PHYTOCHEMICAL AND BIOLOGICAL INVESTIGATION OF *OCHRADENUS ARABICUS* CHAUDHARY, HILLC. & A. G. MILL.

By

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DEPARTMENT OF BIOTECHNOLOGY

FACULTY OF BIOLOGICAL SCIENCES

QUAID-I-AZAM UNIVERSITY

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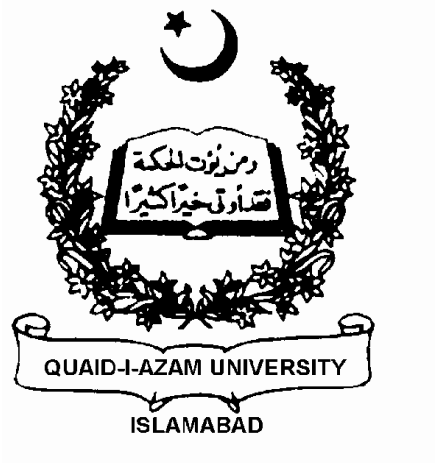
PHYTOCHEMICAL AND BIOLOGICAL INVESTIGATION OF *OCHRADENUS ARABICUS* CHAUDHARY, HILLC. & A. G. MILL.

A thesis submitted in the partial fulfillment of the requirements for the

Degree of Master of Philosophy

In

Biotechnology



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In the name of Allah, the most gracious, the most compassionate

CERTIFICATE

This thesis submitted by **Riaz Ahmad** is accepted in its present form by the Department of Biotechnology, Faculty of Biological Sciences, Quaid-i-Azam University, Islamabad as satisfying the thesis requirements for the degree of Master of Philosophy (M. Phil.) in Biotechnology.

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Dated: 29-12-2014

Declaration of Originality

I hereby declare that the work accomplished in this thesis is the result of my own research carried out in the Molecular Systematics and Applied Ethnobotany Laboratory, Department of Biotechnology, Quaid-i-Azam University, Islamabad and UoN chair of Oman's Medicinal Plants and Marine Natural Porducts, University of Nizwa, Sultanate of Oman. This thesis has not been published previously nor does it contain any material from the published resources that can be considered as the violation of international copy right law. Furthermore, I also declare that I am aware of the terms "copy right" and "plagiarism". If any copy right violation is found in this research work I will be responsible for the consequences of any such violation.

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Name: Riaz Ahmad

Date: 29 Dec, 2014

Dedicated to my

Beloved Parents

And Respected Teachers who are the Nation Builders

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Riaz Ahmad

Abbreviations

Abbreviated name	Abbreviation
Hex	**Hexane**
EtOAc	**Ethyl Acetate**
MeOH	**Methanol**
CHCL3	**Chloroform**
H2O	**Water**
NCEs	**New Chemical Entities**
Mg	**Milligram**
Ng	**Nanogram**
Ml	**Milliliter**
µl	**Microlitre**
M	**Molar**
G	**Gram**
cm	**Centimeter**
mM	**Millimolar**
pH	**Power of Hydrogen ion**
°C	**Centigrade**
NaCl	**Sodium chloride**
$MgCl_2$	**Magnesium chloride**
HCl	**Hydrochloric Acid**
EDTA	**Ethylene diamine tetra acetic acid**
TAE	**Tris Acetate EDTA**
TE	**Tetra Ethylene**
UV	**Ultra Violet**
MoSEL	**Molecular systematics and Applied Ethnobotany lab**

Abstract

Plants are natural factories continuously producing valuable compounds in the most cost efficient way. The term of medicinal plants include a variety of plants used in herbalism and some of these plants have medicinal activities. These medicinal plants considered as rich source of bioactive ingredients can be, used in drug development and synthesis. Numerous approaches have been used to obtain compounds for drug discovery including isolation from plants and other natural sources.

This research project is mainly concerned with the isolation, characterization of bioactive chemical constituents and Biological Evaluation of medicinally important plant *Ochradenus arabicus*. The current research and experimental work carried out at University of Nizwa, Sultanate of Oman and Quaid-i-Azam University, Islamabad, Pakistan.

The plant was collected from Jabal Al-Akhdar, is one of the diverse floral region of Sultanate of Oman. The whole plant of *Ochradenus arabicus* was shade dried and ground into powder. The collected plant materials were extracted with methanol and different fractions of ethyl acetate, hexane, chloroform, butanol and water were made. These fractions in various concentrations were subjected to different biological assays including phenolic, flavonoid, antioxidant, allophatic, alpha-glucosidase inhibitor and anticancer. The fractions ethyl acetate, butanol and water showed anticancer activity, reducing viability of cancer cell (MCF-7, HepG2, HT29, HCT116) lines. In alpha glucosidase inhibition assay only butanol fraction was found little active and showed 12% activity. The ethyl acetate fraction was significantly higher in phenolic and flavonoid contents, which also showed higher antioxidant activity as compared to other fractions. The aqueous fraction exhibited higher phytotoxic activity prevented the germination of lettuce seeds.

The chloroform fraction (85.5g) was selected and then chromatographed over a silica gel column, which results in five known compounds for the first time from *O. arabicus*; 1-Octacosanol, β-sitosterol, β -sitosterol-β-D-glycoside, Ursolic acid and Choerospondin. The structure of these isolated compounds was elucidated using different spectroscopic techniques like NMR, UV, IR, Mass Spectrometry (EI-MS) etc. Our results shows that *O. arabicus* has the potential to discover the potent anticancer agents. Further fractionation of the bioactive fraction can help us to find the lead anticancer compound. Phytochemicals isolated from medicinal plants have been playing a significant role directly or indirectly to combat cancer disorders.

INTRODUCTION

1. Background

The practice of medicinal plants for health care started thousands years ago as traditional medicines and still commonly use in Pakistan, China, Egypt, India and other developing countries. Modern medicines comprise at least 25% of drugs derived from natural resources. On one hand plants contribute in food, fiber & shelter and on the other hand they provide a wide range of secondary metabolites called phytochemicals. These compounds have significant uses in drugs discovery (Cowan, 1999; Lemos *et al.,* 1990).

Plants are natural factories continuously producing valuable compounds in the most cost efficient way. Since the mid of the nineteenth century, several plant based bioactive chemicals have been isolated & structurally elucidated by the scientific and technological approaches. Most of these phytochemical are being practiced as active constituents of the modern drugs to cure a diversity of diseases (Bussmann & Sharon, 2006; Hamburger & Hotettmann, 1991).

1.1 The use of plants as herbal medicines

The term Herbalism most generally used for the study of medicinal purposes of plants. Herbal or traditional medicines have been practiced throughout the human history, and still commonly used today (Mehrabani *et al.,* 2004). According to WHO approximately 80 percent of some of African and Asian countries population currently depend on herbal medicine to meet their primary health care, because herbal remedies can be obtained with simple, easy and low cost practices. Modern medicines or antibiotics are too costly for most of the population of world, in which half of these having daily income below even $2 U.S (Elvin-Lewis, 2001).

Most of the medicines presently accessible in the market having long history of practice as herbal medicines, comprising quinine and aspirin (Satake *et al.,* 2003). World Health Organization estimates about 25 percent of new medicines practice in US have naturally isolated from medicinal plants. Amongst 120 vibrant composites currently obtained from higher medicinal plants and normally practiced in current drugs today, 80 percent display a positive relationship between their modern healing practice and the traditional usage of the plants from which they are isolated (Miceli *et al.,* 2005).

Examples of Naturally Plant-derived Medicines

The following tables comprise a small portion of some plant species used to produce plant based natural medicines.

Table 1.1: Plant-derived Medicines

S.NO	Naturally derived medicines	Origin	Medicinal use
1	Digitalis	Europe	Heart failure treatment
2	Devil's club shrub	Western North America	Infection cure, diabetes and tuberculosis treatment
3	Codeine	Southeastern Europe, western Asia	Pain relief, cough suppression
4	Camphor	Asia	Rheumatic pain relief
5	Colchicine	Eurasia	Cancer and gout treatment
6	Aloe	South Africa, Arabian	Peninsula Treatment of burns and wounds
7	Bloodroot	Southeastern United States	Treatment of skin disorder and cancer
8	Aspirin	Europe	Pain relief, promotion of heart- health, blood thinning
9	Black cohosh	Eastern United States and Canada	Treatment of hormonal disorders, rheumatism
10	Tubocuranine	South America	Surgical muscle relaxation
11	Taxol	North America	Treatment of breast and other cancers Asia
12	Slippery elm bark lining	Southeastern United States	Relief of coughs, gastrointestinal ailments, skin irritations
13	Topotecan	China	Ovarian and lung cancer treatment
14	Hoodia	South Africa	Weight loss
15	Pilocarpine	Amazon Basin	Glaucoma treatment
16	Madagascar Periwinkle	Madagascar	Treatment of leukemia, Hodgkin's disease, and other cancers
17	Gamma-linolenic acid	North America	Eczema, cancer, and nerve-damage treatment

1.2 Natural Products

These are organic compounds, which can be isolated from animals, plants and microorganisms. These compounds are termed phytochemicals when formed by plants. The study of natural products isolation by using plants is known as Phytochemistry. Chemistry of Natural Products deals with the chemistry of metabolites (Hiller *et al.,* 1992). Plants are natural factories continuously producing valuable compounds in the most cost efficient way. The term of medicinal plants include a variety of plants used in herbalism and some of these plants have medicinal activities. These medicinal plants considered as rich source of bioactive ingredients can be, used in drug development and synthesis. In order to obtain sustainable drug discovery, numerous approaches have been used to isolate naturally occurring from different sources (Bhat *et al.,* 2005).

1.3 Types of metabolites

There are two types of metabolites on the basis of metabolic pathways, via: primary metabolites and secondary metabolites.

1.3.1 Primary Metabolites

Primary metabolism involves the chemical process that every plant must carry out every day in order to survive and reproduce its line and is linked with the growth of the plant. Glycolysis Photosynthesis, synthesis of co-enzymes, citric acid cycle (Krebs cycle), synthesis of amino acid, synthesis of protein and enzyme, reproductions of cells, duplications of genetic materials, etc. These processes are collectively defined as primary metabolism, with the compounds formed in the pathways being named primary metabolites, e.g. nucleic acid, carbohydrates, proteins, fats, (Hanson, 2003).

1.3.2 Secondary Metabolites

Secondary metabolites referred as growth non-associated metabolites. These are chemical compounds in organisms that are not directly involved in the normal growth, development or reproduction of an organism. In this sense they are “secondary”. Nature can be seen as a productive biochemical factory for the biosynthesis of both primary and secondary metabolites because natural molecules have different biological functions (Hadian *et al.,* 2006).

Secondary metabolites are mostly linked with an organism’s interaction to its environment, producing novel chemical compounds that stimulate their survival. Secondary metabolites are

found in small amounts and usually work by inhibiting or stimulating biological process in other organisms and are involved in the interaction between organisms, therefore these metabolites often attracted scientific interest due to both the large number of different structures produced by nature and their diverse range of biological activities. In addition, they represent the individuality of the species at a chemical level because they are regularly found to be unique to a particular species (Cragg *et al.*, 2009).

1.4 Screening of Natural Products

Pharmacognosy provides tools for identification, selection and process of natural products for medicinal porpuses. Usually, the natural product compound known as the active principle has some form of biological activity. Plants are natural factories continuously producing valuable compounds in the most cost efficient way. The term of medicinal plants include a variety of plants used in herbalism and some of these plants have medicinal activities .Numerous medicines are achieved openly from a natural source, in one way or the other (Santos *et al.*, 1998).

Various medicines are developed from naturally occurring lead compounds achieved from natural source may comprising the following qualities.

- Manufactured synthetically
- act as a substrate for other semisynthetic compounds
- Act as a model for a structurally unlike synthetic compound

Maximum bioactive naturally occurring products are secondary metabolites having some structural complexity. Which imparts novelty but this complex structure makes the synthesis of several compounds very difficult and the compound generally has to be removed from natural source: slow, inefficient and expensive process (Miller, 2000).

1.5 Drug discovery and the importance of medicinal plants

Many approaches used to obtain phytochemicals for drug discovery comprising isolation from natural sources, Plants, molecular modeling, synthetic and combinatorial chemistry. In spite of the new curiosity in molecular modeling, synthetic chemistry methods by pharmaceutical corporations and funds providing agencies, natural compounds, and mostly medicinal plants persists a significant source for drug discovery. In 2001, approximately one third of the blockbusting medications throughout the world were isolated natural compound (Butler, 2004). Arteether is one of effective antimalarial isolated from artemisinin (Van

Agtmael *et al*., 1999).

1.8 Anti-cancer drug discovery

Above ten million new cases of cancer worldwide along with over 6 million deaths were expected in the year 2000. Plants are natural factories continuously producing valuable compounds in the most cost efficient way. The term of medicinal plants include a variety of plants used in herbalism and some of these plants have medicinal activities. These medicinal plants considered as rich source of bioactive ingredients can be, used in drug development and synthesis. Plants are natural factories continuously producing valuable compounds in the most cost efficient way. The term of medicinal plants include a variety of plants used in herbalism and some of these plants have medicinal activities. These medicinal plants considered as rich source of bioactive ingredients can be, used in drug development and synthesis. In order to obtain sustainable drug discovery, numerous approaches have been used to isolate naturally occurring from different sources (Parkin *et al.,* 2001).

1.9 α- Glucosidase Inhibition

Glycosidases are the enzymes responsible for carbohydrate break down. These enzymes are found in the striated border membrane surface of intestinal cells. The brush border cells are cells are located in two main areas of the body, kidney and the small intestinal tract. Alpha-Glucosidases break down carbohydrates into smaller sugar molecules like glucose; as a result carbohydrates are absorbed. The use of α-glucosidase inhibitors causes competitive and reversible inhibition of these enzymes. The use of these inhibitors is responsible to slow the breakdown of carbohydrates and delay glucose absorption. In results, the rise of blood glucose level is smaller and slower following meals, and remains effective throughout the day (Asano, 2003).

1.10 Total Phenolic contents

These are the secondary metabolites most widely occur in medicinal and aromatic plants. Phenolics comprising simple phenols, coumarins, flavonoids, phenolic acids, stilbenes, lignans, hydrolysable and condensed tannins. These compounds contribute in plant pigmentation. These plant secondary metabolites act as phytoalexins, attractants for pollinators and also play a key role as antioxidants. These compounds also provide protection against UV light (Gottlieb & Borin, 2000).

1.11 Antioxidant

During respiration process biological combustion occurs, which creates oxygen species (ROS). In the body the excess of these reactive oxygen reactive species produce oxidative stress .In a result cumulative damage is caused in lipids, proteins and DNA. Oxidative stress is basically the inequity b/t antioxidants and oxidants cause various diseases in humans. Many medicinal plant species and herbs contain antioxidant constituents in their tissues. Some of medicinal plants vegetables, cereals, fruits, and herbs are rich in phenolics which retard oxidative degradation of lipids and improve the nutritional value of food (Halliwell, 2006).

1.12 Antimicrobial

Medicinal and aromatic plants contain various phytochemicals having significant effects against pathogens. With the passage of time biological evaluation for antimicrobial compounds rationalized based on the new scientific methodologies. In recent years the use of natural antimicrobial plants secondary metabolites has been increased to cure diseases. Because of expensive treatment, resistance of pathogens and side effects of synthetic drugs (Vakalounakis & Chalkias, 2004).

1.13 Allelopathic

Allelopathy is considered a biological phenomenon in which certain organisms release one or more biochemical into the environment that effects the reproduction, survival and growth of other organisms. These biochemicals are also called allelochemicals which have positive or negative allelopathic effect on the target plants. Allelochemicals are secondary metabolites which are not needed during metabolism (development, growth and reproduction). This is a characteristic of some bacteria, plants, fungi, algae and coral. The possible the applications of allelopathy in agriculture sector needs more attention and valuable research. The present research is amid to find out the effects of crops on crops, crops on weeds and weeds on crops (Batish *et al.,* 2001).

1.14 Total Flavonoid contents

Flavonoids are also called bioflavonoids having yellow color in nature. These are plants secondary metabolites having antioxidant activity. Preliminary studies show that flavonoids may affect the anti-inflammatory mechanisms because of their potential to inhibit reactive oxygen or nitrogen compounds. Flavonoids are also proposed to inhibit the activity of those enzymes involved in free radical formation, like as cyclooxygenase and lipoxygenase. The

use of flavonoids in diet helps in reducing gastric carcinoma risk in women. Flavonoids are also helpful to reduce aero digestive tract cancer risk in smokers. Flavonoids also exhibit direct antibacterial and synergistic activity with antibiotics. Flavonoids showed the ability to suppress bacterial virulence in many in vitro and a limited number of in vivo studies (Van Doorn *et al.,* 2007).

1.15 The Family Resedaceae

The Resedaceae family consists of six genera throughout the world. The genera Reseda L. comprise almost sixty species all over the world amongst them. 15 species of the genus are found in Turkey, *R. lutea L*., is one of them (Ikan, 1991). One of the known species of Ochradenus is Ochradenus baccatus which is found growing on sandy and stony places in the Kingdom of Saudi Arabia. *Reseda lutea* is usually found in seconday newyan countries, Finland, UK and Central Europe. (Pettit & Tan, 1995).

1.16 Genus *Ochradenus*

Ochradenus is a genus of plant in family Resedaceae. *Ochradenus* considered as rich source of bioactive ingredients can be, used in drug development and synthesis. In order to obtain sustainable drug discovery, numerous approaches have been used to isolate naturally occurring from different sources. *Ochradenus* comprising eight species of shrubs scattered in desert of South Arabian Peninsula, North East Africa and South West Asia (Taylor *et al.,* 1996). One of the known species of *Ochradenus* is *Ochradenus baccatus* which is found growing on sandy and stony places in the Kingdom of Saudi Arabia. It has also been reported from almost all the deserts of Egypt. This species is medicinally very important as it has already been shown that it can effectively lower blood cholesterol level in rats and showed a strong bioactivity against malarial parasite. This plant being of high medicinal value has been characterized for its composition and has isolated a number of compounds including some novel flavonoids from the leaves of this plant (Cragg *et al.,* 1997).

1.17 *Ochradenus arabicus* Chaudhary, Hillc. & A. G. Mill.

Ochradenus arabicus Chaudhary, Hillc. & A. G. Mill. is a species belong to the *Ochradenus* genus (Kuhlmann, 1997).*O. arabicus* is very important medicinal plant having various medicinal possessions, due to its medicinal importance traditionally it is mostly used to cure diverse ailments in the world, Saudi Arabia is also one of them(Nadeem *et al.,* 2012). *O. arabicus* has the potential to discover the potent anticancer agents. Further fractionation of the bioactive fraction can help us to find the lead anticancer compound. Phytochemicals

isolated from medicinal plants have been playing a significant role directly or indirectly to combat cancer disorders. (Vincken *et al.*, 2007).

Ochradenus arabicus Chaudhary, Hillc. & A. G. Mill.

1.18 Aims and objectives

The purpose of the present study was to assess the phytochemical and biological investigation of medicinally important plant *Ochradenus arabicus*.

The specific objectives of the current study were;

- Collection and identification of medicinally important plant *Ochradenus arabicus*
- Extraction of selected medicinal plant using different solvents such as methanol, ethanol, distilled water and ethyl acetate.
- Biological evaluation of different extracts of the plant
- Bioassay guided isolation of bioactive compounds

Materials and Methods

Materials and Methods

2.1 Experimental

All chemical and instrumental analysis was performed at UoN chair of Omani Medicinal Plants and Marine Natural products, DARES research center, University of Nizwa Sultanate of Oman.

2.2 Sample Collection and Identification

Ochradenus arabicus have been collected from Al-Jabal Al-Akhdar, Sultanate of Oman and have been identified by plant taxonomist. The voucher specimen placed in the Herbarium of University of Nizwa, Sultanate of Oman.

2.3 Extraction and Purification of *Ochradenus arabicus* (JOA)

The plant was collected from Jabal Al-Akhdar, is one of the diverse floral region of Sultanate of Oman. The whole plant of Ochradenus arabicus was shade dried and ground into powder. The collected plant materials were extracted with methanol and different fractions of ethyl acetate, hexane, chloroform, butanol and water were made. These fractions in various concentrations were subjected to different biological assays including phenolic, flavonoid, antioxidant, allophatic, alpha-glucosidase inhibitor and anticancer.

The chloroform fraction (85.5g) was selected and then chromatographed over a silica gel column, which results in five known compounds for the first time from O. arabicus; 1-Octacosanol, beta-sitosterol, beta-sitosterol-beata-D-glycoside, Choerospondin and Ursolic acid. Structure of these isolated phytochemicals conformed by using different spectroscopic techniques like NMR, UV, IR, mass spectrometry (EI-MS) etc. Our results shows that O. arabicus has the potential to discover the potent anticancer agents. Further fractionation of the bioactive fraction can help us to find the lead anticancer compound. Phytochemicals isolated from medicinal plants have been playing a significant role directly or indirectly to combat cancer disorders.

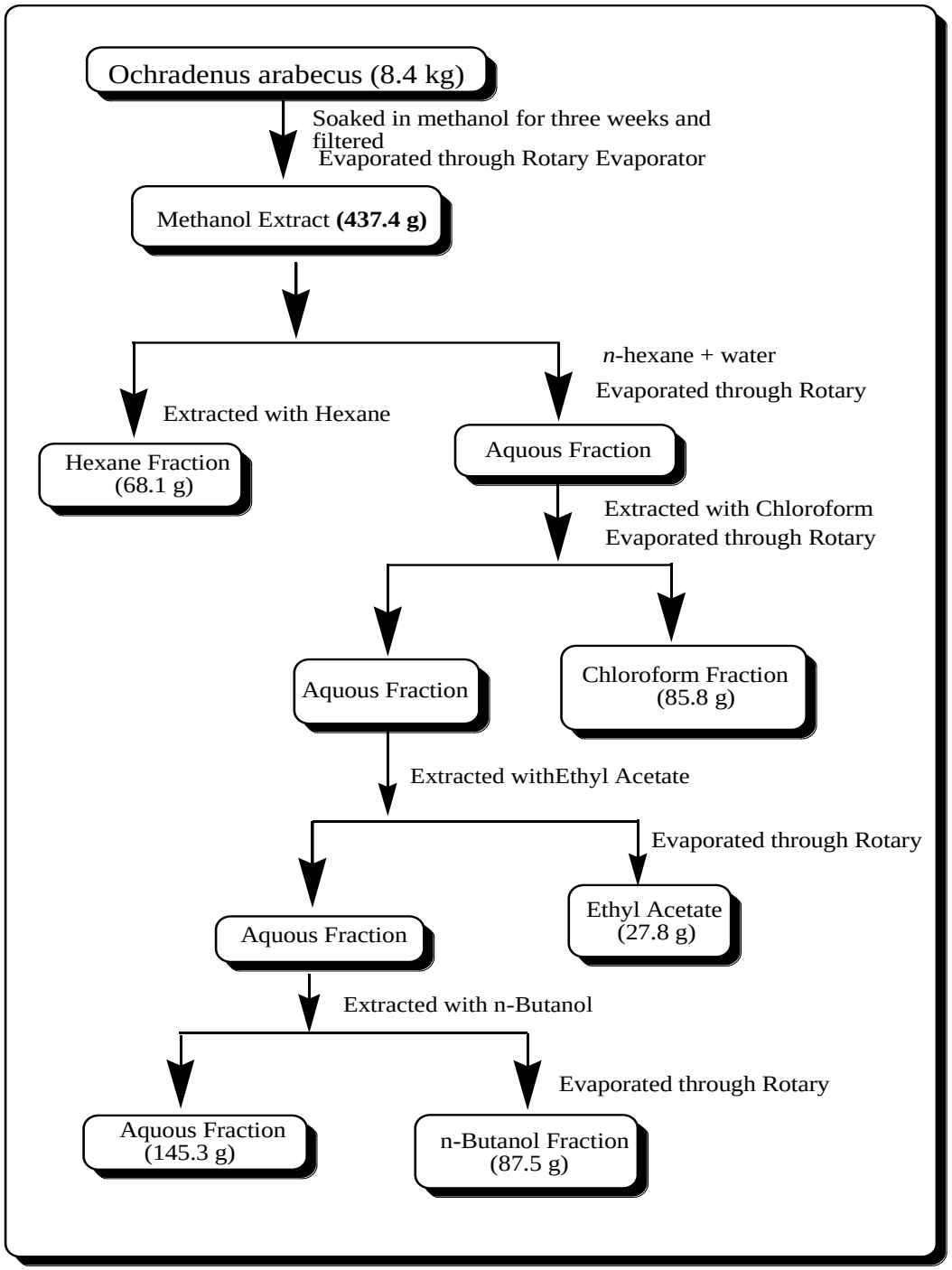


Figure2.1: Fractionation of different solvent fractions including n-hexane, chloroform, ethyl acetate, n-butanol and Aqueous.

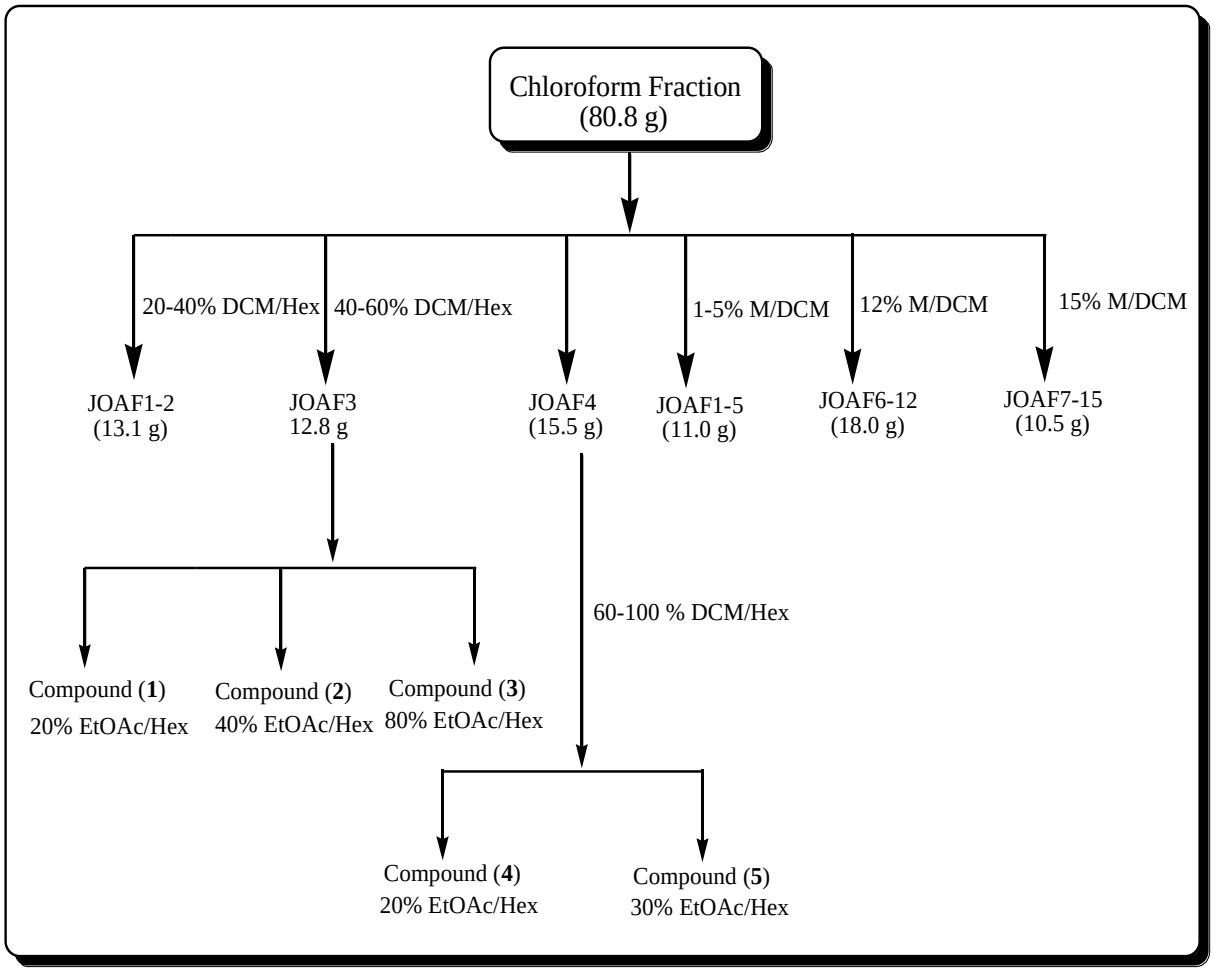


Figure 2.2: Scheme showing the Isolation of compounds 1-5.

2.4 Column Chromatography

Merck silica gel having 70-230 mesh was used for column chroma tography (CC), while silica gel having 230-400 mesh was used for flash chromatography (FC).

2.4.1 Thin Layer Chromatography (TLC)

The thin layer chromatography (TLC) was performed on DC- Micro-Cards SIF 5×10 cm (silica gel with fluorescent indicator of 254 nm on aluminum cards with layer thickness of 0.2 mm), Riedael de Haens, Art no. 37360.. Purity was checked by Thin Layer Chromatography (TLC) with different solvent systems using methanol, acetone, ethyl acetate, chloroform and hexane giving single spot for pure compounds. Spot detections were observed by spraying with 10% solution of Ce (SO4)2 in 2N H2SO4 and heating with hot gun.

2.5 General Instrumentation

2.5.1 Infra Red Spectroscopy

The IR spectra were recorded on a Shimadzu IR-460 (Shimadzu Corporation, Tokyo, Japan) instrument**.**

2.5.2 Ultra Violet Spectroscopy

The UV spectra were recorded on a Shimadzu UV-240 (Shimadzu Corporation, Tokyo, Japan) spectrophotometer.

2.5.3 Mass spectrometry

EI-MS and FAB-MS spectra were recorded on a JMS-HX-110 spectrometer, with a data system.

NMR Spectroscopy

The 13C-NMR (BB and DEPT) spectra were recorded on the same instruments at 75 MHz, respectively. The DEPT experiments were carried out with 45°, 90° and 135°. The quaternary carbons were determined by the subtraction of these spectra from the Broad Band (BB), ^{13}C-NMR spectrum. The chemical shifts are expressed as ppm and coupling constants (J) are in Hz. Tetra Methyl Silane (TMS) is used as an internal standard. HMBC, HMQC and COSY spectra were run on Bruker spectrometers operating at 300 MHz.

2.5.4 Spray Reagents

Ceric sulphate reagent was used for the detection of compounds. Ceric sulphate (0.1g) was dissolved in 4 mL distilled water. The solution was boiled and conc. H_2SO_4 was added drop-wise until the disappearance of turbidity.

2.6 Characterization of 1-Octacosanol (1)

Physical Constants and Spectral Data

Molecular formula: $C_{29}H_{58}O$

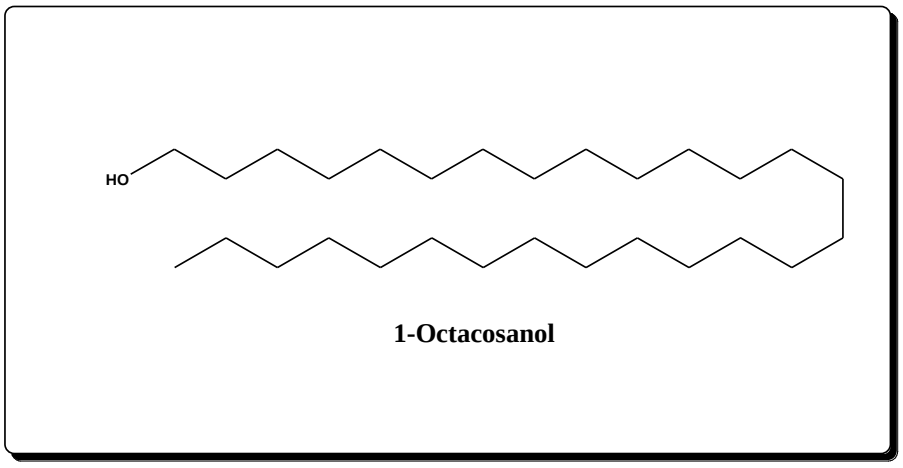
Physical state: Colorless solid

M.P: 204 °C

ESI-MS *m/z*: 447 $[M+Na]^+$, 392, 167, 125, 97 and 57.

^{1}H-NMR (CDCl3, 600 MHz): δ 3.63 (αH, H-1), 0.87 (3H, H-30), 1.53 (2H, C-2), 1.23 (2H, C-4-29).

^{13}C-NMR (CDCl3, 125 MHz): δ 63.1 (C-1), 32.8 (C-2), 31.9 (C-3), 29.0-29.3 (C-4- 29, 14.1 (C-30).



1-Octacosanol

2.7 Characterization of β-sitosterol (2)

Physical Constants and Spectral Data

Molecular formula: $C_{29}H_{50}O$

Physical state: Colorless solid

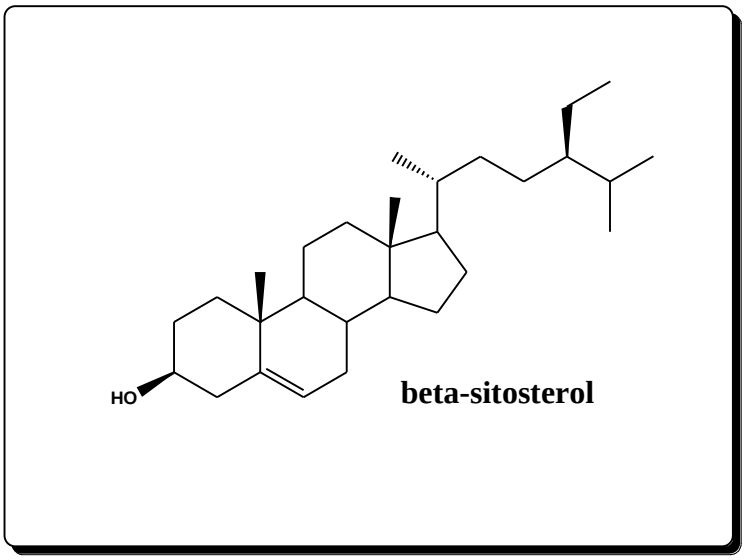
M.P 135 °C

IR ύ *max* ($CHCl_3$) cm^{-1}: 3446 (OH), 3050 (C=C), 1650, and 815.

ESI-MS *m/z*: 437 [M+Na], 396, 381, 329, 371, 303, 273, 255, 99, 85.

1H-NMR ($CDCl_3$, 600 MHz): 5.23 (1H, m, H-6), 3.32 (1H, m, H-3), 1.01 (3H, s, H-19), 0.68 (3H, s, H-18), 0.83 (3H, d, *J* = 6.5 Hz, H-26), 0.81 (3H, d, *J* = 6.5 Hz, H-27), 0.84 (3H, t, *J* = 7.0 Hz, H29).

^{13}C-NMR ($CDCl_3$, 125 MHz): **see table 3.1**



beta-sitosterol

2.8 β -sitosterol-β-D-glycoside (3)

Physical Constants and Spectral Data

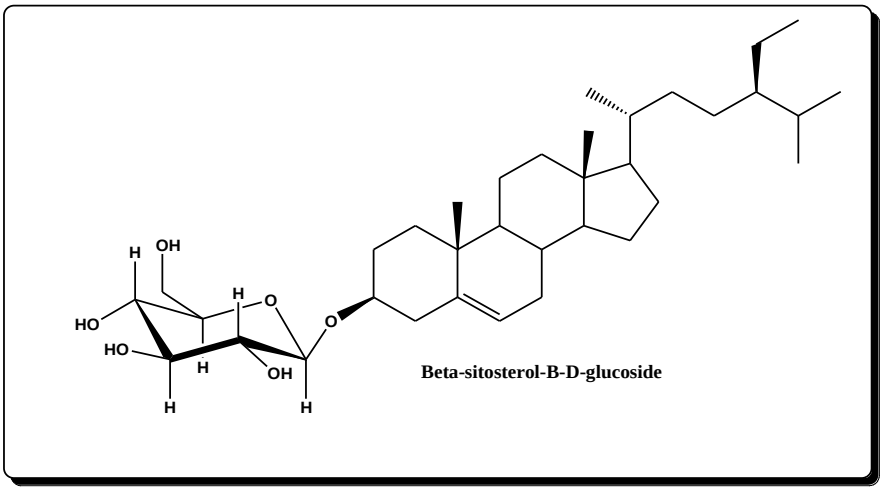
Molecular Formula: $C_{35}H_{60}O_6$

IR ύ *max* ($CHCl_3$) cm^{-1}: 3600-33400 (-OH), 2900-2850 (-CH), 1720 (C=O), 1240, (C–O), 900 (aromatic region), 830 (–CH), 800 cm^{-1} (C=C-H).

ESI-MS *m/z*: 599 $[M + Na]^+$, 396, 381, 329, 371, 303, 273, 255.

^{1}H-Nuclear Magnetic Resonance ($CDCl_3$, 600 MHz): 7.20 (s, 1-H, Hydroxyl group),5.40 (s, 1Hydrogen, Hydrogen-6).

^{1}H-NMR ($CDCl_3$, 150 MHz): **See table 3.2**



2.9 Ursolic acid (4)

Physical Constants and Spectral Data

Molecular formula: $C_{30}\ H_{48}\ O_3$

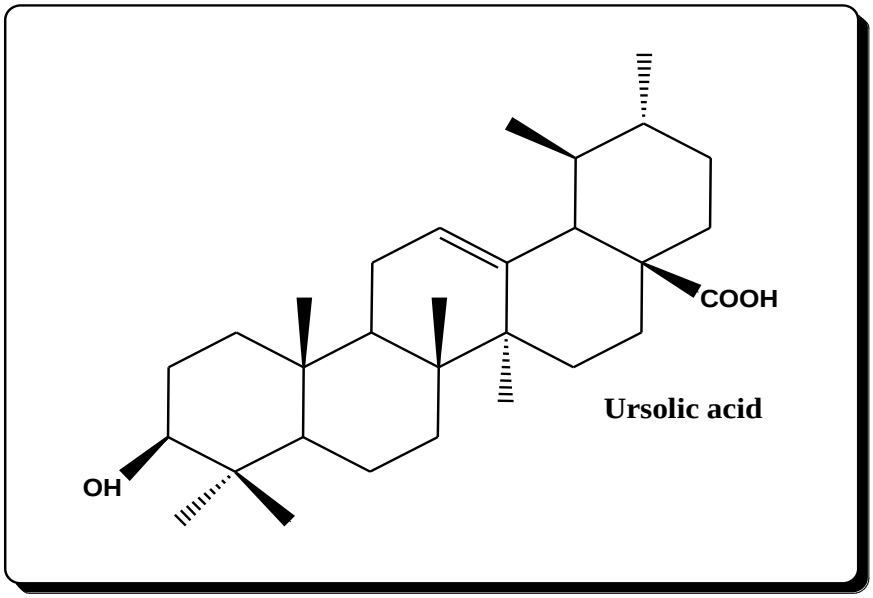
Physical state: Colorless solid

M.P: 283-285 °C

($CHCl_3$) cm^{-1}: 3510 (OH), 3050, 1697 (C=O), trisubstitution double bond at1635 and 820.

ESI-MS *m/z*: 479 $[M+Na]^+$

^{13}C-NMR (CDCl3, 125 MHz): see table 3.3



Ursolic acid

2.10 Choerospondin (5)

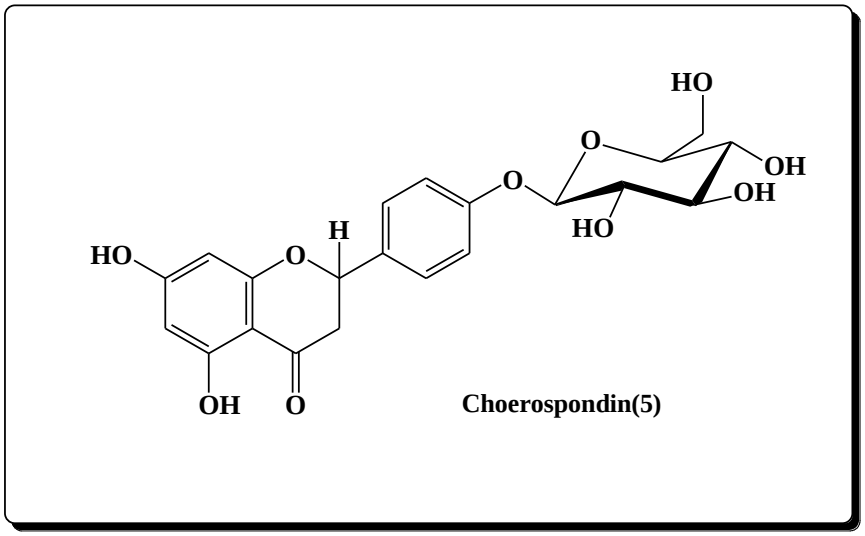
Physical Constants and Spectral Data

Physical State: Yellow amorphous powder

Melting Point: 225 °C

($CHCl_3$) cm^{-1}: 3404, 2890, 1655, 1588, and 1495

ESI-MS: 457 [M+Na]



Choerospondin(5)

^{1}H NMR (C_5D_5N, 300 MHz): δ 12.54 (1H, br s, OH-5

^{13}C Nuclear Magnetic Resonance (C_5D_5N, 75): table 3.4

2.11 Biological Assays

2.11.1 Allelopathic activities

Allelopathic effects of the sub-fractions were assessed by the method of (Khan *et al.,* 2010). Lettuce seed (*Lectuca sativa*) was utilized as an indicator species to know the effective concentration. Three different concentrations of 100, 500 and 1000 ppm of each sub-fraction were ready by thawing it in 5% DMSO(Khan *et al.,* 2009).

2.11.2 Antioxidant activity

The 1, 1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging potential was assessed according to the methods of (Adom *et al.,* 2003). The DPPH solution (0.1 mM DPPH, 50 µL) was prepared in methanol and mixed with plant extract (20 to 1000 µg/mL; 50 µL). The reaction mixture was kept in dark at room temperature for 1 hr min. The absorbance of the reaction was measured at 490 nm. Blank (without plant extract) was used as negative control. Butyl hydroxyl toluene (BHT) and ascorbic acid were used as positive controls. The antioxidant activity was expressed as percentage (%) = (absorbance of control – absorbance of samples/ absorbance of the control) × 100.

2.11.3 Total Phenolic Contents

The total phenolic content was quantified by following Singleton and Slinkard (1977) with some modifications using Folin-Ciocalteau reagent. Briefly, about 20 µl of extract or the gallic acid (positive standard) was diluted with autoclaved distilled water. The mixture was added with 2 N Folin-Ciocalteau reagents. The mixture vigorously vertexed and incubated for 10 minutes. A Na_2CO_3 (5%) was added and vertexed. The reaction mixture was incubated for 30 min at 25°C. The upper supernatant layer was read at 765 nm. The curve for standard was prepared using 25–1000 µg/mL of gallic acid. The total phenolic was shown as gallic acid equivalents (µg/mg of extract). The experiment was repeated three times.

2.11.4 Determination of total flavonoids:

Total flavonoid content was determined after some modification in the assay of (Dixit & Kar, 2009). Briefly, plant extract (50 µL) was mixed with autoclaved distilled water (50 µL) and $NaNO_2$ (5%; 100 µL). This was followed by addition of aluminum chloride (10%; 150 µL of). The reaction mixture was vertexed vigorously and incubated for 15 min. 1 M NaOH (100 µL) was added to the eppendorf tube. The absorbance was read at 510 nm. Quercetin (25–

1000 mg/L) was used as standard. The data was presented as µg equivalents to quercetin of the plant extract.

2.11.5 Anticancer activities:

Four cancer cell lines colorectal adenocarcinoma (HT29), MCF,colorectal adenocarcinoma (HCT116); Human hepatoma derived (HepG2) cell lines. The extracts were assessed for their anticancer activities using different colorectal adenocarcinoma (HT29 and HCT116), hepatoma derived cell (HepG2) and breast cancer cell (MCF-7) lines. Various low concentrations of the extracts were prepared and tested against the grown cell cultures. The screening results showed that JOAE, JOAB and JOAAQ were suppressing the cancer cell growth while other extracts such as JOAH, JOAC and JOAM were inactive as compared to the control. The active fractions were subjected to concentration gradient effects of extracts. According to results of colorectal adenocarcinoma (HT29 and HCT116) cell lines treatments, 100 ug/mL of JOAE, 25 ug/mL of JOAB and 1 ug/ml of JOAAQ has reduced the cancer cell viability as compared to other extracts, their concentrations and control.

2.11.6 Enzyme α-Glucosidase Assay:

According to (Oki et al., 1999) α-Glucosidase enzyme inhibition assay was completed with some minor changes. Spectrophotometer used for the measurement of inhibition. The pH was 6.9 and temperature was kept at 37 °C and PNG used as a substrate.

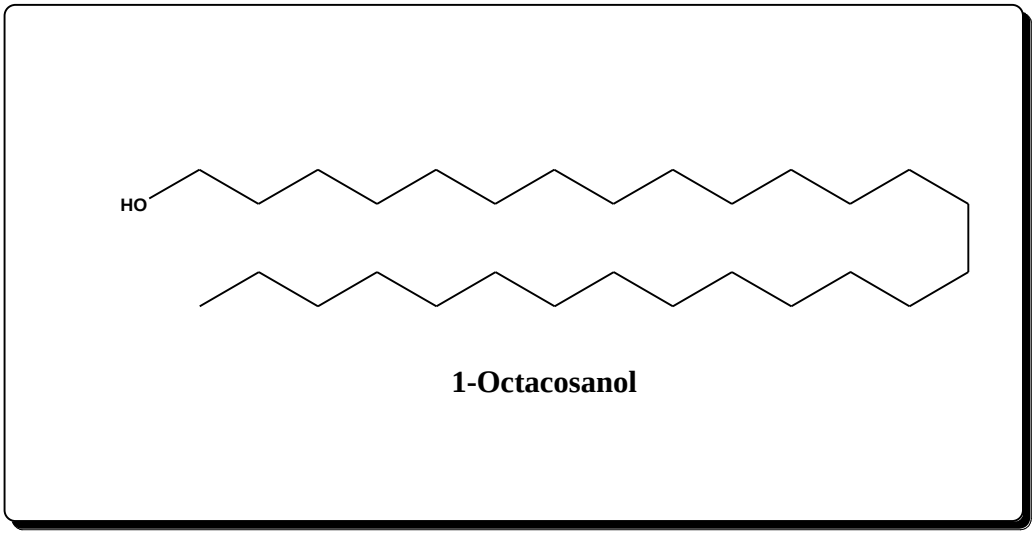
The assay shows that only JOAB was 10% active to inhibit the activity as compared to acrabose while other extracts JOAC, JOAE, JOAH, JOAAQ, and JOAM were inactive to present any inhibitory effect during the reaction.

Results

The powder of the shade dried plant of *Ochradenus arabicus* results into a reasonable amount of extracts including methanol and different fractions of ethyl acetate, hexane, chloroform, butanol and water. Bioassay guided isolation showed that the chloroform extract were the most efficient fraction. It was further processed for column chromatography over silica gel column, which results in five known compounds for the first time from *O. arabicus*; 1-Octacosanol, β-sitosterol, β -sitosterol-β-D-glycoside, Ursolic acid and Choerospondin. The results are described below:

3.1 1-Octacosanol (1)

1-octacosonl was isolated form *Ochradenus arabicus* as colorless solid at 20% EtOAc/Hex. The molecular formula C28 H60 O was recognized on the basis of MS, 1D and 2D NMR spectra.



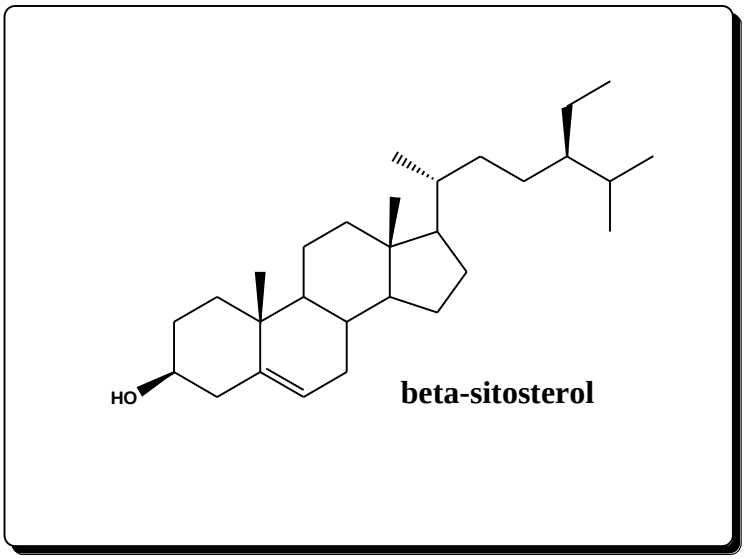
1-Octacosanol

Mass spectrum showed molecular ion peak at m/z 410, proposing molecular formula $C_{28}H_{58}O$. The proton Nuclear Magnetic Resonance spectrum showed a triplet at δ 0.87 (3H) and a broad singlet at δ 1.23 corresponded straight chain hydrocarbon.

3.2 β-Sitosterol (2)

Compound 2 was isolated as white color from the chloroform extract of *Ocradenus arabicus*. The IR spectrum of the compound 2 showed the absorption for OH group (3450 cm -1) and trisubstituted double bonds at position 3446, 3050, 1650 and 815 cm $^{-1}$. C 29 H50 O; the molecular formula was recognized through UPLC/MS/MS (ESI +) presenting molecular ion peak at m/z 437 [M+Na]. Parents to daughter fragments detected at m/z 273 and 255, demonstrating the loss (M +- side chain) and (M +- side chain –H2O).

The proton NMR spectrum of **2** presented six methyl signals comprising two tertiary (δ 0.68 and 1.01), three secondary (δ 0.92, 0.83 and 0.81) and one primary (δ 0.84) methyles. The signals for olefinic and carbinylic proton looked at δ 3.32 (1H, m) and 5.23 (1H, m). The 13 C-NMR (BB, DEPT) spectrum of compound 2 revealed the presence of twenty nine carbon signals for six methyl, eleven methylene, nine methine and three quarternary carbon atoms. Compound 2 was recognized as β- sitosterol through NMR.



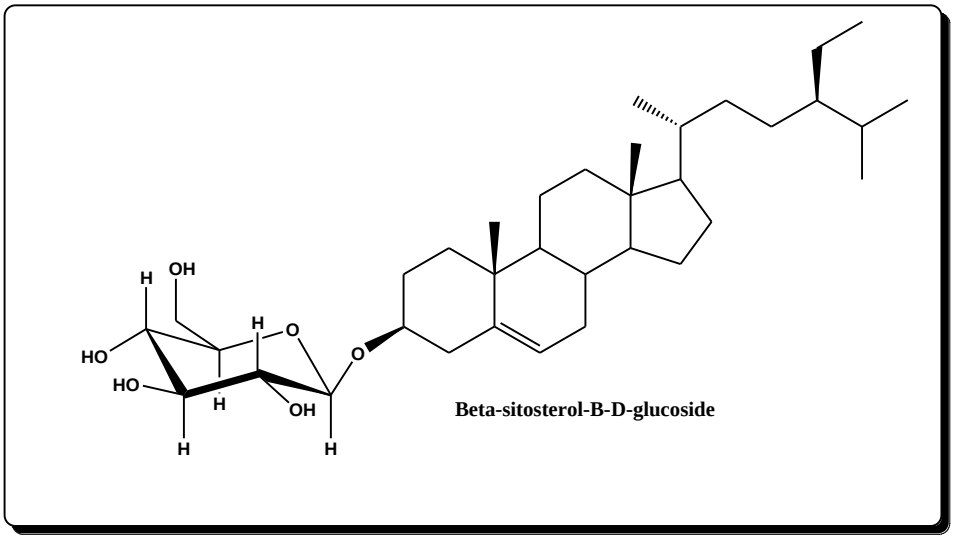
beta-sitosterol

Table 3.1: ^{13}C data for compound 2

C.NO	Multiplicity (DEPT)	^{13}C-NMR	C.NO	Multiplicity (DEPT)	^{13}C-NMR
1	CH2	37	16	CH2	26
2	CH2	27.9	17	CH	56.3
3	CH	71.1	18	CH3	11.1
4	CH2	39.9	19	CH_3	18.5
5	C	140.1	20	CH	35.7
6	CH	120.2	21	CH3	18.2
7	CH2	30.9	22	CH2	33.8
8	CH	31.2	23	CH2	28.1
9	CH	50.1	24	CH	48.1
10	C	35.8	25	CH	28.5
11	CH2	20.9	26	CH3	18.9
12	CH2	39.1*	27	CH3	19.1
13	C	41.7	28	CH2	23.0
14	CH	56.1	29	CH3	11.9
15	CH2	23.9			

3.3 β-sitosterol-β-D-glucoside (3)

Compound (3) was isolated as colorless solid from the chloroform extract of *Ocradenus arabicus*. (3600-33400) cm^{-1} ,the region of the absorption peak of IR spectrum representing group (-OH) of compound 3, 2900-2850 cm^{-1} (stretching and bending of -CH), 1720 cm^{-1} (C=O), 1240 cm^{-1} (C–N stretching), the absorbance peak on 900 cm^{-1} representing aromatic stretching , 830 and 800 cm^{-1} absorption peak showed Carbon and Hydrogen stretching of >C=C-H group.



The chemical shift value of proton NMR of **3** on δ 0.80 and 1.19 revealed the occurrence of 2 CH_3 signals, and at δ 5.35 displayed the existence of one olefinic double bonded proton as a doublet, and another 2 signals on δ 0.80 and 0.82 represented the occurrence of 2 secondary CH_3 at 26 and 27 of basic frame. The chemical shift on δ 0.91 as a triplet apportioned for the terminal CH3 group at C-29. Likewise, the other chemical shift at δ 1.1 assigned secondary methyl group at position 21. The proton NMR at δ 2.00-3.30 multiplet showed the existence 5 protons of sugar moiety and chemical shift at δ 7.22 was allotted for the proton of carboxylic group of glycoside.

^{13}C-NMR of **3** showed 29 carbons, at δ 76.7 and 63.8 presented two separate terminal methyl groups on C-25. The downfield on δ 122.2, 132.00 and 172.1 correspondingly, presented methyl carbons connected on Carbon -18, C-19 and Carbon -21 positions. Similarly down field at δ 48.7, 50.8 and 55.8 represented Carbon number 9, 13 and C-5, respectively. The other shifts represented carbon numbers C-20, 22, 23, 24, 25 and 28. Chemical shift at 143.91 showed sugar moiety's C.

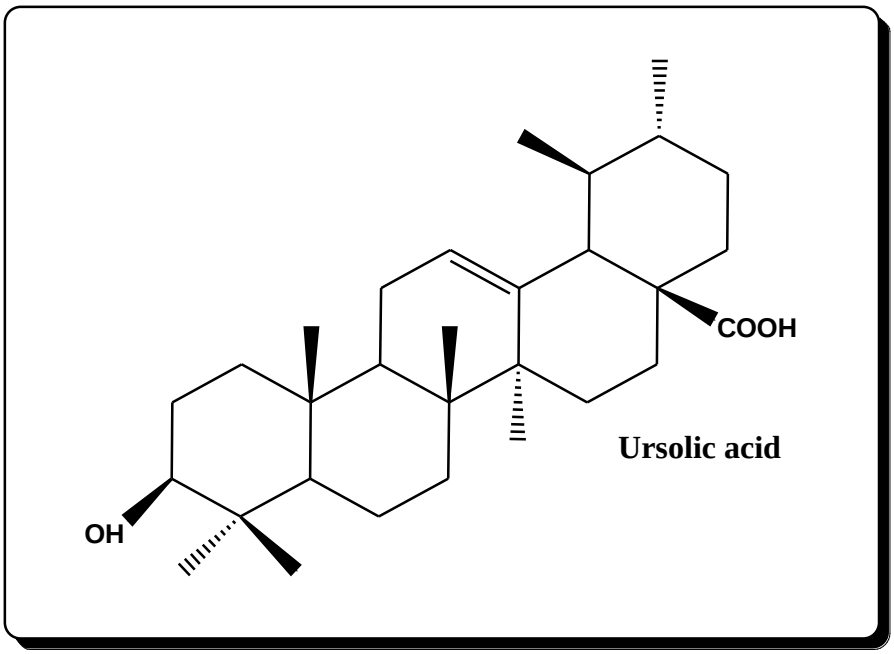
Compound (3) was recognized as β-sitosterol-D-glycoside on the basis of different spectroscopic techniques.

Table 3.2: ^{13}C data for compound 3

C.NO	Multiplicity DEPT	^{13}C-NMR	C.NO	Multiplicity DEPT	^{13}C-NMR
1	CH2	36.48	19		19.4
2	CH2	28.9	20		36.80
3	CH	76.8	21		18.85
4	CH2	39.1	22		34.35
5	C	139.9	23		27.7
6	CH	121.6	24		45.9
7	CH2	32.5	25		29.0
8	CH	24.1	26		18.1
9	CH	49.50	27		19.5
10	C	35.9	28		22.9
11	CH2	21.2	29		11.0
12	CH2	39.8			
13	C	42.1			
14	CH	55.8			
15	CH2	24.9			
16	CH2	27.5			
17	CH	56.9			
18		12.2			

3.4 Ursolic acid (4)

Compound **4** isolated from chloroform soluble fraction of *Ochradenus arabicus* at 20% EtOAc/Hex. The IR spectrum revealed absorptions for carbonyl group at (1697 cm^{-1}) hydroxyl group at (3510 cm^{-1}) and trisubstituted double bond at positions 1635 and 820 cm^{-1}. The molecular formula C30H48O3 was established through MS by giving molecular ion peak at m/z 456. The base peak at position m/z 203 was attributed to the loss of COOH from the fragment at m/z 248. Beside the molecular ion peak, the EI-MS presented other diagnostic peaks at m/z 411 showing the loss of carboxylic group. Another noticeable peak at m/z 248 signified Retro Diel Alder fragmentation, characteristic of Δ12 ursane type triterpenes with carboxylic group at C-17.



Ursolic acid

The 1H-NMR spectrum of **4** demonstrated five tertiary methyl signals at δ 1.27, 1.25, 1.08, 1.05, 0.91 (each 3H, s, H-23, 27, 26, 24 and 25) and two secondary methyl signals at 1.02 (3H, d, J = 6.6 Hz, H-30), 0.97 (3H, d, J = 6.3 Hz, H-29) which were the sign of ursane skeleton. The carbonylic proton that appeared at δ 3.46 (1H, dd, J = 10.3, 4.1 Hz) was in axial configuration as confirmed by a double doublet. The olefinic proton was observed at δ 5.52 as a triplet (J = 3.5 Hz).

Table 3.3: ^{13}C-NMR data for compound (4)

C.NO	Multiplicity DEPT	^{13}C-NMR	C.NO	Multiplicity DEPT	^{13}C-NMR
1	CH2	38	16	CH2	23.2
2	CH2	26.9	17	C	47.3
3	CH	80	18	CH	55.7
4	C	38.1	19	CH	29.8
5	CH	51.9	20	CH	30.5
6	CH2	18.9	21	CH2	27.2
7	CH2	32.9	22	CH2	36.9
8	C	39.1	23	CH3	24.3
9	CH	46.8	24	CH3	15.1
10	C	37	25	CH3	15.5
11	CH2	23.2	26	CH3	17.7
12	CH	125.4	27	CH3	24
13	C	138.1	28	C	177.1
14	C	41.9	29	CH3	2.1
15	CH2	29.8	30	CH3	23.9

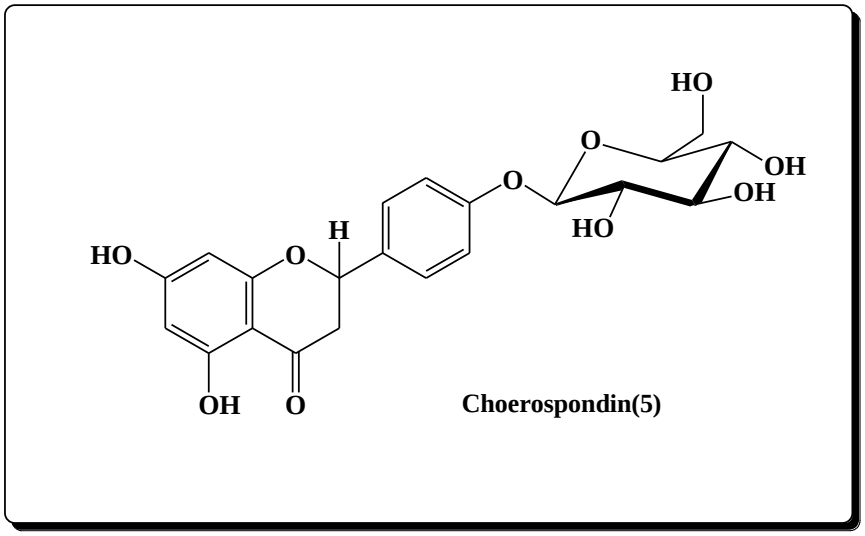
3.5 Choerospondin (5)

Compound **5** was Isolated as yellow amorphous powder from *Ocradenus arabicus* at 30% EtOAc/Hex. The IR spectrum directed the existence of hydroxyl at (3404 cm^{-1}), aromatic functionalities at (2890, 1495 cm^{-1}) and ketone at (1655, and 1588 cm^{-1}). The molecular formula $C_{21}H_{22}O_{10}$ was identified by FAB-MS showing [M^{+}-1] at *m/z* 433 (calcd. for $C_{21}H_{22}O_{10}$, 434.1213).

The existence of flavonoid skeleton was inferred by the aromatic signals between δ 6.56 and 6.61. The proton NMR revealed two doublets each at d 6.56 (1H, J8,6 = 1.6 Hz, H-8), and 6.61 (1H, J6,8 = 1.6 Hz, H-6), which were assigned to H-8 and H-6, respectively. The

downfield resonances of aromatic ring B protons at d 7.19 (2H, d, J3′,2′ = J5′,6′ = 8.2 Hz) and 7.49 (2H, d, J2′,3′ = J6′,5′ = 8.3 Hz), downfield doublet at δ 5.72 (1H, d, J1′, 2′ = 7.4 Hz), was assigned to glucose anomeric proton (H-1″), which obviously showed the presence of β-D-glucopyranoside.

The signal at δ 101.4 in the ^{13}C NMR spectrum along with the HMQC, and HMBC correlations further confirmed the sugar moiety at C-4′ position. The EI-MS further displayed a fragment ion at m/z 434 as molecular ion and other significant fragments m/z 272 due to the loss of glucose moiety and other prominent fragments at m/z at 272 (100%), 271 (62.8%), 255 (11.3%), 153 (96.4%), and 120 (52.7%), which confirmed the naringenin skeleton.



Choerospondin(5)

Table 3.4: ^{13}C-NMR data for compound (5)

C.NO	Multiplicity DEPT	^{13}C-NMR	C.NO	Multiplicity DEPT	^{13}C-NMR
2	CH	79.6	1’	C	129.5
3	CH2	43.1	2’, 6’	CH	128.7
4	C	197.1	4’	C	159.5
5	C	164.4	3’, 5’	CH	116.4
6	CH	97.6	1”	CH	101.4
7	C	166.5	2”	CH	74.6
8	CH	96.3	3”	CH	78.9
9	C	163.5	4”	CH	70.8
10	C	104.2	5”	CH	78.2
			6”	CH2	62.0

3.6 Biolagical Assays

3.6.1 Allelopathic activities

Allelopathy is the effect of a plant on the growth and development of another plant species by secretion of chemical constituents.

In the present study, the allelopathic effect of each fraction (JOAH, JOAE, JOAC, JOAB and JOAAQ) of *O. arabicus* was evaluated by using three concentrations gradient. The results of the assay revealed that aqueous fraction at 1000 ppm have shown significant inhibitory effect towards the development of root and seeds, while at 1000 and 500 ppm, both the aqueous and crude extract of methanol inhibited moderate activity on the root development and lettuce seeds respectively. In case of shoot and propagation of seeds, aqueous fraction revealed the highest activity at 1000 ppm and moderate activity at 500 ppm (Figure 3.1). The actual conc: wanted to show 50 percent seed bigger from high polar (methanol) to low polar (hexane) sub fractions (Figure 3.1).

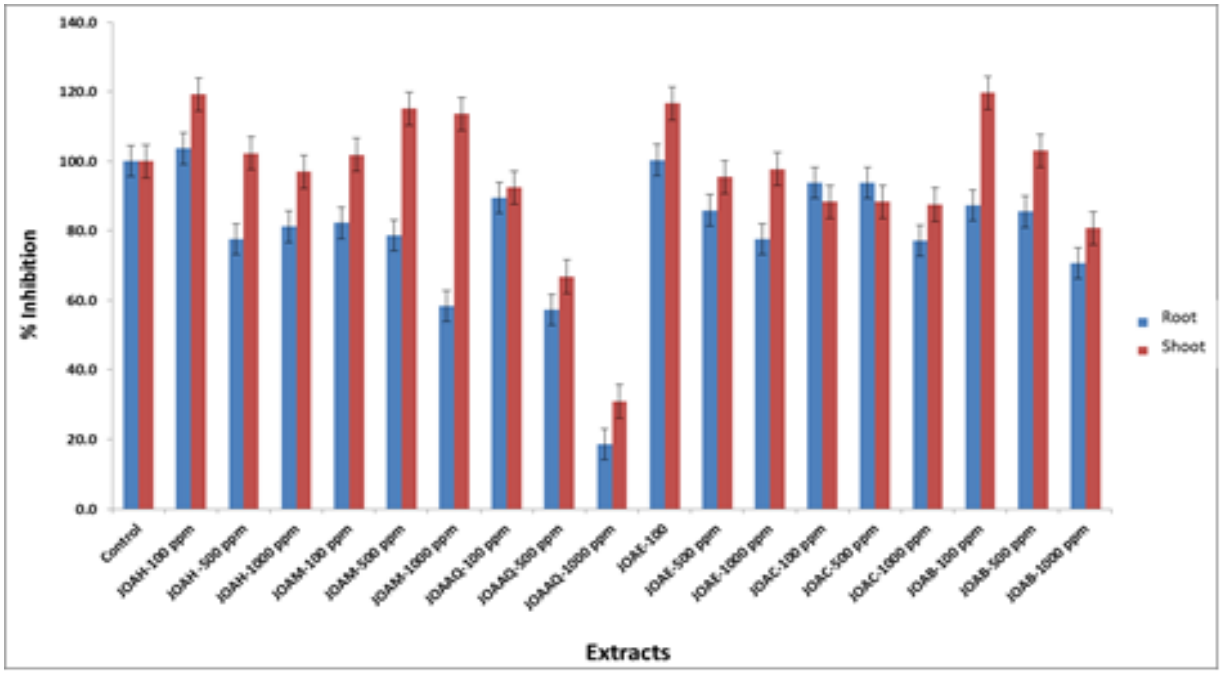


Figure 3.1: Effect of various extracts of JOA on the root and shoot of lettuce seeds. The error bars shows the standard deviation of the mean values. The different letter (s) shows that values of each extract are significantly different (p>0.05) in comparison with control as evaluated by DMRT.

3.6.2 DPPH Radical Scavenging Activity

Antioxidant analysis was performed to evaluate the free radical scavenging properties of six different extracts viz. methanol (JOAM), n-hexane (JOAH), chloroform (JOAM), ethyl acetate (JOAE), n-butanol (JOAB) and aqueous (JOAAQ) of *Ochradenus arabicus* using ascorbic acid as standard antioxidant. The results are presented in Figure 3.2. Among extracts, the ethyl acetate fraction significantly higher activity showed the most promising antioxidant activity followed by methanol and butanol, respectively. DPPH radical scavenging activity of the JOAAQ extracts was significantly lower as compared to other extracts and control. Ascorbic acid being a potent antioxidant and radical scavenger has an important role in metabolism. The JOAE showed a similar level of DPPH radical scavenging in comparison to ascorbic acid. In the present study, the radical scavenging effects were high in JOAE subfraction, suggesting the presence of bioactive metabolites which has oxidative stress mitigation properties.

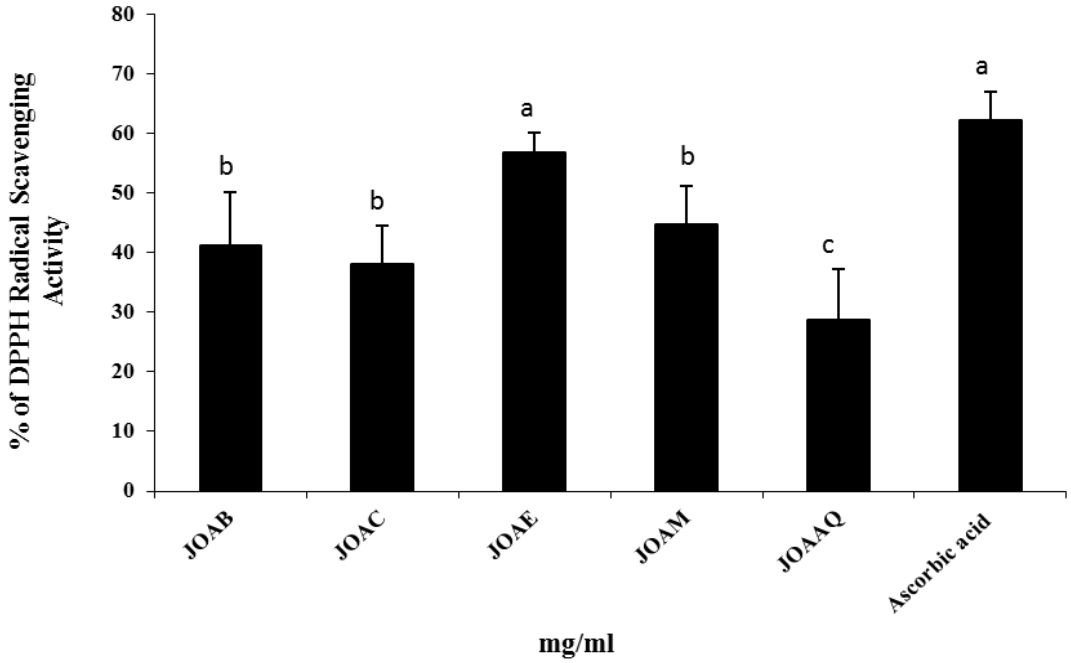


Figure 3.2: DPPH radical scavenging activity of the different extracts of *Ochradenus arabicus*. The error bars shows the standard deviation of the mean values. The different letter(s) shows that values of each extract are significantly different ($p>0.05$) in comparison with positive control as evaluated by DMRT.

3.6.3 Total phenolic contents and total flavonoid contents

Total phenolic contents were evaluated in all the crude methanol extracts and its sub fractions. The total phenolic content was significantly higher (6.43 ug/g) in JOAE which was followed by JOAB (2.2 ug/g) and JOAC (2.3 ug/g) fractions (Figure 4). While in case of JOAH, JOAM and JOAAQ, total phenolic content was significantly lower. The total flavonoid content was significantly higher in JOAE (79.21 ug/g) whilst JOAH, JOAM, JOAAQ, JOAB and JOAC have significantly lower flavonoids content (Figure 3.3).

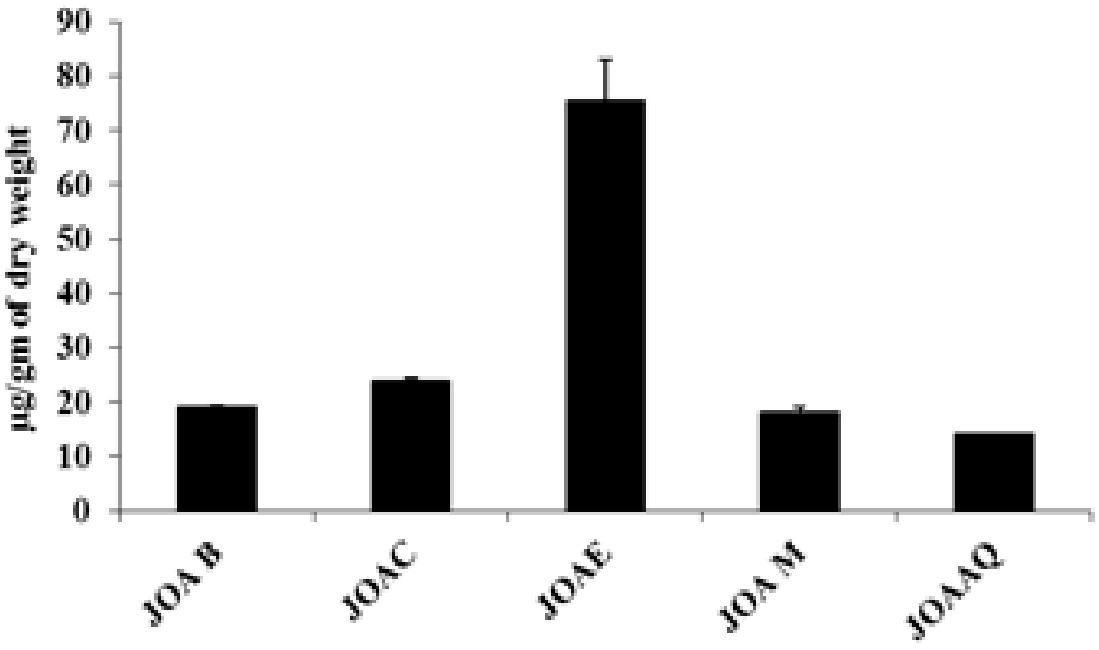


Figure 3.3: Total phenolics and flavonoid contents in various extracts. The error bars shows the standard deviation of the mean values.

3.6.4 Anticancer activities of the extracts:

The extracts were assessed for their anticancer activities using different colorectal adenocarcinoma (HT29 and HCT116), hepatoma derived cell (HepG2) and breast cancer cell (MCF-7) lines. Various low concentrations of the extracts were prepared and tested against the grown cell cultures. The screening results showed that JOAE, JOAB and JOAAQ were suppressing the cancer cell growth while other extracts such as JOAH, JOAC and JOAM were inactive as compared to the control. The active fractions were subjected to concentration gradient effects of extracts. According to results of colorectal adenocarcinoma (HT29 and HCT116) cell lines treatments, 100 ug/mL of JOAE, 25 ug/mL of JOAB and 1 ug/ml of JOAAQ has reduced the cancer cell viability as compared to other extracts, their concentrations and control cells (Figure 3.4).

In case of HepG2 cancer cells, none of the concentrations of JOAE, JOAB and JOAAQ were active to suppress the growing cancer cells. Most of the cellular activity and growth was higher than the control. However, when these extracts were applied to MCF-7 cell lines, the lower concentrations such as 1 ug/mL and 5 ug/mL of JOAE, JOAB, and JOAAQ has suppressed the cancer cells as compared to higher concentrations of the extracts (Figure 3). To know the IC50 activity of the extract, the concentration was increased to 1000 ug/mL. The treatment of JOAE, JOAH, JOAC and JOAM significantly suppressed the cancer cell's viability of HT29 and HCT116 to 42, 15, 14 and 22% respectively. However, the JOAB and JOAAQ did not affect the cancer cell viability. Our results shows that O. arabicus has the potential to discover the potent anticancer agents.

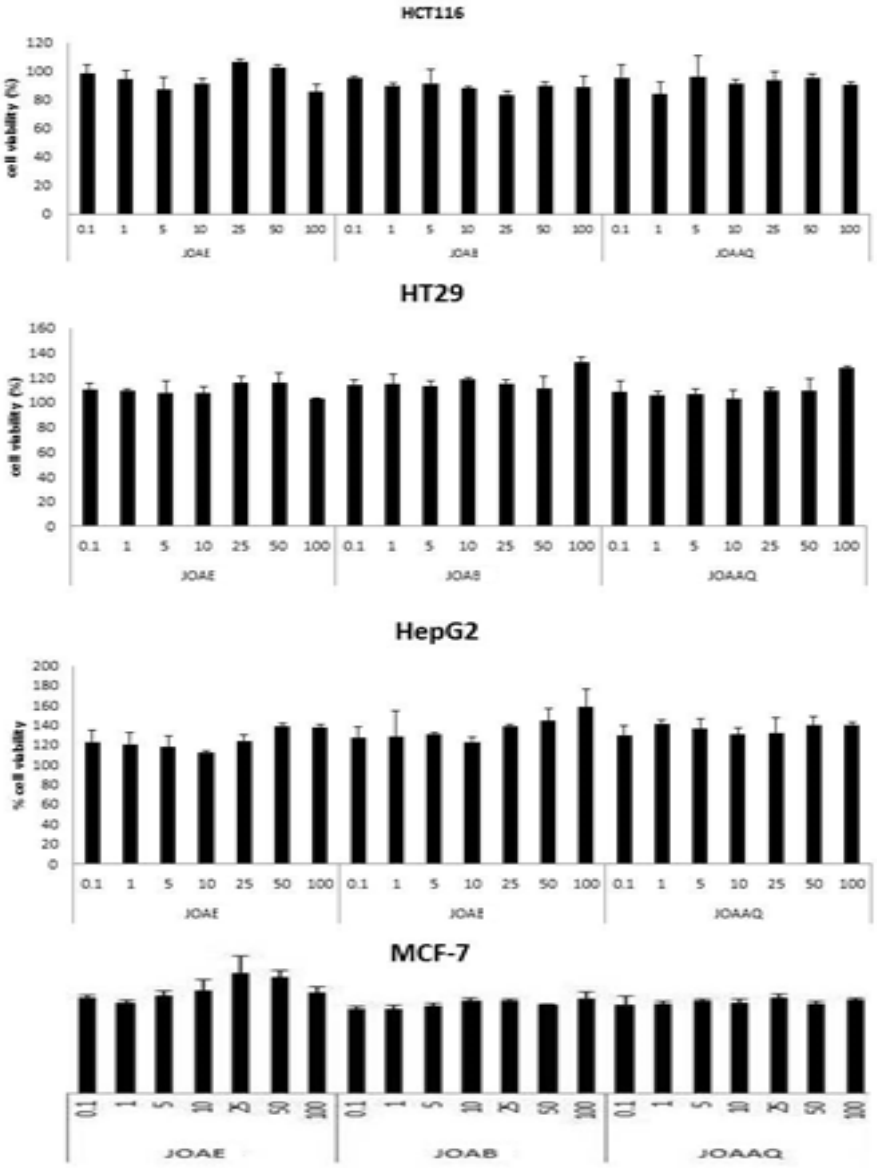


Figure 3.4: Anticancer activities of various concentrations of the extracts against HT29, HCT116, HepG2 and MCF-7lines. The error bars shows the standard deviation of the mean values.

3.6.5 α-Glucosidase activity:

The results of the assay shows that only JOAB was 10% active to inhibit the activity as compared to acrabose while other extracts JOAC, JOAE, JOAH, JOAAQ, and JOAM were inactive to present any inhibitory effect during the reaction.

Discussion

Discussion

Medicinal plants are plants that have a recognized medical use. Medicinal plants considered as rich resources of bioactive phytochemicals, can be used in drug development and synthesis. Numerous approaches have been used to obtain these compounds for drug discovery including isolation from plants and other natural sources. (Kuhlmann, 1997). *Ochradenus arabicus* is a species belong to the Ochradenus genus. *Ochradenus arabicus* has a multitude of medicinal properties, because of which it is widely used locally for curing different ailments in many countries including Saudi Arabia (Nadeem *et al*., 2012). Previous literature revealed that the plants of *Ochradenus baccatus* were tested for antitumor, antimalarial (protozoa) and growth inhibition of wheat-rootlet activities which exhibited strong cytotoxicity (above 97%) against cultured melanoma cell lines (Vincken *et al*,. 2007).

In this study compound 1-octacosanol was isolated from the chloroform fraction of *Ochradenus arabicus* at 20% EtOAc/Hex using column chromatography and the structure was conformed through NMR. The same compound previously reported by (Hou *et al.*, 2002). They subjected methanol extract of *Nepeta prattii* to preparative HPLC and preparative TLC and the structure was conformed through NMR.

Accordingly we have isolated compound 2 from the Chloroform fraction as white color at 20% EtOAc/Hex. Rahmana *et al*., (2009) isolated β-sitosterol and stigmasterol from leaves of *Ocimum sanctum*. The active fraction (containing the major compound b-sitosterol and the minor compound stigmasterol) was isolated and purified by silica gel column chromatography and the structure was conformed using spectroscopic analysis (EIMS, ^{1}H NMR, ^{13}C NMR).

Anderson *et al.,* (1991) previously reported beta-sitosterol-D-glycoside from the methanolic extract of whole plant *Astragalus altaicus.* The compound was characterized by using spectroscopic methods especially 1D and 2D NMR and ESI mass spectrometry. We have isolated the same compound through column chromatography at 80% EtOAc/Hex from the chloroform fraction of the plant. The structure was conformed using different spectroscopic techniques.

We have isolated Ursolic Acid from the chloroform fraction of the plant at 20% EtOAc/Hex. The fraction was subjected to column chromatography and the structure was conformed through NMR. (Hussain *et al.,* 2008) isolated the compound Ursolic Acid from the

Chloroform fraction of the air dried roots of *Nepeta suavis*. They used silica gel chromatography for isolation and the structure was conformed using different spectroscopic methods.

Lu *et al.,* (1983) reported the isolation of Choerospondin from the Ethyl Acetate fraction of the bark of *Choerospondias axillaris.* They subjected the fraction to column chromatography and structure was carried out by spectroscopic methods NMR and ESI mass spectrometry. We have isolated the compound Choerospondin using silica get chromatographic technique at 30% EtOAc/Hex from the chloroform fraction of the plant. The structure was conformed through spectroscopic techniques.

Allelopathy is the effect of a plant on the growth and development of another plant species by emission of different chemicals. Studying the phenomenon of Allelopathy is considered to be very helpful to obtain a sustainable agriculture (Khan *et al.,* 2009). The reason for inhibitory effect is the presence of some Allelochemicals in the extract of *Ochradenus arabicus* affecting the germination potential of seeds. Fujii *et al.,* (2004) also presented similar results. According to their reports, increased concentrations of leaf extracts (Sapindus emarginatus, Terminalia tomentasa, and Vitex negundoi) supressed the growth of some vegetable species. However, this was also observed that reduced concentrations of such extract can also stimulate the germination and growth of seeds (Khan *et al.,* 2009).

In the present study, the radical scavenging effects were high in JOAE sub fraction, suggesting the presence of bioactive metabolites which has oxidative stress mitigation properties. Antioxidants have been noticed in various agricultural and food items including cereals, fruits, vegetables and oil seeds (Adom *et al.,* 2003)

During the cell division becomes uncontrolled and cells grow abnormally. It is not one disease but a group of more than 100 diverse disorders. It is the world's second biggest killer after cardiovascular disease and was responsible for the death of 7.6 million people in 2005 (Shoemaker *et al.,* 2005). Three fractions of *Dorema aucheri* were evaluated for cytotoxic activity. Fractions of *D. aucheri* (petroleum ether fraction and chloroform fraction) showed cytotoxic activity against HepG2 and breast cancer cell (MCF-7) lines. While fractions with methanol were found inactive against these cell lines (Mosaddegh *et al.,* 2012). In our study the HepG2 cancer cells, the concentrations of JOAE, JOAB and JOAAQ were found inactive to suppress the growing cancer cells. However, when these extracts were applied to MCF-7 cell lines, the lower concentrations such as 1µg/mL and 5µg/mL has suppressed the cancer

cells. These medicinal plants considered as rich source of bioactive ingredients can be, used in drug development and synthesis. Numerous approaches have been used to obtain compounds for drug discovery including isolation from plants and other natural sources. *O. arabicus* has the potential to discover the potent anticancer agents. Further fractionation of the bioactive fraction can help us to find the lead anticancer compound. Phytochemicals isolated from medicinal plants have been playing a significant role directly or indirectly to combat cancer disorders. In the present study the fractions of JOAE, JOAH, JOAC and JOAM significantly suppressed the cancer cell's viability of HT29 and HCT116 to 42, 15, 14 and 22% respectively. However, the JOAB and JOAAQ did not affect the cancer cell viability. Our results shows that *O. arabicus* has the potential to discover the potent anticancer agents. Further fractionation of the bioactive fraction can help us to find the lead anticancer compound. Phytochemicals isolated from medicinal plants have been playing a significant role directly or indirectly to combat cancer disorders (Wink *et al.,* 2005).

In our study, we evaluated total phenolic contents in all the crude methanol extracts and its sub fractions. The total phenolic content was significantly higher (6.43 µg/g) in JOAE. The total flavonoid content was significantly higher in JOAE (79.21 µg/g) whilst JOAH, JOAM, JOAAQ, JOAB and JOAC have significantly lower flavonoids content. Numerous flavonoids have been isolated previously from the aerial parts of *Ochradenus baccatus* (Barkat *et al.,* 1991). Flavonoids play a very important role in various pharmacological actives like anti-oxidant and anti-diabetic of plants (Pinent *et al.,* 2008).

Conclusion

Medicinal plants considered as rich source of bioactive phytochemicals, can be used in drug development and synthesis. Numerous approaches have been used to obtain these compounds for drug discovery including isolation from plants and other natural sources. In the present study we performed various biological assays including phenolic, flavonoid, antioxidant, allophatic, alpha-glucosidase inhibitor and anticancer. Some of the fractions showed very good activity against cancer cell lines. This activity correlates with the higher phenolic and flavonoid contents of the extract of the studied plant. This indicates that this plant have a potential bioactive compounds that could be isolated and used for clinical trial for further research in cancer studies.

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