

**DETERMINATION OF THE ANTIBACTERIAL, ANTIOXIDANT ACTIVITY, ISOLATION AND  
CHARACTERISATION OF ACTIVE COMPOUNDS FROM THE LEAVES OF *RHUS*  
*LEPTODICTYA* PLANT**

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Date: February 2014

## DECLARATION BY CANDIDATE

I hereby declare that the dissertation/thesis submitted for M Tech: Chemistry, at Vaal University of Technology has never been submitted to any other institution of higher learning. I further declare that all cited sources are acknowledged by list of referencing.

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## **DEDICATION**

This work is dedicated to my mother, Mrs Masindi Matamela and my son Mutshidzi Maumela.

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## GLOSSARY OF ABBREVIATION

ATCC	American type culture collection
AIDS	Acquired immunodeficiency syndrome
BEA	Benzene ethanol ammonium
BHA	Butylated hydroxyl anisole
BHT	Butylated hydroxyltoluene
ABTS	Azinobis -2,3-ethylbenzo thiozoline -6- sulfonic acid
CEF	Chloroform ethyl acetate and formic acid
DPPH	Diphenyl- 2 – picryl hydrazyl
EMW	Ethyl acetate methanol water
GC	MS – Gas chromatograph mass
H <sub>2</sub> O <sub>2</sub>	Hydrogen peroxide
INT	Iodonitrotetrazolium
MH	Mueller Hinton
MIC	Minimum inhibitory concentration
MVA	Minimum visual angle
NAD	Nicotinamide adenine dinucleotide
NMR	Nuclear magnetic resonance
OH	Hydroxyl group
ROS	Reactive oxygen species
TDH	Threonine dehydrogenase

TLC	Thin layer chromatography
TEAC	Trolox equivalent antioxidant capacity
UV-vis	Ultraviolet-visible
WHO	World health organisation
$^1\text{H}$ NMR	Proton Nuclear magnetic resonance
$^{13}\text{C}$ NMR	Carbon 13 Nuclear magnetic resonance
COSY	Correlation spectroscopy
NEOSY	Nuclear overhauser effect spectroscopy
DEPTH	Distortionless Enhancement by polarisation transfer
HMBC	Heteronuclear multiple-bond correlation
HSQC	Heteronuclear single-quantum correlation spectroscopy

## ABSTRACT

*Rhus leptodictya* commonly known as a mountain karee belongs to the family *Anacardeaceae* and has been used since antiquity in tradition medicine. In the present study antimicrobial and antioxidant potentials of the leaves of *Rhus leptodictya* were studied, followed by isolation of at least one active compound which showed antibacterial and antioxidant potentials. Extractions were performed based on the polarity of the solvent used. The solvents used were hexane, dichloromethane, ethyl acetate, acetone and methanol. Dichloromethane was found to be extracting more compounds than the other used solvents. Thin layer chromatography (TLC) was used to determine the chemical composition of the extracts by employing different solvent systems. The results showed that, of the solvent systems used, namely ethyl acetate: methanol: water (EMW) 40: 5: 1; chloroform: ethyl acetate: formic acid (CEF) 5: 4: 1 and benzene: ethanol: ammonium hydroxide (BEA) 90:10:1, BEA produced better separations.

To determine the antioxidant potential of the leaves, 2,2-diphenyl picrylhydrazyl (DPPH) was used. Different spot with different  $R_f$  values were found to be active by show of yellow colour on the TLC plate. The yellow colour is due to the proton gained by DPPH when it reacts with active compound. Bioutography results showed that different leaves were active against selected bacterium. Minimum inhibitory concentration studies showed that the methanol extract was more active against *B.subtilis* and *S.pnuemonia* at concentration less than 0.1 mg/ml respectively. Dichloromethane extract was found to be the least effective on *S.pneumonia*, as compared to the methanol, acetone and ethyl acetate extracts. In terms of the total activity, the ethyl acetate concentration showed better total activity than the other extracts studied in this research.

A new compound 7, 8-trihydroxy-2-(4'-hydroxy phenyl)-3-5-[5'', 6''-dihydroxy-2''-O-(4'''-hydroxyphenyl)-4-1t-chromen-4''-one]-4-1t-chromen-4-one was isolated and characterized by <sup>1</sup>H-NMR, <sup>13</sup>C-NMR, MS and IR. According to the literature search, this compound has never been isolated from any plant and it has showed both antioxidant and antibacterial activity.

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# CHAPTER 1

## INTRODUCTION

### 1.1 Background

The increasing incidence of multidrug resistant strains of bacteria and the recent appearance of strains with reduced responsiveness to antibiotics raise serious concern of health delivery and accessibility due to untreatable bacterial infections. There is therefore the needed urgency to the search for new antimicrobial agents. Plants are important source of potentially useful structures for the development of new chemotherapeutic agents (Barku et al., 2013). Since time immemorial, plants have been used in preparation of drugs thus, act as sources of medicine.

Various species of plants have been used and consumed due to the presence of high antioxidant and antibacterial phyto-constituents. The extracts of medicinal plants and natural products therefore have become a great source of antioxidant and anti-ageing agents. Many human diseases are caused by oxidative stress that results from imbalance between the formation and neutralization of pro-oxidants (Roberts 2002). To be able to identify the medicinal potency of any plant, the *in vitro* antibacterial activity and antioxidant activity assay must be carried out on the selected plants as the first step. The present study deals with initial phytochemical screening, evaluation of the antioxidant activity and the antibacterial activity of leaf extracts of *Rhus leptodictya* and isolation of one active compound.

Medicinal plants are well known natural sources for the treatment of various diseases since antiquity. About 20 000 plant species used for medicinal purposes are reported by World Health Organization WHO (Taylor 2000). Natural product, either pure compounds, or standardized plant extract, provide unlimited opportunities for new drug leads because of the unmatched availability of chemical diversity (Maregesi 2008). Traditional medicine is a term loosely used to describe ancient and culture-bound health practices that existed before the application of conventional science to health matters in official, modern, scientific medicine or allopath (Fennel et al, 2004).

Natural product from microorganism have been the primary source of antibiotics, but with the increasing acceptance of herbal medicine as an alternative form of health care, the screening of medicinal plants for active compounds has become very important because they serve as a promising sources of novel antibiotic prototypes (Meurer-Grimes et al., 1996). Herbal medicine is the most important medicine for the majority of people on the planet, especially those who cannot afford expensive drugs. It was found that herbal medicine is the oldest and most tried and tested form of medicine. In a sense it forms the basis of all medicine, the mother of all remedy used today. Herbs are widely exploited in the traditional, medicine and their curative potential is well documented (McKenna, 1996). It is estimated that traditional medicine from natural products still forms the basis for primary health care in 80 % of the developing world (Farnsworth 1988; Balick et al., 1994).

Plants have served as a source of new pharmaceutical products and inexpensive starting material for the synthesis of many known drugs. The first chemical substance to be isolated from plant was benzoic acid in 1560 (Cox.1994: Cowan, 1999). The search for useful drugs of known structure did not begin until 1804 when morphine was separated from *papaversomniferum L. pium*. Since then

many drugs from high plants have been discovered, but less than 100 with defined structures are in common use (Angeh, 2006). Considering the great number of chemicals that have been derived from plants as medicine, scientific evaluation of plants used traditionally for the treatment of bacterial infection seems to be logical step of exploiting the anti-microbial compounds, which may be present in plants (Cowan, 1999).

Many medicinal plants produce variety of compounds of known therapeutic properties. Higher plants are still regarded as potential source of new medicinal compounds. It is estimated that there are about 250 000 plant species in the world, and only 5-15% of them have been tested for potentially useful biologically active compounds (McGaw & Eloff, 2008). Medicinal plants can offer a wide range of natural antioxidants due to the structural diversity of their secondary metabolites (Mativandlela, 2005). Medicinal plants have now been recognized as sources of natural antioxidant compounds which are mainly phenolic compounds. The increase in the consumption of market products that accompanies the loss of use of native plants for food or medicine by indigenous people may contribute to change in oxidative status of the plant (Kuhnlein & Receveur, 1996). Many higher plants accumulate extractable organic substances in quantities sufficient to be managing diseases.

Many plants constituents are effective as remedy for some diseases and accounts for large number of pharmaceutical important compounds in Western pharmacopoeia and a number of important drugs (Cowan, 1999). Generally the economically useful plants are sold as commodities of commercial value. Medicinal plants that are sold for medicinal purposes dominate the market. Of the medicinal plants sold for medicinal purposes, those that are used for the treatment of microbial infections constitute the biggest group (Steenkamp, 2006). Traditional lifestyles, in which diet,

exercise and possible antioxidant and hypoglycemic medicine played an important role, may have masked people in a pre-diabetic state in the past (Letitia et al. 2002).

Natural product chemistry is increasingly becoming interdisciplinary in practice. In the study of natural product chemistry, isolation and purification are mandatory first steps encountered. Isolation and characterization of the bioactive agent in a plant leads to the possible synthesis of a more potent drug with reduced toxicity. The pure compound is required to assess the possible toxicity or side effects of the medicinal plant (Saforowa, 1982). Chemicals from plants may possess complex chemical structures that are not available in synthetic compound libraries. There are hundreds of chemical substances that have been derived from plants for use as drugs and medicines. Many more await discovery (Taylor, 2000).

## **1.2 The use of medicinal plant as antioxidant agent**

Oxidation is one of the most important processes, which produce free radicals in food, chemicals and even in living systems (Barku et al., 2013). Catalase and hydroperoxidase enzymes convert hydrogen peroxide and hydroperoxides to nonradical forms and function as natural antioxidants in human body (Halliwell, 1994). Antioxidants are important in maintaining good health and there is growing interest in the investigation of the antioxidant activity of secondary metabolites from medicinal plants for compounds with higher potency and lower toxicities than the synthetic ones currently available (Thabrew et al., 1998). Currently available synthetic antioxidants like butylated hydroxyl anisole (BHA), butylated hydroxyl toluene (BHT), tertiary butylated hydroquinone and gallic esters have been suspected to cause or prompt negative health effect (Barlow, 1990). The

imbalance between intracellular antioxidants and intracellular reactive oxygen species (ROS) is a known contributing factor to over a hundred diseases (Zhang et al., 2009).

Free radicals contribute to more than one hundred disorders in humans including atherosclerosis, arthritis, ischemia and reperfusion injury of many tissues, central nervous system injury, gastritis, cancer and AIDS (Pourmorad et al., 2006). Antioxidants prevent oxidative damage of biomolecules and cells and ROS-induced diseases by reacting with free radicals, scavenging free radicals and chelating free catalytic metals (Tiwari, 2001). Antioxidant potential of plant products is due to the presence of several compounds which have distinct mechanism of action. Some are enzymes and proteins while others are low molecular weight compounds such as vitamins, carotenoids, flavonoids, anthocyanin and other phenolic compounds. Antioxidant action of phenolic compounds is due to their high tendency to chelate metals. Phenolics possess hydroxyl groups, which are used to bind particularly iron and copper. They also inhibit lipid peroxidation by trapping the lipid alkoxyl radical. This activity depends on the structure of the molecules, the number and position of the hydroxyl group in the molecule (Millic.et al .1998).

An easy way, rapid and sensitive method for the antioxidant screening of plant extracts is the free radical scavenging assay using 1, 1-diphenyl-2-picryl hydrazyl (DPPH) stable radical spectrophotometrically. In the presence of an antioxidant, DPPH radical obtains one more electron and the absorbance decreases (Koleva et al, 2002).

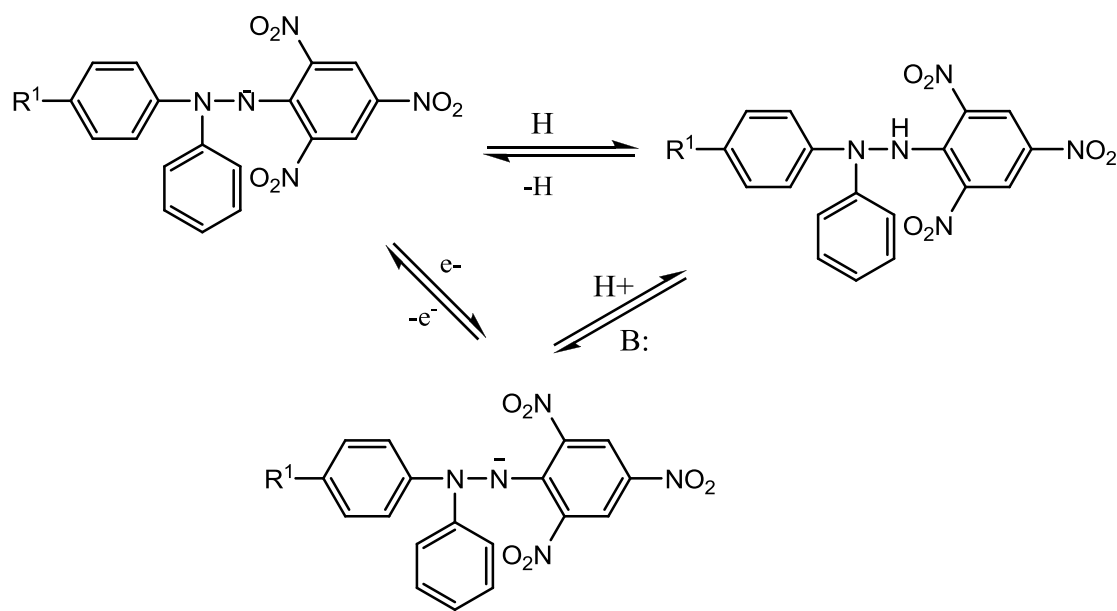


Figure 1: Reaction of DPPH to lose proton

The Trolox equivalent antioxidant capacity (TEAC) assay is widely used in the food and nutraceutical industries to determine the antioxidant capacities of foods, beverages and nutraceutical products. The assay is based upon the ability of antioxidants to decolorize the 2, 2'-azinobis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS) radical cation (ABTS<sup>•+</sup>), which is blue in solution and has an absorbance maximum of 734 nm. (Walker, 2009).

Another major class of antioxidants is the amino- and amidothiols. These include the amino acid cysteine, the endogenous tripeptide glutathione, the antihypertensive drug captopril, the antiarthritic drug penicillamine, the antiurolithic drug N-(2-mercaptopropionyl)glycine and the radio protective drug amifostine (Walker, 2009).

### 1.3 Medicinal Plants as antibacterial agents

Because of the resistance that pathogenic build against antibiotics, there is a great interest in the search for new antimicrobial drugs also from nature (Liouane et al., 2009). The indiscriminate use of antibiotic has led to drug resistance of many bacterial strains (Eloff & Martin, 1998). In 1967, 58% of all antibiotics were produced by Actinomycetes, 18% by other fungi, 12% by higher plants, 9% by bacteria and the remaining 3% by algae, lichens and animals (Edwards, 1980; Eloff and Martin, 1998). The World Health Organization (WHO) estimates that 80% of the people living in developing countries almost exclusively use traditional medicine (Eloff, 1998). Different parts of the plant are used in the indigenous system of medicine for the treatment of various human ailments such as wounds, ulcers, bowels and cholera (Begum et al., 2002). The leaves of *rhus* plants are used for the treatment of wounds, abscesses and externally for the treatment of neuralgia, throat infections and a wide range of skin diseases such as ringworm, ulcers and rashes. The leaves of the pelargonium are used in the treatment of the menstrual and menopausal problems, breast congestion, cellite and fluid retention (Coates, 2002). The leaves can also be used in the childhood ailments such as chicken pox, measles and mumps (Brendler & Van Wyk, 2008).

The medicinal value of medicinal plants lies in some chemical substances that produce a definite physiological action on the human body (Edeoga et al., 2005). The most important of these bioactive constituents of plants are alkaloids, tannins, flavonoids and phenolic compounds (Hill, 1952). The majority of these bioactive compounds are alkaloids, followed by sesquiterpenes, diterpenes, triterpene saponins, triterpene aglycones, flavonoids, sterols, coumarins, quinine's and monoterpenes (Jeruto et al., 2011).

## 1.4 Phytochemical Constituents

Plants produce primary and secondary metabolites which encompass a wide array of functions. Examples of primary metabolites include amino acids, simple sugars, nucleic acids and lipids which are compounds that are necessary for cellular processes (Croteau et al., 2000). The secondary metabolites of plant are compounds with no apparent function in the primary metabolites of the organism, and this substance tends to be of restricted taxonomic distribution. The most common plant secondary metabolites occur in the following groups: alkaloids, anthraquinones, coumarins, essential oils, flavonoids, steroids and terpenoids (Morales et al. 2002).

Phytochemicals are non-nutritive plant chemicals that have protective or disease preventive properties. They are natural bioactive compounds found in medicinal plants. They are nonessential nutrients, meaning that they are not required by the human body for sustaining life. It is well-known that plant produces these chemicals to protect themselves against herbivores, but recent research demonstrates that they can also protect humans against diseases. There are more than thousand known phytochemicals. Some of the well-known phytochemical are flavonoids, tannins, phenols, and many more. Chemical analysis have led to characterization of about 65 metabolites including phenolic acids, cinnamic acids and tannins, flavonoids and coumarins (Roberts 2002).

Medicinal plant parts such as roots, bark, stem, leaves, flowers, and fruits are commonly rich in phenolic compounds such as flavonoids, phenolic acid, stilbenes, tannins, coumarins, lignans and lignins (Survevaran et al, 2006). Medicinal plants constitute an effective source of antimicrobial



natural products. The use of medicinal plants all over the world predates the introduction of antibiotics and other modern drugs into Africa continent (Haslam et al. 1989).

Most phytochemicals have antioxidant activity and protect our cells against oxidative damage and reduce the risk of developing certain types of cancer. Phytochemicals with antioxidant activity are: allylsulfides which includes the (onions, leeks, garlic), carotenoids (fruits, carrots), flavonoids (fruits, vegetables), polyphenols (tea, grapes). Isoflavones, found in soy, imitate human estrogens and help to reduce menopausal symptoms and osteoporosis (Kim et al 2003). Indoles, which are found in cabbages, stimulate enzymes that make the estrogen less effective and could reduce the risk for breast cancer. Other phytochemicals, which interfere with enzymes, are protease inhibitors (soy and beans), terpenes (citrus fruits and cherries). The phytochemical allicin from garlic has anti-bacterial properties. Some phytochemicals bind physically to cell walls thereby preventing the adhesion of pathogens to human cell walls. Proanthocyanidins are responsible for the anti-adhesion properties of cranberry (Ferrara et al 2009). Consumption of cranberries will reduce the risk of urinary tract infections and will improve dental health (Bakker et al., 2004).

### **1.4.1 Flavonoids**

Flavonoids are a group of polyphenolic compounds with known properties which include free radical scavenging, inhibition of hydrolytic and oxidative enzymes and anti-inflammatory action (Frankel, 1995). The basic structure feature of flavonoids compounds is the 2-phenyl-benzo [ $\alpha$ ] pyrane or flavones nucleus, which consist of two benzene rings linked to heterocyclic pyrane ring (fig 1). Flavonoids are ubiquitous in photosynthesizing cells and therefore occur widely in the plant kingdom and they constitute a major group of phenolic compounds in plant. Flavonoids provide pigmentation for fruits, flowers and seeds to attract pollinators and seeds dispersers. Flavonoids

bearing a 3', 4'-dihydroxy-group such as quercetin, myricetin, luteolin, and their glycosides had a vitamin P-like effect. Polymethoxyflavones found in leaves and flower of certain plants and citrus fruits protect them from phytophthorainfestans (Zaprometov, 1993). They are found in fruit, vegetables, nuts, stems and flowers as well as tea, wine, propolis and honey, and represent a common constituent of the human diet (Havsteen, 1983). Flavonoids are becoming the subjects of medicinal research. They have been reported to possess many useful properties, including anti-inflammatory activity, estrogenic activity, enzyme inhibition, antimicrobial activity, antiallergic activity, antioxidant activity, vascular activity and cytotoxic antitumor activity (Middleton et al, 1993). For a group of compounds of relative homogeneous structure, the flavonoids inhibit a perplexing number and variety of eukaryotic enzymes and have a tremendously wide range of activities. In the case of enzyme inhibition, this has been postulated to be due to the interaction of enzymes with different parts of the flavonoids molecule such as carbohydrates, phenyl ring, phenol and benzopyrone ring.

The function of the flavonoids in flowers is to provide colours attractive to plant pollinators. In leaves, these compounds are increasingly believed to promote physiological survival of the plants, protecting it from, for example, fungal pathogens and UV-B radiation. Flavonoids are involved in photosensitization, energy transfer, the action of the plant growth hormones and growth regulators, control of respiration and photosynthesis, morphogenesis and sex determination (Harborne et al. 2000).

Flavonoids can be classified according to biosynthesis origin. Some classes, for example chalcones, flavanones, flavan-3-ols and flavan-3,4-diols, are both intermediate in biosynthesis as well as end product that can accumulate in plant tissues. Two additional classes of flavonoids are

those in which the 2 phenyl side chain of flavanone isomerizes to the 3 position, giving rise to isoflavonones and related isoflavonoids.

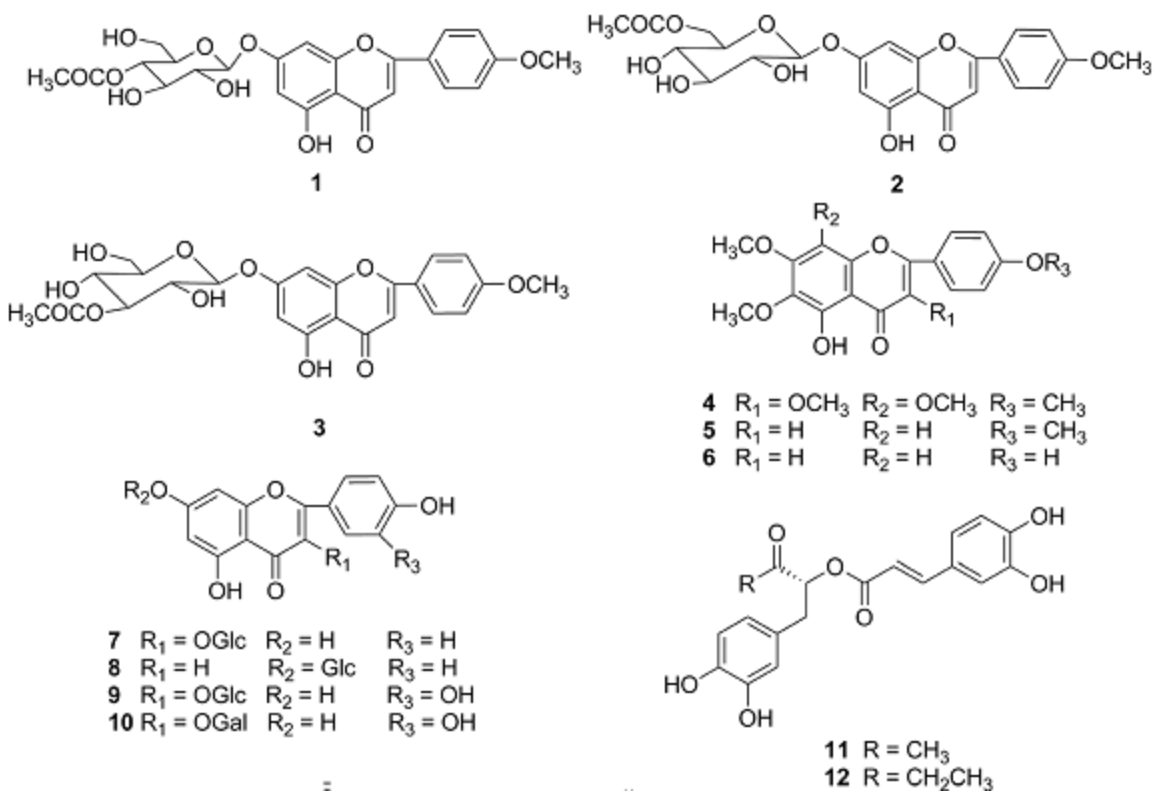


Figure 1.2 Structures of typical flavonoids (Wu et al., 2004)

### 1.4.3 Phenols

Phenols sometimes called phenolic, are a class of chemical compounds consisting of hydroxyl functional group (-OH) attached to an aromatic hydrocarbon group. Phenolic compounds are dietary constituent widely existing in plants and have been considered to have high oxidant capacity and free radical scavenging capacity (Kahkoneon et al., 2001). Phenolic compounds which are synthesized primarily from products of the shikimic acid pathway have several important

roles in plants. Phenolic compounds have attracted more and more attention as potential agents of preventing and treating many oxidative stress-related diseases. Several studies have showed that phenolic compounds were the main antioxidant ingredients in several medicinal plants (Cai et al., 2004; Liu et al., 2008).

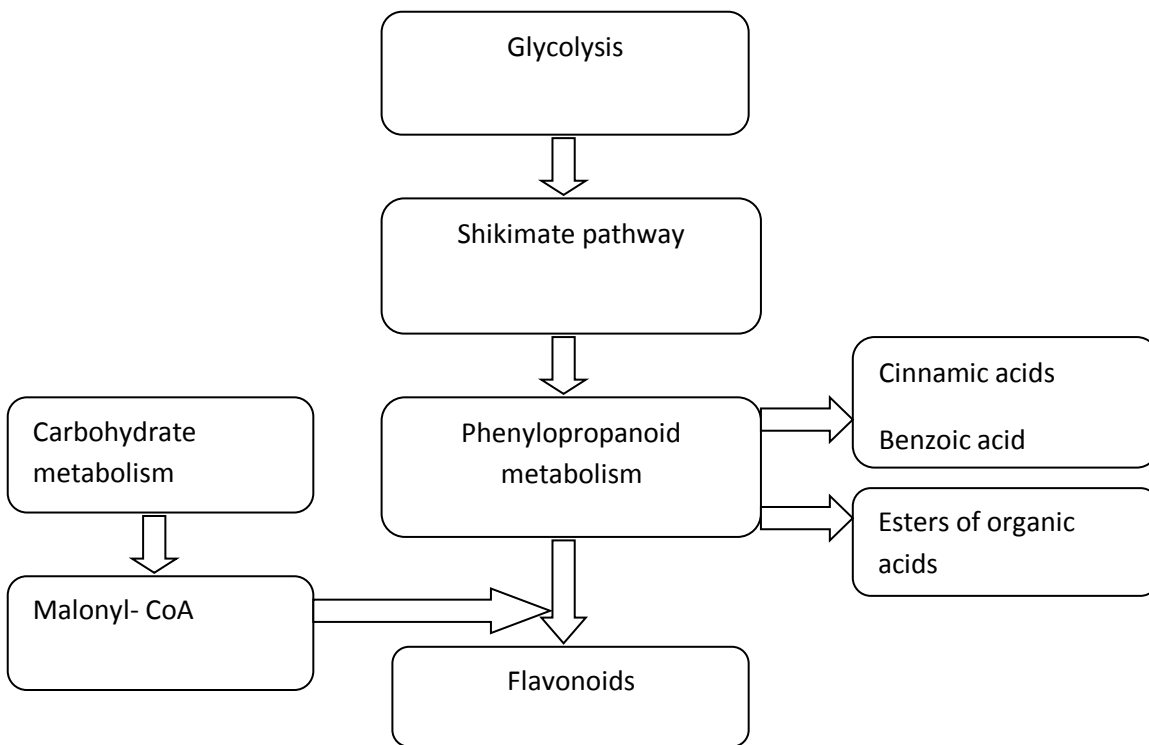


Figure 1.3: Schematic pathway of phenols to the production of flavonoids (Herman et al., 1999).

Phenolic compounds found in medicinal plants are known for their antioxidant potential and their role in prevention of human disease. Phenolic acids are plant metabolites widely spread throughout the plant kingdom. Recent interest in phenolic acids stems from their potential protective role, through ingestion of fruits and vegetables, against oxidative damage disease (coronary heart disease, stroke and cancers) phenolic compounds are essential for the growth and reproduction of plants, and are produced as a response for defending injured plants against pathogens. The

importance of antioxidant activities of phenolic compounds and their possible usage in processed food as a natural antioxidant have reached a new high in recent years (Cai et al., 2004).

As an alternative antioxidant property, some phenolic compounds with dihydroxy groups can conjugate transition metals, preventing metal-induced free radical formation. The redox active metal ions such as  $\text{Cu}^+$  or  $\text{Fe}^{2+}$  interact with hydrogen peroxide ( $\text{H}_2\text{O}_2$ ) through Fenton chemistry (as shown in reaction 3 below) to form hydroxyl radicals ( $\cdot\text{OH}$ ), which is the most reactive ROS known, being able to initiate free radical chain reactions by abstracting hydrogen from almost any molecule.

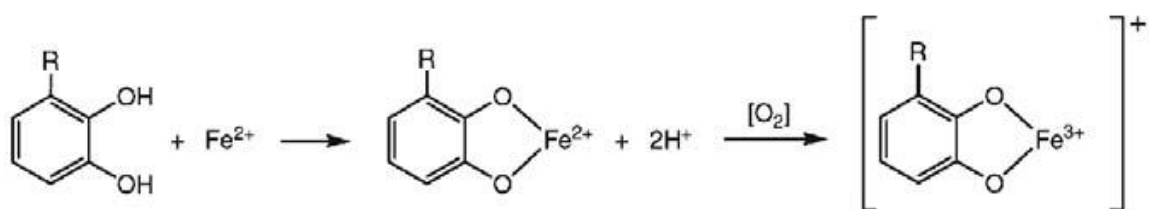


Figure 1.4: Reaction of phenolic compound with redox active metal ions (Fenton chemistry, 2011)

#### 1.4.6 Plant and Antibacterial production

Plants have many ways of generating antibacterial compounds to protect themselves against the pathogens. External plant surfaces are often protected by biopolymers e.g. fatty acid esters such as cutin and suberin and waxes. In addition external tissue can be rich in phenolic compounds such as alkaloids, diterpenoids, steroids and glycoalkaloids and other compounds which inhibit the development of fungi and bacteria. Cell walls of at least some monocotyledons also contain antimicrobial proteins, referred to as thionins (Carr & Klessing, 1989).

It has been reported that Rhus plant are plant cells that contain sequestered glycosides which are released them when ruptured by injury or infection. These glycosides may have antimicrobial activities against the invading pathogens or may be hydrolysed by glycosidases to yield more active aglycones. In the case of phenolic compounds these may be oxidized to highly reactive, antimicrobial quinines and free radicals (Kuc, 1985; Dean & Kuc, 1987).

Angeh 2007 has reported that Plants have several mechanisms to counter anti-microbial attack. Some of the anti-microbial compounds in plants may be exploited for use against bacterial diseases in man (Angeh. 2007). Plants have developed an arsenal of weapons to survive attacks by microbial invasions. These include both physical barriers as well as chemical ones. These are either produced in the plants or induced after infection, the so called phytoalexins. Since phytoalexins can also be induced by abiotic factors such as UV irradiation, they have been defined as antibiotics formed in plants via a metabolic sequence induced either biotically or in response to chemical or environmental factors (Grayer et al. 1994).

## **1.5 Problem statement**

Traditional medicine has been the main form of treatment of disease for many years. However, the medicine has been administered in the form of crude extracts or as a mixture of chemical compounds. These sorts of approach may be dangerous as it does not separate unnecessary compounds which may be poisonous or uncalled for from active chemical constituent. In view of this it is then becomes necessary for the photochemical investigation and biological activity screening of plants with medicinal uses. This allows the platform for the isolation and identification of active compounds and those that are not useful to be discarded.

Nowadays the world is faced with a tremendous problem of pathogens with increase antimicrobial resistance due to the abusive and extensive use of antibiotic. Antimicrobial agents are often unaffordable in developing countries and toxicity of some antimicrobial agents is another factor contributing to this problem. Medicinal plant may provide new therapeutic solution in the form of extracts or compounds which may be active agent against pathogens. They may also be less costly with lower toxicity. There is therefore justification to study the efficiency and the safety of plant extracts.

### **1.6 Aim of the study**

The aim of the research is to study the antibacterial, antioxidant potential and isolation and characterization of active compound from the leaves of the *Rhus leptodictya* plant.

### **1.7 Objectives**

**The objectives of this research are to:**

- Study the phytochemical constituent of the plant by using TLC studies
- Study the antimicrobial and antioxidant potential of the plant by using the 96 well plate method
- To isolate active compound by using column chromatography
- Characterize and name the isolated active compound using NMR, MS, and IR.

The focal point of this research would reside on the phytochemical, antioxidant and antibacterial investigations of the leaves of the *Rhus leptodictya*. The major part of this study will involve

chromatographic separation of the crude extract into pure compounds. This will be followed by the structural elucidation using analytical spectroscopy. At least one isolated pure compounds will be screened for broad biological activity and the outcome compared with the crude extracts.



## 1.8 DISSERTATION OUTLINE

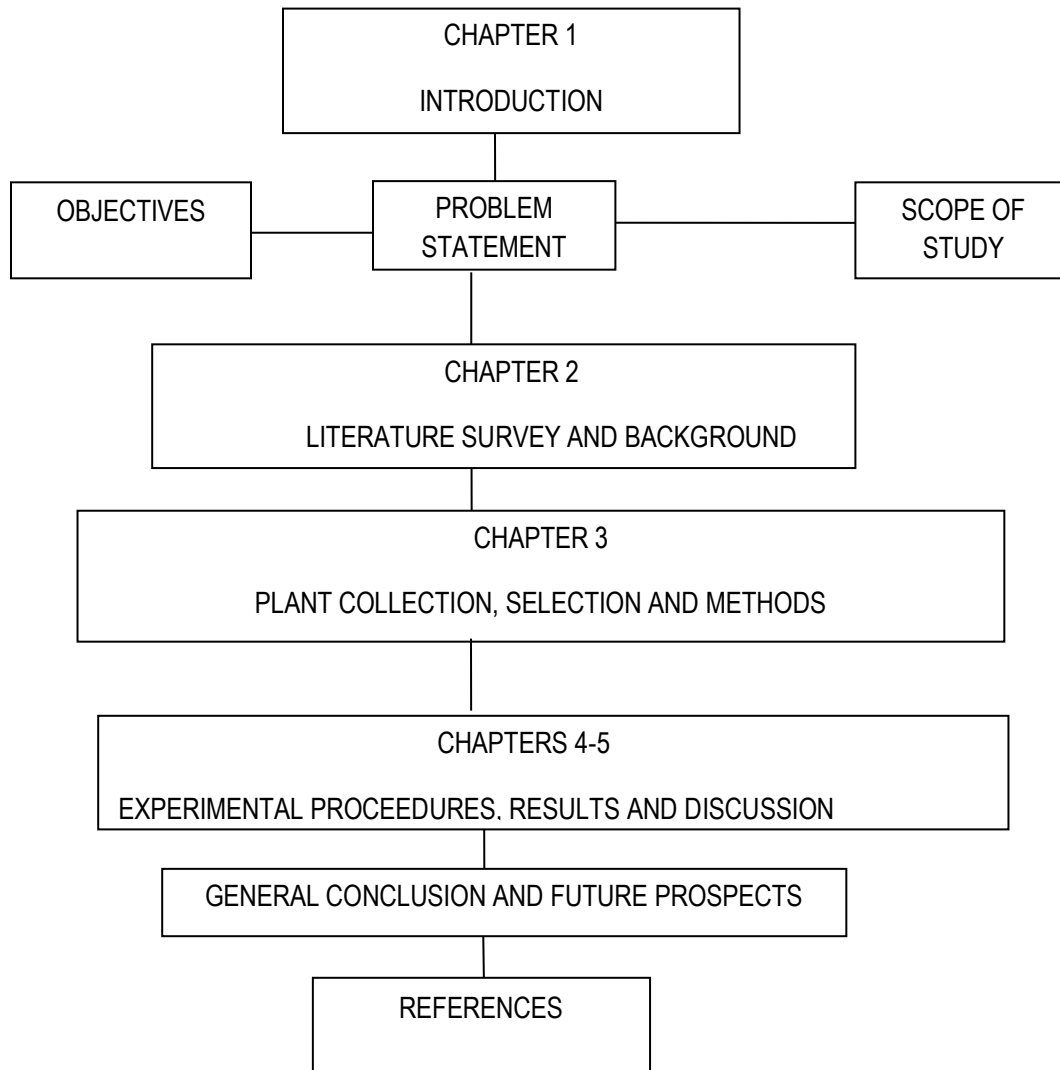


Figure 1.5: The dissertation outline

## CHAPTER 2

### Literature review

#### 2.1 Description of the *Rhus* species

*Rhus* species is a genus that consists of 250 species. It is commonly known as sumac or sumach. These plants are found to be individual species of flowering plants in the family *Anacardiceae*. All sumac have a milky or resinous sap. Some of these *Anacardiceae* are found to be famous poisonous plants for example *Rhus toxicodendron* which is also called poison Ivy. Some of them were used as herbal medicines. *Rhus* is deciduous or evergreen shrubs and shrubby trees (Taylor, 2004). The leaves of sumac or *Rhus* species are three leaflet and they have thin texture. Example of selected member of the representative members of *Rhus* species are as follow: *Rhus chinensis*, *Rhus glabra*, *Rhus leptodictya*, *Rhus verniciflua*, *Rhus toxicodendron*, *Rhus javanica*, *Rhus aromatic*, *Rhus typhina*. Some part of the plants of *Rhus* species are used in South Africa for the treatment of epilepsy. To date the scientist has reported that there are flavonoids and other different compound founds on the sumac genus (Cardinali et al., 2004).

These plants are found in temperate and tropical regions worldwide, often grow in the area of marginal agricultural capacity, and have a long history of use by indigenous people for medicinal and other use. They can grow in non-agriculturally viable regions, and various species has been used by indigenous cultures for medicinal and other purpose, suggesting potential for commercializing the bioactivity of these plants without competing for food production land uses (Van Wyk et al., 2004).

*Rhus Coriaria* also grows in the region from the canary island through mediteranean region to Iran and Afganistan and Middle East. *Rhus javanica* which is also called *Rhus semialata* is also found in the island of Taiwan. *Rhus glabra* which is also called smooth sumac common nonpoisonous shrub of eastern North America with waxy compound leaves and green paniculate flowers followed by red berries found in North America where it has been used by native people for the treatment of bacterial disease. *Rhus toxicodendron* is another genus from the family of *Anacardiceae* family that grows in fields and wooded area in North America (Oh et al., 2003).

## **2.2 Rhus leptodictya**

*Rhus leptodictya* is commonly known as mountain karee, rock karee, in English: bergkaree, klipkaree in Afrikaans; Mohlwehlwe in Sotho and Inhlanguhane in Siswati. It is a large shrub tree native in South Africa. The leaves of *Rhus leptodictya* are divided into three leaflets, bright green with toothed margins. *Rhus leptodictya* need a full sun to light shade. They can grow up to nine meter but is usually a rather shrubby bush of about three to four meter (Mitchell, 2004). Away to distinguish the mountain karee from others is that the two lateral leaflets are at right angles to the terminal one. These plants are distributed in four Northern Province of South Africa and other surrounding countries such as Zimbabwe, Mozambique, Lesotho, Botswana. The mountain karee grows naturally in a variety of habitat types including woodland, forest margins and bushveld (Palgraves, 1988).



Figure 2.1: *Rhus* plant and their leaves

### 2.3 Ethnomedicinal use of *Rhus* species

Some species of the genus *Rhus* are used in traditional medicine either as antimicrobial concoctions or for the cytotoxic properties, while others display insecticidal activities against aphids (Saxena et al., 1994). The research efforts on sumac extracts to date indicating a promising potential for this plant family to provide renewable bio-products with the following reported desirable bioactivities, antifibrogenic, antifungal, anti-inflammatory, antimalaria. The bioactive compounds can be extracted from the plant material using environmental beginning solvents that allow for both food and industrial end-uses (Henk, 1991).

*Rhus chinensis* is a plant that has been used by traditional healer in Asia; it has been used in the treatment of cold fever, malaria, and diarrhea. *Rhus glabra* has been used by native people in the treatment of bacterial disease such as syphilis, gonorrhea, dysentery, gangrene. The leaves of *Rhus semialata* has been used as a folk medicine for the treatment of diarrhea, *spermatorrhea* and

malaria. *Rhus toxicodendron* is the traditional homeopathic name for the plant called toxicodendron pubescent in the current code of nomenclature. *Rhus toxicodendron* was first used in medicine to treat a young man with herpetic eruptions in 1978. Sensitivity to *Rhus toxicodendron* is not innate, it develops through successive contact with plant, and its sensitivity tends to diminished with age. *Rhus toxicodendron* which is also known as poison ivy has been used by traditional healer to heal fever, swollen glands and other disease (Donald, 2008).

Scientific researchers have reported that *Rhus chinensis* compound possess strong antiviral, antibacterial, anticancer and antioxidant activities. Compounds isolated from the stem of *Rhus chinensis* significantly suppressed HIV-1 activity in vitro. Dimethylcaffic acid, isolanciresinol (3-hydroxy-5-methylphenol 1-O-dglucoside are examples of compounds that have been isolated from *Rhus chinensis* (Wang et al., 2006a). This plant is commonly identified by their pointy leaf that grows in trees. *Rhus toxicodendron* is the homeopathy remedy commonly known as poison ivy.

To date it has been reported that *Anacardiceae* has approximately 800 species in 82 genera. *Anacardiceae* are a family of flowering plants bearing fruits that are drupes. They include numerous genera with several economic importance. The wood of *Anacardiceae* has the frequent occurrence of simple small holes in the vessels. The compounds isolated from this family are found to be of chemical interest because they hold the great promise in search of new drug or medicinal or commercial agent (Mithcell, 1990).

## 2.4 Bioactivity of *Rhus* extracts

*Rhus* commonly known as the sumac has showed a wide range of biological activities. They are the most notable for their antimicrobial activities, although there is some information on their antifungal and antiviral activities (Mazza, 2007). Sumac was found to exhibit both the widest zones of inhibition in a disc assay after the screening of 100 medicinal plants in British Columbia. The assay was done using the crude methanolic extracts of *Rhus Glabra*. The bacteria that were used during the screening were *Bacillus subtilis*, *Escherichia coli*, *staphylococcus aureus*, *Pseudomonas aeruginosa*, *Salmonella typhimurium* TA98. The literature strongly suggests the potential for useful antimicrobial, antifungal and antiviral agent to be obtained from sumac extract (McCutcheon et al., 1992).

## 2.5 Compounds isolated from Sumac plants by other Scientists

The search for biologically active extracts based on traditionally used plants is still relevant due to the appearance of microbial resistance of many antibiotics and the occurrence of fatal opportunistic infections (Tshikalange et al., 2005). Six bioflavonoids which are robustaflavone, amentoflavone, agathiflavanone, volkensiflavanone, succedaneaflavanone, and *rhus flavonone* are compounds isolated from *Rhus succedanea* seeds and tested for inhibitory activities against the number of viruses including respiratory viruses (Kosar et al., 2006).

Five flavonoids garbanzol, sulfuretin, fisetin, fustin, and mollisacasin have been isolated from the stem of *Rhus verniciflua* (Lee et al., 2004). Pentagalloyl glucose, butein, fisetin, morinhydrate, sulfuretin, 3,4,7,8- tetrahydroxyflavone isomaterial were isolated from the bark of the *Rhus verniciflua*. The methanol extract from *Rhus glabra* was subsequently fractionated and monitored by bioassays leading to the isolation of three antibacterial compounds, the methyl ester of 3,4,5-

trihydroxybenzoic acid (methyl gallate), 4-methoxy-3,5-dihydroxybenzoic acid and gallic acid (Saxen, 1994). 6-pentadecylisyllic acid were isolated from the stem of *Rhus semialata*, three cyclolignan esters and seven known compounds including two cyclolignan isolariciresinol, lynoiresinol, and five aromatic compounds methyl ferulate, vanillin, 4-hydroxy-3,5-dimethoxybenzaldehyde, 4-methoxygallic acid, gallic acid were isolated from n-butanol extract of *Rhus semialata* (Cutillo et al., 2003) .

Researches also reported that abenzofuran lactone, ruscholide, 5-hydroxy-7-(3,7,11,15-tetramethylhexadeca-2,6,10,11-tetraenyl)-2(3 H)-benzofuranone, betulin, betulonic acid, moronic acid , 3-oxo-6 beta-hydroxyolean-12-en-28-oic acid and 3-oxo-6 beta-hydroxyolean-18-en-28-oic acid were isolated from the stems of *R. chinensis* (Gu et al., 2007). *Bioassay-directed* fractionation of the n-hexane extract of the stem of *R. semialata* (Anacardiaceae) has led to the isolation of 6-pentadecylsalicylic acid (Kuo et al., 1999). Two biflavonoids with activity in the H-Ro 15-1788 (flumazenil) binding assay were isolated by high pressure liquid chromatography (HPLC) from fractionation of the ethanol extract of the leaves of *R. pyroides*. The extracts of *R. pyroides* contain agathisflavon and amentoflavone (Svenningsen et al., 2006). The biflavanone(2S,2"S)-7,7"-di-O-methyltetrahydroamentoflavone and five flavonoids, 7-O-methylnaringenin, 7 , 3'-O-dimethylquercetin, 7-O-methyleluteonlin and eriodictyol are present in the leaves of *R. retinorrhoea* (Ahmed et al., 2001).

Table 2.1: Biological activities of compounds and fraction extracted from *Rhus* plants (Mazza, 2007)

Rhus species	Plant part	Biological activity	compounds
R. verniciflua	Bark	Antifibrogenic	butein
Rhus. Retinorrhoea	Leaves	Antimalarial	7,3'-o-dimthylquercetin
Rhus retinorrhoea	Leaves	Antimicrobial	7-o-methylnaringenin
Rhus glabra	Branches	Antimicrobial	Methyl galate
Rhus verniciflua	Brances	Antioxidant	Fustin, quercitin
Rhus succedanea	Fruits	Antiviral	Robustaflavone
Rhus verniciflua	Branches	Cytotoxic	Benzenediol/oxygen oxidoreductase
Rhus vernificera	Sap	Leukopenic	Polysaccharide extracts



## CHAPTER 3

### METHODOLOGY

#### 3.0 Methodology

Most of the traditional healers use the dried plant material for healing. The dried plant material can be used as a source for the extraction of secondary plant components. Most of the researchers use the dry plant material for several reasons.

In this study the dry leaves were used for direct extraction for the preliminary screening steps. Different solvent were used depending on their polarity, from low polar (non-polar) to the polar solvent hexane, Dichloromethane, ethyl acetate, acetone, and methanol.

#### 3.1 Materials and methods

##### 3.1.1 Plant collection

The leaves of *Rhus leptodictya* were collected from Pretoria under the guidance of Prof Eloff. The plant leaves were identified by the university of Pretoria botanical garden. Then it was transported to Vaal university of Technology.

##### 3.1.2 Plant preparation

The leaves of *Rhus leptodictya* were dried in the room temperature in the natural product laboratory at the Vaal University of Technology for three weeks. The dried leaves were

grounded to fine powder using a macasalab mill (model 200 Lab) from the University of Pretoria. The fine powder materials were store in a closed container at room temperature in the laboratory until they were needed.

### 3.1.3 Preliminary extraction procedure

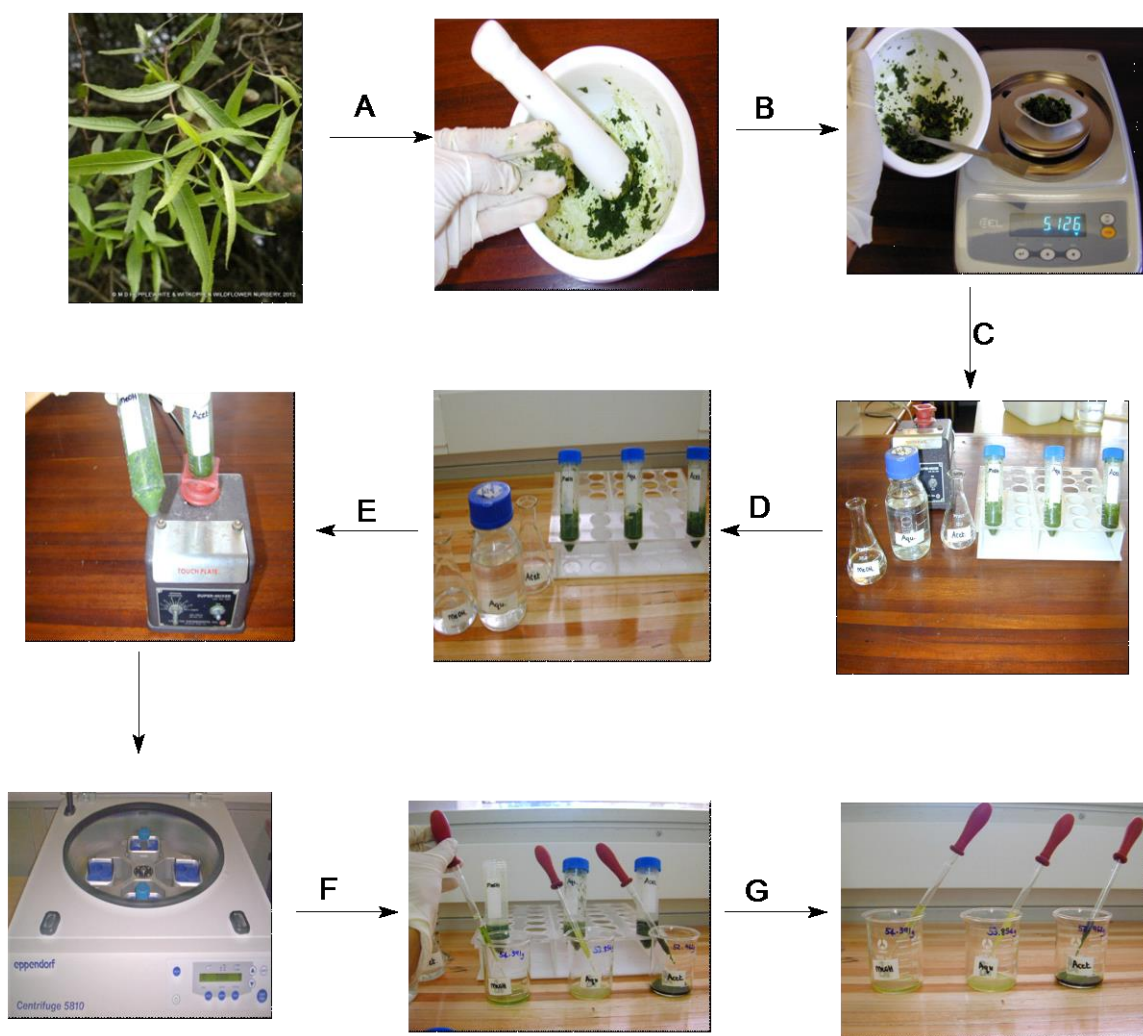


Figure 3.1: Stages of extractions

Five different aliquots of 3 g of the fine powder materials of *Rhus leptodictya* were extracted with 30 ml of five different solvent of increasing polarity (hexane, dichloromethane, ethyl acetate, acetone, and methanol) in a 50 ml centrifuge tubes. The tubes were shaken in a rotary shake for about four hours. The extracts were centrifuged using an EBA 30 Hetich centrifuge at 6000 xg for 20 minutes and the supernatant was filtered through whatman No.1 filter paper into pre-weigh glass vials and placed under a stream of cold air to dryness and the mass of the extracts was determined. Concentration of 10 mg/ml was prepared in acetone for biological assays.

### **3.2 Chromatographic analysis**

After the extraction was done, the chemical profile of extracts was determined by the TLC method using aluminum backed thin layer chromatography plates (silica gel 60, F254, Merck). The following three solvent system were used to develop the plates: ethyl acetate/methanol/water (40:5:1) [EMW] polar, chloroform/ethyl acetate/formic acid 5:4:1 [CEF] (intermediate polarity/acidic), Benzene/ethanol/ammonium hydroxide: 90:10:1 [BEA] non-polar/basic). The solvent systems have been optimized to separate components of each extracts of *Rhus* members. The development of the chromatogram was done in a closed TLC developing tank in which the atmosphere has been saturated with eluent. Samples were applied rapidly and developed without delay to minimize the possibility of oxidation or photo-oxidation of constituents. The developed TLC plates were visualized under UV light at 254 and 365 nm to detect UV absorbing or fluorescing bands. The plates were then sprayed with vanillin spray reagent (0.1 g of vanillin dissolved in 28 ml of methanol, add 1 ml of sulphuric acid) and heated at 1100° C to optimal color development.

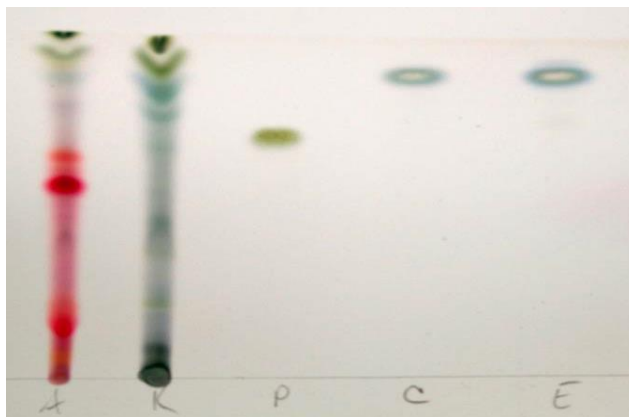


Figure 3.2: TLC plate sprayed with vanillin

### 3.2 Antioxidant assay of the *Rhus* extracts (free radical scavenging)

The method used for measuring antioxidant is the one that involve the generation of free radical species. An atom or molecule that has at least one unpaired electron is called free radical. They generally abstract electrons from other molecules, thereby inducing a chain reaction of electron abstraction and radical formation (Masoko, 2007).

The free radical scavenging was done using the 2, 2-diphenyl-picrylhydrazyl (DPPH). The DPPH radical is reduced from a stable free radical, which is purple in color to diphenylpicryl hydrazine, which gives a yellow colour. Different extracts of *Rhus* were separated by TLC, air dried and then sprayed with 0.2% of DPPH in methanol. The chromatogram was examined for colour change over 30 minutes. If the antioxidant activity is present the colour changes from purple to yellow.

### **3.4 Phytochemical analysis**

#### **3.4.1 Qualitative analysis**

Chemical tests for the screening and identification of bioactive chemical constituents were carried out in extracts as well as powder specimens using the standard procedures described by Savithramma et al., 2011). The test was done to find the presence of the active chemical constituents such as alkaloids, glycosides, terpenoids and steroids, flavonoids, reducing sugar, saponins and tannins by the following procedure. The test was done in triplicate.

#### **3.4.2 Reducing sugars**

The aqueous extracts of *Rhus* plant were added to boiling solution of fehling solution (A & B) in a test tube and the color change was observed.

#### **3.4.3 Glycoside**

Glycosides are compound which upon hydrolysis give rise to one or more sugars and a compound which is not sugar. To the solution of the extract in glacial acetic acid, few drops of ferric chloride solution and concentrated sulphuric acid are added, and a reddish brown coloration at the junction of the two layers was observed.

#### **3.4.4 Terpenoids and steroids**

Four milligram of *Rhus* extracts was treated with 0.5 ml of acetic anhydride and 0.5 ml of chloroform. After, concentrated solution of sulphuric acid was added slowly and a redish violet color was observed for terpenoids and green bluish color for steroids.

### **3.4.5 Flavonoids**

This test was done in three different ways; firstly, A few drops of 1% aluminium solution was added to the aqueous extracts .a yellow coloration indicates the presence of flavonoids. Secondly three milliliters of extracts solution was treated with 1.5 ml of 50% methanol solution. The solution was warmed and metal magnesium was added. 506 drops of concentrated hydrochloric acid was added. Red color was observed for flavonoid s and orange color for flavones. Third, three ml of extracts was mixed with four ml of 1% potassium hydroxide in a test tube and the colour was observed. A dark yellow colour indicates the presence of flavonoids (Sofowara, 1993).

### **3.4.6 Tannins**

This test was done in two methods: firstly, 2 ml of the extracts was added to few drops of 1% lead acetate, a yellowish colour indicate the presence of tannins. Secondly, 1 ml of extracts was mixed with 2 ml of water and 2-4 drops of ferric chloride solution was added. Blue colour was observed for gallic tannins and green black for tannins.

### **3.4.7 Saponins**

1 ml of the extracts was mixed with 5 ml of distilled water. The mixture was shaken for 10 minutes. The formation of foam indicated the presence of saponin.

### **3.5 Quantitative analysis**

#### **3.5.1 Determination of total flavonoids content**

The method used for this test was the aluminum chloride calorimetric with some modification. 1 ml of methanol extract was mixed 3 ml of methanol, 0.2 ml of 10% aluminum chloride, 0.2 ml of 1M potassium acetate and 5.6 ml of distilled water and remains at room temperature for 30 minutes. The absorbance was measured at 420 nm. Quercetin was used as standard. Five standards with different concentration from 12.5, 25, 50, 75, 100 ppm. All the tests were performed in triplicate. Flavonoids contents were determined from the standard curve and were expressed as quercetin equivalent (Aiyegboro, 2010).

### **3.6 Biological assays for screening**

#### **3.6.1 Bacterial culture**

Five different organisms were used for the screening tests; the organisms were obtained from the microbiology laboratory at Vaal University of Technology. The following organisms were used for the bioautography and MIC assay of the plant extracts, *Staphylococcus aureus* (gram-positive), *Pseudomonas Aeruginosa* (gram-negative ATCC 27853), *Escherichia coli* (gram-negative ATCC 25922), *Enterococcus faecalis* (gram-positive ATCC 29212). The cultures were maintained on Muller Hinton (MH) agar at 4 °C and were inoculated in MH broth at 37 °C and incubated for 18 hours prior to bioautography and MIC assay.

### **3.6.2 Bioautography assay of the extracts**

The TLC plates were prepared for the bioautography; the plates were loaded with the 100mg of each extracts, and dried in the stream of air before developing in different mobile phase. After running with the solvent system the plates were dried in a stream of air to allow the solvent to evaporate before they were sprayed with an actively growing culture of bacteria. After they were sprayed with the culture of bacteria the chromatograms were then incubated for 24 hours at 37 under 100% relative humidity to allow the microorganism to grow on the plates. After 24 hours incubation the bioautogram were sprayed with an aqueous solution of 2 g/ml p-iodonitrotetrazolium violet INT which is clear in colour and incubated for 24 hours for colour development. The clear zone against a red background indicates the inhibition of bacterial growth by bioactive compounds in the extracts.

### **3.6.3 Reaction of p-iodonitrotetrazolium violet**

The reaction is based on the transfer of electron from NADH. A product of the threonine dehydrogenase [TDH] catalysed reaction, to the tetrazolium dye [p-iodonitrotetrazolium violet]. Threonine dehydrogenase [TDH] from bacteria catalyses the NAD-dependent oxidation of the threonine to form 2-amino 3-ketobutyrate and NADH. During the active growth of bacteria, an electron is transferred from NADH which is transparent in the visible range] to p-iodonitrotetrazoliumviolet, aformazan dye which is purple-red in colour. The clear zone indicates the areas of inhibitions.



### **3.7 Microplate dilution assay**

Microplate dilution method was used to determine the minimum inhibitory concentration values of the extracts against each test bacterial species (Eloff 1998a). This assay was done in triplicate. The plants extracts was serially diluted two-fold with water in a 96-well microplates a 100 µl aliquot of test bacteria culture was added to each well. Acetone was used as a solvent control and distilled water was used as a negative control. Gentamicin was used as appositve control. This was done in a twofold serial dilution of extracts beyond the level where no inhibition of growth of organisms was observed. The micro plate were sealed and incubated for 24 hours at 37 degree. After incubation 40 µl of 0.2 mg/ml of INT was added to each well ants the plate were incubated for further 2 hours before observation in antibacterial activity assays. The development of red colour, resulting from the formation of the red/purpleformazan, was indicate of growth (posit indication of cell viable). The MIC values were regarded as the lowest concentrations of the extracts that inhibit the growth of the test organism.

### **3.8 Separation and Isolation of bioactive compound**

Column chromatograph is one of the most techniques used in the isolation of chemical compound. TLC was found to be an important method for the isolation, purification and conformation of natural product. Column chromatography was performed on the sample that shows high antibacterial activity because of the apparently simple nature of the components that was seen on the TLC plate. Plant constituent are distributed between the solid phase which is the silica gel or the sephadex and the mobile phase, which comprise the eluting solvent. In silica gel the separation of compounds from each other in an extracts is based on the number of factor including the polarity of

compounds, hence compounds are eluted from the column with the solvent systems of different polarity. Silica gel has polar ends which interact strongly with polar compounds and they are eluted later from the column. In the sephadex gel filtration the separation of constituent in an extracts depend on the size of the molecules. Constituent with a small molecular weight interact strongly with the matrix of the gel and tend to move slowly through the gel and they are eluted later while the large molecular weight constituents are eluted early because they move fast through the column.

The successful isolation of bioactive compounds from indigenous medicinal plants will validate indigenous knowledge adding value to the plants and support plant conservation and knowledge. This study may also contribute to research and development in the production of new pharmaceutical drugs for the treatment of various diseases.

### **3.8.1 Bulk extraction of plant material**

200 grams of powdered plant material was exhaustively extracted with 2 litres of acetone. The process was repeated three times, using a fresh aliquot solvent of the acetone every time. The mixture was shaken vigorously for twelve hours using a labotech shaker instrument. The supernatant was filtered using cotton wool and the filtered again using filter paper No1 whatman with a Buchner funnel. The extract was concentrated using the rotary evaporator. The reduced extract was transferred to a clean pre weighed honey jar container and placed under a stream of air at room temperature to dryness. The mass of the extract was determined.

### 3.8.2 Solvent- solvent fractionation

The researcher has reported that the purpose of solvent-solvent partitioning is to simplify extracts by fractionating the chemical compounds into broad groups based on their solubility. The separation was undertaken with immiscible solvents to fractionate compounds with different polarities. The method used here was the one employed. Partitioning is to simplify extracts by fractionating the chemical compounds into broad groups based on their solubility. The separation was undertaken with immiscible solvents to fractionate compounds with different polarities. The method used here was developed by the national cancer institute and applied in the analysis of anthocleista grandiflora by Eloff (1998a).

Two grams of ethyl acetate extracts *Rhus leptodictya* obtained from serial exhaustive extraction were used. These extracts were dissolved in equal volume of 1:1 mixture of chloroform and water and the two phases were separated in a separating funnel. The water fraction was mixed with equal volume of n-butanol in a separating funnel and yields the water [W] and [B] fraction. The chloroform fraction was dried in a rotary evaporator under reduced pressure and extracted with an equal volume of hexane and 10% water/methanol mixture.

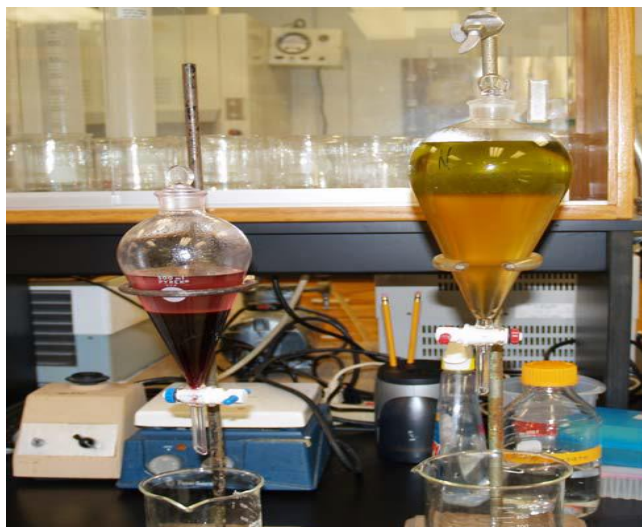


Figure 3.3: Chloroform fraction and water fraction in two different separating funnel

### 3.9.1 Extraction and isolation of pure compound

Dried leaves of *Rhus leptodictya* were powdered and extracted with ethyl acetate by maceration at room temperature. The extracts were concentrated under vacuum to yield residues. A mass of 3.882 g of the ethyl acetate fraction was dissolved in a minimum amount of ethyl acetate and silica gel to produce slurry with the solution and dried under a fan at room temperature. A column was set up for chromatography with 180 g of silica gel 60. After adding the ethyl acetate fraction impregnated on silica, elution was commenced with hexane: ethyl acetate in a ratio 95:5 to 60:40. The solvent system was changed with polarity ratio progression of elution as illustrated on table 5.2. Isolated compound were obtained as mixtures at the elution of 70:30. The 55-62 fractions were prepared for further purification by smaller column purification. Fraction 55-62 gave flavonoid, 5, 7, and 8-Trihydroxy-2-(4'-hydroxyphenyl)-3-[5", 6"-dihydroxy-2"-(4"-hydroxyphenyl)-4"*H*-chromen-4"-one]-4*H*-chromen-4-one, isolated as an amorphous yellow powder.

Hexane	Ethyl acetate
100	0
95	5
90	10
85	15
80	20
75	25
70	30
65	35
60	40

**Table 5.1:** Solvent ratio used for elution of *Rhus Leptodictya* of ethyl acetate fraction

## CHAPTER 4

### RESULTS AND DISCUSSION

#### 4.1 Quantity Extracted

The quantities and percentage yields extracted from 3 g of the finely powdered leaves of *Rhus leptodictya* using different solvents are given in Table 4.1, Figures 4.1 From the graphical data, it is clear that dichloromethane (DCM) had the highest percentage extractive potential compared to the other solvents used. The lowest extractive potential was observed with hexane, which showed about 3% yield relative to the 35% observed with DCM.

This therefore suggests that, the leaves of *Rhus leptodictya* contain more polar compounds than non-polar compounds since more compounds are found in the Methanol and DCM which are moderate and more polar respectively.

Table 4.1: Total amount extracted from 3 g of powered leaves of *Rhus leptodictya*

Plant extracts	Weight of extracts (mg)	% yield
Hexane extract	53	2.65
DCM extract	589	31
ETOAC extract	105.9	5.8
Acetone extract	112.3	6.6
Methanol extract	389	24

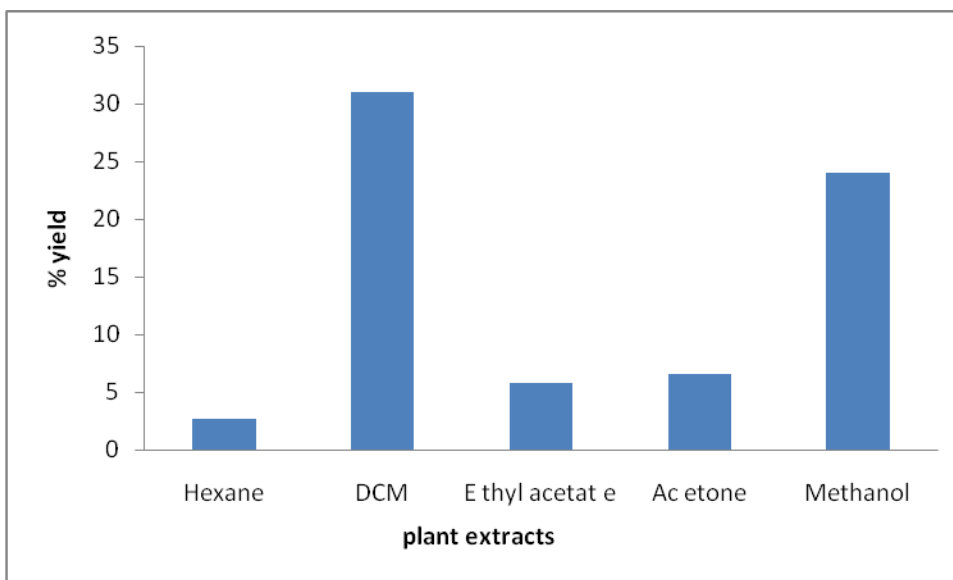


Figure 4.1: Percentage yield extracted from 3 g powdered leaves of *Rhus leptodictya*

## 4.2 Thin layer chromatography Finger Printing

The results of the TLC fingerprinting are shown in figure 4.2 and Table 4.2. In this research, three mobile phase systems were used to separate compounds based on their polarities. The mobile phase systems were chloroform/ethyl acetate/formic acid (CEF) in the ratio 5:4:1; (BEA) which is benzene/ethanol/ammonia hydroxide in the ratio 18:2:0.2 and EMW, which is ethyl acetate/methanol/water in the ratio 40:5.4:5. The mobile phase which showed good separation was found to be CEF, followed by EMW. Therefore, for further work with regard to separation of different compounds from the leaves of *Rhus leptodictya*, CEF was taken as a system of choice.

Table 4.2: R<sub>f</sub> values of different compounds from development of the TLC plates using different solvent systems

	Hexane	Dichloromethane	Ethyl acetate	Acetone	Methanol
BEA (18:2:0.2)	0.35	0.35	-	-	-
	0.434	0.434	-	-	0.434
	0.652	0.652	-	-	-
	-	0.797	-	-	-
	-	0.89	0.89	0.89	-
EMW (40:5:4.5)	-	-	0.366	-	-
	0.9758	0.563	0.563	-	-
	-	-	0.648	-	-
		0.887	0.815	-	-
			0.944	-	-
CEF (5:4:1)	-	0.743	0.622	0.243	0.333
	-	0.878	0.743		0.432
	-	-	0.	0.797	0.797
	-	-	0.919	0.919	0.919
			-	0.959	0.959

BEA =Benzene, Ethanol, Ammonia)      EMW =Ethyl acetate, Methanol, Water

CEF =Chloroform, Ethyl acetate, Formic acid

The developed TLC plates were sprayed with vanillin/H<sub>2</sub>SO<sub>4</sub> solution after which it was heated at 100°C to allow color development. The identification of separated components was achieved on the basis of the retention factor (R<sub>f</sub>) values and colours as can be seen in Table 4.2. Separated



compounds visualization was achieved by natural colour in daylight or by fluorescent quenching on 254 nm (for conjugated double bonds or extended  $\pi$  electron systems) or 366 nm (Ahmed, 2013).

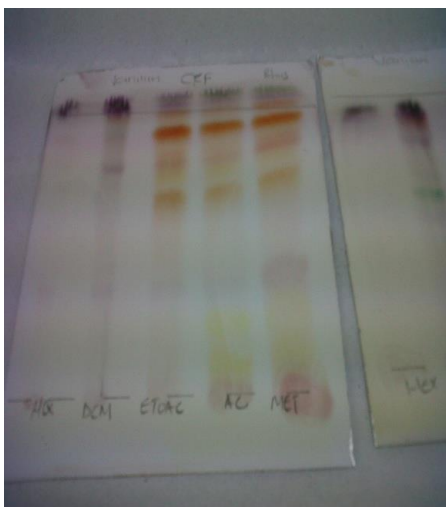
The observed chromatograms developed from different solvents systems showed complex mixture of compounds confirmed by different colours when reacting with vanillin/ $\text{H}_2\text{SO}_4$  spray reagent. The classes of compounds observed included terpenoids (purple or bluish purple (Ahmed, 2013; Taganna et al., 2011); flavonoids which are phenolic in nature (pinkish, yellow or orange); stilbenes (bright red to dark pink colour) and proanthocyanidins (pink colour). To confirm the presence of phenolic compounds, blue-black spots with ferric chloride-potassium ferric cyanide reagent were observed (Wettasinghe et al., 2001; Ahmed, 2013). The presence of flavonoids was confirmed by yellow spots when the spots reacted with aluminium chloride/acetic acid spray reagent ( $\text{AlCl}_3/\text{CH}_3\text{COOH}$ ) (Rijke et al., 2006). Characterization of the phytochemical profile of the extracts indicated that the extraction method and the extractants used resulted in splitting the complex mixture in the way that the flavonoids shown as yellow colour in Figure 4.2 were concentrated in the ethyl acetate, acetone and methanol extracts.



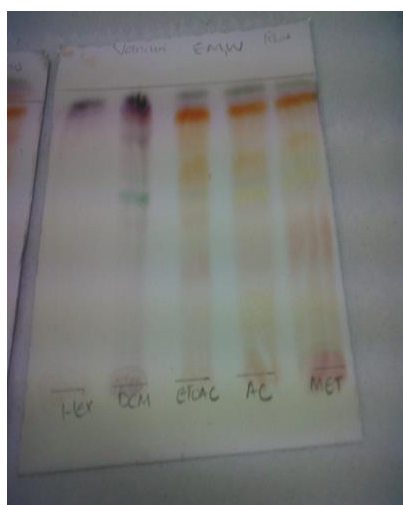
Solvent system: BEA (18:2:0,2)



Solvent system : CEF (5:4:1)



Solvent system: CEF (5:4:1)



Solvent system: EMW (40:2:0,2)

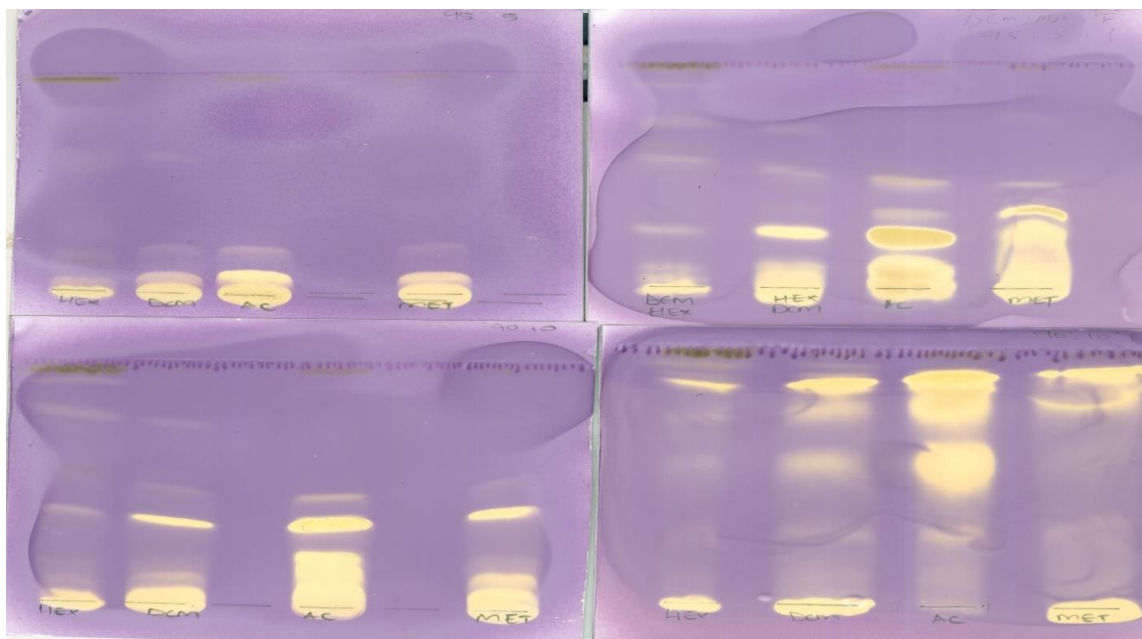
Figure 4.2: Chromatograms of the hexane (Hex), dichloromethane (DCM), ethyl acetate (EtOAc), Acetone (Ac) and Methanol (Met) under different solvent systems sprayed with vanillin.

### 4.3 Antioxidant activity

The therapeutic benefit of medicinal plants is often attributed to their antioxidant property (Vinay et al., 2010). Antioxidants play very important roles in coetaneous tissue repair as they significantly prevent tissue damage that stimulates wound healing process (Koleva et al., 2002). The antioxidant capacities of the leaves of *Rhus leptodictya* plant extracts as measured by the DPPH method are presented in Figure 4.3.

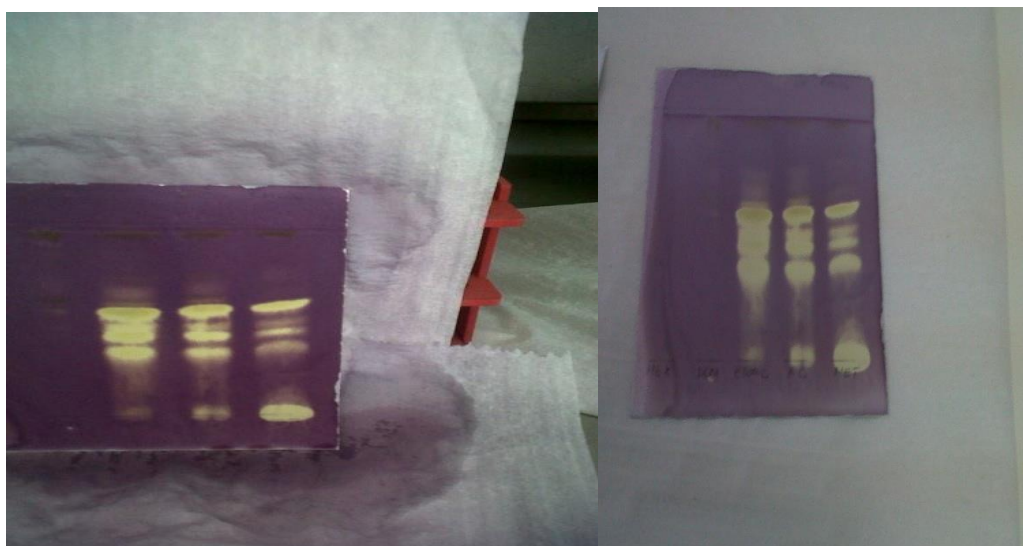
A: Hexane, Ethyl acetate (95:5)

B: Hexane: ethyl acetate (90:10)



C: Hexane :ethyl acetate( 85:15)

D Hexane:ethyl acetate (80:20)



E: Hexane:ethyl acetate(75:25)

F: Hexane ;ethyl acetate 70:30)

Figure 4.3: Chromatograms of antioxidant activity sprayed with DPPH in different solvent system.

The leaves extracts were run under the following solvent system, i.e. BEA (18:2:0,2), CEF (5:4:1) and EMW (40:2:0,2) which showed the activity at the origin of the TLC plates with the exception of acetone and methanol extracts and other extracts with no activity. In the chromatograms eluted with CEF (ratio) and EMW (ratio) there are some activity at the top of the plate of the polar fractions. The polar extractants, methanol and acetone showed some activity in all solvents systems compared to other extracts. This is due to the polarity of the components of the compounds present in the extractant.

In the antioxidant potential determined by DPPH reagent acetone extract had the highest activity followed by *the* methanol extract. DPPH is a free radical that forms a stable molecule on accepting an electron or a hydrogen atom as can be seen in Figure 4.5.

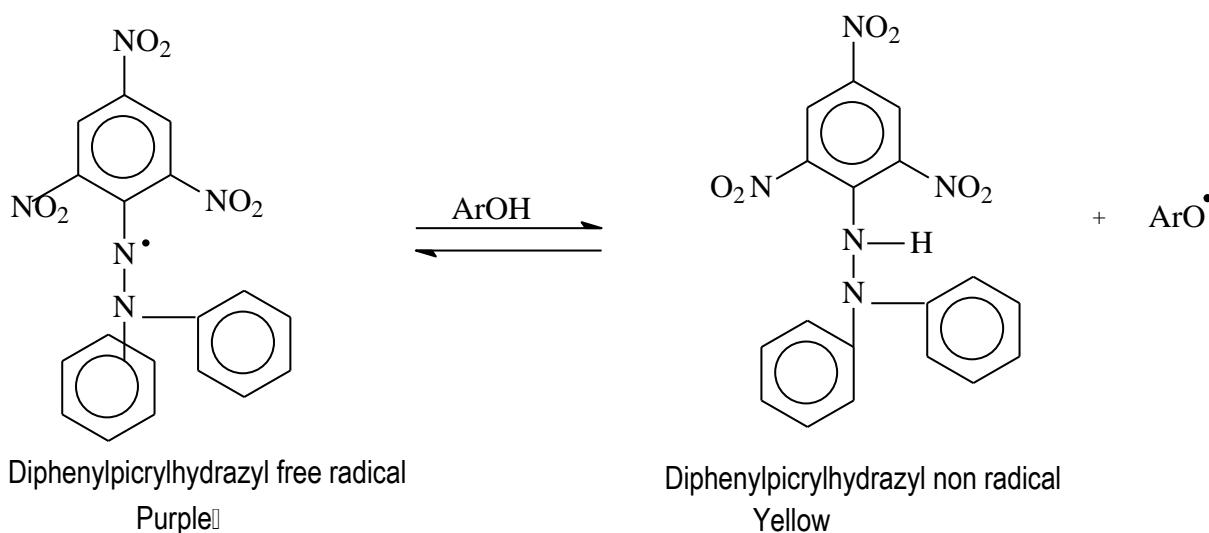


Figure 4.4: The DPPH redox reaction

The free radical scavenging activities of the plant extracts was done using the 2, 2-Diphenyl-1-picryl hydrazyl radical and it was determined using the UV Perkin Elmer Lambda at 517 nm.

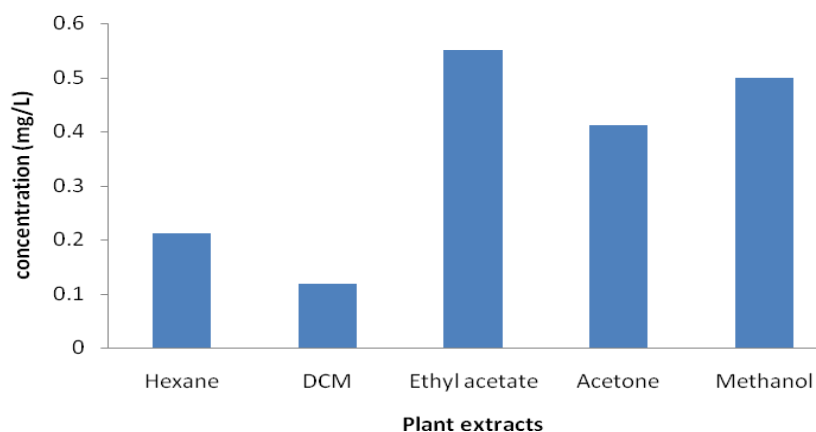


Figure 4.5: Determination of free radical scavenger using DPPH

Free radicals induce oxidative stress *in vivo* that may lead to oxidative modification or damage of some biological structures such as lipids, proteins, DNA and may give rise to degenerative diseases (Muleya et al., 2013). The *in vitro* study looks encouraging as the plant extracts studied have some radical scavenging effect.

The human body frequently produces reactive oxygen species (ROS) which are beneficial in small amounts. However, large amounts of these ROS are produced during increased oxidative stress encountered in the body due to either environmental hazard, or impairment in the body metabolism due to varying disease conditions including drugs or having insufficient amount of dietary antioxidants. This situation may be dangerous and has to be curbed by exogenous supply of antioxidants as a choice of therapy or preventive measure (Muleya et al., 2013). The natural sources are much safer to use due to less toxicity and side effects (Akharaiyi, 2011).

### 4.3.1 Quantification of the phenolic constituents of the leaves extracts of *Rhus leptodictya*

Natural antioxidants from plants are normally found in the form of phenolic compounds such as flavonoids, phenolic acids, tocopherols etc. (Ali, 2008). Several studies have shown that the higher antioxidant activity associated with medicinal plants is attributed to the total phenolic compounds (Cheung et al., 2003). Therefore, the content of total phenolics in leaves of *Rhus leptodictya* plant extracts was determined as the calibration curve can be seen in Figure 4.6 with standard curve equation:  $y = 0.00048x + 0.0055$ ,  $r^2 = 0.9873$ . Phenolics are important mainly because of their function to scavenge the free radicals in the human body and to help maintaining healthy body by scavenging or removing the reactive oxygen species (ROS). The results revealed that the leaves of *Rhus leptodictya* are rich in phenolics. The potential beneficial effects of the high antioxidant activity and protections of cells from free radical attack seem clear (Halliwell, 1997).

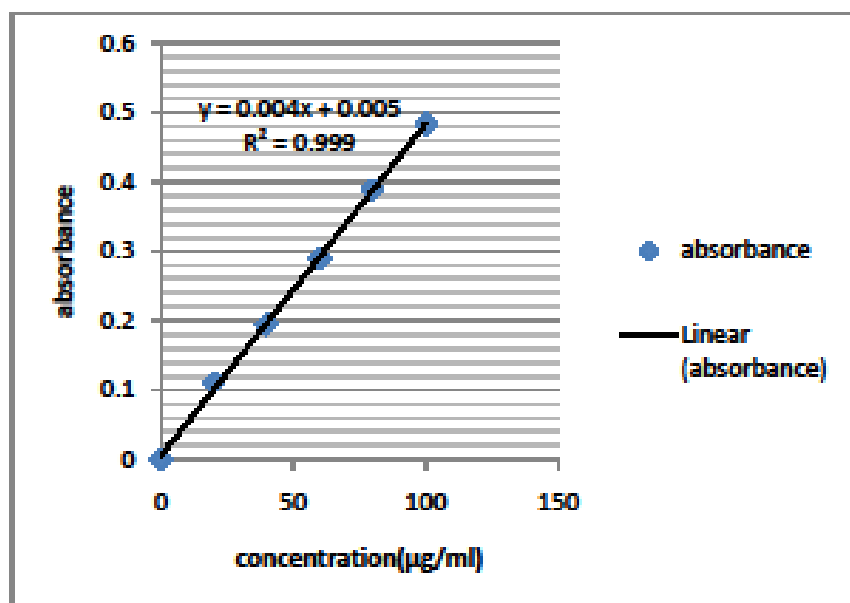


Figure 4.6: Calibration curve of phenolic constituents

Table 4.3: Total Phenolic compounds in *Rhus* plant

Extract	Total Phenolics (mg/g)
Methanol	0.38
Acetone	0.21
Ethyl acetate	0.18
Dichloromethane	0.003
Hexane	0.12

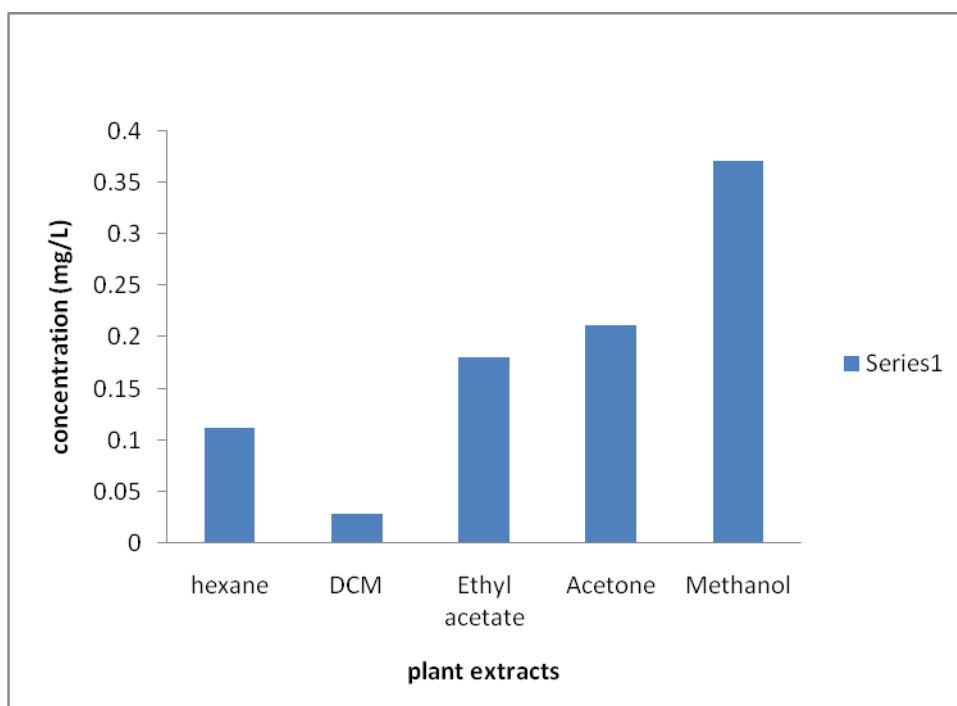


Figure 4.7: Bar graph of quantification of total phenolic constituent by Uv-Vis

The total phenol content was  $0.38 \pm 0.002$ ;  $0.21 \pm 0.0007$ ;  $0.18 \pm 0.0002$ ;  $0.003 \pm 0.000$  and  $0.12 \pm 0.0001$  mg g<sup>-1</sup> in the methanol, acetone, ethyl acetate, dichloromethane and hexane extracts respectively (Table 4.2 and Figure 4.7). In all, methanol extract generally, exhibited the higher

values of antioxidants. The result clearly shows that the solvent influences the extractability of the phenolic compounds. The antioxidant activity has a positive correlation ( $R = 0.49$ ) with phenolic content of all the solvent extracts of the plant. This confirms the assertion that phenolic content of plants contribute directly to their antioxidant properties. The values recorded for all extracts of the plant, even though lower than the standard antioxidant (ascorbic acid), showed that the leaves of *Rhus leptodictya* are a relatively good source of antioxidant activity.

The leaves of *Rhus leptodictya* contain flavonoids in the hexane, dichloromethane, acetone, ethyl acetate and methanol extracts (Figure 4.7 and Figure 4.8). Flavonoids are natural antioxidants.

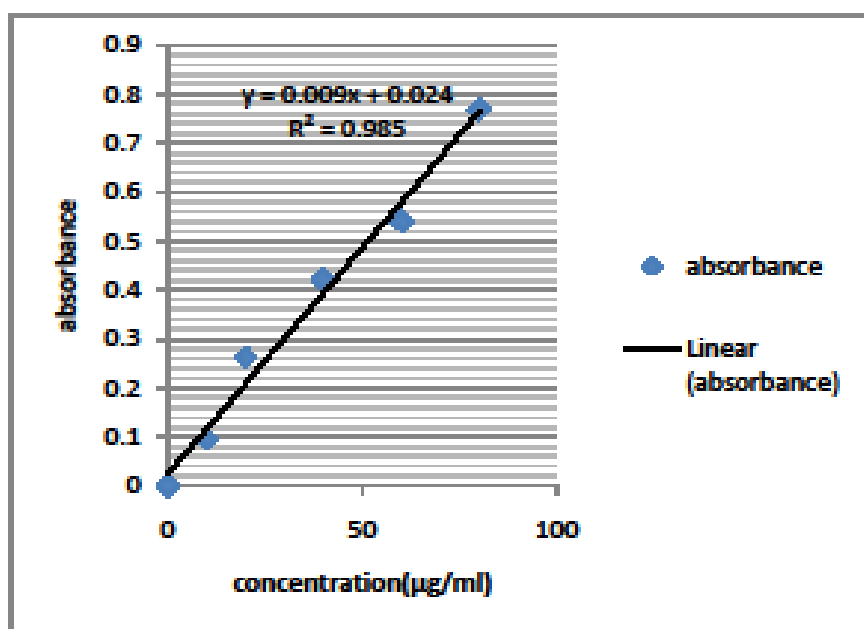


Fig 4.8: Calibration curve of total flavonoids content from leaves of *Rhus* plant



Table 4.4: Total Flavonoids in *Rhus* plant

Extract	Total Flavonoids (mg/g)
Methanol	11.547
Acetone	18.221
Ethyl acetate	8.3006
Dichloromethane	10.039
Hexane	2.350

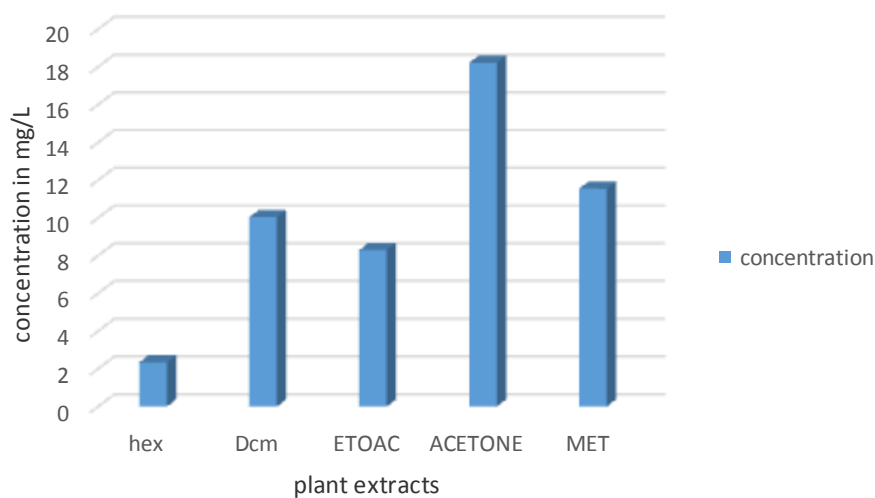


Figure 4.9: Total Flavonoids content of different solvent extracts of *Rhus* plant

The flavonoid content of the extracts in terms of quercetin equivalent (the standard curve equation:  $y = 0.0092x + 0.0249$ ,  $r^2 = 0.985$ ; Figure 4.8 ) were  $11.54 \pm 1.7$ ;  $18.22 \pm 2.1$ ;  $2.35 \pm 0.2$ ;  $10.03 \pm 3.3$  and  $8.30 \pm 1.4$  mg g<sup>-1</sup> for methanol, acetone, hexane, dichloromethane and ethyl acetate extracts respectively (Table 4.3).

Flavonoids are well-known dietary biochemical agents, which show pH dependent antioxidant behaviour in human body. They are hydroxylated phenolic substances which are synthesized by plants in response to microbial infection and they have been found to be antimicrobial substances against wide range of microorganisms. Their activity are said to be due to their ability to complex with extracellular and soluble proteins and to complex with bacterial cell wall (Marjorie, 1996). They are also known to be effective antioxidant and show strong anticancer activities (Salah et al., 1995). These molecules are also effective for cardiovascular system and work as cardio protective agents. The total flavonoid contents in methanolic extracts of the leaves of *Rhus leptodictya* implies that extracts with the higher concentration of total phenols is also having more concentrations of flavonoids.

#### **4.4 Phytochemical constituents of the leaves extracts of *Rhus leptodictya***

The Phytochemical screening of the leaves of *Rhus leptodictya* was done and the results are presented in a Table 4.3. The results showed that the leaves of *Rhus leptodictya* are rich in phenols, tannins, saponins and flavonoids. However, the hexane and dichloromethane extracts showed the absence of tannins. The leaves of *Rhus leptodictya* contain phenolic compounds which are the largest and most abundant groups of plant metabolites (Singh, et al., 2007). They have biological properties such as antiatherosclerosis, cardiovascular protection and improvement of endothelial function as well as inhibition of angiogenesis and cell proliferation activities (Han, et al., 2007).

Table 4.5: Phytochemical constituents of the leaves of *Rhus leptodictya*

Plant extracts	phenols	tannins	saponins	flavonoids
Hexane extract	+ve	-ve	+ve	+ve
DCM extracts	+ve	-ve	+ve	+ve
ETOAC extracts	+ve	+ve	+ve	+ve
Acetone extracts	+ve	+ve	+ve	+ve
Methanol extracts	+ve	+ve	+ve	+ve

Phytochemicals such as saponins and tannins have anti-inflammatory effects (Liu, 2003; Manach et al., 1996). Tannins are known to bind proline rich protein and interfere with protein synthesis, whilst on the other hand, saponins are known to produce inhibitory effect on inflammation (Just, et al., 1998). They are also known to have the property of precipitating and coagulating red blood cells (Sodipo, et al., 2000).

#### 4.5 Bioautography activity of leaves extract of *Rhus leptodictya*

The bioautography assay was used to determine the antibacterial activity of active compounds present in leaves of the plant extracts. The clear zones on the chromatogram indicate the inhibition

of growth by the plant extract (Figure 4.10). Due to being unable to get other bacteria tested before for this assay, the plant was tested against *staphylococcus aureus* only.

Acetone and Ethyl acetate fractions showed the highest number of antibacterial active compounds against *staphylococcus aureus* followed by crude extract of the dichloromethane. Hexane, Chloroform and Methanol showed only two spots which are active. The results obtained in this assay showed that the leaf extracts of *Rhus leptodictya* possess good antibacterial activity against *staphylococcus aureus* in the acetone and ethyl acetate fractions respectively since each has about five spots which are active against *staphylococcus aureus*.

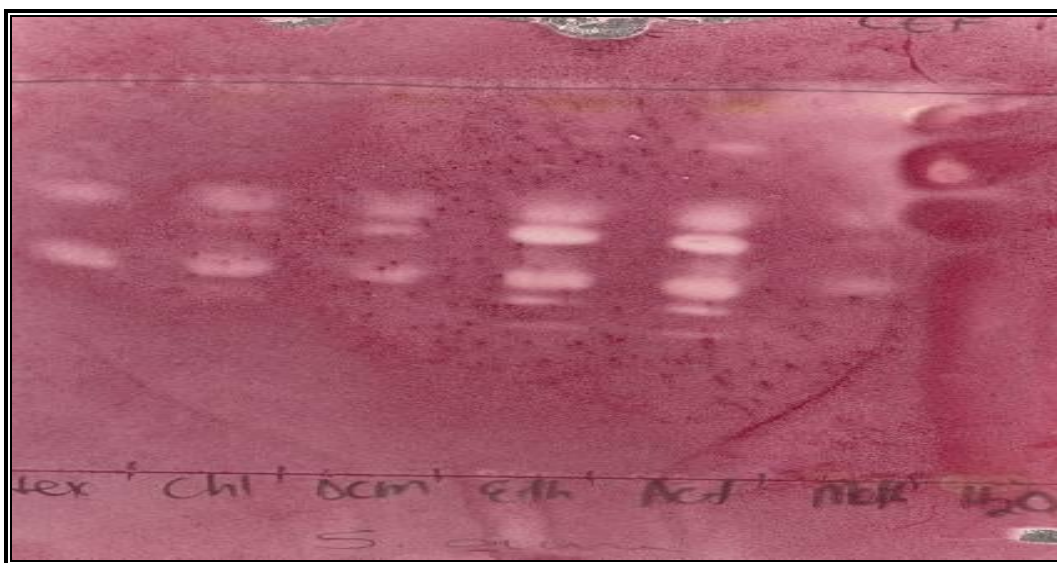


Figure 4.10: Bioautography of crude extracts developed in (CEF:5;4;1 ) solvent system and sprayed with *staphylococcus aureus*

Table 4.6: R<sub>f</sub> values of active spots against *staphylococcus aureus*

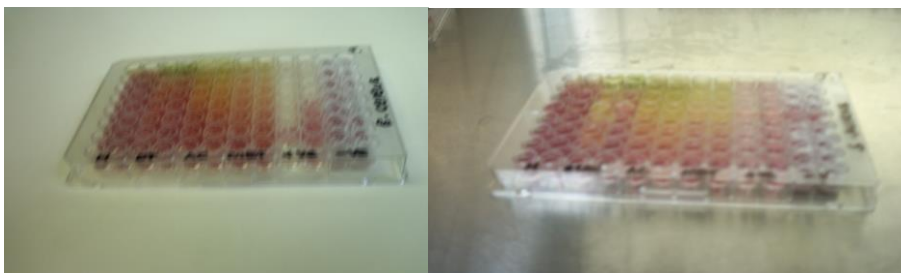
Bacteria Tested	Dichloromethane	Hexane	Ethyl acetate	Acetone
<i>staphylococcus aureus</i>	0.42	0.42	0.42	0.42
	0.5		0.5	0.5
	0.68	0.68	0.68	0.68
	*	*	0.81	0.81
	*	*	*	0.87

\*Means no activity

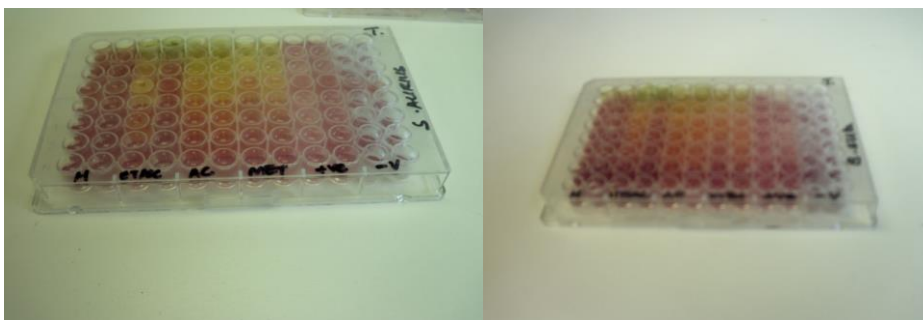
#### 4.6 Minimum Inhibitory Concentrations (M.I.C) of different leaves extracts of

##### *Rhus leptodictya* plant against different bacteria

The methanol and acetone extracts of the leaves of *Rhus leptodictya* showed antimicrobial activity against Gram positive bacteria and Gram negative bacteria, although, both extracts were more active against Gram positive bacteria - *S. aureus* (Table 4.4 and Figure 4.11). This suggests that these fractions have broad spectrum of antimicrobial activity. Gram negative bacteria have been known to show resistance to antimicrobial agents due to the composition of their cell membrane (Wiley, 2008).



**MIC plates of extracts with INT and bacteria after 12hours**



**MIC plate of extracts with INT and bacteria after 48hours**

Figure 4.6: Typical 96 well plates with of extracts INT

The bacterial growth in the microtitre plates were indicated and detected with the use of a bacterial growth indicator, INT. Spectrophotometric readings of the microtitre plates before and after the addition of INT facilitated the analysis of antibacterial activity of the plant extracts (Figure 4.11, A - D). The INT indicates the presence of viable bacteria in the microtitre plate suspensions by acting as an electron acceptor for the bacteria to form a red-coloured formazan product. After INT addition and incubation of the microtitre plates the presence of antibacterial activity could be assessed with the naked eye. Antibacterial activity was indicated by the absence of the red-coloured formazan.

The methanol and acetone extracts displayed significant antimicrobial activity against such recalcitrant pathogenic bacteria like *S. aureus* and *E. coli* that are increasingly becoming more difficult to treat due to the development of resistance to known antibiotics including the newer ones. These pathogens are known to cause majority of community and hospital acquired infections and are capable of elaborating several virulence factors. The methanol fraction had the lowest M.I.C. value of 0.04 mg/ml and 0.008 mg/ml against *S. pneumoeae* and *S.aureus*. The methanol extract is followed by the acetone extract in term of activity against the selected bacteria and furthermore, acetone extract showed more activity against *S. pneumoneae* at concentration less than 0.1 mg/L. Dichloromethane extract is less active against *B. subtilis*, *E. coli* and *S. aureus* when compared to the acetone and methanol extracts (Table 4.4 and Figure 4.11). It is however active against *S. pneumonia* since its M.I.C is about 0.31 mg/l. The ethyl acetate extract was also found to be active against *S. pneumonia* and *B. subtilis* with the M.I.C's of 0.08 and 0.16 mg/l respectively. From the results, it was clear that all the extracts were very active against *S. pneumoneae*

Table: 4.7: MIC value of plant extracts against different bacteria

Plant extracts	MIC values (ml/g)			
Hexane	<i>S. aureus</i>	<i>E. coli</i>	<i>S. pneumoneae</i>	<i>B. subtilis</i>
Dichloromethane	0.6	0.63	0.31	0.63
Ethyl acetate	0.29	0.63	0.08	0.16
Acetone	0.16	0.16	0.04	0.16
Methanol	0.08	0.16	0.04	0.08

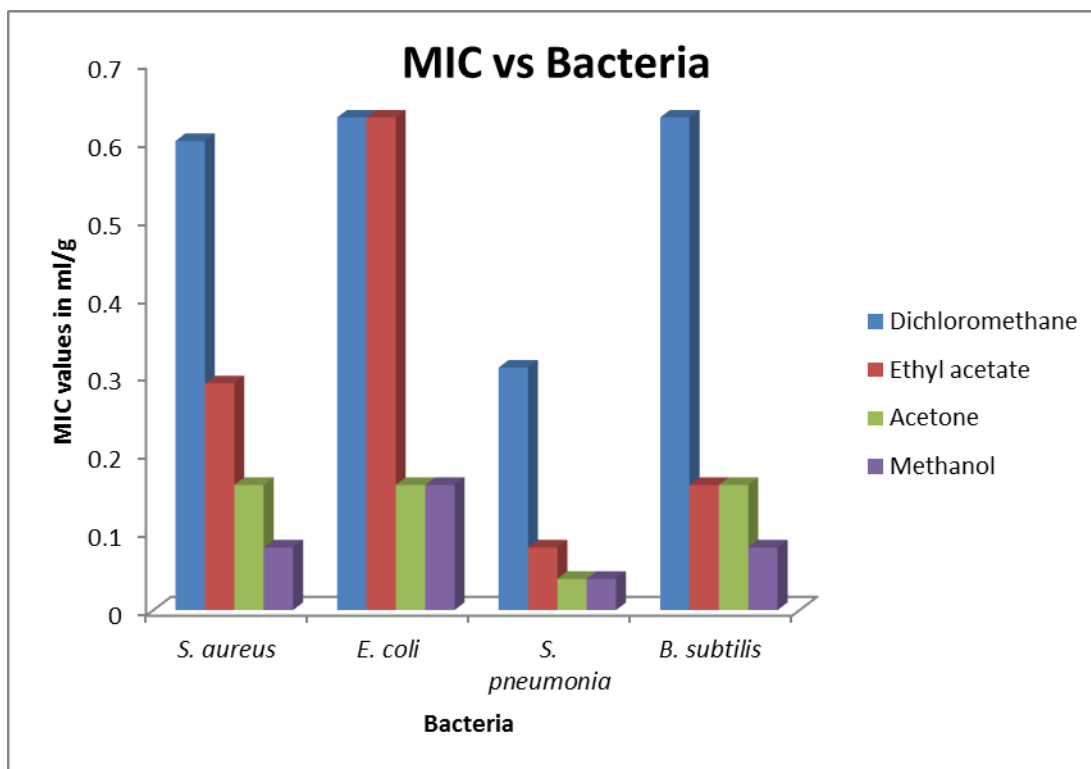


Figure 4.11: Graph of MIC versus the bacteria using different solvent system

#### 4.7 Total activity of plant extracts against selected bacteria

Table 4.5 and Figure 4.12 show total activity values which were obtained by dividing the quantity of the extract in mg from 1 g of the crude material of different extracts by the Minimum Inhibitory Concentration (M.I.C) given in Table 4.4. Total activity indicates the volume that which active constituents found in 1 g of the plant extract can be diluted and still be active or potent to kill the pathogen. Although the studied plant extracts gave poor M.I.C values, some good total activity values were shown by some extracts as observed.



Table 4.8: Total activity of plant extracts against the bacteria

Plant Extract	Total activity (ml/g)			
	<i>S. aureus</i>	<i>E. coli</i>	<i>S. pneumoneae</i>	<i>B. subtilis</i>
Hexane				
Dichloromethane	68	68	139	68
Ethyl acetate	6031	1532	12063	6031
Acetone	875	438	1750	438
Methanol	2025	1013	4050	2025

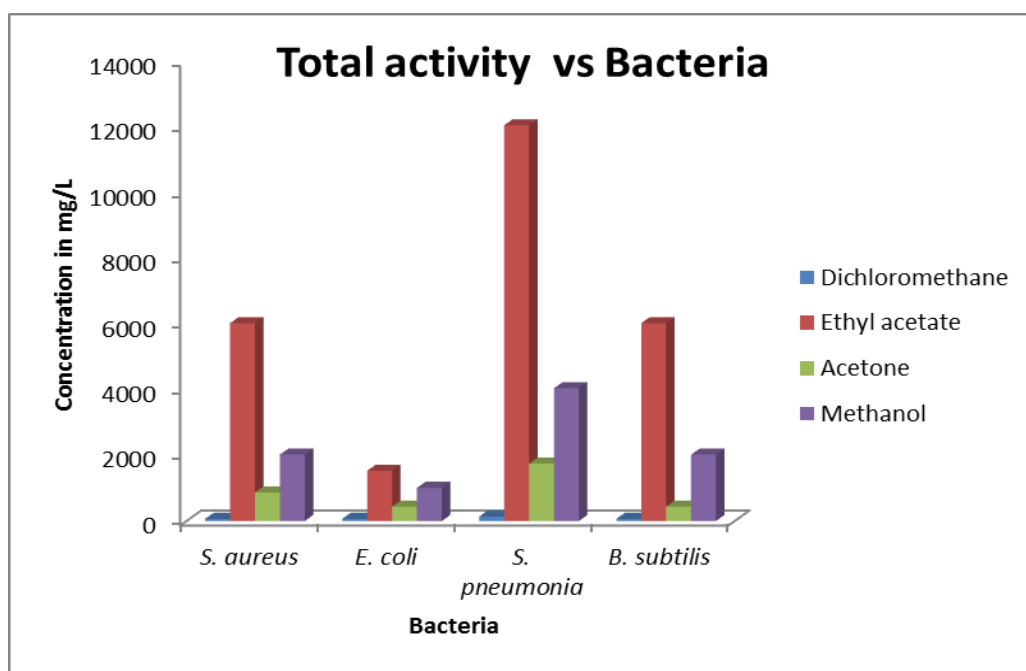


Figure 4.12: Graph of total activity vs bacteria from *Rhus leptodictya* leaves

## CHAPTER 5

### ISOLATION AND CHARACTERIZATION

#### 5.1 Isolation and characterization of the isolated compounds

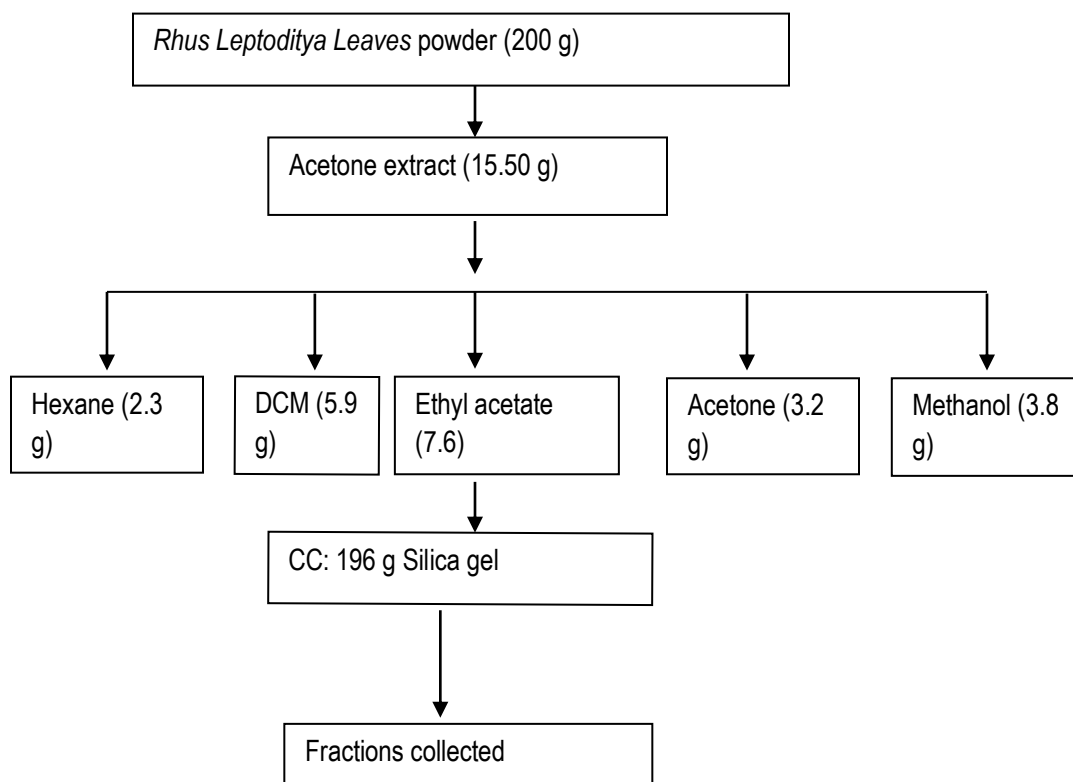


Figure 5.1: Isolation protocol used to isolate pure compounds from the leaves extract

## 5.3 Spectroscopic analysis of isolated compounds.

### 5.3.1 General

The different interactions of electromagnetic radiation with organic compounds based on their structural features form the basis of the application of spectroscopy in the structural elucidation of organic compounds. The identification of compounds involves a diverse range of analytical techniques and methods such as nuclear magnetic resonance (NMR), ultraviolet (UV) and infrared (IR) spectroscopy, and mass spectroscopy (MS). In this study NMR and MS techniques were used as tools for the analysis and identification of the compounds isolated from *R. leptodictya*. Structure elucidation of isolated compounds was achieved by a combination of nuclear magnetic resonance (NMR) and mass spectrometric (MS) analysis.

One dimensional NMR spectra were recorded for the isolated compounds on Varian 300 Spectrophotometer using DMSO as solvent. 2D NMR (COSY, HMBC and HMQC) data were also obtained. Heteronuclear multiple quantum correlation (HMQC) was used for the determination of substitution patterns at the different carbon atoms, correlation of carbon shifts, multiplicity and proton shifts were compiled from a two dimensional heteronuclear correlation spectrum. The combined spectral information allowed a view of carbon atoms showing different functionalities and substitutions.

Two dimensional spectra were obtained by recording a series of conventional NMR in which two parameters are changed incrementally. The most readily established connections between the individual carbon atoms were derived from connectivity's through couplings between the protons as

compiled from a COSY spectrum. From these correlations, the main part of the proton spin systems was outlined and several structural fragments were identified.

Heteronuclear bond correlation (HMBC) experiment provided information on the direct  $^{13}\text{C}$  and  $^1\text{H}$  heteronuclear connectivity. The method relies on the indirect detection of the  $^{13}\text{C}$  by observing their effects on the more sensitive proton nuclei to which they are coupled. It not only shows the connection of unprotonated carbon atom to the proposed elements, but also indicated vicinal proton relationships not resolved in the COSY spectrum.

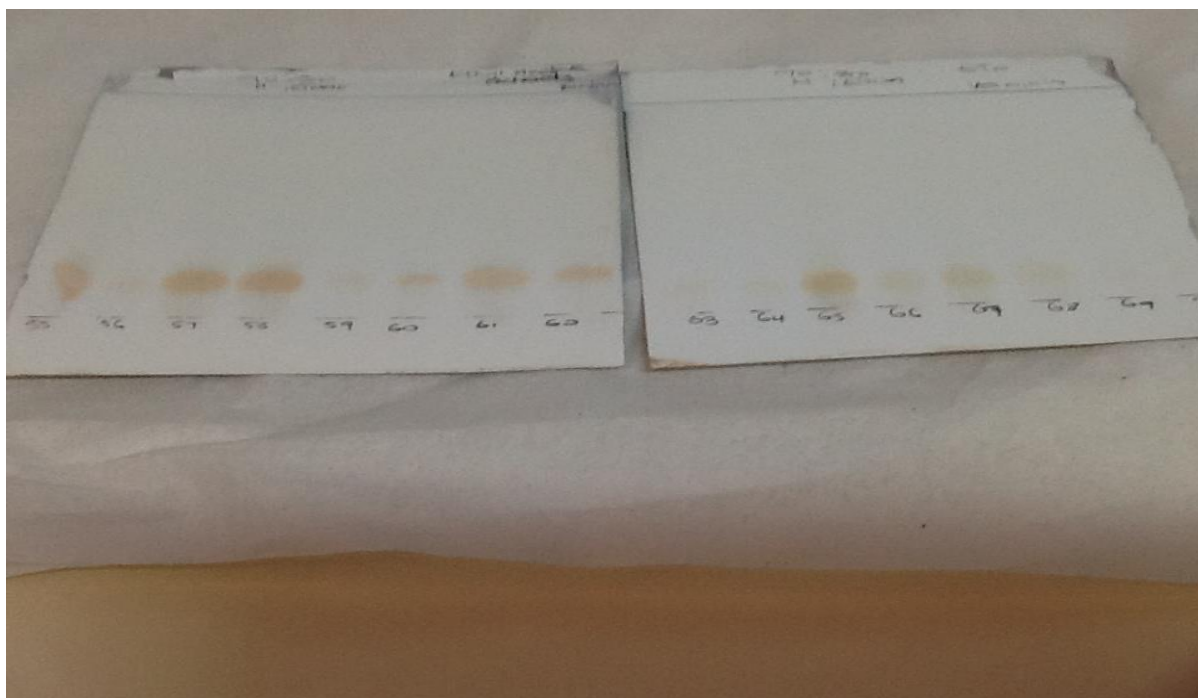


Figure 5.2: TLC plate of ethyl acetate fraction combined together.

## 5.4. Characterization of the isolated compound

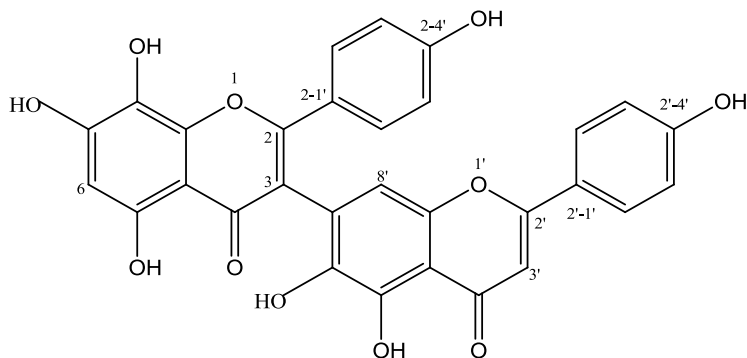


Figure 5.3: Structure of the isolated compound

$^1\text{H}$  NMR (DMSO  $-d_6$  500MH) H:  $\delta$  13.18(1H, s, 5-OH), 11.55(1H, s, 5'-OH or 6'-OH), 11.38(1H, s,

6'-OH or 5'-OH), 10.13 (1H, s, O H),

9.70(1H, s, OH), 9.69(1H, s, OH),

9.67(1H, 3 x s,  $A_2B_2$  patterns [7.32(2H, d; J=6.2Hz; 2-2'/2-6'), 7.27

(2H, d; J=6.8Hz; 2'-2'/6'), 6.83(2H, d; J=6.2; 2-3'/2-5')],

6.65(d; J=6.8Hz; 2'-3'/2'-5'), 5.99-6.00(3H, 2 aromatic H-8; and

olefin H-3')

$^{13}\text{C}$  NMR (DMSO- $d_6$  500MH):  $\delta$  185.4(s, C=O), 176 (s, C=O),  $A_2B_2$  patterns 8C for

1,4-disubstituted phenyl; [130.8(2XC, d)

130.4 (2 x C, d), 116.1 (2 XC, d), 115.5 (2 X C, d)],

169.23; 165.69; 164.37, 162.7,

159.8, 158.31, 151.6, 147.1, 136.5, 124.8, 121.5,

107.2, 105.4, 103.5, 99.3, 97.35(d), 95.57(d),

95.00 (d).

IR (Cm<sup>-1</sup>)  $V_{\max}$  (cm<sup>-1</sup>) 3326 (OH); 1746 and 1606 aromatic, 1512, 1470 cm<sup>-1</sup>

## 5.5 Structural elucidation

The novel natural biflavone, [7, 8-Trihydroxy-2-(4'-hydroxyphenyl)-3-[5'', 6''-dihydroxy-2''-(4'''-hydroxyphenyl)-4''*H*-chromen-4''-one]-4*H*-chromen-4-one], was identified mainly by <sup>1</sup>H NMR, <sup>13</sup>C NMR and MS analyses. The positive ion of HRMS of the new compound showed a peak at *m/z* 555 [M+H]<sup>+</sup> which is consistent with the molecular formula of C<sub>30</sub>H<sub>18</sub>O<sub>11</sub>. Evidence of the presence of 30 carbons is in good agreement with the <sup>13</sup>C NMR spectrum.

The IR spectrum exhibited absorption bands at max. 3672 cm<sup>-1</sup> (broad) indicating the presence of strongly H-bonded OH groups as well as carbonyl groups (C=O) with peaks at 1704 and 1640 cm<sup>-1</sup> while a peak at 1512 and 1463 cm<sup>-1</sup> are assigned to the aromatics moieties .

The <sup>1</sup>H NMR spectrum run in DMSO-d<sub>6</sub> indicated the presence of 7 OH signals, 3 of which are strongly deshielded at δ 13.18 for the 5-OH and δ 11.56 and 11.38 for 5''-OH and 6''-OH. The remaining 4 signals of OH groups resonated at δ 10.14, 9.70, 9.67 and 8.88. Additionally, the <sup>1</sup>H NMR spectra showed the presence of 11 protons at δ 5.99 - 7.24 attributable to 8 x aromatic CH ( 2 x *p*-hydroxyphenyls), 2 x aromatic CH ( 8'-H and 6-H) and 1 x olefin CH (3'-H).. The absence of the 7 OH signals due to deuterium exchange with CD<sub>3</sub>OD in the <sup>1</sup>H NMR spectra run in CD<sub>3</sub>OH was

evident and thus further supporting the information obtained from the  $^1\text{H}$ NMR (DMSO) and IR spectra. The  $^1\text{H}$ NMR further reveals the presence of signals assignable two different AB aromatic spin systems evident by two superimposed doublets at  $\delta$  7.22 (4H for H-2'/6' and H-2'''/6'''), a 2-proton doublet at  $\delta$  6.82 ( $J = 8.5$  Hz for H-3'/5') and a 2-proton doublet at  $\delta$  6.69 ( $J = 8.5$  Hz for H-3'''/5'''). An additional 3-proton sharp multiplet at  $\delta$  6.01 is assigned to the remaining 3 protons viz., H-3'', H-8'' and H-6.

The DEPT spectrum indicate the presence of 7 signals and illustrated the expected 8xC-H signals of the two 1,4-disubstituted para-hydroxyphenyl systems at  $\delta$  129.9 (x2), 129.3(x2), 115.7(x2) and 115.3(x2) with the overlap of the signals clearly being ascribed due to the symmetry of the molecule. The remaining three C-H signals at  $\delta$  97.3, 95.6 and 95.0 are assigned for C-8', C-6 and C-3' respectively. The COSY spectrum confirmed the two aryl 1, 4-disubstituted spin systems with correlation between to signals at  $\delta$  7.22 with both doublets at  $\delta$  6.82 and 6.69. The HSQC spectrum clearly shows these same peaks correlating with the  $^{13}\text{C}$  signals at  $\delta$  129.9 and 129.3 for C-2'/6' and C-2'''/6''' while the signals at  $\delta$  115.7 and 115.3 are assigned to C-3'/5' and C-3'''/5''' respectively.

The HMBC spectrum illustrates the correlation between the 3 aryl CHs at  $\delta$  6.01 with the same three DEPT signals at  $\delta$  97.3, 95.6 and 95.0 and in addition that each of these is associated with a C-O signal at  $\delta$  162.8, 164.4 and 169.2. The two OH signals at  $\delta$  10.14 (4'-OH/4'''-OH) and 9.70 (4'''-OH/4'-OH) are correlated with the signals at  $\delta$  115.7 and 115.3 which are thus the C-3'/5' and C-3'''/5''' sets of Cs which in turn correlate with the two pairs of doublets at  $\delta$  6.82 and 6.69. The 4-proton signal at  $\delta$  7.22 correlates with the signals at  $\delta$  129.3 and 129.9 as expected and has been assigned. These in turn correlate to the two signals at  $\delta$  158.3 and 159.8 for the C2- and C2'' of the

two C rings. To the best of our knowledge, the molecule is a novel compound and according to SciFinder the compound has not been isolated and identified previously. Preparation of a publication is underway.



## CHAPTER 6

### CONCLUSION AND RECOMMENDATIONS

#### 6.1 CONCLUSION & RECOMMENDATIONS

In conclusion it shows that *Rhus leptodictya* can act as an important source of phenolic compounds and antibacterial agents. The results of this study showed that the leaves extracts of this plant contain high amount of flavonoids and exhibited a great antioxidant and antibacterial activities. The bioautography study conducted showed that ethyl acetate have the most antibacterial activity followed by the acetone and methanol. In this context, *Rhus leptodictya* can be used as an easily accessible source of natural antioxidants and antibiotic in commercial food products and drugs. From the ethyl acetate extracts, a flavonoid compound was successfully isolated. The compound was characterized by NMR and MS as 5, 7, 8-Trihydroxy-2-(4'-hydroxyphenyl)-3-[5'', 6''-dihydroxy-2''-4hydroxyphenyl)-4''H—chromen-4''-one]-4H-chromen-4-one. The isolated flavonoid compound exhibited good antioxidant activity. Based on the structural activity studies, good antioxidant activity is believed to bring about by the presence of a catechol (3', 4'-OH) on ring B, the presence of 2, 3 unsaturation along with 3-OH and a keto group in position 4. To date, Scifinder has revealed that this natural product has not been isolated and identified previously. Furthermore, an extensive literature search conducted could not find the structure of this molecule in reported literature.

It is recommended that further research on the *Rhus* plant be conducted in order to fully identify other chemical constituent present in the plant since TLC plates of the crude extracts from ethyl acetate fraction showed the presence of other unidentified natural products. The study can be pursued as a separate M-tech or D-Tech programme.

## APPENDICES

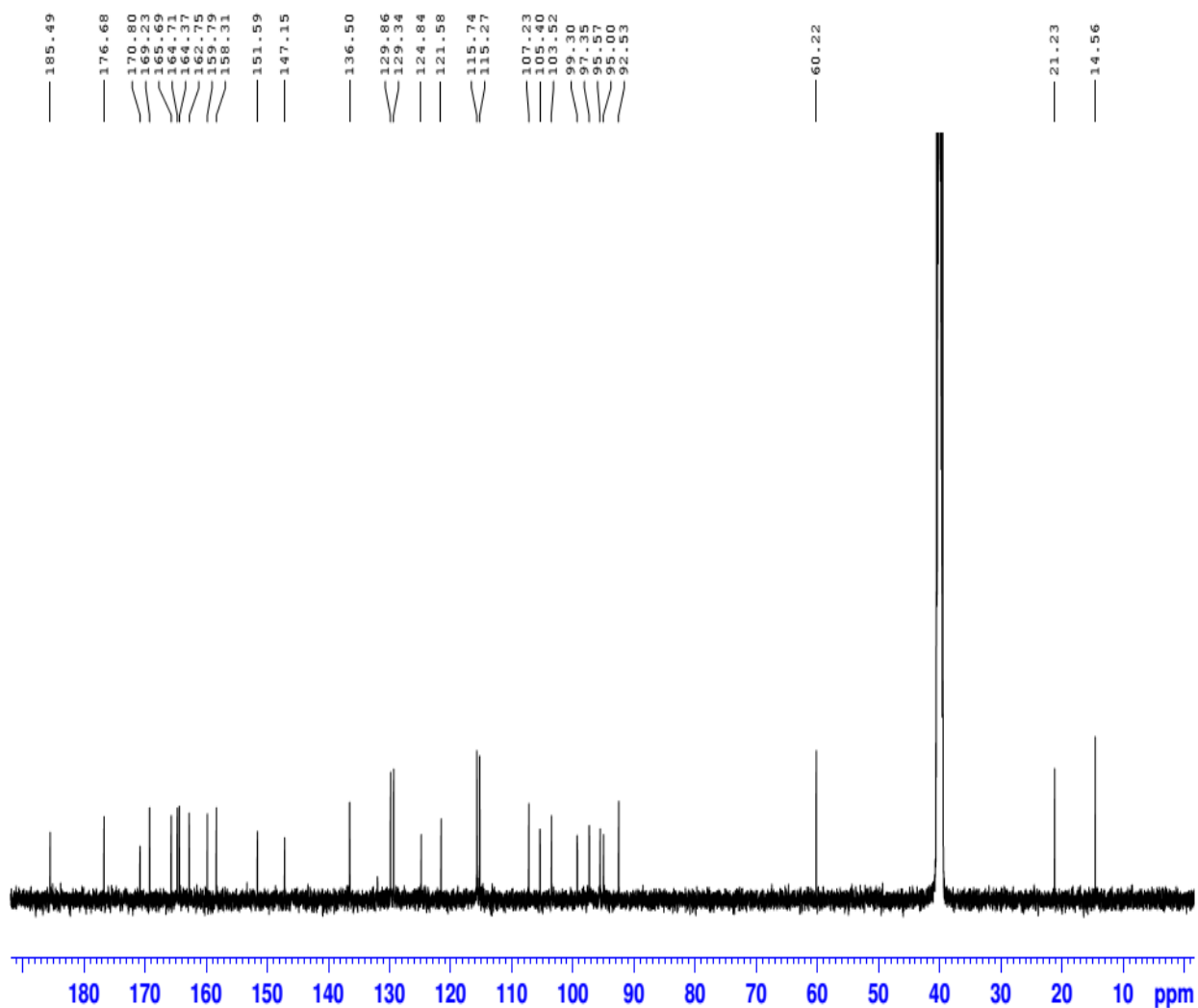


Figure 7.1:  $^{13}\text{C}$  NMR spectrum for ethyl acetate in DMSO

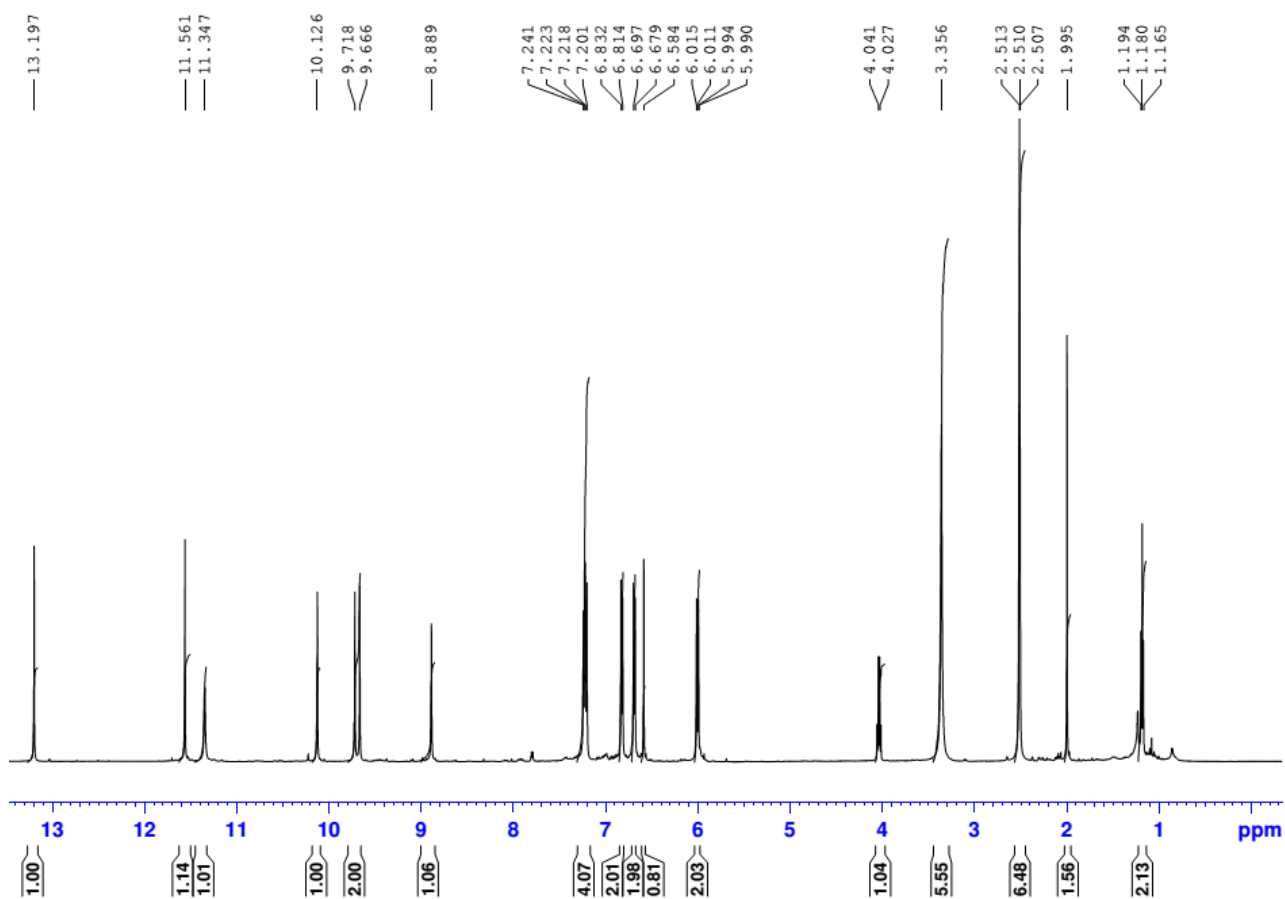


Figure 7.2:  $^1\text{H}$  spectrum of ethyl acetate in DMSO

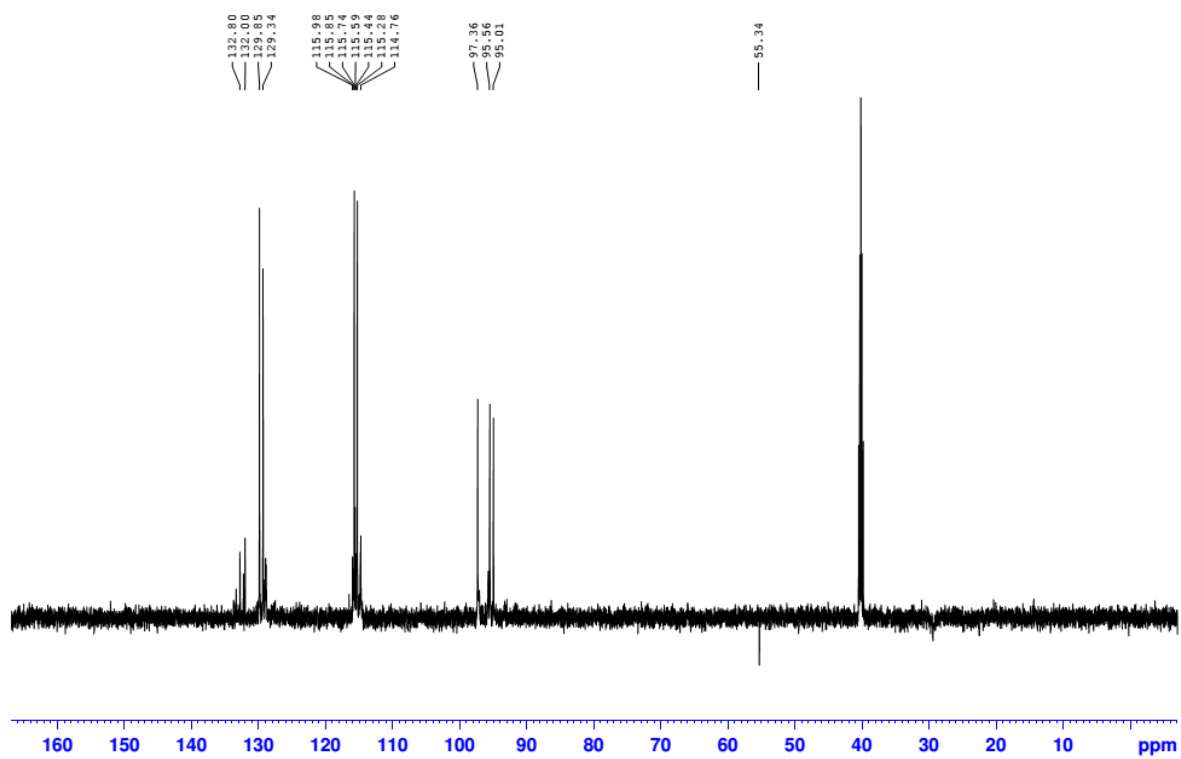


Figure 7.3: DEPT NMR spectra of ethyl acetate in DMSO

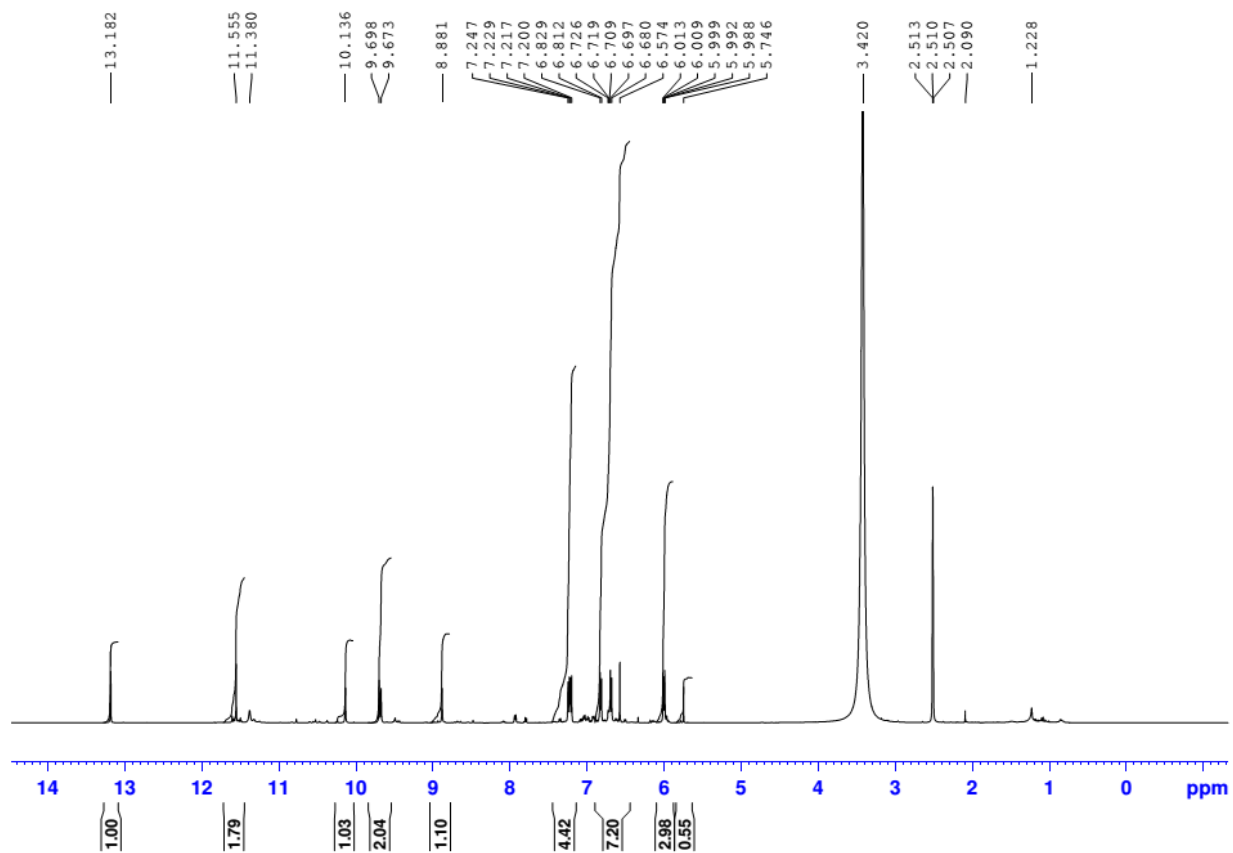


Figure 7.4:  $^1\text{H}$  NMR spectrum for ethyl acetate

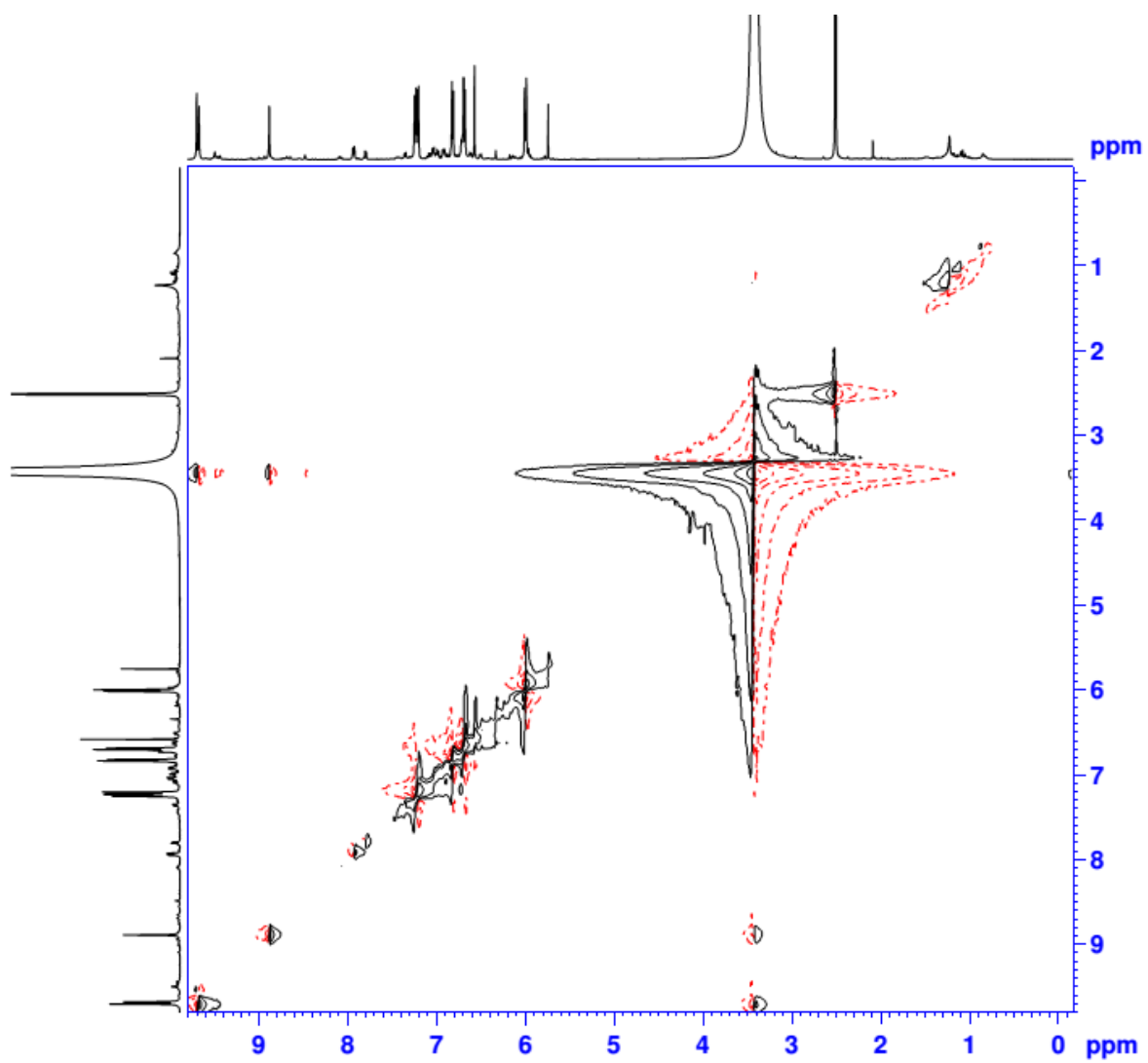


Figure 7.5: Nosy spectrum for ethyl acetate

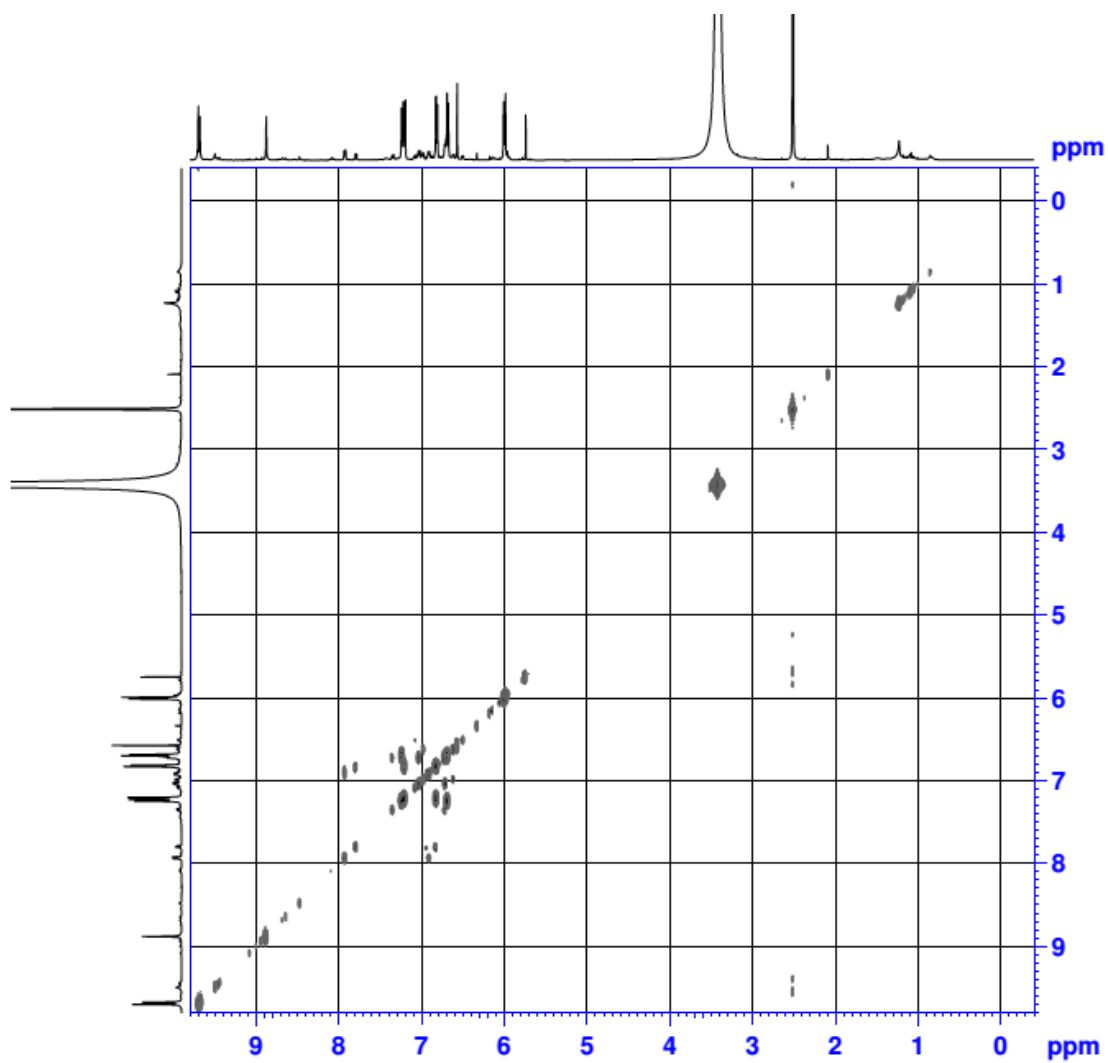


Figure 7.6: COSY spectrum for ethyl acetate

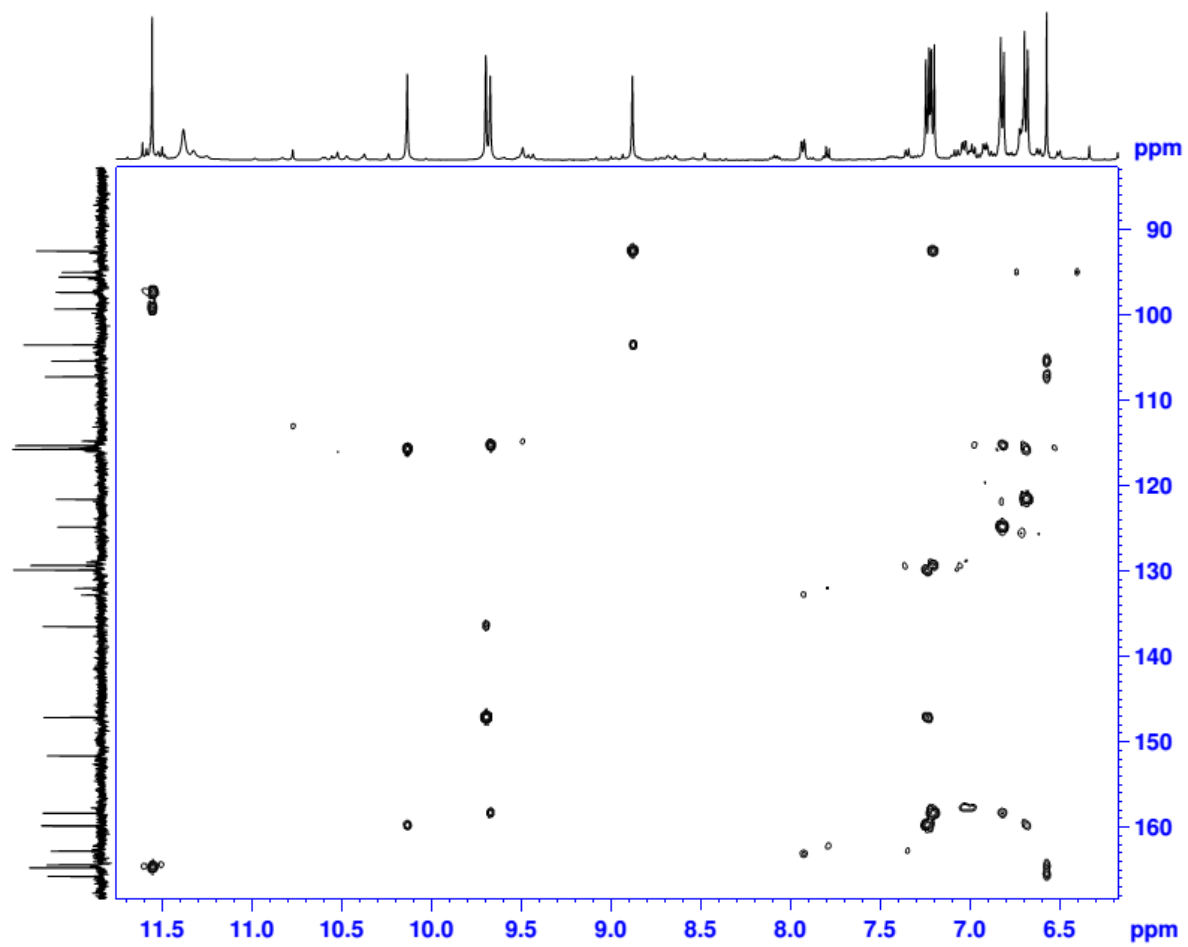


Figure 7.7: Figure: HMBC spectrum ethyl acetate



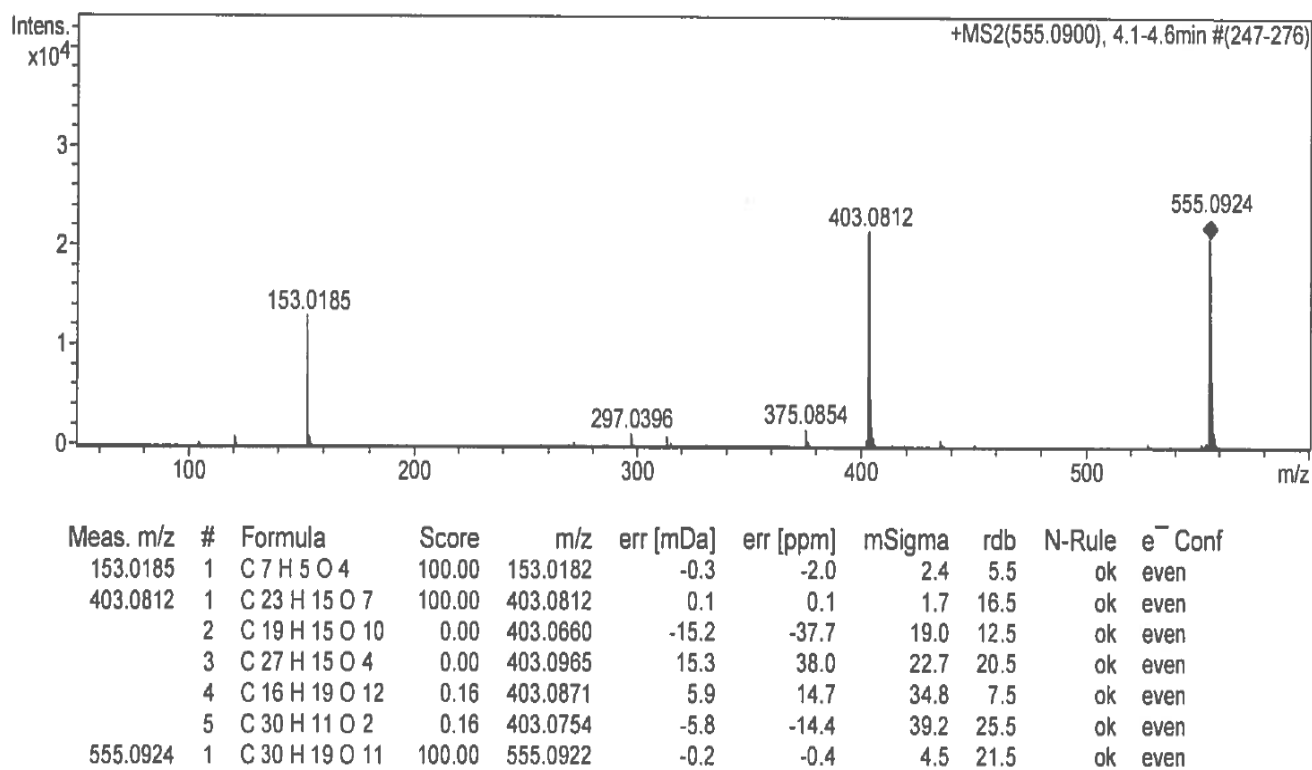


Figure 7.8: Accurate mass spectra scan ESI-MS spectrum

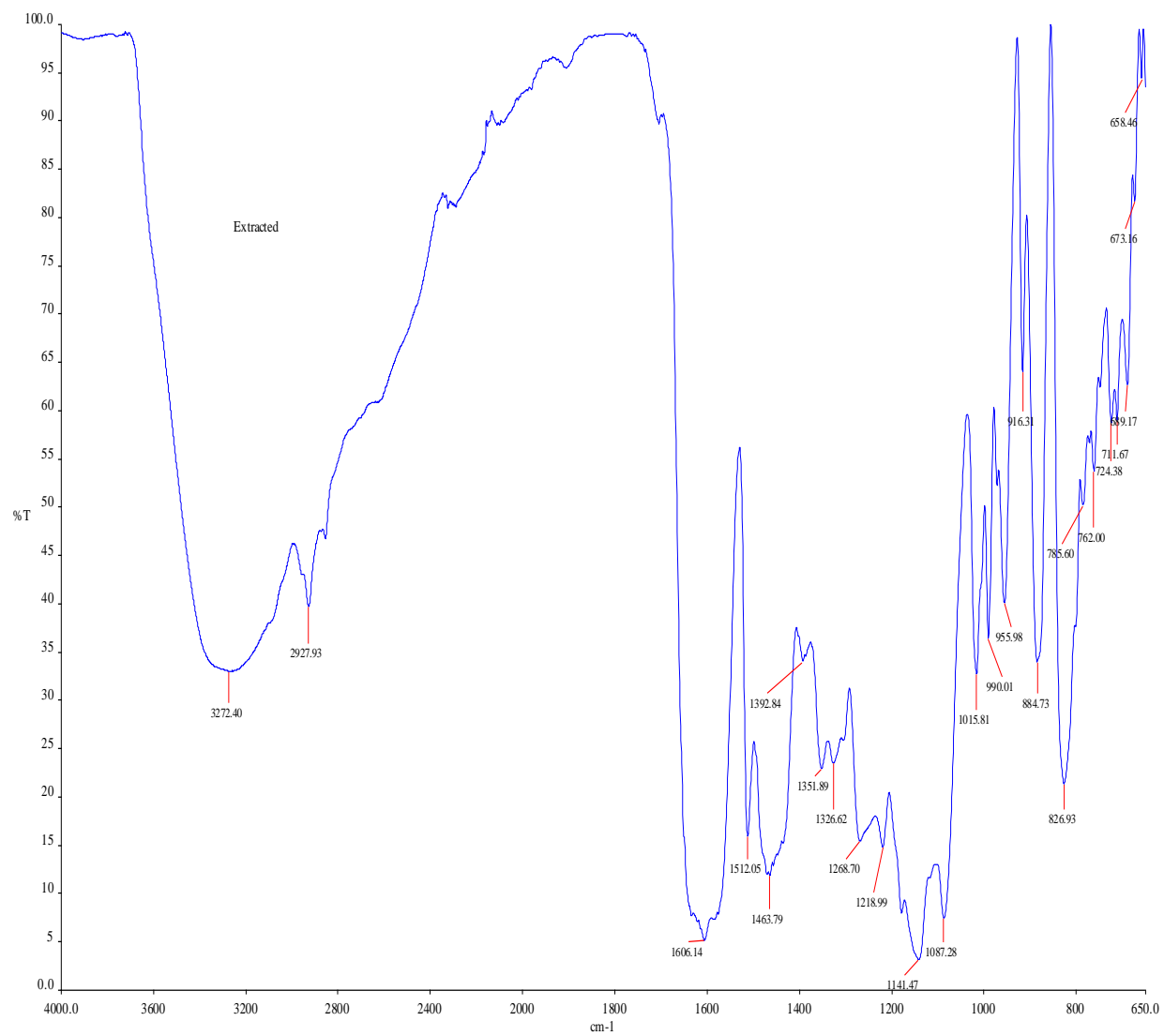


Figure 7.9: FTIR spectrum of the leaf extracts

## CHAPTER 8

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