Phytochemical composition, antioxidant and potential anti-cancer activity of extracts from Drumstick (*Moringa oleifera*) and Quinine tree *(Rauwolfia caffra*)

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in Biotechnology, University of Nairobi

By

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JULY 2013

DECLARATION

I the undersigned, declare that this thesis is my original work and to the best of my knowledge has not been presented for the award of a degree in any other university.

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DEDICATION

This thesis is dedicated to my parents, John Milugo and Hellen Milugo for playing a vital role in my upbringing and encouragement to pursue my Master in Science (MSc.) studies.

ACKNOWLEDGEMENT

My most sincere gratitude goes to my supervisors, Dr. Leonidah Kerubo, Dr. Fred Wamunyokoli, Dr. Julius Oyugi and Prof. James Ochanda for their guidance, excellent advice and patience throughout the period of my research work. I will forever be grateful to Bethwel Owuor for always ensuring that I had enough plant materials for my research work.

I acknowledge the German Academic Exchange Services (DAAD) and Gandhi Smarak Nidhi Fund for the scholarship award for my MSc studies. I sincerely thank Dr. Wallace Bullimo for giving me the opportunity to undertake training on tissue culture in his laboratory, and together with Prof. Philip Nyaga for their assistance with the cell lines.

I greatly appreciate Drs. Isabella Ocholla and Joel Ochieng for their support and encouragement throughout the course of my study. Many thanks go to the technical staff of the Department of Medical Microbiology, Institute of Tropical and Infectious Diseases, and the Natural Product group, Department of Chemistry, University of Nairobi. I also commend the assistance by my colleagues at the Center for Biotechnology and Bioinformatics (CEBIB).

Finally, I thank my family for financial and moral support throughout my study. Their love and care gave me the will to carry on to the end.

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LIST OF ABBREVIATIONS

$AlCl_3$	Aluminum chloride
BHT	Butylated hydroxytoluene
CO_2	Carbon dioxide
CH_2Cl_2	Dichloromethane (DCM)
C_6H_{12}	*n*-hexane
DMEM	Dubelcos Minimum Essential Medium (DMEM)
DMSO	Dimethyl sulphoxide
DNA	Deoxyribonucleic acid
DPPH	1, 1-diphenyl-2-picrylhydrazyl
ELISA	Enzyme Linked Immunosorbent Assay
FBS	Fetal Bovine Serum
H_2SO_4	Sulfuric acid
HCl	Hydrochloric acid
Hep-G2	Hepatocellular carcinoma cell line
HPTLC	High Performance Thin Layer Chromatography
HRMS	High Resolution Mass Spectrometry
IC_{50}	Half Maximal Inhibitory Concentration
KEMRI	Kenya Medical Research Institute
Km	Kilometers
MeOH	Methanol
Min	Minutes
Ml	Mililiters
Mg	Magnesium

NCD	Non Communicable Diseases
NMR	Nuclear Magnetic Resonance
OD	Optical Density
PenStrep	Penicillin/streptomycin antibiotic
RD	Rabdomyosarcoma cell line
ROS	Reactive Oxygen Species
RSA	Radical Scavenging Activity
TLC	Thin Layer Chromatography
USA	United States of America
UV	Ultra Violet Light
WHO	World Health Organization

ABSTRACT

This study had the aim of phytochemically evaluating the usefulness of two plants (*Moringa oleifera* and *Rauwolfia caffra*), used in traditional health care to manage cancer and diseases related to oxidative stress. To achieve this, the leaves of *M. oleifera* and the leaves and stem bark of *R. caffra* were extracted with different solvent systems and subjected to Radical Scavenging Activity (RSA) assay to determine the level of antioxidant activity. Establishing the antioxidant activitiy of the extracts of the two plants is important since strong antioxidants are normally associated with the prevention of cancer.

RSA was assessed using 1, 1-diphenyl-2-picrylhydrazyl (DPPH) method and the commercially available quercetin was used as a positive control. The 50 % CH_2Cl_2: MeOH extracts of the stem bark of *R. caffra* exhibited the highest RSA of 96.03 % at 0.2 mg/ml while the MeOH extract of the leaves of *M. oleifera* showed RSA of 83.84 % at 0.2 mg/ml. The *R. caffra* stem bark fractions obtained by fractionation of the total extract (CH_2Cl_2: MeOH; 1:1) revealed possible antagonistic effect in at least two classes of secondary metabolites: co-occurrence of alkaloids and saponins reduced antioxidant activity (all phytochemicals = 58 %; alkaloids only = 63 %; alkaloids plus saponins = 15 %). However, synergistic activity was observed for a combination of steroids, terpenoids and cardiac glycosides, but without alkaloids (82 %).

Anti-poliferative activity was assessed using crystal violet assay where human hepatocellular carcinoma (Liver cancer; Hep-G2) and rhabdomyosarcomas (Muscle cancer; RD) were used as model cell lines, while Vero cells were used as control, and to test for possible cytotoxicity to normal cells. The MeOH extract of the leaves of *M. oleifera* displayed significant anti-proliferative activity ($p < 0.05$) against Hep-G2 and RD cell lines with limited activity on Vero cells. Comparatively, proliferation of RD cell lines was more affected than Hep-G2. The 50 % MeOH/CH_2Cl_2 extract of the stem bark of *R. caffra* did not show significant activity against proliferation of RD and Hep-G2 cells, but it exhibited activity against proliferation of Vero cells.

Overally, *M. oleifera* leaf extracts were less toxic and displayed anti-proliferative activity while extract of the stem bark of *R. caffra* was not target specific. However, the latter exhibited significant antioxidant activity. The usefulness and risk levels associated with drumstick and

quinine tree; plants used in traditional medicine to manage cancer and diseases related to oxidative stress, require further investigation as the solvent systems used in this study, may not exactly mirrow those used by traditional healers. Phytochemicals have been shown to act in a solvent specific manner.

CHAPTER ONE

INTRODUCTION

1.0 GENERAL

Cancer remains a major global health burden and is currently ranked as the third leading cause of death worldwide after cardiovascular and infectious diseases (WHO, 2013). A recent situational analysis showed cancer cases in Kenya to stand at 22,000 deaths annually (Ministry of Public Health and Sanitation and Ministry of Medical Services, Kenya 2011). Lack of effective chemotherapeutic drugs for cancer treatment may be a major contributor to the high fatality rate. Most drugs that are currently used in cancer treatment and management are costly and their success rates are low. For instance, chemotherapy which is the most common mode of cancer treatment today involves the use of chemotherapeutic drugs which tend to kill normal and cancerous cells alike, thus causing severe side effects (National Cancer Institute,USA 2011). Such challenges have made it necessary to continuously search for alternative drugs for cancer management. A number of compounds with anticancer activity have previously been isolated from plants and synthesized for use in contemporary medicine, indeed most promising anticancer drugs contain a bioactive compound that can be traced to plants (Heinig and Jennewein, 2009). Kenya has a wealth of indigenous knowledge in traditional medicine; for instance, the Kuria community uses certain plants (*Moringa oleifera* and *Rauwolfia caffra*) in traditional management of multiple tumors (Owuor B, personal communication). As such, it is possible that these plants contain bioactive molecules that can suppress tumor. This study investigated the anticancer properties of *Moringa oleifera* and *Rauwolfia caffra*.

1.1 Cancer prevalence and therapy

Cancer is a class of disease that is characterized by uncontrolled division of cells and is one of the most common diseases with a high incidence and mortality rate globally (World Health Organization (WHO), 2013). Current statistics indicate that over 7.9 million people succumb to cancer related illness every year and this is expected to rise to 13.1 million by 2030 if immediate interventions are not put in place (WHO, 2013). To lower the mortality rate of cancer, several cancer treatment methods such as chemotherapy, radiation therapy and surgery have been developed. In chemotherapy, drugs are designed to arrest the cell cycle or cause apoptosis of

cancerous cells. However, their mode of action involves targeting rapidly dividing cells, hence they are known to causes severe side effects to some cells in the body such as; bone marrow cells, immune cells and hair follicle cells that portrays similar characteristics (National Cancer Institute, USA 2011). Radiation therapy works by damaging the deoxyribonucleic acid (DNA) of the cancerous cells, but this may also damage the DNA of adjacent normal cells leading to adverse side effects (National Cancer Institute,USA 2011). Surgery is yet another form of cancer treatment; the kind of surgery varies depending on the type of cancer and the patients' health (National Cancer Institute, USA 2011).

1.2 Oxidative stress and antioxidants

Oxidative stress is a condition that occur when the level of free radicals in the body increases beyond the scavenging ability of the antioxidants in the body, resulting in severe consequences such as DNA damage, protein oxidation or lipid peroxidation (Paliwal *et al.,* 2011; Turrens, 2003). Free radicals are unstable and very reactive molecules that are produced as byproducts of normal biological processes such as mitochondrial oxidative phosphorylation, prostaglandin synthesis and phagocytosis (Halliwell, 1989), as well as on exposure to environmental factors such as pollution, radiation and cigarette smoke (Hamid *et al.,* 2010). Therefore, the human body is usually exposed to plenty of free radicals that may interfere with the functioning of the cells. The body has developed natural antioxidant defense mechanism to help guard against oxidative stress (Halliwell, 1989; Paliwal *et al.*, 2011).

Antioxidants are molecules with high affinity for free radicals and they are of two types; natural and synthetic antioxidants. Natural antioxidants such as vitamin A **(1)** (Figure 1), C **(2)** (Figure 1), and E **(2)** (Figure 1), are obtained from herbs (Paliwal *et al.*, 2011), grains (Hodzic, 2009), spices (Ghasemzadeh and Rahmat, 2010; Maizura and Wan Aida, 2011), fruits (Ramamoorthy and Bono, 2007) and vegetables (Kunyanga *et al,* 2012) while synthetic antioxidants such as Butylated Hydroxytoluene (BHT) **(4)** (Figure 1)**,** and propylgallate **(5)** (Figure 1), are commercially produced. Synthetic antioxidants are associated with carcinogenesis whereas natural antioxidants are considered safe and have been isolated and used as ingredients in dietary supplements for management of various diseases such as cancer, coronary heart diseases and many others (Hamid *et al.,* 2010).

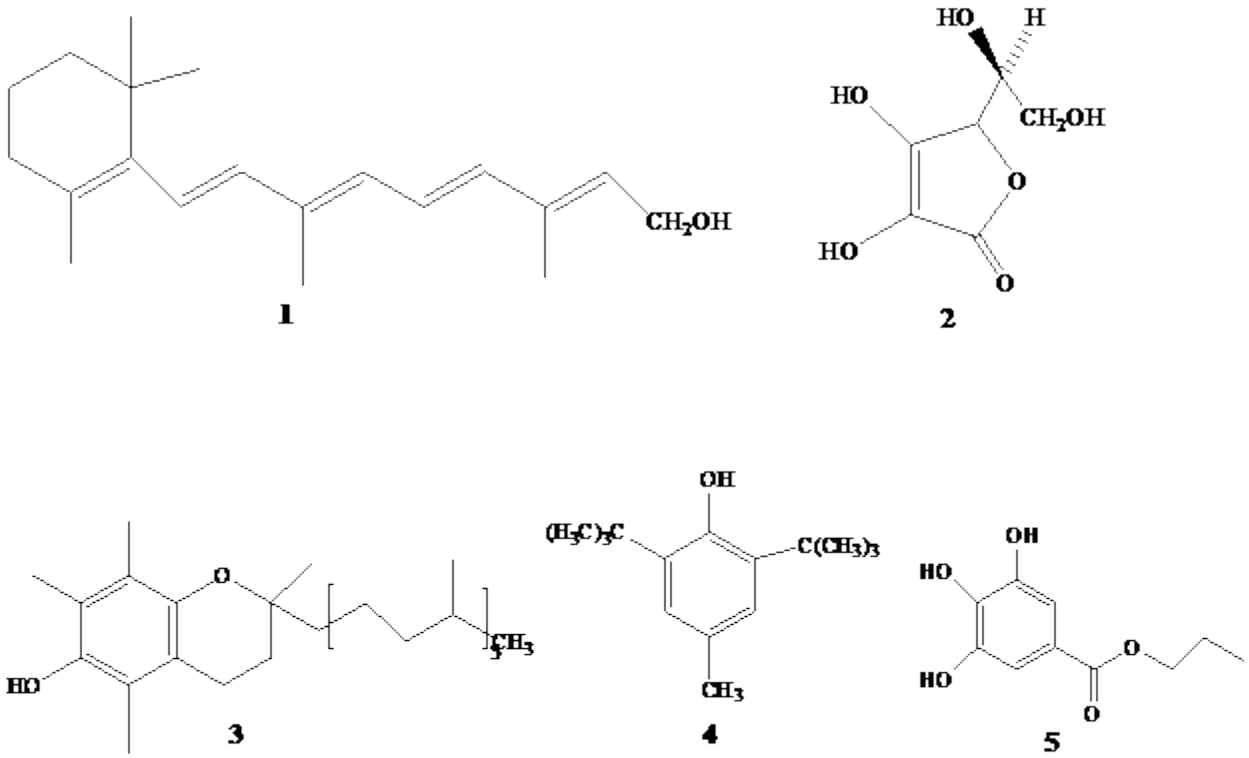

Figure 1: Structure of compounds with antioxidant activity. Vitamin A **(1)**, C **(2)** Vitamin E **(3),** BHT **(4)** and Propylgallate **(5)**

1.3 PROBLEM STATEMENT

Medicinal plants are commonly used by many communities across the world to treat various ailments; however, there is very little scientific evidence to verify the efficacy of bioactive compounds present in these plants. This is because ethno-medical knowledge is normally held in secrecy by traditional herbalists, making it difficult to conduct any scientific investigations. The Kuria community of Kenya for instance, uses certain medicinal plants to manage tumor and related ailments. Despite such promise in cancer treatment, the biochemical mode of action of these plants remains unknown. As such, it is difficult to design a novel anticancer drug from the extracts of these plants since doing so requires background knowledge of the identity, composition and structure of the bioactive phytochemicals. Thus, assessing the efficacy of bioactive compounds present in medicinal plants and determining their specificity against certain cancer types is essential. To facilitate an immediate uptake of these results by pharmaceutical industries, the active pharmaceutical compounds present in these plants needs to be isolated and described. Further, assessment on side effects and biosafety of the pure compounds is also required.

1.4 RESEARCH OBJECTIVES

1.4.1 General objective

To biochemically assess the usefulness of phytochemicals present in drumstick and quinine tree in management of cancer and other diseases related to oxidative stress.

1.4.2 Specific objectives

I. To determine whether drumstick and quinine tree can be used to suppress cell proliferation and kill cancerous cells.

II. To asses the level of risk associated with the use of drumstick and quinine tree in cancer management in traditional medicine.

III. To understand the mechanism of action of active compounds from the two plants by identifying major phytochemicals present.

1.5 JUSTIFICATION

Exploring locally available therapies for management of chronic diseases such as cancer should be encouraged since most currently available anticancer drugs are costly and have low efficacy. In other regions of the world, search for new therapeutic alternatives for treatment of diseases such as cancer have focused on plants. Kenya is home to great biological diversity including indigenous plants from which potential lead structures for use in formulation of novel drugs can be obtained. Drumstick (*M. oleifera*) and quinine tree (*R. caffra*) are plants used in traditional medicine to manage cancer and diseases related to oxidative stress such as rheumatism, inflammations and diabetes. These diseases are usually managed in formal hospitals but people in many parts of the world are now increasingly getting attracted to herbal medicine. These are cost effective, easily available and acceptable to local communities. It is risky; however, if these herbs don't really work yet people suffering from various tumor-related ailments fail to seek formal medical attention. It needs to be established scientifically that these herbs indeed have bioactive compounds that can cure cancer. There is also need for safety assessments to evaluate if any risk is associated with their consumption. Such knowledge will inform pharmaceutical industry on the medicinal value of these plants, and will also validate indigenous knowledge as valuable resource for medicine and pharmaceutical explorations.

1.6 HYPOTHESIS

Drumstick (*M. oleifera*) and quinine tree (*R. caffra*) have phytochemicals with tumor suppressing ability.

CHAPTER TWO

LITERATURE REVIEW

2.1 Botanical information

2.1.1 The family *Moringaceae*

Drumstick (*M. oleifera*) also known as the horse-randish tree, belongs to the family *Moringaceae*; a monogeneric family that has thirteen species consisting of shrubs or trees (Navie and Csurhes, 2010). The members of this family are recognized by their compound leaves which have a unique unpleasant smell when crushed. The flowers have five stamen held on one side of the flower while the fruits contain winged seeds and are often pollinated by wind, bees and birds (Navie and Csurhes, 2010). They can be used as food, fodder, or as ingredients in traditional medicine (Fahey, 2005; Navie and Csurhes, 2010).

2.1.1.1 The genus *Moringa*

The genus consist of the following species; *M. arborea* (endemic to Kenya), *M. borziana*, *M. concanensis*, *M. drouhardii, M. hildebrandtii, M. longituba*, *M. oleifera, M. ovalifolia, M. peregrine, M. pygmaea, M. rivae, M. ruspoliana* and *M. stenopetala* (Navie and Csurhes, 2010). The species are further categorized into three groups; the first group "bottle neck tree" consists of four species of trees characterized with swollen trunks and radially symmetrical flowers. The second group "tuberous clade" consists of six species with thick and fleshy tuberous roots and the third group "slender tree clade" consists of three species with slender-trunked trees and bilaterally symmetrical flowers (Navie and Csurhes, 2010)**.** *M. oleifera* which is the most widely cultivated species belongs to the "slender tree clade".

2.1.1.1.1 Ethno-medicinal uses of the genus *Moringa*

Plants in the genus *Moringa* have been used by many communities in the world to manage a number of ailments as listed in table 1.

Table 1: Ethno-medical uses of the genus *Moringa*

Plant species	Plant part	Uses	Refrences
M. oleifera	Leaves	Inflammation	Chuang *et al.*, 2007
		Urinary tract infection, epstein-barr virus, herpes simplex virus	Fahey, 2005
		Bronchitis, throat infection, anaemia	Fuglie, 2001; Fahey, 2005
		Hypertensive agent	Faizi and Siddiqui, 1992
		Antibacterial, antifungal agent	Omino and Kokwaro, 1993; Fahey, 2005
	Leaves with roots	Pulmonary diseases, headache,	Omino and Kokwaro, 1993; Fahey, 2005
	Leaves with oils	Purgative, gout and inflamation	Fuglie, 2001; Fahey, 2005; Chuang *et al.*, 2007
	Leaves with roots	Diarrhoea	Fuglie, 2001; Fahey, 2005
	Leaves with gum	Dysentery	Fuglie, 2001; Fahey, 2005
	Gum	Typhoid, syphilis, erache	Fuglie, 2001; Fahey, 2005
	Roots	Rheumatism	Navie and Csurhes, 2010
		Flatullence, kidney pain	Fuglie, 2001; Fahey, 2005
	Root bark with gum	Dental caries, tooth ache, asthma	Fuglie, 2001; Fahey, 2005
	Pods	Joint pains	Fuglie, 2001; Fahey, 2005; Navie and Csurhes, 2010
	Bark	Stomach disorders	Navie and Csurhes, 2010
		Snake bite	Fahey, 2005; Fuglie, 2001
	Flowers	Inflammations	Navie and Csurhes, 2010
	Flowers with root barks	Common cold	Fuglie, 2001
	Seeds	Warts, inflammation	Fuglie, 2001 Chuang *et al.*, 2007
	Leaves, flowers, seeds with bark	Anti-tumour, inflammation	Fahey, 2005; Faizi, *et al.*, 1998

2.1.1.2 *Moringa oleifera*

Drumstick (*M. oleifera*) also known as Miracle tree in English and Mronge in Kiswahili is a fast growing evergreen tree that grows to the height of 15 meters. It has swollen underground rootstocks that have a taste of horse-radish hence, its normally called horse-radish tree (Navie and Csurhes, 2010). It produces large elongated capsules containing numerous seeds; these seeds contain large quantities of oil which are used as medicine (Table 1), fuel, perfume or as lubricants (Fahey, 2005; Navie and Csurhes, 2010). Although *M. oleifera* is native to the Himalayan tracts of India, Pakistan, Bangladash and Afghanistan, it has currently become naturalized in many locations in the tropics (Fahey, 2005). In Kenya for instance, it is found in Malindi, Likoni, Kitui, Kibwezi (Muluvi *et al.,* 1999), Kuria and Suba counties (Owuor B, personal communication).

Figure 2: A typical *M. oleifera* tree growing in a natural environment. Photo by Price, 2007

2.1.2 The family *Apocynaceae*

Quinine tree (*R. caffra*) belongs to the family *Apocynaceae* which is a family of flowering plants that consist of 164 genera and 1,500 species most of which are herbs, trees, lianas and shrubs. Most of these plants are known to be poisonous and to produce milky latex when injured (Watson and Dallwitz, 1992)**.** The family is divided into five subfamilies; *Apocynoideae, Rauvolfioideae, Asclepiadoideae, Periplocoideae* and *Secamonoideae.* Plants of this family have flowers that are bisexual while the leaves are usually evergreen, alternate or opposite, simple or whorled and lucks stipules. The seeds are flat and winged and may have a tuft of hairs at one end (Watson and Dallwitz, 1992). The plants can be used as food, fodder, ornaments, medicine or as arrow poison (Watson and Dallwitz, 1992)**.**

2.1.2.1 The genus *Rauwolfia*

The genus *Rauwolfia* is named after Leonhard Rauwolf a German physician who was specialized in plants collection (Orwa *et al.,* 2009). This genus has 42 species most of which are poisonous hence their use as medicinal plants is highly discouraged (www.prota.org). Examples of species in this genus are; *R. serpentina, R. caffra, R. vomitoria, R. stricta, R. media* and *R. mombasianna* which is native to Kenya (www.prota.org).

2.1.2.1.1 Ethno-medical uses of the genus *Rauwolfia*

The plants in the genus *Rauwolfia* are characterized by poisonous shrubs and small trees that are rich in alkaloids; basic organic substances containing at least one nitrogen atom in their structure (Hamid *et al.,* 2010). Plants in this genus have been used extensively by many communities in the world to treat a number of ailments, their ethnobotatical uses are described in table 2.

Table 2: Ethno-medical uses of the genus *Rauwolfia*

Plant species	Plant part	Uses	Refrences
R. caffra	Bark	Inflammation, rheumatism, pneumonia, coughs	Orwa *et al.*, 2009; www.prota.org
	Root	fractures, abdominal pain, fever	Omino and Kokwaro, 1993; Orwa, *et al.*, 2009;www.prota.org
	Stem with root bark	Internal parasites such as Roundworms, tapeworms	Orwa *et al.*, 2009; www.prota.org
	Root bark	Hypertension, psychoses	Orwa *et al.*, 2009; www.prota.org
		Abscesses	www.prota.org
	Flowers	Wounds	Orwa *et al.*, 2009; www.prota.org
	Leaves	Headache	Omino and Kokwaro, 1993, www.prota.org
R. vomitoria	Roots	Malaria, tumor, diabetes, skin diseases and opportunistic infections in HIV/AIDS patients	Erasto *et al.*, 2011
	Root with stem bark	Root and stem bark	Saeed *et al.*, 1993
	Leaves	Diabetes	Campbell and Mølgaard, 2006
R. sellowii	Roots	Hypertension	Batista *et al.*, 1996
R. serpentina	Root	Inflammation	Falkenhagen *et al.*, 1993; Saeed *et al.*, 1993
		Tumors	Wachsmuth and Matusch, 2002
R. sandwicensis	Root	Anti-arrhythmic agent	Saeed *et al.*, 1993
R. mauiensis	Root	Anti-arrhythmic agent	Saeed *et al.*, 1993
R. stricta	Leaves	Tumors	Henry, 1932
R. media	Root	Pimples and itching	Omino and Kokwaro, 1993
R. mombasianna	Leaves	Abscess	Omino and Kokwaro, 1993
	Root with bark	Malaria, scabies and venereal diseases	Omino and Kokwaro, 1993 Muthaura *et al.*,2007

2.1.2.2 *Rauwolfia caffra*

Quinine tree (*R. caffra*) also known as mwembe mwitu or mkufu in Kiswahili is an evergreen tree that grows to the height of 35 meters, forming a dense crown. It has simple leaves which are shinny green on the upper surface but paler on the lower surface. The flowers are bisexual and have a cup shaped calyx with the anthers slightly above the stigma (Orwa *et al.*, 2009). *R. caffra* grows at altitudes of 0 to 1,500 meters and is usually found along streams or in swampy forest. In Kenya, *R. caffra* is commonly found along the coastal region (Orwa *et al.*, 2009).

Figure 3: A typical *R. caffra* tree in natural environment. Adopted from www.prota.org.

2.1.3 Biological activity of extracts and compounds from *M. oleifera*

Drumstick is used in traditional medicine to treat respiratory tract infections (Mehta and Agrawal, 2008), bacterial and fungal infections, and sexually transmitted diseases (Rahman *et al.,* 2009). The leaves have strong antioxidant activity (Chumark *et al.*, 2008; Khalafalla *et al.*, 2010) while the seeds contains high levels of monosaturated oils and proteins, hence, the plant is commonly given to infants and nursing mothers suffering from malnutrition to boost their immune systems (Fahey, 2005; Tsaknis *et al.*,1999). The seeds contain compounds of pharmaceutical importance and this might explain the extensive use of *M. oleifera* seeds in the treatment of various diseases (Table 1). Additionally, the seeds are used as water disinfectant since they contain polypeptide molecules that act as cationic polymers (Lantagne *et al.*, 2008).

Informed by its wide traditional medicinal uses, chemical investigations have been carried out on *M. oleifera* and several bioactive compounds isolated and identified: Examples of such compounds include; the antioxidant kampeferol **(6)** (Figure 4) which was isolated from the leaves (Bushra and Anwar, 2008), and pterygospermin **(7)** (Figure 4) a potent antibiotic and antifungal agent isolated from the flowers and roots (Horwath and Benin, 2011; Navie and Csurhes, 2010)**.** The root bark contains very toxic alkaloids and is rarely used for medicinal purposes (Navie and Csurhes, 2010).

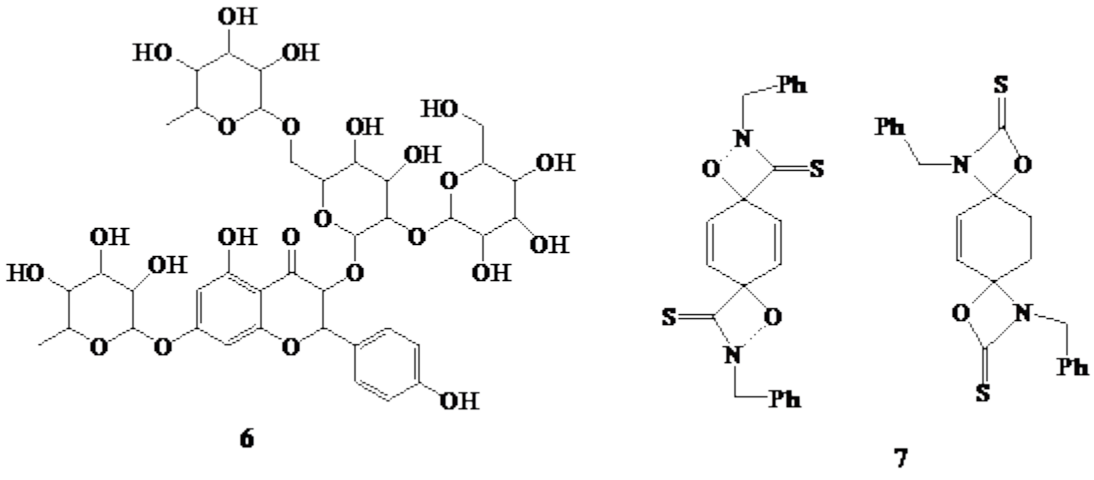

Figure 4: Structure of bioactive compounds from *M. oleifera*; kaempferol **(6)**, pterygospermin **(7)**.

2.1.4 Biological activity of extracts and compounds from *R. caffra*

Previous studies (e.g Erasto *et al.*, 2011; Gbonjubola, 2010; Nasser and Court, 1984) have revealed the presence of pharmacologically important compounds from *R. caffra.* For instance, alkaloids from the root extracts have been shown to have a very strong antimicrobial and antioxidant activity (Erasto *et al.*, 2011). Macrocaffrine (**26)** and yohimbine **(27)** are compounds of medicinal importance found in *R. caffra* (Ohba and Natsutani, 2007). Yohimbine **(27)** for instance, is a potent antidepressant used in treatment of hypertension (Marion, 1952).

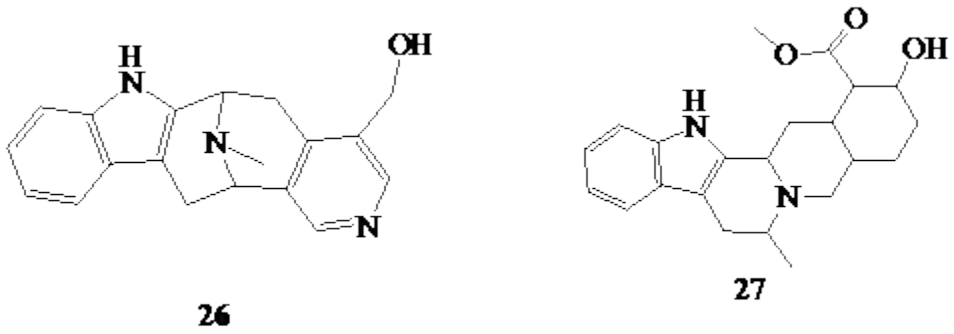

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Figure 5: Structures of bioactive compounds from *R. caffra;* macrocaffrine **(26)**; yohimbine **(27)**

2.1.5 Phytochemistry of the genus *Moringa*

The genus *Moringa* is known to contain a number of phenolic compounds as shown in table 3 and the respective structures are found in **Appendix 1**. The basic structure of phenols consists of a hydroxyl group attached to a benzene ring **(8).**

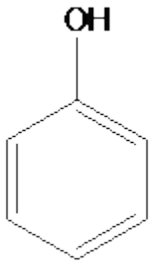

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Table 3: Compounds previously described in *Moringa* species

Compounds	Source	Reference
Phenols		
Amino methoxysulfinyl pentasilfide **(9)**	*M. oleifera*	Faizi *et al.,* 1998
3,4-Dihydro-3-8-dihydroxy-3-1H-2-benzopyran-1-one **(10)**		Assante *et al.,* 1977
2,6-Dimethylbenzoic acid glucopyranosyl ester **(11)**		Memon and Memon, 1985
3,3´,4,4´,5,5´,7 heptahydro3-0-(β-D-galactopyranose,D-glucopyranose) **(12)**		Asem and Laitonjam, 2008
4-hydroxybenzaldehyde O-(4-O-acetyl-α-rhanmopyranoside) **(13)**		Faizi *et al.,* 1994; Faizi *et al.,* 1998
p-Salcylic acid **(14)**		Strohl and Seikel, 1965
4-Hydroxybenzyl glucosinolate **(15)**	*M.oleifera M.peregrina*	Fahey *et al.,* 2001
4-Hydroxybenzyl isothiocynate **(16)**	*M.oleiferaM.peregrina, M.stenopetala*	Kær *et al.,*1979; Eilert *et al.,* 1981
(4-hydroxybenzyl) carbamic acid **(17)**	*M. oleifera*	Faizi *at al.,* 1998; Tiwari *et al.,* 2011
(4-hydroxybenzyl) thiocarbamic acid**(18)**		Francis *et al.,*2004
4-Hydroxyphenylacetic acid **(19)**		Faizi *et al.,* 1995
4-Hydroxyphenylthiocarbamic acid **(20)**		Faizi *et al.,* 1995
Kaempferol 3,7-diglycopyranosyl (β-D-glucopyranosyl-(1-2) -(αrhamnopyranosyl-(1-6)-*β*-d-glucopyranoside), 7-O-α-L-rhamnopyranoside **(6)**		Faizi *et al.,* 1995; Bushra and Anwar, 2008
Rhamnose; α-l-pyranose-form, Ph glycoside **(21)**		Francis *et al.,* 2004
3-(2-Heptenyloxy)-1,2-propanedioal-3-undecanoyl **(22)**		Faizi *et al.,* 1994; Faizi *et al.,* 1998
Niazidin **(24)**		Francis *et al.,* 2004
4´,5,7-Tryhydroxyflavonon 5- Me ether, 4´-O-(α-L-rhamnopyranosyl-(1-2)-β-D- glucopyranoside), 7-O-β-D- glucopyranoside **(25)**		Albach and Redman, 1969

2.1.6 Phytochemistry of the genus *Rauwolfia*

The genus *Rauwolfia* is known to contain a number of alkaloids (table 4 and **Appendix 2)**. Alkaloids are a large group of nitrogen-containing secondary metabolites of plant, microbial or animal origin (Hamid *et al.*, 2010). The term originally implied pharmacologically active bases of plant origin, but the definition has subsequently been broadened to include majority of nitrogen containing natural products with the exception of simple aminoacids, proteins and nitrogen-containing substances of polyketide origin such as the aminoglycoside antibiotics. They are bitter and some are very toxic and are normally classified according to their pharmacological properties e.g. analgesic, stimulant or anti-malarial alkaloids, or according to their sources e.g opium, vinca and cinchona alkaloids (Kashani *et al.*, 2012). Alkaloids from the genus *Rauwolfia* can be classified into a number of skeletal classes. The main alkaloid types identified from the stem bark of *R. caffra* includes; ajmaline (**8**) which contains both 5, 16 and 7, 17 bonds. Almost all the bases in this group contain the same skeleton, however, perakine (**93**) and raucaffrinoline (**94**) afford a rare structural variation in which the 21, N bond has been replaced by a 19, N bond; heteroyohimbines (**29**, **31**, **117-122**); E-seco indole (**69-73**); indolenine (**93-94**) and indole (**59-63, 96**).

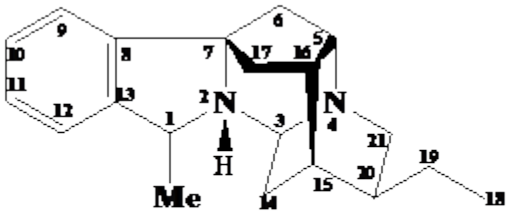

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Table 4: Compounds previously described in *Rauwolfia* species

Class of compound	Source	References
Ajmalicine; (-)- form **(29)**; Ajmalicini al; N^{+}-Methoxycarbonyl **(30)**	*R. serpentina*	Henry, 1932; Nasser and Court, 1984;
Ajmalicinine **(31)**	*R. caffra*	Nasser and Court, 1984
Akuammicine; (-)-form **(32)**	*R. volkensii*	Henry, 1932
Akuammicine; (-)-form, 19,20 *S*-Dihydro **(33)**	*R. caffra*	Habib, 1974; Henry, 1932; Nasser and Court, 1984
Akuammidine **(34)**	*R. vomitoria*	Silvers and Tulinsky, 1962

Akuammiline **(35);** Akuammine; Me ether **(36)**	*R. oreogiton*	Akinloye and Court, 1980
Alkaloid RMB 10 **(37)**	*R. mombasiana*	Iwu and Court, 1979
Alstonine **(38)**	*R. obscura*	Timmins and Court, 1976
Alstonine; 19, 20- Diepimer, 11-methoxy **(39)**	*R. vomitoria*	Bader *et al.,* 1954; Timmins and Court, 1976
Alstonine; 19, 20- Diepimer, **(40)**	*R. cumminsii*	Bader *et al.,* 1954;
Arbutin; 6-0-β-D-xylopyranosyl **(41);** Arbutin; 4´-Me ether, 6-0-β-D-xylopyranosyl **(42)**	*R. serpentina*	Durkee *et al.,*1968; Sakar *et al.,* 1991
Arcine (**43)**	*Rauwolfia spp*	Stoll and Hofmann, 1955
Arcine19, 20- Diepimer, **(44)**	*R. vomitoria*	Stoll and Hofmann, 1955
Caboxine A; 7-Epimer **(45)**	*R. vomitoria*	Amer and Court, 1980
Carapanaubine **(46);**Carapanaubine; 3,7-Diepimer **(47)**;Carapanaubine; *N*-oxide**(48)**	*R. vomitoria*	Shamma *et al.,* 1967; Iwu and Court, 1977 a
1-(β-Carbolin-1-yl)-3,4,5-Trihydroxy-1-pentanone **(49)**	*R. serpentina*	Kitajima, 1966
Cathafoline; 19,20-Epoxide **(50)**	*R. salicifolia*	Kam *et al.,* 1999;Kam,2004
Corynantheine **(51)**	*R. canescens*	Bartlett *et al.,* 1962
Corynantheine ; 17-*O*-De-Me **(52)**	*R. mombasiana*	Bartlett *et al.,* 1962;
Deserpideine **(53)**	*R. nitida*	Amer and Court, 1981
Deserpideine; 11-Methoxy **(54)**	*R. canescens*	Smith, 1967
Deserpidine **(55)**	*R. canescens*	Varchi *et al.,* 2005
16,17, -Didehydro-17-Hdroxy-16 (hydroxymethyl) corynan-5-carboxylic acid **(56)**	*R. caffra*	Nasser and Court, 1984
19,20-Dihydroyohimbine **(57)**	*R. obscura*	Timmins and Court, 1976
Dihydrocorynatheol **(58)**	*R. cumminsii*	English and Williams, 2010
Dihydroperaksine **(59)**	*R. caffra*	Nasser and Court, 1983
Dihydroperaksine; 19- Aldehyde, 17-Ac **(60)**	*R. volkensii*	Akinloye and Court, 1979; Nasser and Court, 1983
Dihydroperaksine; 20,21-diepimer, 17-Aldehyde **(61)**;Dihydroperaksine; 20,21-Diepimer, 10- Hydroxy **(62)**; Dihydroperaksine; 20,21-Diepimer **(63)**	*R. serpentina*	Akinloye and Court, 1979; Nasser and Court, 1983
Vomifoliol **(64)**	*R. vomitoria*	Park and Maldonado, 1979
18-hydroxyyohimbine **(65)**	*R. mombasiana*	Amer and Court, 1981
Methyldeserpidate **(66);** Isoraunescine **(67);**Raunescine **(68)**	*R. canescens*, *R. lingustrina*	Huebner *et al.,*1955; Amer and Court, 1981

Geissoschizine **(69)**	*R. volkensii*	Lounasmaa and Hanhinen, 1996
Geissoschizol **(70);** *O*- acetylgeissoschizol **(71);** 10-hydroxygeissoschizol **(72);** 10-Methoxygeissoschizol **(73)**	*R. vomitoria*	Bartlett *et al.,* 1963; Gilbert *et al.,* 1965; Heimberger and Scott, 1973; Jahodář *et al.*,1974
2-hydroxybenzaldehyde **(74)**	*R. caffra*	Morishige, 1974
Indobine **(75)**: Indobinine **(76)**	*R. serpentina*	Okabe and Adachi, 1998
Isoboonein **(77)**	*R. grandiflora*	Bianco *et al.,* 1994
Isositsirikine **(78)**	*R. yunnanensis*	Robert *et al.,* 1983
Lankanescine **(79)**	*R. canescenes*	Arambewela *et al.,* 2001
7-Epiloganin **(80)**	*R. serpentina*	Itoh *et al.,* 2005
Macrocaffrine **(26)**; Macrophylline **(81)**	*R. caffra, R. macrophyla*	Nasser and Court, 1983; Ohba and Natsutani, 2007
Macrospegatrine **(82)**	*R. verticcillata*	Lin, 1987
12-Methoxyaffinisine **(83)**; 12-methoxyvellosimine **(84)**; 12-Methoxyaffinisine; 17-Aldehyde **(85)**	*R. bahiensis*	Kato, 2002
11-Methoxyyohimbine **(86)**	*R. nitida, R. capuroni*	Amer and Court, 1981
Methuenine; 19,20β-Dihydro **(87)**	*R. discolor*	Bui *et al.,* 1977
Obscuridine: Obscurine; Obscurifoline **(88)**; Usambarine **(89)**	*R. obscura*	Roland, 1959; Angenot *et al.,* 1978
Amerovolficine **(90)**	*R. cubana*	Martinez, 1989
10-Methoxyamerovolficine **(91)**	*R. yunnannensis*	Hu, 2006
Papaverine **(92)**	*R. serpentina*	Han *et al.,* 2010
Perakine **(93)**	*R. caffra, R. volkensi,*	Amer and Court, 1980
Raucaffrinoline **(94)**	*R. caffra, R. nitida, R. vomitoria*	Amer and Court, 1980; Batista *et al.,* 1996
Pekerakine dimethyl acetal **(95)**	*R. sellowii*	Amer and Court, 1980;
Peraksine **(96)**	*R. parakensis*	Nasser and Court, 1984
Picrinine **(97)**	*R. vomitoria*	Akinloye and Court, 1980
Quaternine **(98)** Picrinine; 10,11-Dimethoxy **(99)**	*R. volkensii, R. oreogiton*	Akinloye and Court, 1980
Polyneuridine **(100)**	*R. suaveolens*	Joule *et al.,* 1965
Gustastatin **(101)**	*R. mattfeldiana*	Pettit *et al.,* 2004
Raumacline **(102)**; Isoraumacline **(103)**; 6 α-hydroxyraumacline **(104)**	*R. serpentina*	Endreß *et al.,* 1993; Endreß *et al.,* 2007
Rauvidridine **(105)**	*R. viridis*	Renner *et al.,*1963

Rauvolcinine **(106)**	*R. volkensii*	Akinloye and Court, 1981
Rauwolfine **(107)**; Rescinnaminol **(108)**; Reserpenediol **(109)**	*R. serpentina*	Bose, 1952, 1954; Uddin, 1978; Siddiqui, 1986
Reserpic acid **(110)**	*R. vomitoria*	Rosen and Shoolery, 1961
Raugustine **(111)**; 3-Epirescinnamine **(112)**	*R. ligustrina, R. vomitoria*	Awang and Ekiel, 1990
Reserpic acid; Me ester **(113)**	*R. vomitoria, R. macrophylla*	Woodward *et al.*, 1958; Rosen and Shoolery, 1961
Rescidine **(114)**	*R. vomitoria*	Woodward *et al.*, 1958
Rescinnamine **(115)**	*R. vomitoria, R.serpentina,R.caffra*	Woodward *et al.*, 1958; Rosen and Shoolery, 1961
Rescinnamidine **(116)**	*R. serpentina*	Rosen and Shoolery, 1961
Reserpline **(117)**	*R. canescens*	Sabri and Court, 1978; Cancelieri *et al.*, 2002
Reserpiline; 3,20-Diepimer **(118)**	*R. cumminsii*	Woodward *et al.*, 1958; Cancelieri, *et al.*, 2002
Reserpiline; 3-Epimer, 18,19-Didehydro **(119)**	*R. grandiflora*	Sabri and Court, 1978; Cancelieri, *et al.*, 2002
Reserpiline; 3-Epimer **(120)**	*R. discolor*	Cancelieri, *et al.*, 2002
4-Methylreserpiline **(121)**	*R. confertiflora*	Kiang *et al.*, 1964; Sabri and Court, 1978
Neoreserpiline **(122)**	*R. perakensis*	Kiang *et al.*, 1964
Rauvanine **(123)**	*R. vomitoria*	Stoll and Hofmann, 1955; Kiang *et al.*, 1964
Renoxydine **(124)**	*R. vomitoria, R. canescens*	Woodward *et al.*, 1958; Rosen and Shoolery, 1961
Pseudoresrpine **(125)**	*R. canescens*, *R.nitida*	Martin *et al.*,1987
Reserpinine **(126)**	*R. serpentina*	Shamma and Richey, 1963; Taylor and Farnsworth, 1973
Serpagine **(127)**	*Rauwolfia spp*	Stoll and Hofmann, 1955
Serpagine; 18-Hydroxy, 10-Me ether **(128)**	*R. biauriculate*	Stoll and Hofmann, 1955; Khan and Khan, 1965
Serpagine; 10-Me ether **(129)**	*R. macrophylla, R. nitida*	Khan and Khan, 1965; Timmens, 1974
Sellowiine **(130)**	*R. selowii*	Batista *et al.*, 1996
Sempervirine **(131)**	*Rauwolfia spp*	Gribble *et al.*, 1988
Seredine **(132)**	*R. vomitoria*	Sequin, 1982
Sitsirikine **(133);** Norsauveoline **(134)**	*R. caffra*	Nasser and Court, 1984
21-Hydroxycyclolochnerine **(135)**	*R. biauriculate*	Garnick and Le Quesne, 1978

3,4,5,6,-Tetrahydrogeissoschizol **(136)**; 3,4,5,6, Tetrahydrogeissoschizol *β-D*-Glucopyranoside **(137):** 3,4,5,6,-Tetrahydroyohimbine **(138)**	*R. serpentina*	Wachsmuth and Matusch, 2002
Tetraphyllicine **(139)**	*R. tetraphylla*	Amer and Court, 1981
Tetraphyllicine; *N*-De-Me, *O*-Ac **(140)**	*R. nitida*	Amer and Court, 1981
Nor rauvomitine **(141)**	*R. vomitoria*	Iwu and Court, 1977a
Nortetraphyllicine **(142)**	*R. vomitoria*	Sabri and Court, 1978
12-Hydroxymauiensine **(143)**	*R. media*	Kan *et al.,*1986
17-Epinoseredamine **(144)**	*R. cumminsii*	Gorman *et al.,*1963
Reflexine **(145)**	*R. reflexa*	Chatterjee *et al.,*1976
17-Epitetraphyllicine **(146)**	*R. mauiensis*	Gorman *et al.,* 1957
10-Hydroxynortetraphyllicine **(147)**;Normitoridine**(148);**Mitoridine**(149)**	*R. vomitoria*	Sabri and Court, 1978
Rauflorine **(150)**	*R. confertiflora*	Jokela and Lounasmaa, 2007
Endolobine **(151)**	*R. cumminsii, R. mombasiana*	Amer and Court, 1981; Sabri and Court, 1978
Norpurpeline **(152)**	*R. vomitoria*	Iwu and Court, 1977a,1977b
Rauflexine **(153)**	*R. reflexa*	Chatterjee *et al.,* 1976
Purpeline **(154)**	*R. cumminsii, R. reflexa,R.vomitoria*	Chatterjee *et al.,* 1976; Iwu and Court, 1977b
Seradamine **(155)**	*R. vomitoria*	Sabri and Court, 1978
Rauvomitine **(156)**	*R. vomitoria*	Iwu and Court, 1977a
Tombozine **(157)**	*Rauwolfia spp*	Patel *et al.,* 1973
Vellosimine **(158)**	*R. vomitoria, R.nitida*	Timmens, 1974
Pericyclivine **(159)** Tombozine; Me ether **(160)**	*R. cumminsii*	Patel *et al.,* 1973; Amer and Court, 1981
Venoterpine **(161)**	*R. verticillata*	Arthur and Loo, 1966
Vincarine **(162)**	*R. discolor*	Gorman *et al.,* 1957
Yohambinine **(163)**	*R. serpentina*	Lohse, 2002
Acetylalloyohimbine **(164)**	*R. nitida*	Marion, 1952; Itoh *et al.,* 2005
Alloyohimbine **(165)**	*Rauwolfia spp*	Amer and Court, 1981
Yohimbic acid **(166);** Isorauhimbic acid **(167);** Isoraumbine **(168)**	*R. serpentina, R. nitida*	Amer and Court, 1981; Robert *et al.,* 1983
Reserpine **(169)**	*R. serpentina*	Rosen and Shoolery, 1961

CHAPTER THREE

METHODOLOGY

3.1 Collection of plant materials

The leaves of *M. oleifera* and *R. caffra*, and stem bark of *R. caffra* were collected from Kuria County in Western region of Kenya, approximately 200 Km from Kisumu city in March 2012. The plant materials were identified by a botanist; Mr. Bethwel Owuor and deposited at the University of Nairobi herbarium, School of Biological Sciences, College of Biological and Physical Sciences.

3.2 Extraction of plant materials

3.2.1 Extraction from the leaves of *M. oleifera*

The fresh healthy leaves of *M. oleifera* were washed, air dried in the tissue section of the laboratory at room temperature for one week and ground into fine powder. The powder (200 g) was weighed and serially extracted in the following order of increasing polarities; 100 % *n*-hexane (C_6H_{12}); CH_2Cl_2:C_6H_{12} (1:1); 100 % CH_2Cl_2; MeOH: CH_2Cl_2 (1:1) and 100 % MeOH for 24 hours with frequent stirring. The extract was filtered using cotton wool to remove particulate matter and the solvent removed *in vacuo* using a rotary evaporator to obtain crude extracts.

3.2.2 Extraction from the stem bark and leaves of *R. caffra*

The stem bark and leaves of *R. caffra* were extracted as described under the section 3.2.1 (Extraction from the leaves of *M. oleifera).*

3.2.2.1 Fractionation of *R. caffra* stem bark extract

The MeOH: CH_2Cl_2 (1:1) extract (15 g) of the stem bark of *R. caffra* was adsorbed onto 15 g of silica gel (70-230 mesh) and then loaded onto 150 g silica gel column in 50 % CH_2Cl_2: *n*-C_6H_{12}. The column was eluted with the following solvent systems in increasing polarities; CH_2Cl_2: *n*-C_6H_{12}; MeOH: CH_2Cl_2 yielding 120 fractions (50 ml each). Fractions with similar TLC profile were combined and concentrated to dryness on a rotary evaporator giving a total of 16 fractions coded as F_A - F_P.

3.3 Phytochemical analysis of the plant materials

The different classes of phytochemicals present in the extracts of the two plants were detected using standard procedures and the phytochemicals analyzed were; flavonoids, coumarins, phenols, alkaloids, steroids, saponin, terpenoids and cardiac glycosides.

3.3.1 Detection of flavonoids: MeOH extract of *M. oleifera* leaves, *R. caffra* leaf extracts (MeOH: CH_2Cl_2 1:1), *R. caffra* stem bark extract (MeOH: CH_2Cl_2 1:1) and *R. caffra* stem bark fractions were tested for the presence of flavonoids using the following tests;

3.3.1.1 Ammonia test: 0.5 g of the crude extract was dissolved in minimum quantities (5 ml) of the solvent (CH_2Cl_2) and then spotted on a TLC plate. The spot on the plate was fumigated with ammonia and appearance of a yellow spot or yellow green fluorescence under ultraviolet light (UV) was interpreted to show the presence of flavonoids (Cai *et al.*, 2011).

3.3.1.2 Aluminum chloride ($AlCl_3$) test: 0.5 g of the crude extract was dissolved in minimum amounts of the solvent (5 ml CH_2Cl_2) and spotted on a filter paper which was then dried and sprayed with $AlCl_3$ reagent. The appearance of yellow spots or yellow green fluorescence under UV was interpreted to indicate the presence of flavonoids (Cai *et al.*, 2011).

3.3.1.3 Hydrochloric acid-Mg reaction: Approximately 0.5 g of magnesium turnings were added into a test tube with 3 ml test samples, and a few drops of concentrated HCl added. Change in color to red indicated the presence of flavonoids (Cai *et al.,* 2011).

3.3.2 Detection of coumarins and lactones: For identification of coumarins and lactones the opened loop-closed loop response was used. Two drops of 1 % sodium hydroxide solution was added to a test tube containing 5 ml solution of the extract. This mixture was incubated for 3 min in boiling water, after which 4 drops of 2 % HCl were added. Turbidity implied the presence of either coumarins or lactones (Cai *et al*., 2011).

3.3.3 Detection of phenolics and tannins

The extracts and fractions were tested for the presence of phenolic compounds using the following tests;

3.3.3.1 Ferric chloride test: The extract (50 mg) was dissolved in 5 ml of distilled water, few drops of 5 % ferric chloride solution was added to the test tube containing 5 ml of the extract. A dark green or bluish green color indicated the presence of phenolic compounds (Cai *et al.,* 2011)**.**

3.3.3.2 Vanillin-HCl reaction: 0.5 g of crude extract was dissolved in 5 ml CH_2Cl_2 and a drop of the extracts was placed on a filter paper, dried and sprayed with vanillin HCl reagent. Appearance of varying degrees of red color indicated the presence of phenols (Cai *et al.,* 2011).

3.3.4 Detection of terpenoids

To detect terpenoids the following tests were performed;

3.3.4.1 Acetic anhydride-sulphuric acid (H_2SO_4) test: 2 ml of the extract was added to 2 ml of a mixture of acetic anhydride and concentrated H_2SO_4. Formation of green rings indicated the presence of terpenoids (Savithramma *et al.,* 2011).

3.3.4.2 *p*-anisaldehyde test: 0.5 g of the crude extract and fractions was dissolved in the solvent (CH_2Cl_2) and spotted on TLC plate. The plate was developed using the most appropriate solvent (3 % MeOH/CH_2Cl_2) as mobile phase and dried. Plates were then sprayed with *p*-anisaldehyde reagent and dried at 105 °C for a few minutes. The formation of colored bands on the plate indicated the presence of terpenoids.

3.3.5 Detection of saponin

The following classification tests were performed to detect the presence of saponins.

3.3.5.1 Vanilin-H_2SO_4 tests: 0.5 g of the crude extract was dissolved minimum amounts of the disolving solvent (5 ml of CH_2Cl_2) and spotted on TLC plate. The plate was developed in 3 % MeOH/CH_2Cl_2 as mobile phase and dried. Plates were then sprayed with 1 % vanilin and then 5 % H_2SO_4 reagent and dried at 105 °C for a few minutes. The appearance of a dark bluish spot indicated the presence of saponins (Sindhu, 2011).

3.3.5.2 Froth test: 0.5 g of the extract was dissolved in 10 ml of distilled water in a test tube and shaken vigorously for 30 seconds, and then allowed to stand for 45 min; the appearance of persistent frothing indicated the presence of saponins (Savithramma *et al*., 2011).

3.3.6 Detection of pytosterols

Salkowskis test was used to classify the compounds. 10 ml of chloroform (CH_3Cl) was added into a test tube containing 1 ml of test samples, equal volume of concentrated H_2SO_4 was added at the sides of a test tube. Appearance of yellowish colour with green fluorescence in the H_2SO_4 layer indicated the presence of phytosterols (Savithramma *et al.*, 2011).

3.3.7 Detection of alkaloids

Wagners test was used. To 3 ml of the solution of the extract and fractions, few drops of Wagner's reagent were added by the side of the test tube. Formation of a reddish brown precipitate confirmed the test as positive (Tiwari *et al.,* 2011).

3.3.8 Detection of cardiac glycosides

The Keller-Killani test was used. 5 ml of each extract was treated with 2 ml of glacial acetic acid containing one drop of ferric chloride solution. This was underlayed with 1 ml of concentrated sulphuric acid. A greeninsh ring in the upper layer and a brown ring at the interface indicated the presence of cardiac glycosides (Cai *et al.,* 2011).

3.4 Biological Activity Studies

3.4.1 Radical Scavenging Test

3.4.1.1 DPPH radical scavenging test using TLC

The crude extracts obtained from the leaves of *M. oleifera,* leaves and stem bark of *R. caffra* were subjected to preliminary antioxidant activity on TLC plate and developed using different solvent system (*n*-C_6H_{12}, CH_2Cl_2 and MeOH) to obtain well resolved spots on the plate. The TLC plates were air dried and compounds detected under UV lamp at 254 nm. The plates were sprayed with DPPH reagent (prepared by dissolving 12 mg of DPPH reagent in 50 ml of analytical grade MeOH) to detect the presence of antioxidant compounds in the extract. The colour discharge of DPPH (purple) to white at the spots was an indication of radical scavenging properties of the crude extract and fractions (Wang *et al.,* 2012).

3.4.1.2 DPPH radical scavenging test using spectrophotometry

Following preliminary antioxidant assay, the leaf extract (MeOH) of *M. oleifera*, leaf extract of *R. caffra* (CH_2Cl_2: MeOH; 1:1 and MeOH only) and the extract of the stem bark of *R. caffra* (CH_2Cl_2:MeOH; 1:1 and MeOH only) were selected for DPPH radical scavenging activity assay as described by Erasto *et al.,* (2011). RSA assay was also performed on the fractions obtained from the stem bark of *R. caffra*. A total of 200 µg of each extract was weighed, dissolved in double distilled methanol and serially diluted to the desired concentrations (3.13 µg/ml-200 µg/ml). 0.5 ml was added to 3 ml of 0.1 mM DPPH that had been dissolved in methanol and incubated for 30 min at room temperature. Quercetin (at a concentration of 100 µg/ml) a commacially available standard was used as a control and the absorbance was measured at 517 nm using a thermo UV spectrophotometer. The decrease in optical density (OD) indicated the presence of RSA and the percent RSA was calculated as follows:

$$RSA\ (\%) = ((A_{blank} - A_{sample}) / A_{blank})* 100$$

Where A_{blank} refers to the OD of the control while A_{sample} refers to the OD of the test sample (Erasto *et al.,* 2011).

3.5 Anti-proliferation assay

3.5.1 Cell lines

Rhabdomyosarcoma (RD) cell lines (from human muscles) passage number 2 and hepatocellular carcinoma (Hep-G2) cell lines (from human liver) passage number 3 were obtained from KEMRI while Vero cell lines passage number 2 was obtained from the department of Veterinary Services, Kenya. The Vero cell lines were used as control cells for comparison with RD and Hep-G2 model (Machana *et al.,* 2012).

3.5.2 Cell Culture preparation and maintenance

The cells were grown in medium containing Dubelcos Minimum Essential Medium (DMEM) from Sigma-Aldrich, 10 % (v/v) Fetal Bovine Serum (FBS) from Gibco, 2 mM L-glutamine and 1 % penicillin/streptomycin (penstrep) from Kobian. The cells were maintained in a humidified incubator at 37 °C, 5 % CO_2 and those that had reached cellular confluence were trypsinized with 0.25 % trypsin, 2 mM EDTA and re-suspended in the medium.

3.5.3 Anti-proliferative assay

Cytotoxicity assay was carried out according to the method of Rakad and Jumaily (2010). The extracts were dissolved in Dimethyl Sulfoxide (DMSO) and diluted with DMEM medium to give concentration ranging from 31.25-500 µg/ml. The cells were plated at a density of $1x10^4$ cells/well in a 96-well plate, incubated for 24 hrs at 37 °C and 5 % CO_2, after which they were treated with crude extracts (leaf extract (MeOH)) of *M. oleifera* and stem bark extract of *R. caffra* (MeOH: CH_2Cl_2; 1:1) at various concentrations and incubated for 24, 48 and 72 hrs. Four replicate wells were prepared for each individual concentration and negative control cultures contained DMEM only. 50µl of crystal violet stain was added to the wells and the plates were incubated in a CO_2 incubator for 30 min at 37 °C. The cells were washed gently with distilled water three times and air dried. The optical density (OD) was recorded on ELISA reader at 450 nm. The inhibitory rate of cell growth was calculated as follows;

Inhibition (%) = ((OD of control wells-OD of test wells)/OD of control wells)*100

The IC_{50} value was calculated using SPSS version 16 and the significant difference between control and sample means was assessed using student *t* test; *p* values ≤ 0.05 was considered to be statistically significant.

CHAPTER FOUR

RESULTS

4.1 Classes of compounds identified from extracts of *M. oleifera*

Phytochemical screening of the extract of the leaves of *M. oleifera* obtained by using methanol revealed the presence of the following classes of compounds; flavonoids, coumarins, steroids, cardiac glycosides, alkaloids, terpenoids, saponin, phenols and tannins. Phenolics, steroids and cardiac glycosides were the most abundant classes of compounds (Table 5). To ensure reproducibility of the results, more than one classification test was carried out for the same class of compounds. These tests showed consistency for each class of compound with insignificant differences in abundance in some cases.

Table 5: Classes of phytochemicals detected in the MeOH extract of the leaves of *M. oleifera*

Class of Compounds	**Classification Test**	**Abundance *M. oleifera* leaves**
Alkaloids	Wagnes test	+
Terpenoids	*p*-anisaldehyde test	+
	Salkowski test	+
Saponin	Vanilin/sulphuric acid test	+
	Foam test	-
Steroids	Chlorofoam/sulphuric acid test	++
Cardiac glycosides	Keller-Killani test	++
Flavonoids	HCl-Mg reaction test	++
	$AlCl_3$ reaction	++
	Ammonia test	++
Coumarin	Open loop-close loop response test	++
Phenols and tannins	$FeCl_3$ test	++
	Vanillin-HCl reaction	++

Legend: ++ Present in high concentration

\+ Weakly present

\- Absent

4.2 Classes of compounds identified from the extracts of the leaves and stem bark of *R. caffra.*

Phytochemical screening conducted on the leaf extracts (MeOH: CH_2Cl_2; 1:1) and extract of the stem bark (MeOH: CH_2Cl_2; 1:1) of *R. caffra* revealed the presence of the following classes of compounds: alkaloids, terpenoids, saponin, cardiac glycosides and steroids (Table 6).

Table 6: Class of phytochemicals in leaves (MeOH: CH_2Cl_2; 1:1) and stem bark (MeOH: CH_2Cl_2; 1:1) of *R. caffra*.

Class of Compounds	**Classification Test**	**Abundance**	
		R. caffra	
		Leaves	**Stem bark**
Alkaloids	Wagnes test	++	++
Terpenoids	*p*-anisaldehyde test	+	++
	Salkowski test	++	++
Saponin	Vanilin/sulphuric acid test	+	++
	Foam test	++	++
Steroids	Chlorofoam/sulphuric acid test	++	++
Cardiac glycosides	Keller-Killani test	++	++
Flavonoids	HCl-Mg reaction test	-	-
	$AlCl_3$ reaction test	-	-
	Ammonia test	-	-
Coumarin	Open loop-close loop response	-	-
Phenols and tannins	$FeCl_3$ test	-	-
	Vanillin-HCl reaction	-	-

Legend: ++ Present in high concentration

\+ Weakly present

\- Absent

4.3 Biological activity

4.3.1 Antioxidant activity

4.3.1.1 Antioxidant activity of the extracts of *R. caffra* leaves and stem bark and leaves of *M. oleifera*

Preliminary RSA using 1, 1-diphenyl-2-picrylhydrazyl (DPPH) free radical as a spray reagent on TLC plates; of the leaves and stem bark extracts of *R. caffra* (50 % MeOH/CH_2Cl_2), the leaves and stem bark extracts of *R. caffra* (100 % MeOH) and the MeOH extract of the leaves of *M oleifera;* indicated the presence of compounds with RSA. Quantitative antioxidant activity assay revealed the extract of the stem bark of *R. caffra* obtained using 50 % MeOH/CH_2Cl_2 to have the highest RSA of 96.03 % at 0.2 mg/ml while the methanol extract of *M. oleifera* showed the least RSA of 83.84 % at 0.2 mg/ml (Table 5). It is important to note that even at lower concentrations (0.05 mg/ml) the RSA of *R. caffra* (50% MeOH in CH_2Cl_2) was 91.57 % almost as high as that observed for 0.2 mg/ml (Table 7).

Table 7: % RSA of the extracts of the leaves (MeOH; MeOH: CH_2Cl_2; 1:1), stem bark (MeOH; MeOH: CH_2Cl_2; 1:1) of *R. caffra* and leaves of *M. oleifera* (MeOH)

Conc. of extract µg/ml	**% RSA**					
	R. caffra				***M. oleifera***	
	Leaves		**Stem bark**		**Leaves**	
	Neat MeOH	**50 % MeOH:CH_2Cl_2**	**Neat MeOH**	**50 % MeOH:CH_2Cl_2**	**Neat MeOH**	**Querc**
003.13	00.25	56.42	06.21	46.00	33.00	42.18
006.25	01.03	58.44	09.61	46.94	33.00	69.31
012.50	01.33	74.88	10.48	72.39	33.00	89.71
025.00	02.23	77.51	16.75	78.79	37.78	92.89
050.00	14.16	85.50	18.90	91.57	60.37	95.29
100.00	46.32	86.42	52.12	93.15	61.00	96.39
200.00	86.11	88.44	84.64	96.03	83.84	-

% RSA are the means of triplicate measurement (n = 3); the concentration of the extract was double that of the standard compound, quercetin

4.3.1.2 The relationship between Radical Scavenging Activity (RSA) and the composition of fractions of the stem bark extract (50 % MeOH/CH_2Cl_2) of *R. caffra*

The 50 % MeOH/CH_2Cl_2 extract of the stem bark of *R. caffra* and the MeOH extract of the leaves of *M. oleifera* exhibited % RSA of 96.03 and 83.84 respectively at the initial tested concentration of 0.2 mg/ml (Table 7). These extracts were subjected to serial dilution to yield minimum concentrations of 0.003 mg/ml which were active with % RSA of 46.00 for *R. caffra* stem bark and 33.00 for *M. oleifera* extracts (Table 7). Having shown activity even at lower concentrations, the extract of the stem bark of *R. caffra* was fractionated by column chromatography. The fractions (sixteen) were analyzed for their RSA using DPPH reagent and the composition of classes of compounds present was detected using multiple functional group tests.

Modest blend effect was observed in the following family of compounds; alkaloids, steroids, cardiac glycosides, saponins and terpenoids had a % RSA of 58.99±1.9 at 0.2 mg/ml. The absence of saponins led to reduction of % RSA to 38.18±3.6 at the same concentration (Table 8). The highest activity (82.39±1.4 %) was observed when two classes of compounds (alkaloids and saponins) were not detected; as exhibited by the fraction eluted with 5-7 % MeOH/CH_2Cl_2 consisting of only steroids, cardiac glycosides and terpenoids (Table 8).

Table 8: Relationship between the phytochemistry of the fractions of the extract (50 % MeOH/CH_2Cl_2) from the stem bark of *R. caffra* and RSA

Fraction	Alkaloids	Steroids	Cardiac glycosides	Saponins	Terpenoids	% RSA
F_A 5-10% CH_2Cl_2/*n*-C_6H_{12}	+	+	+	+	-	41.82±3.3
F_{BandC} 10-20% CH_2Cl_2/*n*-C_6H_{12}	+	-	-	+	-	15.68±2.2
F_{DandE} 30-40% CH_2Cl_2/*n*-C_6H_{12}	+	-	-	-	-	*
F_F 50-60% CH_2Cl_2/*n*-C_6H_{12}	+	-	-	-	-	62.99± 3.7
F_G 70-80 % CH_2Cl_2/*n*-C_6H_{12}	+	+	+	-	-	43.8 ±2.4
F_{HandI} 90-100 % CH_2Cl_2/*n*-C_6H_{12}	+	-	-	-	-	*
F_J 0.5-1% CH_3OH/CH_2Cl_2	+	-	-	-	-	*
F_{KandL} 1-5 % CH_3OH/CH_2Cl_2	+	+	+	-	+	38.18±3.6
F_M 5 -7% CH_3OH/CH_2Cl_2	-	+	+	-	+	82.39±1.4
F_N 7-9% CH_3OH/CH_2Cl_2	+	+	+	+	+	58.99±1.9
F_O 9-15% CH_3OH/CH_2Cl_2	+	+	+	+	+	*
F_P 100% CH_3OH	-	+	+	-	+	*

Legend: + = present; - = absent; * not determined; *n*-C_6H_{12} = *n*-hexane; CH_3OH = methanol; CH_2Cl_2 = dichloromethane

4.3.2 Anti-proliferative activity

4.3.2.1 Anti-proliferation activity of the methanol extract of *M. oleifera* leaves

Anti-proliferative activity of *M. oleifera* leaf extracts (MeOH) was carried out on Hep-G2, RD and Vero cell lines and monitored over a period of 72 hrs at different concentration (Table 9). *M. oleifera* leaf extracts (MeOH) significantly inhibited the growth of Hep-G2 and RD cell lines ($p < 0.05$), and the optimum quantity required to suppress the growth of 50 % of cancer cell lines (IC_{50}) is presented in table 10. Further analysis showed *M. oleifera* leaf extracts (MeOH) to inhibit the growth of all the cell lines in a dose and time dependent manner and the highest inhibition was recorded after every 72 hrs on all the cell lines (Table 9); the proliferation of Hep-G2 and RD cell lines was also significantly affected (Table 10).

Table 9: Percentage inhibition of Hep-G2, RD and VERO cell lines by methanol extract of *M. oleifera* leaves after 24, 48 and 72 hrs of exposure

Concentration (µg/ml)	Inhibition %								
	Hep-G2			RD			VERO		
	24 Hours	48 hours	72 hours	24 Hours	48 hours	72 hours	24 hours	48 hours	72 hours
31.25	4.27	18.44	24.35	12.73	16.00	36.82	0.00	0.00	0.00
62.50	6.71	21.72	-	14.55	16.00	-	2.00	4.08	-
125.00	8.23	29.51	33.44	14.55	25.33	43.64	6.00	6.12	11.08
250.00	22.87	29.51	39.60	16.36	34.67	48.18	6.00	6.12	14.73
500.00	24.70	30.33	53.51	25.46	52.00	63.18	6.00	16.33	24.93
-ve control	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

Legend: -ve control; negative control (medium only)
Empty cells represent negative values.

Table 10: IC_{50} values for Hep-G2, RD and Vero cell lines after 72 hrs exposure to methanol extract of *M. oleifera* leaves

	IC_{50} (mg/ml)		*P* - value
Hep-G2	0.50	**Hep-G2 vs VERO**	0.004
RD	0.17	**RD vs VERO**	0.005
VERO	3.78		

Legend: IC_{50} Half maximal inhibitory concentration

4.3.3.2 Anti-proliferation activity on cancer cell lines by *R. caffra* stem bark extracts (**MeOH: CH_2Cl_2; 1:1)**

The anti-proliferative activity of *R. caffra* stem bark extracts **(**MeOH: CH_2Cl_2; 1:1) was carried out on Hep-G2, RD and Vero cell lines. The bioactivity of crude extract from the stem bark **(**MeOH: CH_2Cl_2; 1:1) of *R. caffra* on Hep-G2 and RD cell lines as well as the optimum quantity required to suppress the growth of 50 % of the cells (IC_{50}) is presented in tables 11 and 12. The anti-proliferative activity of *R. caffra* stem bark extracts **(**MeOH: CH_2Cl_2; 1:1) against the three cell lines was not statistically significant ($p > 0.05$). The extracts inhibited the growth of all the cell lines in a dose dependent manner and the highest inhibition was recorded after 72 hrs (Table 11).

Table 11: Percentage inhibition of the growth of Hep-G2, RD and Vero cell lines by *R. caffra* stem bark extracts (MeOH: CH_2Cl_2; 1:1) after 24, 48, and 72 hrs of exposure

Concentration (µg/ml)	Inhibition %								
	Hep-G2			RD			VERO		
	24 hours	48 hours	72 hours	24 hours	48 hours	72 hours	24 hours	48 hours	72 hours
031.25	15.85	13.60	13.04	0.00	0.00	23.41	0.00	1.72	23.78
062.50	17.68	15.64	19.57	0.00	5.33	37.05	2.00	-	34.19
125.00	17.68	26.13	16.09	3.64	16.00	49.77	2.00	10.35	35.00
250.00	21.65	20.76	27.39	10.91	28.00	51.59	4.00	13.45	37.46
500.00	31.71	34.82	42.17	18.18	50.67	61.14	20.00	32.59	51.00
Control	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

Legend: -ve control; negative control (medium only)
Empty cells represent negative values.

Table 12: IC_{50} values for Hep-G2, RD and VERO cell lines after 72 hrs exposure to *R. caffra* crude stem bark extract (MeOH: CH_2Cl_2; 1:1)

	IC_{50} (mg/ml)		*P* value
Hep-G2	0.89	**Hep-G2 vs VERO**	0.059
RD	0.19	**RD vs VERO**	0.081
VERO	0.60		-

IC_{50} Half maximal inhibitory concentration

CHAPTER FIVE

DISCUSION

5.1 Overview

This study evaluated the efficacy of phytochemicals present in two plants (*R. caffra* and *M. oleifera)* used in traditional health care to manage cancer and related illnesses. A clear disparity was observed in the phytochemical composition of the extracts of the two medicinal plants although they are used to manage the same ailment. The 50 % MeOH/CH_2Cl_2 extract of stem bark of quinine tree displayed substantial antioxidant activity as compared to the leaves of drumstick extracted with methanol, which exhibited better anti-proliferative activity against Hep-G2 and RD cell lines. These results imply that the composition of the extract in terms of the class of compounds present determines the activitiy of the extracts, as some compounds antagonize while others boost each others activities.

5.2 Classes of compounds identified from extract of *M. oleifera*

Phytochemical screening of medicinal plants is key to the identification of new sources of therapeutically and industrially important compounds (Savithramma *et al.,* 2011). Phytochemical analysis was performed on crude extracts of *M. oleifera* leaves (MeOH) and the extracts were found to be rich in phenolic compounds but to contain low levels of alkaloids (Table 5). This is consistent with Rajula and Ujwala, (2010) findings which showed *M. oleifera* to contain low level of alkaloids. The class of phytochemicals present in a plant may assist in predicting its biological activities; for instance, flavonoids and tannins are strong antioxidant agents due to their ability to scavenge free radicals (Dai and Mumper, 2010; Si Heung Sung, 2012; Zhang *et al.,* 2010) while coumarins are anti-mutagenic agent (Lacy and O'Kennedy, 2004; Mirunalini and Krishnaveni, 2011). Thus, *M. oleifera* is generally expected to have antioxidant and antitumor properties.

The identification of cardiac glycosides (Table 5) in the current study is consistent with previous studies (Rajula and Ujwala, 2010). Cardiac glycosides are molecules used in treatment of heart diseases (Kashani *et al.,* 2012) hence, *M. oleifera* extracts may be used to manage heart diseases as is currently practiced by many communities across the world. These results therefore reaffirm the value of indigenous knowledge in identification of plants for pharmaceutical use.

5.3 Classes of compounds identified from extract of *R. caffra*

The stem bark extract (MeOH: CH2Cl2; 1:1) of quinine tree was found to be rich in alkaloids (Table 6). The detection of alkaloids in the stem bark extract (MeOH: CH_2Cl2; 1:1) of *R. caffra* further reinforces the presence of alkaloid in this species as already outlined by other independent studies (Erasto *et al.,* 2011; Nasser and Court 1984). This class of compounds have wide pharmacological effects and has been used extensively as drugs in medical field; examples of alkaloids of pharmaceutical importance from *R. caffra* include; ajmalicine **(29),** reserpinine **(126)** and reserpine **(169)** which are used as antihypertensive and anti-inflammatory agents (Kashani *et al.,* 2012; Vakil, 1955). Saponins; the other group of phytochemicals detected in *R. caffra,* are compounds with a wide array of biological effects. They have been shown to have antimicrobial, antiviral, antioxidant and cytotoxic properties, however, this compounds are known to interfere with the digestion of protein and uptake of vitamins and minerals (Francis *et al.,* 2007). Hence, they are mainly used as detergents and surface active agents in industries (Savithramma *et al.,* 2011).

One unanticipated finding was the absence of phenolic compounds in the extracts of *R. caffra*. The leaves of this plant contain wax which is rich in phenolic compounds. Surprisingly, these were not detected even by using multiple tests. It is possible that most of the polyphenols were lost in the solvent system (hexane and DCM) before the extracts were subjected to subsequent analysis on methanol.

5.4 Biological activities

5.4.1 Radical scavenging activity of extract from *M. oleifera*

In the current study, methanol was used as a negative control in all the tests and it had a 0.00% radical scavenging capacity. The commercially available quercetin; used as a positive control had a RSA of 96.39 % at 0.1 mg/ml, implying that the method used was reliable for determining antioxidant activity. The methanol extract of the leaves of *M. oleifera* had a low RSA of 61.00 % at 0.1 mg/ml (Table 8). This low antioxidant activity exhibited by *M. oleifera* extracts in the current study was indeed unanticipated since phytochemical analysis revealed the presence of phenols which are known to be rich antioxidants (Mirunalini and Krishnaveni, 2011; Si Heung Sung, 2012). The low antioxidant activity of *M. oleifera* extracts can be attributed to the

reduction in the efficacy of antioxidant molecules in the plant post harvesting; the long duration between sample collection and analysis could have influenced the molecules capacity to scavenge the free radicals. Moreover, other confounding factors such as; geographical distribution, soil composition and the age of the plants may have influenced the antioxidant activity of the plant (Mokale *et al.*, 2011). Indeed, a study by Iqbal and Bhanger, (2006) established that seasons and geographical locations may influence the concentrations of antioxidants in *M. oleifera* leaf extract.

5.4.2 Radical scavenging activities of extract from *R. caffra*

Extracts from the stem bark of *R. caffra* revealed a strong dose-dependent RSA against DPPH (Table 7) which is consistent with previous findings (Erasto *et al.*, 2011; Gbonjubola, 2010). The % RSA of *R. caffra* stem bark extracts was 93.15 % at 0.1 mg/ml while that of the standard quercetin was 96.39 % at 0.1 mg/ml, suggesting *R. caffra* stem bark extract to be a competitively strong antioxidant. The results show that *R. caffra* was tested at double the concentration of quercetin and from the table the activity of the standard is higher but comparable to that of the extract of *R. caffra.*

Further analysis performed on the fractions obtained from the stem bark of *R. caffra* showed activity variations in RSA depending on the phytochemical composition. For instance, the alkaloids exhibited improved RSA in the absence of other classes of phytochemicals (Table 8). RSA was consistently lower in fractions exhibiting the presence of alkaloids and other phytochemicals concurrently pointing to a possibility of this compounds having antagonistic effect with alkaloids. For instance, alkaloids and saponins appeared to have antagonistic interaction, at least with regards to RSA. This potentially lowers their activity as antioxidants, and possibly the potency of extracts containing both compounds. Although the antagonistic interaction of biomolecules in drumstick was not determined, it is probable that the low RSA of the MeOH extract of *M. oleifera* is due to antagonistic effect of the classes of compounds in the extract, rendering the whole extract less effective with respect to antioxidant activity.

5.4.3 Anti-proliferative activity

5.4.3.1 Anti-proliferation activity of *M. oleifera* leaves extracts

The MeOH extract of *M. oleifera* demonstrated prominent anti-proliferative activity on both Hep-G2 and RD cell lines. This findings are consistent with those of Khalafalla *et al.,* (2010) who found hot water extracts of *M. oleifera* to have significant cytotoxic activity against Hep-G2 cell lines. The authors attributed the strong anticancer activity of *M. oleifera* extracts to the presence of phenolic compounds (Khalafalla *et al.,* 2010), it is likely that the same class of compounds were responsible for the anti-proliferative activity observed in the current study. Another important finding is the low cytotoxicity of MeOH extract of *M. oleifera* leaves against Vero cells. After 72 hrs of exposure of the cells to high concentrations of the extract (500 µg/ml), the cytotoxic effect was found to be less than 50 % (Table 9), an indication that the extract is less toxic to normal cells. Previous studies have found *M. oleifera* to be potentially non-toxic (Kasolo *et al.,* 2011) validating its traditional use as a vegetable (Fahey, 2005), water disinfectant (Lantagne *et al.,* 2008) and as a medicinal herb (Fahey, 2005).

5.4.3.2 Anti-proliferation activity of *R. caffra* stem bark extracts

The MeOH: CH_2Cl_2 (1:1) extracts of *R. caffra* stem bark inhibited the growth of all the cell lines in a dose dependent manner and as anticipated, the highest concentration (500 µg/ml) had the highest anti-proliferative effect (Table 11). An important finding in the current study, was the fact that the extracts greatly inhibited the growth of control cell lines (Vero cells); there was no significance difference in the anti-proliferative activity of *R. caffra* stem bark extract (MeOH: CH_2Cl_2; 1:1) on Hep-G2 cells and normal Vero cells ($p > 0.05$) cells, implying that the extracts could be toxic to normal cells (Table 12). The cytotoxicity observed in the MeOH: CH_2Cl_2 (1:1) extract of *R. caffra* could be attributed to the presence of alkaloids such as akuamicine **(33)** (Nasser and Court, 1984), dihydroperaksine **(59)** and macrocaffine **(26)** (Nasser and Court, 1983) detected in earlier studies. Further, a comparative analysis revealed RD cell lines to be more sensitive than Hep-G2 when exposed to extracts of the two plants, however this was more prominent in the case of *R. caffra* extracts where Hep-G2 cell lines gave inconsistent results due to the resilience nature of the cell line, indeed a study by Mahavorasirikul, *et al.,* (2010) found Hep-G2 cell lines to be resistant to most of the plant extracts tested.

5.5 General discussion

A comparison of the phytochemical component of the two plants revealed the leaves of *M. oleifera* (MeOH) to be rich in phenolic compounds while *R. caffra* stem bark extracts (MeOH: CH2Cl2; 1:1) was found to be rich in terpenoids and alkaloids (Tables 5 and 6). The variation in phytochemical composition may be responsible for the disparity in the antioxidant and anti-proliferative activity of the two plants; the MeOH extract of the leaves of *M. oleifera* was found to have better anti-proliferative activity than *R. caffra* stem bark extract (MeOH: CH_2Cl_2; 1:1). Apart from its anti-proliferative properties, *M. oleifera* leaf extracts also have hepato-protective (Das *et al.,* 2012; Verma *et al.,* 2012), antioxidant (Das *et al.,* 2012; Paliwal, *et al.,* 2011; Verma *et al.,* 2012) and antibacterial activities (Peixoto *et al.,* 2011). The stem bark of *R. caffra* (MeOH: CH_2C_{l2}; 1:1) on the other hand exhibited the highest antioxidant activity making it a good source of natural antioxidants. However, these extracts were also found to be toxic to vero vells, the problem of cytotoxicity can be resolved by adopting modern biotechnology during extraction to minimize toxicity and to optimize the bioactivity of compounds from *R. caffra*.

Phytochemicals in the stem bark extract (MeOH: CH_2C_{l2}; 1:1) of *R. caffra* were observed to have synergistic and antagonistic activity. For instance, saponins and alkaloids showed possibilities of having a negative blend effect on steroids, cardiac glycosides and terpenoids. A combination of saponins and alkoloids antagonized each other resulting to a substantial reduction in activity; the fraction with alkaloids had a RSA of 62 % and inclusion of saponin reduced activity to 15 %. This findings support previous suggestions that synergistic and antagonistic activity in medicinal plants may lower the concentration of bioactive ingredient to suboptimal levels or increase their concentrations to toxic levels resulting in severe side effects (Doughari *et al.,* 2009). In traditional medicine as practiced by the Abakuria community of Kenya, *R. caffra* is normally administered as a concortion: It's possible that the activity of the toxic molecules is antagonized by other compounds that are incorporated in the herbal concoction thus lowering their toxicity.

CHAPTER SIX

6.1 Conclusion

This study has shown that the leaves of *R. caffra* and the stem bark of *M. oleifera* indeed have phytochemicals of known health benefits, for instance;

- The extracts of the leaves of *M. oleifera* (MeOH) were found to be rich in cardiac glycosides and phenolic compounds such as flavonoids, coumarins and tannins.
- The extracts of the stem bark of *R. caffra* (MeOH: CH_2C_{l2}; 1:1) were found to contain phytochemicals such as cardiac glycosides, alkaloids, saponins, steroids and terpenoids.

With reference to bioactivity the major findings were;

- The bioactivity of the phytochemicals in the stem bark of *R. caffra* crude extract fractions was found to be suboptimal in certain instances due to a possibility of antagonistic effects between alkaloids and saponins.
- The extracts of the stem bark (MeOH: CH_2C_{l2}; 1:1) of *R. caffra* was toxic to vero cells (representetative of non cancerous mammalian cells), the same extract however, was found to have high RSA an indication that it may be effective in managing diseases related to oxidative stress if administred in the appropriate doses.
- Extracts from the leaves of *M. oleifera* (MeOH) on the other hand exhibited significant anti-proliferative activity against RD and Hep-G2 cell lines an indication that it might be a suitable source of chemotherapeutic molecules.

6.2 Recommendation

- To determine the antioxidant and anti-proliferative activity of drumstick and quinine tree, crude extracts were used; such extracts contain a mixture of compounds that may have synergistic and antagonistic activity. These effects can be overcomed by using pure compounds isolated using preparative High Performance Thin Layer Chromatography (HPTLC) and High Perfomance Liquid Chromatography (HPLC), and their structure resolved using high resolution mass spectrometry (HRMS) and Nuclear Magnetic Resonance (NMR).

- In view of the revelation that antagonistic interactions influence bioactivity (perhaps explaining the lack of correlation between anticancer and antioxidant activity), testing pure compounds isolated from the two plants can shed insights into skeletol structure-activity relationship.

- This study utilized only two cell lines (RD and Hep-G2) though cell lines respond differently to the same treatment. This may have limited the true value of these plants as potential for cancer treatment. Pure isolates from the two plants should be tested against other types of cancers such as prostrate or breast cancer.

- In the current study the anti-proliferative activity was performed on cell lines. However, bioactivity of the extracts may vary between in-vivo and in-vitro models. It would be valuable to test these extracts and constituent compounds *in-vivo* using a model such as mice.

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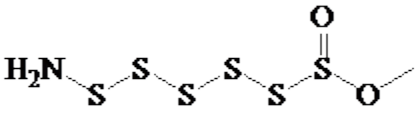
Appendix 1: Structures of compounds previously described in *M. oleifera*



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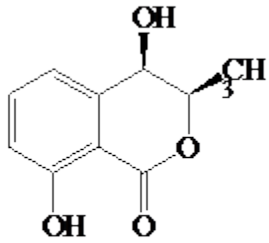

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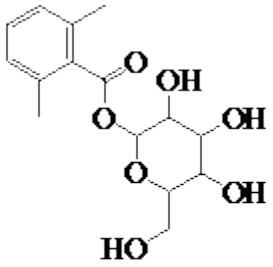

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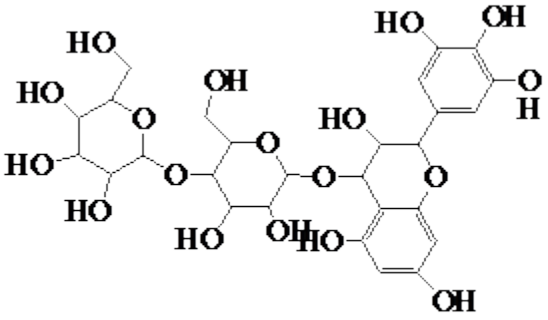

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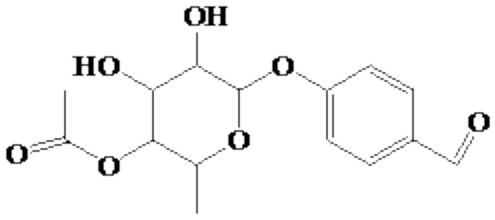

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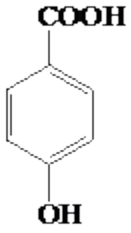

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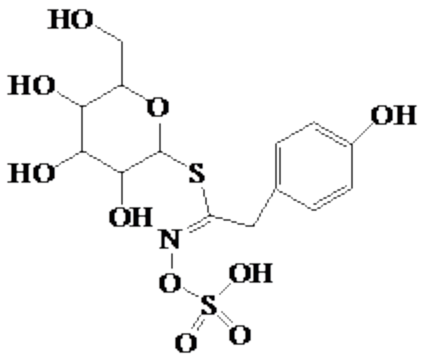

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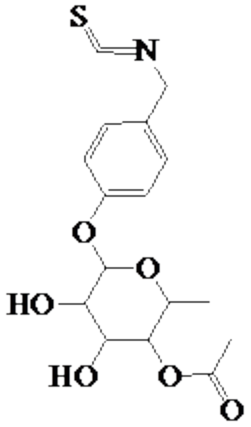

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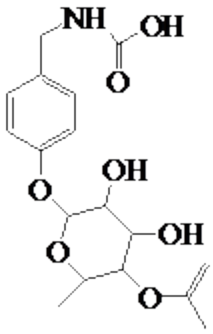

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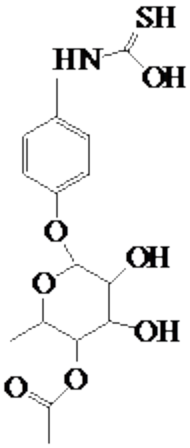

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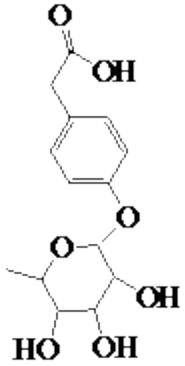

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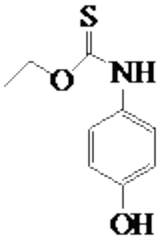

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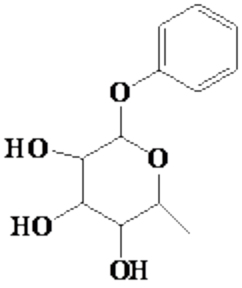

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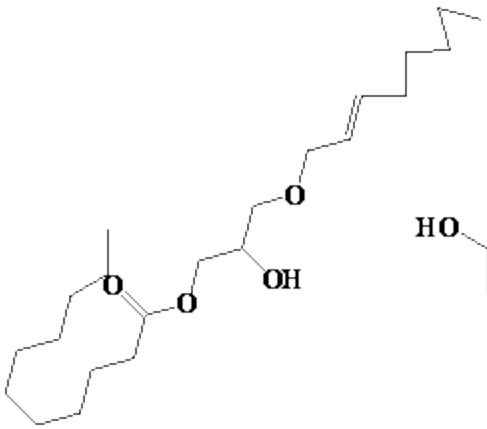

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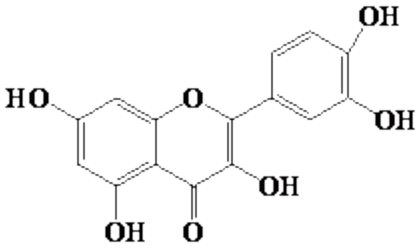

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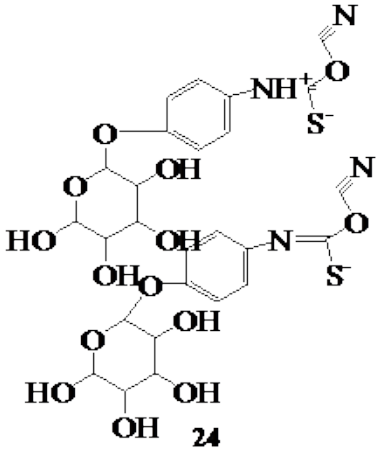

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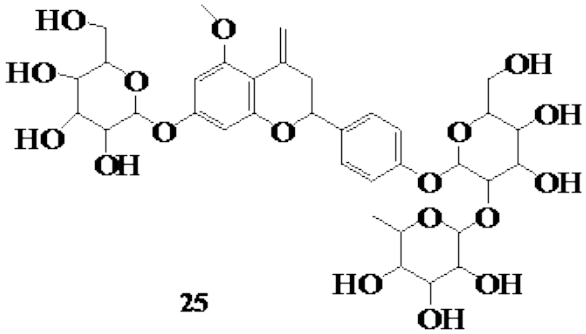

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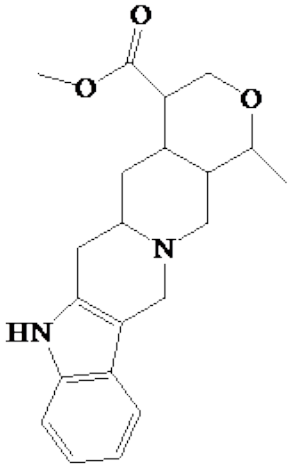
Appendix 2: Structures of compounds previously described in *R. caffra*



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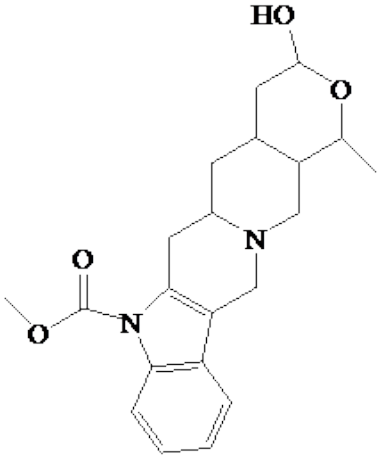

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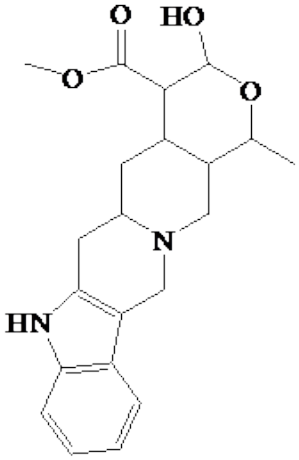

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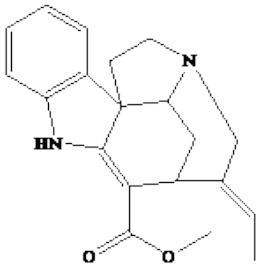

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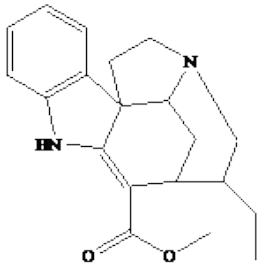

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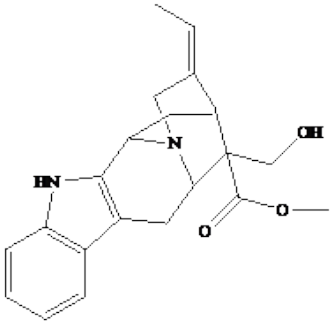

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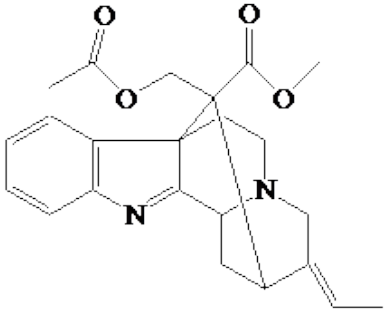

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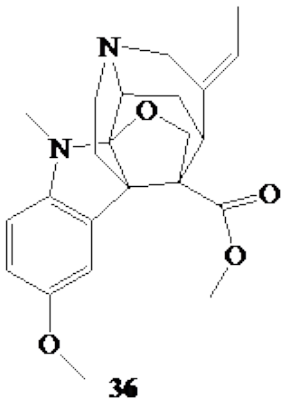

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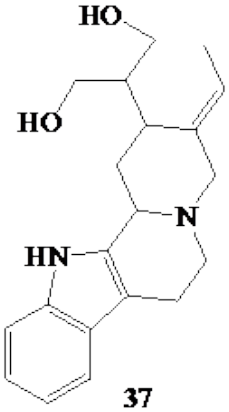

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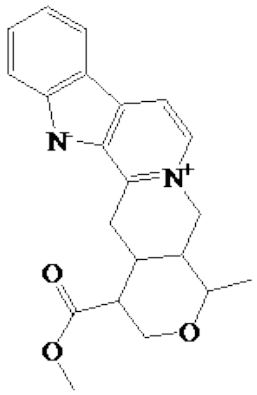

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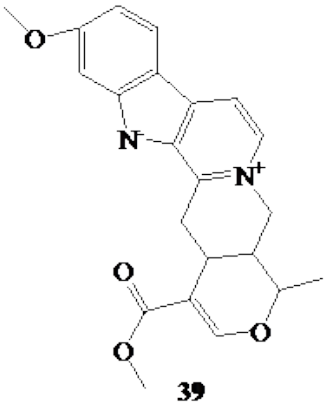

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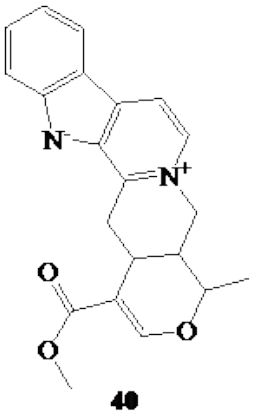

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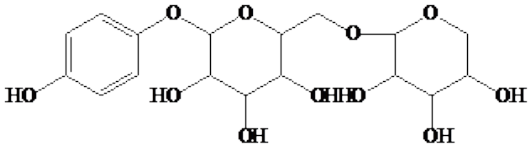

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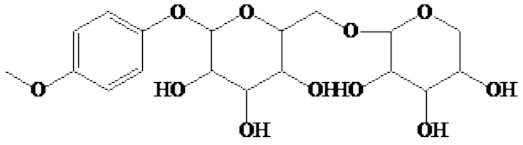

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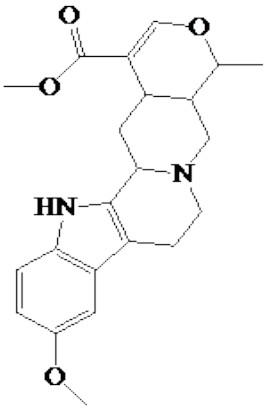

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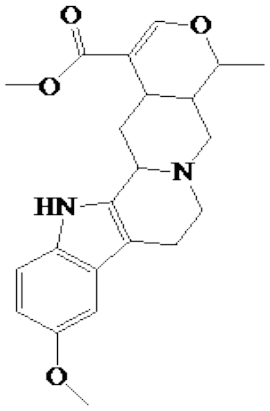

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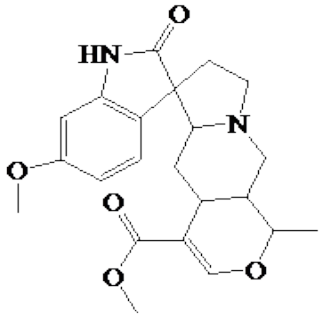

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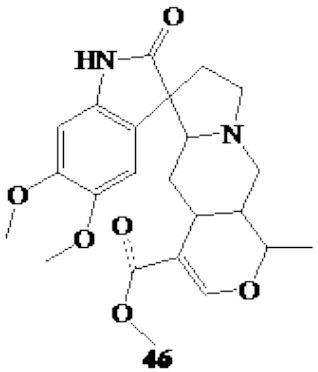

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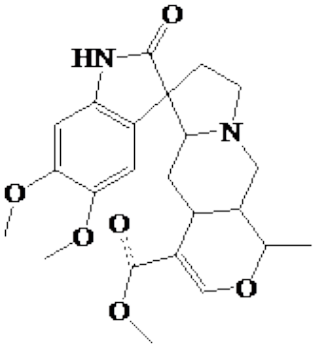

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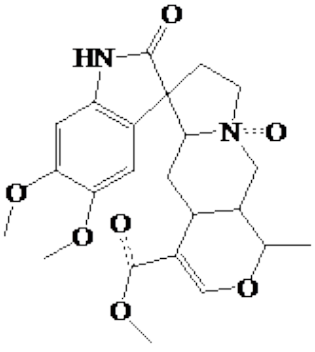

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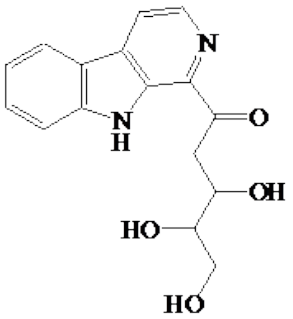

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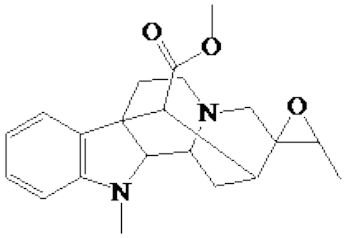

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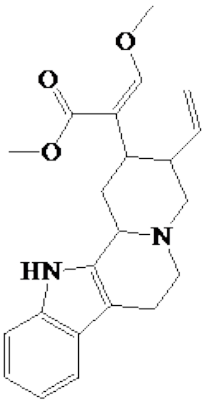

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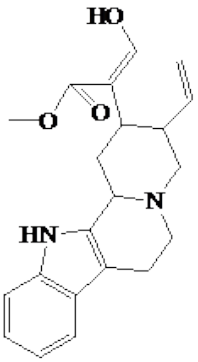

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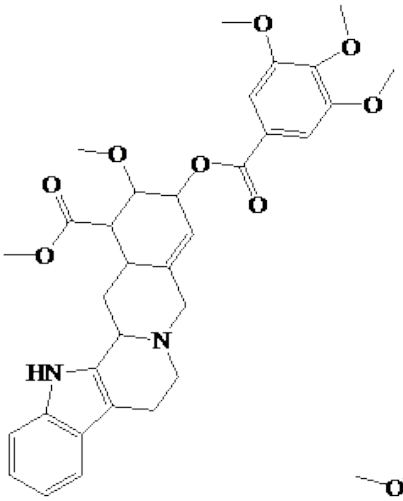

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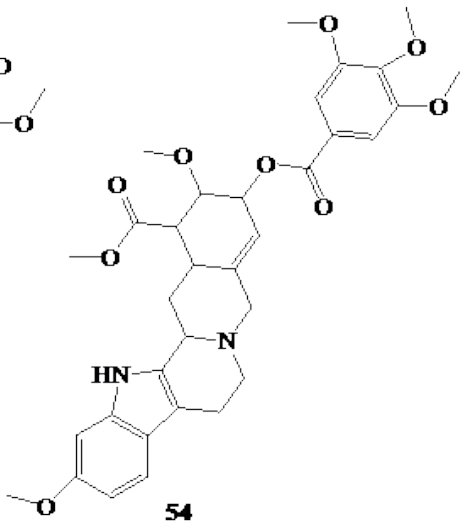

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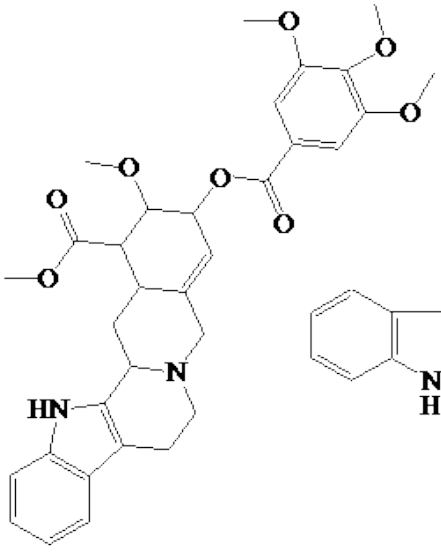

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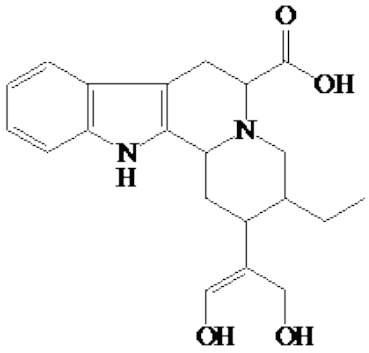

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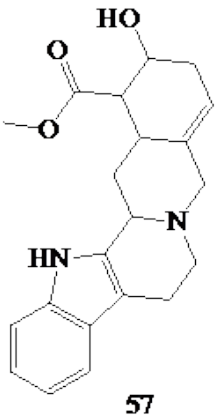

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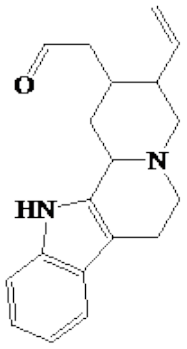

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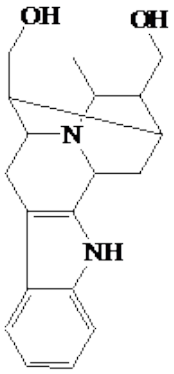

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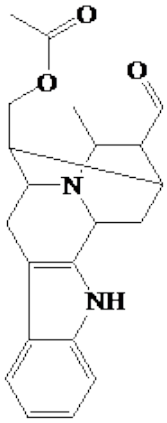

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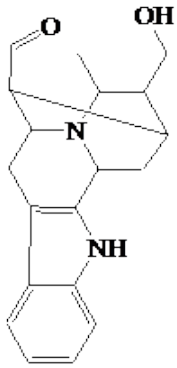

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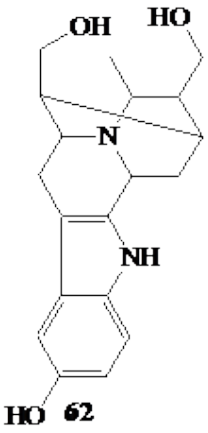

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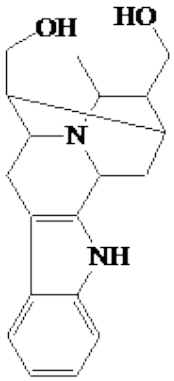

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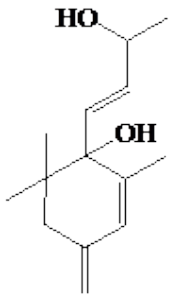

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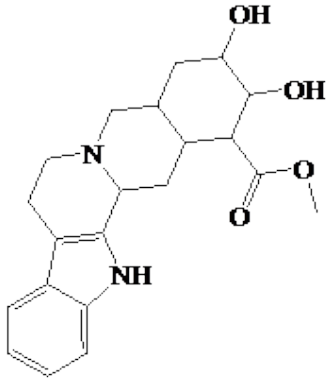

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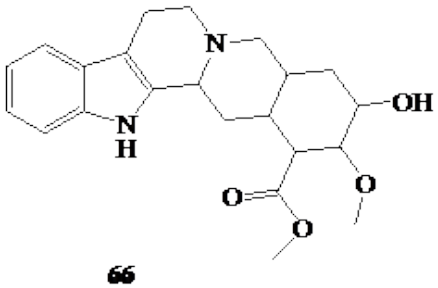

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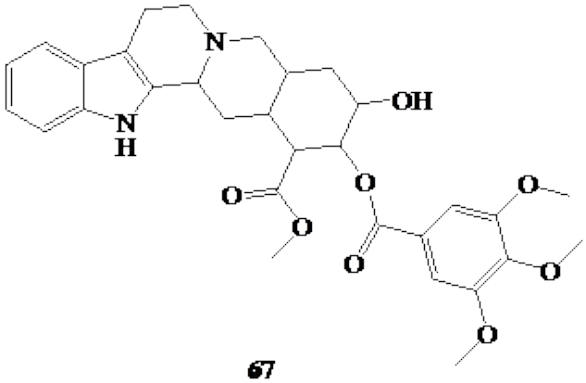

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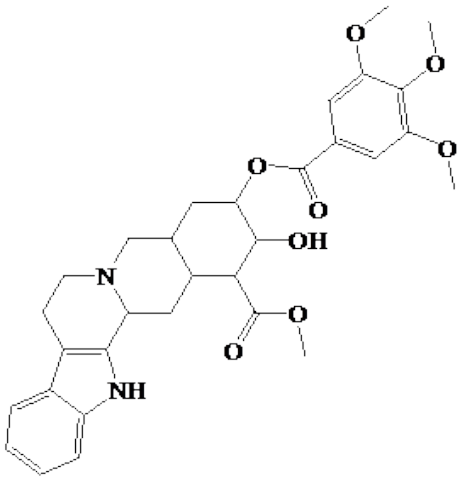

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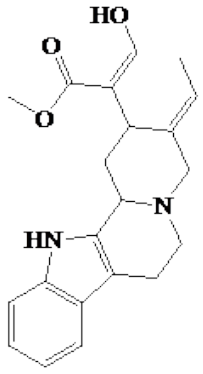

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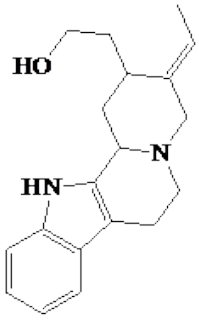

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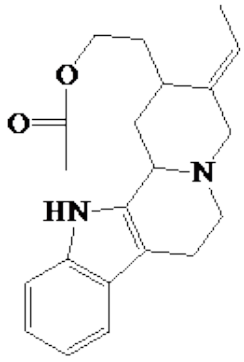

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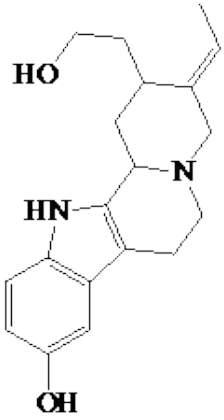

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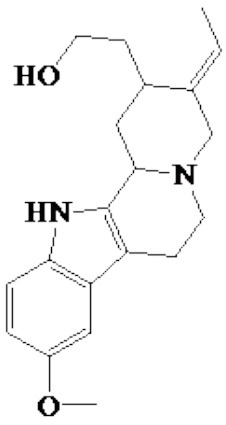

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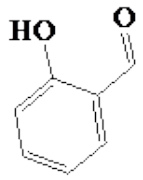

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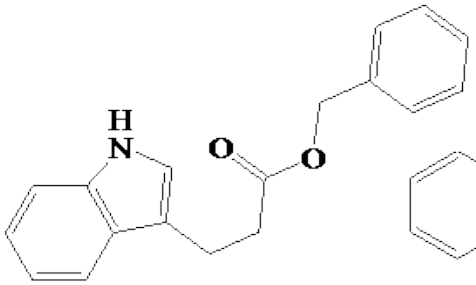

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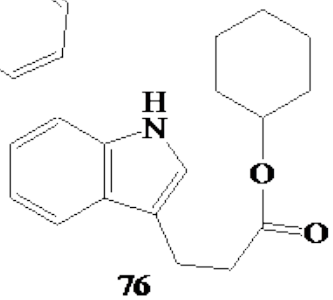

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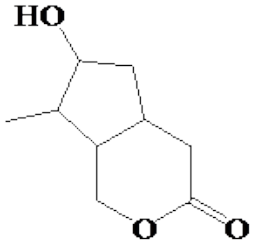

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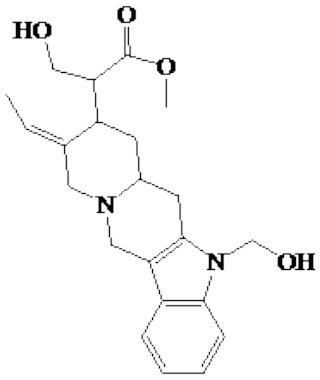

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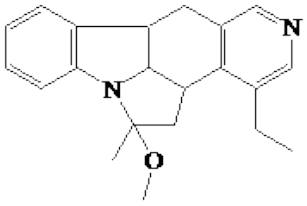

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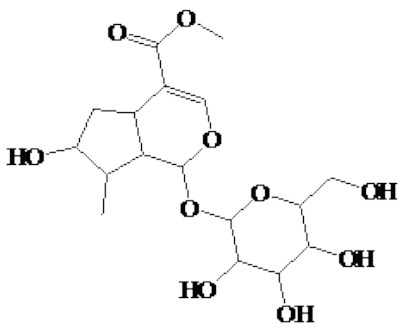

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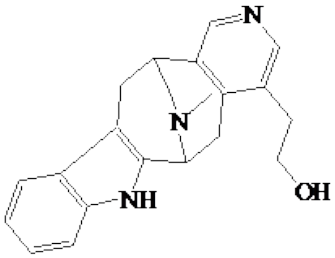

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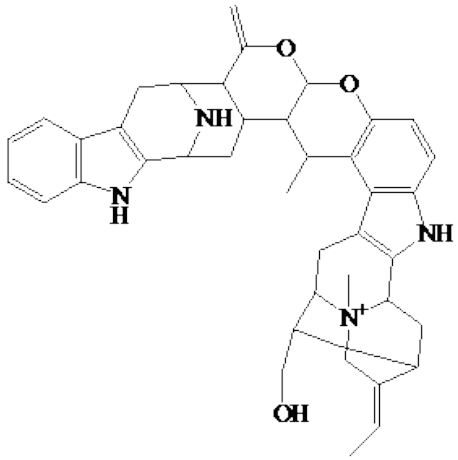

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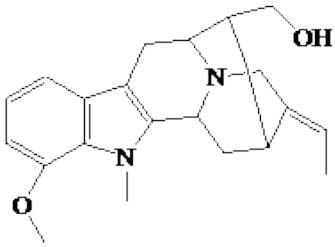

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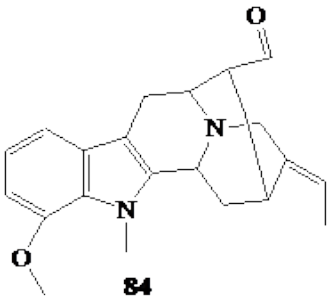

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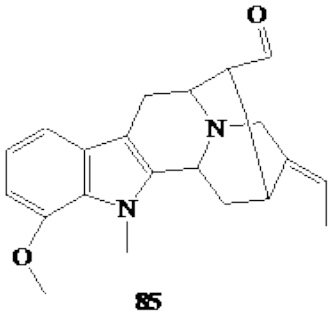

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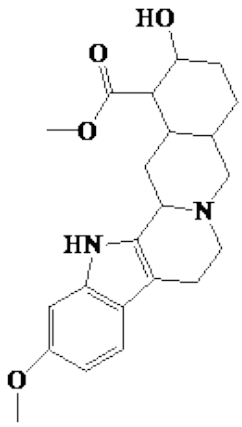

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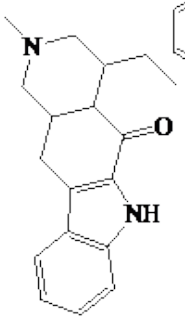

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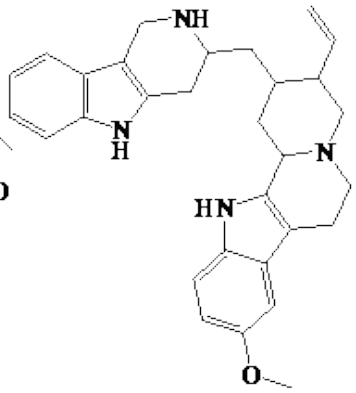

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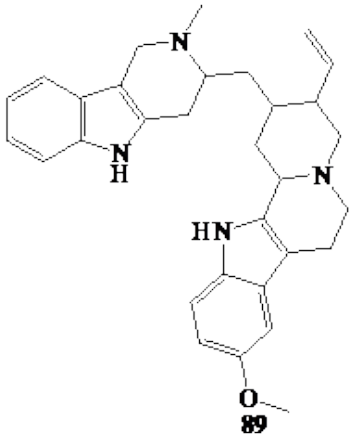

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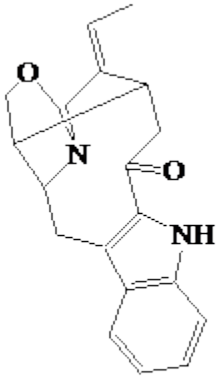

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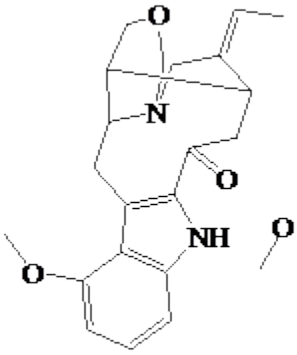

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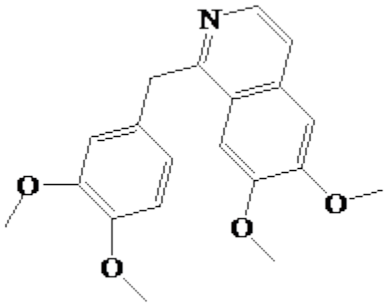

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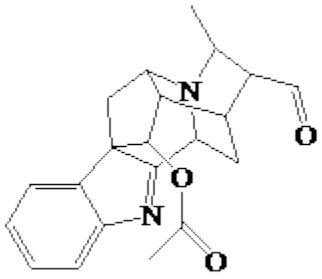

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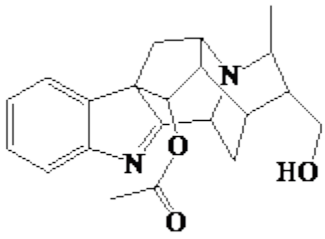

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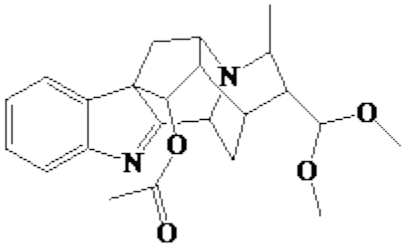

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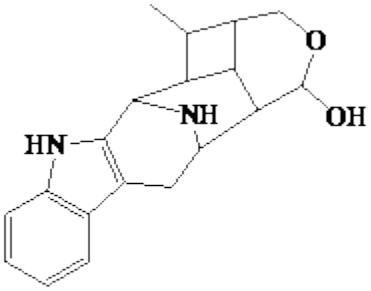

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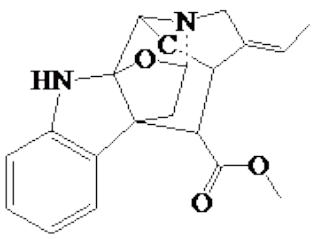

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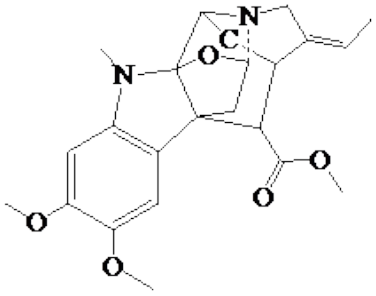

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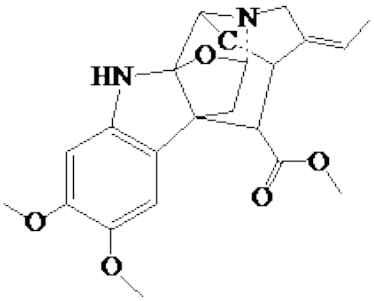

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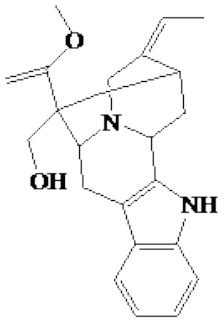

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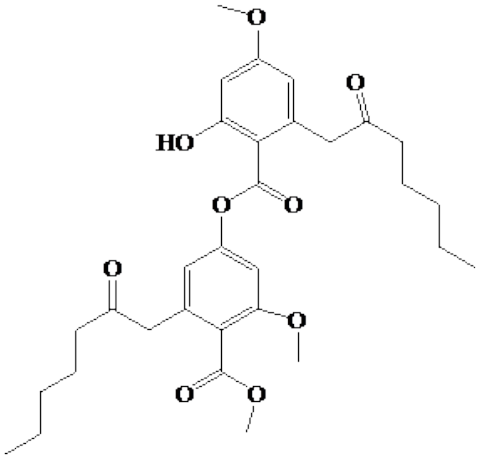

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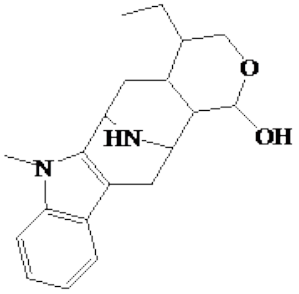

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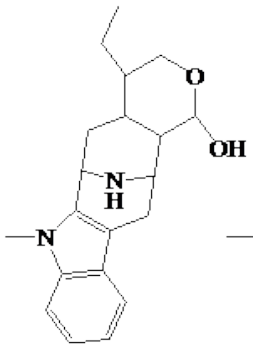

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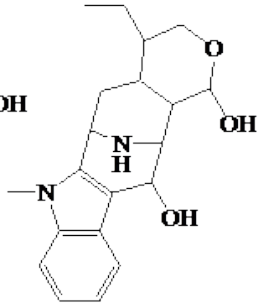

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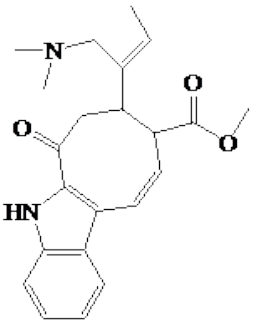

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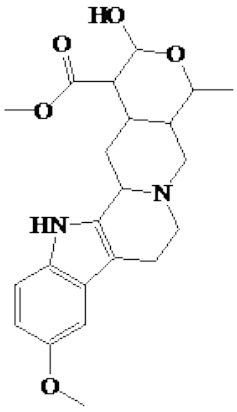

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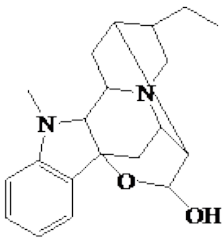

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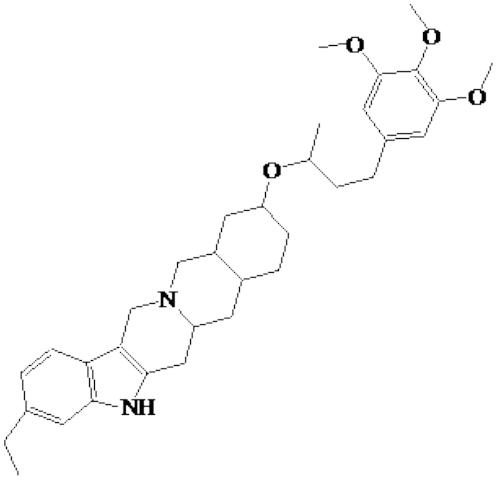

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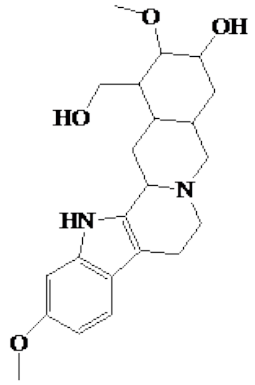

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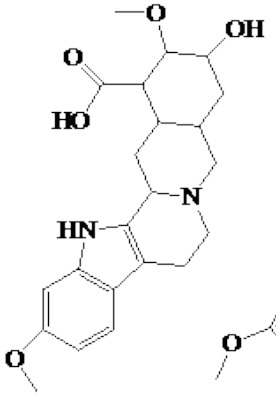

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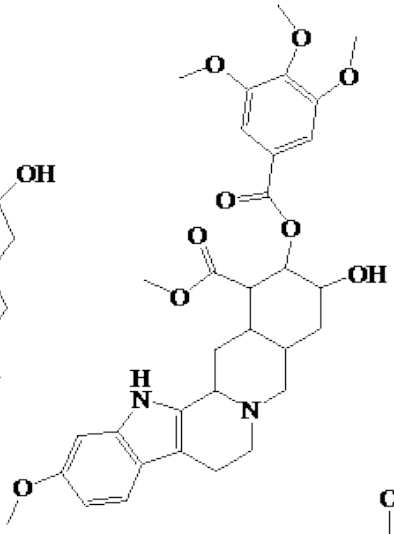

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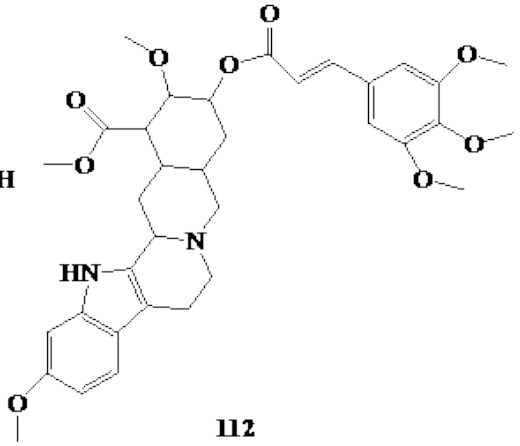

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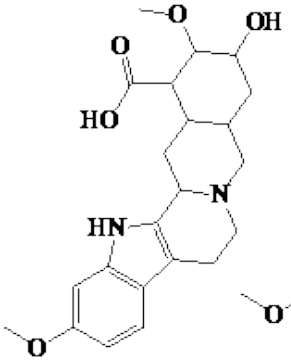

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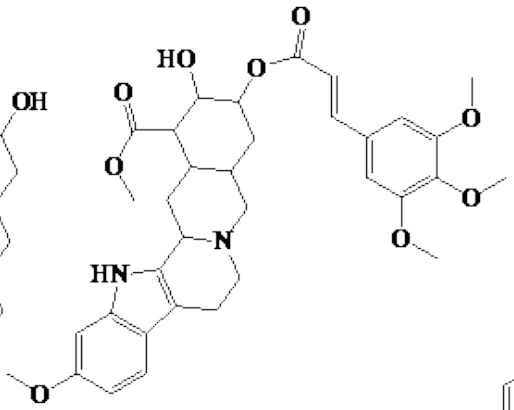

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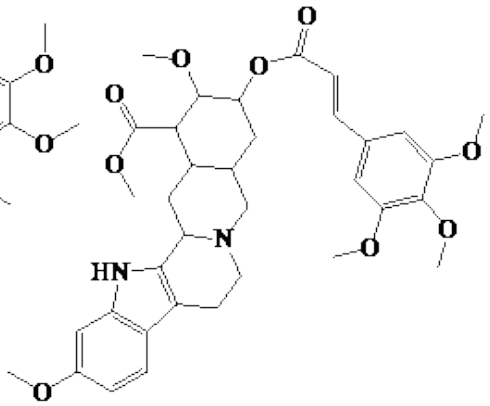

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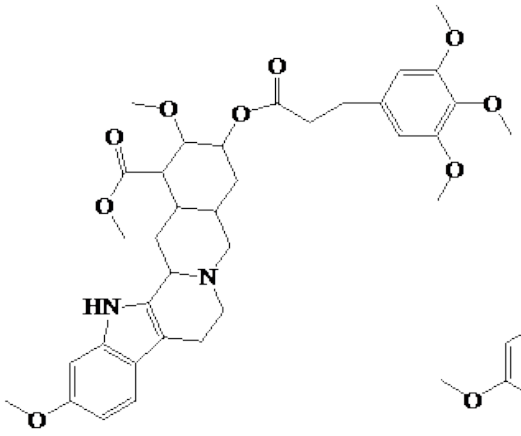

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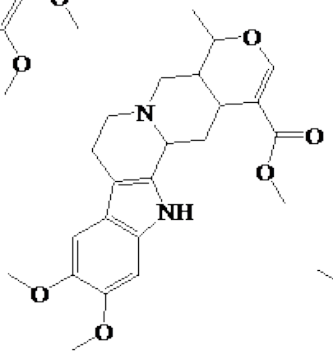

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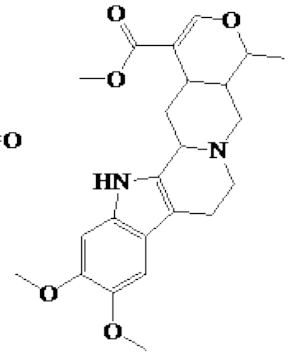

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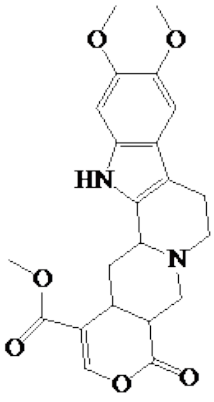

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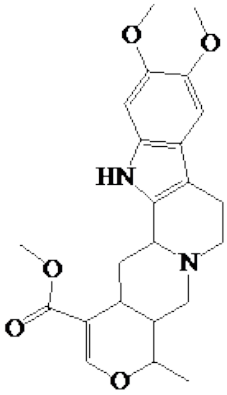

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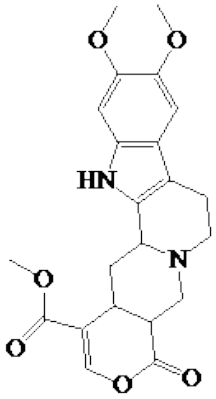

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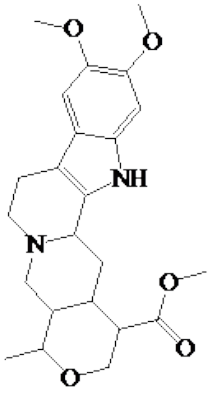

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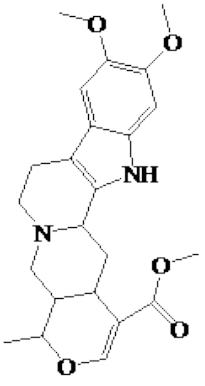

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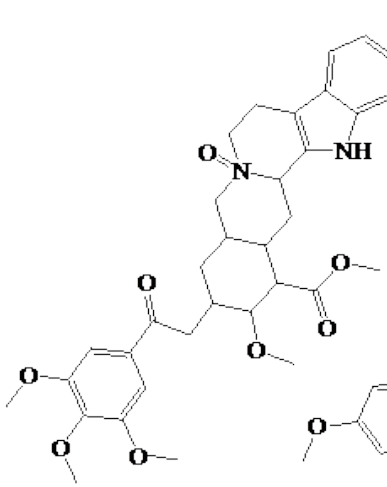

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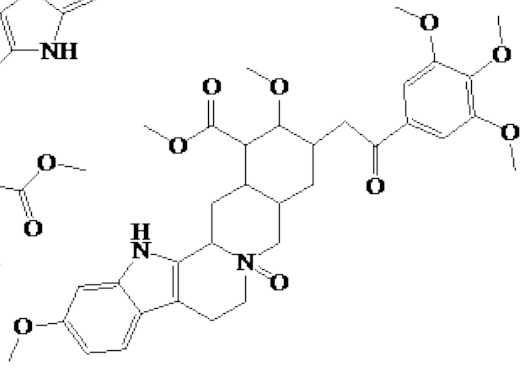

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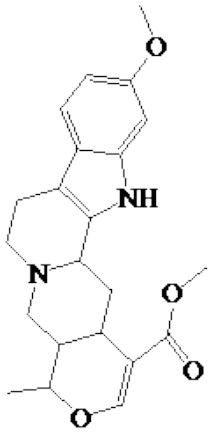

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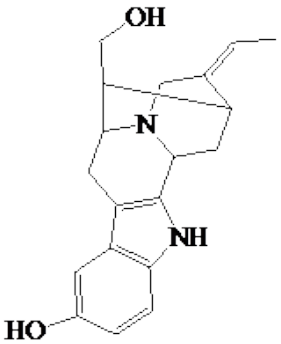

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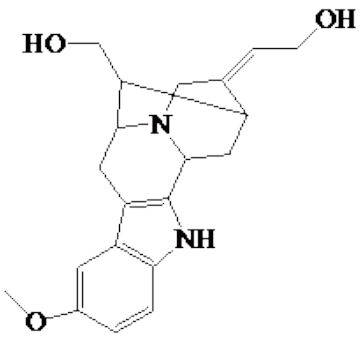

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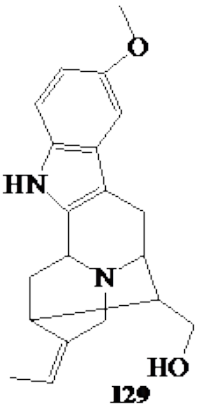

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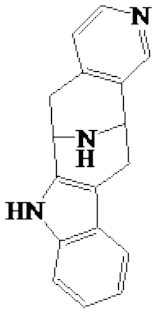

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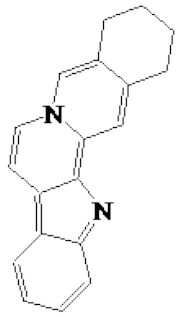

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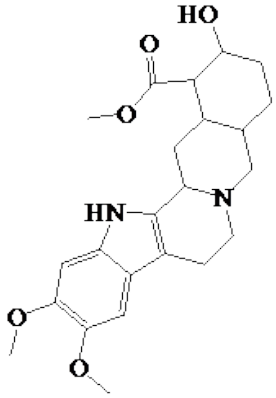

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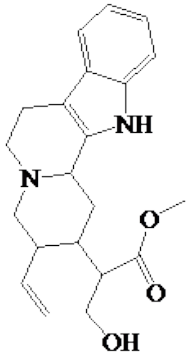

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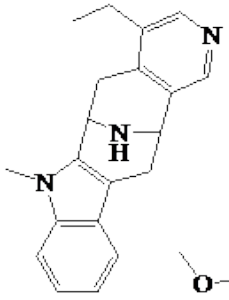

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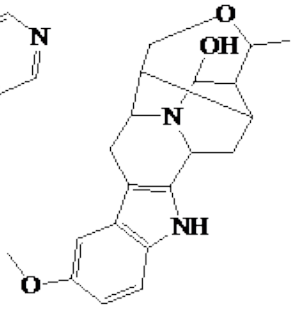

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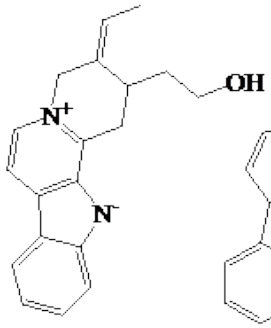

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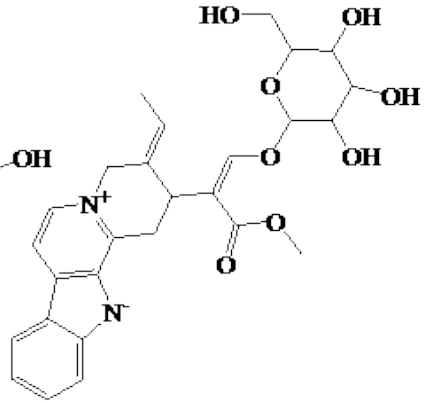

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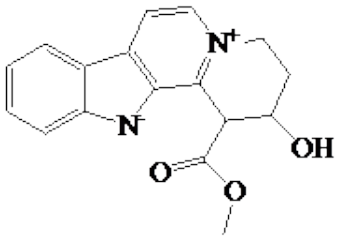

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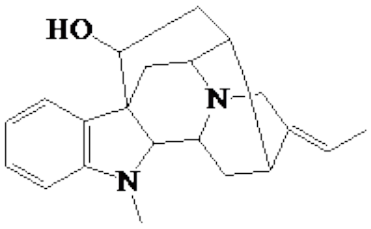

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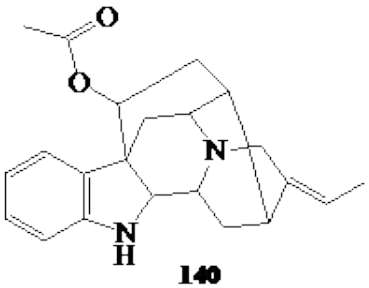

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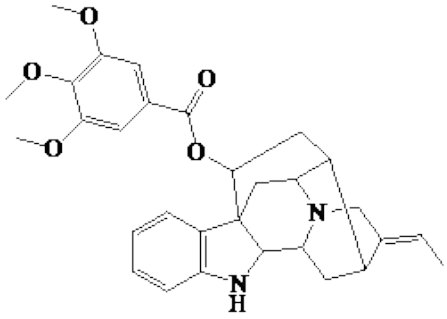

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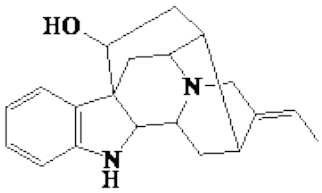

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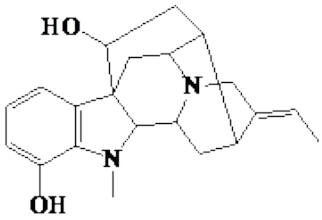

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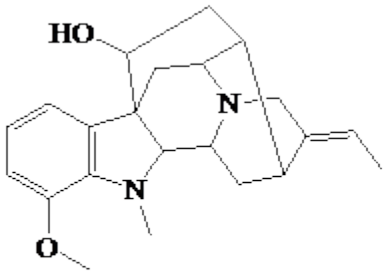

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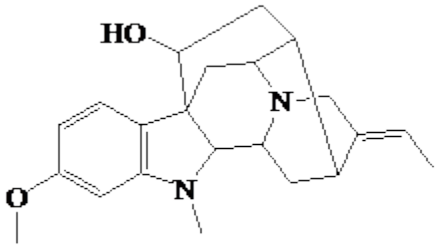

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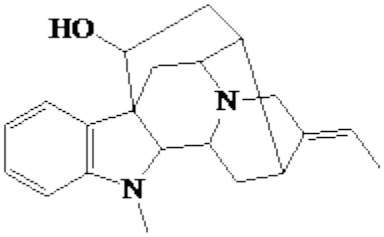

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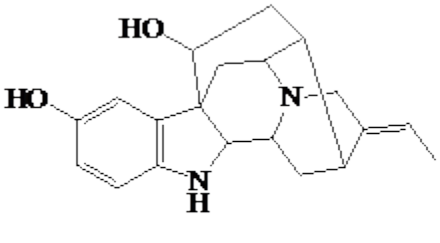

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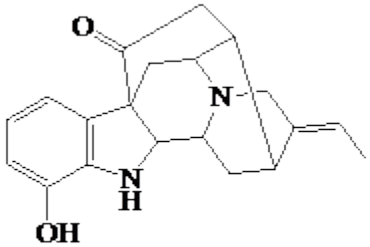

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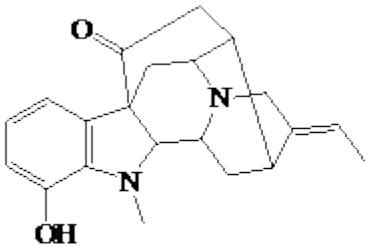

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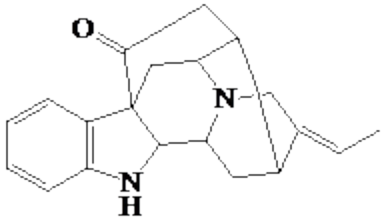

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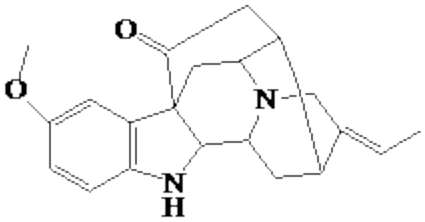

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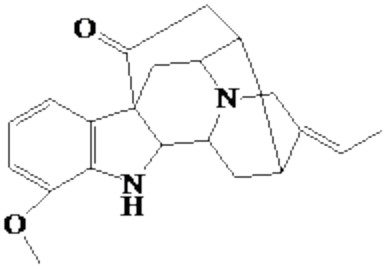

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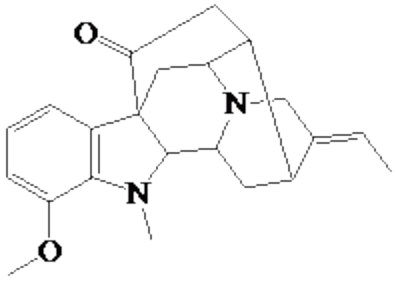

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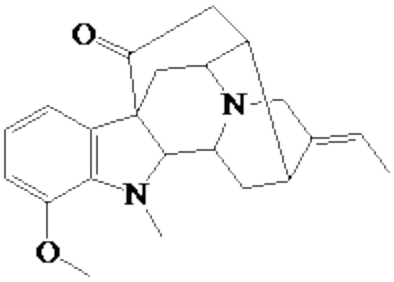

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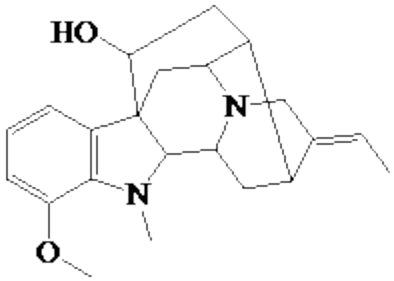

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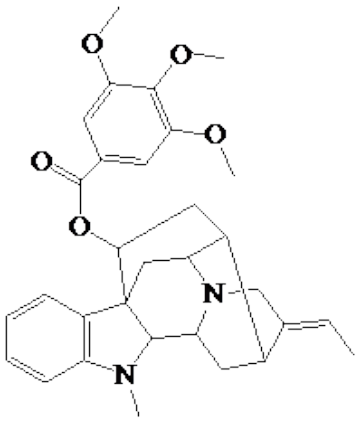

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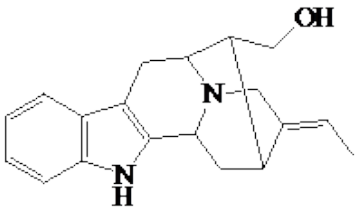

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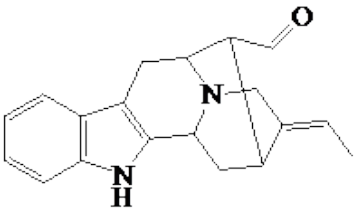

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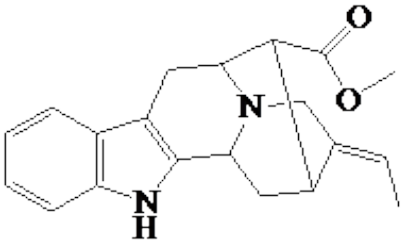

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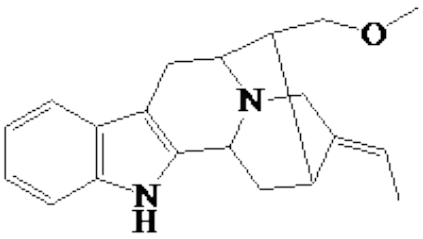

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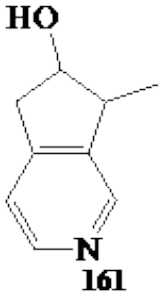

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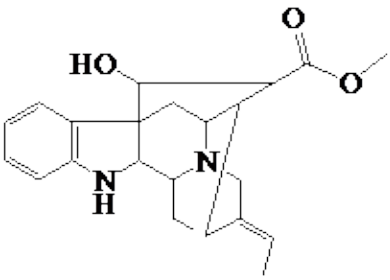

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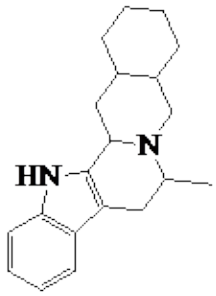

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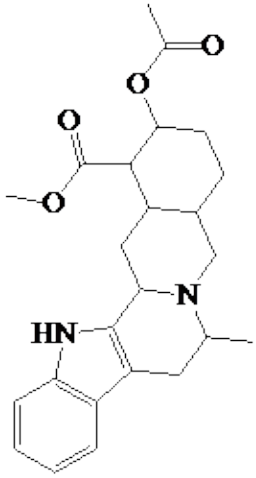

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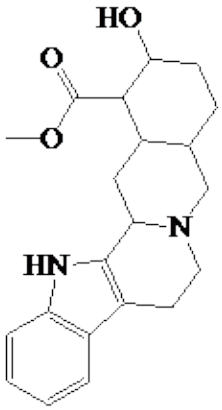

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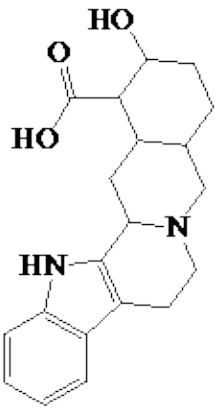

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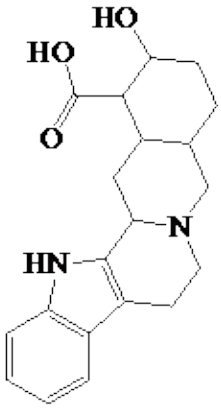

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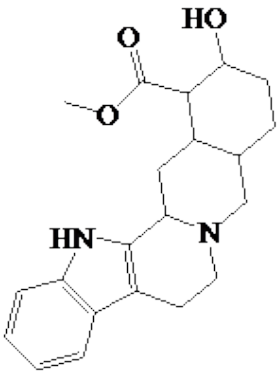

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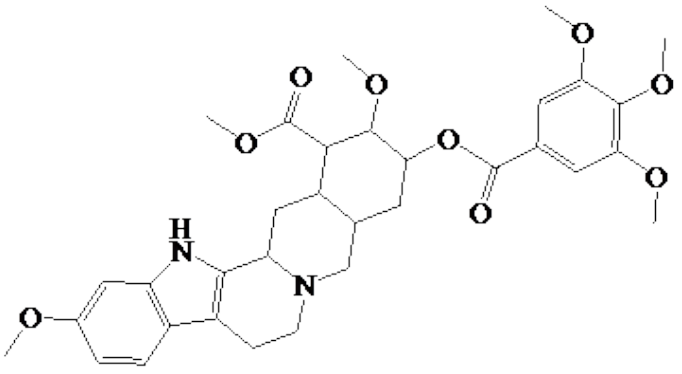

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