EXTRACTION AND CHARACTERISATION OF PHYTOCHEMICALS FROM WHITE SERINGA (KIRKIA ACUMINATA) BARK EXTRACTS

BY

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ABSTRACT

The intention of this study was to extract and characterise phytochemicals with analgesic effect from *Kirkia Acuminata* bark extracts. Soxhlet extraction and Steam distillation were used for the extraction of compounds. Methanol,dichloromethane and hexane were used as solvents. Classes of phytochemicals were identified by qualitative tests and Thin Layer Chromatography using UV light.The qualitative tests of the phytochemical screening indicated the presence of alkaloids, anthraquinones, glycosides, flavonoids, phenols, tannins to name a few. Alkaloids, flavonoids, phenols and tannins were also observed on Thin Layer Chromatography. Menthol, catechol, 1,2 benzenediol 4 methyl, nitro phenyl salicylate, phenol dimethoxy,tau-cadinol, Isopropenyl,8 dimethyl, menthone and levomenthone were identified using Gas Chromatography-Mass Spectrometer.. The hexane fraction which is highly a non-polar solvent showed that very few phytochemicals were taken up in it. The polar solvents showed compatibility with the various chemical classes. The presence of these compounds gives *Kirkia acuminata* its characteristic property of being an analgesic. It thus finds application in the field of medicine.

Key Words: *Kirkia acuminata*, phytochemicals, Thin Layer Chromatography, Gas Chromatography-Mass Spectrometer.

DECLARATION

I, Rosa Mhlanga Chinheya hereby declare that this work is my original dissertation and to my knowledge, it has not been submitted anywhere else for the award of a degree at any other University.

Date: 03/06/2016 Signed:

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DEDICATION

To My Family,

To My Friends,

for the unequalled company.

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CHAPTER 1: INTRODUCTION

1.1 Background

Kirkia acuminata belong to the Kirkiaceae family. Its common names are Mubvumira, Mutsakatidze and Mutuhwa (Shona), Umvumile (Ndebele) and White seringa (English). It is found all over African Countries; South Africa, Botswana, Namibia, Mozambique, Malawi, Zimbabwe and Tanzania. *Kirkia acuminata* is a drought resistant plant, therefore it grows very well in hot and dry areas, and it is quite sensitive to frost. It may be found on various soil types from fertile soils (alluvial flats) and sandy or loamy soils near rivers to sandy and dry soils and rocky slopes, but well-drained, basic soils are more preferred. It reproduces itself using seed or stem parts and it is fast growing.The plant has got different uses but in Africa it is mainly known for its medicinal uses. An infusion of the bark is taken against vomiting and abdominal pains, infusion of the root is taken to treat coughs. The fruit sap is applied on wounds and as an antidote on snake bites. Pulverized roots are a remedy for toothache (Hweywood *et al*., 2007).

Fig 1.1: *Kirkia acuminata* plant

Natural products have been considered effective for maintaining a good health especially in humans. The interest in medicinal plants and their biologically active ingredients has increased in the past few years, in relation to the possible development of novel potential drugs. Quite a number of natural plants are used as herbal medicines and these herbs contain some major active ingredients with different effects.

A medicinal plant is any plant who's some of its parts contains substances that can be used for healing uses, or which are used during the chemo-pharmaceutical semi synthesis according to the World Health Organisation. The plant parts including roots, leaves, rhizomes, stems, barks, flowers, fruits, grains or seeds, are the ones used in the control or treatment of a disease condition and they contain some chemical components or substances that are have medicinal effects. These chemical substances from plants are commonly known as phytochemicals. These chemical substances are responsible acting against microbial infections or attack by pests (Abo *et al*., 1991; Liu, 2004; Nweze *et al*., 2004; Doughari *et al*., 2009). According to Doughari and Obidah, 2008; Doughari *et al*., 2009, isolation and characterization of fruits and vegetables was carried out.

1.1.1 Classification of phytochemicals

Alkaloids

Alkaloids are considered as one of the largest and well known groups of secondary metabolites. Alkaloids are made from ammonia compounds consisting of nitrogen bases prepared from amino acid blocks. They behave a basic in most reactions and also turn red litmus paper to blue. Nitrogen atoms in the compound are the ones responsible for the basicity. The presence and location of the functional groups in the compound contributes to a greater extend the degree of basicity (Sarker & Nahar, 2007). According to Firn, 2010 alkaloids were reported to react with acids forming some crystalline salts without the production of water.

Alkaloids have pharmacological applications such as anesthetics and Central Nervous System stimulants (Madziga *et al*., 2010). Close to 20% of the plant species contain more than 12 000 alkaloids.

Glycosides

According to (Kar, 2007 and Firn 2007) glycosides are produced from the condensation products of sugars. Glycosides are classified basing on the type of sugar component, chemical nature of aglycone as well as their pharmacological action. There are C-glycosides and O-glycosides. O-glycosides are tested by boiling plant samples with hydrochloric acid and water inorder to hydrolyse the anthraquinone glycosides to respective aglycones, and a base, such as sodium hydroxide is then added. C-glycosides are tested by hydrolysing plant samples using $FeCl_3$or HCl, and an aqueous base, such as NaOH solution is added to it. A pink or violet colour in the base layer after adding the aqueous base is observed in both cases indicating the presence of glycosides in the plant sample under investigation.

Flavonoids

Flavonoids are made up of more than one benzene ring in structure, and several researches support them for being used as antioxidants (Kar, 2007). The parent compounds of flavonoids are known as flavans. More than four thousand flavonoids exist and most of them are pigments in higher plants.

Phenolics

Polyphenol extracts occur everywhere as natural colour pigments that are responsible for the colour of fruits in plants. Phenolics found in plants are prepared from phenylalanine via the action of an enzyme called phenylalanine ammonia lyase. They are very important to plants and have quite a number of functions. The most important role of plant phenolics are in defending plants against pathogens and herbivore predators, and are also applied in controlling human pathogenic infections (Doughari, 2012). They are classified into (i)

phenolic acids and (ii) flavonoid polyphenolics that are flavonones, flavones, xanthones and catechins and (iii) non-flavonoid polyphenolies.

Saponins

Saponins have the characterisc of soap in water that is they produce foam. During saponins hydrolysis, an aglycone is formed, which is known as sapogenin. There are two known types of sapogenin namely steroidal and triterpenoidal. Usually, the sugar is attached at Carbon 3 in saponins, whilst in most sapogenins there is a hydroxyl group at Carbon 3. The two major classes of saponins are steroid and triterpene saponins. Saponins dissolve in water and do not dissolve in ether, and they produce aglycones upon hydrolysis. According to (Kar, 2007), saponins are very poisonous, and are known to cause heamolysis of blood and cattle poisoning. Besides causing irritation to membranes of the mucous they also have a bitter taste.

Tannins

Tannins are phenolic compounds with very high masses. They dissolve in water and alcohol. Phenolics and carboxylic groups present in tannins contribute to their acidic behavior (Kar, 2007). Hydrolysable and condensed tannins are two major types of tannins. Antiseptic activity of tannins is due to the presence of the phenolic group.

Terpenes

Terpenes are unsaturated hydrocarbons and are flammable. According to (Firn, 2010) terpenes are found in liquid form especially in essential oil and resins. Terpenoids have the general formula $(C_5H_8)_{n.}$ The classification of terpenes depends on the number of carbon atoms (Martinez *et al.*, 2008). The numbers of isoprene units involved in the formation are considered when classifying terpenoids.

Anthraquinones

Anthraquinones are derived from phenolic and glycosidic compounds. According to (Maurya *et al*., 2008 and Firn, 2010), anthraquinones are solely derived from anthracene giving variable oxidized derivatives like anthrones and anthranols. Other derivatives such as chrysophanol, aloe-emodin, rhein, salinos poramide, luteolin and emodin have in common a double hydroxylation at positions Carbon 1 and Carbon 8. Anthraquinones are tested by mixing powdered plant material with organic solvent and filter, and an aqueous base, for example NaOH or NH_4OH solution, is added to it. The presence of anthraquinones is indicated by a pink or violet colour in the plant sample under investigation (Sarker and Nahar, 2007).

Essential oils

They are referred to as volatile oils or ethereal oils because they evaporate on exposure to air even at ambient conditions**.** They mostly contribute to the odor of the aromatic plants that are used to improve the aroma of spices (Martinez *et al.,* 2008). Essential oils have been associated with different parts of the plants including leaves, stems, flowers, roots or rhizomes. According to (Firn, 2010) a single volatile oil is known to comprise of approximately two hundred different chemical substances. They can be prepared from plant material either by direct steam distillation or hydrolysis with the use of enzymes. Distillation process depends on the type and nature of the plant material.

CHEMICAL CONSTITUENTS OF ESSENTIAL OILS:

An essential oil consists of more than two hundred chemical components. Essential oils consist of chemical compounds which have hydrogen, carbon and oxygen as their building blocks. Essential oils are classified into two groups that are volatile oils and non-volatile residue.

Volatile fraction: This fraction constitutes of 90–95% of the oil in weight, containing monoterpene and sesquiterpene hydrocarbons.

Non-volatile residue: Non-volatile residue contains 1–10% of the oil.

Steroids

According to (Firn, 2010) plant steroids have found applications in medicine as cardiac drugs. Cardiac glycosides are steroids with a strong ability to afford a specific and powerful action especially on the cardiac muscle when being administered into humans through injection. According to Maurya *et al.,* 2008 and Madziga *et al.,* 2010 anabolic steroids were reported to promote nitrogen retention in osteoporosis. Examples of plant steroids include diosgenin and cevadine.

1.1.2 Modes of action for phytochemicals

Mechanisms of action of phytochemicals may prevent microorganisms to interfere with some metabolic processes (Kris-Etherton *et al*., 2002; Manson, 2003 and Surh, 2003). They may be used in two different forms as chemotherapeutic or chemo preventive agents (D'Incalci *et al*., 2005; Sarkar and Li, 2006). Plant extracts and essential oils have different modes of action against bacterial strains like how they interfere with the phospholipids bilayer of the cell membrane. In general mechanism of action of phytochemicals is regarded as a disturbance of the cytoplasmic membrane (Kotzekidou *et al.,* 2008).

Antioxidants

Antioxidants protect cells by acting against damaging effects of reactive oxygen species. These reactive oxygen species are known as free radicals (Mattson & Cheng, 2006). Natural antioxidants play a key role in health maintenance and preventing chronic and degenerative diseases (Uddin *et al*., 2008 and Jayasri *et al*., 2009). Free radicals are responsible for causing quite a number of diseases such as infertility, cystic fibrosis etc. (Chen *et al*., 2006 and Uddin *et al*., 2008). Inadequate amounts of antioxidants are produced by the human body which are

required for preventing oxidative stress (Sen, 1995)**.** Natural antioxidants, like vitamin C, E and natural products in plants have to be used to cater for antioxidant shortage in the body (Madsen & Bertelsen, 1995; Rice- Evans *et al*., 1997; Diplock *et al*., 1998).

Anticacinogenesis

According to (Liu, 2004) polyphenols are amongst phytochemicals that are capable of inhibiting carcinogenesis. Formation of the specific cancer-promoting nitrosamines from the nitrites and nitrates dietary is mimised by phenolics acids. Glucosinolates present in different vegetable sources like cabbage and cauliflower have a significant protective support against colon cancer.

Antimicrobial activity

Phytoconstituents used by plants to protect them from pathogenic insects such as bacteria, fungi or protozoa are also applied in human medicine (Nascimento *et al*., 2000). phenolic acids help in the reduction of particular adherence of organisms to the cells lining the bladder, and the teeth, which then lowers the incidence of urinary-tract infections and dental caries (Jakhetia *et al.*, 2010). *A. flavus*, which is known to produce toxins, was found to be the most resistant microorganism. It is worthy of note that antimicrobial activity results of the same plant part tested most of the time varied from researcher to researcher. This is possible because concentration of plant constituents of the same plant organ differ from one geographical location to another depending on the age of the plant, differences in topographical factors, nutrient concentrations of the soil, extraction method as well as method used for antimicrobial study. It is therefore important that scientific protocols be clearly identified and adequately followed and reported.

Anti-inflammatory

Anti-inflammatory refers to the property or ability of a substance and treatment that reduces inflammation or swelling. Anti- inflammatory drugs make up about half of analgesics,

remedying pain by reducing inflammation as opposed to opioids, which affect the central nervous system. Essential oil of *C. osmophloeum* twigs has excellent anti- inflammatory activities and cytotoxicity against HepG2 (Human Hepatocellular Liver Carcinoma Cell Line) cells (Jakhetia *et al.*, 2010).

Anti-diabetic

Drugs used to treat diabetes mellitus lower glucose levels in the blood. Cinnamaldehyde, a phytoconstituent extracts have been reported to exhibit significant antihyperglycemic effect resulting in the lowering of both total cholesterol and triglyceride levels and, at the same time, increasing cholesterol in induced diabetic rats. This investigation reveals the potential of cinnamaldehyde for use as a natural oral agent, with both hypoglycaemic and hypolipidemic effects (Jakhetia, *et al*, 2010).

1.1.3 Methods of studying phytochemicals

No single method is sufficient to study the bioactivity of phytochemicals from a given plant.

An appropriate assay is required to first screen for the presence of the source material, to purify and subsequently identify the compounds in the plant material. Assay methods differ depending on what bioactivity is targeted and these may include antimicrobial, anti-malarial, anticancer and mammalian toxicity activities. Purification steps may involve simple crystallization of the compound from the crude extract, further solvent partition of the co extractives or chromatographic methods in order to fractionate the compounds basing on their acidity, polarity or molecular size.

Extraction

Extraction is very necessary when analysing medicinal plants. Chemical components extracted from the plant materials are then separated and characterized. Extraction process involves pre-washing, drying of plant materials, grinding to obtain a uniform sample. This

improves the rate of reaction during analytic extraction and also increasing the contact between sample surface and solvent medium. The processing of the plant material should be perfomed in a way that the desired active constituents are not lost, distracted or destroyed during the preparation of extracts from plant materials. According to (Fabricant and Farnsworth, 2001), a plant that is selected basing on the traditional uses should be prepared in a way described by the herbalist in order to produce as closely as possible the traditional medicine. The nature of the bioactive compound under investigation has got an impact on the selection of the solvent system to be used. Various solvent medium are available for extraction of phytochemicals from plant samples. Solvents such as methanol, ethanol or ethyl-acetate are used for the extraction of polar compounds. Dichloromethane or a mixture of dichloromethane and methanol in ratio of 1:1 are used for the extraction of lipophilic compounds (Cosa *et al*., 2006). The suitability of the methods to be used during extraction must also be considered since the target compounds maybe non-polar to polar. Different extraction methods, such as sonification, refluxing, soxhlet extraction and so on are commonly used (United States Pharmacopeia and National Formulary, 2002; Pharmacopoeia of the People's Republic of China, 2000; The Japanese Pharmacopeia, 2001).

Solid-phase micro-extraction, supercritical-fluid extraction, microwave-assisted extraction, solid-phase extraction, and so on, have got some advantages over traditional techniques. Some of the advantages include reduction in organic solvent consumption and in degrading the sample (Huie, 2002).

Soxhlet Extraction

A Soxhlet extractor is a piece of laboratory apparatus (Harwood and Moody, 1976) designed by Franz von Soxhlet in 1879 (Soxhlet, 1879). This type of extraction is required when the desired compound has a limited solubility in a solvent used solvent. Plant material with the desired compound is placed in a thimble. The solvent to be used for the extraction is placed in

a flask and heated to reflux. This process takes place for hours or days. After many cycles the desired compound is concentrated in the distillation flask. After extraction the solvent is concentrated on a rotary evaporator. The insoluble portion of the extracted solid remains in the thimble and discarded.

Steam Distillation

Steam distillation is employed when essential oils are to be extracted from the plant material. It is used when separating temperature sensitive materials such as oils, resins, hydrocarbons, to name a few which are insoluble in water and also decompose at their boiling point. Steam distillation enables a compound or mixture of compounds to be distilled at a temperature below that of the boiling points of the individual constituents.

1.1.4 Identification and characterization

Separation of plant extracts is a big challenge for the process of identification and characterization since they usually occur as a combination of different types of bioactive compounds with different polarities. Several separation techniques such as TLC, column chromatography, gas chromatography, High Performance Liquid Chromatography and so on, are be used to inorder to obtain pure compounds. Pure compounds obtained from the separation process are then used for the determination of structure and biological activity. Non-chromatographic techniques like immunoassay, phytochemical screening assay and Fourier-transform infrared spectroscopy, can also be used to obtain and help in the identification of the phytochemicals (Sasidharan *et al*. 2011)

Thin Layer Chromatography

Analysis using chromatography plays a very important role amongst the chemical methods for examination. Due to the numerous advantages of the chromatographic techniques (like specificity and a possibility to use them for analysis), they comprise an essential part of the medicinal plant analysis (Waksmundzka- Hajnos *et al*., 2008).

Summing up, TLC is a principal separation technique in plant chemistry research. It can be used in a search for the optimum extraction solvents, for identification of known and unknown compounds, and what is at least equally important for the selection of biologically active compounds. TLC also plays a key role in preparative isolation of compounds, purification of the crude extracts, and control of the separation efficiency of the different chromatographic techniques and systems. TLC has many advantages in plant chemistry research and development. These include single use of stationary phase (no memory effect), wide optimization possibilities with the chromatographic systems, special development modes and detection methods, storage function of chromatographic plates (all zones can be detected in every chromatogram by multiple methods), low cost in routine analysis, and availability of purification and isolation procedures (Waksmundzka- Hajnos *et al*., 2008)

Gas Chromatography- Mas Spectroscopy

Gas chromatography-mass spectrometry (GC-MS) is an analytical method that combines the features of gas-liquid chromatography and mass spectrometry to identify different substances within a test sample. GC can separate volatile and semi-volatile compounds with great resolution, but it cannot identify them. MS can provide detailed structural information on most compounds such that they can be exactly identified, but it cannot readily separate them.

Gas Chromatography is a type of chromatographic technique whereby the mobile phase used is a carrier gas. The most commonly used carrier gases are helium and nitrogen and the stationary phase is a microscopic layer of liquid or polymer on an inert solid support, inside glass or metal tubing, called a column. The capillary column contains a stationary phase; a fine solid support coated with a non-volatile liquid. The solid can itself be the stationary

phase. The sample is swept through the column by a stream of helium gas. Components in a sample are separated from each other because some take longer to pass through the column than others. Mass Spectrometry (MS), the detector for the GC is the Mass Spectrometer (MS). As the sample exits the end of the GC column it is fragmented by ionization and the fragments are sorted by mass to form a fragmentation pattern. Like the retention time (RT), the fragmentation pattern for a given component of sample is unique and therefore is an identifying characteristic of that component. It is so specific that it is often referred to as the molecular fingerprint.

Principle of Gas Chromatography – Mass Spectrometer

GC/MS is a combination of two different analytical techniques, Gas Chromatography (GC) and Mass Spectrometry (MS), is used to analyze complex organic and biochemical mixtures (Skoog *et al.,* 2007). The GC-MS instrument consists of two main components. The gas chromatography portion separates different compounds in the sample into pulses of pure chemicals based on their volatility (Oregon State University, 2012) by flowing an inert gas (mobile phase), which carries the sample, through a stationary phase fixed in the column (Skoog *et al*., 2007). Spectra of compounds are collected as they exit a chromatographic column by the mass spectrometer, which identifies and quantifies the chemicals according their mass-to-charge ratio (m/z). These spectra can then be stored on the computer and analyzed (Oregon State University, 2012).

Instrumentation and working of GC-MS

The Fig 1.2 is the schematic diagram of GC-MS. Its different parts and their functions are discussed below.

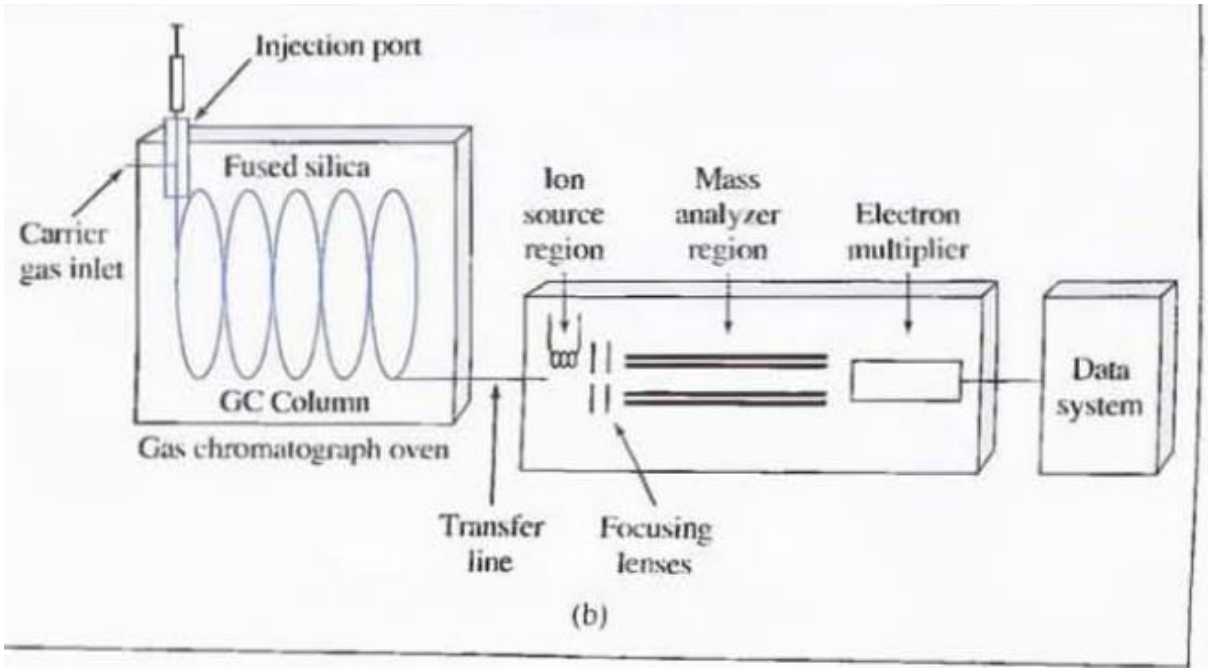


Fig 1.2: Schematic of GC-MS instrument

Gas Supply

Carrier gas is fed from the cylinders through the regulators and tubing to the instrument. It is usual to purify the gases to ensure high gas purity and gas supply pressure (Gas Supply and Pressure Control from theory and Instrumentation of GC-GC Channel.

Injector

Here the sample is volatilized and the resulting gas entrained into the carrier stream entering the GC column (Sampling Techniques and Sample Introduction from Theory and Instrumentation of GC-GC Channel).

Column

Gas Chromatography uses a gaseous mobile phase to transport sample components through columns either packed with coated silica particles or hollow capillary columns containing, the stationary phase coated onto the inner wall. Capillary GC columns are usually several meters long (10-120 m is typical) with an internal diameter of 0.10-0.50 mm, whilst packed GC columns tend be 1-5 meters in length with either 2 or 4mm internal diameter (GC columns from Theory and Instrumentation of GC).

Oven

Gas chromatography have ovens that are temperature programmable, the temperature of the gas chromatographic ovens typically range from 5^0C to 400^0C but can go as low as -25^0C with cryogenic cooling (GC Temperature Programming from the 'Theory and Instrumentation of GC.

Mass Spectrometer: The separation of the phase ions is achieved within the mass spectrometer using electrical and/or magnetic fields to differentiate ions.

Ion source: In the ion source, the products are ionized prior to analysis in the mass spectrometer.

Mass analyzer: There are several very popular types of mass analyzer associated with routine GC-MS analysis and all differ in the fundamental way in which they separate species on a mass-to-charge basis**.**

Vacuum system: Mass analyzers require high levels of vacuum in order to operate in a predictable and efficient way.

Detector: The ion beam that emerges from the mass analyzer, have to be detected and transformed into a usable signal. The detector is an important element of the mass spectrometer that generates a signal from incident ions by either generating secondary electrons, which are further amplified, or by inducing a current (generated by moving charges).

Control Electronics: The MS parameters can be selected and controlled from this panel. Modern instruments will also allow controlling MS parameters from a computer by using specially designed software. The mobile-phase called as carrier gas, must be chemically inert. The helium gas is most commonly used; however, argon, nitrogen, and hydrogen are also used. These gases are held in pressurized tanks and use pressure regulators, gauges, and flow meters to control the flow rate of the gas. Flow rates usually range from 25-150 mL/min with packed columns and 1-25 mL/min for open tubular capillary columns, and are assumed to be constant if inlet pressure is constant. This is often accompanied by a molecular sieve to purify the gas before it is used. Samples are introduced as a plug of vapor. Liquid samples are introduced using calibrated micro syringes to inject sample through a septum and into a heated sample port which should be about 50°C above the boiling point of the least volatile constituent of the sample. After the sample is introduced, it is carried to the column by the mobile phase. The temperature of the column is an important variable, so the oven is equipped with a thermostat that controls the temperature to a few tenths of a degree. Boiling point of the sample and the amount of separation required determines the temperature the sample should be run with. As the mobile phase carrying the sample is passed through the stationary phase in the column, the different components of the sample are separated. After being separated, the sample is run through a detector (Skoog *et al*., 2007), which ionizes the sample and then separates the ions based on their mass-to-charge ratio. This data is then sent to a computer to be displayed and analyzed. The computer linked to the GC-MS has a library of samples to help in analyzing this data (Agilent Technologies, 2012). Data for the GC-MS is displayed in several ways. One is a total-ion chromatogram, which sums the total ion abundances in each spectrum and plots them as a function of time. Another is the mass spectrum at a particular time in the chromatogram to identify the particular component that

was eluted at that time. Mass spectra of selected ions with a specific mass to charge ratio, called a mass chromatogram, can also be used.

This study was designed to extract and characterise phytochemicals with analgesic effect in *Kirkia acuminata* barks. The traditional healers/herbalists use the selected plant barks to act against vomiting and abdominal pains.

1.2 AIM

- To extract and find out the active compounds with analgesic effects from *Kirkia acuminata* bark extracts

1.3 Objectives

- Sample collection and preparation
- To extract the phytochemicals by soxhlet extraction and steam distillation
- To determine the components present in the extracts by Thin Layer Chromatography
- To carry out phytochemical screening of secondary metabolites using qualitative tests
- To carry out instrumental analysis of the extracted components using Gas Chromatography- Mass Spectrometer (GC-MS)

1.4 Problem Statement

For many decades phytochemistry drew attention of chemists for the search of active compounds/phytochemicals in natural products since traditional herbs are being accepted worldwide even among the literates in urban areas. This is due to the increasing inefficiency of most drugs used for the control of diseases.

Traditional plants are still used by most people worldwide, for the treatment of common diseases like cough, diarrhoea, sexually transmitted infections, and tooth infections.

A lot of researches were done on different plants that are used as medicines. However, no previous studies have been conducted on the extraction and characterisation of phytochemicals from the bark extracts of *Kirkia acuminata*. Yet it is well known that the

plant barks are used by herbalists and the community nowadays to act against stomach pains and vomitting. Data collected in the present paper illustrates the phytochemicals with analgesic effect present in *Kirkia acuminata* bark extracts that is used as medicine by traditional healers/herbalists.

1.5 Justification

Characterisation is a must; we need to know the active compounds in the plants that we use as medicines. For the past years phytochemistry occupies a key position in medicinal plant research and indigenous knowledge systems. Sharing of such information is important for maintaining options for the use of traditional medicines and also because of reasonable costs and increasing faith in herbal medicines.

This research will help the community to know the active compounds present in the medicinal plant and this information will be used for the manufacturing of conventional drugs. The drug synthesised will have the same pharmacological effect as the medicinal plant. Knowing the active compounds with medicinal effects in the drug also adds value and market to the plant.

CHAPTER 2: Literature Review

Masoko, P (2013), carried out a research on ethnobotanical study of ten medicinal plants including *Kirkia Acuminata* used by traditional healers in Limpopo Province (South Africa). Hexane, dichloromethane (DCM), acetone and methanolic extracts of these plants were screened for antibacterial activity against *Staphylococcus aureus* ATCC 29213, *Pseudomonas aeruginosa* ATCC 27853, *Enterococcus faecalis* ATCC 29212 and *Escherichiacoli* ATCC 25922. Ampicillin was used as the positive control. Methanol extract of *Kirkia acuminata*, acetone extracts *of Maytenus senegalensis* and *Millettia stuhlmannii* were the most active with average minimum inhibitory concentration (MIC) values of 0.32 to 0.33 mg/ml. Average total activity, a measure of potency, was highest for acetone and methanol extract of *Maytenus udanta* 1352 and 2022 ml/g respectively and followed by acetone extract of *Maytenus udanta* (1640 ml/g). Most of the selected plants have shown great potential as antibacterial agents.

Aluminium-backed TLC plates were used for extraction of chemical constituents. Three mobile solvents were used to develop TLC plates. Solvents used were, ethyl acetate/methanol/water in the ratio of (40:5.4:5) respectively (Kotze and Eloff, 2002). Chromatograms were developed in a closed tank and it was lined with a filter paper wetted with the eluent.

Visible bands were marked under daylight and ultraviolet light at wavelength of around 250 nm, and then sprayed with *p*-anisaldehyde or vanillin spray reagents (Stahl, 1969). The TLC plates were then heated at a temperature of 105ºC. TLCs were used to separate extracts as described earlier. After that the plates were dried in the fumehood. For the detection of antioxidant activity, chromatograms were sprayed with 2-diphenyl-2-picrylhydrazyl in

methanol (Derby and Margotteaux, 1970). Yellow spots against a purple background were observed on TLC plates sprayed with 0.2% DPPH in methanol this indicated the presence of antioxidant compounds.

According to Eloff, 1998 inorder to determine the minimum inhibitory concentration of extracts against S. aureus, P. aeruginosa, E. faecalis and E. coli the microplate serial dilution method was used. The total activity was determined and given in ml/g .It was obtained by dividing the minimum inhibitory concentration value with the extracted quantity from 1 g of plant material (Eloff, 2004).

A research on separation and characterisation of anthraquinone derivatives from *Cassia Fistula* using chromatographic and spectral techniques was carried out by Mehta, (2012). The study involved characteristic evaluation of medicinally important ingredients such as anthraquinone derivatives from *Cassia Family*. *Cassia* family is well-known source of anthraquinone glycosides and its derivatives in the various parts of plants. A simple high performance liquid chromatography (HPLC) method was developed and validated for the determination of three anthraquinone derivatives (rhein, emodin and chrysophanic acid) in the extracts from *Cassia fistula.* The extracts were analyzed on C-18 column isocratic mobile phase in HPLC equipped with UV detector at 254 nm. The identification of each analyte was performed by use of standards. The limits of detection obtainedfor the analyte were in the range of 2.5-15 μg/mL. All three components present in the extracts of *cassia fistula* were also characterized by the 1H-NMR and mass spectroscopic analysis.

Compositional characterization of traditional medicinal plants: Chemo-metric approach was carried out by Prasad, (2010). The study was undertaken to investigate the compositions of

some common medicinal plants. The medicinal plant samples (seeds/steams/leaves) were analyzed for crude fiber, fat, moisture, protein, acid soluble and total ash content. The energy content of the samples was obtained using Atwater factors. The obtained data were evaluated using multivariate methods: Principal Component Analysis (PCA) and Hierarchical Cluster Analysis (HCA).

Deba *et al*., (2008) researched on the activities and composition of essential oils from *Bidens pilosa* Linn. Var.*Radiata*. The study described on the chemical composition, antibacterial and antifungal activities of essential oils from the plant. Caryophyllene and tau-cadinene were the main compounds in the leaves and flowers respectively. Essential oils and aqueous extracts of leaves and flowers were subjected to screening for antioxidant activities. The oils from B.pilosa exerted significant antibacterial and antifungal activities against six bacteria and three fungal strains. The results from the research demonstrated that the essential oils and aqueous extracts of B. pilosa possess antioxidant and antimicrobial activities that might be responsible for the preservation of food.

Isolation and characterization of antimicrobial compounds from the leaf extract of *Combretum zeyheri* was carried out by Mukanganyama *et al*., (2011). The leaves of *C. zeyheri* have been used as a herbal medicine and have pharmacological activity which includes anti-bacterial, anti-fungal, anticancer and antioxidant properties. The goal of the study was to isolate, identify and characterise compounds from C. zeyheri leaves which are responsible for its anti-microbial activity. The preliminary isolation of *C. zeyheri* active compounds was carried out using chromatographic techniques which includes Sephadex gel column chromatography, silica gel column chromatography and thin-layer chromatography (TLC). The study resulted in isolation of four pure compounds (A-D). It was concluded that

C.zeyheri, may serve as a potential source of lead compounds that can be developed as antifungal phytomedicines.

Joabe, *et al*., (2011) researched on Phytochemical and pharmacological notes of plants indicated to treat tumors in Brazil. The plants used in traditional medicine have been considered an important source of molecules with pharmacological activity, including antitumor. The aim of the research was to present a pharmacological description and the phytochemical components related to antitumor activity of thirty plants commonly cited in Brazil to treat tumors as well as offering an overview of approaches that are necessary for the development of herbal medicines from these resources. In the search for studies with these plants, five database were used (SciELO, Scirus, Scopus, Biological Abstracts and Web of Science), with the following keywords: tumor AND Species AND cancer. Twenty one species (70%) have at least one evaluation of a class of molecule or metabolite isolated against a pharmacological model. Most species (60%) has *in vivo* studies. It was concluded that of the thirty plant species, two stood out for having pharmacological studies *in vitro, in vivo* and clinical with positive results: *Chelidonium majus* L., Papaveraceae, and *Aloe arborescens* Mill, Xanthorrhoeaceae. Although there was generally a good activity of species presented, there is a need for further studies in order to evaluate the possibility of developing some by-product.

A research was carried out by Abebe Habtamu *et al*., (2014) on Phytochemical Investigation on the Roots of *Solanum Incanum*, Hadiya Zone, Ethiopia. Solanum incanum (Solanaceae) is bushy herb up to 3 m tall, native to Northern and north-eastern Africa including Ethiopia. It is a well-known medicinal plant. Throughout tropical Africa a sore throat, angina, stomach-ache, colic, headache, painful menstruation, liver pain and pain caused by onchocerciasis,

pleurisy, pneumonia and rheumatism are treated with Solanum incanum. This research project is aimed at isolating, Phytochemical screening and characterizing the chemical constituents of Solanum incanum, Hadiya Zone, Ethiopia. The root of Solanum Incanum (650 g) was macerated and extracted with 1.5 L 80% methanol at room temperature for 48 hours with occasional shaking. This process was repeated twice at room temperature, filtered and concentrated using Rota vapor to give yellowish extracts and partitioned was done with n-hexane, ethylacetate and butanol. Then the resulting extract was filtered using filter paper (What man No 1.5, what man Ltd., England). The methanol extract was evaporated to dryness in vacuum using Rota-vapour at 40^{o}C to yield 17 g of crude extract. Phytochemical screening revealed the presence of alkaloids, polyphenols, flavonoids, glycosides, phytosterols, saponins, triterpens, tannins and steroids as a major class of compounds. Phytochemical investigation on the methanol extract of the roots of this plant resulted in the isolation of one steroid derivative SIE2. The structure of compound SIE2 was determined by means of spectroscopic methods (IR, 1H, 13C and 2D NMR, UV and DEPT).

Powdered root of *Solanum incanum* was macerated in 1.5 L 80% methanol at room temperature for 48 hours with occasional shaking. This process was repeated twice. Then the resulting extract was filtered using filter paper (What man No 1.5, what man Ltd., England). The methanol extract was evaporated to dryness in vacuum using Rota-vapour at 40^{o}C to yield 17 g of crude extract. The obtained crude extract was dissolved in distilled water and defatted with petroleum ether to remove fats, then successively partitioned with hexane, ethyl acetate and butanol in this order by using separator funnel (100 ml). Then the TLC chromatograms for both ethyl acetate and butanol fractions were developed in chloroform/ethyl acetate/ethanol (40: 40:20) and the plate was visualized by UV light (254 and 365 nm). Then the profile was checked for both ethyl acetate and butanol.

The ground *Solanum incanum* root was extracted with 80% MeOH at room temperature, filtered and concentrated using Rota vapor to give yellowish extracts and partitioned with n-hexane, ethylacetate and butanol. TLC of the EtOAC soluble part using Hexane/EtOAC/EtOH (1:1:1) solvent system revealed presence of four spots when visualized by UV light (254 and 365 nm). While TLC of n-butanol soluble part using $CHCl_3$/EtOAC/MeOH (30:50:20) revealed presence of five spots when visualized by UV light (254 and 365 nm). The spot with the highest R_f value was labelled as SIE1 and SIB1 the lowest as SIE4 and SIB5 respectively. The EtOAC soluble part was subjected to column chromatography and fractions were monitored by TLC. Those fractions that had similar retention factor were combined and purified by precipitation. In this work it was possible to isolate from the EtOAC soluble part of methanol extract two compounds exhibiting R_f value of 0.36 SIE2 and R_f value 0.75 SIE3 by using chloroform:ethanol:buthanol (4.0:1.1:4.9). Characterization of this compound was based on IR, UV, 1H, 13C and 2D NMR spectra.

The investigation was conducted because of the importance of compounds in the genus Solanum, especially the steroids which are of great interest for their cytotoxic properties. Since many steroids, steroidal alkaloids and steroidal saponins are also antimicrobial agents, it is possible that they also exert their action by altering the microbial composition. One compound (SIE2) were isolated and characterized from the root extract of *Solanum incanum*. Moreover the structural elucidation of this compound was done by using spectroscopic methods NMR, IR and UV-vis.

Interest in traditional plants has grown throughout the world because of the high demand for chemical diversity in screening programmes. Sasidharan *et al*, (2014) focused on the analytical methods such as extraction, isolation and characterization of phytochemicals in

herbal preparations. Extraction is the most crucial step in the analysis of constituents present in herbal preparations.

CHAPTER 3: EXPERIMENTAL

3.1 METHODOLOGY

Kirkia acuminata barks were collected from the plant at the University of Zimbabwe close to the Agriculture department. The plant barks were taken to the First Year Laboratory in the Chemistry Department. All the chemicals and materials used were taken to the First year Laboratory Chemistry Department. The plant was selected based on its uses by traditional healers and the society in Zimbabwe as a pain reliever especially to act against vomiting and abdominal/stomach pains.

3.2 Chemicals and reagents used are:

Methanol (HPLC grade), hexane (HPLC grade), dichloromethane (HPLC grade), acetonitrile (HPLC), de-ionised water

Medicinal plant : *Kirkia acuminate* barks

3.3 PROCEDURE

3.3.1 Sample Preparation

The *Kirkia acuminata* barks were air dried in the laboratory over a period of two weeks. The barks were then ground into very fine powder with a pulveriser at the Institute of Mining Research University of Zimbabwe.

Fig 3.1: Powdered barks

Extraction

3.3.2 Soxhlet Extraction

A mass of 50g samples were placed into three separate thimbles and each of the samples were separated separately. The first sample was extracted with 150ml of hexane, the second with 150ml of dichloromethane and the third was extracted with 150ml of methanol.The extractions were aalowed to run for 6 hours. After extraction the samples were evaporated on the rotary evaporator. The samples were then taken for analysis on the Gas Chromaography-Mass Spectrometer.

Fig 3.2: soxhlet extraction

3.3.3 Steam Distillation

A mass of 200g sample was weighed and placed in a 1000ml round bottomed flask and 500 ml of water was added. This was steam distilled for 8 hours. The distillate was extracted with hexane, followed by dichloromethane by liquid-liquid solvent extraction using three 200ml portions in each extraction. The hexane fraction and dichloromethane were evaporated on the rotary to separate the oil from the solvents. The sample oil obtained was analysed by Gas Chromatography-Mass Spectrometer.

Fig 3.3: steam distillation

3.3.4 Phytochemical screening of secondary metabolites

Table 1 shows a summary of the experimental procedures for the various phytochemical screening methods for the screening metabolites. After obtaining the crude extract or active fraction from the plant material by soxhlet extraction and steam ditillation, phytochemical screening was performed with the appropiate tests as shown in the Table 3.1.

Table 3.1: Phytochemical Screening of secondary metabolites procedures

Secondary metabolite	**Name of test**	**Procedure**	**Positive identification of test**
Alkaloid	Wagner test	To 2 ml extract add 1 % HCl. Then add 6 drops of wagners reagent,	Brownish-red precipitate
Anthraquinone	Borntrager's test	Heat about 50mg of extract with 1ml 10% ferric chloride solution and 1ml of conc HCl. Cool the extract and filter. Shake the filtrate with equal amount of diethyl ether. Further extract the ether extract with strong ammonia.	Pink or deep red Coloration of aqueous Layer
	Borntrager's test	Add 1 ml of dilute (10 %) ammonia to 2 ml of chloroform extract.	A pink-red color in the Ammoniacal layer
Cardiac glycosides	Kellar Kiliani Test	Add 2ml filtrate with 1ml of glacial acetic acid, 1ml ferric chloride and 1ml concentrated sulphuric acid	Green-blue coloration of Solution
Flavanoid	NaOH test	Treat the extract with dilute NaOH, followed by addition of dilute HCl	A yellow solution with NaOH, turns colourless with dilute HCl
Phlobatannin	Test	2 ml extract was boiled with 2 ml of 1% hydrochloric	Formation of red

		acid HCl.	Precipitates
Phenol	Ferric chloride test	Add 3-4 drops of ferric chloride into 2 ml of extract	Blue black colour
Reducing sugar	Fehling test	Add dilute H_2SO_4 to water extract in a boiling tube and then boil for fifteen minutes. Cool and then neutralize with 10% sodium hydroxide to pH 7 and then add 5ml of Fehling solution.	Brick red precipitate
Saponin	Frothing test / Foam test	To 5ml of distilled water add 0,5 ml of the filtrate and shake well	Persistence of frothing
Steroid	Liebermann-Burchardt test	1ml of methanolic extract is added to 1ml of chloroform followed by 2-3ml of acetic anhydride and 2 drops of concentrated sulphuric acid.	Dark green coloration
Tannin	Braemer's test	10% alcoholic ferric chloride is added to 2-3ml of extract (1:1)	Dark blue or greenish grey colour
Terpenoid	Salkowski test	To 5 ml extract add 2 ml of chloroform and 3 ml of concentrated sulphuric acid H2SO4.	Reddish brown color of Interface
Volatile oil	-	To the extract add dilute NaOH and small quantity of dilute HCl then shake the solution.	Formation of white Precipitates
Proteins and amino acids	Xanthaproteic test	2ml of extract treated with few drops of conc nitric acid	Formation of yellow colour

Preparation of reagents

Wagner Reagent

- Dissolve 2g of iodine add 6g of KI in 100ml of distilled water

Fehling Solution: mixture of Fehling 1 and 2

- Fehling solution 1: Dissolve 63g of copper sulphate crystals in distilled water and make up to the mark.
- Dissolve 352g pf potassium sodium tartrate (Rochelle salt) and 154g of NaOH in water and make the solution up to 1 litre.

Chromatographic determination of phytochemicals in extracts

3.3.5 Thin Layer Chromatography

The four extracts were run on a Thin Layer Chromatography plate. A small spot from each extract was applied to a different plate, about 1,5 centimeters from the bottom edge. The solvent was allowed to completely evaporate off to prevent it from interfering with sample's interactions with the mobile phase in the next step. This step was repeated to ensure there is enough analyte at the starting spot on the plate to obtain a visible result. Different volume ratios of the solvents to be used were used in order to get the best separation. The best separation was obtained using hexance/dichloromethane/methanol (10:25:35) The solvent was poured into a glass beaker and the TLC plate was then placed in the chamber in such a way that the spot of the sample do not touch the surface of the eluent in the chamber and the lid was closed. The solvent moved up the plate by cappillary action, met the sample and carried it up the plate (eluted the sample). The plate was removed from the chamber before the solvent front reached the top of the stationary phase and dried. After drying the TLC plateswere visualised using ultraviolet light at a wavelength of 254 to 365nm.

Thin Layer Chromatography Conditions:

- Stationary phase : silica gel
- Chromatographic mechanism : adsorption
- Mobile phase : hexane/ ethylacetate/methanol
- Spotting : pipette drop
- Visualisation technique : UV light

3.3.6 Gas Chromatography-Mass Spectrometer

Extracts from the soxhlet extraction and essential oils from the steam distillation were analysed by Gas Chromatography- Mass Spectrometer. The sample extracts were injected into the instrument. The gas chromatography utilised a cappillary column which depended on the column's dimensions as well ass the phase properties. The molecules were retained by the column and then eluted from the column at different times called the retention time. After that the mass spectrometer captured,ionized, accelerated,deflected and detected the ionised molecules separately. The mass spectrometer does that by breaking each molecule into ionised fragments and detecting these fragments using their mass to charge ratios. The NIST library in the system was then used to give information on the name of the compounds analysed and also giving their retention times and quantities

Gas Chromatography- Mass Spectrometer Conditions:

- Instrument : Agilent 7890A coupled to a Agilent 5975c VL MSD
- Detector : triple axis mass spectrometer
- Mobile phase : helium
- Injection Volume : 1µL
- Pressure : 24,175kPa

- Flow : 0,68099mL/min
- Average Velocity : 30cm/sec
- Holdup Time : 1,6667 minutes
- Run Time : 28,5 minutes
- Date : 13/05/2016

Fig 3.4: GC-MS

CHAPTER 4 : RESULTS

Table 4.1: Phytochemical screening assay

NB: Positive result indicated by (+), Negative result indicated by (-)

Secondary metabolite	**Name of test**	**Methanol Extract**	**Dichloro methane extract**	**Hexane extract**	**Essential Oils**
Alkaloid	Wagner test	+	+	-	+
Anthraquinone	Borntrager's test	+	+	-	+
Cardiac glycosides	Kellar Kiliani Test	-	-	-	-
Flavanoid	NaOH test	+	-	-	+
Phlobatannin	Test	-	-	-	-
Phenol	Ferric chloride test	+	+	+	+
Reducing sugar	Fehling test	+	+	-	-
Saponin	Frothing test / Foam test	+	+	-	-
Steroid	Liebermann-Burchardt test	+	+	-	+
Tannin	Braemer's test	+	+	-	+
Terpenoid	Salkowski test	+	+	-	+

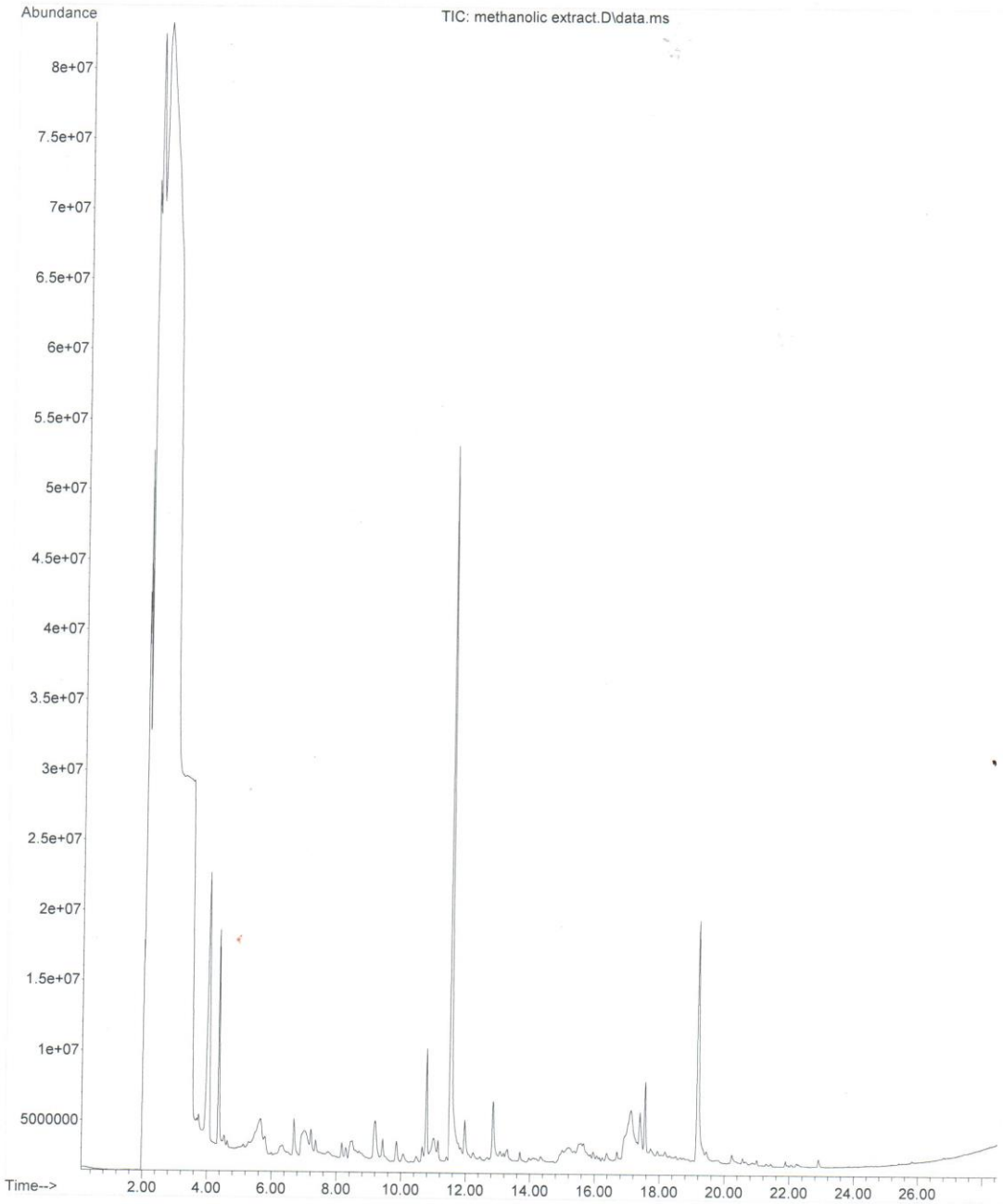
Volatile oil	-	-	-	-	-
Proteins and amino acids	Xanthaproteic test	-	-	-	-

TABLE 4.2: THIN LAYER CHROMATOGRAPHY

	Alkaloid (orange)	Tannins (brownish grey)	Phenol (green)	Flavonoids (orange)
Methanol	+	+	+	+
Hexane	-	-	+	-
Dichloromethane	+	+	-	-
Essential Oils	+	+	+	+

4.2 Gas Chromatography-Mass Spectrometer Results

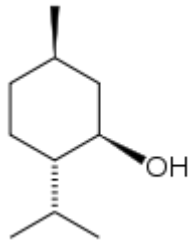
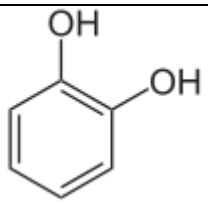
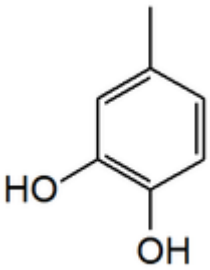
File :D:\DATA\KS 2016-05-13\methanolic extract.D
Operator : hbg
Acquired : 13 May 2016 14:24 using AcqMethod PLANT EXTRACT.M
Instrument : SIRDC
Sample Name: methanolic extract
Misc Info :
Vial Number: 114

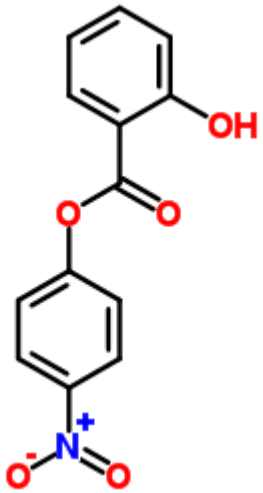
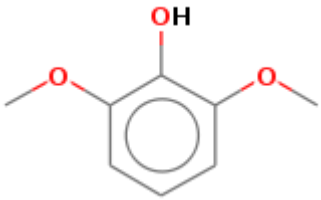
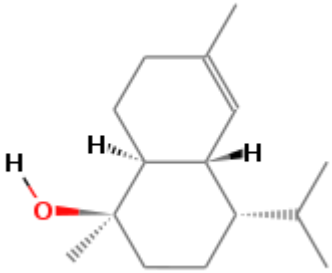


NB: Quantity of compound in sample = (volume of solvent used/mass of sample)*% quantity

%age of compound in sample = (quantity of compound in g/mass of sample) * 100

Table 4.3: Methanolic extracts

Compound name	**Chemical structure**	**Retention Time/Minutes**	**Quantity of compound in 50g sample**	**%age of compound in sample**
Menthol		11,141	2,85	5,70
Catechol		11,524	2,88	5,76
1,2benzenediol-4 methyl		12,852	2,94	5,88

Nitro phenyl salicylate		13,306	0,27	0,54
Phenol dimethoxy		13,677	2,4	4,8
tau-cadinol		17,398	2,97	5,94

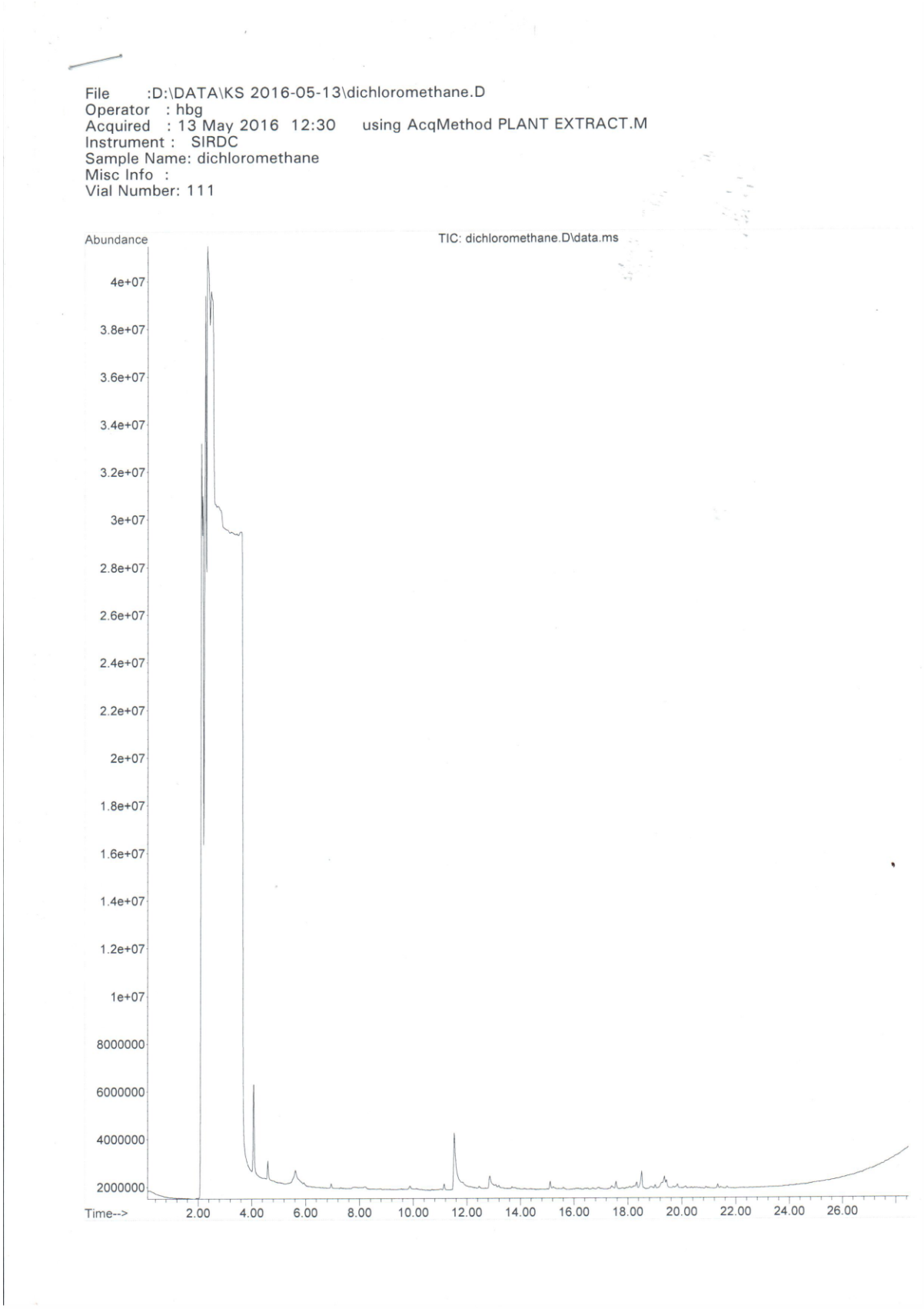
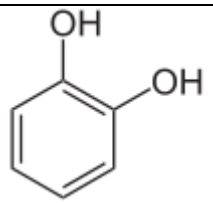
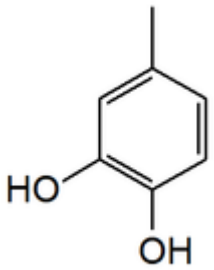
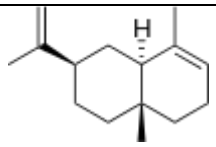


Fig 4.2: Dichloromethane extract Chromatogram

Table 4.4: Dichloromethane extracts

Compound Name	**Chemical Structure**	**Retention Time/minutes**	**Quantity of the compound in 50g sample**	**%age of compound in sample**
Catechol		11,524	2,88	5,76
1,2 benzenediol 4-methyl		12,852	2,88	5,76
Isopropenyl,8 dimethyl		19,348	2,70	5,40

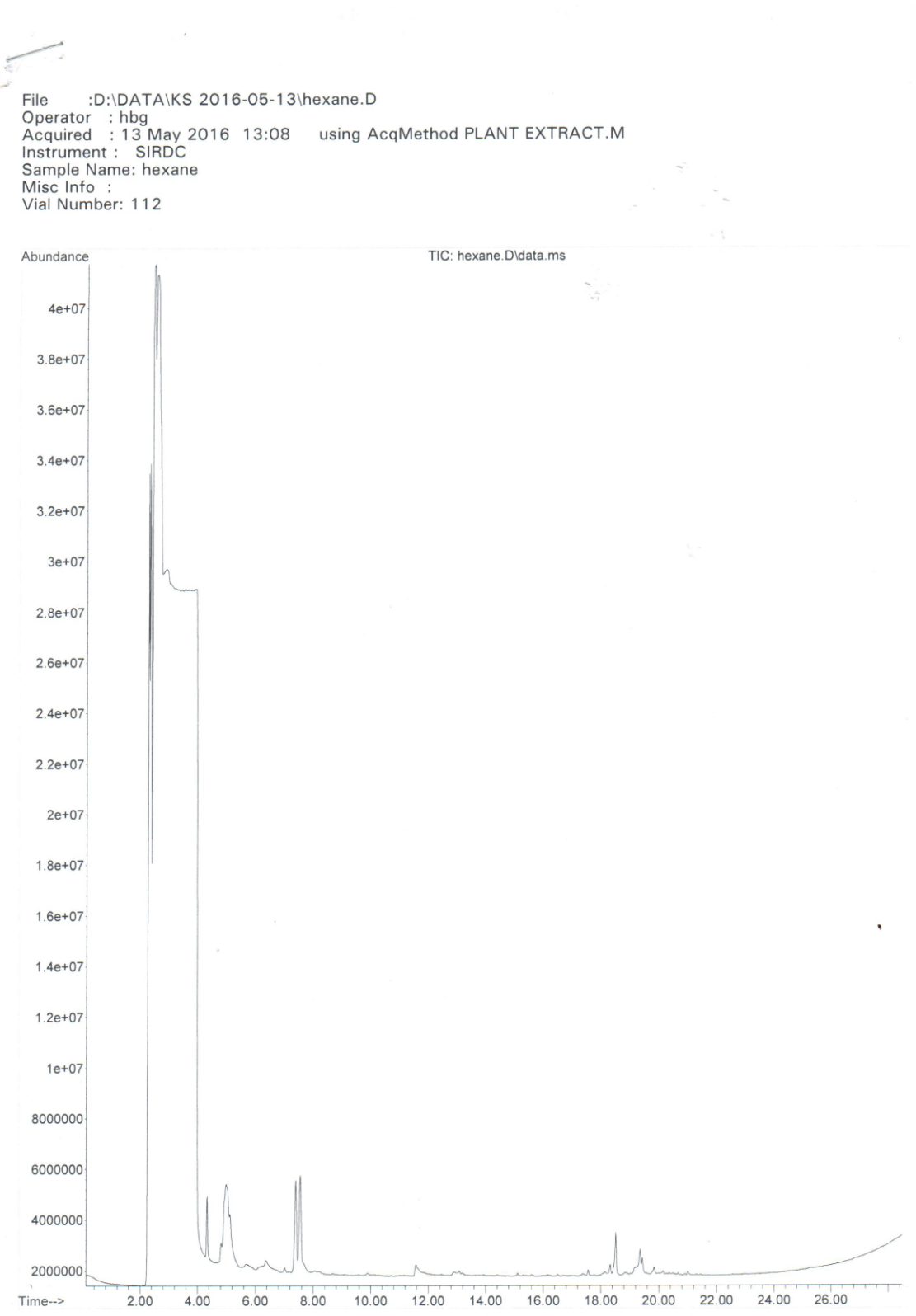
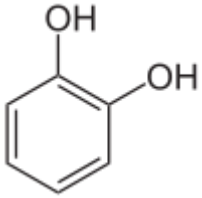
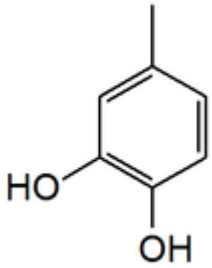


Fig 4.3: Hexane Extract

Table 4.5: Hexane extracts

Compound Name	Chemical Structure	Retention Time/minutes	Quantity of compound in 50g sample	%age of compound in sample
Catechol		11,572	2,79	5,58
1,2benzenediol-4 methyl		12,900	2,61	5,22

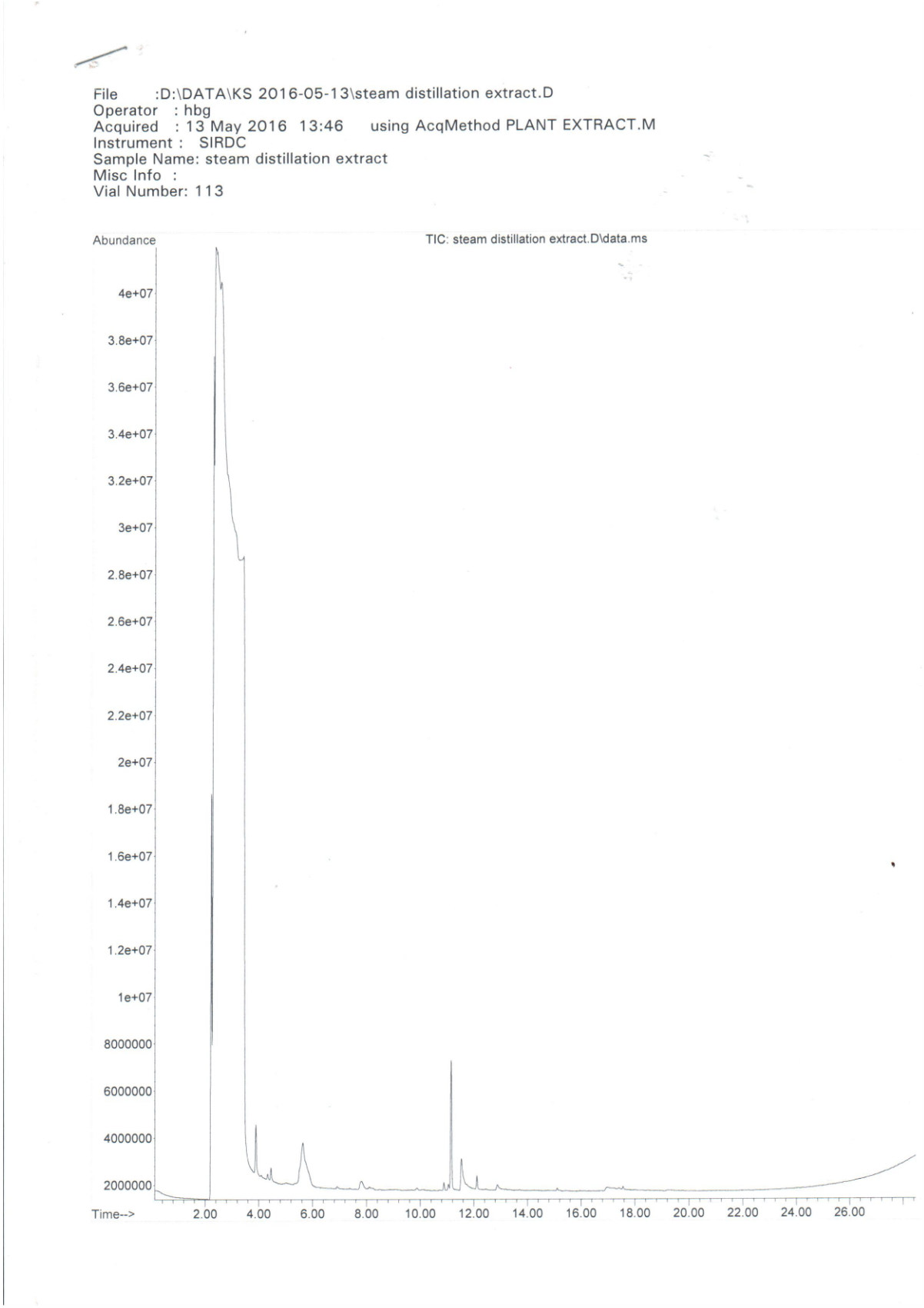
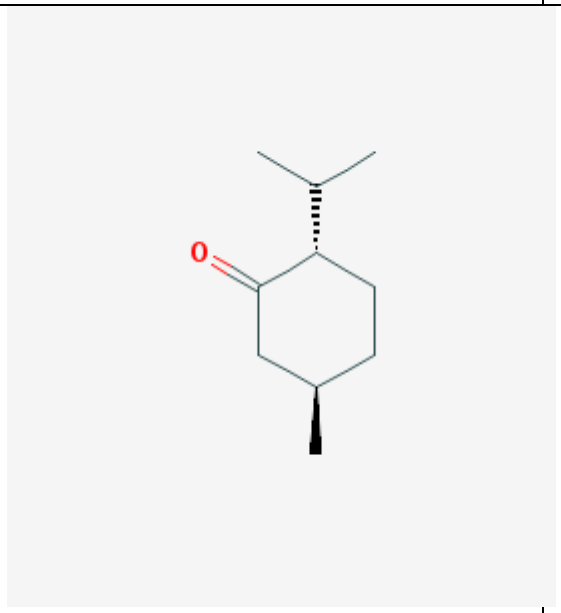
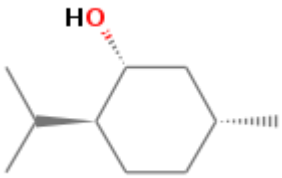
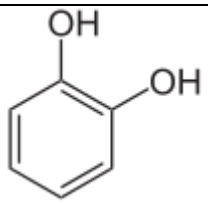
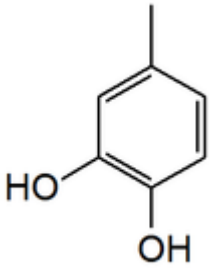
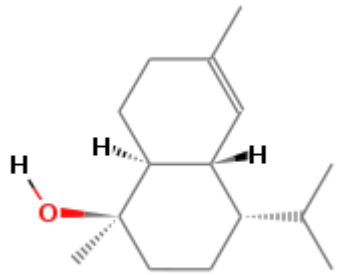


Fig 4.4 : Steam Distillation (Essential oils) Chromatogram

Table 4.6: Steam Distillation Extracts (Essential oils)

Compound Name	Chemical Structure	Retention Time/minutes	Quantity of compound in 200g sample	% age of compound in sample
menthone		10,878	2,45	1,225
levomenthol		11,141	2,25	1,125
Catechol		11,548	2,25	1,125
1,2 benzenediol ,4 methyl		12,864	2,40	1,2

tau- cadinol		17,565	2,375	1,1875

CHAPTER 5: DISCUSSION

During the study *Kirkia acuminata* barks were extracted using different solvents of different polarities (methanol,dichloromethane and hexane), essential oils were also extracted by steam distillation. Methanol was acting as the polar solvent, dichloromethane medium polar and hexane as the non polar solvent.The extracts were subjected to three different tests; phytochemical screening by qualitative tests, Thin Layer Chromatography analysis and instrumental analysis wherby Gas Chromatography-Mass Spectrometer was used.

Results from **Table 4.1 for phytochemical screening** showed that non polar solvent (hexane) has a low compatibility with most of the phytochemicals. This was shown by the negative results obtained from the qualitative tests, only phenols were found to be present out of all the phytochemicals that were tested for in hexane extract. This also showed that phytochemicals found in *Kirkia acuminata* are polar compounds.Polar solvent (methanol) and medium polar (dichloromethane) however dissolved most of the phytochemicals in the plant barks.This was indicated by the positive results from the analysis performed by the qualitative tests. Essential oils extracted by steam distillation showed the prescence of most phytochemicals that were tested for.Results showed that phenol was present in all the four extracts. Glycosides were absent in all the extracts. Saponnins and reducing sugars were only found in the methanol and dichloromethane extracts. Flavanoids were present in methanol extract and essential oils only. Alkaloids, anthraquinones, streoids, tannins and terpenoids were found to be present in other extracts except for the hexane extract. The aqueous extract which is typically used by the traditional healers for treatment of various ailments showed prescence of practically 70% of the tested phytochemicals. The results from qualitative tests gave information on different classes of phytochemicals that are present in the plant barks. These classes account for different activities : analgesic, antipyretic, antimicrobial,

antispasmodic to name a few. *Kirkia acuminata* thus has a broad spectrum of activities due to the presence of all these phytochemicals.

Results from **Table 4.2 for Thin Layer Chromatography** analysis only the coloured compounds were observed. The coloured compounds observed under UV light were then compared with the colours from literature of phytochemicals. Four classes of phytochemicals were observed on the TLC plates for methanolic extracts and esssential oils from steam distillation. These phytochemicals observed were alkaloids (orange), tannins (brownish grey), phenols (green) and flavonoids (orange). The coloured compounds observed using TLC also gave positive results when tested for by qualitative analysis. When TLC plate for the hexane extract was visualised a green spot was observed showing the presence of phenols. This proved to be correct since phenols also gave a positive result when tested for by qualitative analysis. Two spots coloured orange and brownish grey were observed when the TLC plate for the dichloromethane plate was visualised under UV light.

The present study indicated that for the qualitative and Thin Layer Chromatography analysis methanol and steam distillation have the highest compatibility with most of the phytochemicals , followed by dichloromethane and hexane having a low compatibilty.

Results from **Table 4.3 to 4.6** showed that **Gas Chromatography-Mass Spectrometer** identified a lot of compounds in each of the extracts but the compounds that were being investigated in this study are the ones with analgesic effects since the plant barks are mainly now for treating stomach pains. The information obtained by the GC/MS was solely dependent on the NIST library in the system.The compounds with analgesic effect that were identified from the methanolic extract include menthol, catechol, 1, 2 benzenediol 4 methyl, nitro phenyl salicylate, phenol dimethoxy and tau- cadinol. Catechol, 1, 2 benzenediol 4 methyl and Isopropenyl, 8 dimethyl were identified from dichloromethane extract.

Compounds identified from the hexane extract were catechol and 1, 2 benzenediol, 4 methyl. Menthone, levomenthol, 1, 2 benzenediol, 4 methyl catechol and tau- cadinol were identified in essential oils. These compounds were identified at different retention times. These compounds account for analgesic activity of the plant *Kirkia acuminata.* The results from the study indicated that catechol was identified in all the extracts. Therefore catechol is the major component in the plant barks of *Kirkia acuminata*. Catechol is known as a compound that is formed from the hydroxylation of phenols and also it is a component of castoreum. Castoreum is used as an analgesic, anti-inflammatory and antipyretic (Philipson, 2002). This shows that catechol belongs to the class of phenols. 1, 2 benzenediol, 4 methyl was also identified in all the extracts. It is also known as 4 methyl catechol. So it's a methylated catechol therefore it also belongs to the class of phenols. Menthone, menthol and levomenthol were identified from methanol and essential oils, these compounds belong to the class of terpenes (mono terpenes). They are known as topical analgesics, used to relieve minor aches and pains (Sarker and Nahar, 2007). Isopropenyl, 8 dimethyl was identified from the dichloromethane extract belong to the terpenoids it's a sesquiterpenes. Cadinol was identified from the methanol extract and essential oils it is a sesquiterpernoid alcohol. It is mainly found in essential oils and extracts of many plants (Chen *et al*., 2006). Nitro phenyl salicylate is also known as salol and it can be formed by heating salicylic acid with phenol. It has been used as an antiseptic based on the antibacterial activity upon hydrolysis in the small intestines and it acts as a mild analgesic (Sen, 1995). From its synthesis this shows that salol has got phenolic properties as well as salicylates properties. Therefore its presence in the barks has got some activities in analgesic effects.

The results indicated that some of the classes obtained from the phytochemical screening and Thin Layer Chromatography have other activities other than analgesic effect since the

compounds identified that belong to those classes do not have analgesics activities. They might have other activities like repellent, larvicidal, antimicrobial to name a few. However to a larger extent the findings demonstrated that essential oils and aqueous extracts from *Kirkia acuminata* bark extracts possess the analgesic activity although they have a small percentages ranging from 0, 54 to 5, 88%.

The results obtained may vary from researcher to researcher. It is known that the active constituents of medicinal plants are affected by many factors and may vary during the course of plant growth. Proper time of collection is very important to obtain a drug of a good quality. Factors that are known to affect the presence or absence of active constituents in a plant are time of the year, time of the day and stage of maturity and age when the plant parts are collected. A plant may contain a substance in winter that is not present in summer or its amount varies markedly, same applies with the time of the day. The value and content of active ingredients of many drugs depends on the stage of maturity and age.

CHAPTER 6: CONCLUSION

The qualitative tests of the phytochemical screening indicated the presence of alkaloids, anthraquinones, glycosides, flavonoids, phenols, tannins to name a few. Alkaloids, flavonoids, phenols and tannins were also observed on Thin Layer Chromatography using UV Light. Menthol, catechol, 1,2 benzenediol 4 methyl, nitro phenyl salicylate, phenol dimethoxy,tau-cadinol, Isopropenyl,8 dimethyl, menthone and levomenthone were identified using Gas Chromatography-Mass Spectrometer.. The hexane fraction which is highly a non-polar solvent showed that very few phytochemicals were taken up in it. The polar solvents showed compatibility with the various chemical classes. The presence of these compounds gives *Kirkia acuminata* its characteristic property of being an analgesic. It thus finds application in the field of medicine.

Steam distillation technique was found to be one of the best methods for the extraction of essential oil from plants since various phytochemicals were identified in the extract. Analysis using Gas Chromatography-Mass Spectrometer was found to be the best technique to identify various components of oils and aqueous extracts along with major components.

CHAPTER 7: RECOMMENDATIONS

Results obtained need further investigations. Bioassay guided fractionation of the constituents of the plant as well as acute toxicity studies are needed inorder to conclude on the plant activity.

Researches have to be done on other parts of the *Kirkia acuminata* plant (roots and fruit sap) since they are also used as medicines.

More research must be carried out on the phytochemicals of plants that are used as herbal medicines.

CHAPTER 8: REFERENCES.

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