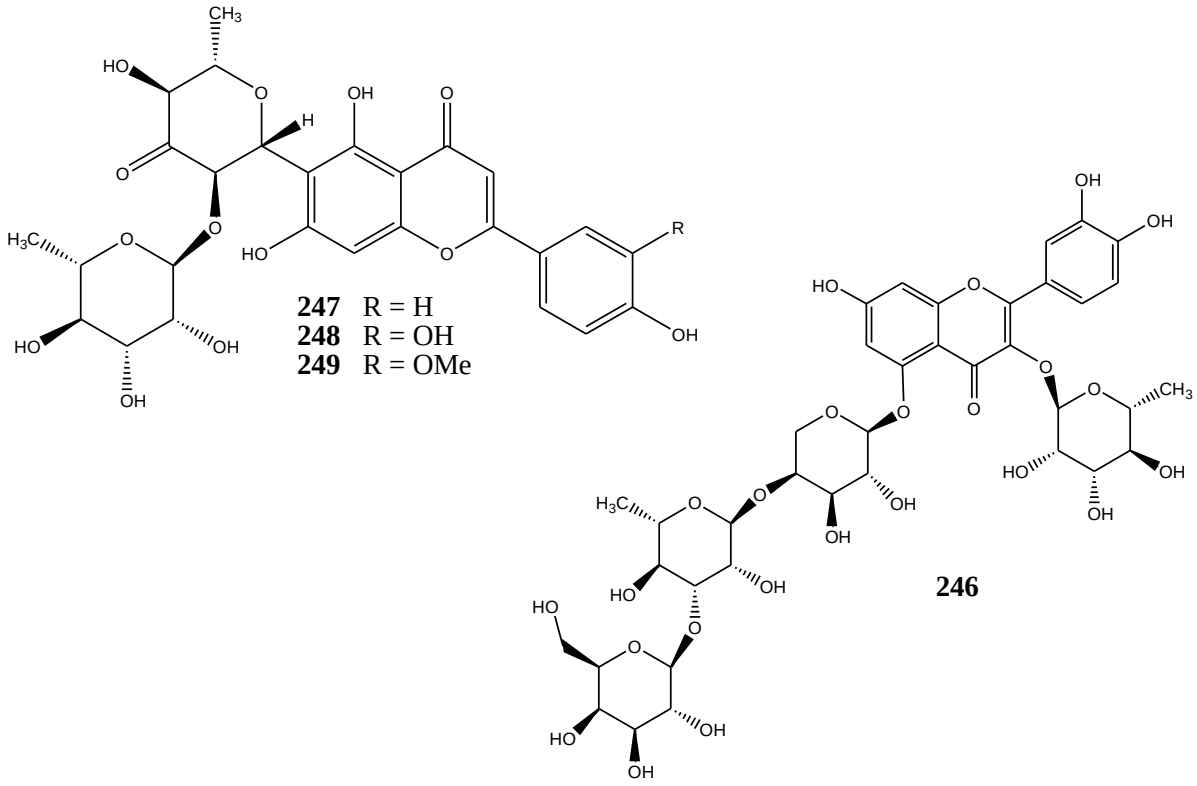
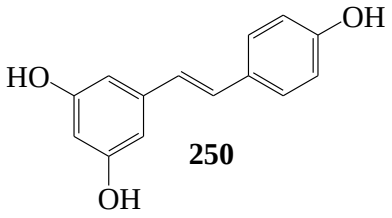


Senna italica, is widely used in South African traditional medicine to treat a number of diseases. From the acetone root bark extract has been reported the isolation of a compound that was identified as 3, 4', 5-trihydroxystilbene (resveratrol) (**250**) (Mokgotho*et al*., 2013). Gupta and Singh (1991) reported chrysoeriol-7-*O*-*β*-D-mannopyranosyl (2``-1''')-*β*-D-allopyranoside (**251**) from seed extract of the plant. From the roots of *S. alata* the isolation of kampferol (**231**) was documented by Fernand *et al.,* (2008). In another report compiled by Juan-Badaturuge *et al* (2011), the isolation of kaempferol- 3-*O*-rutinoside (**252**), quercetin (**253**) and luteolin (**254**) together with kampferol (**231**) from S. *auriculata* aerial parts. Phytochemical study on *S. angustifolia* leaves methanol extract led to isolation and characterization of three flavonoid glycosides: quercetin- 3-*O*-gentiobioside (**255**), kaempferol- 3-*O*-gentiobioside (**256**), isorhamentin 3-*O*-gentiobioside (**257**) (Ganapaty *et al*., 2002).



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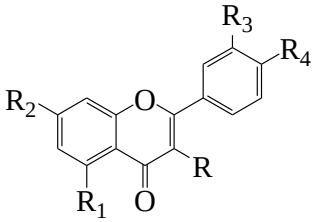
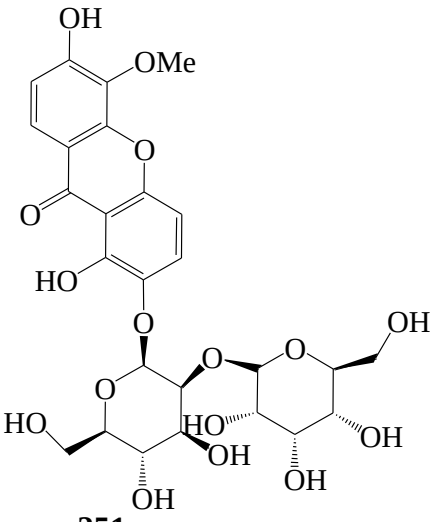


252 R = O glu(1-6)rha, $R_1 = R_2 = R_3 = R_4 = OH$

253 $R = R_1 = R_2 = R_3 = R_4 = OH$

254 R = H, $R_1 = R_2 = R_3 = R_4 = OH$



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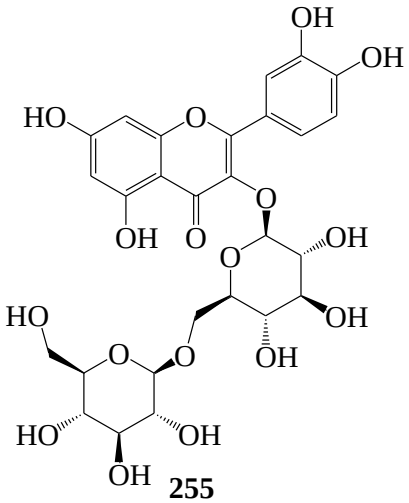

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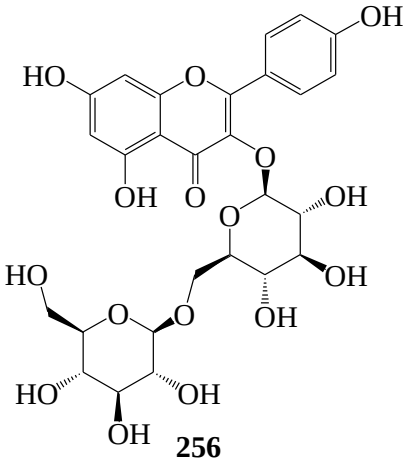

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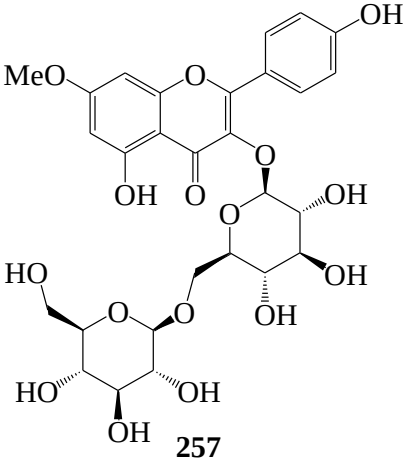
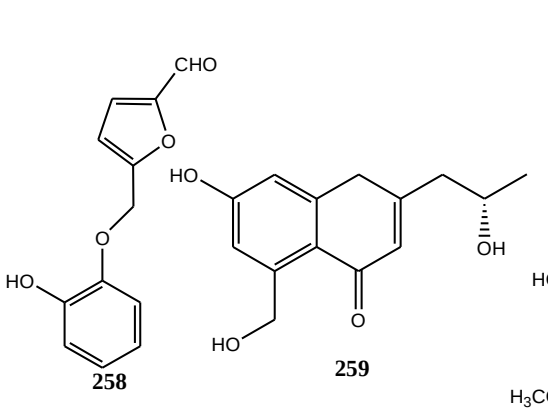
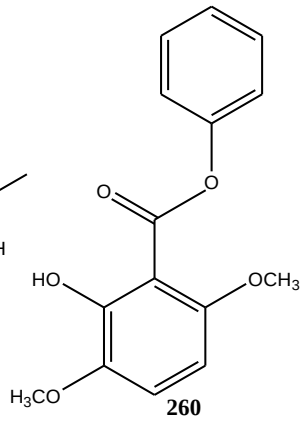


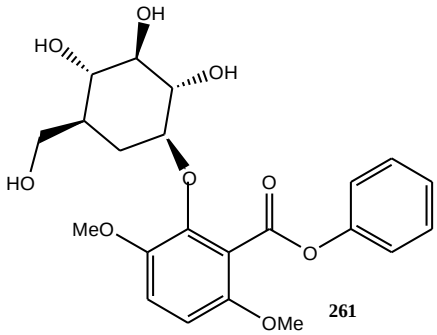
257

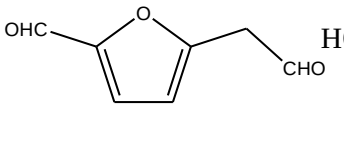
On the other hand, the leaves extract of *S. fistula* uponexploration for phenolic constituents resulted in the isolation and identification of 5-(2-hydroxyphenoxymethyl)furfural (**258**), (2'S)-7-hydroxy-5- hydroxymethyl-2-(2'-hydroxypropyl)chromone (**259**), benzyl 2-hydroxy-3,6-dimethoxybenzoate (**260**), benzyl –*O*-2β-D-glucopyranosyl-3,6-dimethoxybenzoate (**261**), 5-

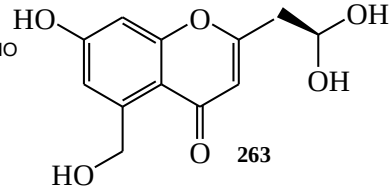
hydroxymethylfurfural (**262**) and (2'S)-7-hydroxy-2-(2'-hydroxypropyl)-5-methylchromone (**263**) (Kuo *et al*., 2002). Another species which has been phytochemically investigated for its favonoid constituents is *S. glauca*. From the leaves of this plant apigenin (**242**), luteolin (**254**), quercetin (**253**), quercetin-3-*O*-*β*-D-glucopyranoside (**264**), kaempferol-3-*O*-rutinoside (**252**) and rutin (**240**) has been reported (El-Sayed *et al*., 2013). In a separate study El-Sawi and Sleem (2010) documented the isolation of quercetin-3-*O*-glucoside-7-*O*-rahmnoside (**265**) from the leaves of *Senna surattensis*.

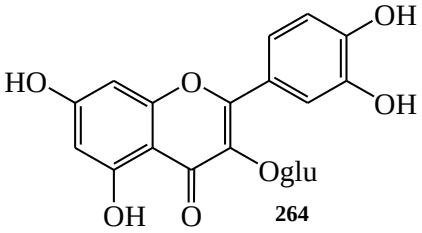


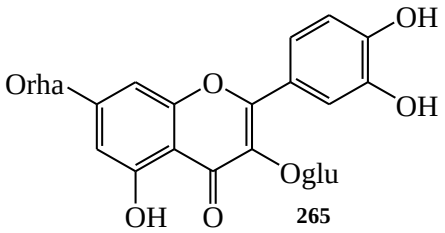






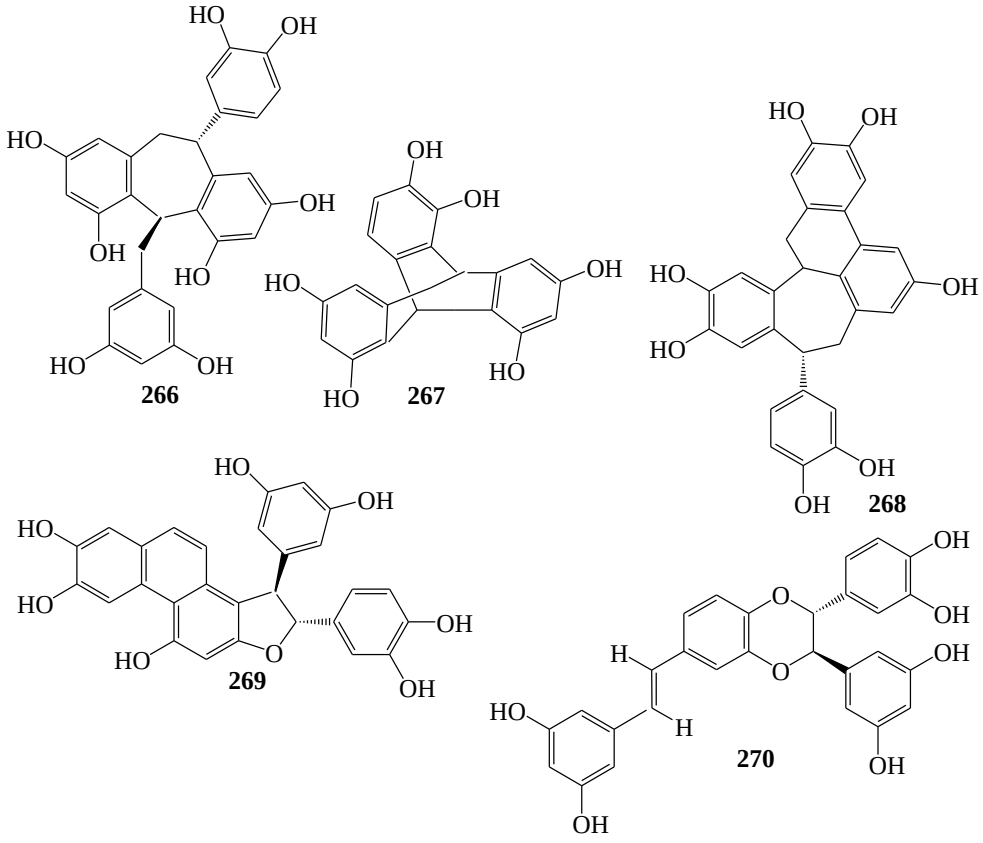


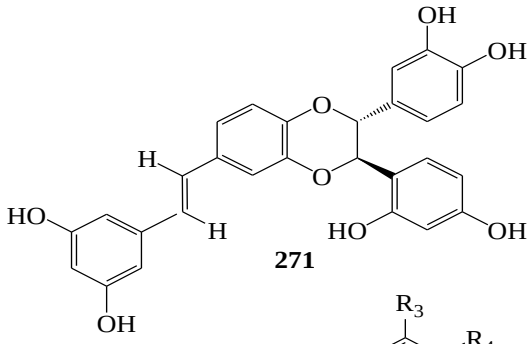




A search for phenolic component of *C. garrettiana* heart wood by Murakami *et al* (1992) led to the isolation of stibernoids namely, cassigarol A (**266**). In the same year compound (**266**) together

with cassigarol B (**267**), cassigarol C (**268**) and Cassigarol D (**269**) were extracted from the same plant heartwood (Baba *et al*., 1992). Two years later, polyphenol stilbernoids named cassigarol E (**270**), Cassigarol F (**271**) and cassigarol G (**272**) were isolated from the plant heartwood (Baba *et al*., 1994). From *S. italic* aerials parts, an array of phenolic compounds have been reported and these included apeginin-7-O-*β*-D-glucoside (**273**), kampferol-7-*O*-*β*-D-glucoside (**274**), quercetin-7-*O*-*β*-D-glucopyranoside (**275**), tamarixetine-7-*O*-*α*-L-rhamnopyranoside (**276**) andisorhamentin-7-*O*-*β*-D-glucopyranoside 3-*O*- rutinoside (**277**) El-sayed *et al*., 1992).





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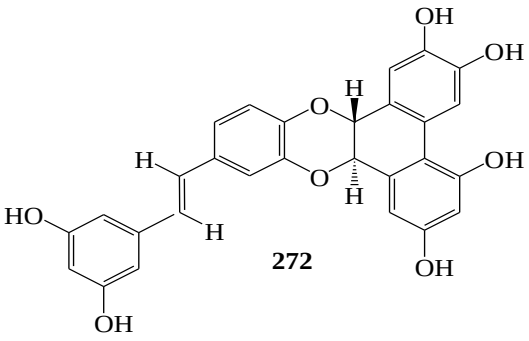

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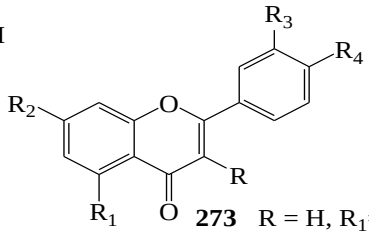


273 R = H, R_1= R_4 = OH, R_2 = Oglu

274 R = R_1 = R_4 = OH, R_2 = Oglu, R_3 = H

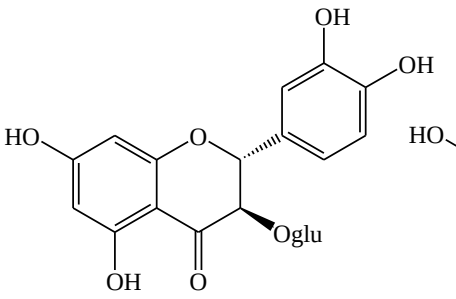
275 R = R_1= R_3 = R_4 = OH, R_2 = Oglu

276 R = R_1 = R_3 = OH, R_2= Orha, R_4 = OMe

277 R = O glu (1-6) rha, R_1= R_4 = OH, R_2 = Oglu, R_3 = OMe

Similarly, search for phenolic compounds in the flowers of *S. javanica* afforded dihydrorhamnetin-3-*O*-*β*-D-glucopyranoside (**278**) and leucocyanidin- 4'-*O*-methyl ether-3-*O*-*β*-D-galactopyranoside (**279**) (Bhuvaneswari and Gobalakrishnan, 2015). Elsewhere phenolic constituents vitexin (**280**) four flavonoid derivatives demethyltorosaflavone C (**281**), demethyltorosaflavone D (**282**) and luteolin 7- *O*-*β*-D-glucopyranoside (**283**) have been found in *S. nomame* aerial parts (Kitanaka. and Takido, 1992). As part of isolation of bioactive phenolic constituents with regards to *S. obtusifolia* seeds Wu *et al* (2012) reported the isolation of 2-benzyl-4,6-dihydroxy benzoic acid (**284**), 2-benzyl-4,6-dihydroxy benzoic acid-6-O-*β*-D-glucopyranoside (**285**), 2-benzyl-6-dihydroxy benzoic acid-4-O-*β*-D glucopyranoside (**286**), 2-benzyl-4-hydroxybenzoic acid-6-*O*-[2`,6`-*O*-diacetyl]-*β*-D-glucopyranoside (**287**), 2-benzyl-4-dihydroxy benzoic acid-6-*O*-[3`,6`-*O*-diacetyl]-*β*-D-glucopyranoside (**288** and 2-benzyl-4-dihydroxy benzoic acid-6-*O*-[4`,6`-*O*-diacetyl]-*β*-D-glucopyranoside (**289**). From the unripe seeds of *S. torosa*, flavonoid derivatives namely, demethyltorasaflavone C (**290**) and torosaflavone D

(**291**) along with 2, 5-dimethyl -8,10-dihydroxy-4H naphtho[l,2-b]pyran-4-one (**292**) have been reported (Takidoand Kitanaka, 1991).



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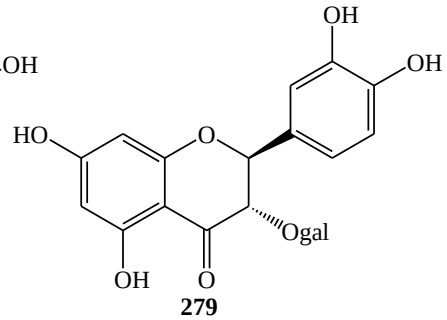

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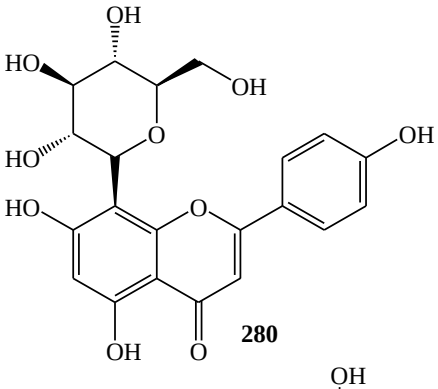

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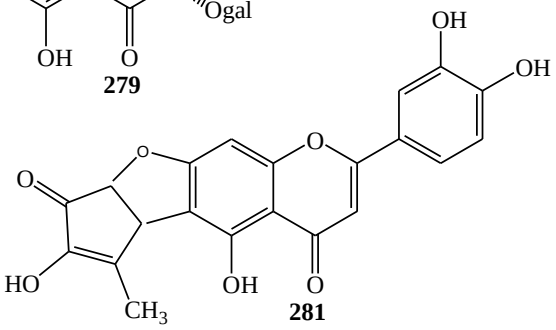

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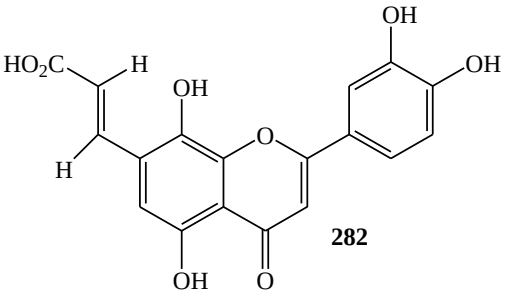

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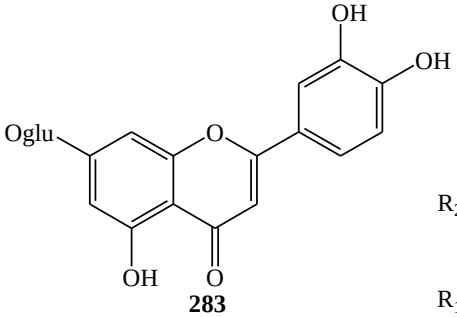

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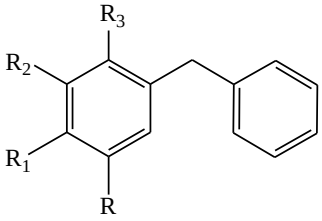
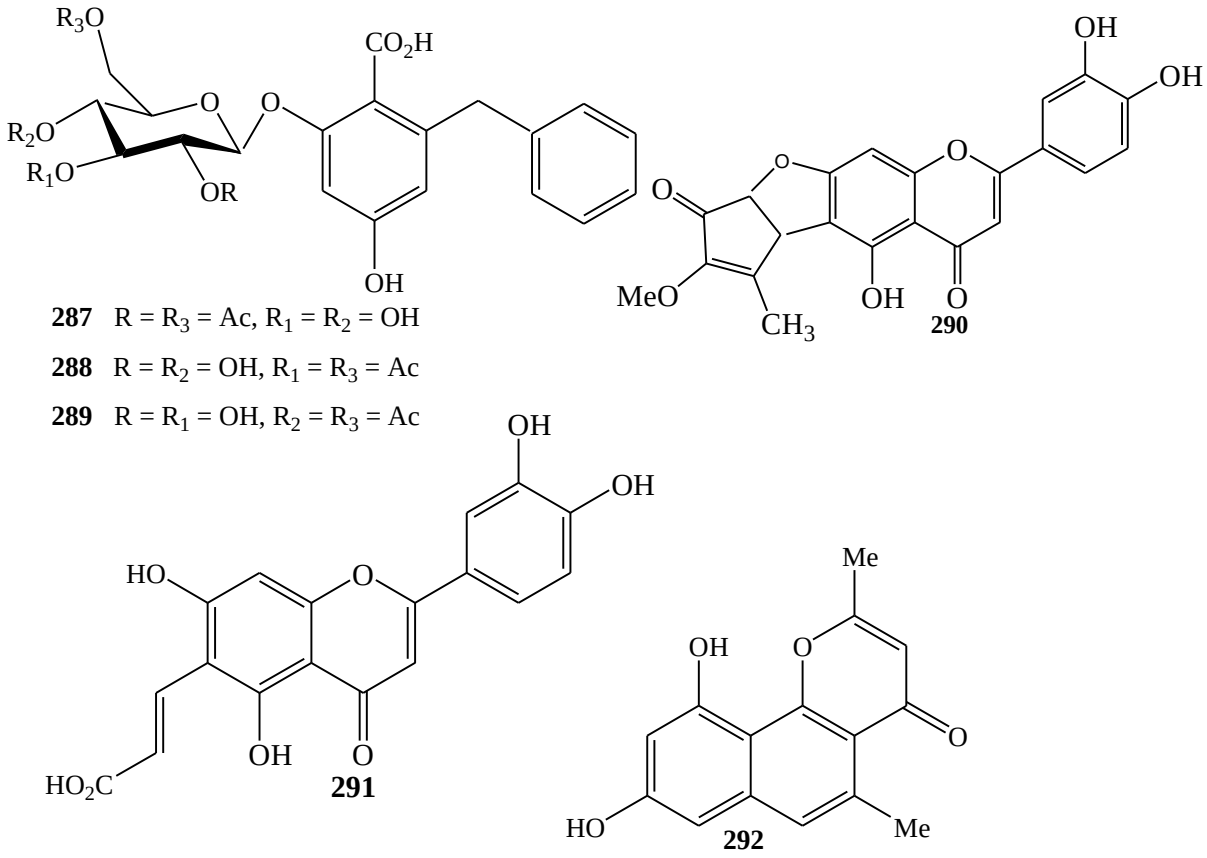


284 R = R_2 = OH, R_1 = H, R_3 = CO_2H

285 R = OH, R_1 = H, R_2 = Oglu, R_3 = CO_2H

286 R = Oglu, R_1 = H, R_2 = OH, R_3 = CO_2H



287 R = R_3 = Ac, R_1 = R_2 = OH

288 R = R_2 = OH, R_1 = R_3 = Ac

289 R = R_1 = OH, R_2 = R_3 = Ac

290

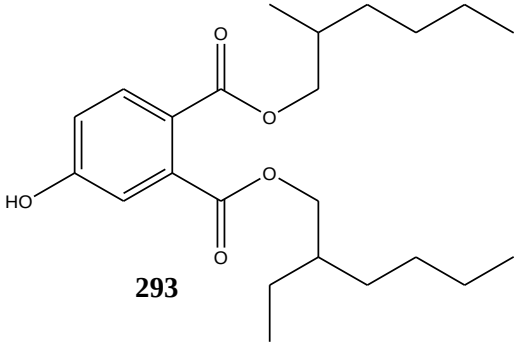
291

292

2.6.3 Other Miscellaneous Classes of Secondary Metabolites Isolated from Senna Species.

Chemical analysis of *S. auriculata* leaves by Rao *et al* (2000) led to the isolation di-(2-ethyl)-hexylphthalate (**293**) while from the leaves *of S. floribunda* a compound named N1,N8-dibenzoylspermidine (**294**) has been reported (Singh *et al*., 2013). In a study to determine fatty acid composition and characteristics of *S. glauca* seed oil, Dixit and Tiwari (1990) characterized six fatty acids namely, palmitic acid (**295**), stearic acid (**296**), oleic acid (**297**), linoleic acid (**298**), linolenic acid (**299**) and arachidic acid (**300**). From the stem bark of the plant, β -sitosterol- β –D-glucoside (**301**) has been reported (Dave and Ledwani, 2012). β -sitosterol (**302**), stigmasterol (**303**) and galactomannan (**304**) have been reported from *S. italica* leaves (Kazmi *et al*., 1994; Jones *et al*., 2000). Chemical investigation of *S. laevigata* leaf extractled to the isolation of

cinnamic acid (**305**), 3-hydroxy-1-(4-hydroxy-3-methoxyphenyl)-propan-1-one (**306**), 2,3-dihydroxy-1-(4-hydroxy-3-methoxyphenyl)-propan-1-one (**307**), 3-hydroxy-1- (4-hydroxy-3,5-dimethoxyphenyl)-propan-1-one (**308**), syringic acid (**309**) and vanillic acid (**310**) (Jones *et al*., 2000).



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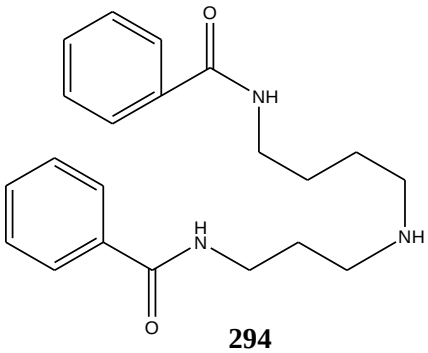


294

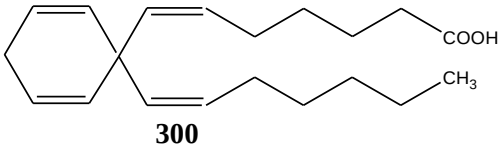
$CH_3(CH_2)_{13}CH_2CO_2H$
295

$CH_3(CH_2)_{16}CO_2H$
296

$CH_3(CH_2)_7CH{=}CH(CH_2)_7CO_2H$
297

$CH_3(CH_2)_4CH{=}CHCH_2CH{=}CH(CH_2)_7CO_2H$
298

$CH_3CH_2CH{=}CHCH_2CH{=}CH(CH_2)_7CO_2H$
299



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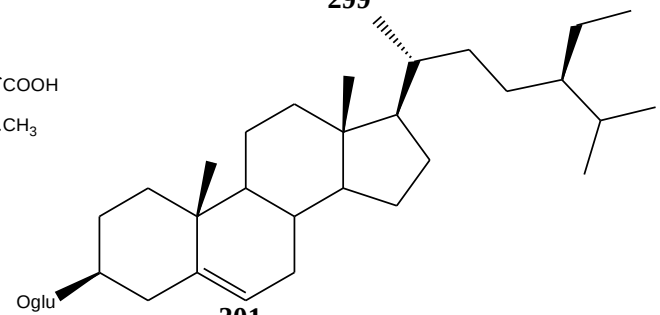

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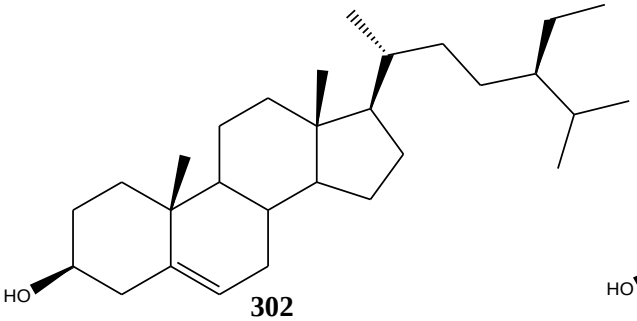

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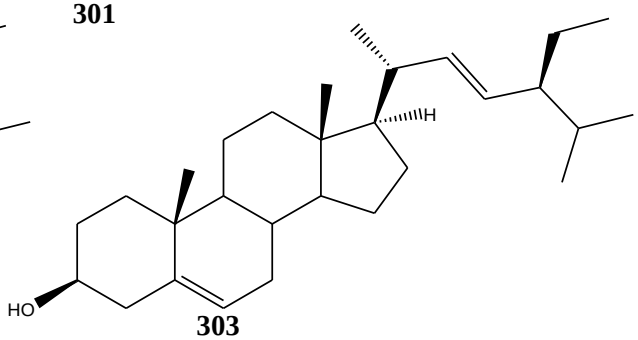

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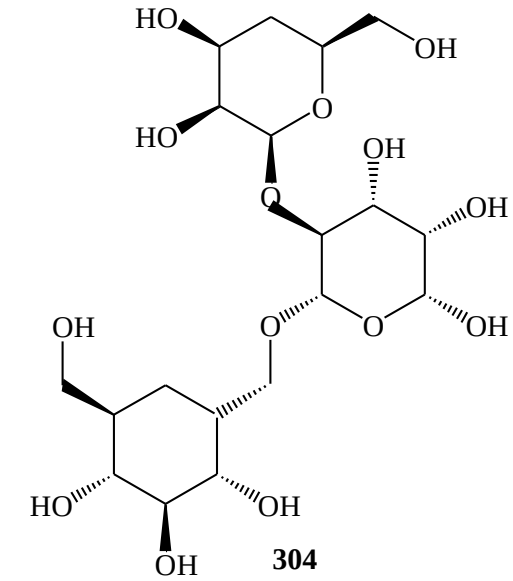

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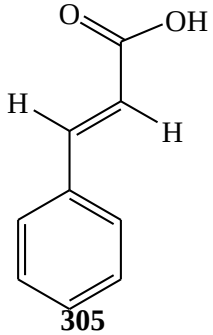

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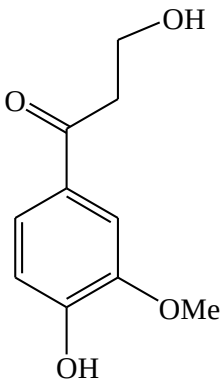

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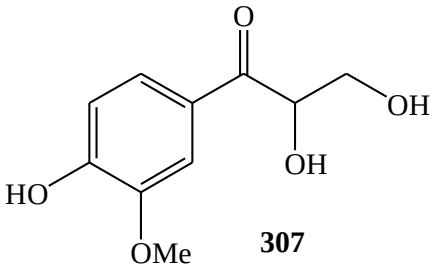

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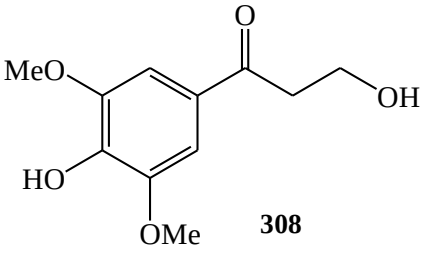

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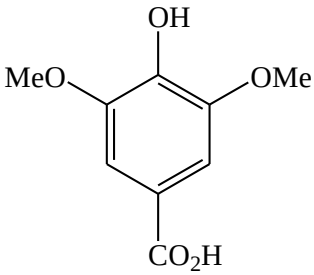

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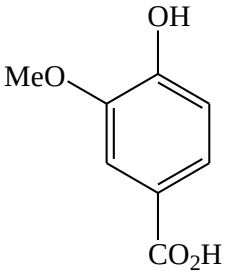
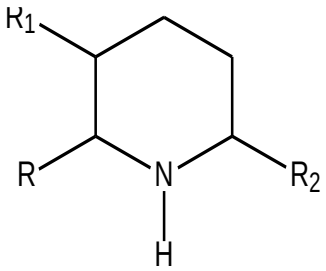


310

On the other hand, piperidine alkaloids documented from *S. leptophylla* leaves by Bolzani *et al*. (1995) included (-)-spectaline (**311**), (-)-spectalinine (**312**), canavaline (**313**), leptophyllin A (**314**), 3-acetylleptophyllin A (**315**), iso-6-canavaline (**316**) and leptophyllin B (**317**). The leaf extract of *S. multijuga* yielded 5-acetonyl-7- hydroxy-2-methylchromone (**318**) and 5-acetonyl-2-methylchromone-7-*O* - *β* - D-glucopyranoside (**319**) as phenolic compound derivatives (Zhao *et al*., 2016) while chemical investigation of *S. nodosa* stem bark nodolidate (**320**) (Jain *et al*., 2013).



311 R = α-Me, R_1 = α-OH, R_2 = α-$(CH_2)_{12}COCH_3$

312 R = α-Me, R_1 = α-OH, R_2 = α-$(CH_2)_{12}$ CH (OH) CH_3

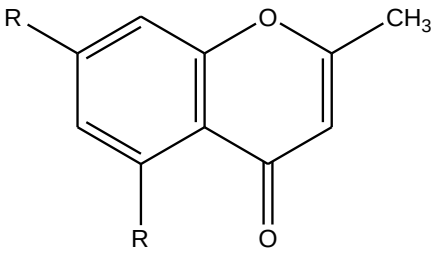
313 R = α-Me, R_1 = α-OH, R_2 = α-$(CH_2)_{10}$ CH (OH) CH_3

314 R = β-Me, R_1 = β-OH, R_2 = β-$(CH_2)_{10}$ CH (OH) CH_2OH

315 R = β-Me, R_1 = β-OAc, R_2 = β-$(CH_2)_{10}$ CH (OH) CH_2OH

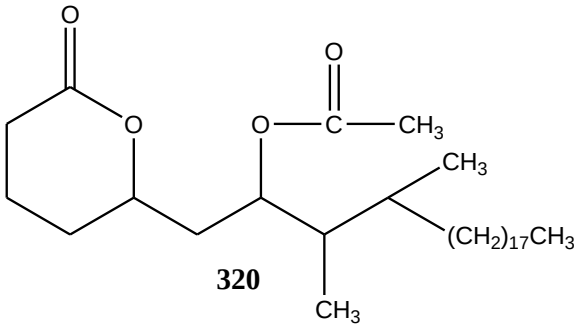
316 R = α-Me, R_1 = α-OH, R_2 = β-$(CH_2)_{10}$ CH (OH) CH_3

317 R = α-Me, R_1 = α-OH, R_2 = β-$(CH_2)_{10}CO2H$



318 R = CH_2COCH_3, R_1 = OH

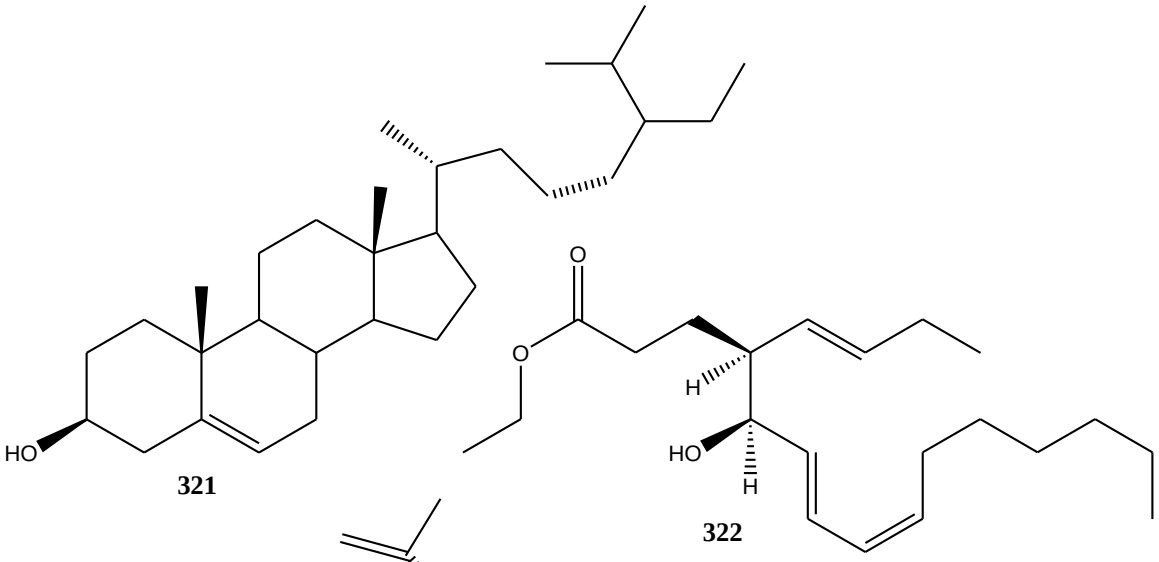
319 R = OH, R_1 = Oglu



320

A A sterol derivative, phytosterol (**321**) has been isolated from *S. occidentalis* roots (Yadav *et al*., 2010) while from *C. obtusifolia* leaves the isolation of (4R, 5S, 6E, 8Z)-ethyl-4-[(E)-but-1-enyl]-5-hydroxypentadeca-6,8- dionate (**322**), stigmasterol (**303**), lupeol (**323**), betulinic acid (**324**) and friedelin (**325**) has been reported (Sob *et al*., 2010). Rahman *et al* (2017) reported the isolation of *β*-sitosterol (**302**) from *S. sophora* leaves and from the seeds of *S. tora*, a compound named cassitoroside (**326**) was isolated (Choi *et al*., 1995). A compound named barakol (**327**) has

been reported from *S. siamea* leaves (Oshimi *et al*., 2008) while from the leaf extracts of the same plant, Ravi*et al* (2013) documented the presence of chrobisiamone A (**328**) and cassiarin A (**329**). Similarly, from the flowers of the plant the isolation and characterization of secondary metabolites cassiadinine (**330**), 5-acetonyl-7- hydroxy-2-methylchromone (**331**), (+)-6-hydroxymellein (**332**), (+)-6-hydroxymellein diacetate (**333**) and chaksine (**334**) (Thongsaard *et al.*, 1996).



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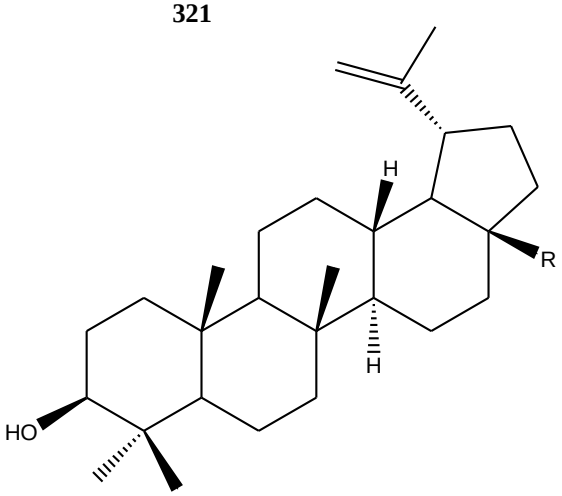
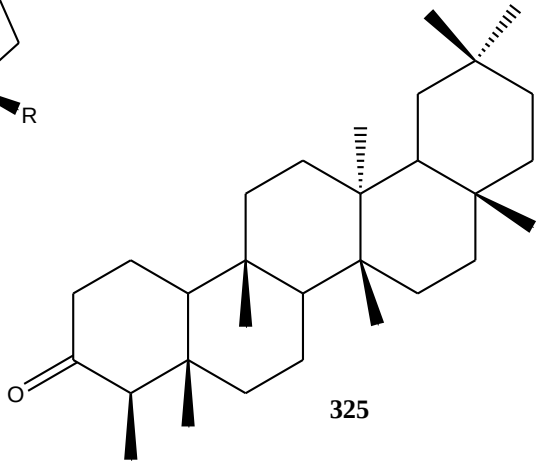


323 R=Me

324 R=CO_2H



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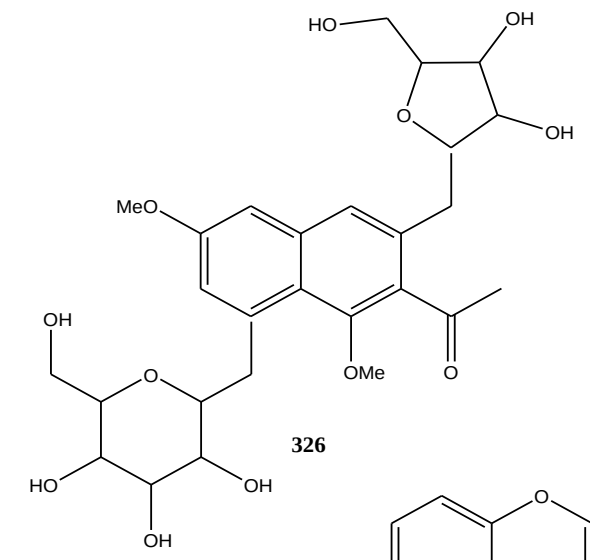

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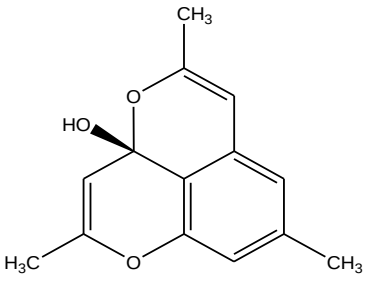

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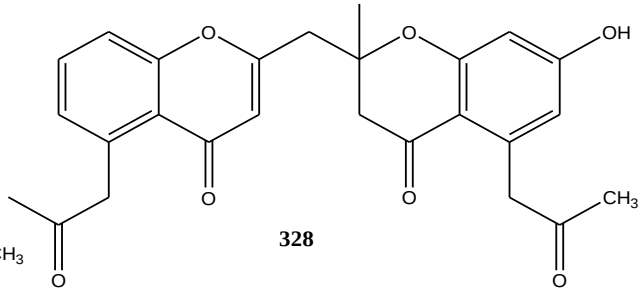

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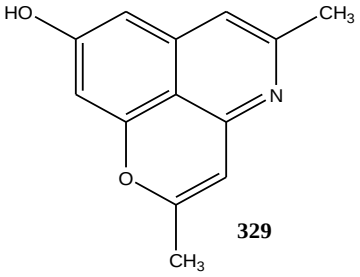

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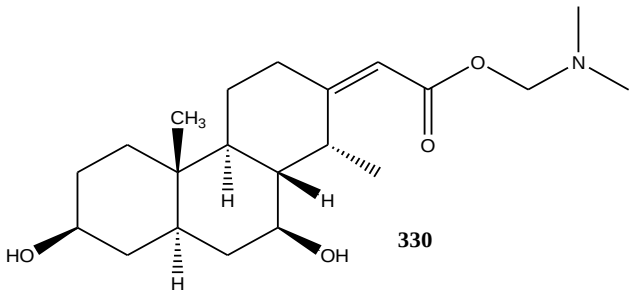

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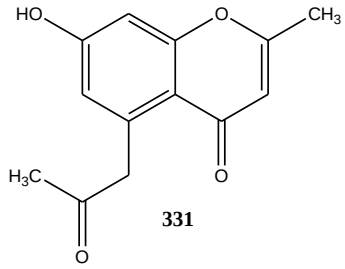

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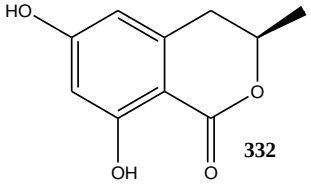

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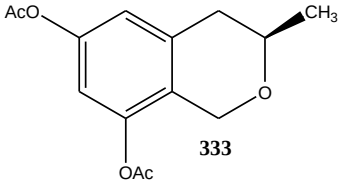

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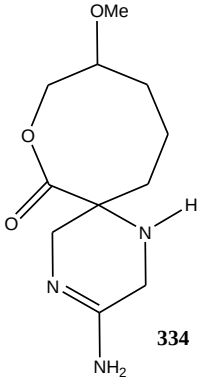

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CHAPTER THREE

MATERIALS AND METHODS

3.1 General Experimental Procedure, Solvents and Fine Consumables

Melting points were determined using Gallenkamp melting point apparatus. NMR data were measured in $CDCl_3$ and DMSO-d_6 on a JOEL NMR instrument operating at 600 and 150 MHz, respectively. Some NMR data were done using Brucker AM 300 spectrometer operating at 360 and 90 MHz, respectively. Tetramethylsilane (TMS) was used as reference standard. The mass spectral data were obtained using a Varian MAT 8200 A instrument. Electron-impact (EI) mass spectra (70 eV) were measured on a Hewlett-Packard 5989B mass spectrometer. Silica gel 60 (63-200 μm, Merck, Darmstadt, Germany) was used for gravity column chromatography (CC). TLC was performed on precoated DC Alufolien 60 F254 sheets and UV lamp, conc. ammonia, conc. sulphuric acid and vanillin were used as visualizing agents. Solvents used were all of analytical grade.

3.2 Collection and Prepation of Plant Materials

Collection of the flowers was done from Kisumu County Nyahera region (Lat: 0.0268ºS and Long: 34.7243ºE) in the month of February 2014. The selection of the region was due to ethnobotanical use of *Senna didymobotrya* flowers in management of diarrhoea and skin diseases, which are microbial infections, by the people from this region (P. Atieno, "personal communication," January, 2014). The plant twigs were identified at Maseno University, Botanical Garden Herbarium after comparison with authentic samples where the voucher specimen (2014/02/HAO/CHEMMK) was deposited. The flowers were dried under shade at room temperature for one week and crushed into fine powder using a grinding mill model 4 E.

3.3 Sequential Extraction of Plant Materials Using Organic Solvents

Dry powdered flowers (1 kg) was sequentially cold extracted with *n*-hexane, ethyl acetate and methanol (each 2L) lasting 24 hours while under mechanical stirring. The extracts were separately filtered and the filtrates concentrated under *vacuo* to yield 10 g, 25 g and 40 g, respectively. The *n*-hexane extract was yellow, ethylacetate extract was brown while methanol extract was dark brown in colour.

3.4 Test Microorganisms

Staphylococcus aureus (ATCC 43320), *Streptococcus faecalis* (ATCC 25925) and clinical isolate *Bacillus anthracis* were representatives for Gram-positive bacteria while *Klebsiela pneumaoniae* (ATCC 90028), *Escherichia coli* (ATCC 25922), *Salmonella typhimurium* (ATCC 25927) and *Pseudomonas aeruginosa* (ATCC 27853) represented Gram-negative bacteria in the antibacterial assays. The fungal pathogens *Candida albicans* (ATCC 90028), and the clinical isolates *Microsporum gypseum* and *Trichophyton mentagrophyte* were representatives of yeast and filamentous fungi used for antifungal tests. The test organisms were obtained from the stock kept at the Microbiology Section of the Jaramogi Oginga Odinga Teaching and Referral Hospital in Kisumu County, Kenya.

3.5 Preparation of Antimicrobial Agent Discs

Punch paper discs of 6 mm diameter were cut off from Whatman paper No. 3 and put in clean, dry capped bottle and sterilized at 120 ^{0}C for 20 minutes. The discs were stored in a refrigerator at 4°C for future use (Baur *et al*., 1966).

3.6 Preparation of Mueller Hinton Agar (MHA) Medium

The Mueller-Hinton agar (Oxoid, UK) medium was prepared according to Baur *et al*., (1966). The prepared medium was autoclaved and then allowed to cool after which it was poured into petri dishes on a level surface to produce a depth of approximately 4 mm (25 ml of medium for 100 mm plates). The plates were allowed to cool to room temperature. The same procedure was used to prepare Sabouraud dextrose agar.

3.7 Preparation of bacterial inoculum for antibacterial test

Disc diffusion assay procedure that involves impregnation of antibacterial substances on filter discs was used (Baur *et al*., 1966; Murray *et al*., 1999). The inocula were prepared for both Gram positive and Gram negative bacteria. Five colonies of each bacterium of the same morphological type were picked using an inoculating loop from overnight growth on agar medium and suspended in sterile saline. The mixture was shaken on an orbital shaker and turbidity adjusted to yield 0.5 McFarland standards (approximately 1-2 x 10^8 colony forming units per milliliters).

3.8 Preparation of Fungal Inoculum for Antifungal Test

The procedure for preparation of fungal inoculation was similar to that for bacteria (Baur *et al*., 1966). The inocula were prepared for both yeast and filamentous fungi whereby approximately four colonies of each fungal strain was picked by a sterile wire loop from Sabouraud dextrose agar and suspended in sterile saline in separate petri dishes. The turbidity of the suspension adjusted to 0.5 McFarland to give inoculum concentration of 1-5 x 10^6 colony forming units per milliliters (Shadomy and Pfaller, 1985).

3.9 Impregnation of Paper Discs with Plant Extracts

Baur-Kirby procedure which uses known concencentrations of antimicrobial substances impregnated on paper discs was used (Baur *et al*., 1966). The anti-biogram patterns were studied using *n*-hexane, EtOAc, and MeOH extracts, and isolates of *S. didymobotrya* flowers. The plant extracts 100 µg were dissolved in 2.0 ml dimethyl sulfoxide (DMSO) to give a final concentration of 50 µg/ml. Sterile paper discs (6 mm of diameter) were impregnated with 10µl of each solution of extracts. For each extract subjected to the antimicrobial tests, three discs impregnated with the test substances were used per plate for each microorganism. The discs were placed each time alone with the help of forceps and gently pressed down onto the agar surface for uniform contact. The impegnated discs were kept at room temperature for 30 minutes for proper diffusion of extracts and isolates before putting them on the petri dishes. Similarly, paper discs containing standard concentrations of antibiotics kanamycin and antifungal fluconazole were used as positive control while 95% DMSO was used as a negative control.

3.10 Incubation of the Impregnated Discs

After approximately 30 minutes, petri dishes containing Mueller-Hinton agar and Sabouraud dextrose agar seeded with bacterial and fungal inocula, respectively were incubated. Dishes containing *S. aureus* (ATCC 43320), *S. faecalis* (ATCC 25925), *B. anthracis, K. pneumaoniae* (ATCC 90028), *E. coli* (ATCC 25922), *S. typhimurium* (ATCC 25927) and *P. aeruginosa* (ATCC 27853) were incubated at 37 ^{0}C for 24 hours while the yeast fungus, *C. albicans* was incubated at 35 ^{0}C for 24 hours. The filamentous fungi, *T. mentagrophyte* and *M. gypseum* were incubated at 25 °C for 72 hours (Peterson and Shanhaltzer, 1992). After incubation period the plates were observed for the zone inhibitions which corresponds to the antimicrobial activity of tested extracts. The zones of inhibition observed were measured in mm. The tests were done in triplicate for all

the organisms and values obtained were subjected to Fisher's protected least significant difference test.

3.11 Determination of Minimum Inhibitory Concentrations (MIC) for Extracts.

The minimum inhibitory concentration (MIC) is a measured parameter that evaluates the effect of antimicrobial agent and it is defined as the lowest concentration of an antimicrobial drug that inhibits the growth of a microbe after an incubation period of time at a given temperature (Peterson and Shanholtzer, 1992). Stock solutions of extracts were constituted according to Luvonga (2007) method whereby the extracts were dissolved in DMSO to give final concentration of 500 µg/ml. Serial dilution was done according to the method of Washington and Sutter (1980) whereby the extracts stock solutions were put in labelled sterile test tubes, serially diluted and thereafter arranged from the highest to lowest concentrations. Using sterile pipette, 2 ml of DMSO was added to each of the nine tubes and to the first tube was added 500 µg/ml of the extract and the mixtures agitated. An aliquot 2 ml solution of this tube was transferred to the second one and a similar procedure repeated up to the eighth tube. Thus, this process afforded the concentrations 500 µg/ml, 250 µg/ml, 125.0 µg/ml, 62.0 µg/ml, 31.30 µg/ml, 15.0 µg/ml, 7.80 µg/ml and 3.90 µg/ml. The content of nineth tube was only DMSO and it acted as negative control experiment.

3.12 Isolation of Compounds from *Senna didymobotrya* Flowers

3.12.1 Thin Layer Chromatography (TLC) Analysis of the extracts of *S. didymobotrya* flowers

TLC analyses of *n*-hexane extract using *n*-hexane-CH_2Cl_2 (3:2) as a mobile phase showed three spots of R_f values 0.49, 0.45 and 0.32. The first two yellow spots appeared dark brown when visualized under UV light (254 nm) while the latter spot was inactive under UV light. Upon exposure to concentrated ammonia vapour, the first two yellow spots turned purple but later

changed to their original yellow colour signifying the presence of anthraquinones while the spot with R_f 0.32 was inactive to concentrated ammonia. Lastly the spots were sprayed with vanillin-concentrated H_2SO_4 acid mixture followed by heating on hot plate at 100 ºC for 1 minute whereby the spot with R_f value 0.32 turned purple suggesting it could be either sterol or terpenoid. The EtOAc extract when subjected to TLC analysis using *n*-hexane-EtOAc (1:1) afforded additional five more spots of R_f values 0.56, 0.54, 0.48, 0.30 and 0.21 which were absent in the *n*-hexane extract. The yellow spot with $R_f = 0.48$ turned purple on exposure to concentrated ammonia vapour and with time reverted to its original yellow colour indicating the presence of anthraquinone. The remaining five spots turned purple upon spraying with vanillin-sulphuric acid mixtures indicating the presence of sterols or terpenoids. On the other hand, the MeOH extract run on TLC plate using dichloromethane-methanol (9:1) followed by the same solvent system in the ratio 4:1 afforded five faint yellow spots R_f values 0.61, 0.58, 0.56, 0.31 and 0.26, all showed dark yellow colours upon exposure to conc ammonia and upon spraying with sulphuric acid which suggested the presence of flavonoid derivatives.

3.12.2 Separation and Purification of Components from S. didymobotrya Flower Extracts.

3.12.2.1 Fractionation of *n*-Hexane Extract

Approximately 8.5 g of the *n*-hexane extract mixed with 20 ml hexane was charged onto a glass column (3.5×60 cm) packed with a slurry of 200g of silica gel. Elution was performed using mixtures of solvents in order of increasing polarity starting with *n*-hexane, *n*-hexane-dichloromethane, 95:5, 9:1, 4:1 and 3:2 and lastly the column was eluted with CH_2Cl_2. A total of 150 fractions, each 20 ml were sampled and their TLC profiles monitored using *n*-hexane-CH_2Cl_2 (3:2). Fractions (1-20) afforded an orange spot that moved with the solvent front which after some time faded in colour indicating deterioration in presence of light. Fractions 25-80 (2.5 g) afforded

two spots of R_f values 0.49 and 0.45 (solvent system: *n*-hexane-CH_2Cl_2, 3:2). The two constituents in the fractions were resolved into individual components using medium pressure chromatography (2.5×60 cm, SiO_2 100g, pressure approximately 1.5 bar) to afford a yellow crystalline solid **A** (R_f = 0.49, 100.0 mg) and yellow needle-like solid **B** (R_f = 0.45, 75.5 mg). Fractions 82-148 showed two spots of R_f values 0.45 and 0.32 and after evaporation of the solvent gave 3.5 g of residue. The mixture was resolved into individual components by repeated column chromatography (2.5 x 60 cm, SiO_2) using *n*- hexane-CH_2Cl_2, 3:2) to afford a further 30.0 mg of **B** and white crystalline solid (**C) (**R_f= 0.32, 150.0 mg).

3.12.2.2 Fractionation of Constituents in EtOAc Extract**.**

Approximately 20.0 g of EtOAc extract was loaded on top of silica gel column (4.5 x 80 cm, SiO_2 350g, pressure = 1.5 bar). Column elution was effected with mixtures of solvents in order of increasing polarity starting with *n*-hexane, *n*-hexane-EtOAc (95:5, 9:1, 4:1, 2:1, and 1:1), EtOAc neat and elution concluded with EtOAc-MeOH (4:1), each 1.5 L of solvent. A total of 300 fractions each 20ml were sampled and depending on their TLC profiles, were grouped into four pools (pool **I –IV**). Pool **I** (fractions 10-50, 1.5 g) eluted with *n*-hexane-EtOAc (9:1) produced further solid **A** (45.2 mg) and **B** (54.0 mg). Pool **II** (fractions 55-95) mainly from *n*-hexane-EtOAc (4:1) were combined, solvent removed and the residue further purified by medium pressure chromatography using *n*-hexane-EtOAc (9:1) to afford white powder **D** (R_f = 0.56, 75.5 mg) and another white amorphous powder **E (**R_f **=** 0.54**,** 50.0 mg). Fractions from more polar ratios 2:1, and 1:1, eluted mixtures of components with R_f values 0.48, 0.30 and 0.21 (solvent system: *n*-hexane-EtOAc, 3:2), respectively, which constituted pools III and IV. Pool III (4.5 g) was further subjected to silica gel column chromatography using solvent system *n*-hexane-EtOAc (3:2) to afford an orange solid **F** (R_f = 0.48, 60.1 mg) and white crystalline solid **G** (R_f = 0.30, 85.4 mg).

Pool IV (1.5 g) was similarly fractionated over silica gel packed column and elution effected with *n*-hexane-EtOAc (1:1) resulting in the isolation of white amorphous powder **H** (R_f = 0.21, 90.0 mg).

3.12.2.3 Fractionation of Methanol Extract of *S. didymobotrya* Flowers**.**

Approximately 35.0 g of the MeOH extract was loaded onto silica gel packed column (5.0 x 80 cm, SiO_2 300g, pressure = 1.5 bar) and developed starting with CH_2Cl_2-MeOH (98:2) and then with increasing concentrations of MeOH in CH_2Cl_2 and finally column elution was concluded with 100% methanol. A total of 150 fractions each 50 ml were sampled and their composition monitored by TLC using dichloromethane-MeOH (95:5, 9:1 and 4:1). The chromatograms were developed on the plates with ammonia, concentrated H_2SO_4 acid and colour reactions observed after spraying with acidified vanillin and heating which gave the following results. Fraction 20-55 showed a spot of R_f value 0.61(CH_2Cl_2-MeOH, 9:1) which turn purple upon spraying with acidified vanillin and heating. Further purification by crystallizing in *n*-hexane-EtOAc (1:2) afforded further **H** in 72.5 mg. Fractions 60-100 (CH_2Cl_2-MeOH, 9:1) gave two yellow spots R_f values 0.58 and 0.56, respectively. The yellow colours intensified upon exposure to concentrated ammonia vapour indicating they could be flavonoid derivatives. The compounds were resolved into individual components by further fractionating over silica gel column using CH_2Cl_2-MeOH (9:1) to give compounds pale yellow amorphous powder **I** (R_f = 0.58, 65.4mg) and yellow amorphous powder **J** (R_f =0.56,41.0 mg). Fraction 105-120 (3.5g) showed a single spot of R_f = 0.31 which upon removal of the solvent was crystallized in aqueous MeOH (95%) to give a pale yellow amorphous powder **K** in a yield of 65.5 mg. The remaining fractions (fractions 124-140, 2.0 g) also showed a single spot (CH_2Cl_2-MeOH, 2:1) which upon evaporation of the solvent followed by crystallization in 2% aqueous MeOH afforded pale yellow amorphous powder **L** (52.0 mg, R_f = 0.26).

3.13 Physical and Spectroscopic Data of Compounds Isolated From *Senna didymobotrya* Flowers.

Compound **A** with R_f 0.49 (*n*-hexane-CH_2Cl_2, 3:2), is a yellow substance with m.p. 196 -197^{0}C; ^{1}H and ^{13}C NMR ($CDCl_3$) data are in Table 5.0 (Appendix A1 and A2, respectively); MS (70 ev); *m/z* (rel.int.) 255 $[M+H]^+$(Appendix A3).

Compound **B,** R_f = 0.45 was yellow needle like crystals after crystallization in *n*-hexane-CH_2Cl_2 (3:2), m.pt 200-203 ^{0}C ; ^{I}H and ^{13}C NMR ($CDCl_3$) see Table 6.0, Appendix B1 and B2; MS (70 ev): *m/z* (rel. int.) 285 $[M+H]^+$ (Appendix B3).

Compound **F** was isolated as an orange compound, R_f= 0.48 (solvent system: *n*-hexane-EtOAc, 3:2); m.p. 258-260 ^{0}C, ^{1}H NMR ($CDCl_3$) δ ppm: see Table 7.0 (Appendix C1), ^{13C} NMR ($CDCl_3$) see Table 4.3 (Appendix C2); EI-MS *m/z*: 271 (Appendix C3).

Compound **K,** R_f = 0.31 (solvent system: CH_2Cl_2-MeOH, 4:1) was isolated as yellow amorphous powder, m.p. 290-294 ^{0}C; IR ν_{max} (KBr) cm^{-1}: 3360 (OH), 2869, 2298, 1783, 1140; ^{1}H and ^{13}C NNR (DMSO-d_6) δppm: see Table 8.0 (Apppedix D1 and D2); MS (70ev): *m/z* (rel. int.) 303 $[M]^+$ (100), 273(10), 244(18), 153(12), 69(15) (Appendix D3).

Compound **D,** was isolated as white powder, R_f = 0.56 (solvent system: *n*-hexane-ethyl acetate 4:1), m.p. 134-135 ^{0}C; ^{1}H and ^{13}C NMR ($CDCl_3$) δ ppm: see Table 9.0 (Appendix E1 and E2);E-IMS *m/z* (rel. int): 414.5 $[M+1]^+$ (5), 412.4$[M]^+$ (20), 397.4 (10), 369.4 (8), 327.2(8), 300.4 (10), 281.2 (25), 271.3 (70), 255.3 (30), 253.2 (25), 213.3 (10), 207.1 (50), 191.0 (10), 173.2 (7), 161.2 (11), 147.1 (25), 135.2 (22), 95.2 (35), 81.1(60), 69.1 947), 55.1 (100), 43.1 (89), 42.1 (55) (Appendix E3).

Compound **E** was isolated as white amorphous powder, R_f = 0.54 (solvent system: *n*-hexane-EtOAc, 4:1); m.p. 170-171^{0}C. ^{1}H and ^{13}C NMR (CD Cl_3) δ ppm: see Table 10.0 (Appendix F1 and

F2); EI-MS *m/z* (rel. int.): 412.4 $[M]^+$(20), 382.6 (10), 355.2 (15), 314.3 (2), 281. 1 (50), 241.3 (2), 207.1 (100), 161.1 (25), 133.1 (30), 95.2 (20), 57.1 (5) (Appendix F3).

Compound **C,** R_f =0.32 (*n*-hexane-CH_2Cl_2: 3:2), m.p. 216-218^{0}C; ^{1}H and ^{13}C NMR data (CDCl3): see Table 11.0 (Appendix G1 and G2); EI-MS: *m/z* (rel. int): 426 [M]+ (100), 411 (20), 383 (10), 254 (30), 254 (3), 339 (10), 315 (10), 315 (33), 298 (20), 284 (40), 257 (35), 234 (4), 218 (30), 207 (20), 189 (80), 175 (25), 136 (75), 123 (28), 121 (68), 107 (60), 67 (93), 56 (45), 55 (81), 43 (70) (Appendix G3).

Compound **G** was obtained aswhite crystals with R_f = 0.30(*n*-hexane-ethyl acetate, 1:1), m.p. 280-282 ^{0}C; ^{1}H and ^{13}C NMR($CDCl_3$) δ ppm: see Table 12.0 (Appendix H1 and H2);ESIMS *m/z* (rel. int): 456 $[M]^+$ (100), 437 (10), 283 (10), 269 (40), 254 (15), 207 (5) 189(10) (Appendix H3).

Compound **H** was isolated as white amorphous powder, m.p. 174-176 ^{0}C; ^{1}H and ^{13}C NMR ($CDCl_3$) δppm: see Table 13.0 (Apppedix **I**1 and **I**2); EI-MS (70 eV): *m/z* 455 $[M-H]^+$ (100), 407 (10), 297 (10), 283 (40), 269 (8), 256 (30). (Appendix **I**3).

Compound **L** was isolated as pale yellow amorphous powder, mp. 223-225 ^{0}C; IR ν_{max} (KBr) cm^{-1}: IR ν_{max} (KBr) cm^{-1}: 3400 (OH), 1586, 1025, 745; ^{1}H and ^{13}C NMR data: see Table 14.0 (Appendix J1 and J2 respectively); EI-MS: *m/z* (rel. Int.) 390 $[M]^+$ (100) (Appendix J3).

Compound **I**, R_f = 0.58 (solvent system: CH_2Cl_2-MeOH, 9:1) was obtained as pale yellow amorphous powder; mp. 178-180 ^{0}C; ^{1}H and ^{13}C NMR ($CDCl_3$) δ ppm: see Table 15.0 (Appendix K 1 and K 2); EI-MS (70 eV): *m/z* (rel. int.): 239 $[M]^+$(Appendix K3).

Compound **J**, R_f =0.56 (solvent system: CH_2Cl_2-MeOH, 9:1) was was isolated as yellow amorphous powder, m.p. 174-176 ^{0}C; ^{1}H and ^{13}C NNR ($CDCl_3$) δppm: see Table 16.0 (Apppedix L1 and L2 respectively); EI-MS (70 ev): *m/z* 239 $[M]^+$ (Appendix L3).

3.14 Determination of Minimum Inhibitory Concentrations (MIC) for Isolates

Stock solutions of isolates were constituted according to Luvonga (2007) method whereby the isolates were dissolved in DMSO to give final concentration of 500 µg/ml. Serial dilution was done according to the method of Washington and Sutter (1980). The process afforded the concentrations 500 µg/ml , 250 µg/ml, 125.0 µg/ml, 62.50 µg/ml, 31.3 µg/ml, 15.60 µg/ml, 7.80 µg/ml, 3.90 µg/ml and 1.95 µg/ml and 0.97 µg/ml. 95% DMSO was used as negative control .

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 Antimicrobial Activity of Crude Extracts

n-Hexane, ethyl acetate and methanol extracts of flowers of *S. didymobotrya* were subjected to antimicrobial activity tests against *S. aureus* (ATCC 43320), *S. faecalis* (ATCC 25925) and clinical isolate *B. anthracis* which represented Gram-positive bacteria and *K. pneumaoniae* (ATCC 90028), *E. coli* (ATCC 25922), *S. typhimurium* (ATCC 25927) and *P.aeruginosa* (ATCC 27853) as representatives of Gram-negative bacteria. The fungal pathogens *C. albicans* (ATCC 90043) and the clinical isolates *M. gypseum* and *T. mentagrophyte* were used for antifungal tests. Kanamycin and Fluconazole were used as standard antibacterial and antifungal positive controls, respectively. DMSO was used as a negative control.

4.2 *In vitro* Antibacterial Activities of Extracts

The antibacterial activities of *n*-hexane, EtOAc and MeOH crude extracts of *S. didymobotrya* flowers were done by measuring the zones diameters of growth inhibition on both Gram-positive and Gram-negative bacteria and the results are displayed in Table 3.0. The test microorganisms showed varying degree of susceptibility to flower extracts and was observed to be dependent on the bacteria strains. A similar trend in susceptibility of bacteria to plant extracts where inhibition zone diameters vary significantly ($P \leq 0.05$) according to strains has been reported (Karou *et al.*, 2006). In this study methanol extract had a significantly higher ($P \leq 0.05$) antibacterial activities as compared to the *n*-hexane and EtOAc extracts. Significantly high ($P \leq 0.05$) growth inhibition zone of 18.1 mm was realized for *K. pneumaoniae* (ATCC 90028). Methanol extract was also observed to significantly ($P \leq 0.05$) inhibit the growth of *S. typhimurium* (ATCC 25927) 16.7 mm, *S. faecalis* (ATCC 25925) 16.3 mm, *P. aeruginosa* (ATCC 27853) (13.3 mm), *Bacillus anthracis* (13.1 mm)

and *S. aureus* (11.5 mm) as compatred to *n*-hexane and EtOAc. On the other hand, EtOAc extract had significantly (P≤0.05) lower inhibition zones at same concentration as compared to MeOH. From the antibacterial activity tests, the *n*-hexane was the least active extract. The extract showed moderate activities against *S. faecalis* (ATCC 25925) and *S. aureus* (ATCC 43320) with inhibition zone diameters of 12.1mm and 10.1mm, respectively. The extract showed weak activities against *E. coli* (7.7 mm), *B. anthracis* (7.1 mm), *P. aeruginosa* (7.3 mm), *K. pneumaoniae* (8.1 mm) and *S. typhimurium* (9.3 mm).

From the antibacterial results it can be envisaged that *K. pneumaoniae* (ATCC 90048) was regarded as susceptible to the methanol extract as the zone of inhibition was within the zone diameter breakpoints for standard antibiotic kanamycin (inhibition diameter 18-25 mm), as reported by the Clinical and Laboratory Standards Institute (CLSI, 2017). The methanol extract was the most effective antibacterial than both EtOAc and *n*-hexane extracts and the large zone inhibitions is due to the potency of secondary metabolites associated with it. The activities of the extracts against the pathogens were significantly (P≤0.05) lower compared to the activities exhibited by kanamycin which was used as standard positive control. The antibacterial activities of flower extracts of this plant against the test microorganisms except *S. aureus* and *E. coli* were done for the first time.

4.3 *In vitro* Antifungal Activities of Flower Extracts

The *n*-hexane, EtOAc and MeOH flower extracts of *S. didymobotrya* were also tested for antifungal activities against the pathogens *Candida albicans* (ATCC 90043) and the clinical isolates *M. gypseum* and *T. mentagrophytes* at a concentration of 50 µg/disc. The zone of inhibition varied for the fungi with respect to the type of plant extract used. As exhibited in Table 3.0, the three crude extracts showed antifungal activities against the three fungi tested. Methanol crude

extract exhibited significantly higher (P≤0.05) activity on the growth of *M. gypseum* and *T. mentagrophyte* with inhibition zones of 15.0 mm and 14.0 mm, respectively. The ethyl acetate extract showed significantly lower (P≤0.05) activities compared to methanolic extract with zone inhibition values of 10.2 mm, 12.0 mm and 12.1 mm against *C. albicans*, *M. gypseum* and *T. mentagrophytes*, respectively. In comparison to the antibacterial tests, the *n*-hexane exhibited fairly moderate activities against the three fungi with the highest activity being recorded for *M. gypseum* with zone inhibition 10.0mm. This was followed by *C. albicans* which gave inhibition diameter of (10.6) and lastly *T. mentagraphytes* (8.1mm). Activities of the extracts against the tested fungi were observed to be significantly (P≤0.05) lower compared to the activities exhibited by Fluconazole which was used as standard positive control. The findings reveals that the flower extracts in this study could be effective antibiotics since they inhibited the growth of fungal causing agents of skin diseases.

Table 3.0. Zones of inhibition (mm) of flower extracts against bacterial and fungal strains.

Flower extracts	Conc (µg/ml)	Inhibition zone in diameter (mm)									
		Gram-positive bacteria			Gram-negative bacteria				Fungi		
		S.a	S.f	B.a	K.p	E.c	S.t	P.a	C.a	T. m	M.g
MeOH	50	11.50b	16.27b	13.10b	18.10b	9.73b	16.70b	13.33b	12.83b	14.03b	15.00b
EtOAc	50	10.70c	13.13c	12.27c	16.27c	9.40c	13.33c	10.90c	10.17c	12.13c	12.00c
n-hexane	50	10.10d	12.10d	7.13d	8.13d	7.70d	9.27d	7.30d	10.60c	8.10d	10.03d
Fluconazole	50	ND	ND	ND	ND	ND	ND	ND	28.27a	26.13a	26.20a
Kanamycin	50	28.10a	25.07a	24.13a	30.07a	21.97a	26.03a	32.10a	ND	ND	ND
LSD(≤0.05)		0.18	0.18	0.17	0.25	0.14	0.26	0.17	1.26	0.22	0.15
CV (%)		0.8	0.7	0.8	0.9	0.7	1.1	0.7	1.1	1.0	0.6

Means followed by different letters down the column are statistically different at P≤0.05 by Fisher's protected least significant difference test. S.a = *Staphylococcus aureus*, S.f = *Streptococcus faecalis*, B.a = *Bacillus anthracis*, K.p = *Klebsiela pneumaoniae*, *E.c = Escherichia coli*, S.t = *Salmonella typhimurium*, P.a = *Pseudomonas aeruginosa*, C.a = *Candida albicans*, M.g = *Microsporum gypseum*, T.m = *Trichophyton mentagrophytes.* ND = not done

4.4 Minimum Inhibitory Concentration (MIC) of Flower Extracts.

According to Kuete (2010), a plant extract exhibiting a MIC value less than 100 μg/ml has a significant (P≤0.05) antimicrobial activity while those giving MIC values in the range 100-250 μg/ml are considered moderate. Extracts affording MIC values beyond 250 μg/ml are considered inactive. Therefore on the basis of this analysis, it can be argued that the flower extracts showed antimicrobial activity because MIC values (Table 4.0) of 250 μg/ml and below were achieved with each extract on at least one microorganism strain. The *n*-hexane extract showed significantly (P≤0.05) high antibacterial activity against *S. aureus* and *S. faecalis* with MIC value of 125 μg/ml each. On the other hand, the extract showed significantly (P≤0.05) low antibacterial activity against *B. anthracis, K. pneumaoniae, S. typhimurium* and *P. aeruginosa* with MIC value of 250 μg/ml. MIC value above 250 μg/ml was obtained with *E. coli* which indicated that the extract was not active against the microorganism. Ethyl acetate extract of the flowers showed significantly (P≤0.05) high potency against the bacteria tested as compared to *n*-hexane extract. The extract was observed to produce significantly (P≤0.05) high activity against Gram positive bacteria *S. faecalis* and *B. anthracis* with MIC value of 62.5 μg/ml while a value of 125 μg/ml was registered for *S. aureus*. However, significantly (P≤0.05) low activities were observed when the extract was tested against the *S. typhimurium, K. pneumaoniae* and *P. aeruginosa* (250 μg/ml). *n*-Hexane extract and the EtOAc had significantly (P≤0.05) low activities against *E. coli* (>250 μg/ml). Out of the three extracts that were evaluated against bacteria, methanol extract proved to have significantly (P≤0.05) high potency as compared to *n-hexane* and the EtOAc. The extract showed significantly (P≤0.05) low MIC value against *K. pneumaoniae* (31.3 μg/ml) while for both *S. faecalis* and *B. anthracis* the extract registered MIC value of 62.5 μg/ml.

From the antifungal test, significantly (P≤0.05) low MIC value (3.90 µg/ml) was obtained with MeOH extract against *M. gypseum*. The same extract also showed significantly (P≤0.05) low MIC values against *T. mentagrophytes* and *C. albicans* with MIC values of 7.80 µg/ml and 31.3µg/ml, respectively. The ethyl acetate extract of the plant flowers also showed significantly (P≤0.05) low MIC values which ranged from 15.6 µg/ml to 62.5 µg/ml. In this case the fungus *M. gypseum* was the significantly (P≤0.05) susceptible with MIC value of 15.6 µg/ml. Similarly, evaluation of *n*-hexane extract for antifungal activities revealed that extract was potent against all the tested fungal pathogens with MIC values ranging between 125 µg/ml to 250 µg/ml. As was observed from the accrued data the three extracts were more potent to fungal pathogens than the bacteria.

Table 4.0. The MIC's of the extracts against bacteria and fungi strains

Flower extracts	Concentration (µg/ml)									
	Gram-positive bacteria			Gram-negative bacteria				Fungi		
	S.a	S.f	B.a	K.p	E.c	S.t	P.a	C.a	T.m	M.g
n-hexane	125a	125a	250a	250a	500a	250a	250a	250a	125a	125a
EtOAc	125a	62.5b	62.5b	250a	500a	250a	250a	62b	62.5b	15.6b
MeOH	125a	62.5b	62.5b	31.3b	250b	125b	125b	31.3c	7. 8 c	3.0c
kenamycin	10b	15c	16c	18 c	30c	20c	20c	ND	ND	ND
Fluconazole	ND	ND	ND	ND	ND	ND	ND	4d	2.2 d	2.0c
LSD (P≤0.05)	1.88	1.88	4.98	7.25	7.25	6.56	6.79	5.18	4.20	4.98
C.V	1.04	1.50	2.70	1.98	1.98	20.33	2.23	3.16	3.20	7.20

Means followed by different letters down the column are statistically different at P≤0.05 by Fisher's protected least significant difference test. S.a = *Staphylococcus aureus*, S.f = *Streptococcus faecalis*, B.a = *Bacillus anthracis*, K.p = *Klebsiela pneumaoniae, E.c = Escherichia coli*, S.t = *Salmonella typhimurium*, P.a = *Pseudomonas aeruginosa* C.a = *Candida albicans*, M.g = *Microsporum gypseum*, T.m = *Trichophyton mentagrophytes.* ND = not done

4.5 Structural Elucidation of Compounds from *S. didymobotrya* Flower Extracts

Compound **A** was isolated as a yellow pigment, R_f =0.49 (solvent system: *n*-hexane-CH_2Cl_2, 3:2). It showed a purple colour on TLC when exposed to concentrated ammonia vapour and after a few minutes the colour reverted to its original yellow colour signifying the presence of anthraquinone derivative. The ^{1}H NMR spectrum (Table 5.0, Appendix A1) showed two sharp singlets down-field at δ_H 12.11 and 12.01 ascribable to two chelated hydroxyl groups. The spectrum also displayed five aromatic proton signals at δ 7.83 (1H, dd, *J* =8.5,1.0 Hz, H-5), 7.68 (1H, d, *J*= 1.2 Hz. H-4), 7.64 (1H, t, *J*=8.5 Hz, H-6), 7.30 (1H, dd, *J*=8.5, 1.2 Hz, H-7) and 7.10 (1H, d, *J* =1.0 Hz, H-2) together with an aromatic methyl as a singlet at at δ 2.46. These ^{1}H NMR data were supported by the ^{13}C NMR spectra (Table 5.0, Appendix A2). The ^{13}C NMR showed one methyl carbon at δ 22.27, two oxygenated carbons at δ 162.82 (C-8) and 163.10 (C-1), two carbonyl carbons at δ 182.32 (C-10) and 192.78 (C-9) and one methyl substituted carbon at 149.35 (C-3). On the other hand five unsubstituted carbons which appeared at δ 124.57 (C-2), 121.37 (C-4), 119.94 (C-5), 136.96 (C-6) and 124.37 (C-7) along with other carbon peaks at δ 133.69 (C-4a), 133.31 (C-10a), 113. 76 (C-9a) and 115.90 (C-8a) suggested that compound **a**is chrysophanol (Mining *et al*., 2014). The mass spectrum showed molecular ion peak at *m/z* 255 which is consistent with the molecular formula $C_{15}H_{10}O_4$. Thus, on the basis of spectral data and by comparing with those reported in the literature (Ochieng, 2012), compound **A** was confirmed to be chrysophanol (**7**) (1,8-dihydroxy-3-methylanthracene-9-10-dione).

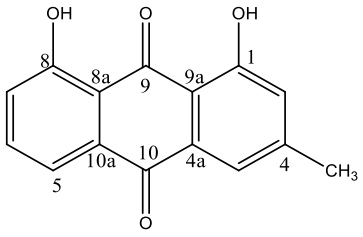


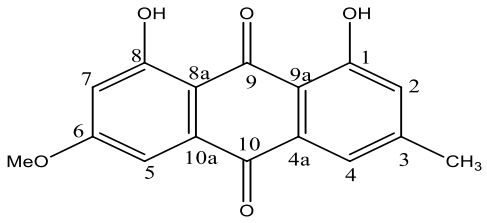
Table 5. 0**:** ^{1}H ($CDCl_3$, 600 MHz) and ^{13}C ($CDCl_3$, 150 MHz) NMR data of chrysophanol **(7)**

	^{1}H (δ_H, mult, *J* in Hz)		**^{13}C (δ_C)**	
C#	*Reported lit. values	Observed values	*Reported lit. values	Observed values
1			162.4	163.10
2	7.1 (br s)	7.10 (d, *J* =1.0)	124.3	124.56
3			149.3	149.35
4	7.7 (br s)	7.68 (d, *J* = 1.2)	121.3	121.37
4a			133.2	133.69
5	7.8 (d, *J*=7Hz)	7.83 (dd, *J* = 8.5, 1.0)	119.9	119.94
6	7.7 (t, *J*=8H_Z	7.64 (t, *J* = 8.5)	136.9	136.96
7	7.3 (d, *J*=8H_Z	7.30 (dd, *J* = 8.5, 1.2)	124.6	124.37
8			162.7	162.82
8a			115.8	115.90
9			192.5	192.78
9a			113.7	113.76
10			181.9	182.32
10a			136.9	133.31
3-Me	2.5 (s)	2.46 (s)	22.2	22.27
1-OH	12.0 (s)	12.09 s		
8-OH	12.1 (s)	12.11 s		

*Literature data (Ochieng, 2012)

Compound **B**,yellow needle like crystals with m.p. 200-203 ^{0}C and R_f = 0.45 (solvent system; *n*-hexane-CH_2Cl_2, 3:2). Like compound **A**, it also turned purple on TLC when exposed to conc. ammonia vapour and the colour on exposure to air reverted to the original colour suggesting the presence of anthraquinone derivative. The ^{1}H NMR spectrum (Table 6.0, Appendix B1) revealed the presence of two chelated hydroxyl resonances at δ_H12.32 and 12.12 accounting for OH-8 and OH-1, respectively. Also four aromatic protons centered at δ7.64 (d, *J* = 1.2 Hz), 7.38 (d, *J*= 2.4 Hz), 7.09 (d, *J* = 1.2 Hz)) and 6.70 (d, *J*= 2.5Hz) represented H-4, H-5, H-2 and H-7, respectively. The spectrum also exhibited singlet signals at δ_H3.91 and 2.45 for methoxy and methyl groups, respectively (Tripathi *et al*., 2014). The ^{13}C NMR spectrum (Table 6.0, Appendix B2) revealed the presence of twelve aromatic carbons including four methines and eight quarternary carbons of which five bore oxygen, two carbonyls, and one bonded to methyl carbon (Ochieng, 2012). In fact both the ^{1}H and ^{13}C NMR data further evidenced an anthraquinone derivative. The mass spectral

data showed molecular ion peak at *m/z* 285.0, consistent with the molecular formula $C_{16}H_{12}O_5$ and on this basis, the structure of compound **B** was deduced to be physcion (**9**) (1,8-dihydroxy-6-methoxy-3-methylanthracene-9,10-dione).



9

Table 6. 0**:** ^{1}H ($CDCl_3$, 600 MHz) and ^{13}C ($CDCl_3$, 150 MHz) NMR data of physcion **(9)**

	^{1}H (δ_H, mult, *J* in Hz)		**^{13}C (δ_C)**	
	Reported lit.values	Observed	*Reported lit.values	Observed
1			162.44	162.55
2	7.3 (brs)	6.70 (d, *J* = 2.5)	119.88	121.32
3			148.41	148.47
4	7.6 (brs)	7.64 (d, *J*=1.2)	121.33	122.09
4a			133.30	133.50
5	7.4 (d, *J*=3Hz)	7.38 (d, *J*=2.4)	108.12	108.24
6			166.23	166.51
7	7.0 (d, *J*=3Hz)	7.09 (d, *J*=1.2)	106.82	106.40
8			162.76	163.23
8a			115.92	115.72
9			192.52	192.46
9a			117.62	117.58
10			181.80	182.40
10a			135.20	135.51
6-OMe	3.9 s	3.91 (s)	56.08	56.09
3-Me	2.5 s	2.45 (s)	22.03	22.47
1-OH	12.1s	12.12 (s)		
8-OH	12.3 s	12.32 (s)		

*Literature data (Tripathi *et al.*, 2014)

Compound **F** was isolated as orange crystals, m.p. 258-260 ^{0}C. Its ^{1}H NMR (Table 7. 0, Appendix C1) exhibited four sets of aromatic peaks at δ 7.25 (d, *J* = 2.5 Hz, H-5), 7.16 (d, *J* = 1.5 Hz, *J* = 1.5 Hz), 7.15 (d, *J* = 1.4 Hz, H-7) and 6.56 (d, *J* = 2.1 Hz, H-4), three sets of hydroxyl groups (at δ 12.03, 11.97 and 11.88, all singlets) and one methyl singlet at δ 2.45 attached to aromatic ring.

The ^{13}C NMR chemical shifts (Table 7. 0, Appendix C2) revealed the presence of 12 aromatic carbons at δ 161.45 (C-1), 108.35 (C-2), 148.40 (C-3), 120.80 (C-4), 132.84 (C-4a), 108.82 (C-5), 166.55 (C-6), 107.85 (C-7), 165.43 (C-8), 109.31 (C-8a), 114.00 (C-9a) and 135.01 (C-10a), two carbonyl at δ 190.00 (C-9) and 182.24 (C-10) and a benzylic methyl carbon at δ23.10 (C3-Me). The accrued data suggested that compound **F** is an anthraquinone derivative with three hydroxyl substituents possibly at position 1, 6 and 8 (Khan *et al*., 2019; Zelalem and Dula 2019). The mass spectrum gave a molecular ion peak at *m/z* 272.0 consistent with the molecular formula $C_{15}H_{10}O_5$.Therefore on the basis of physical and spectroscopic data as well as comparison with literature data (Khan *et al*., 2019), compound **F** was identified as emodin (**10**).

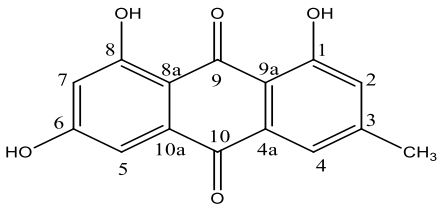


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Table 7. 0 ^{1}H (DMSO-d_6, 300 MHz) and ^{13}C (DMSO-d_6), 75 MHz) NMR data of emodin **(10)**

C#	^{1}H (δ_H, mult, *J* in Hz**)**		^{13}C (δ_C)	
	Reported lit.values	Observed values	*Reported lit.values	Observed values
1			162.03	161.45
2	7.16 (d, *J*=1.2)	7.16 (d, *J*=1.5)	124.03	124.00
3			148.63	148.40
4	6.68 (d, *J*=2.4)	6.56 (d, *J* =2.1)	120.58	120.80
4a			131.31	132.84
5	7.27 (d, *J*=2.4)	7.25 (d, *J*=2.5)	108.74	108.82
6			165.41	166.55
7	6.68 (J = 1.3)	7.15 (d, J = 1.4)	107.97	107.85
8			165.37	165.43
8a			109.58	109.31
9			190.79	190.00
9a			113.55	114.00
10			181.18	182.24
10a			135.70	135.01
1-OH	12.12 (s)	11.88 (s)		
6-OH	12.21 (s)	12.03 (s)		
8-OH	12.30 (s)	11.97 (s)		
3-CH_3	2.46 (s)	2.45 (s)	21.10	23.10 s

*Literature data (Khan*et al.*, 2019)

Compound **K** was obtained as an amorphous yellow powder with R_f value 0.31 (solvent system, CH_2Cl_2-MeOH, 4: 1, on deactivated silica gel) and m.p. > 300^0C. The yellow colour intensified on TLC when exposed to concentrated ammonia vapour. The yellow colour also turned dark brown when sprayed with ferric chloride solution on TLC. The two tests were indicative that compound **K** is a flavonoid derivative (Mabry *et al*., 1970). Its IR spectrum determined as KBr pellet (Appendix DI) showed significant absorption at 3360 cm^{-1} indicative of OH moiety. The ^{1}H NMR spectral data (Table 8. 0; Appendix D2) showed two sets of aromatic peaks: AX system type of peaks at δ_H 6.18 (d, *J* = 1.8 Hz, H-6) and 6.41 (d, *J* = 1.8 Hz, H-8) while the ABX type of peaks were centered at δ 7.67 (d, *J* = 1.8 Hz, H-2), 7.53 (dd, *J* = 8.4, 2.4 Hz, H-6') and 6.89 (d, *J* = 8.4 Hz, H-5'). Besides these, there was a singlet peak at δ 12.48 which represented hydrogen bonded C-5 hydroxy group (Batterham and Highet, 1964). The ^{13}C NMR spectrum (Table 8.0; Appendix D3) exhibited a total of fifteen carbon skeleton including five aromatic methines and ten quaternary

carbons as evidenced by 135 Dept spectrum (Appendix D4). Both the ^{1}H and ^{13}C NMR data suggested that compound **K** is quercetin, a fact that was confirmed by the EIMS peak at *m/z* 303 (Appendix D5) consistent with a molecular formula $C_{15}H_{10}O_7$. Thus on the basis of physical and spectroscopic data, compound **K** was concluded to be quercetin (**253**).

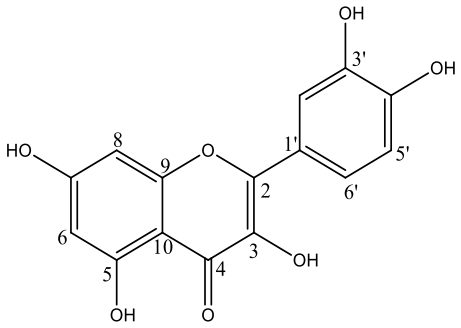

Table 8. 0 ^{1}H (DMSO-d_6, 600 MHz) and ^{13}C (DMSO-d_6), 150 MHz) NMR data of quercetin **(253)**

Position	^{1}H (δ_H, mult, *J* in Hz)		^{13}C (δ_C)	
	*Reported lit.values	Observed	*Reported lit.values	observed
2			147.3	146.8
3			136.1	135.7
4			176.3	175.8
5			161.1	160.7
6	6.20 (d, *J* = 2.1)	6.18 (d , *J*=1.8)	98.7	98.1
			164.3	163.8
8	6.40 (d J = 2.1)	6.41 (d, *J*=1.8)	93.85	93.3
9			156.6	156.1
10			103.5	103.0
1`			122.4	121.9
2`	7.70 d (2.1)	7.67 d (*J*=1.8)	115.5	115.0
3’			145.5	145.0
4`			148.1	147.7
5`	6.90 d (8.4)	6.89 (d, *J* = 8.4)	116.1	115.6
6`	7.50 dd (8.4, 2.1)	7.53 (dd, *J* = 8.4, 2.4)	120.5	120.0
5-OH		12.48 s		

*Literature data (Zhang *et al*., 2014)

Compound **D** was obtained as a white amorphous powder with m.p. 134-135 ºC. It responded to Liebermann-Burchard test suggesting the presence of sterol and/ or triterpenoid derivative (Chaturvedula and Prakash, 2012). The ^{1}H NMR spectrum (Table 9.0, Appendix E1) of the

compound showed two angular methyl singlets at δ 0.68 (Me-19) and 1.01 Me-18), and three methyl doublets which appeared at δ 0.92 (J = 6.1 Hz), 0.85 (*J* = 6.4 Hz), 0.82 (*J* = 6.6 Hz) representing Me-29, Me-26 and Me-27, respectively. These together with the methyl triplet at δ 0.87 (*J* = 6.3 Hz), one olefinic proton at δ 5.35 (t, *J* = 3.6 Hz) and an oxymethine hydrogen which appeared at 3.54 (m) suggested that the compound is a sterol derivative (Ochieng, 2012).The ^{13}C NMR (Table 9.0, Appendix E2) exhibited twenty nine carbon signals including six methyls, ten methylenes, ten methines and three quaternary carbons as evidenced by 135 DEPT experiments. The SP^2 hybridizedcarbons that appeared downfield at δc 140.77 and 121.73 in the ^{13}C NMR are characteristic of *β*-sitosterol (Mining *et al*., 2014). Thus, on the basis of both ^{1}H and ^{13}C NMR compound **D** was proposed to be *β*-sitosterol. This was confirmed by electron ionization mass spectrum which showed a molecular ion peak at *m/z* 414.5 (Appendix E3) consistent with the molecular formula $C_{29}H_{50}O$ and the fragmented daughter ions at *m/z* 396 and 381 in mass spectrum (Figure 1.0). Therefore from the accrued spectroscopic and literature data, compound **D** was concluded to be *β*-sitosterol (**302**).

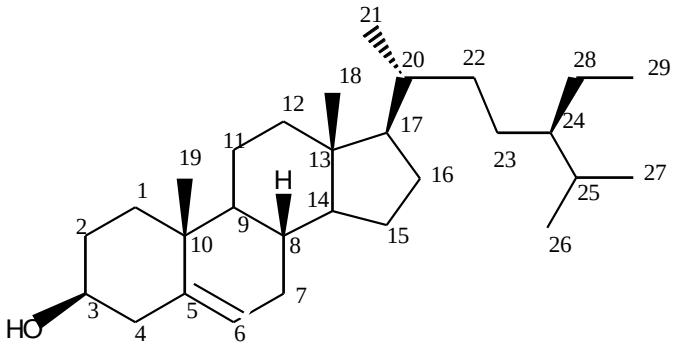

Table 9.0: ^{1}H ($CDCl_3$, 600 MHz) and ^{13}C ($CDCl_3$, 150MHz) NMR data for *β*-sitosterol **302)**

	^{1}H (δ_H, mult, *J* in Hz)		**^{13}C (δ_C)**	
C#	Reported lit.values	Observed values	Reported Lit.values	Observed values
1			37.5	37.27
2			31.9	31.92
3	3.53 (tdd, *J* = 4.5, 4.2, 3.8)	3.54 m	72.0	71.93
4			42.5	42.32
5			140.9	140.77
6	5.36 (t, *J* = 6.4)	5.35 (t, *J* = 3.6)	121.9	121.73
7			32.1	32.43
8			32.1	31.92
9			50.3	50.15
10			36.7	36.52
11			21.3	21.10
12			39.9	39.79
13			42.6	42.34
14			56.9	56.79
15			25.3	24.31
16			28.5	28.25
17			56.3	56.13
18	1.01 s	1.01 s	12.0	12.25
19	0.68 s	0.68 s	19.0	19.05
20			36.3	36.52
21	0.93 (t, *J* = 6.5)	0.92 (d, *J* = 6.1)	34.2	33.97
22		-	31.9	31.90
23		-	26.3	26.11
24			46.1	45.86
25			29.4	29.29
26	0.83 (d, *J* = 6.5)	0.85 d, *J* = 6.4)	20.1	20.21
27	0.81 (d, *J* = 6.4)	0.82 (d, *J* = 6.6)	19.6	19.62
28			23.3	23.09
29	0.84 (t, *J* = 6.5)	0.87 (t, *J* = 6.3)	12.2	12.40

*Literature data (Chaturvedula and Prakash, 2012)

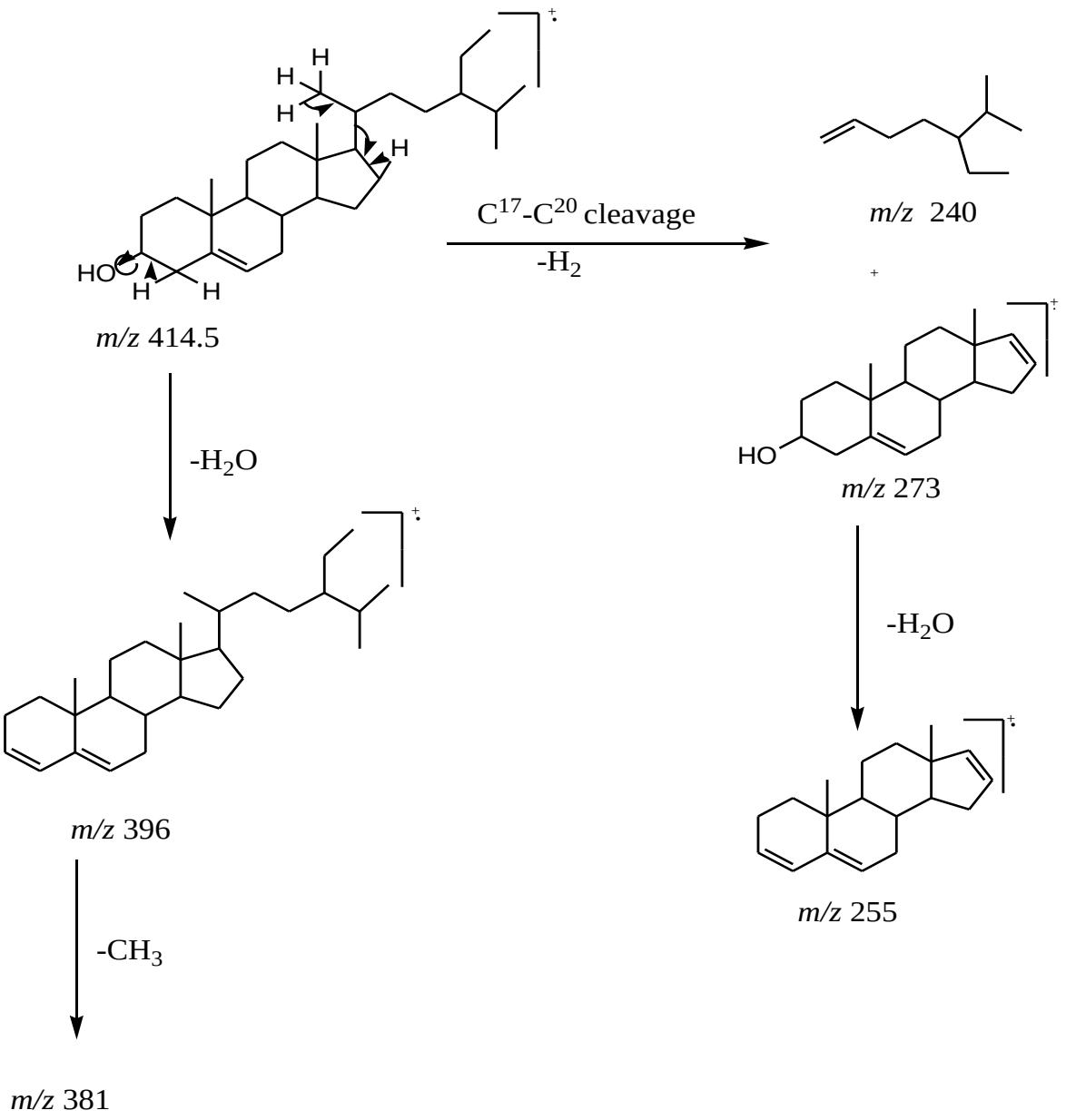

Figure 1.0: Proposed fragmentation pattern of compound **302** in EI-MS

Compound E was isolated as white powder with R_f =0.54 (solvent system: *n*-hexane-EtOAc, 4:1) and m.p. 170-171 ^{0}C. It gave a positive test (purple colouration) with Liebermann-Burchard reagent which suggested that the compound is a sterol and /or triterpenoid derivative (Kandati *et al*., 2012; Raju *et al*., 2012). The ^{1}H NMR and ^{13}C NMR data (Table 10.0, Appendix F1 and F2) of the compound showed the presence of two methyl singlets at δ_H 0.68 (Me-19) and 1.05 (Me-

28), three methyl groups which appeared as doublets at δ 0.81 (*J* = 6.3 Hz, Me-27)), 0.83 (d, *J* =6.6 Hz, Me-26), and 0.90 (d, *J* =6.2 Hz, Me-21); and a methyl triplet at δ_H 0.84 (t, *J* =7.2 Hz, Me-29). The ^{1}H NMR spectrum also exhibited peaks for a trisubstituted olefinic bond at δ 5.35 (t, *J* = 5.4 Hz, H-6) while peaks centered at δ 5.02 and 5.20 appearing as multiplets corresponded to an isolated disubstituted olefinic moiety (Chaturvedula and Prakash, 2012). These together with an oxymethine multiplet at δ 3.52 (H-3) suggested that compound **E** is a sterol derivate (Chartuvedula and Prakash 2012; Mining *et al*. 2014). The ^{13}C NMR exhibited a total of 29 resonances which were sorted out into into six methyls, nine methylenes, eleven methines and three quaternary carbons. In the spectrum, peaks at δ_c 140.76 (C-5) and 121.72 (C-6) confirmed the existence of endocyclic olefinic bond while those that appeared at δ 138.32 and 129.39 were indicative of isolated carbon-carbon double in the molecule. The signal which appeared at 71.80 confirmed the presence of oxymethine carbon in the molecule. The ^{1}H and ^{13}C NMR data supported the presence of sterol derivative with a hydroxyl group at C-3, endocyclic double bond at C-5/C-6 and an isolated double bond at C-20/C-21 which is characteristic of stigmasterol (Chartuvedula and Prakash 2012). The mass spectrum showed a molecular ion peak at *m/z* 412.5 which is two a.m.u less that of compound **D** (Appendix F3) was consistent with $C_{29}H_{48}O$ formula. This together with characteristic fragmentation pattern in EI-MS (Figure 2.0) confirmed that the compound was stigmasterol. Thus, on the basis of physical and spectral data as well as reported data (Habib *et al*., 2007; Jamal *et al*., 2009) compound **E** was concluded to be stigmasterol (**303**).

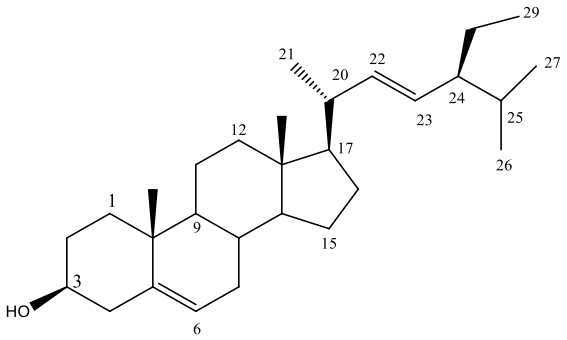

Table 10.0: ^{1}H ($CDCl_3$, 600 MHz) and ^{13}C ($CDCl_3$, 150 MHz) NMR data for stigmasterol **(303)**

	^{1}H (δ_H, mult, *J* in Hz)		**^{13}C (δ_C)**	
	Reported lit.values	Observed values	*Reported lit.values	Observed values
1			37.6	37.27
2			32.1	32.43
3	3.51 (tdd, *J* = 4.5, 4.2, 3.8)	3.52 m	72.1	71.80
4			42.4	42.34
5			141.1	140.76
6	5.31 (t, *J*= 6. 1Hz	5.35 (t, *J* =5.4 Hz	121.8	121.72
7			31.8	31.92
8			31.8	31.69
9			50.2	50.16
10			36.6	36.52
11			21.5	21.22
12			39.9	39.79
13			42.4	42.32
14			56.8	56.86
15			24.4	24.31
16			29.3	29.18
17			56.2	56.79
18	1.03 s	1.05 s	12.2	12.22
19	0.71 s	0.68 s	18.9	18.99
20			40.6	40.49
21	0.91 (d, *J*=6.2)	0.90 (d, *J* = 6.3)	21.7	21.22
22	4.98 m	5.02 m	138.7	138.32
23	5.14 m	5.20 m	129.6	129.39
24			12.1	12.32
25			29.6	31.89
26	0.82 (d, *J*=6.6)	0.83 (d, *J*= 6.6)	20.2	21.1
27	0.80 (d, *J*=6.6)	0.81 (d, *J* = 6.3)	19.8	19.40
28			25.4	25.41
29	0.83 (t, *J* =7.1)	0.84 (t, *J* = 7.2)	12.1	12.44

*Literature data (Chaturvedula and Prakash, 2012)

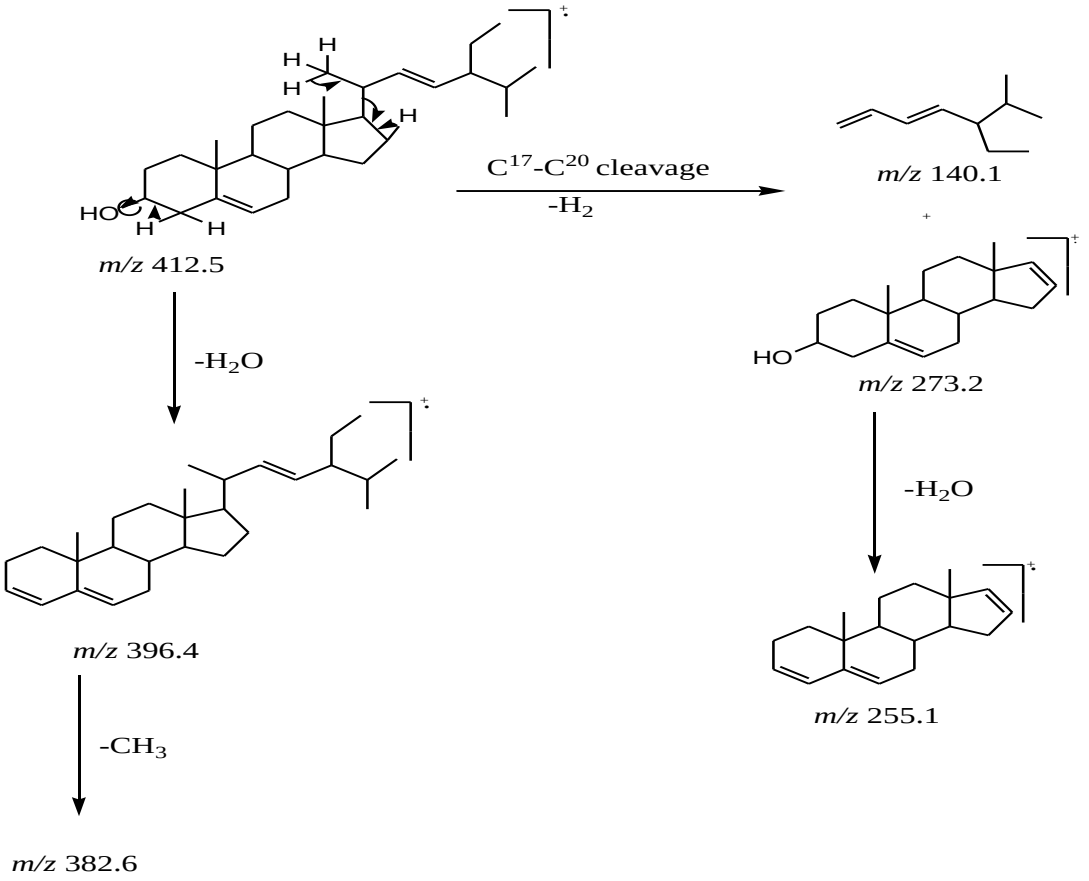

Figure 2.0: Proposed fragmentation pattern of compound **303** in EI-MS.

Compound **C** was obtained as white needle like crystals with R_f =0.32 (*n*-hexane-CH_2Cl_2: 3:2) and m.p. 216-218 ^{0}C. It afforded a positive Libermann-Burchard test suggesting the presence of sterol and /or triterpenoid derivative (Attarde *et al*., 2010). The ^{1}H and ^{13}C NMR spectra data of compound **C** are displayed in Table 11.0 (Appendix G1 and G2). In the ^{1}H NMR spectrum, the presence of seven tertiary methyl protons (integrated for 3H each) were observed at δ 1.25, 1.03, 0.94, 0.83, 0.80, 0.79, 0.76 and 0.75. These together with a pair of doublets which resonated at δ 4.60 (*J*= 0.5 Hz) and δ 4.70 (*J*= 0.6 Hz) suggested the presence of a pentacylic triterpene of lupane type (Pavia et al., 2009). This was further evidenced by a multiplet peak centered at δ 2.32 assignable to 19 *β*-H and a doublet of doublet peak at δ 3.23 (dd, *J*=11.5, 4.7 Hz) ascribable to H-3. In fact the peak at δ 2.32 is typical of lupeol trtiterpene (Abdullahi *et al*., 2013). The ^{1}H NMR

was further coroborated by the ^{13}C NMR spectrum which exhibited seven tertiary methyl groups at δc 27.50 (C-23), 18.00 (C-28), 15.9 (C-25), 16.13 (C-26), 14.56 (C-24), 15.40 (C-27) and 19.30 (C-30) while the exomethylene group which resonated at δc 108.10 and 150.50 represented C-29 and C-20, respectively. Also in the spectrum the deshielded signal at δc 77.7 was assigned an oxymethine C-3. This was further evidenced by EI-MS (Appendix G3) which showed a molecular ion at *m/z* 426 $[M]^+$ corresponding to $C_{30}H_{50}O$. Confirmation of structure of compound **c** further substantiated by analysis of HMBC (Appendix G4) and significant daughter ions observed in the EI-MS (Figure 3.0). Thus, on the basis of physical and spectroscopic data as well as comparison wth literature data (Abdullahi *et al*, 2013), compound **C** was identified as lupeol (**323**).

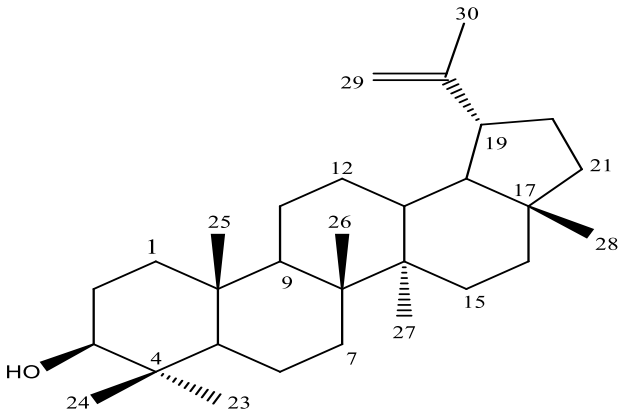

Table 11.0: ^{1}H ($CDCl_3$, 600 MHz) and ^{13}C ($CDCl_3$, 150 MHz) NMR data for lupeol **(323)**

C#	**^{1}H (δ_H, mult, *J* in Hz)**		**^{13}C (δ_C)**	
	Reported lit.values	Observed values	*Reported lit.values	Observed values
1			37.80	38.70
2			27.40	27.40
3	3.20 (1H, dd, *J* = 11.0, 3.4)	3.20 (1H, dd, *J* =11.5, 4.7)	79.00	77.20
4			38.90	38.80
5			55.50	55.30
6			18.50	18.34
7			34.20	34.30
8			40.90	40.80
9			50.50	50.50
10			37.20	37.20
11			21.00	20.95
12			25.20	25.20
13			38.10	38.00
14			42.90	42.90
15			27.10	27.40
16			35.50	35.60
17			43.00	42.90
18	3.15 (dd, J=11.5, 5.3 Hz)	3.18 (dd, *J* = 11.0, 3.4 Hz)	48.30	49.20
19	2.29 m	2.35 m	48.00	48.30
20			150.0	151.0
21			29.90	29.80
22			40.00	40.00
23	0.77 s	0.76 s	28.00	27.50
24	0.80 s	0.79 s	15.50	14.56
25	0.82 s	0.83 s	16.10	15.90
26	0.94 s	0.94 s	16.00	16.13
27	1.02 s	1.03 s	14.80	15.40
28	0.76 s	0.75 s	18.00	18.02
29	4.57 (d, J = 0.4, H_α-29), 4.67 (d, J =0.5, H_β-29)	4.60 (d, *J* = 0.5, H_α-29), 4.70 (d, *J*= 0. 6, H_β-29)	109.0	109.3
30	1.20, s	1.25 s	19.50	19.32

*Literature data (Abdullahi *et al*., 2013)

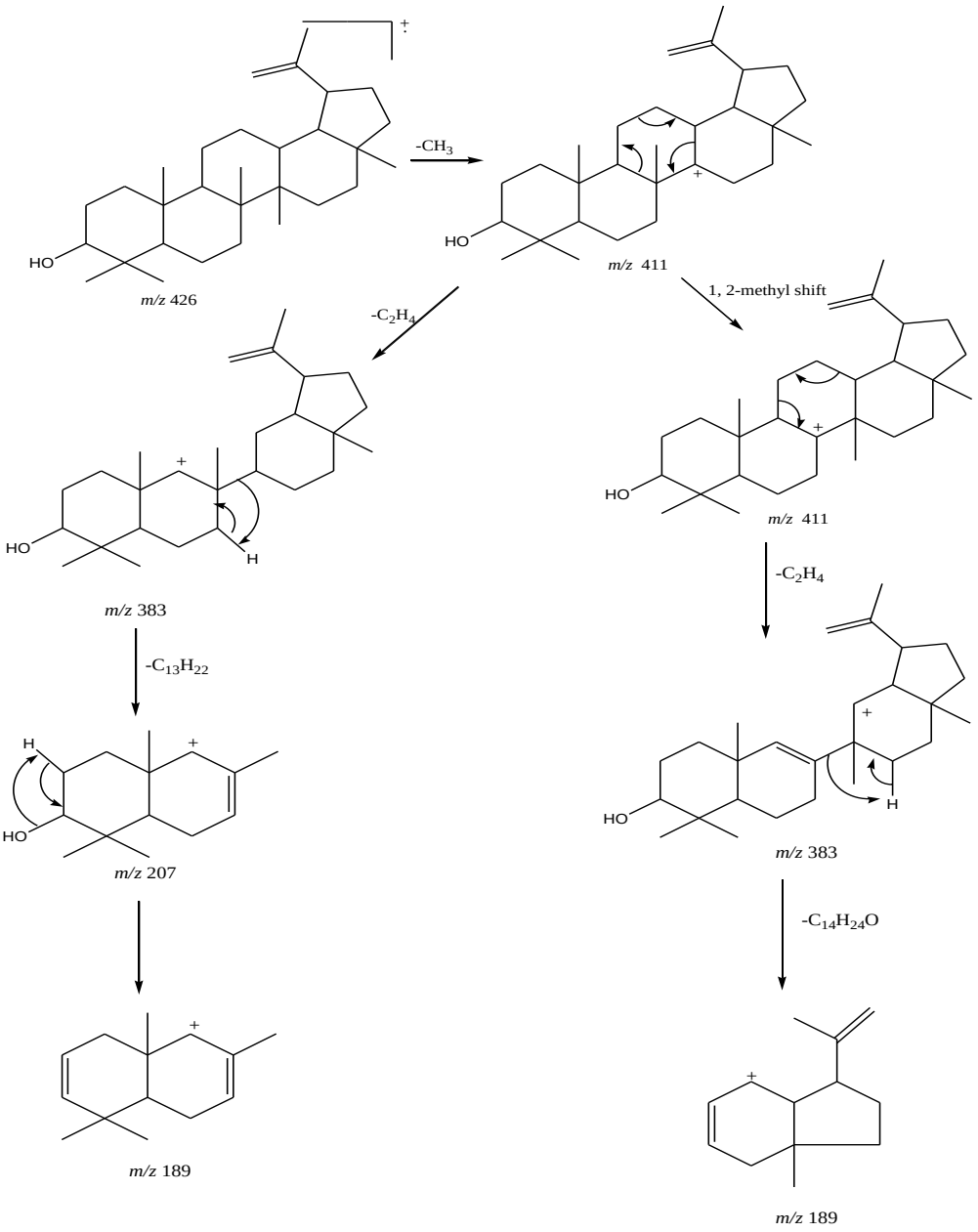

Figure 3.0. Proposed fragmentation of compound **323** in EI-MS (70 eV)

Compound **G** was isolated as white powder, m.p. 280-282 ^{0}C. It gave a positive Libermann-Burchard test suggesting that it could be a sterol or a triterpenoid derivative (Attarde *et al*., 2010). In the ^{1}H NMR spectrum (Table 12.0, Appendix H1) six tertiary methyl groups appearing as singlets were observed at δ 1.64, 1.11, 0.96, 0.94, 0.82 and 0.75. This together with olefinic protons which appeared at δ 4.73 and 4.60 as broad singlets and a doublet of doublet peak at δ 3.22 (*J*=11.1,

5.4 Hz) signified the presence of pentacyclic lupane-type triterpene containing a hydroxyl group (Egbubine *et al.,* 2020).The ^{13}C NMR (Table 12. 0, Appendix H2) showed a total of thirty signals sorted out into six methyls, eleven methylenes, six methines and seven quartenary carbons. In the spectrum, the downfield signal at δc 177.49 was assigned to the carbon of the carboxylic group while signals at δc 150.52, 109.82 and 77.04 represented a quarternary vinyl carbon, a terminal methylene carbon and an oxymethine carbon, respectively. The signals that corresponded to five SP^3 hybridized quarternary carbons were observed at δc 56.63 (C-17), 42.19 (C-14), 34.13 (C-7), 38.71 (C-4) and 36.93 (C-10) whereas those that represented five methine carbons excluding an oxymethine carbon were centered at δ δ55.12 (C-5), 50.15 (C-9), 48.73 (C-19), 46.84 (C-18) and 37.78 (C-13). Similarly, peaks for ten methylene carbons excluding terminal methylene resonated at δc 39.43 (C-1), 36.57 (C-22), 34.13 (C-7), 31.93 (C-16), 30.60 (C-15), 29.43 (C-21), 27.36 (C-2), 25.29 (C-12), 21.00 (C-11) and 18.17 C-6) while the remaining peaks were for the six quaternary methyl carbons and were observed at δ 28.30 (C-23), 19.14 (C-30), 16.14 (C-25), 16.09 (C-26), 15.94 (C-24) and 14.58 (C-27). Both the ^1H and ^{13}C NMR datacombined together account for 30 carbon signals characteristic of betulinic acid (Egbubine *et al.*, 2020), a fact further supported by the EIMS spectrum (Appendix H3) which showed a molecular ion peak at at *m/z* 455 $[M-H]^+$ corresponding to $C_{30}H_{50}O_3$ formula. From the accrued physical and spectroscopic data as well as comparison with available literature data (Ghias *et al.*, 2011) compound **G** was concluded to be betulinic acid (**324**).

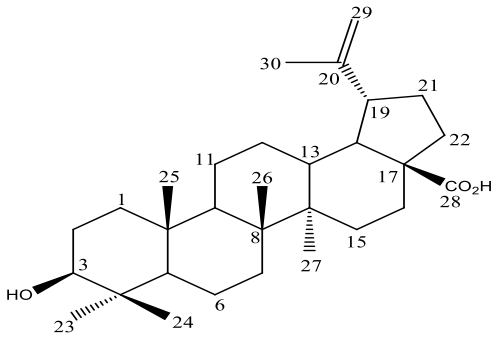

Table 12.0: ^{1}H ($CDCl_3$, 360 MHz) and ^{13}C ($CDCl_3$, 90 MHz) NMR data for betulinic acid (**324**)

	^{1}H (δ_H, mult, *J* in Hz)		**^{13}C (δ_C)**	
C#	Reported lit.values	Observed values	Reported lit.values	Observed values
1			38.3	39.43
2			27.3	27.36
3	3.18 (dd, *J*=10.2,5.5)	3.22 (dd, J = 11.1, 5.4)	78.2	77.04
4			38.4	38.71
5			55.0	55.12
6			18.6	18.17
7			33.9	34.13
8			40.2	39.97
9			50.4	50.15
10			36.7	36.93
11			20.8	21.00
12			25.1	25.29
13			37.9	37.78
14			41.9	42.19
15			29.0	30.60
16			30.1	31.93
17			55.8	55.63
18			48.8	46.84
19			47.6	48.73
20			150.2	150.52
21			29.2	29.43
22			34.6	36.57
23	0.96 s	0.96 s	27.4	28.30
24	0.75 s	0.75 s	15.3	15.94
25	0.80 s	0.82 s	15.5	16.14
26	0.97 s	0.94 s	17.1	16.09
27	0.99 s	1.11 s	14.7	14.58
28			178.6	177.49
29	4.70 br s, 4.58 br s	4.73 br s, 4.60 br s	108.9	109.82
30	1.7 s	1.64 s	20.4	19.14

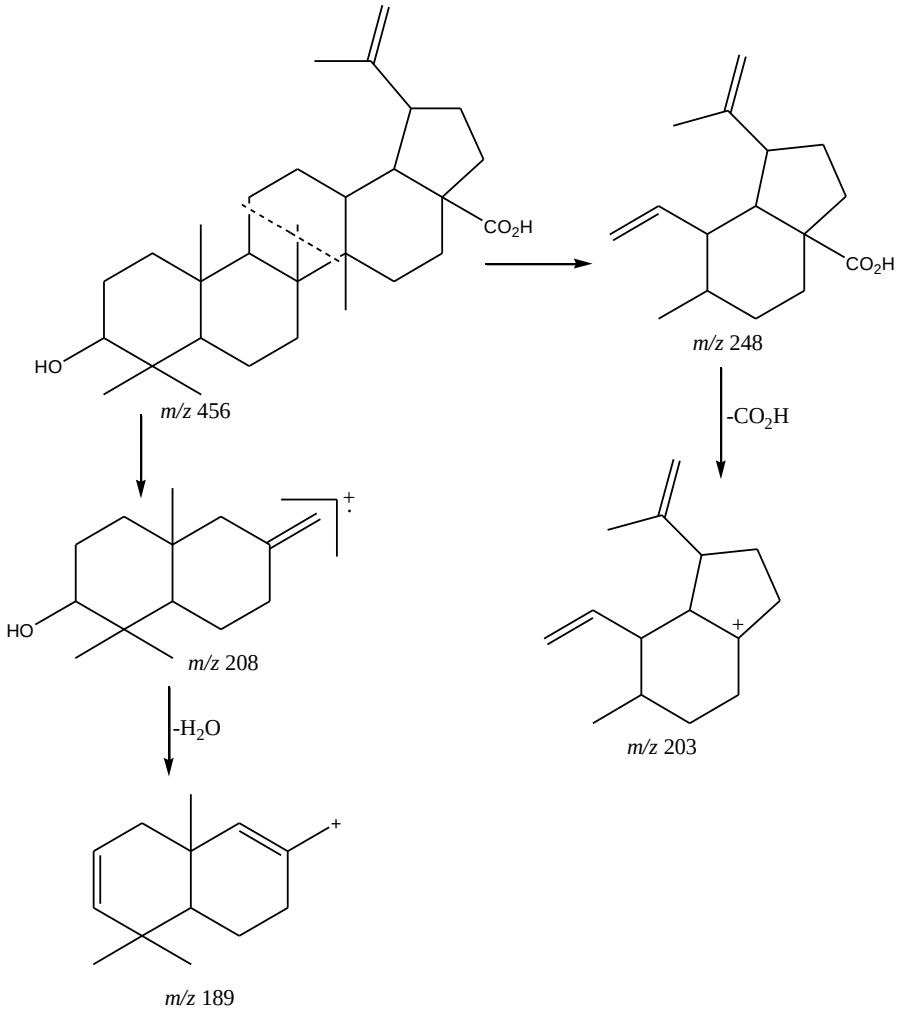

Figure 4.0 Proposed fragmentation pattern of betulinic acid (**324**)

Compound **(H)** acid was obtained as white powder with m.p. > 300 ^{0}C and R_f value of 0.21 (solvent system: *n*-hexane-EtOAc 3:2). It showed a purple colour with acidified anisaldehyde after heating on hot plate at 100^{o}C suggesting the presence of a sterol or a terpenoid derivative. The compound responded positively to Liebermann-Buchard and ceric sulphate tests which further supported the presence of a terpenoid derivative (Sukumar *et al.*, 1995). The ^{I}H NMR spectrum **(**Table 13.0; Appendix **I**1**)** exhibited a triplet peak at δ_H 5.23 (J = 5.8 Hz) assignable to H-12 whereas the peak that appeared at δ_H 3.26 (dd, J = 9.9, 4.4 Hz) was assigned to oxymethine proton H-3. The position of the hydroxyl group was suggested to be at C-3 and was equatorially oriented

based on the coupling constants (Seebacher*et al*, 2003).This together with seven tertiary methyl groups on quaternary carbons centered at δ_H 1.27 (C-27), 1.20 (C-23), 1.08 (C-26), 1.01 (C-25), 0.97 (C-30), 0.96 (C-29) and 0.95 (C-24) were suggestive of oleanane type of triterpenes (Seebacher *et al*., 2003). The ^{13}C NMR spectrum **(**Table 13.0; Appendix **I**2**)** exhibited a total of 30 carbon signals which were resolved by DEPT-135 into seven methyls, ten methylenes, five methines including one olefinic and one oxygen bearing carbons as well as eight quaternary carbons. The olefinic carbons C-12 and C-13 appeared at δ_C 121.42 and 142.94, respectively and the down field value of C-13 supported the compound to be an oleanane derivative rather than an ursane derivative (Doddrell *et al.*, 1974; Sukumar *et al.*, 1995). Similarly, the chemical shift at δc 179.75 was assigned to carbon of the carboxylic acid C-8 while the deshielded oxymethine carbon that resonated at δc 77.57 was assigned to C-3 (Seebacher*et al*, 2003). Both the ^{1}H and ^{13}C NMR spectra displayed data that were characteristic of oleanolic acid (Seebacher *et al*., 2003). This was further corroborated by EIMS molecular ion peak at *m/z* 455 corresponding to $C_{30}H_{48}O_3$ formula and the fragmentation pattern as displayed in Figure 5.0. Therefore, from physical and spectroscopic data as well as comparison with literature data (Seebacher *et al*., 2003), compound **H** was established to be oleanolic acid (**335**).

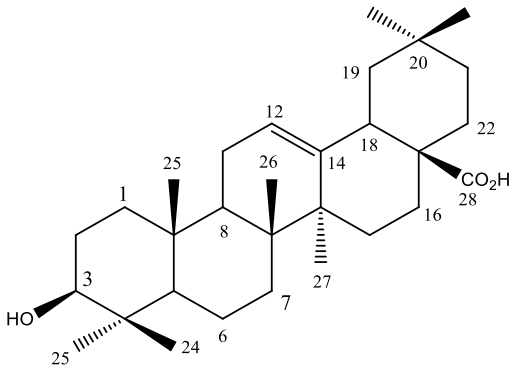

Table 13.0: ^{1}H ($CDCl_3$, 300 MHz) and ^{13}C ($CDCl_3$, 75 MHz) NMR data for oleanolic acid (**335**)

Position	**^{1}H (δ_H, mult, *J* in Hz)**		**^{13}C (δ_C)**	
	*Reported lit.values	Observed	*Reported lit.values	Observed
1	1.02 m, 1.57 m		39.0	37.68
2	1.82 m, 1.82 m		28.1	26.68
3	3.4 (dd,*J*=10.2, 5.5 Hz	3.26 (dd, *J*=9.9, 4.4)	78.2	77.57
4			39.4	38.34
5	0.88 m		55.9	54.51
6	1.39 m, 1.58 m		18.8	17.35
7	1,36 m, 1.53 m		33.4	32.81
8			39.8	40.73
9	1.71 m		48.2	47.97
10			37.4	36.00
11	1.96 m, 1.96 m		23.8	24.52
12	5.49 br s	5.23 (t, *J* = 5.8)	122.6	121.42
13			144.8	142.94
14			42.2	40.45
15	1.22 m, 2.19 m		28.4	26.74
16	1.96 m, 2.12 m		23.5	22.37
17			46.7	46.23
18	3.30 (dd, *J* = 12.9, 4.2)		42.5	40.73
19	1.32 m , 1.83 m		44.8	45.44
20			31.0	31.14
21	1.23 m, 1.46 m		34.1	32.81
22	1.82 m, 2.04 m		32.3	31.63
23	1.24 s	1.20 s	28.8	29.50
24	1.02 s	0.95 s	16.5	15.65
25	1.04 s	1.01 s	17.5	17.34
26	1.04 s	1.08 s	19.5	21.90
27	1.30 s	1.27 s	26.2	25.73
28			180.0	179.75
29	0.97 s	0.96 s	33.4	32.84
30	1.02 s	0.97 s	23.8	22.38

*Literature data (Seebaccher *et al*., 2003)

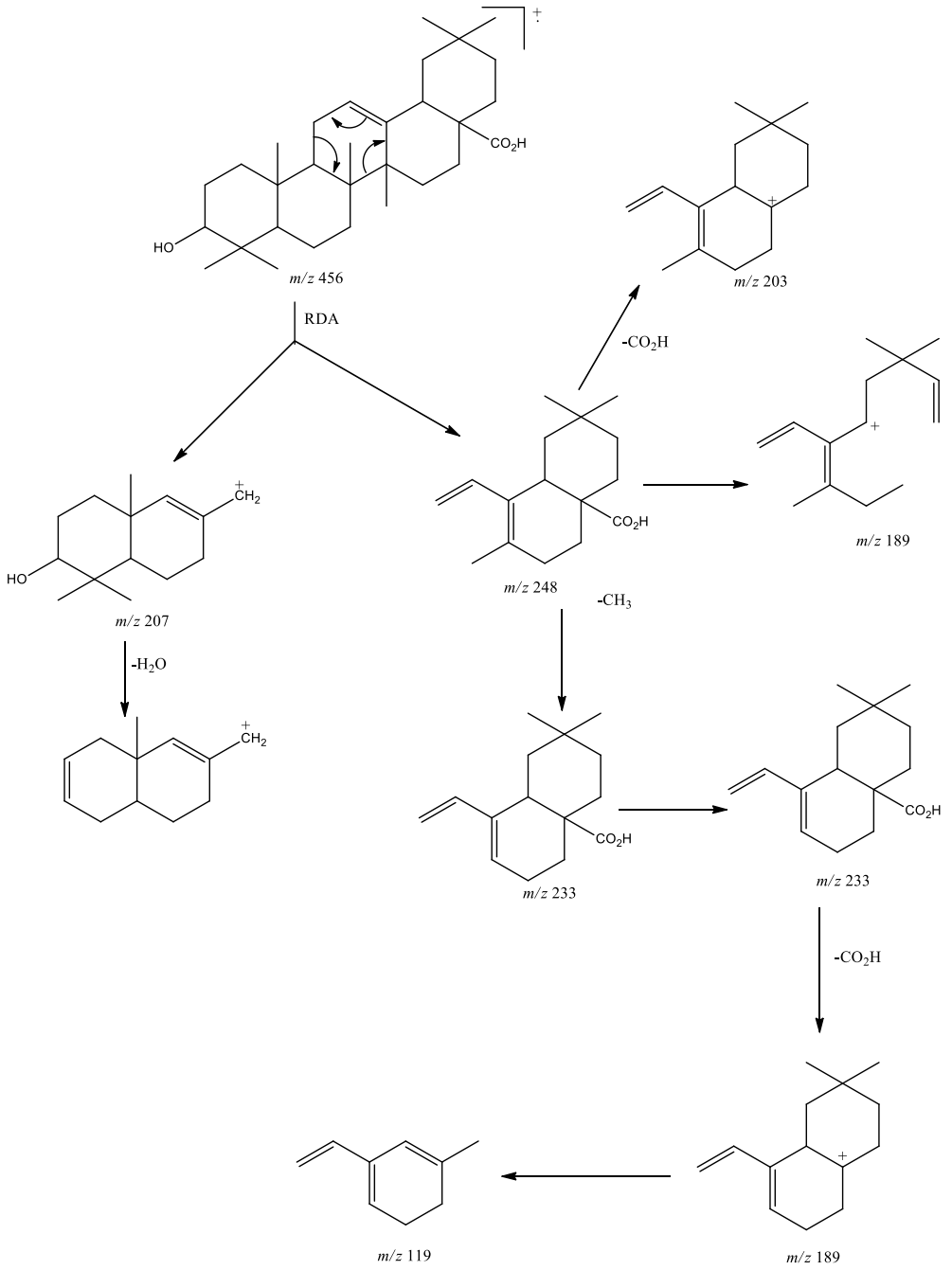

Figure 5.0. Proposed fragmentation pattern of oleanolic acid (**335**)

Compound **L** was isolated as pale reddish powder, m.p 220-222 ^{0}C. It exhibited a significant IR peak at 3400 cm^{-1} which signified a hydroxyl functionality.The ^{1}H NMR spectral data (Table 14.0, Appendix J1) of the compound showed characteristic aglycone pattern of resveratrol derivative namely: three *meta*-coupled aromatic protons of ring A appearing at δ 6.62 (H-6,1H,s), 6.45 (H-2,

d, *J*=1.2 Hz), and 6.77 (t, *J*=2.2, 1.2 Hz, H-6) Vastano *et al*, 2000). The other set of aromatic protons signifying AA`XX` spin system at δ7.35 (d, *J*=9.0 Hz, H-2`/H-6`) and 6.79 (d, *J*=10.2, 6.6 Hz, H-3`/H-5`) respectively. These were supported by δc signals at 128.9 C-2`, 116.5 C-3`, 116.2 C-5`, and 128.6 C-6`. These together with an isolated olefinic protons observed at δ7.00 (d, *J*=16.8 Hz, H-8) and 6.83 (d, *J*=15.7 Hz, H-7) suggested that the aglycone is resveratrol (Park and Boo, 2013). The anomeric proton signal in the ^{1}H NMR spectrum appeared at δ 4.83 as a doublet with coupling constant *J*=7.2 Hz which is in accordance with the axial-axial coupling between protons on C-1`` and C-2`` in a *β*-linked hexose (Vastano *et al*, 2000). The other signals in the relatively upfield region of the spectrum at δ 3.47, 3.37, 3.31, 3.39, 3.54 and 3.70 corresponding to one proton each accounted for H-2, H-3, H-4, H-5 and H-6 respectively. The^{13}C NMR (Table 14.0, Appendix J2) displayed 20 signals which were resolved into one methylene δ 62.6 (C-6), 14 methines and five quarternary carbons at δ 141.6 C-1), 159.6 (C-5) 126.7 (C-1`) and 158.5 (C-4`) and 158.5 (C-4). The position of the hydroxyl groups as well as attachment of the sugar to the aglycone was established from HMBC experiments (Appendix J3) which indicated a three H-C correlations: H-1 correlated with C-3 (δc 160.5) whereas H-2` and H-6` showed cross peaks with C-4` (δc 158.5) indicating that the sugar was attached at C-3, while a hydroxyl group was placed at C-4`. In the mass spectrum (Appendix J4), fragment ion at *m/z* 229 $[M + H - C_6H_{10}O_5]^+$ corresponding to the aglycone was due to loss of a hexose moiety. Acid hydrolysis of the compound gave resveratrol and glucose, confirmed by the TLC and paper chromatography with authentic samples. Thus, the above data for the compound are consistent with the structure of 4`, 5-dihydroxystilbene-3-*O*-glucoside (**336**).

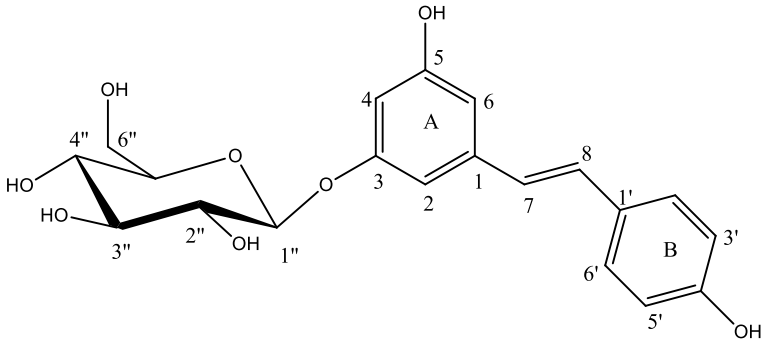

Table 14: ^{1}H (600 MHz) and ^{13}C (150 MHz) NMR (DMSO-d_6) data for 4`, 5-dihydroxystilbene-3-*O*-glucoside **(336)**

C#	^{1}H Multiplicity, (*J* in Hz)	^{1}H* Multiplicity, (*J* in Hz)	^{13}C	^{13}C*
1	-	-	141.6	141.1
2	6.45 (d, *J*=1.2)	6.46 s	102.4	102.7
3	-	-	160.5	159.1
4	6.77, (t, *J*=2.2, 1.2)	6.46, (t, *J*=2.3, 1.7)	104.1	105.6
5	-	-	159.6	158.3
6	6.62 bs	6.59 s	107.1	107.0
7	6.83, (d, *J*=15.7)	6.45, (d, *J*=16.6)	130.0	128.6
8	7.00, (d, *J*=16.8)	6.62 (d, *J*=16.6)	126.7	125.3
1`	-	-	130.4	129.0
2`	7.35, (d, *J*=9.0)	7.35, (d, *J*=8.6)	128.9	127.6
3`	6.79, (d, *J*=10.2, 6.6)	6.75 (t, *J*=10.1, 8.6)	116.5	115.1
4`	-	-	158.5	156.9
5`	6.79 (d, *J*=10.2, 6.6)	6.75 (t, *J*=10.1, 8.6)	116.2	115.1
6`	7.35 (d, *J*=9.0)	7.35 (d, *J*=8.6)	128.6	127.6
1``	4.83 (d, *J*=7.2)	3.91 (dd, *J*=7.1, 1.7)	102.4	101.1
2``	3.47 (dd, *J*=9.4, 6.8)	3.41	75.0	73.6
3``	3.37 (dd, *J*=9.0, 4.2)	3.36	78.1	76.7
4``	3.31 (bt, *J*=9.8)	3.35, s	71.5	70.1
5``	3.39 (dd, *J*=9.8, 6.0)	3.38, s	78.3	76.9
6``	3.54 (H_α, dd, *J*=13.2, 6.6) 3.70 (H_β, dd, *J*=12.0, 6.6)	3.44 (H_α, t, *J*=12.4, 6.3) 3.69 (H_β, dd, *J*=10.7, 6.0)	62.6	61.2

^{1}H* and ^{13}C* NMR data (Vastano *et al*, 2000)

Compound **I** was obtained as yellow crystals in a yield of 65.4 mg with mp. 175-176^{0}C. Its ^{I}H NMR spectrum (Table 15.0, Appendix K1) showed three aromatic proton peaks at δ 7.59 (t, *J* =8.4 Hz,), 6.81 (d, *J* = 4.4) and 7.15 (d, *J* =8.5 Hz) which are characteristics of monohydroxylated ring A of a flavone derivative at C-5 (Uivarosi *et al*., 2013). The presence of a flavone was further

supported by a singlet peak that was observed at δ 7.14 assigned to H-3. Besides these peaks, another set of five aromatic signals associated with ring B were observed: the ortho coupled doublet at δ 8.06 (d, *J* = 7.6 Hz) represented H-2' and H-6'whereas a multiplet signal integrating into three proton represented H-3', H-4' and H-5` observed at δ 7.60, further indicated that compound **i** is a flavone derivative with only one hydroxyl substitution in ring A. Similarly, the ^{13}C NMR (Table 15.0, Appendix K2) showed the presence of fifteen carbon signals including eight aromatic CH, an olefinic CH (δ 106.64) and six quartenary carbons including α, β-unsaturated carbonyl at δ 185.18 (C-4) as evidenced by 135 DEPT spectrum (Appendix K3). The position of hydroxyl group attachment was evidenced by HMBC correlations (Appendix K4) between H-7 (δ 7.59) and C-5 (δ 161.83). The EIMS spectrum (Appendix K5) gave a molecular ion peak at 238.0 consistent with a molecular formula $C_{15}H_{10}O_3$. Thus, on the basis of spectroscopic data, the compound was concluded to be 5-hydroxyflavone (primuletin) (**337**).

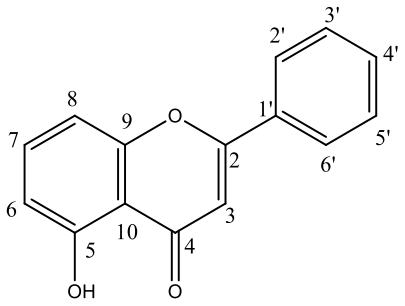

Table 15.0:^{1}H (600 MHz) and ^{13}C (150 MHz) NMR (DMSO-d_6) data for 5-hydroxyflavone (**337**)

C#	^{1}H multiplicity, (*J* in Hz)		^{13}C NMR (δc)	
	Reported lit. value	Observed values	Reported value	observed value
2			164.1	166.52
3	7.10 s	7.14 s	105.7	106.64
4			183.2	185.18
5			159.8	161.83
6	6.80 (d, *J* = 8.3)	6.81 (d, *J* =8.5)	110.0	111.71
7	7.60 (t, *J* =8.1)	7.59 (t, *J* =8.4)	135.9	136.99
8	7.20 (d, *J* =8.1)	7.15 (d, *J* = 4.4)	107.5	108.42
9			155.9	157.97
10			110.1	112.32
1’			130.5	131.31
2’	8.10 (dd, *J* = 7.8,1.7)	8.06 (d, *J* = 7.6, 2.0)	126.6	127.68
3’	7.60 m	7.60 m	129.2	130.31
4’	7.60 m	7.60 m	132.3	132.44
5’	7.60 m	7.60m	129.2	130.31
6’	8.1 (dd, *J*=7.9, 1.7)	8.06 (d, *J* =7.6, 2.0)	126.6	127.68
5-OH	12.55 s	12.60 s		

*Literature data (Uivarosi *et al*., 2013)

Compound **J** was also obtained as a pale yellow powder with m.p.249-252^{0}C and R_f value 0.56 (solvent system; CH_2Cl_2-MeOH, 9:1). The yellow colour intensified on TLC when exposed to conc ammonia solution vapour. The yellow colour also turned dark brown when sprayed with ferric chloride solution on TLC suggesting the presence of flavonoid derivative (Manguro *et al*., 2020). The compound afforded a HREI-MS molecular ion peak at *m/z* 238.0 $[M]^+$ corresponding to $C_{15}H_{10}O_3$ molecular formula. Its ^{1}H NMR spectrum (Table 16.0, Appendix L1) exhibited two meta coupled aromatic protons centered at δ 6.94 (dd, *J* = 8.8, 2.5 Hz) and 6.96 (d, *J* = 2.4 Hz) representing H-6 and H-8, respectively while an ortho coupled aromatic proton that resonated at 7.99 (d, *J* =8.8 Hz) was assigned to H-5. In addition to these, a singlet peak at δ 6.81 typical for flavone derivatives was observed in the ^{1}H NMR (Vijay *et al*., 2011; Kostrzewa-Susłow and Janeczko, 2012). This together with a set of aromatic proton signals representing ring B were observed at δ 8.02 (d, *J* = 7.6 Hz, H-2’ and H-6’), δ 7.56 (m, H-4’ and H-3’) and δ 7.57 (m, H-5’).

The accrued ^{1}H NMR results suggested that 7-hydroxyflavone was similar to 5-hydroxyflavone with the major difference being the position of hydroxyl group in ring A. Changes of diagnostic values were observed in the ^{13}C NMR spectrum (Table 16.0, Appendix L2) of 7-hydroxyflavone in comparison with 5-hydroxyflavone whereby C-5 shifted upfield at δ 127.88 due to the absence of OH group while C-7 peak was observed down field at δ 165.29 arising from OH substitution at this position. This was further evidenced by ^{1}H-^{1}H COSY (Appendix L3)which showed correlation between H-5 and H-6, a fact further supported by HMBC correlations (Appendix L4) between H-5 and C-7, a fact that was further confirmed by EI-MS (Appendix L5) peaks at *m/z* 138 and 90. Therefore from the spectroscopic data compound **J** was identified as 7-hydroxyflavone (**338**), a compound isolated for the first time as a natural product.

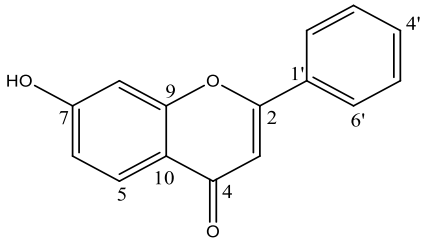

Table 16.0: ^{1}H (600 MHz) and ^{13}C NMR (150 MHz) (DMSO-d_6) data for 7-Hydroxyflavone **(338)**

C#	^{1}H multiplicity, (*J* in Hz)		^{13}C NMR (δc)	
	Reported lit. value	observed	Reported value	observed value
2			162.8	165.42
3	6.86 s	6.81 s	106.6	107.31
4			176.4	180.24
5	7.86 (d, *J* = 8.7)	7.99 (d, *J* =8.8 Hz)	126.5	127.88
6	6.91 (dd, *J* = 8.7, 2.3)	6.94 (dd, *J* =8.8 Hz, 2.5Hz)	115.1	116.63
7			161.9	165.29
8	6.97 (d, *J* =2.2)	6.96 (d, *J* = 2.4 Hz)	102.6	103.60
9			157.5	159.88
10			116.1	117.35
1’			131.3	132.88
2’	8.02 (d, *J* = 7.9)	8.06 (d, *J* = 7.6 Hz)	126.2	127.43
3’	7.53 m	7.56 m	129.1	130.24
4’	7.53 m	7.56 m	131.6	132.99
5’	7.53 m	7.57 m	129.1	130.24
6’	8.02 (d, *J*=7.9)	8.06 (d, *J* =7.6 Hz)	126.2	127.43
7-OH	10.52 s	10.60 s		

*Literature data (Kostrzewa-Susłow and Janeczko, 2012)

4.6 Minimum Inhibitory Concentration of the Isolated Compounds

The MIC values for the isolated compounds are displayed in Table 17.0. A total of twelve compounds isolated from *n*-hexane, EtOAc and MeOH extracts were evaluated for antimicrobial activities against three Gram-positive and four Gram-negative bacteria as well as three fungi strains. According to Gibbsons (2004), compounds showing antimicrobial activities in the range 64-100 µg/ml are considered as clinically relevant. In addition to this, it has also been documented that compounds exhibiting MIC values of 10 µg/ml or less are not active while compounds showing antimicrobial activities in the range of 100-250 µg/ml are considered moderate (Rios and Recio, 2005). Three anthraquinone derivatives namely, chrysophanol (**7**), physcion (**9**) and emodin (**10**) have been reported to occur in at least over seventeen plant families worldwide and have been screened for antimicrobial activities (Hazra *et al*., 2005). The compounds were evaluated for

antimicrobial activities and according to the results, compound **10** had significantly (P≤0.05) high antimicrobial activities. The compound showed significantly (P≤0.05) high inhibitory activities against bacteria strains *S. aureus* (31.3 μg/ml), *S. faecalis* (62.5 μg/ml), *B. anthracis* (62.5 μg/ml) and *K. pneumoniae* (62.5 μg/ml). Lu *et al*., (2011) in a similar study documented a MIC value of 20 μg/ml for emodin against *S. aureus* but it was observed that the compound did not show any activity when tested against *K. pneumoniae* which is contrary to the current findings. On the other hand, evaluation of compound **10** for antibacterial activity against *P. aeruginosa and S. typhimurium* showed significantly (P≤0.05) higher activity with a MIC value of 125 μg/ml each, however, the compound did not show any inhibitory activity against *E. coli* (250 μg/ml). The other anthraquinone metabolites, chrysophanol (**7**) and physcion (**9**) showed significantly (P≤0.05) higher MIC values against *S. aureus, S. faecalis, K. pneumoniae, P. aeruginosa* and *B. anthracis* with MIC values 125 μg/ml each as compared to compound **10**. However, the microrganisms, *E. coli* and *S. Typhimurium* were observed to be inactive to the compounds.

The antifungal mechanisms of anthraquinones is yet to be fully understood but it is believed to be associated with cell wall integrity disruption and suppression of glucan synthesis in microorganism cells. Compound (**10**) caused 100% inhibition of *T. mentagrophytes, M. gypseum and C. albicans* growth at MIC values 15.6 μg/ml, 31.3 μg/ml and 62.5 μg/ml, respectively. The antifungal activity of emodin (**10**) against *C. albicans* has been reported to be related to the inhibition of (1,3)-*β-D*-glucan synthase activity, leading to disruption of (1,3)-*β-D*-glucans in the fungal cell wall (Janeczko, 2018). The foregoing evidence had been earlier substantiated by Agarwal *et al* (2000) who reported that chrysophanol (**7**) and physcion **(9)** isolated from the rhizomes of *Rheum emodin* exhibited antimicrobial activities against fungal species *C. albicans, T. mentagrophytes* and *T. gypseum* with MIC values in the range 62.5-250 μg/ml. The three anthraquinones physcion,

chrysophanol and emodin have also been documented to show antifungal activity (MIC 90-100 mg/ml) and the inhibition effect depends on the presence of substituents OH at C-1 and C-8) (Manojlovic *et al*., 1998). Compound **7** has also been reported to be active against *Bacillus subtilis*, *Staphylococuss epidermidis* and *E. coli* (García-Sosa *et al*., 2006). Hatano *et al* (1999) reported MIC of chrysophanol (**7**) against *E. coli* to be greater than 1024 µg/ml, while the value for methicillin resistant *S. aureus* was 256 µg/ml.

Compounds **302** and **303** were obtained from EtOAc crude extract of *S. didymobotrya* flowers. The two compounds were subjected to both antibacterial and antifungal tests and their antimicrobial activities were observed to be significantly ($P \leq 0.05$) low as compared to the standard drug used (kanamycin). From the data obtained, the negative growth inhibitory activity of compounds was observed to be almost the same for all strains of bacteria and fungal species. The compounds exhibited inhibitory activities against *S. aureus* with MIC of 250 µg/ml which is in agreement with already reported antibacterial activity of the compounds (Tamokou *et al*, 2011). The compounds did not show any inhibitory activity to the remaining microorganisms tested.

Compound (**253**) is a flavonoid derivative that has been reported to have broad-spectrum antimicrobial properties (Yang *et al*., 2020). The current research evaluated antimicrobial activity of **253** and from the findings the MIC values ranged between 62.5-250 µg/ml. Using the compound, *S. aureus* and *P. aeruginosa* together with filamentous fungi *M. gypseum* and *T. mentagrophytes* showed inhibition of 62.5 µg/ml while moderate activity was observed with *S. faecalis, K. pneumoniae, S. typhimurium, B. anthracis, C. albicans* (125 µg/ml each) and *E. coli* (250 µg/ml). The current results are in complete agreement with earlier investigations reported by Jaisinghani (2017) and Perera *et al* (2022). Elsewhere, studies have shown that compound **253** has a good inhibitory effect on the growth of *P. aeruginosa, S aureus* and *E. coli* (Qin *et al*., 2009).

Triterpenes are applied in treatment of microbial infections due to their lipophilic properties which help them to interact with the bacteria cell wall, thus interfering with the biosynthesis of its components. The compounds also penetrate the bacterial cell and interfere with protein synthesis and DNA replication and repair mechanisms (Karaman *et al*., 2003; Tene, 2009). In the current study, three triterpenoids namely, betullinic acid (**324**), lupeol (**323)** and oleanolic acid (**335**) were tested for their antimicrobial activities and the results indicated that the antimicrobial activities may be due to the difference in the substituent groups. Compound **335** significantly ($P \leq 0.05$) showed potent antimicrobial activity compared to compounds **323** and **324.** It showed a significantly ($P \leq 0.05$) high inhibitory activity with a MIC value of 62.5 µg/ml against *S. aureus, B. anthracis,S. faecalis, K. pneumaoniae, M. gypseum, T.* mentagrophytesand *C. albicans.* The compound was observed to show significantly ($P \leq 0.05$) low inhibitory growth to *E. coli* (250 ug/ml). Haraguchi *et al* (1999) reported the antibacterial activity of oleanolic acid for *S. aureus* (>200 µg/ml), *E. coli* (>200 µg/ml), *P. aeruginosa* (100 µg/ml). Similarly, the diversity of the antibacterial properties of compound **335** has also been demonstrated with methicillin-resistant *S. aureus, B. anthracis, B. cereus, E. faecalis* and *E. faecium* (Jesus *et al*., 2015). Compound **324** was another triterpene isolated for the first time from *S. didymobotrya*. The compound has been reported to exhibit good to moderate antimicrobial activities against pathogenic microorganisms. The compound recorded MIC values which ranged from 62.5-250 µg/ml except with *E. coli* (MIC value > 250 µg/ml). The highest significantly ($P \leq 0.05$) high activity was recorded for Gram-positive bacteria, *S. aureus* and *S. faecalis* as well as with fungal strain *C. albicans* (62.5 µg/ml). The compound afforded significantly ($P \leq 0.05$) lower MIC vlues 125 µg/ml against *B. anthracis, S. typhimurium, K. pneumoniae, P. aeruginosa* as well as *M. gypseum* and *T. mentagrophytes* with a MIC value of 125 µg/ml as compared to *S. aureus* and *S. faecalis* . However, this compound did

not affect the growth of *E. coli* (> 250 µg/ml). In a previous study the non-activity of the compound was reported for *Bacillus subtillis, C. albican* and *S. aureus* which does not corroborate the current findings except for *E. coli* (Girma *et al*., 2003). Elsewhere the compound has been reported to show moderate activity (MIC > 128 ug/ml) against *Bacillus cereus, Enterococcus fecalis, E. coli, Literia monocytogene, P. aeruginosa, Salmonella enterica* and *S. aureus* (Fontanay *et al*., 2008). Chung *et al* (2014) reported constrasting results in which MIC values were found to be in the range 4 to 64 µg/ml against different strains of *S. aureus*. Compound **324** showed significantly ($P \leq 0.05$) high antifungal activity against the fungal strains tested. It was found to inhibit the growth of *C. albicans* (62.5 µg/ml) while with *M. gypseum* and *T. mentagrophytes* moderate activity was observed with MIC value of 125 µg/ml each. Compound **324** has been reported to inhibit the growth of fluconazole sensitive strain of *C.albicans* at MIC value 16 µg/ml, a finding that corroborated the present study (Shai *et al*., 2008). The compound was reported to show moderate antifungal activities against dermatophytes (*M. gypseum* and *T.* mentagrophytes) with a MIC value of 125 µg/ml each. The results accrued in this study are in agreement with the finding of Machado *et al*., (2009) (100 µg/ml).

Compound **323** is lupane-type triterpene widely distributed in many plants. In the current study, the compound was observed to show significantly ($P \leq 0.05$) high activity against *S. aureus* with a MIC value of 62.5 µg/ml, a finding which agreed with an earlier report indicating that the compound has considerable activity against *S. aureus* with a MIC value of 100 µg/ml (Ghosh *et al*., 2010). Compound **323** also demonstrated moderate activity when tested against *B. anthracis,S. faecalis, K. pneumoniae, M. gypseum, T. mentagrophytes* and *C. albicans* with a MIC value of 125 µg/ml. However, the compound did not show any inhibitory activity against *E. coli, S. typhimurium* and *P. aeruginosa.* In a previous study, Amoussa *et al.,* (2016) reported the compound to be active

against *S. aureus* and *P. aeruginosa* (MIC 25 µg/ml), methicillin-resitant *S. aureus* and *E. faecalis* (MIC 50 µg/ml each). The promising MIC value against *P. aeruginosa* was of interest considering that it is a Gram-negative bacterium. Similarly, Abas *et al* (2015) documented the antibacterial activity of the compound with MIC value of 100 µg/ml against *B. anthracis*, *S. feacalis* and *K. pneumaoniae*. The significantly ($P \leq 0.05$) low fungistatic nature of the compound **323** against *M. gypseum*, *T. mentagrophytes and C. albicans* (each 125 µg/ml) exemplified the fungicidal nature of the compound as previously documented by Emaikwu *et al* (2020). The results accrued and those obtained by Abas *et al* (2015) and Emaikwu *et al* (2020) demonstrated the importance of the three triterpenes as antimicrobials.

Flavonoid compounds are widely distributely secondary metabolites playing significant roles as natural protective substances. Compound (**337**) exhibited significantly ($P \leq 0.05$) high antimicrobial effect on the Gram positive bacteria *S. aureus* and *B. anthracis* as well as on the fungal species *M. gypseum*, *T. mentagrophytes* and *C. albicans* with each exhibiting a MIC value of 62.5 µg/ml. However, when the same compound was tested on *S. feacalis*, *P. aeruginosa*, *S. typhimurium* and *K. pneumaoniae*, it showed significantly ($P \leq 0.05$) low activity with MIC value of 125 µg/ml. No activity was observed when tested against *E. coli*. The antifungal activity of compound **337** against *C. albicans* has been investigated (Kuete *et al*., 2010). From the study, it revealed that the compound showed lowest MIC value of 39.06 µg/ml against the fungus. In a similar study, Montenegro *et al* (2017) reported a MIC value of 200 µg/ml when the compound was tested on *S. aureus*, *E. coli* and *P. aeruginosa*. Similarly, Resende *et a*l., (2015) documented MIC values of 100 µg/ml and 200 µg/ml for *S. aureus* and *E. coli*, respectively.

Compound (**338**) was another flavonoid derivative that gave significantly ($P \leq 0.05$) high antimicrobial activity against the microorganisms tested with MIC values in the range 31.3-125

μg/ml except for *E. coli* (250 μg/ml). The promising antimicrobial activity associated with the compound had been supported by Resende *et al* (2015), whereby *S. aureus and E. coli* recorded MIC values of 50 μg/ml and 100 μg/ml.

Compound (**336**) is commonly found in food and drinks and has been reported to possess antimicrobial activity (Paulo *et al*., 2010). The minimum inhibitory activity of compound (**336**) showed MIC values ranging between 62.5 and 250 μg/ml against all the microbes tested. In current study, the compound afforded a MIC value of 62.5μg/ml against *S. aureus*, *S. typhimurium,T. gypseum*, *T. mentagrophytes* and *C. albicans* while with the remaining microorganisms, the compound exhibited moderate activities.

Table 17.0 The MIC's of the isolated compounds against bacteria and fungi strains

Compounds/Standards	Microorganisms and Concentration of isolates in µg/ml.									
	Gram-positive bacteria			Gram-negative bacteria				Fungi		
	S. aureus	*S. faecalis*	*B. anthracis*	*K. pneumoniae*	*E.coli*	*S. typhymurium*	*P. aeruginosa*	*C. albicans*	*T. mentagrophyte*	*M. gypseum*
Chrysophanol (**7**)	125b	125b	125b	125b	500a	500a	125c	250b	125b	62.5c
Physion (**9**)	125b	125b	125b	125b	500a	500a	125c	125c	62.5c	62.5c
Emodin (**10**)	31.3d	62.5c	62.5c	62.5c	500a	125b	125c	62.5d	15.6e	31.3d
Quercetin (**253**)	62.5c	125b	125b	125b	250b	125b	62.5d	125c	62.5c	62.5c
β-sitosterol (**302**)	250a	500a	500a	500a	500a	500a	500a	500a	500a	500a
Stigmasterol (**303**)	250a	500a	500a	500a	500a	500a	500a	500a	500a	500a
Lupeol (**323**)	62.5c	125b	125b	125b	500a	500a	500a	125c	125b	125b
Betullinic acid (**324**)	62.5c	62.5c	125b	125b	500a	125b	125c	62.5d	125b	125b
Oleanolic acid(**335**)	62.5c	62.5c	62.5c	62.5c	250b	125b	125c	62.5d	62.5c	62.5c
4', 5-dihydroxystilbene 3-*O*-glucoside (**336**)	62.5c	125b	125b	125b	250b	125b	250b	62.5d	62.5c	62.5c
5-hydroxyflavone (**337**)	62.5c	125b	62.5c	125b	500a	125b	125c	62.5d	62.5c	62.5c
7-hydroxyflavone (**338**)	31.3d	62.5c	62.5c	125b	250b	125b	125c	62.5d	31.3d	62.5c
Kenamycin	10e	15d	16d	18d	30c	20c	20e	ND	ND	ND
Fluconazole	ND	ND	ND	ND	ND	ND	ND	4e	2.2f	2.0e
LSD (P≤0.05)	1.77	1.73	1.73	1.71	1.685	1.68	1.70	1.70	1.69	1.00
C.V	1.06	0.82	0.82	0.75	0.4	0.56	0.62	0.95	0.95	0.99

Means followed by different letters down the column are statistically different at P≤0.05 by Fisher's protected least significant difference test. ND= Not Done.

CHAPTER FIVE

SUMMARY, CONCLUSSIONS AND RECOMMENDATIONS

5.1 Summary

- Extraction of 1.0 kg of pulverized *S. didymobotrya* flowere using n-hexane, EtOAc and MeOH afforded 10.0 g, 25.0 g and 40.0 g representing 1%, 2.5 % and 4.0 %, respectively. From the data obtained, the yields increased with polarity of the solvent. MeOH being polar solvent presented the highest yield followed by ethyl acetate and the least was n-hexane.

- In the antimicrobial inhibition growth assays, the methanol extract was the most potent of the three extracts. It exhibited strong growth inhibition zones of 18.1mm, 16.7 mm and 16.3 mm against *K. pneumoniae, S. tyhimurium* and *S. faecalis*, respectively. The extract showed moderate activity to the rest of the bacteria microorganism. Evaluation of the extract against the fungal pathogens did reveal that it was fairly active to *M. gyseum* and *T. mentagrophyte* with growth inhibition zones of 15.0 mm and 14.0 mm, respectively. On the hand, EtOAc extract fairly afforded promising growth inhibition of 16.3 mm for *K. pneumonia*with the least recorded for *E. coli* (9.4 mm). With fungi susceptibility tests, the extract showed moderate zone inhibition growth values of 12.0 mm, 12.1 mm and 10.2 mm against *M. gypseum*, *T.mentagrophyte* and *C. albicans*, respectively. The *n*-hexane was the least active extract. It registered fairly moderate activity of inhibition growth of 12.1 mm for *S. faecalis* while against *S. aureus*, it afforded a value of 10.1 mm, respectively. The rest of bacteria tested showed inhibition growth in the range 7.1 mm to 9.3 mm.

- The Minimum Inhibitory Concentration (MIC) is the lowest concentration of an antimicrobial ingredient to prevent the visible growth of a microbe. The extracts were also evaluated for MIC against both Gram-positive and Gram-negative bacteria as well as fungal microbes.

MeOH extract was observed to be more potent against the fungi pathogens compared to the bacteria ones. In this case the lowest MIC value (3.90 µg/ml) was registered for *M. gypseum* while values of 7.8 mm and 31.3 mm were recorded for *T.mentagrophyte* and *C. albicans*, respectively. Using the bacteria pathogens, the lowest MIC value (31.3 µg/ml) was observed with *K. pneumoniae*. The remaining microbes presented MIC values in the range 62.5-250 µg/ml. The EtOAc extract was also observed to be more active to fungi pathogens than bacteria. The activities of the extract against the three fungi was in the range 15.6 µg/ml to 62.5 µg/ml while for bacteria pathogens MIC values in the range 62.5 µg/ml to 250 µg/ml were recorded except for *E. coli* (> 250 µg/ml). As was observed in the growth inhibition assays, *n*-hexane extract registered the least activity as evidenced by the accrued MIC values. The extract gave MIC values in the range 125 µg/ml to 250 µg/ml for both bacteria and fungi except for *E. coli* (MIC > 250 µg/ml). In both growth inhibition and MIC assays the activities of the extracts were observed to be lowercompared to those exhibited by kanamycin and fluconazole used as positive controls against bacteria and fungi.

- Gravity column chromatography of *n*-hexane, EtOAc and MeOH extracts using both deactivated and active silica gel as stationary phase and different organic solvents as eluents afforded twelve compounds namely; chrysophanol (**7),** physcion **(9),** emodin (**10**)**,** quercetin **(253),** *β*-sitosterol **(302),** stigmasterol (**303**),lupeol **(323),** betullinic acid **(324),** oleanolic acid **(335),** 4', 5-dihydroxystilbene-3-*O*-glucoside **(336),** 5-hydroxyflavone **(337)** and7-hydroxyflavone **(338).** Betullinic acid (**324**), 5-hydroxyflavone (**337**) and 7-hydroxyflavone (**338**) are secondary metabolites being reported for the first time from *S. didymobotrya*. Infact compound **338** is being reported for the first time as natural product, though it has been synthesized.

- All the compounds isolated from *S. didymobotrya* flowers were assayed for antimicrobial activities. From the data obtained compound **10** was observed to be the most active. It was effective against *S.aureus* and *M. gypseum* with MIC value of 31.3 µg/ml. It also registered an impressive MIC value of 15.6 µg/ml against *T. mentagrophyte*. The remaining compounds showed varying degrees of activities depending on the microorganism tested except for *β*-Sitosterol **(302)** andstigmasterol (**303**) which showed weak activity against *S. aureus* with MIC value of 250 µg/ml. Both compounds were inactive to the rest of microorganisms.

5.2 Conclusions

- The *n*-hexane, ethyl acetate and methanol extracts of the flower exhibited antimicrobial activities against all the tested microorganisms. This validates the traditional use of theflowers in management of microbial infections.
- Among the three extracts, methanol extract had the highest activities. This may be due to the presence polar compounds with high biological activities or due to the synergistic effect of the compounds present.
- From the three extracts a total of twelve compounds namely; chrysophanol (**7),** physcion **(9),** emodin (**10**)**,** quercetin **(253),** *β*-sitosterol **(302),** stigmasterol (**303**), lupeol **(323),** betullinic acid **(324),** oleanolic acid **(335),** 4', 5-dihydroxystilbene-3-*O*-glucoside **(336),** 5-hydroxyflavone **(337)** and7-hydroxyflavone **(338)** were isolated. Compound **338** is being reported for the first time as natural product.

  All compounds obtained from the *S. didymobotrya* flower extracts were subjected to antimicrobial activity against seven bacterial strains including three Gram positive *S. aureus, S. faecalis* and *B. anthracis* and four Gram negative *K. pneumoniae, S. typhimurium, P. aruginosa* and *E.coli* as well as three fungal strains *C. candidas, T. mentagrophyte* and *M.*

gypseum. Compound **10** was observed to be the most active. It was effective against *S.aureus* and *M. gypseum* with MIC value of 31.3 µg/ml. It also registered an impressive MIC value of 15.6 µg/ml against *T. mentagrophyte*. The remaining compounds tested exhibited moderate activities with MIC in the range 62.5-250 µg/ml except and *β*-sitosterol (**302**) stigmasterol (**303**) (MIC > 250 µg/ml). The activities of isolated compounds were lower compared to kanamycin and fluconazole used as positive controls for bacteria and fungi respectively.

5.3 Recommendation

- Use of flowers of *S. didymobotrya* should be encouraged as an alternative remedy to antimicrobial infections particularly in control of dermatophyte fungi.
- Compounds that exhibited high antimicrobial activities such as emodin (**10**), 5-hydroxyflavone (**337**), 7-hydroxyflavone (**338**), oleanolic acid (**335**), betullinic acid (**324**) and 4', 5-dihydroxystilbene 3-*O*-glucoside (**336**) may be developed into formulations to be used as antimicrobials.

5.4 Suggestions for Future Studies

- Antibacterial and antifungal activities of the extracts and the active isolates should be tried *in vivo* todetermine their potential application.
- The structural modification (including methylation and esterification) studies on active isolated compounds should be done to determine the pharmacophores and to evaluate if their activity can be enhanced.
- Synergistic evaluation of isolated compounds should be done to find out if this process can enhance microbial activity.

5.5 Significance of the Study

- Data accrued from this study afforded antimicrobials from flowers of *S. didymobotyra* that can be used to control microbial infections affecting human beings and animals.
- The results manifest the ethnomedicinal use of these plants in control of microbial infections in human beings.

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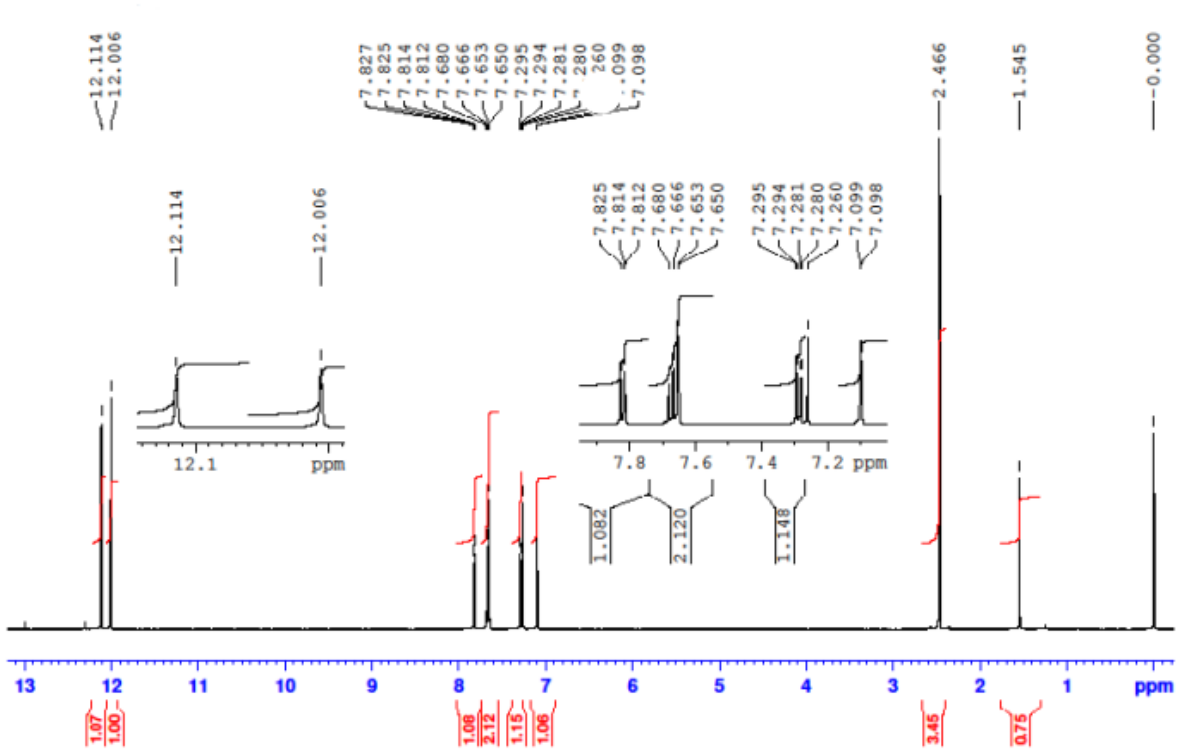
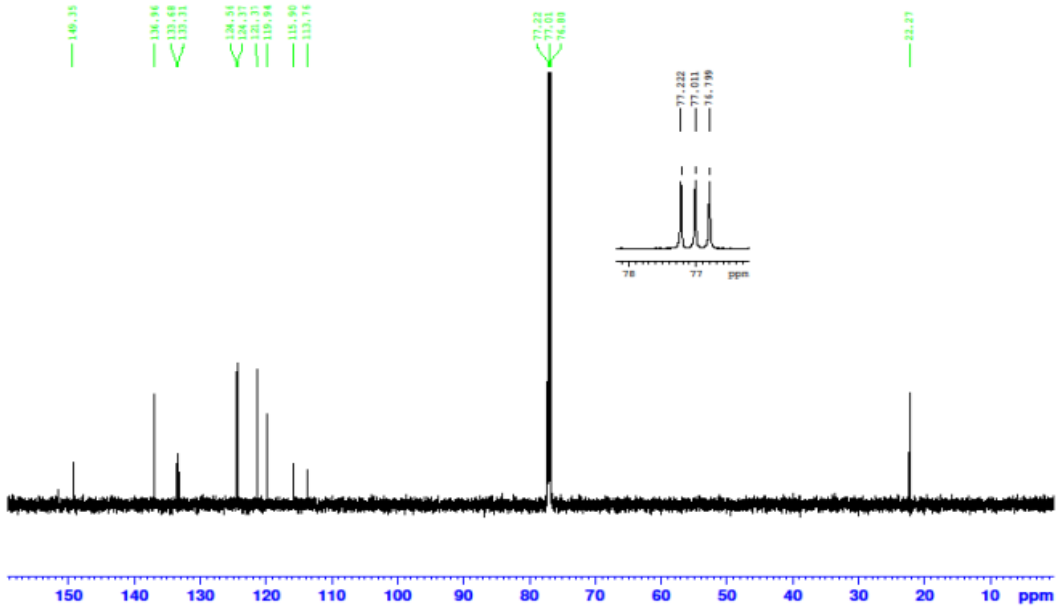
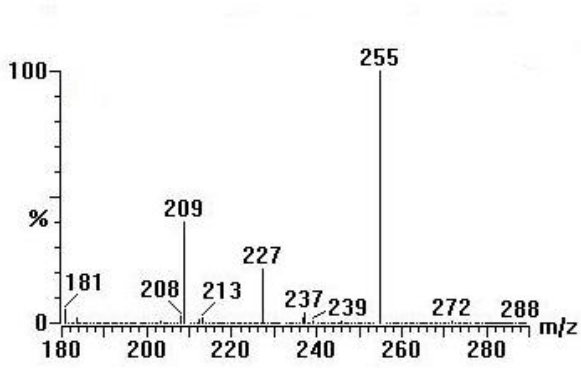
APPENDICES



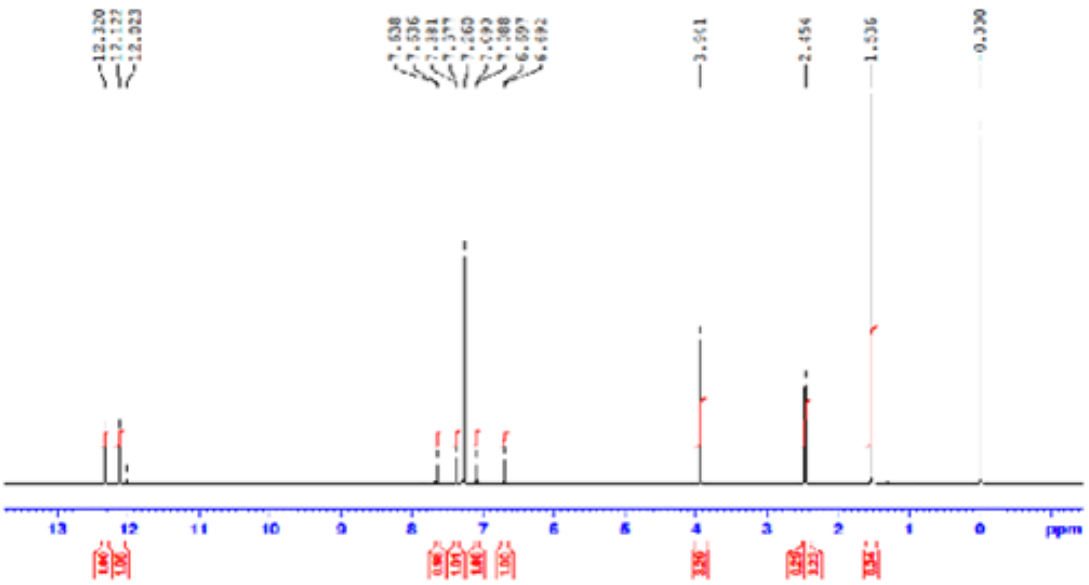
Appendix A1 ^{1}H NMR spectra for chrysophanol (**7**)



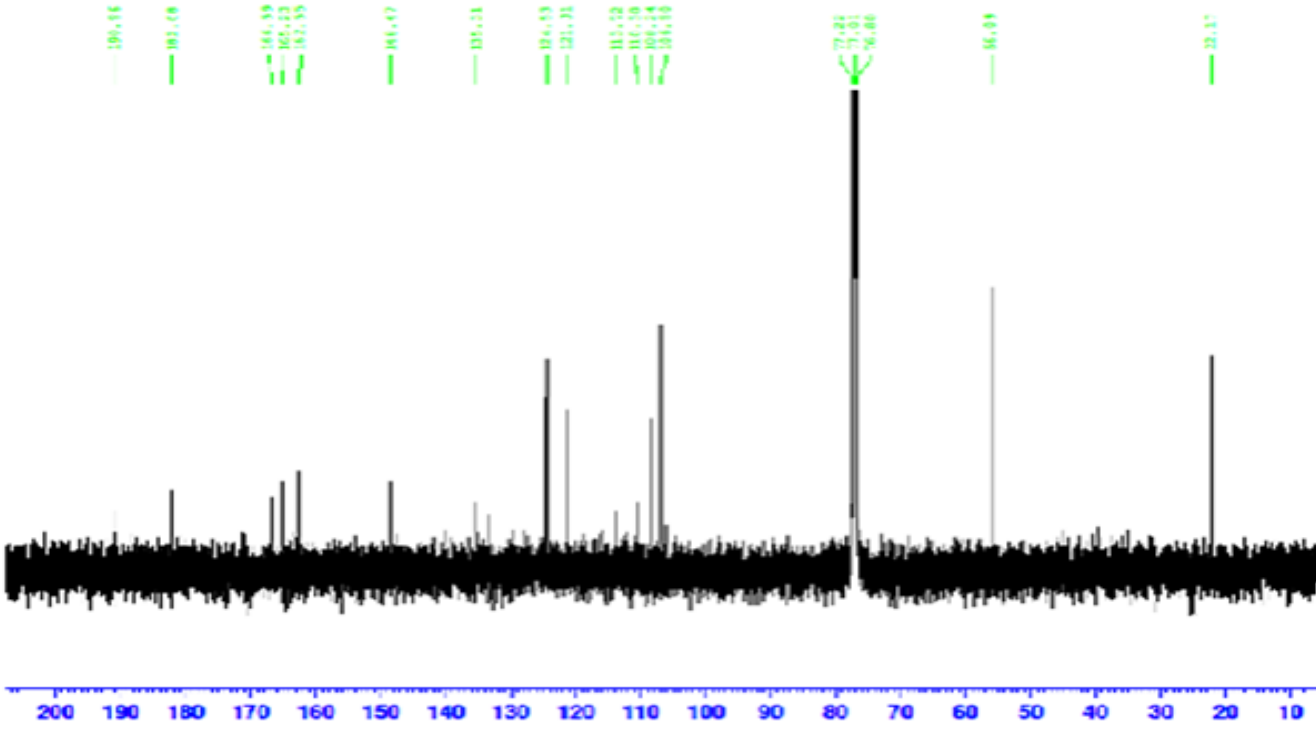
Appendix A2 ^{13}C NMR spectra for chrysophanol (**7**)



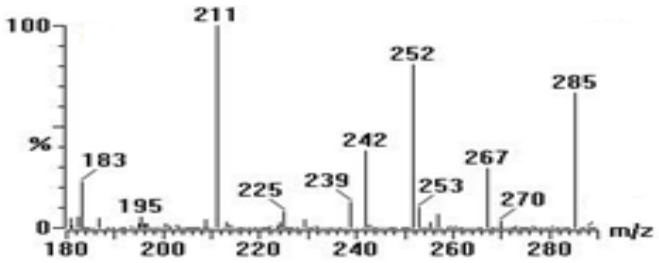
Appendix A3 Mass spectrum for chrysopanol **(7)**



Appendix B1 ^{1}H NMR spectra for physcion (**9**)



Appendix B2 ^{13}C NMR spectra for physcion (**9**)



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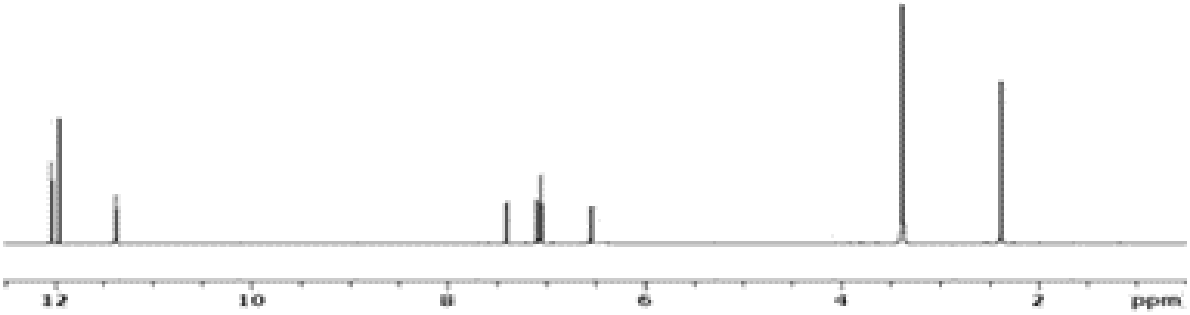
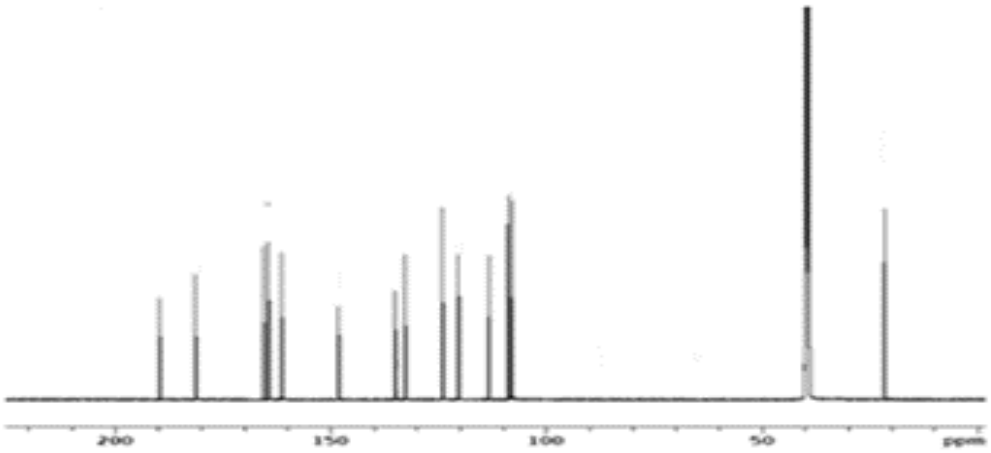
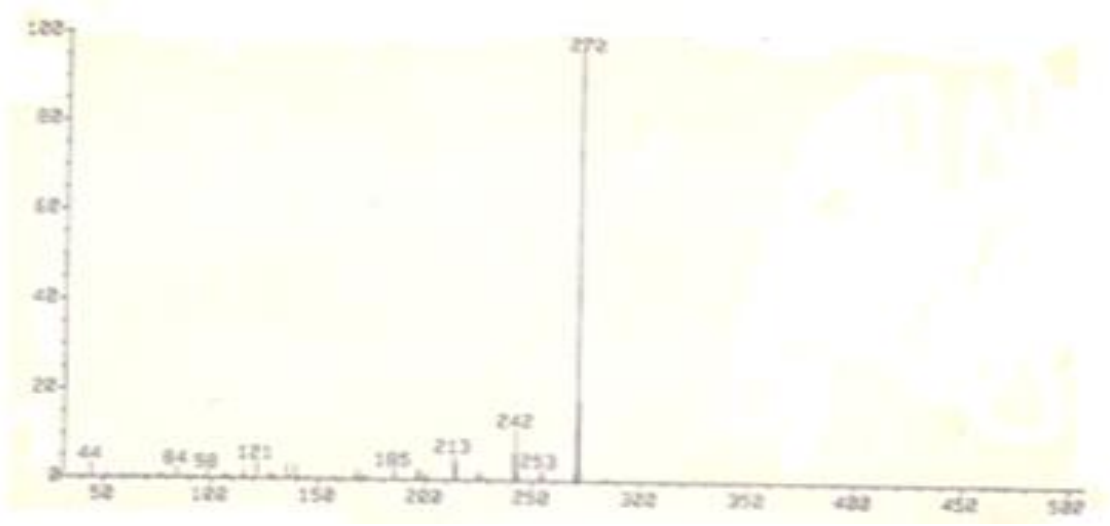


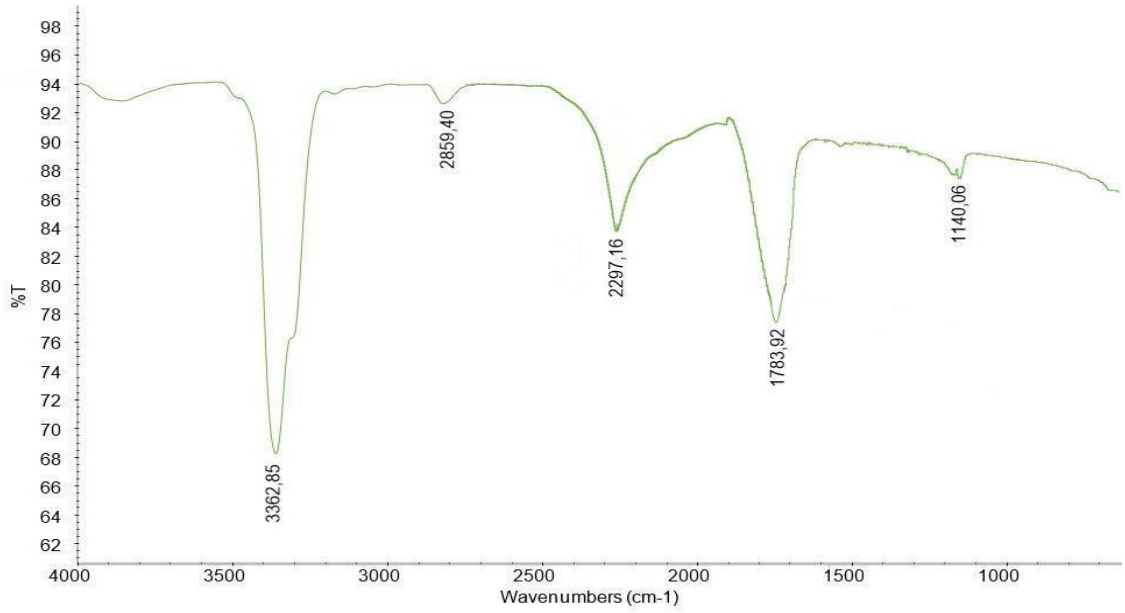
Appendix C1 ^{1}H NMR spectra for emodin (**10**)



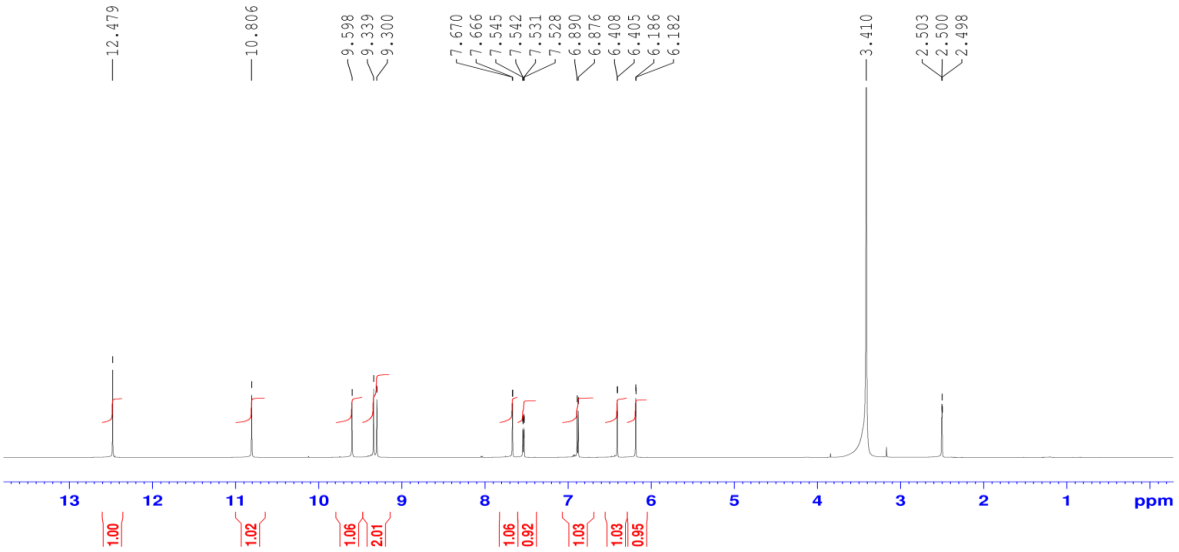
Appendix C2 ^{13}C NMR spectra for emodin (**10**)



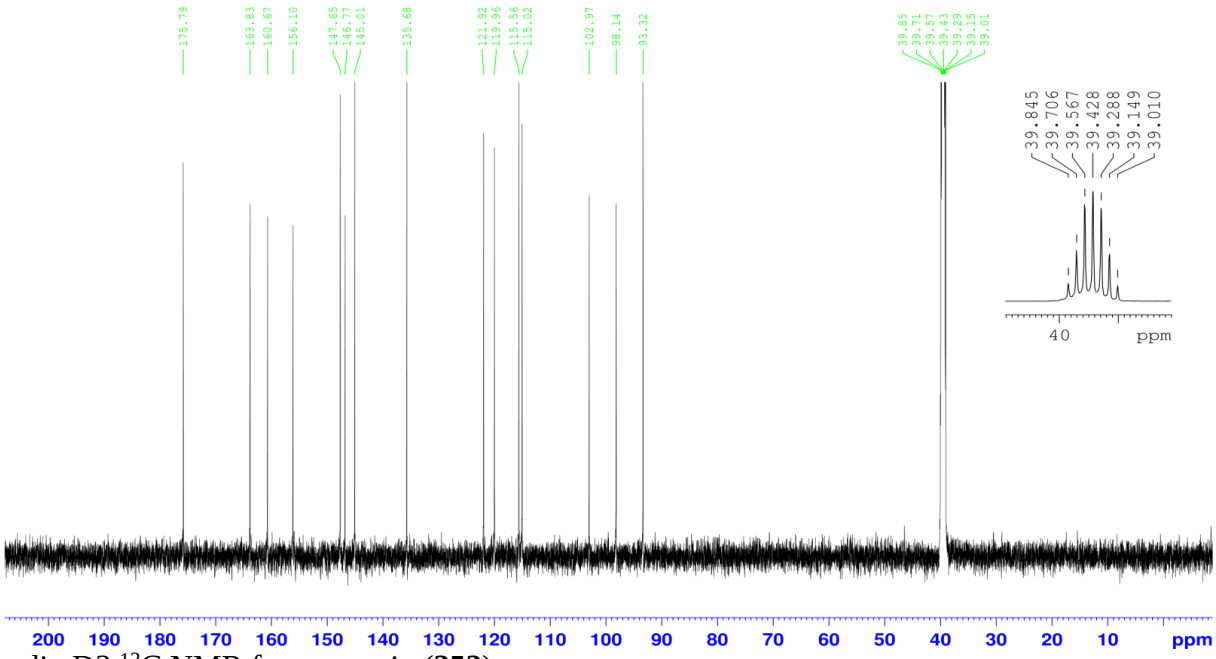
Appendix C3 Mass spectrum for emodin (**10**)



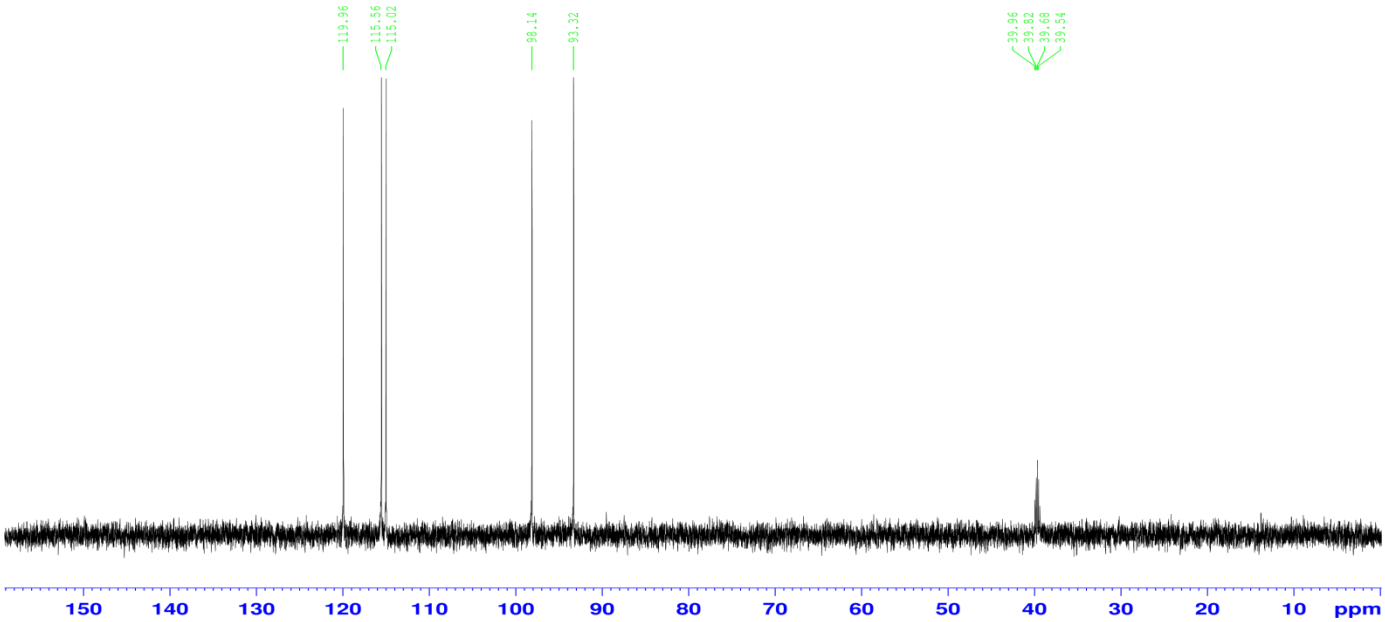
Appendix D1 IR Specrum for quercetin (**253**)



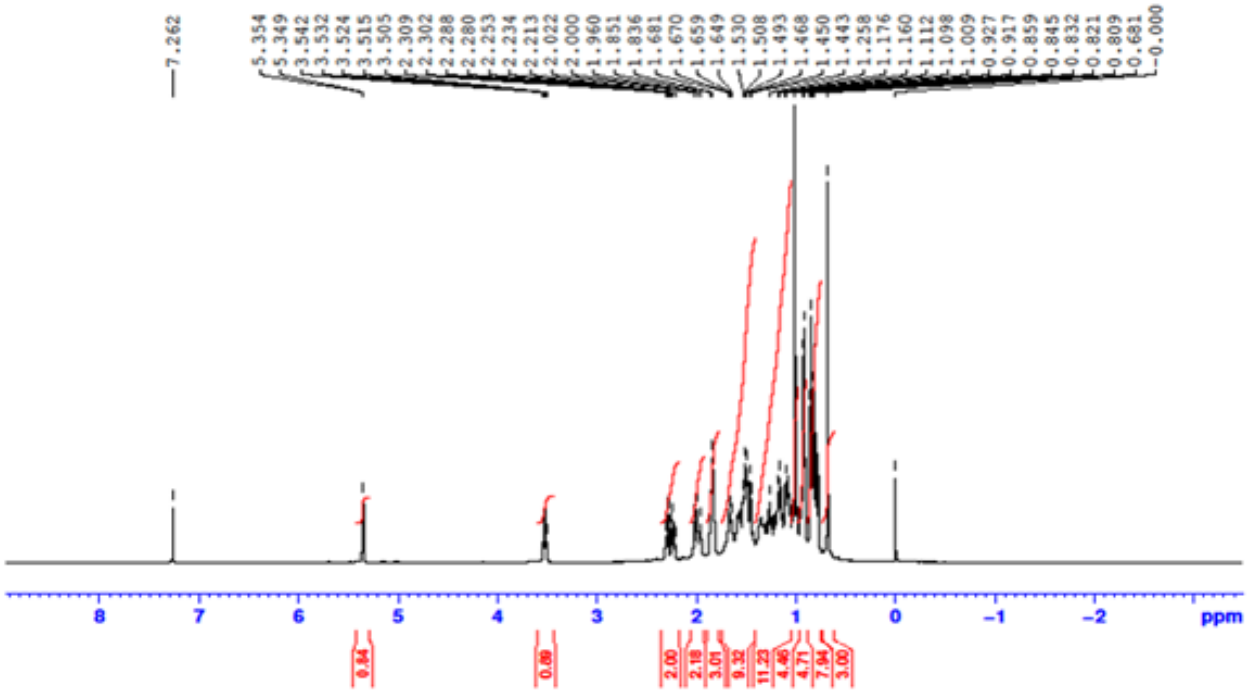
Appendix D2 ^{1}H NMR for quercetin (**253**)



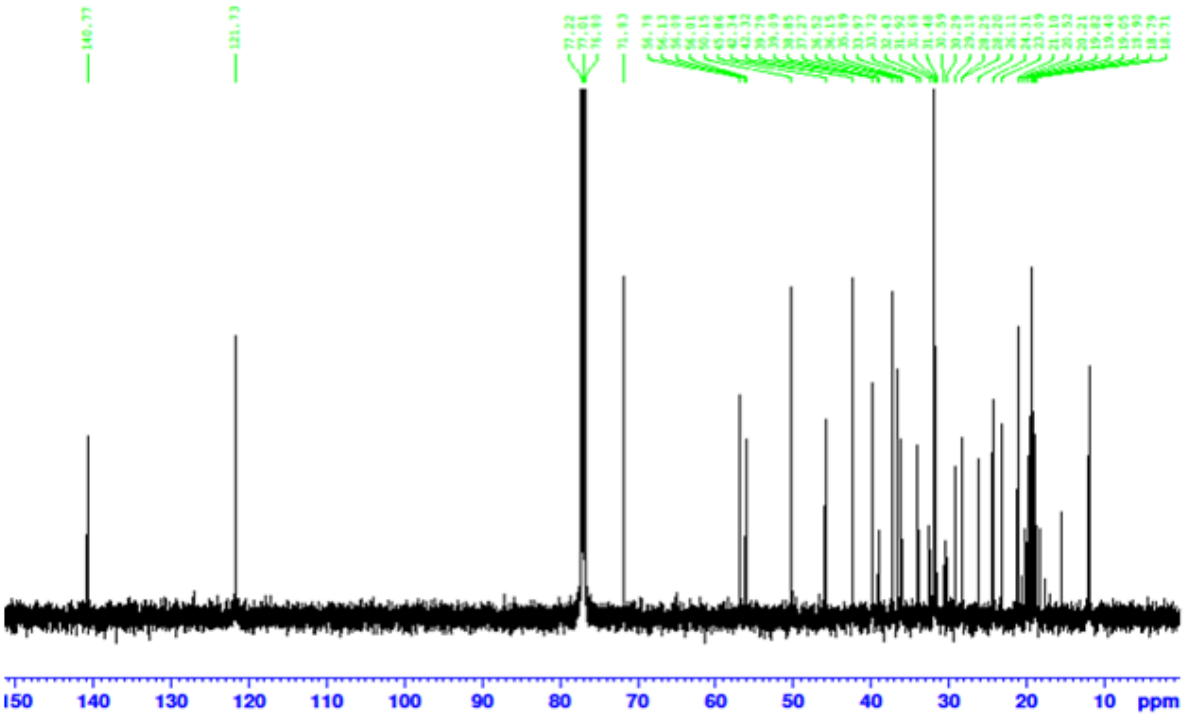
Appendix D3 ^{13}C NMR for quercetin (**253**)



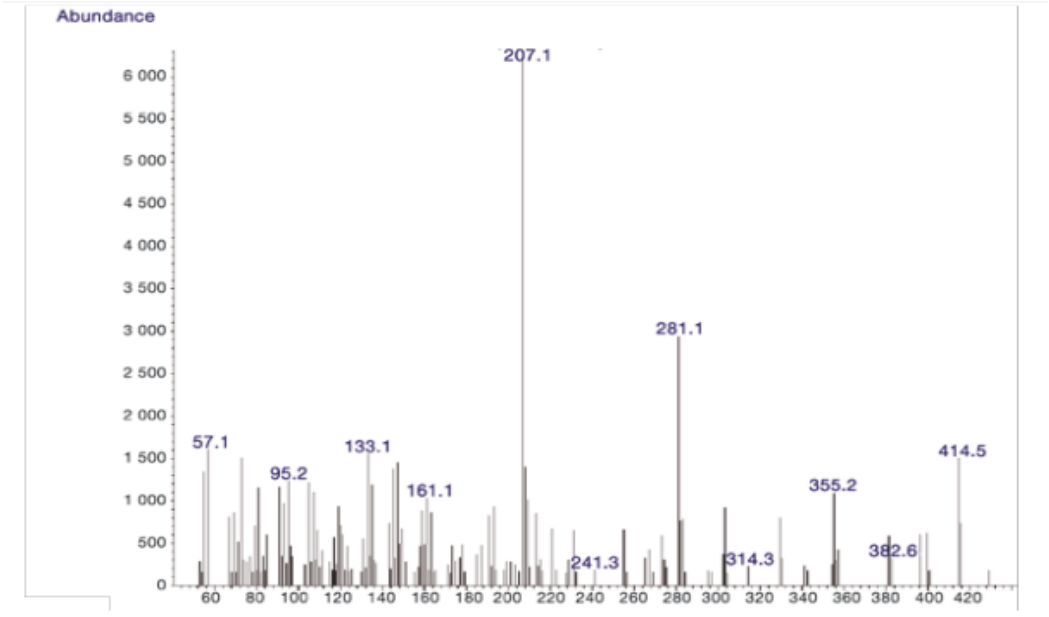
Appendix D4 DEPT 135 spectrum for quercetin (**253**)



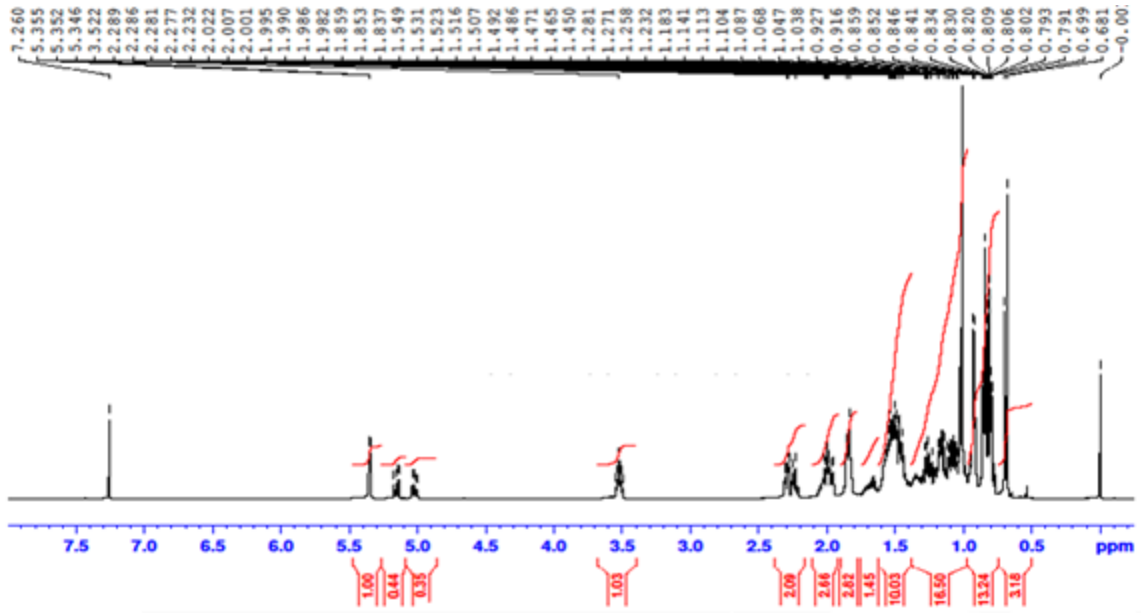
Appendix E1 ^{1}H NMR for *β*-sitosterol **(302)**



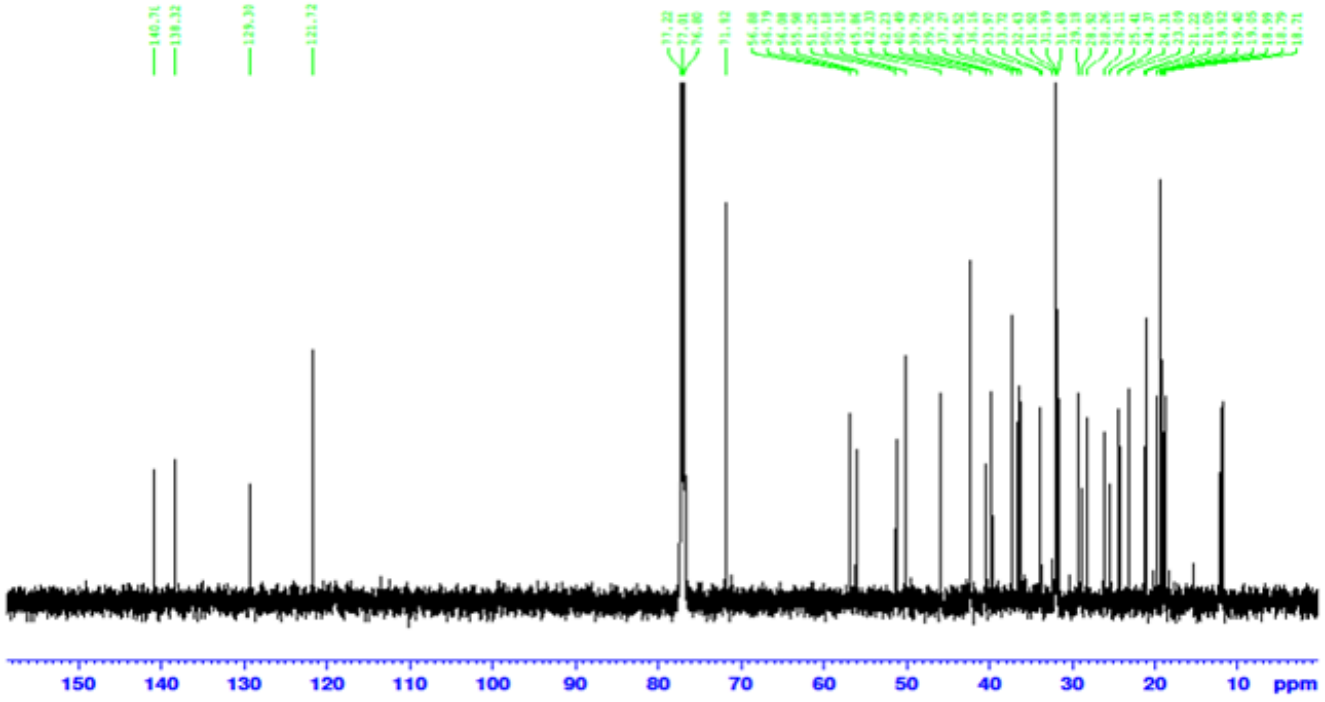
Appendix E2 ^{13}CNMR for *β*-sitosterol **(302)**



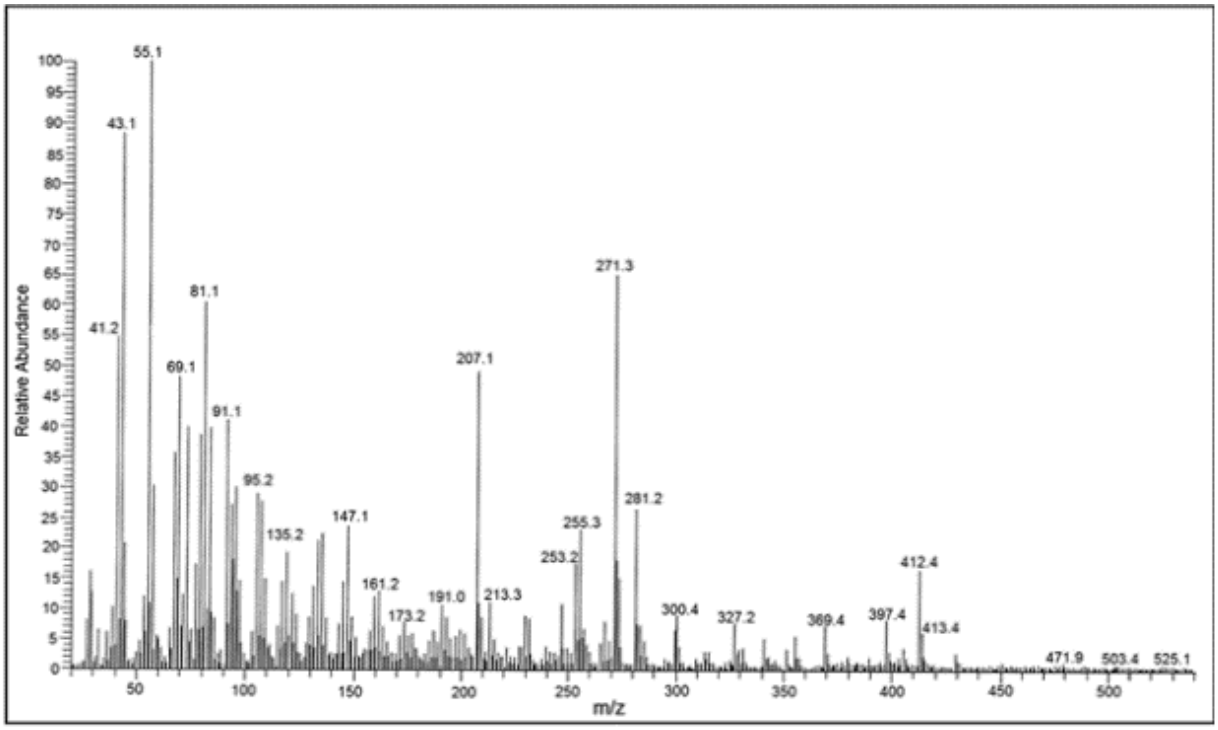
Appendix E3 Mass spectrum for *β*-sitosterol (**302**)



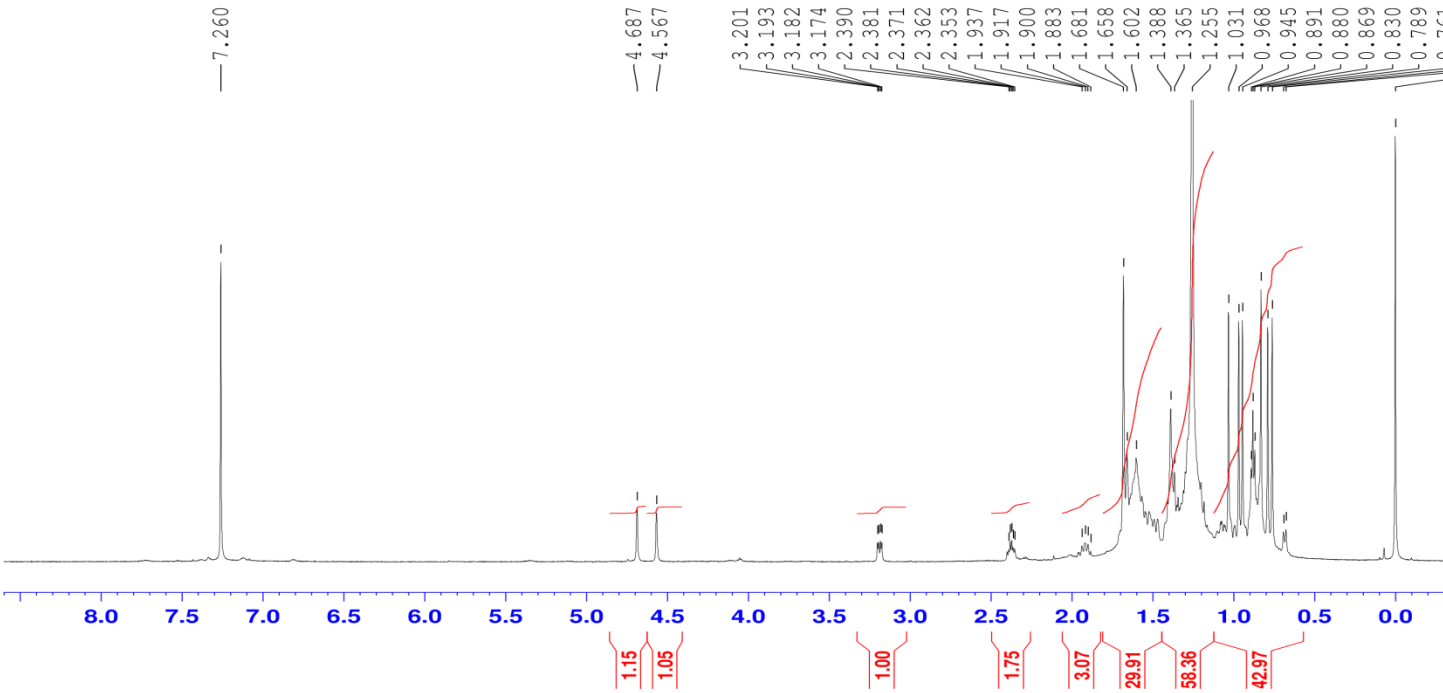
Appendix F1 ^{1}H NMR spectrum for stigmasterol (**303**)



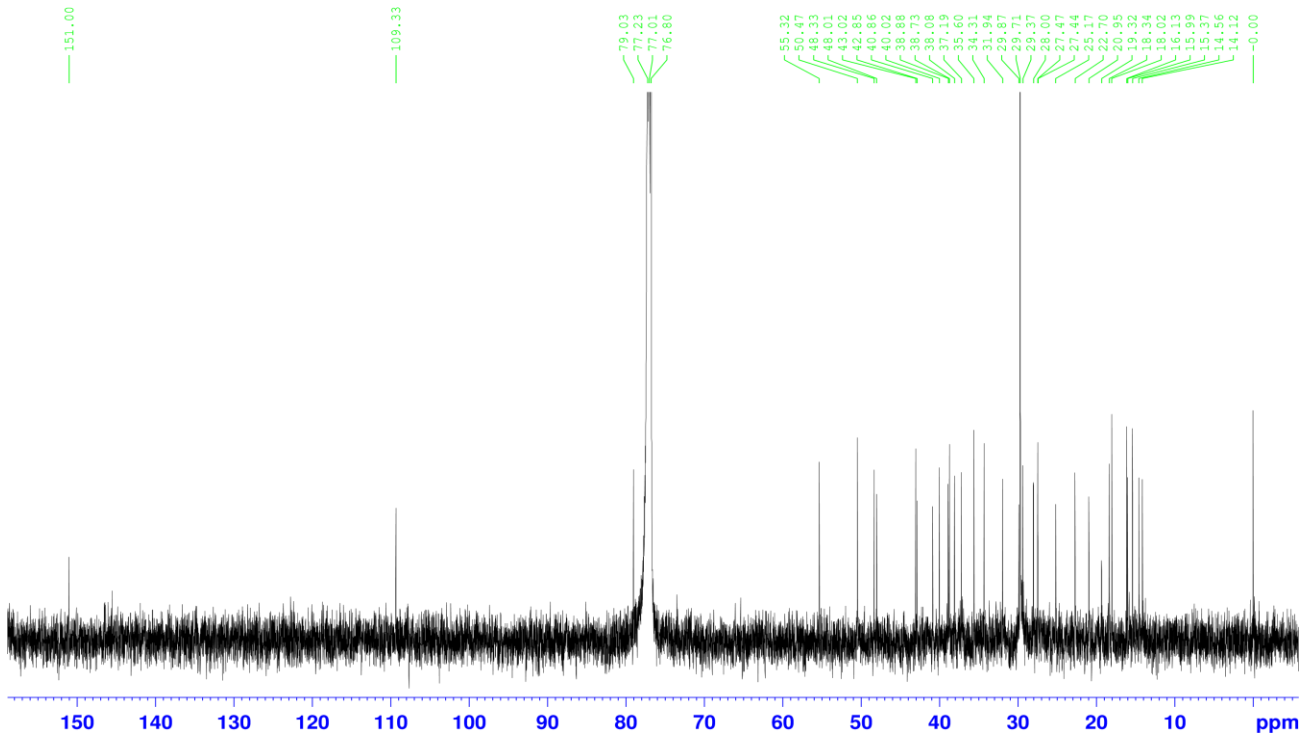
Appendix F2 ^{13}C NMR spectrum for stigmasterol (**303**)



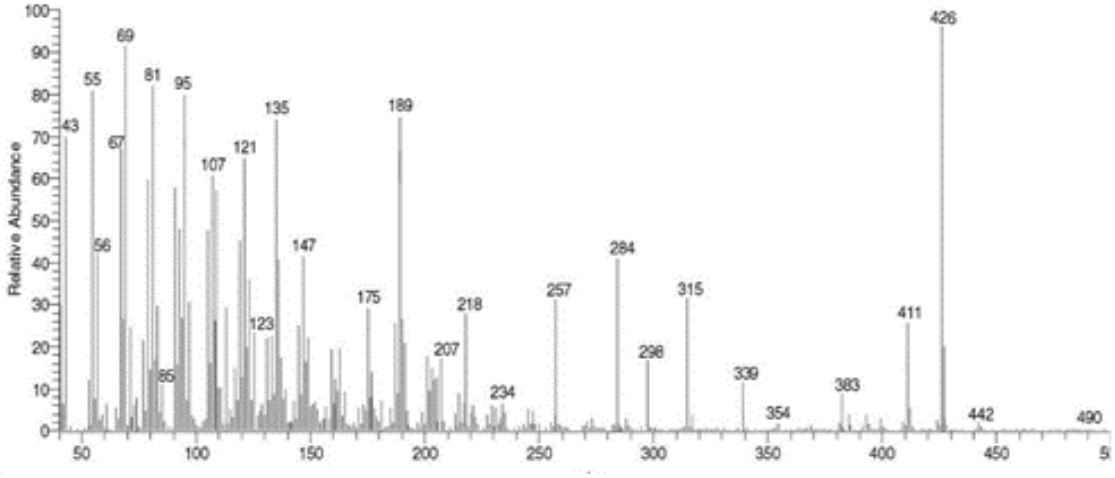
Appendix F3 Mass spectrum for stigmasterol (**303**)



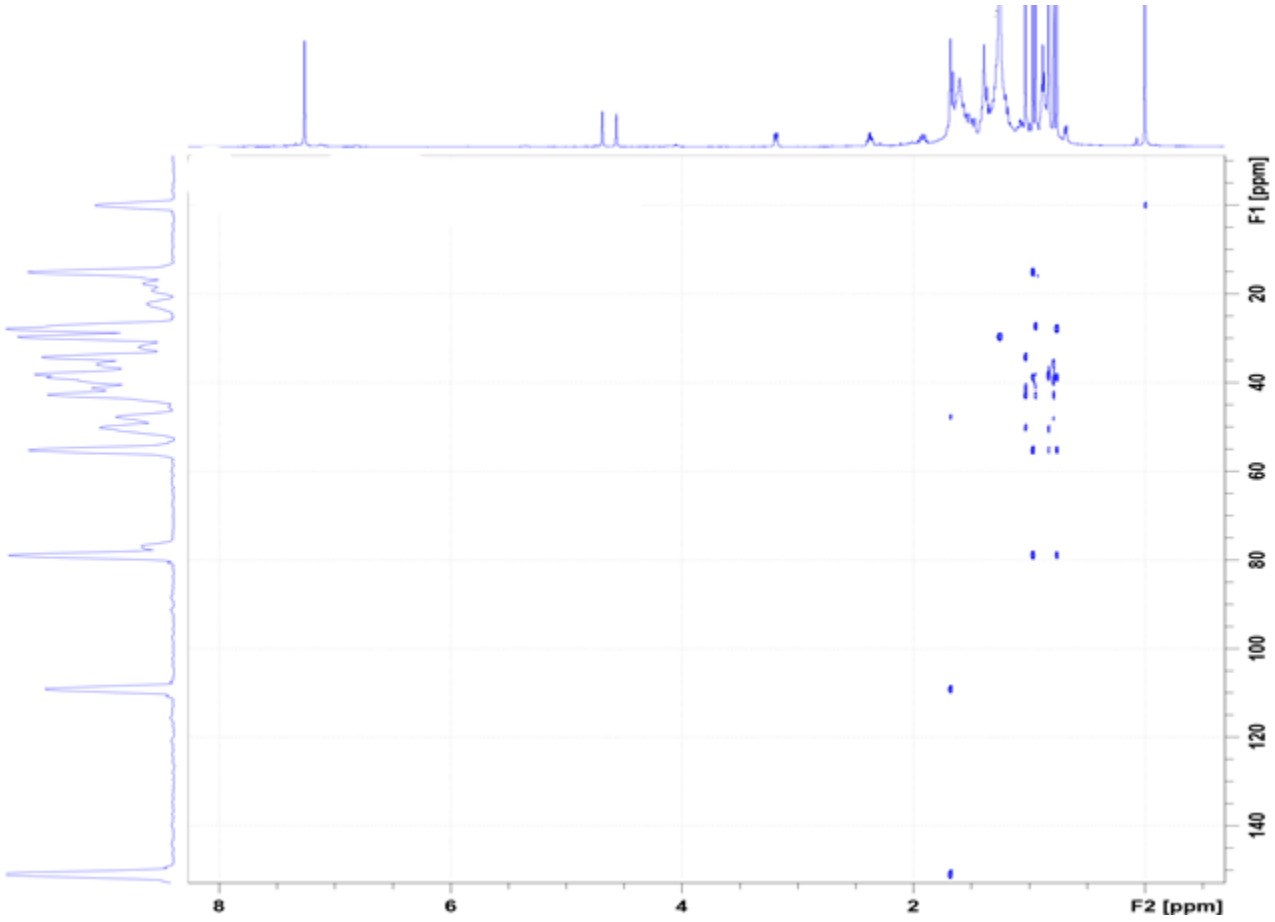
Appendix G1 ^{1}H NMR spectrum for lupeol (**323**)



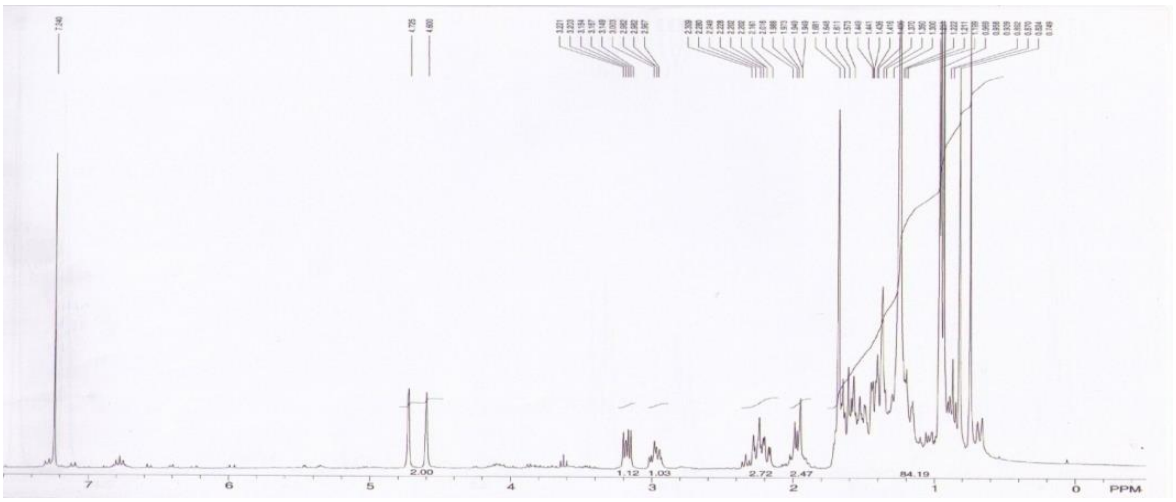
Appendix G2 ^{13}C NMR spectrum for lupeol (**323**)



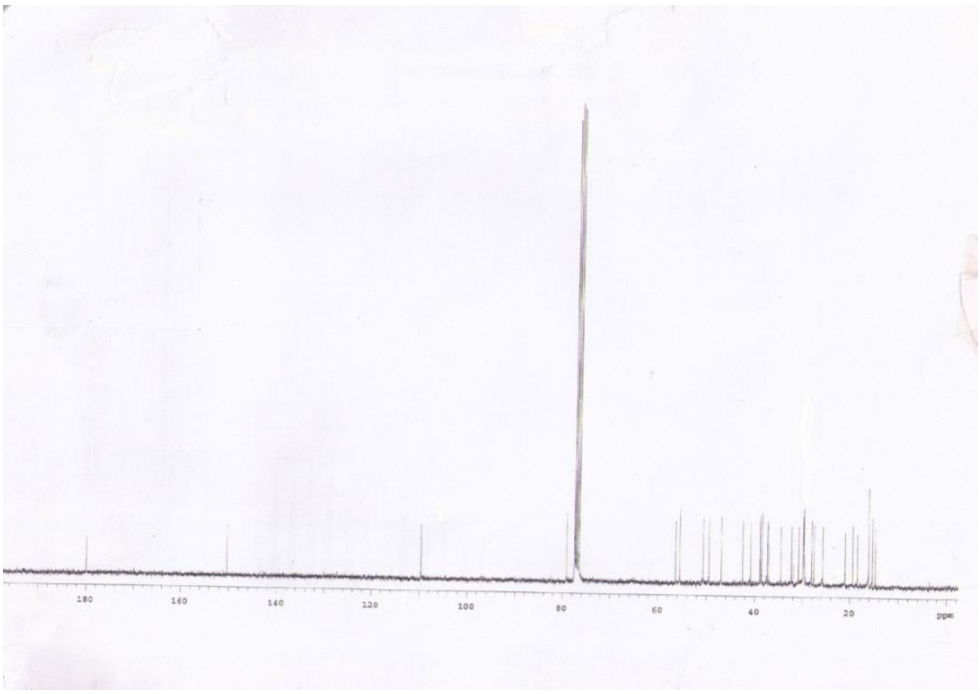
Appendix G3 Mass spectrum for lupeol (**323)**



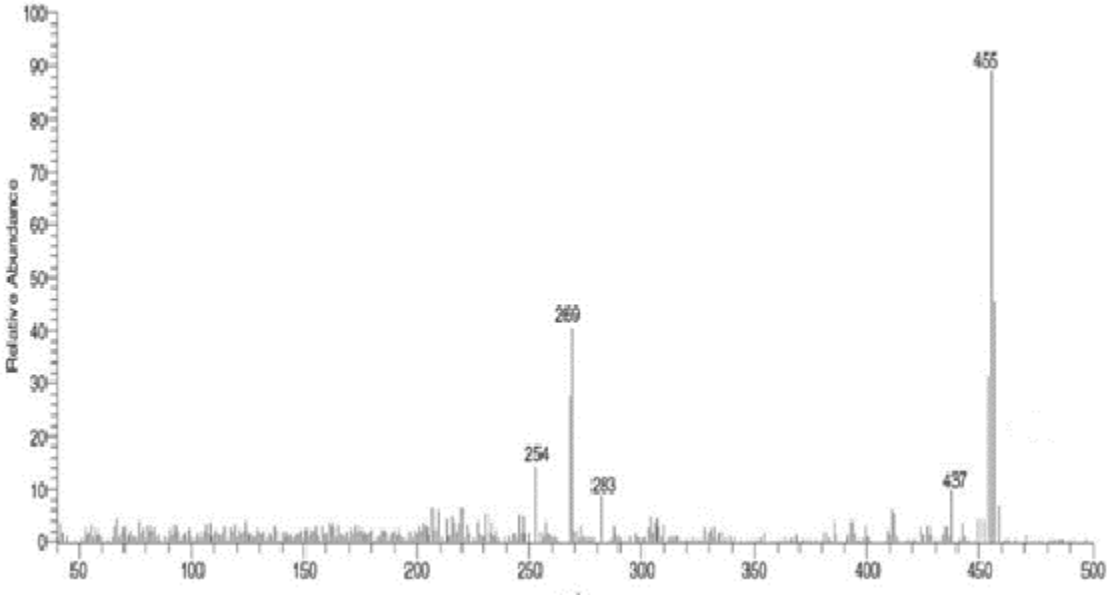
Appendix G4 HMBC for lupeol (**323**)



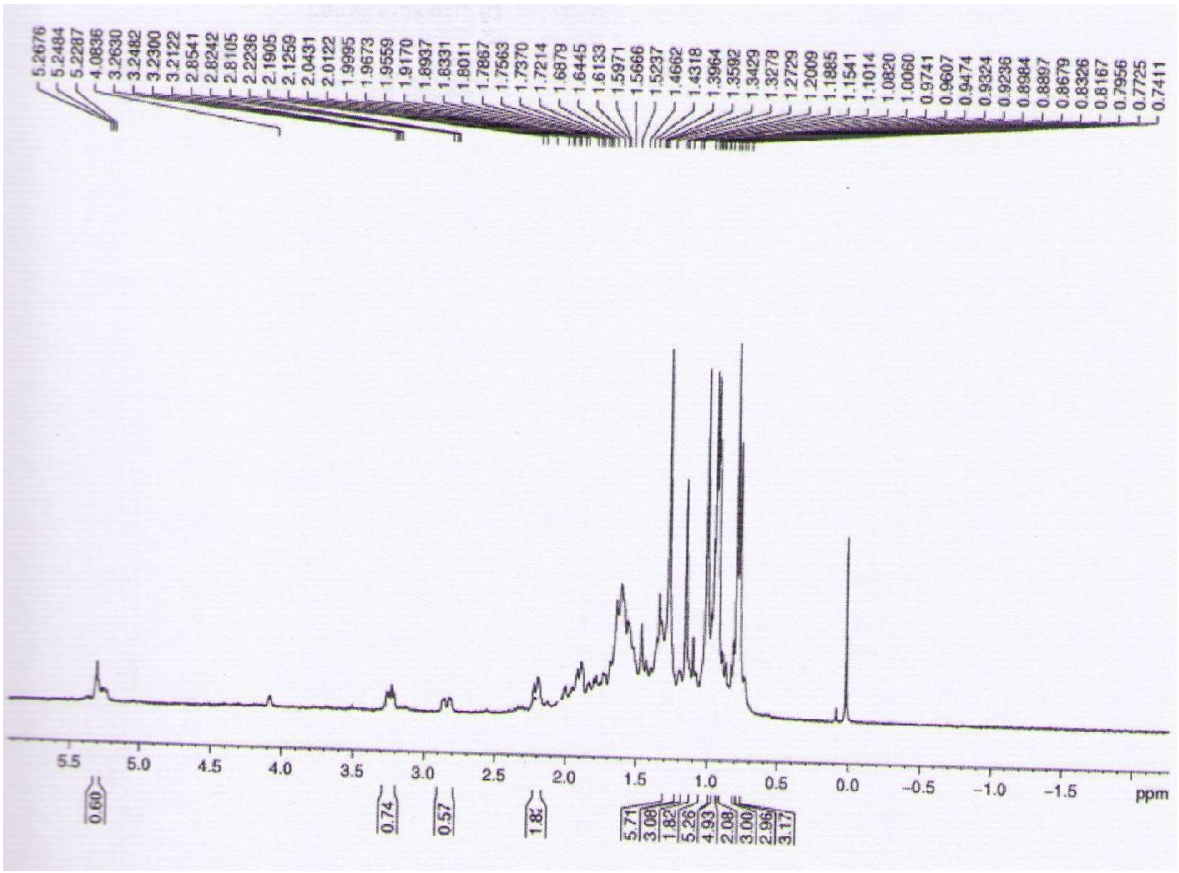
Appendix H1 ^{1}H NMR for betulinic acid (**324**)



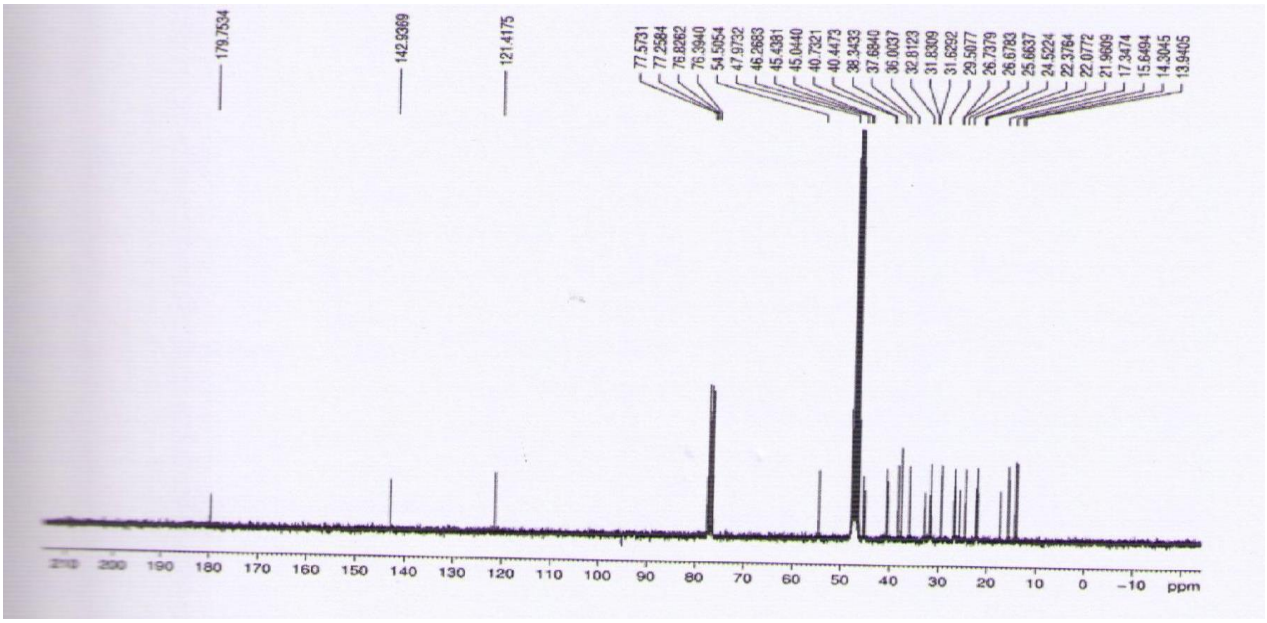
Appendix H2 13C NMR spectrum for betulinic (**324**)



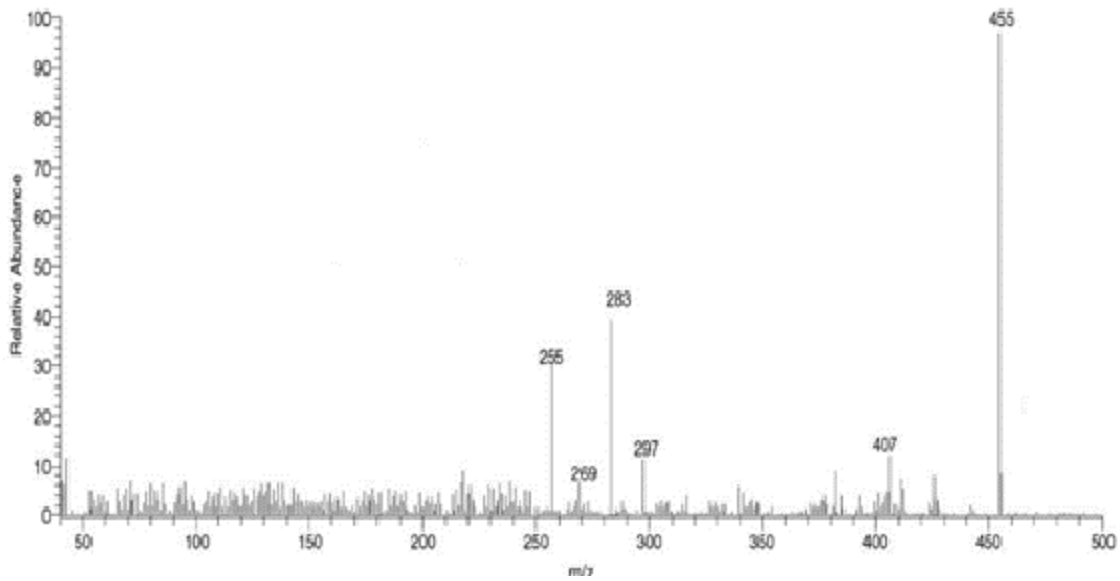
Appendix H3 Mass spectrum for betulinic acid (**324**)



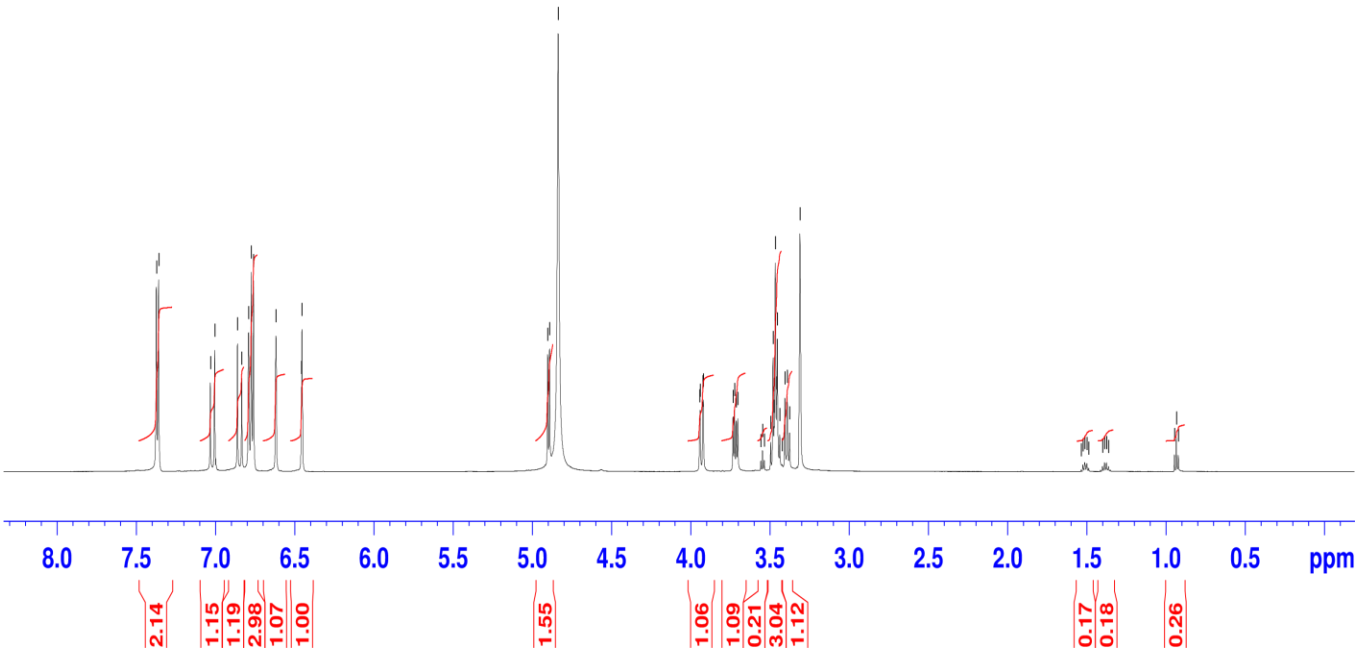
Appendix I 1 ^{1}H NMR for oleanolic acid (**335**)



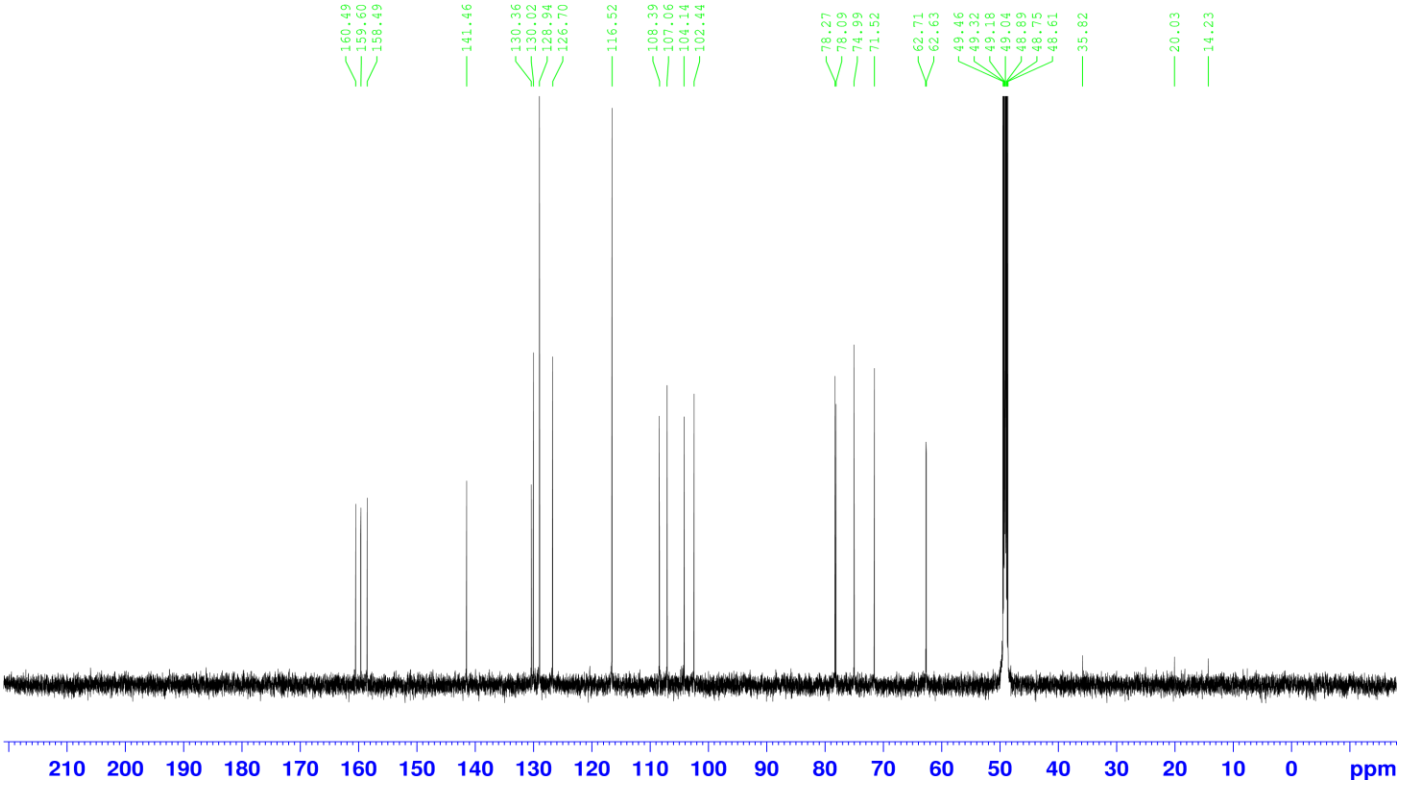
Appendix I 2 ^{13}C NMR for oleanolic acid (**335**)



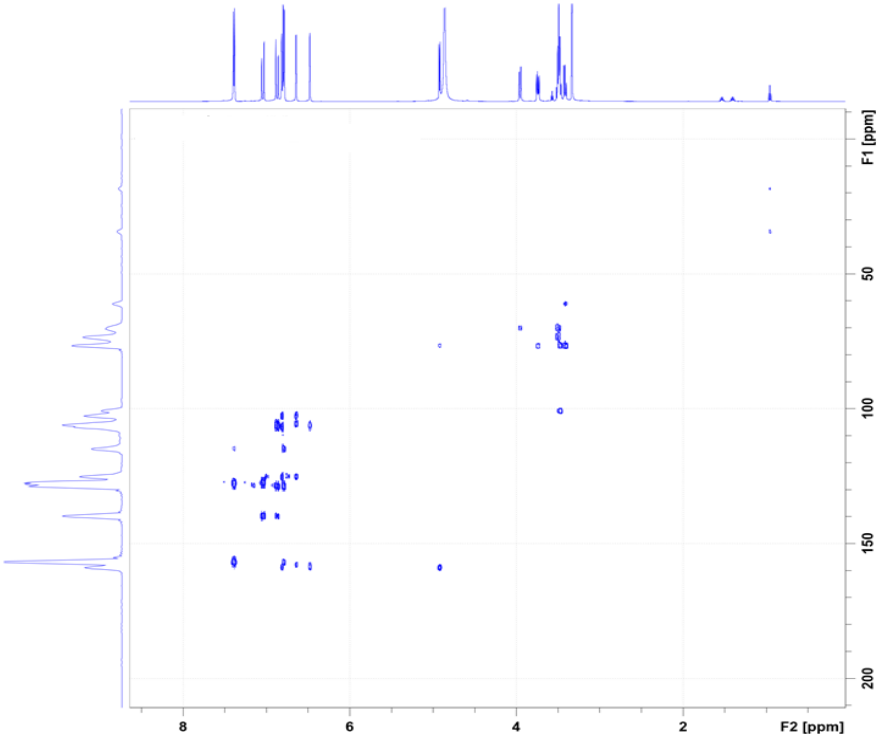
Appendix I 3 Mass spectrum for oleanolic acid (**335**)



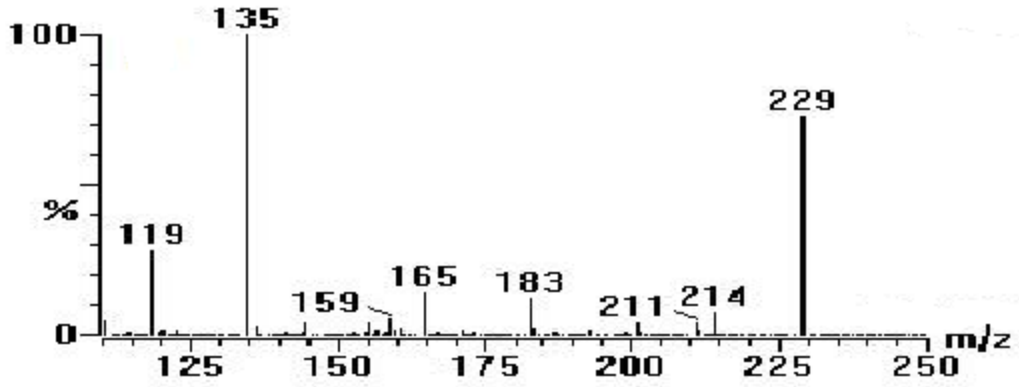
Appendix J1 ^{1}H NMR for 4`,5-dihydroxystilbene (**336**)



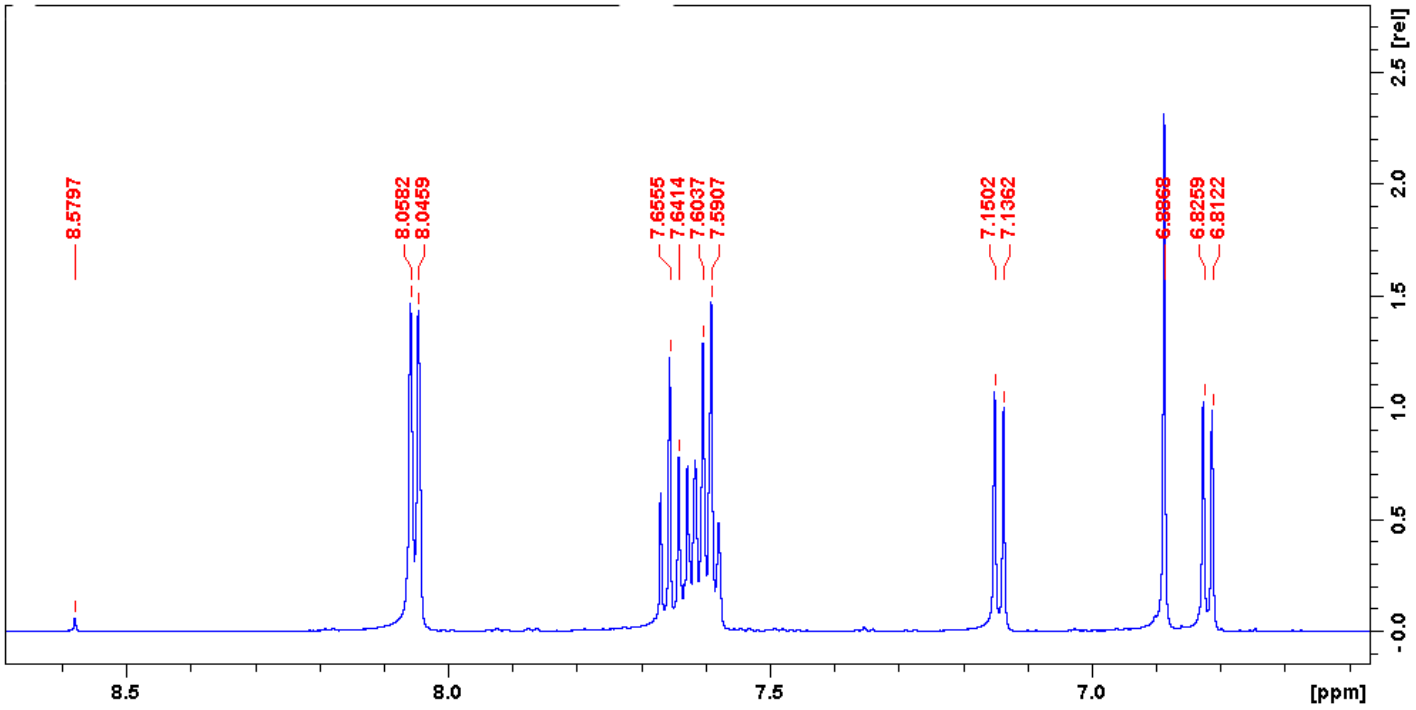
Appendix J2 ^{13}C NMR for 4`,5-dihydroxystilbene (**336**)



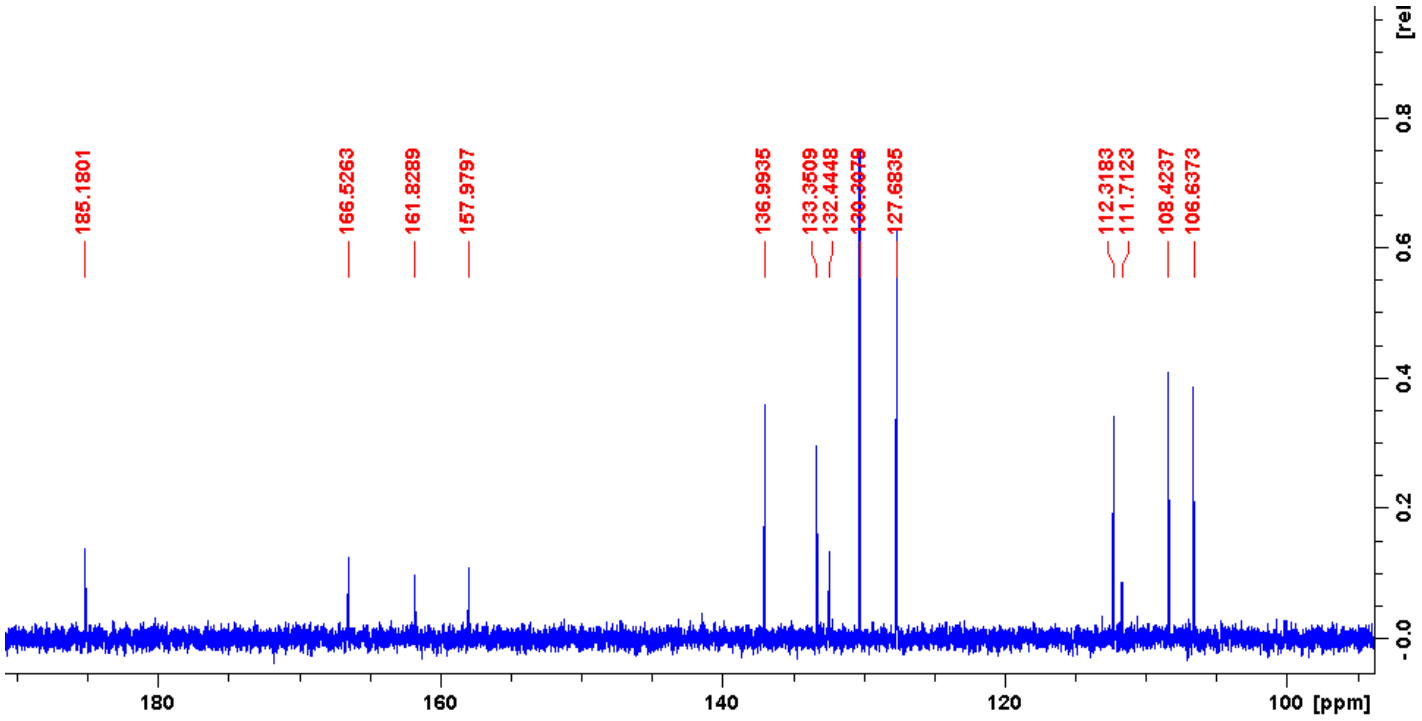
Appendix J3 HMBC spectrum for 4`,5-dihydroxystilbene-3-*O*-glucoside (**336**)



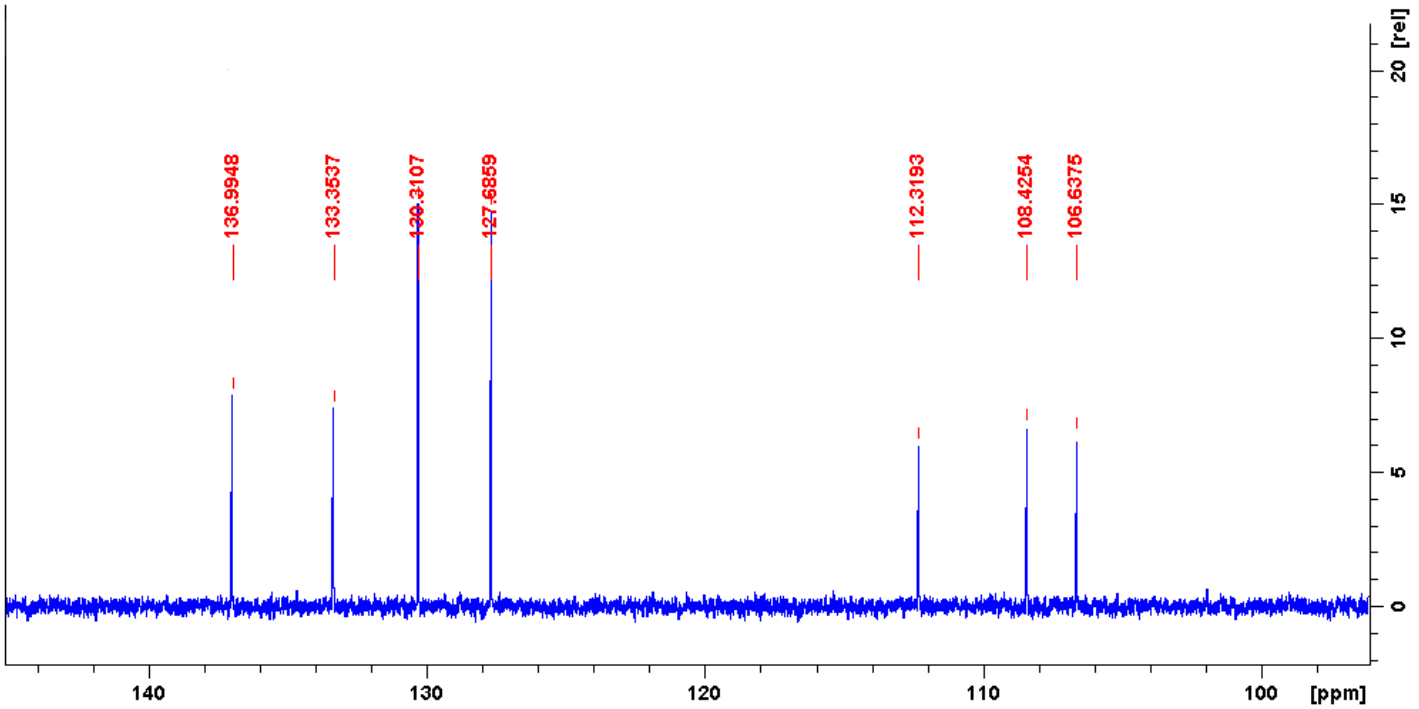
Appendix J4 Mass spectrum for 4`,5-dihydroxystilbene (**336**)



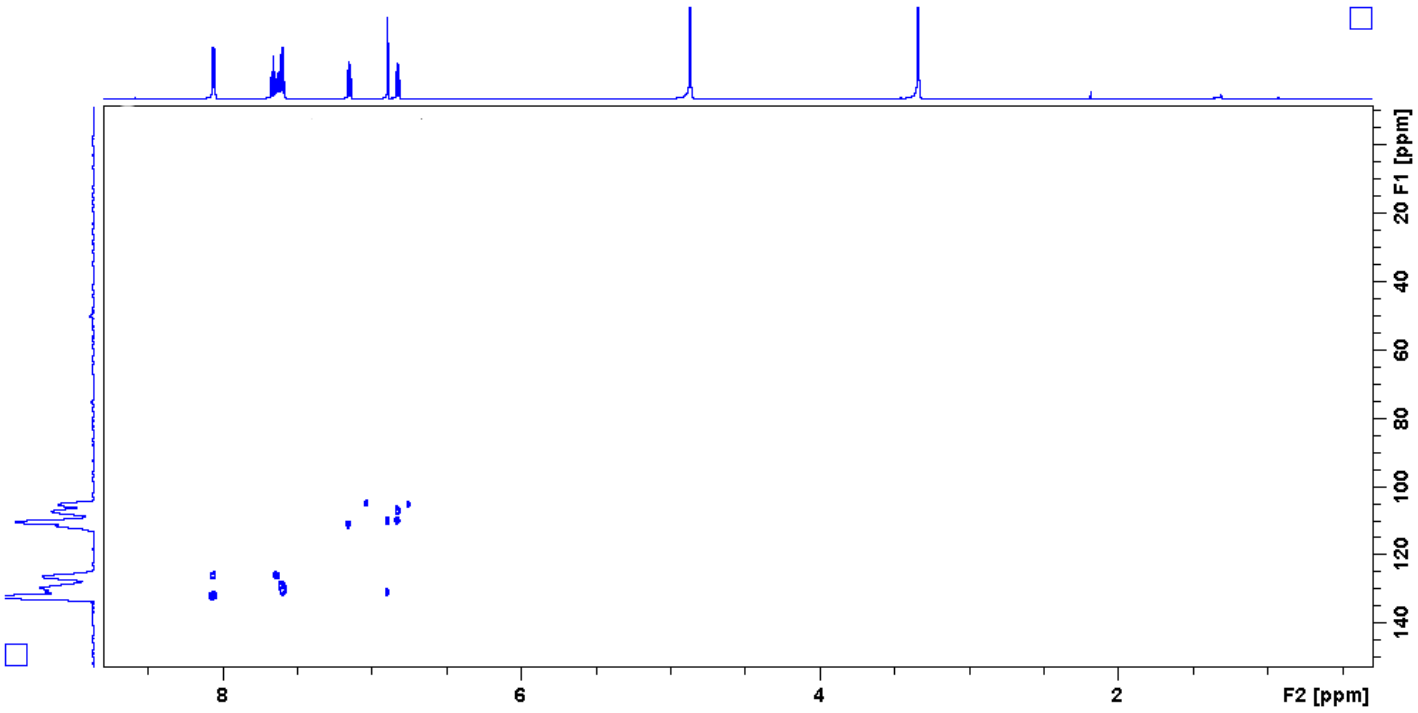
Appendix K1 ^{1}H NMR for 5-hydroxyflavone (**337**)



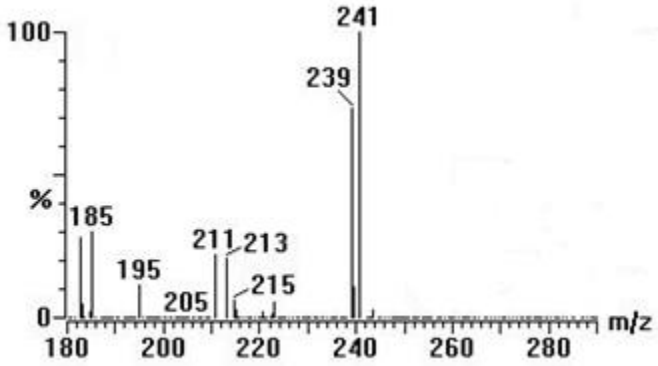
Appendix K2 ^{13}C NMR for 5-hydroxyflavone (**337**)



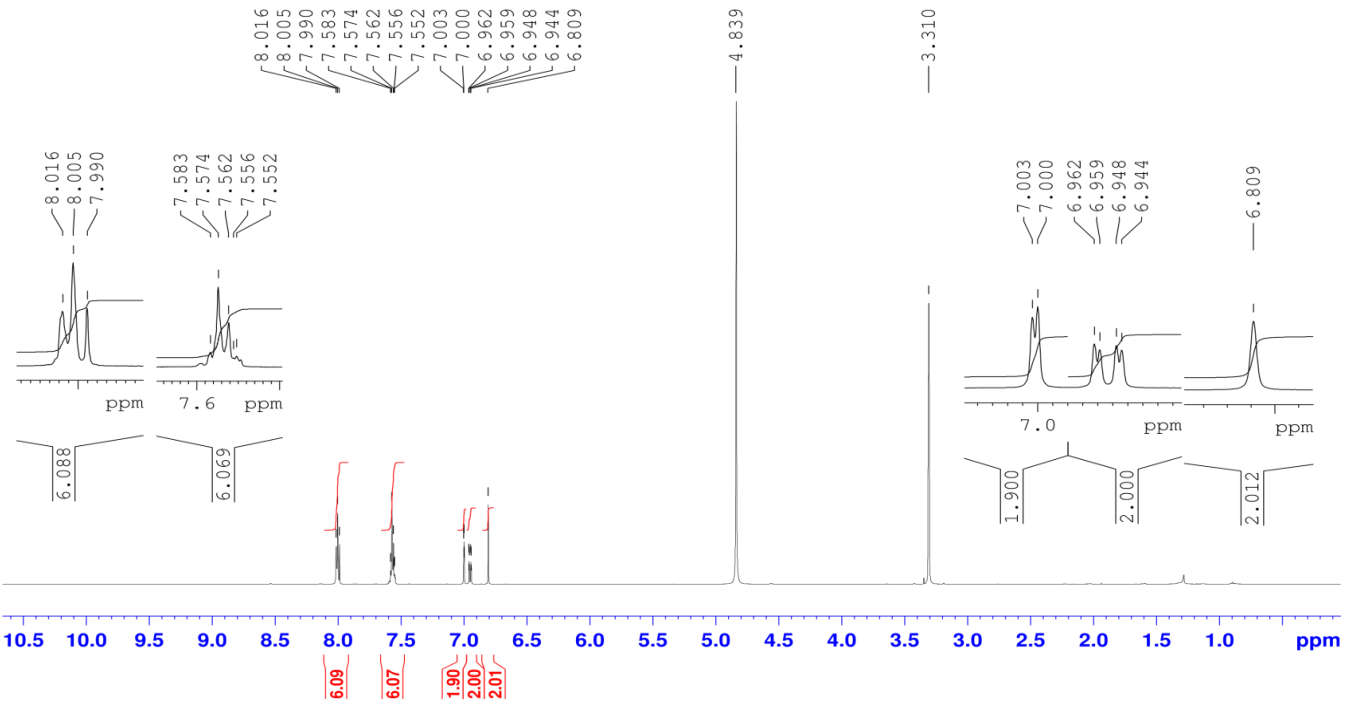
Appendix K3 DEPT 135 for 5-hydroxyflavone (**337**)



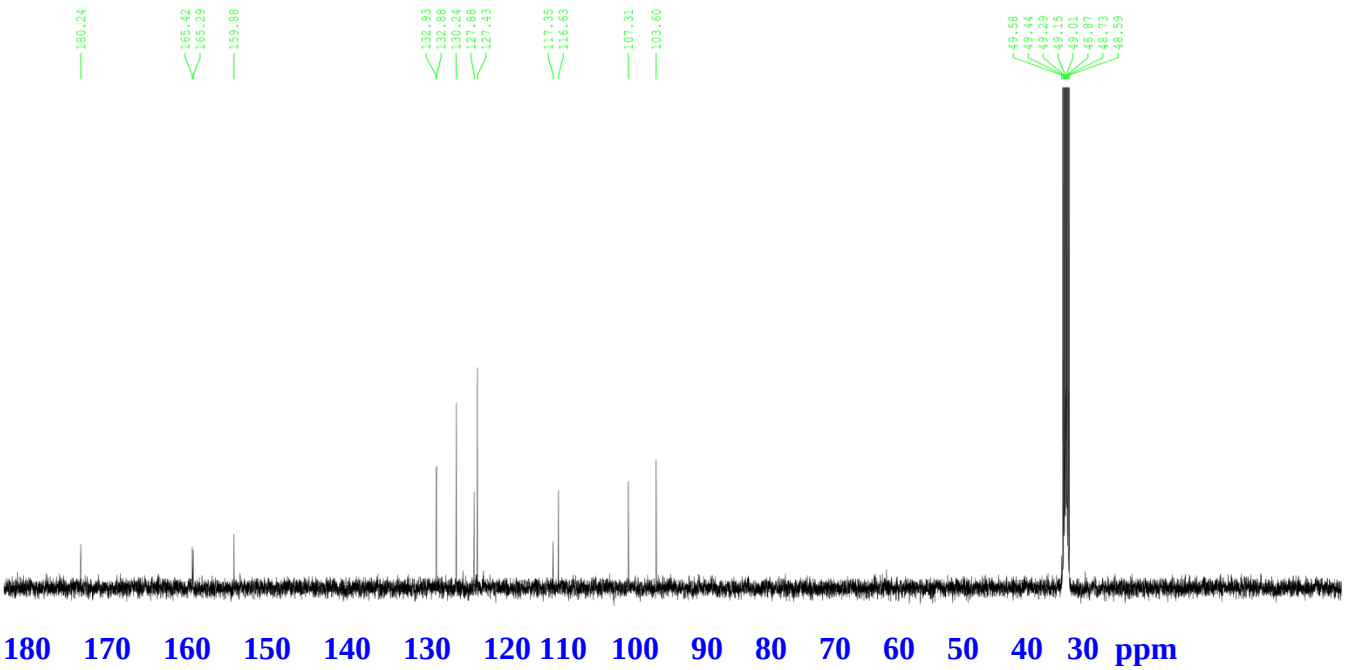
Appendix K4 HMBC spectrum for 5-hydroxyflavone (**337**)



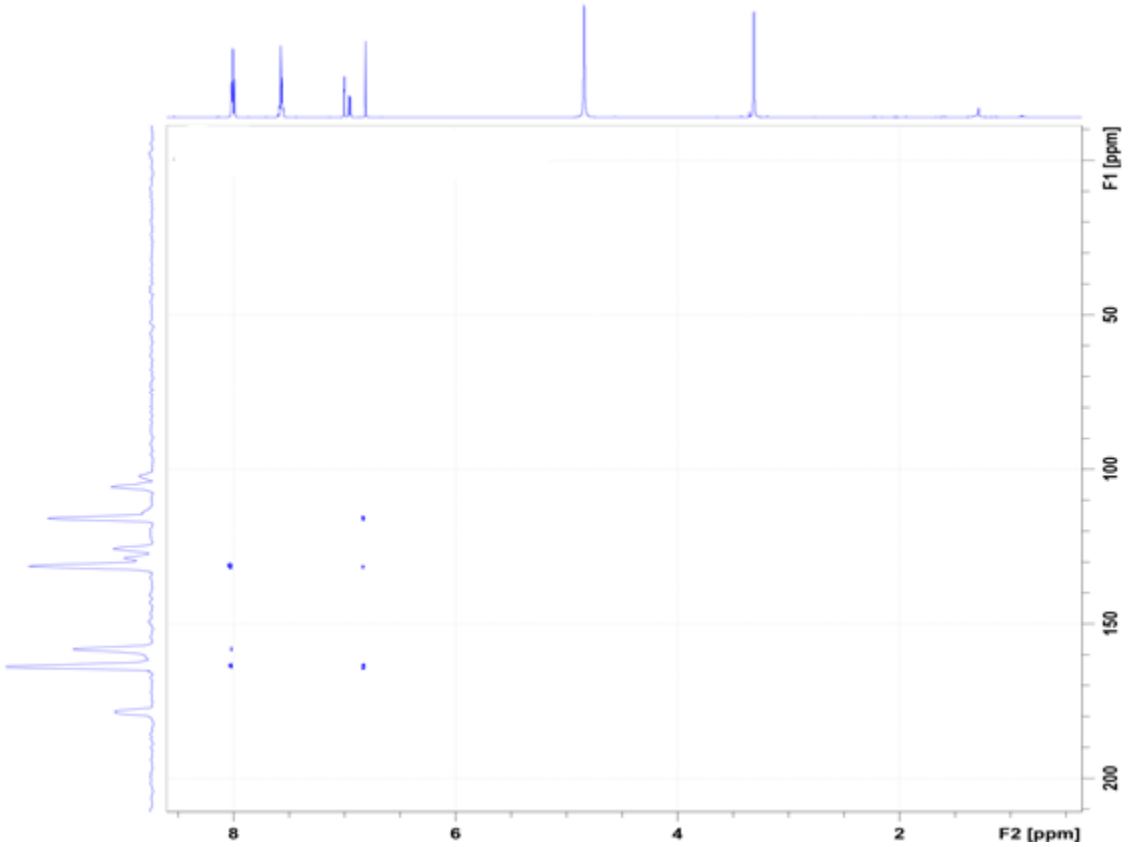
Appendix K5 Mass spectrum for 5-hydroxyflavone (**337**)



Appendix L1 ^{1}H NMR for 7-hydroxyflavone (**338**)



Appendix L2 ^{13}C NMR for 7-hydroxyflavone (**338**).



Appendix L4 HMBC spectrum for 7-hydroxyflavone (**338**)