Phytochemical Composition, Characterization and Termiticidal Activity of *Acacia tortilis* Heartwood Extracts

By

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DECLARATION

Declaration by Candidate

This thesis is my original work and has not been submitted for a degree in any other University.

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DEDICATION

I dedicate this work to my family members who loved and supported me during my studies. I also dedicate this work to my classmates and best friends.

I also dedicate this work to everyone who helped me; morally, financially, or by counseling, for successful achievement of this work.

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ABSTRACT

Termites are commonly known as “silent destroyers” as they have the ability to attack wood undetected. Globally, the economic loss incurred from termites’ destruction in the agricultural sector is approximately US $ 50 billion annually. In Kenya, termites cause 11-20 % crop yield losses and synthetic chemicals used in control of termites’ infestation are non-eco-friendly contributing to lots of environmental problems. This study therefore aimed at investigating phytochemical composition and anti-termiticidal properties of *Acacia tortilis* heartwood extracts. The specific objectives were to (1) determine the phytochemical composition of *A. tortilis* heartwood extracts, (2) characterize *A. tortilis* heartwood extractives using spectroscopic methods and (3) evaluate termiticidal activity of *A. tortilis* heartwood extracts. The ground heartwood samples were extracted using hexane, ethyl acetate (EtOAc), acetone and ethanol solvents by the Soxhlet method. Phytochemical screening of these extracts was done using standard methods. Characterizations of the extractives were done using Ultraviolet-Visible (UV-Vis), Gas chromatography Mass spectroscopy (GC-MS), Liquid Chromatography-Photodiode-array-Mass spectroscopy (LC-PDA-MS) and Fourier transform Infrared (FTIR). To determine termiticidal activity, wood blocks of *A. tortilis* and *Pinus sylvestris* measuring 10 cm × 1.5 cm × 0.5 cm (longitudinal × radial × tangential) were prepared. Extracted, unextracted wood blocks of *A. tortilis* (negative control) and *Pinus sylvestris* (positive control) was used to provide evidence if the experimental setup was working. Wood blocks were randomly exposed to *Macrotermes natalensis* for 5 months at Cheptebo in Kerio valley, Kenya. Phytochemical screening showed presence of phenols, tannins, flavonoids, cardiac glycosides, terpenoids and alkaloids. UV-Vis spectra showed presence of chromophores due to peaks at 338, 338, 339 and 341 nm. Total phenolic content (TPC) was of the order $10.7 \pm 0.10 < 157.6 \pm 0.06 < 169.3 \pm 0.15 < 350.1 \pm 0.10$ mg GAE/g for hexane, EtOAc, acetonic and ethanolic extracts. Total flavonoid content (TFC) was of same order of solvent polarity as mentioned in TPC; $0.7 \pm 0.06 < 14.7 \pm 0.15 < 19.2 \pm 0.15 < 21.1 \pm 0.17$ mg QE/g respectively. GC-MS with NIST library revealed presences of catechin, epicatechin and pinitol in acetonic, EtOAc and ethanolic extracts. Pinitol was the prevalent compound exhibited in ethanolic (84%), acetonic (59%) and EtOAc (28%). Fatty acids and terpenoids were also identified in EtOAc and hexane extract. LC-PDA-MS, further confirmed the presence of catechin with charge/mass ratio (*m/z*) of 289 with maximum wavelength of 278 nm. Afzelechin was observed with *m/z* of 273. Two compounds with *m/z* of 817 and 801 were identified as hydrolysable tannins. FTIR analyses showed peaks between 3254 - 3272 cm^{-1} (OH stretch), 1701 cm^{-1} (C=O stretch) and 1604, 1513 and 1445 cm^{-1} due to vibration of C=C of aromatic skeleton. The weight loss of unextracted *A. tortilis* heartwood blocks was 0.4 % and *P. sylvestris* (100%) after 5 months of exposure. Weight losses of extracted *A. tortilis* blocks were dependent on the extractive; hexane (1.9%), EtOAc (3.7%), acetone (3.9 %) and ethanol (4.8 %). In conclusion, *A. tortilis* contains useful secondary metabolites and exhibited anti-termiticidal activity which the study attributes to the identified compounds. The study recommends that isolation of identified compounds from *A. tortilis* and testing the termiticidal activity of isolated compounds to be done.

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ACRONYMS

AWPA	- American Wood Protection Association
ASTM	- America Society for Testing and Materials
BSTFA--N, O	- bis (Trimethylsilyl) trifluoroacetamide
FTIR	- Fourier Transform Infrared Spectroscopy
GC-MS	- Gas Chromatography Mass spectroscopy
LC-PDA-MS	- Liquid Chromatography Photodiode Array Mass spectroscopy
NIST	- National Institute of Standard and Technology
PCBS	- Polychlorinated biphenyl
POPs	- Persistence Organic Pollutants
TMCS	- Trimethylchlorosilane
UNEP	- United Nations Environment Programme
WHO	- World Health Organization

CHAPTER ONE

INTRODUCTION

1.1 Background Information

1.1.1 Termites and their economic importance

Termites are insects which commonly possess a stable and very organized colony in warm environment, they are known to be communal insects (Daniel & Emana, 2014). They belong to different families namely-: Hodotermitidae (*Anacanthotermes* and *Hodotermes*), Kalotermitidae (*Neotermes*), Rhinotermitidae (*Coptotermes*, *Heterotermes*, and *Psammotermes*), and Termitidae such as *Amitermes, Cornitermes, Macrotermes, Microcerotermes, Microtermes, Odontotermes, Procornitermes*, and *Syntermes* (Rana *et al*., 2021).They are highly distributed in tropical and subtropical parts of the world (Ghaly, 2011). All over the world, termites are known as soil engineers since they influence decomposition of organic matter and nutrient cycling (Materu *et al*., 2013). However, they still pose a serious menace to crops, buildings, wood and finished products such as books and furniture. Over 300 million dollars in a year is used to repair damages caused by termites in New Orleans, U.S.A (Verma *et al*., 2009). In India, they are attributed for the loss of 15-20% of the maize crop and about 1478 million rupees are lost every year (Pandey *et al*., 2012). While in Kenya, Uganda and Tanzania they are reported to be responsible for maize yield losses of 10-30% and above 50% maize yield losses in Ethiopia and Nigeria (Otieno, 2018).

1.1.2 Wood and its preservatives against bio degraders

Wood is linked to economic development of man and it is regularly used for construction purpose worldwide for both in-door and out-door services (Olufemi *et al*., 2011). However, their service life and durability is reduced by infestation of bio-degraders like termites, bacteria and fungi (Mubeen *et al*., 2019). Therefore, different

methods are adopted to modify wood so as to increase their service life. Wood modification is the process of application of chemical, physical, or biological methods to alter the properties of the wood. Woods are normally modified with intention of improving performance of wood, resulting in improvement in dimensional stability, increase decay resistance to pathogens, and weathering resistance (Lahtela *et al*., 2013). Chemical method uses synthetic pesticides to increase the service life of wood by either decreasing or repelling biological agents such as termites from attacking the wood (Hassan *et al*., 2016). The most used synthetic pesticides for controlling termites include synthetic inorganic and organic chemical agents such as creosote, chromated copper arsenate, tri-butyl-tin oxide and pentachlorophenol (Hassan *et al*., 2016).

1.1.3 Environmental effects of chemical preservatives

Synthetic pesticides are very effective for controlling termites because they are chemically intended to target and interrupt specific biological functions of termites resulting to dead of termites. However on the other hand, they are non-biodegradable, toxic to non-target organisms and their continuous use causes termites resistance and resurgence (Ekhuemelo *et al.*, 2017;; Tascioglu *et al.*, 2012). Due to all these problems there is a great need for research and development for alternative methods that are environmentally friendly (Watanabe *et al*., 2005). Such eco-friendly methods includes use of microbes like fungi, nematodes, bacteria and viruses as biocontrol agents and use of plant derived compounds (biopesticides) (Mishra *et al*., 2017; Mubeen et al., 2019). Utilization of biopesticides as alternative approach of controlling termites has gained attention in the field of wood preservation and it has been accepted worldwide (Sudrajat *et al*., 2018; Tascioglu *et al*., 2012). Biopesticides are less harmful hence potentially better than the conventional chemical method of controlling termites infestation (Alshehry *et al*., 2014; Kadir *et al*., 2014).

1.1.4 Wood extractives as potential termiticidal

Wood, which is part of the plant, is predominantly made up of cellulose, hemicelluloses, lignin and extraneous chemicals known as extractives (Lahtela *et al*., 2013). Some of these extractives play pivotal role in natural durability and protection of wood against biological agents like wood rot fungi, termites and bacteria (Mburu *et al*., 2007; Scheffer & Morrell, 1998). Whereas Babar *et al*. (2016) reported that wood extractives exhibit toxic and repellant activity against many species of termites. However, these extractive content can vary from family of trees and also within the parts of tree (Kadir *et al*., 2014).

1.1.5 Seeking alternatives to chemical control of termites

With increase in the aim of replacing the conventional synthetic pesticides with biopesticides, plants which are reported to be resilient to termites' attack have been studied (Sudrajat *et al*., 2018). This is because woods that are resistant to termites attack are reported to contain compounds like flavonoids and terpenoids that are naturally either repellent or has toxic activity towards termites (Mishra *et al*., 2017). Hence, evaluating the extractive content of the heartwood in the tree species that are resistant to termites is vital (Kadir *et al*., 2014).

Approximately 1,200 Acacia species have been identified and they are mainly found in the warmer and drier parts of the world mostly in Arabia, Australia and Africa (Yadav & Kant, 2013). In Kenya *Acacia tortilis* is found mostly in the Eastern and Northern parts of the country (Otieno *et al*., 2005). This tree is a rich source of chemical constituents and in accordance to a review paper by Verma (2016), it is reported that *A. tortilis* contain oleic acid, linoleic acid, hydrolysable tannins, vicein, rutin, uronic acid, galactose, arabinose, rhamnose, mannose and protein content. *A. tortilis* has been reported to possess anthelmintic, anti-asthmatic, antifungal properties and is used for

treatment of pulmonary diseases (Ogunwande et al., 2008). Apart from therapeutic properties of *A. tortilis* its wood, has been utilized as source of charcoal, firewood and timber is for construction of furniture and wagon wheels (M. Srivastava & Kumar, 2013). It is believed that Noah of Old Testament used wood from *A. tortilis* to build his ark (Yadav & Kant, 2013). In a case study in Ethiopia *Acacia tortilis* was among the trees that were claimed to be resistant to termites attack (Fenetahun & Yong-dong, 2019).

1.2 Statement of the Problem

Termites are extremely devastating pests which cause damage to plants, furniture and even buildings. This destruction, contributes to high economic losses globally since it has been recorded that destruction caused by termites' infestation all over the world is nearly 50 billion USA dollars annually. Since 1900, synthetic pesticides have been used to manage damages caused by termites. However, management of termites has been really a challenge from the beginning because most synthetic termiticides have been continuously banned from the market. This is due to their hazardous effect like -: they are harmful to humans and animals, environmental contamination and lethal effect to non-target insects.

Apart from using synthetic pesticides to control wood infestation by termites, the degraded woods or houses by termites are either replaced or repaired by using new woods, which eventually leads to deforestation. A factor that contributes to global warming later leading to climate change. Due to climatical change, human beings and wild animals face new challenges for survival such as frequent and intense drought, shifting of wildlife populations and habitats, rising seas among many other impacts. Besides infestation of woods, buildings and finished products for examples, furniture

and books termites also destroy crops like sugarcane, soybeans, groundnut, cotton and maize which lead to food insecurity across the world.

Replacement of synthetic pesticides with biopesticides has been accepted globally. Plants exhibit bioactive chemicals such as phenols, flavonoids, terpenes and tannins, which are either toxic or repellant to subterranean termites making the plant to be resistant to termites. Plants which are reported to be resilient to termite attack have been scientifically studied while others have not been scientifically studied up to date. Therefore, the study aimed at investigating the anti-termite activity of *A. tortilis* heartwood which in future, may be a source of ecofriendly biopesticide.

1.3 Justification

Increase in environmental awareness, has resulted to development of monitoring measures that purpose to safeguard environment from exploitation and pollution in future. To achieve this, many pesticides are categorized into less and the more hazardous forms and those that are more hazardous i.e persistent organic pollutants (POPs) identified by the United Nations Environment program (UNEP) have been eliminated from the market. Examples of the POPs includes; Aldrin, chlordane, dichlorodiphenyl trichloroethane (DDT), dieldrin, dioxins, endrin, furans, heptachlor, hexachlorobenzenes, mirex, polychlorinated biphenyls (PCBs), and toxaphene. Among these POPs heptachlor is used as a termicides. Therefore, there is need to develop alternative biopesticides which are biodegradable in nature. Biopesticides that are used to control termites infestation are obtained from plants that are resistant to termite attack due to their toxicity and repellency activity (Adfa *et al.*, 2020). Hence plants with these properties have gained attention in the field of wood preservation. Therefore, evaluating the extractives content of the tree species that are resistant to termites is vital (Kadir *et al*., 2014). *A. tortilis* is used traditionally by locals of Kerio valley as

fencing posts purporting that they show resistance to termites attack. However, till date there is no scientific report to validate its resistance against termites which have been documented. Hence the current study is designed to investigate phytochemical composition, characterization and anti- termite activity of *Acacia tortilis*.

1.4 Objectives of the Study

1.4.1 General objective

To investigate phytochemical composition, characterization and anti-termiticidal properties of *Acacia tortilis* extracts.

1.4.2 Specific objectives

The intended specific objectives of the study were to;

1. Determine phytochemical composition of *A. tortilis* heartwood extractives of hexane, ethyl acetate, acetone and ethanol solvents.
2. Characterize *A. tortilis* heartwood extractives.
3. Evaluate the anti-termite activity of *A. tortilis* heartwood extractives in the field.

1.5 Research Questions

1. Which are the major phytochemicals in *A. tortilis* heartwood extractives?
2. What are some compounds in *A. tortilis* that contributes to its anti-termiticidal properties?
3. Do extracted and unextracted heartwood blocks of *A. tortilis* have similar anti-termiticidal properties?

CHAPTER TWO

LITERATURE REVIEW

This chapter covers the literature adopted from the various review papers and research conducted by different scholars. Several topics covered include; existence of termites, advantages and disadvantages of termites in the ecosystem, how to control destruction caused by termites considering utilization of biopesticides, distribution of *Acacia tortilis* globally, chemical constituents reported to be found in *A. tortilis* in the previous studies, various uses and pests that infest this tree.

2.1 Termites

Termites belong to the order Isoptera in the animal kingdom. They are large and diverse group of insects, globally consisting of over 2800 species and about 185 are considered as pest (Jouquet *et al*., 2011). Termites are present all over the warmer regions of the world and plays important role in the ecosystem because they take part in nutrient cycling. However, on the other hand they are among the main dangerous insects in the ecosystems because termites are best known for their potential to cause economic losses globally (Bacci *et al*., 2015).

2.1.1 Type of termites

Globally, there are three different types of termites-: subterranean, dry wood and damp wood termites (Osipitan *et al*., 2017) (Figure 1). The division to these three groups depends on the conditions of the habitat.

2.1.1.1 Damp wood termites

Damp wood termites do require the moisture from underground to survive. In order to feed on the woods, it must be damp. This is why most damp wood termites are found in forest coastal regions. They seldom attack homes, unless there is a source of water

dripping on wood. So, in most cases, eliminating the source of water will cause the colony to desiccate and die.

2.1.1.2 Dry wood termites

Dry wood termites are mostly found in arid areas. These termites don't dry out in open air like their subterranean cousins. They also create much smaller colonies. Most are 2,500 or less. These termites burrow into dead and dry woods to build their colonies. It is difficult to detect them, because they live in the center of a piece of wood. The key is looking for the frass holes. If you find these holes, you will see the frass pellets on the ground outside of them. These termites form hexagonally shaped frass. This is an important distinction from other termites.

Dry wood termites are treated either locally or as a whole structure. Whole structure treatments are tented and then fumigated. This is the most effective way of treating these pests as it will not only eliminate the colonies found, but also any hiding ones. Local treatments use a foam to fill the insides of termite colonies.

2.1.1.3 Subterranean termites

Subterranean termites are the most common termites. These termites build colonies of millions of termites underground. They, by far, do the most damage to homes and other structures. These termite's fragile exoskeletons require high humidity air around them at all times. Without this moisture, they will dry out and die. For this reason, these termites must remain underground, where the air is more humid, at all times.

But, in order to find for food, they must often forage for food above the surface. Subterranean termites accomplish this by building mud tubes. These tubes are built using mud, sawdust and their feces. These tubes will not only carry the termites, but the humid underground air as well. They have four different types of tubes. The

foraging tubes are smaller and extend up the sides of buildings and other structures in search of food. Once food is found, they will build utility tubes. These tubes are larger and allow the workers to transport the food underground. Return tubes go back to the ground. They are often lighter in color because they are using materials from the building. And lastly there are swarming tubes. These open-ended tubes are strictly for the purpose of allowing reproductive out of the colony to swarm.

Figure 1: a: subterranean termites, b: damp wood termites and c: dry wood termites

https://www.peststrategies.com/pest-guides/termite-guides/termite-types/

2.1.2 Termites' habitat

Termites are eusocial insects which live in mound (Figure 2) with division of labor depending large colonies that is formed basing on a hierarchy or caste structure (Mahdi *et al.*, 2020)

Figure 2: Termite mound

A photo of termite mound taken at Cheptebo in Kerio Valley, Kenya

In the termite mound, the three major castes of adult termites are-: (1) worker termites, (2) soldier termites and (3) reproductive termites form colonies. Each caste has different roles within the colony. The reproductive group are responsible for expansion of colony group, while workers search for food, feed younger ones, construct and repair of the nest whereas soldiers have mandibles to provide security to the colony members (Materu *et al*., 2013).Figure 3 below shows image of worker, soldier and reproductive termite.

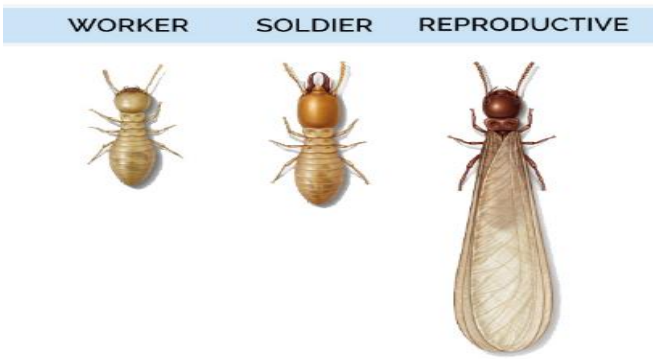


Figure 3: Three major castes of adults termites

https://www.shutterstock.com/image-vector/caste-system-termites

2.1.3 Role of termites in the ecosystem

Termites are very important insects in the ecosystem because they contribute to gas exchange, nitrogen fixation, soil stability and quality of soil (As & Kalleshwaraswamy, 2018). They are also a source of food for some organism like ants, woodpeckers, skunks, bears, chimpanzees, wasps, spiders and scorpion. Edible termites are fried by human beings and used as food while for farmers termites are source of poultry feeds (Figueirêdo *et al*., 2015). Besides the nutritional value of termites, it is also as a source of medicine for the treatment of wounds, malnutrition and heart condition, anemia and diarrhea (Govorushko, 2019).

Although termites are very useful, they are also major cause for economic losses (Bacci *et al*., 2015). They destroy wood, agricultural crops, household material and finished products like furniture, books and magazines (Bakaruddin *et al*., 2018). Wood is majorly attacked by termites because they contain lignocellulose as main structural polymer and is digested by hindgut bacteria or protozoa of termites (Babar Hassan *et al*., 2016; Mubeen *et al*., 2019).

2.1.4 Economic losses caused by termites

Termites are a threat to the world because they contribute to economic losses. Kadir (2014) approximated that destruction caused by termites activity all over the world is nearly 50 billion USA dollars annually. According to these authors, subterranean termites are known to be very destructive type of termites. In Africa Countries like Nigeria, Cameroon, Democratic Republic of Congo, Gambia, Ghana, Guinea, Ivory Coast, Mozambique, Senegal, Angola, Sierra Leone, Somalia, Sudan, Tanzania and Uganda, subterranean termites are known to be major detrimental species (Okweche *et al*., 2021).

Ibrahim & Adebote (2012) found out that 51% of traditional houses in Indonesia were destroyed by termites. Similarly, 42.7% of houses were infested by termites in Southern Brazil (Bacci *et al.*, 2015). In South America it has been reported that termites from the genus Heterotermes attacks sugarcane, soybeans, groundnut, cotton and maize while in Brazil they have been projected to cause economic loss of US$ 2.1 million (Mayra *et al*., 2015). Likewise, 15-25% yield loss of maize in India is caused by termites whereas 10-30% loss of harvested groundnuts were destroyed by termites in Nigeria, Mali, Burkina-Faso and Niger (Alamu et al.,2018). In USA the damages caused by termites alone is over 3 billion every year with the 80% contributed by subterranean termites (Ojewumi *et al*., 2017). Termites are attributed for the losses of up to trillions per year (Arif *et al*., 2019). It has been reported that 11-20% maize yield loss is caused by termites in western part of Kenya (Anyango *et al*., 2019). Therefore, different control methods have been embraced to mitigate the losses caused by termite's destruction worldwide.

2.1.5 Control of termites

Destruction caused by termites is massive and alarming; therefore, there is necessity to control these damages in order to reduce the economic loss across the world. Globally, different control methods of termites have been used such as chemical method, non-chemical method and use of termites baits (Alamu *et al*., 2018).

Non-chemical method which is also known as traditional method involves control of termites without use of chemicals. Some of these strategies are application of mulch, intercropping, ploughing and digging out mounds while chemical method involves application of formulation which contains different active ingredients that kills termites. This include use of wood ash, plant extracts and synthetic termiticides (Otieno *et al*., 2018). Lastly is the monitoring method where termites baits are used, termite bait is a

method where a structural component of wood is combined with a slow-acting termicides which disrupts the normal growth process in termite (Majid & Hafiz, 2020). The active ingredients used in termite baiting systems affect only the targeted pest species and use a relatively small amount of termicides compared to standard chemical soil treatment. Chouvenc *et al*. (2011) reported that chitin synthesis inhibitors (CSI) have been invented as termite baiting system and they are available in the markets. CSI disrupt synthesis of termite chitin, the main component of termite exoskeletons leading to disruption termite molting, causing death of termites.

Many traditional methods of controlling termites are available but use of synthetic termiticides still remain to be highly preferred method of controlling termites. This is because synthetic termites kill termites within a short period hence effective method of eliminating termites. Globally, there are two types of synthetic termicides i.e repellent and non-repellent. Repellent termicides deter the termites from approaching the treated parts of soil, constructed structures or building tunnels towards some crop farms, buildings and even fencing poles. The major types of repellent termiticides are cypermethrin, bifenthrin, fenitrothion, permethrin, fenvalerate and chlorpyrifos while non-repellent chemicals are imidacloprid, Aldrin, heptachlor, copper naphthenate, boric acid and chlordane. These non-repellent termicides are non-detectable by termites and enables the termites to enter into treated soil to an extend that they are affected and after some time they die (Kamble & Jayram, 2014). On the other hand, continuous use of these termiticides have adverse effects to human and other non-target organism beings such as death, nausea, headaches, shortness of breath and seizures, neurotoxicity, muscle weakness among many other problems (Ojewumi *et al*., 2017). While the long term effect is the bioconcentration and biomagnification of these chemicals in the ecosystem which leads to hazardous effects to both human beings and animals because

most these termicides are persistent organic pollutants (WHO, 2008). In another paper by Gupta *et al*. (2011) stated that continuous use of these synthetic chemicals leads to an increase in pesticide resistance. Hence, many restrictions are being placed on the control of termites using termicides.

Therefore, efforts by researchers are being made all over the world to substitute these synthetic chemicals with biological alternatives (biopesticides), which are ecofriendly (Aziz *et al*., 2016). Examples of some bio pesticides are the pyrethroids and azadirachtins which have been developed from the *Chrysanthemum genus* and *Azadirachta indica* respectively. All these bio-pesticides are biodegradable (Adfa *et al*., 2020). So, naturally derived plants products provides an alternative to conventional pesticides for present and near future (Alshehry *et al*., 2014). Bioactive compounds in termite resistant plants may have contact toxicity to termites or act as antifeedants, repellents, or even protozoacides (Kard et al., 2007). Many plants have been tested for their anti-termite activity.

2.1.6 Termiticidal activity in plants

2.1.6.1 Durable timber from wood species that are resistant to termite attack

Natural durable woods species with antitermitic activity, like *A. nilotica* and *A. monticola* have been used for long as source of timber because they are resistant to termites (Fagg & Stewart, 1993). Alaskan yellow cedar (*Chamaecyparis nootkatensis*), Western red cedar (*Thuja plicata*) Tung tree (*Aleurites fordii*), Red wood (*Sequoia sempervirens*), Western juniper (*Juniperus occidentalis*) Hook and Teak (*Tectona grandis*) are known to be very resilient to termites and are good replacements to commonly used commercial timbers like southern yellow pine (Pinus spp.) and cotton wood (Populus spp.) which are readily attacked by termites (Babar *et al*., 2017)**.** It has been reported that the leaves and seeds of *Jatropha curcas, Maesa laceolata,*

Chenapodium ambrosoids, Vernonia hymenolepis, Azadirachta indica and leaves of *Lanta camara*, the rhizome of *Alpinia galangal* showed potential plants species to control termites (Bakaruddin *et al*., 2018). In accordance to Garcia (2016) some plants extracts have been confirmed to possess termiticidal properties. The use of natural plant extracts is known to be alternative approach for wood preservation to increase the durability of non-durable wood species as in the case of extracts from *Acacia dealbata* which is known to protect non-durable wood species (Yildiz *et al*., 2018). Plants extracts can be potential termicides because it contains bioactive compounds such phenols, flavonoids, terpenes, tannins, amines and alkaloids which have been reported to contain anti insect and anti-feeding activity against termites (Yildiz *et al*., 2018).

2.1.6.2 Termiticidal activity of essential oils (Eos)

Investigation on insecticidal activity of essential oils (EOs) has been there since 1972. Verma *et al*. (2009) reported that evaluation of many EOs against termites has been conducted and found out that the essential oils possess toxicity, repellence and deterrence activity against termites. In addition to these three properties of EOs, they are also volatile due to their low molecular weight which makes it to be easily eliminated from the environment once they are released to the environment hence are eco-friendly (Mayra *et al*., 2015). According to study by Alamu *et al*. (2018) plant oils from neem seed oil, *Jatropha* seed oil and palm kernel oil showed a higher termites mortality when applied to soil than when it was directly applied on the sugarcane strips in plastic cages.

According to research conducted by Pandey *et al*. (2012), compounds such as eugenol, thymol and carvacrol exhibited anti termites activity when these bioactive constituents derived from seven essential oils-: *Cymbopogon citratus, Eucalyptus globulus, Syzygium aromaticum, Origanum vulgare, Rosmarinus officinalis, Cinnamomum*

verum and *Thymus vulgaris* were tested for their termiticidal activity. While when "no-choice" bioassay of mint oil, lemongrass and ajwain oils were evaluated against *Odontotermes obesus* by Gupta *et al*. (2011) they found out that mint oil gave the best results (100% mortality was observed in 30 min with 10% oil and in 10 hours with 0.12% oil) followed by the lemongrass and ajwain oils. According to the authors the toxicity of mint and ajwain oils against termites is due to presence of menthol and thymol respectively which are phenolic components.

In another investigation, the termiticidal activity of plant essential oils from *Corymbia citriodora*, *Croton sonderianus*, *Cymbopogon martini*, *Lippia alba, Lippia. gracilis, Lippia. sidoides* and *Pogostemon cablin* on termites (*Nasutitermes corniger*) was carried out. This study showed that all these plants showed toxicity against *N. corniger* however *L. sidoides* and *P. cablin* showed higher toxicity than other plants. Their higher toxicity was attributed to different chemicals compounds such as thymol, p-cymene and methyl thymol ether in *L. sidoides* and patchoulol, α-bulnesene and α-guaiene in *P. cablin* which work either additional or synergistically (Lima *et al*., 2013).

2.1.6.3 Different concentrations of plant extracts showing potential anti-termite activity

In accordance to research conducted by Mishra *et al*. (2017), it was found out that quercetin identified from the *Punica granatum* fruit rind extract at different concentrations possess antitermiticidal activity against *Microtermes beesoni.* The authors also added that partially purified flavonoid shows activity as post-harvest pesticide against legumes and grains.

Figure 4 below shows examples of phenolic compounds that have anti termite activity

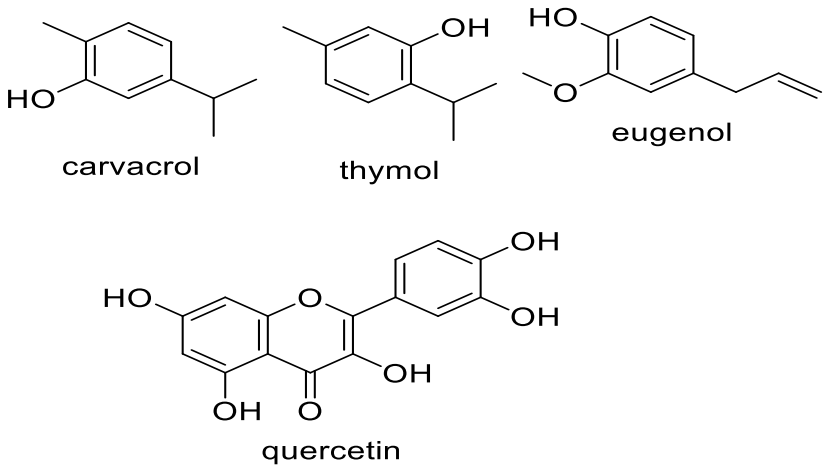


Figure 4: Structures of compounds having termiticidal activity carvacrol, thymol and eugenol from *Cymbopogon citratus*, *Eucalyptus globulus*, *Syzygium aromaticum*, *Origanum vulgare*, *Rosmarinus officinalis*, *Cinnamomum verum* and *Thymus vulgaris* and quercetin from *Punica gratanum* fruit rind.

According to the research conducted by Wititsiri (2011) when the plywood was coated with tar from the wood vinegar obtained from coconut shell showed high level of termiticidal property than the wood vinegar prepared from coconut shell and coir and wood vinegar obtained from mixture of coconut shell, coir and holy basil. From their study it was reported that high termiticidal activity of wood vinegar from coconut shell can be related to chemical composition present in the coconut shell.

Termiticidal activity of 7-methyljuglone (5-hydroxy-7-methylnaphthalene-1,4-dione) and isodiospyrin isolated from *Diospyros virginiana* L. wood extracts showed that they were toxic to *Recticulitermes flavipes* termites species (Carter *et al.*, 1978). Adedeji *et al*. (2018) reported that *Khaya ivorensis* stem bark have reasonable termiticidal property in the 5%, 10% and 15% concentrations. According to Mburu *et al*. (2007) the heartwood of *P. africana* is resistant to termites and the activity is attributed to the presence of wood extractives triterpenic acids like oleanolic and ursolic acid or even

their glycosides. The results showed that the free extractive wood was attacked by *Macrotermes natalensis* while unextracted wood was resistant to termites.

Figure 5 below show chemical structures of compounds identified to possessing anti-termiticidal properties.

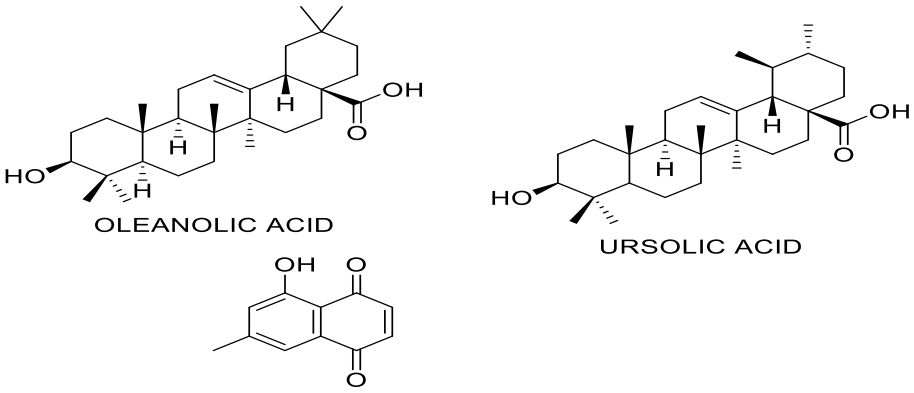


Figure 5: Compounds with anti-termite activity from *P. africana* (oleanolic acid and ursolic acid) and 5-hydroxy-7-methylnaphthalene-1,4-dione from *Diospyros virginiana* L

2.1.6.4 Antitermite activity of various chemical constituents identified in different parts of different plants

Rutin which is known for its insecticidal property was found to be dominant in Quebracho extract which showed lower mass loss of the extract treated wood block and high termite mortality when the study was conducted by Tascioglu *et al*. (2012). The research attributed rutin and Gallic acid for anti-termite activity of Quebracho extract. In another study by Bakaruddin *et al.* (2018) rutin was among the compounds that were identified in methanolic extract of *Phyllanthus niruri*. This plant showed high repellency against *Globitermes sulphureus* and *Coptotermes gestroi* termite's species. *Acacia crassicarpa* and *Acacia mearnsii* 70 % acetone extracts showed feeding deterrent against *Coptotermes gestroi* whereas both ethyl acetate and water extracts

obtained from *A. mearnsii* prevailed higher feeding deterrence than *A. crassicarpa*. The feeding deterrent was due to 5-hydroxyfisetinidol-(4α-8)-galocatechin and 5-hydroxy-robinetinidol-(4α-8)-galocatechin in *A. crassicapa* while fisetinidol-(4α-8)- catechin and Robinetinidol-(4α-8)-catechin in *A .mearnsii* (Ismayati *et al*., 2014).

Kadir *et al*. (2014) investigated the effect of wood extracts as antitermitic agents in two Malaysian timber species, namely *Madhuca utilis* and *Neobalanocarpus heimii*. Termiticidal activity of *M. utilis* was attributed for the presence of γ-tepinen, terpinen-4-ol, eicosane and p-cymene in the heartwood while farnesol and thymol methyl ether in the bark. Identified compounds in the *N. heimii* heartwood were eicosane (C20) and 2-prenyl cyclopentanone as well as Benzyl carbinol and benzyl isoamyl ether in the bark. In another study the bark extracts of *Scorodocarpus borneensis* revealed the presence of hexadecanoic and decanoid while the heartwood extract of white mulberry exhibited resorcinol. Some of the identified compounds were responsible for anti - termites activity (Hassan *et al*., 2018; Sudrajat *et al*., 2018).

The woods that are resilient to termite attack are reported to contain allelochemicals like quinines, flavonoids and terpenoids (Mishra *et al*., 2017). These components contain natural repellent and toxic activity. When anti termite activity of sapwood and heartwood are compared it is evidential that heartwood is more resistant to termite attack than sapwood. This is because sapwood is made of sugars and starches that makes them easily attacked by termites (Ghaly, 2011; Kardi *et al*., 2007; Santana *et al*., 2013). While heartwood for some plants is resistant to termite attack due to presence of chemical constituents that are synthesized during heartwood formation Extractives present in the heartwood that can be extracted either using polar or nonpolar solvents, cover a large number of different compounds such as terpenes and terpenoids and phenolic compound which are reported to possess anti-termite activity (Céspedes

et al., 2006). Extraction which is the most crucial step needs to be performed on well prepared sample from plant parts in order to obtain the afore mention extractives (Truong *et al*., 2019). In accordance to (Seidel, 2006) extraction is always conducted on the plant sample so that to separate the soluble secondary metabolites from insoluble ones. However, during extraction process there are several factors like method of extraction, sample to solvent ratio, temperature, type and concentration solvent that affects the quantity of extractable compounds from plants (Alara *et al*.,, 2020; Houda *et al*., 2020).

2.2 Acacia tortilis

2.2.1 *Acacia tortilis* distribution

The genus Acacia is widely spread worldwide (Noumi *et al*., 2012). There are almost 1,300 Acacia species that have been identified and reported to be found mainly in the warmer and drier parts of the world mostly in Arabia, Australia and Africa (Kubmarawa, 2012). The genus Acacia is an indigenous plant which is found in Kenya (Otieno *et al*., 2001). Acacia spp have been reported to exhibit different phytoconstituents which includes amines, alkaloids, cyanogenic glycosides, cyclitols, fatty acids, seed oils, fluoroacetate, gums, non-protein amino acids, terpenes, hydrolysable tannins and flavonoids (Mahmoud *et al*., 2016).

In Baringo–Kerio Valley, Kenya *Acacia tortilis* is found in the ecozone V with the following conditions; annual rainfall between 500 and 850 mm with fine textured soil (Onyango, 2016; Thom & Martin, 1983). *A. tortilis* is among the 135 African Acacia species with six intra-specific taxa with recognized four subspecies (*tortilis, spirocarpa, heteracantha,* and *raddiana) (Ogunwande et al., 2008). Acacia tortilis* is normally called ‘umbrella thorn because it forms umbrella like crown. In India, it is locally called Israeli babool (Orva) whereas it is called Samar, Sammar, Smor, Samra

or Sayyal in Arabic, haak-en-steek in Afrikaans, Kindil in Kanuri, Gabaruwa in Hausa, and Chilluki in Fulani Dhadachaa in Ethiopia (Verma, 2016; Kubmarawa, 2012; Fenetahun & Yong-dong, 2019). In Kenya Luos called it (Otiep); Kipsigis (Chebitet); Maasai (Oltepesi, Sagararam); Marakwet (Ses); Mbeere (Mugaa); Nandi (Sesya) and Swahili (Mgunga) (https://infonet-biovision.org/EnvironmentalHealth/Trees/Umbrella-thorn-acacia).

Acacia tortilis is drought resistant tree grows up to 4-20 m in height but once it matures it forms deep roots and a spreading umbrella-shaped crown. This tree remains ever green while other trees become dry and fruitless during the long period of drought. Therefore, mature tree serve as the source of forage and source of shade in the arid and semi- arid regions for animals (Al Jabr Ahmed, 2008).

2.2.2 Botanical description

Stem and branches of *A. tortilis* are dark brown when it matures and while young are reddish brown with grey lenticels. Leaves can reach up to 1 to 7 cm long. It has 2 to 14 pinnae each with 6 to 22 pairs of leaflets that are smooth to densely pubescent. Flowers are either white or pale yellowish that are round heads, solitary or fascicles in shape with fragrance. They also have spines that are in pairs, either short or hooked up to 5 mm long or long straight slender which is up to 10 cm long. The bark can be grey, brown or black in color that are rough and fissured. The pod of *A. tortilis* is semi-dehiscent that are twisted, yellow brown that is 5 to 15 cm, with longitudinal veins and it is slightly limited between 5 to 18 seeds per pod (Verma, 2016).

Figure 6 shows pictures of *A. tortilis* tree and its parts

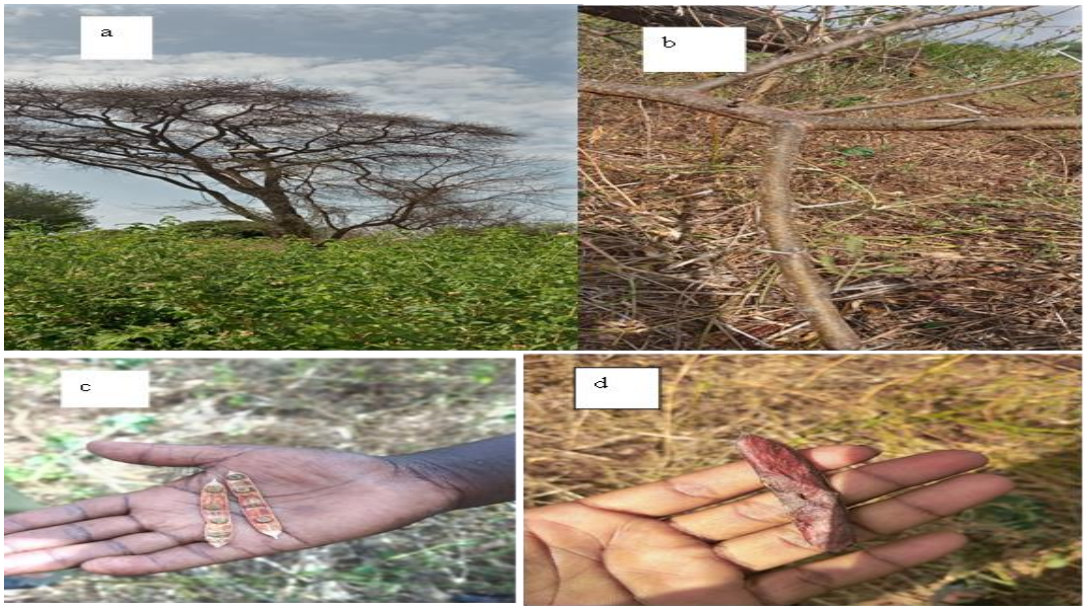


Figure 6: (a) is *Acacia tortilis* tree, (b), is *Acacia tortilis* thorns, (c) is *Acacia tortilis* seeds and (d) is *Acacia tortilis* pod.

Photographs of *A. tortilis* different parts taken at Cheptebo in Kerio valley, Kenya

2.2.3 Chemical constituents of *Acacia tortilis*

Plants naturally grow with accumulation of both secondary and primary metabolites which are useful to the plants growth and protection against biotic and abiotic factors. Ouafae *et al*. (2017) reported that *Acacia tortilis* is reputed for treatment of wounds and stomach ache and according to their results the leaves of *A. tortilis* revealed presence of flavonoids, tannins, coumarins and anthraquinones. According to a study done by Suleiman *et al*. (2017), characterization of stem bark extracts of *A. tortilis* using Gas chromatography-mass spectrometry (GC-MS).The GC-MS analysis revealed presence of 53.97% 1, 2, 3-Benzenetriol as the major compounds, followed by 4-O-Methyl mannose with 38.28% and 11, 13-Tetradecadien-1-ol of 3.53%. When unifloral honey from *A. tortilis* was characterized, it revealed presence of Gallic acid (4-hydroxy-3-methoxybenzoic acid), syringic acid, p-coumaric acid, ferulic acid, cinnamic acid, catechin, epicatechin and rutin (Ciulu *et al*. (2016). Verma (2016) reports that *A. tortilis*

contain oleic acid, linoleic acid, hydrolysable tannins, vicenin, rutin, uronic acid, galactose, arabinose, rhamnose, mannose and protein content.

Oil constituents from *Acacia tortilis* leaves contained monoterpenes, sesquiterpenes, aromatic compounds and aliphatic compounds (Ogunwande *et al*., 2008). According to the research conducted by Kubmarawa *et al*. (2012) using hexane and water as solvents, they found out that there were presence of saponins, tannins, volatile oil, alkaloids, phenols and flavonoids expect in N-hexane fraction flavonoids were completely absent. Hagos *et al*. (1987) reported that the aqueous bark of *A. tortilis* exhibited a new compound quracol, both 1-(2, 4-dihydroxyphenyl)-3-(3-hydroxyphenyl)-propan-2-ol and 1-(2, 4-dihydroxyphenyl)-3-(3, 4-dihydroxyphenyl)-propan-2-ol. According to analysis of fatty acid composition of *A. tortilis* seed oil the gas chromatography revealed the presence of linolenic acid (50.43%), linoleic acid (36.74%) and oleic acids. Because of presence of useful bioactive compounds that are present in *A. tortilis* various biological activity including anti-nociceptive, anti-oxidant and antifungal activity have been conducted on this tree.

2.2.4 Tested activity of *Acacia tortilis*

Various activities have been conducted pertaining *Acacia tortilis*. Ackacha & Elsharif, (2012) studied the leaves of *Acacia tortilis* adsorptivity of Lead. The study showed that the leaves of *Acacia tortilis* was able to adsorb Lead from aquatic environment. The adsorptivity was very fast in the first 15 minutes and it reached equilibrium at 60 min. Pain is a component of virtually all clinical pathologies and management of pain is a primary clinical concern in medical practice. Drugs like Nonsteroidal anti-inflammatory drug (NSAIDs), antidepressants and anticonvulsants are used to manage pains. Anti-nociception is that action or process of hindering the detection of a painful or injurious stimulus by sensory neurons. Anti-nociceptive activity of *A. tortilis* seed

extracted with water was investigated (Agrawal et al., 2018) The results of the study revealed that the seed extract showed anti-nociceptive effect in acetic acid induced writhing response and formalin induced paw licking response in inflammatory phase alone.

The methanolic trunk bark extract of *A. tortilis* revealed anti-oxidant activity with IC_{50} value of 0.01 ± 0.01 µg/mL. The anti-oxidant activity was attribute to total phenolic content (383.19 ± 0.07 mg GAE/g), condensed tannins (18.21 ± 0.04%) and flavonoids (66.09 ± 0.06 mg QE/g) (Alphonsine *et al*., 2019). Dawit (2020) reported that *A. tortilis* fiber can be used as green fiber to reinforce composite based materials due to its light weight and high strength.

Recently, Mezouari *et al*. (2019) evaluated the antifungal activity of *A. tortilis* subsp r*addiana* against *Fusarium oxysporum* f.sp. *albedinis* (Foa). The results revealed that *A. tortilis* was efficient in inhibiting the growth of Foa *in vitro* with a minimum inhibitory concentration of 3 µg/ml. Therefore, suggesting that *A. tortilis* can be used to control Bayoud, a damaging disease of date palm caused by *Fusarium oxysporum* f.sp. *albedinis* (Mezouari *et al*., 2019). In another empirical investigation, aqueous extract of *A. tortilis* showed moderate inhibitory activity against the three selected phytopathogenic fungi *Alternaria alternata, Helminthosporium rostratum* and *Fusarium solani* at high concentrations (Fatimah, 2019).

2.2.5 Various uses of *Acacia tortilis*

2.2.5.1 Medicinal value

Traditional medicine is a complete discipline involving indigenous herbalism and African spirituality, normally involving diviners, midwives and herbalists. Traditional healers use prepared concoction and decoction of *A. tortilis* for medicinal values such

as treatment of dysentery, pharyngitis, diarrhea, tuberculosis, hemorrhage, relief burn pains, stomach ache, malaria and digestive problem. It is also used as sedatives, analgesic and disinfectant (Subhan *et al*., 2018). The seeds of *A. tortilis* are usually combined with *Vigna unguiculata* seeds to make a concoction used for curing skin disease like edema/allergic dermatitis or antiparasitic (Fraga-Corral *et al*., 2021).

2.2.5.2 Human and animal use

Acacia tortilis is a very important plant to animals and human beings. Seeds from *A. tortilis* are used as source of fodder for animals like goats and camels. The use of *A. tortilis* seeds as animal feed was justified by the research that was conducted by Embaby & Rayan (2016)., they reported that the seeds of *A. tortilis* contains protein, lipids, fibers and mineral which are significant food ingredients. The seed flour from this plant has physicochemical and functional properties for example-: excellent water holding index, swelling index, foaming capacity and foam stability. These properties make it suitable for food formulation after elimination or reducing of anti-nutrients such as phytic acid, tannins and protease inhibitors. These anti- nutrients have beneficial health effects at only low concentrations because they reduce cancer risk, blood glucose and insulin responses to starchy foods. While wood from this tree is a source of firewood, charcoal and timber is used for construction purpose. *A. tortilis* is also used as a source of shelter around villages. Honey is produced by honeybees from floral nectar of *A. tortilis,* is red to black in color with strong odor and if well stored it can even stay fresh for many years while still maintaining its therapeutic properties (https://www.althenayanhoney.com/productDetails.aspx?pr=102). The bark of *A. tortilis* is a potential source for dyes. The bark has been also used as a string medium. Gums obtained from this tree is edible and can be used as food additive (Muturi *et al*., 2001).

2.2.6 Plant pest and diseases

Seeds and pods of A*cacia tortilis* are susceptible to bruchids in India (Orwa *et al*., 2009). Similarly, it has been reported that in India also *Sinoxylon anale* and *S. crassum* (power pest beetles) attack *A. tortilis* timber and they can reduce timber into dust in few weeks (Muturi *et al*., 2001). While in Tanzania *Bruchidius spadiceus* attack the seeds (Wahbi *et al*., 2013). Although, *A. tortilis* is susceptible to these pests, on the other side it is claimed to be resistant to termites attack in Ethiopia this is in accordance to a case study by (Fenetahun & Yong-dong, 2019).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study Design

Random experimental design was employed where both qualitative and quantitative analysis was conducted.

3.2 Study Area

The selected County for study was Elgeyo Marakwet County, Kenya, which is located along the basin of the Kerio River in the Rift valley province of Kenya (Figure 7). It borders Trans Nzoia County to the Northwest, while to the West is Uasin Gishu County, to the East is Baringo County and to the North is West Pokot County. The selected area of sample collection site was Cheptebo which is found in Kerio valley in Elgeyo Marakwet County, Kenya. This area was selected because the area is infested with termites and *A. tortilis* is widely used in this area for construction and as a fencing post but it is not attacked by termites. Cheptebo is found in the lowland part of the County with the following coordinates (Latitude: N 0˚ 31’ 26. 484’’ and Longitude: E 35˚ 36’11.441”). The area lies 900 m above the sea level with annual mean temperature that ranges from 25 ˚C to 28 ˚C. The area receives an average rainfall of 700 mm annually.

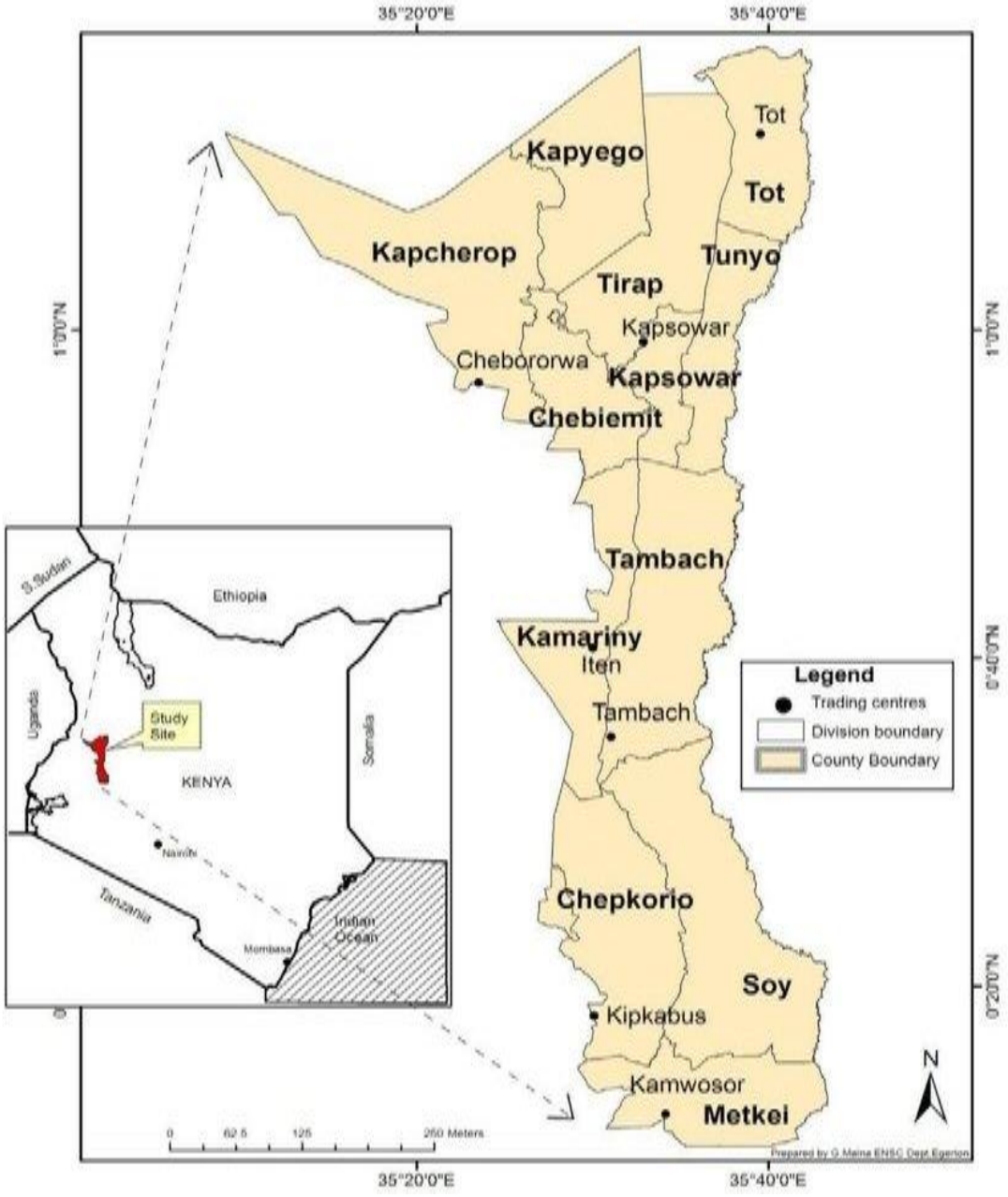


Figure 7: Map of Kenya showing the position of Elgeyo https://www.researchgate.net/figure/Map-of-Elgeyo-Marakwet

3.3 Materials and methods

3.3.1 Chemicals and reagents

Hexane, ethyl acetate, acetone, ethanol, sodium hydroxide, ferric chloride, glacial acetic acid, Folin Ciocalteu reagents (FCR), Sodium carbonate (Na_2CO_3), Gallic acid, Aluminium chloride, Quercetin, Sodium Nitrate, Sulphuric acid, iodine, potassium

iodide, N, O-bis (Trimethylsilyl) trifluoroacetamide with Trimethylchlorosilane (BSTFA + TMCS), acetonitrile, Methanol (HPLC grade) were purchased from Sigma –Aldrich, Germany. All chemicals and reagents used in this study were of analytical grade.

3.3.2 Sample preparation

Acacia tortilis growing in Cheptebo, Kerio Valley was authenticated by botanist in Kitale Museum and it was randomly selected from forest. The tree trunk was cut and it was transported to Moi University. Its heartwood was separated from other parts and it was cut into small pieces, dried, ground and sieved using 115-mesh sieve to obtained fine powder which was later oven-dried at 60 ˚C and stored in a well closed bag

3.3.3 Extraction of crude extract

Extraction was performed in accordance to the procedure by (Syofuna *et al.*, 2012) with slight modification. Each Soxhlet extraction was carried out separately with 250 ml of solvent on 20 g of *A. tortilis* heartwood fine powder for 15 hours at a rate of 12 cycles per hour with hexane, ethyl acetate, acetone and ethanol. After each extraction, the different extracts were concentrated using a vacuum rotary evaporator with a pressure apparatus at the temperature of 50 ˚C under reduced pressure. Different concentrated extracts were further air dried and stored in the refrigerator at 4 ˚C awaiting further analysis.

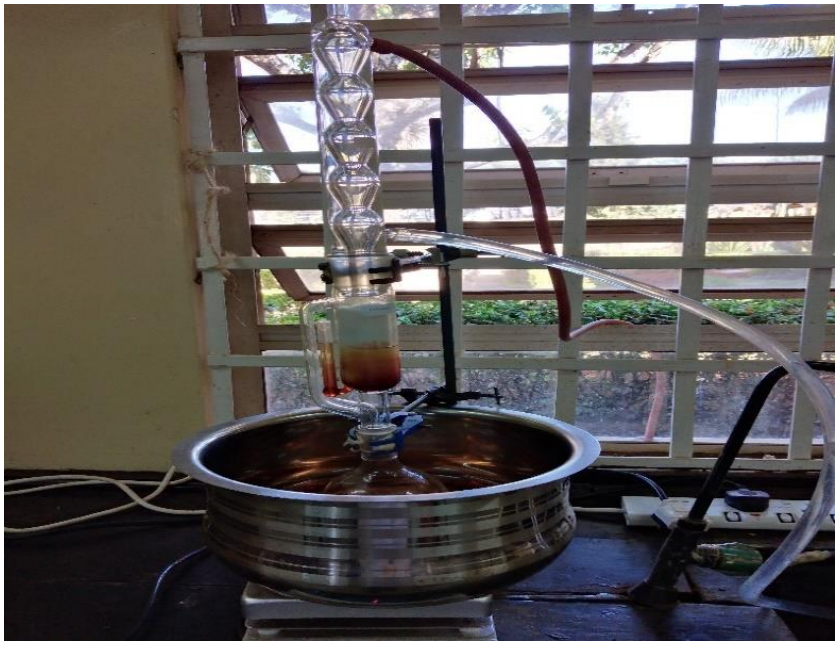


Figure 8: Soxhlet extraction of fine powder from *A. tortilis* heartwood

3.3.4 Qualitative determination of phytochemical composition of crude extracts

3.3.4.1 Determination of extraction yield

After extraction the relationship between polarity of different solvents (ethanol, acetone, ethyl acetate and hexane) and amount of yield was evaluated. The yield of different extracts was calculated using the formula below: -

$$\text{Percentage yield \%} = \frac{yield\ mass\ (g)}{mass\ of\ sample\ subjected\ to\ extraction\ (g))} \times 100 \ldots\ldots \text{ Equation 1}$$

3.3.4.2 Phytochemical screening procedure

Phytochemical screening was done to assess the qualitative chemical composition of the crude extracts using commonly employed precipitation and coloration chemical tests to identify the major secondary metabolites like flavonoids, saponins, tannins, phenol, alkaloids, terpenoids, and cardiac glycosides according to the protocol by Sonam *et al*. (2017).

3.3.4.2.1 Analysis of flavonoids/ the alkaline reagent test

Two ml of plant extract was treated with two drops of 1% sodium hydroxide. Formation of yellow color indicated presences of flavonoids.

3.3.4.2.2 Analysis of phenol/ ferric chloride test

Ferric chloride test was used. Plant extract (2 ml) was treated with two drops of neutral 5% ferric chloride solution. A dark green color showed the presence of Phenols.

3.3.4.2.3 Analysis of alkaloids

Wagner's reagent test was used. To 2 ml of plant extract, two drops of Wagner's reagent was added along the sides of the test tube. A reddish-brown precipitate indicated positive results.

3.3.4.2.4 Analysis of tannins

To 5ml of plant extract, two drops of 1% iron (iii) chloride was added. The appearance of intense blue- black color indicated the presence of tannins in the test samples.

3.3.4.2.5 Analysis of cardiac glycosides

Keller-kiliani test was used. Measured 50 mg of extract was treated with 1ml glacial acetic acid and two drops of 5% ferric chloride. To this solution two drops of concentrated Sulphuric acid was added. Appearance of reddish-brown color ring at the junction of two layers revealed presence of glycosides.

3.3.4.2.6 Analysis of saponins

Frothing test was used. The plant extract (50 mg) was diluted with distilled water up to 20 ml and shaken for 15 minutes in a graduated cylinder. Formation of 1 cm layer of foam revealed the presence of saponin.

3.3.4.2.7 Analysis of terpenoids

Five ml of extract was dissolved in chloroform (2 ml) and then 3 ml of concentrated Sulphuric acid was added to the solution. Formation of reddish-brown colored at the interface showed the presence of terpenoids.

3.3.4.3 Ultraviolet -Visible spectroscopy analysis

The UV-Vis Spectroscopy was used to evaluate existence of conjugated system in the crude extract. The extract was centrifuged at 3000 rpm for 10 min and filtered through Whatman filter paper No.1. The sample was diluted to 1:10 with solvent of extraction. The extract was scanned at wavelength ranging from 200 to 800 nm using Perkin Elmer spectrophotometer and the characteristic peaks were recorded (Patle *et al*., 2020).

3.3.5 Quantitative determination of phytochemical composition of crude extracts

3.3.5.1 Preparation of reagents and standard solution used in determination of Total phenolic content (TPC)

3.3.5.1.1 Preparation of Folin Ciocalteu reagents (FCR)

Using measuring cylinder, 10 ml of FCR was taken in a volumetric flask and diluted to 100 ml with distilled water to obtain 10 % FCR.

3.3.5.1.2 Preparation of 7.5 % Sodium carbonate solution

Measured 7.5 g of sodium carbonate was place in 100 ml of volumetric flask and small amount of water was added and shaken to dissolve Na_2CO_3 thereafter the distilled water was top up to the mark.

3.3.5.1.3 Preparation of standard solution (Gallic acid solution)

Accurately weighed 250 mg of Gallic acid was dissolved in 250 ml of distilled water to obtain stock solution of 1000 µg/ml. Serial dilution was then performed to prepare solution of different concentration (10, 20, 40, 80 and 160 µg/ml).

3.3.5.1.4 Preparation of blank

Blank consisted of 5 ml of FCR, 1 ml of ethanol and 1 ml of sodium carbonate.

3.3.5.1.5 Preparation of sample

Measured 10 mg of each extract hexane, ethyl acetate, acetone and ethanol were taken into test tube and dissolve in 10 ml of ethanol.

3.3.5.1.6 Calibration of standard curve

Using a measuring cylinder, 1 ml of prepared standard solutions were placed in different test tubes. To each test tube 2.5 ml of FCR and 2.5 ml of sodium carbonate were added and incubated for 20 minutes at 25 ˚C. The curve was determined by running in the UV-Vis to obtain the fixed wavelength.

3.3.5.2 Determination of total phenolic content

Total phenolic content in hexane, ethyl acetate, acetonic and ethanolic extract were quantified following the protocol by Khalil *et al*. (2009) with slight modification. The quantification was based on the reduction reaction where phosphomolybdate ion of FCR was reduced by the phenolate ion of the extract. One ml of each extract was placed in the test tubes then 2.5 ml of 10% FCR followed by 2.5 ml of 7.5 % sodium carbonate. Thereafter the test tubes were incubated at 25 ˚C for 20 minutes before the absorbance was measured at 760 nm using UV spectrophotometer. The TPC was calculated from standard Gallic acid calibration curve and the TPCs of each extract were expressed as milligram Gallic acid equivalents per gram of the extract (GAE mg/g of extract) considering dilution factor. Using the formula below:

$$C = \frac{C \times V}{M} \quad \text{……Equation 2}$$

calculation of GAE mg/g of extract was done. The same method was repeated in triplicates.

Where: C = Concentration of GAE mg/g of dry extract, c= Concentration of Gallic acid obtained from the graph, V= volume and M= mass used

3.3.5.3 Preparation of reagents and standard solution for determination of Total flavonoid content (TFC)

Total flavonoid content of heartwood extracts of A*cacia tortilis* were determined by Aluminium chloride calorimetric method (Sulaiman *et al*., 2014), with slight modification. Quercetin was used as a standard.

3.3.5.3.1 Preparation of standard solution

Weighed 250 mg of quercetin was dissolved into 250 ml distilled water to make the concentration be 1000 µg/ml stock solution from which different concentrations were prepared (10, 20, 40, 80 and 160 µg/ml).

3.3.5.3.2 Preparation of 1M Sodium hydroxide solution

Four grams of Sodium hydroxide was taken in 100 ml volumetric flask and small amount of distilled water was added to dissolve it. Then the final volume was made to the mark by adding distilled water.

3.3.5.3.3 Preparation of 5% Sodium Nitrate

Five grams of Sodium Nitrate was taken in 100 ml volumetric flask and small amount of distilled water was added and shaken to dissolve it. Final volume was made to the mark by adding distilled water.

3.3.5.4 Experimental procedure for determination of TFC

Aluminum chloride colorimetric method was used to determine total flavonoid content of *Acacia tortilis* heartwood extracts (ethanol, acetone, ethyl acetate and hexane extracts). Using 10 ml measuring cylinder, 1 ml of plant extracts or standard solutions of quercetin with concentration of 10, 20, 40, 80 and 160 µg/ml were measured

thereafter 4 ml of distilled water was added followed by 0.30 ml of 5% $NaNO_2$ and after 5 minutes 0.3 ml of 10% $AlCl_3$ was also added. After 5 minutes of reaction, 2 ml of 1M NaOH was added and the volume was made up to 10 ml with distilled water. The solution was mixed, and absorbance was measured against the blank at wavelength of 420 nm using UV-Vis spectrophotometer. The TFC content was calculated from the regression equation of the standard curve and expressed as milligram of quercetin equivalent (mg QE/g). The procedure was in triplicates (Thivakar *et al*., 2020).

Total flavonoid content to total phenolic content ratio was determined for each extract using formula:

$$\frac{Total\ flavonoid\ content}{Total\ phenolic\ content} \quad \text{.....Equation 3}$$

3.3.5.5 Statistical analysis

For statistical analysis the samples were carried out in triplicates and all results were expressed as mean ± standard deviation (SD). Correlation between TFC and TPC were established using Pearson's correlation with confidence interval of 95%.

3.3.6 Characterization of *Acacia tortilis* heartwood extractives

3.3.6.1 Fourier transform infrared (FTIR) analysis

Heartwood extracts of *A. tortilis* were qualitatively analyzed using FTIR spectroscopy (Nicolet- 6700, Thermo Scientific, USA). Extracts of *A. tortilis* heartwood were characterized using FTIR spectrophotometer and functional groups of the components were identified by analyzing the obtained peaks in the region of 4000-500 cm^{-1}.

3.3.6.2 Gas chromatography-mass spectrophotometry analysis

3.3.6.2.1 Preparation of samples for GC-MS analysis

The hexane extract was analyzed without derivatization because compounds extracted by hexane solvents are volatile. It was prepared by re-dissolving 1 mg of the extract in

1 ml of hexane. Acetonic, ethyl acetate, ethanolic extracts were first derivatized with intention of converting nonvolatile compounds or volatile products. In a screw-cap vial, a sample of approximately 1 mg of extracts were dissolved in 0.5 ml of N, O-Bis (trimethylsilyl) trifluoroacetamide with 1% Trimethylchlorosilane (BSTFA/ 1% TMCS). The obtained solutions in screw capped vials were kept in the oven at 60°C for 8 hrs. Afterwards vials were opened and left in the oven for 3 hours to remove excess derivatizing agent and the residue was diluted using 1 ml of ethyl acetate thereafter analyzed in the GC-MS.

3.3.6.2.2 Sample analysis in the GC-MS

Gas chromatography-mass spectrophotometry (GC-MS) analysis of the hexanoic and derivatized extracts were performed on a Clarus A® 680 gas chromatograph (Perkin Elmer Inc., USA) coupled to a Clarus A® 500 MS quadrupole mass spectrometer (Perkin Elmer Inc., USA). Gas chromatography separations were carried out on a 5% diphenyl / 95 % dimethyl polysiloxane fused-silica capillary column (Elite-5ms, 60 m x 0.25 mm, 0.25 mm film thickness, Perkin Elmer Inc, USA). The gas chromatograph was with an electronically controlled split /split less injection port was used. The injection (injection volume of 1 µl) was performed at 250 °C in the split mode (split flow of 20 ml/min). The carrier gas (Helium) with a constant flow of 1.2 ml per min. The oven temperature program was as follows: 200 °C constant for 4 min, 200 °C to 330 °C at a rate of 5 °C/min and then constant for 330 °C. Ionization energy of 70 eV was achieved under the electron impact mode. The scan mode was *m/z* 45 to *m/z* 750 in mass spectrophotometer. The detector was switched off in the initial 2.8 minutes (solvent delay). Compounds were identified by comparison with spectra from National Institute of Standards and Technology (NIST) mass spectral library and comparison with those in literature.

3.3.6.3 Liquid Chromatography-Photo diode array Mass Spectrometry (LC-PDA-MS) analysis

The analysis was performed using a Supercosil ™ LC-18 column (250 mm x 4.6 mm, i.d) fixed with a binary pump. The LC was interfaced with a PDA and Q-TOF mass spectrometer fitted with an ESI source. Full-scan mode from *m/z* 100 to 2000 was performed with a source temperature of 140°C. A mass of 0.5 mg of acetonic, ethyl acetate and ethanolic extracts were diluted in ethanol afterwards 5 µl was injected in the LC-PDA-MS. To achieve separation, linear gradient elution with (A) HPLC water containing 0.1 % of formic acid, (B) methanol (HPLC grade) and (C) acetonitrile was performed. The Photo diode array detector was set at 280 nm. The MS spectra were acquired in both positive ion mode and negative mode. The temperature of the drying gas (Nitrogen) was 350 °C, at a gas flow rate of 6 ml/min, and a nebulizing pressure (Nitrogen) of 25 psi.

3.3.7 Evaluation of Anti-termite activity of *Acacia tortilis*

3.3.7.1 Wood block preparation

The study adopted American Wood Protection Association, AWPA: E7-93 (1993) methodology of evaluating resistance of wood to subterranean termites. A total of 120 heartwood blocks of *Acacia tortilis*, measuring 10 cm × 1.5 cm × 0.5 cm (longitudinal × radial × tangential) were prepared. Also 24 blocks of the same dimension were cut from *Pinus sylvestris* that was used as positive control. *P. sylvestris* is usually used as a positive control because it is known to be easily attacked by termites and therefore it ascertains that the experimental set-up is working properly. To obtain 96 extracted wood, 24 blocks of *A. tortilis* were extracted using Soxhlet apparatus (Figure 9) separately in 200 ml of hexane, ethyl acetate, acetone and ethanol for 48 hours and the siphoning was adjusted to be 10 cycles per hour. After extraction the wood blocks were

well labelled including 24 unextracted blocks of *A. tortilis* and 24 blocks of *P. sylvestris*. All wood blocks (144) were oven dried at 103 °C to a constant weight (ASTM D-1413-2007) and the weight was recorded. The blocks were kept in a desiccator prior to exposure to termite attack.

Figure 9: Extraction of *A. tortilis* heartwood blocks

3.3.7.2 Field determination of anti-termite activity of *A. tortilis*

AWPA: E7-1993 was adopted during this study. Active termite nest composing of *Macrotermes natalensis* was identified at Cheptebo, Kerio Valley in Elgeyo Marakwet County, Kenya. Cellulosic materials were cleared from the identified nest by digging. The prepared wood blocks were then setup in a randomized complete block design with four replicates for each tested sample. The test samples were arranged so that the distance between each block was 20 cm and 30 cm from the termite nest and were buried in the ground as shown in figure 10 for 5 months. The positions of the installed

test samples were mapped in the field to facilitate inspection and record keeping. After every one month, four replicates each from hexane, ethyl acetate, acetone and ethanol extracted wood blocks, *P. sylvestris* and unextracted *A. tortilis* were collected, dried to constant mass and weighed. Samples were not collected in the first month because the termites' nest was inactive after it was disturbed by digging when clearing the termite nest.

Thereafter the degree of attack was evaluated by calculating the weight loss using the formula below-:

$$\% \text{ Weight loss} = \frac{DX-DY}{DX} \times 100 \quad \text{.....Equation 4}$$

Where; DX: oven dried weight before field exposure and DY: oven dried weight after field exposure

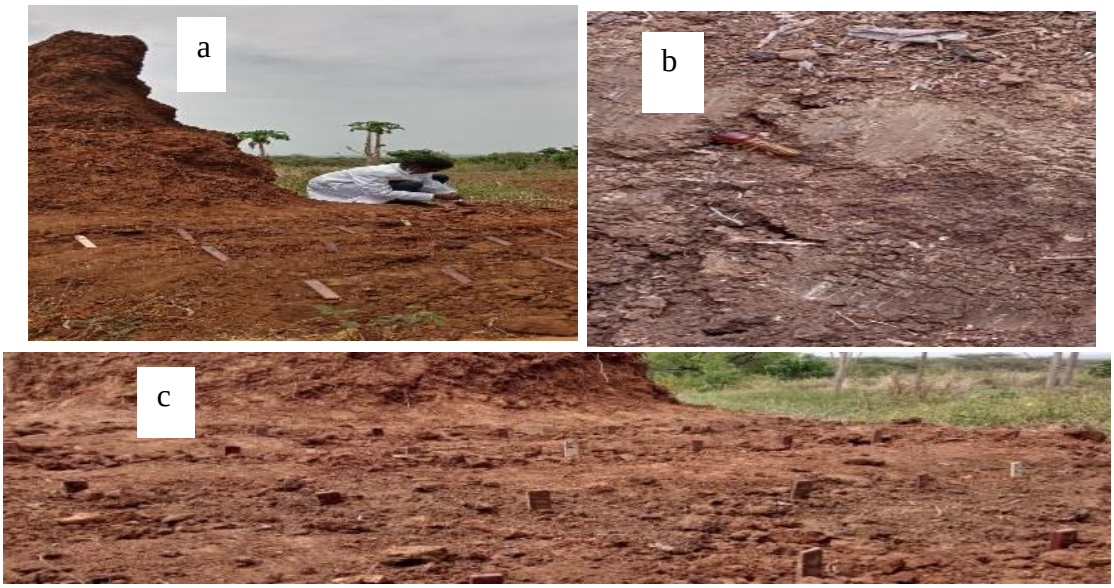


Figure 10: (a): Arrangements of wood blocks around termite nest, (b): *Macrotermes natalensis* and (c): buried wood block arranged so that the distance between each block is 20 cm and 30 cm from the termite nest.

CHAPTER FOUR

RESULTS AND DISCUSSION

In this chapter results obtained on extraction yield, phytochemical screening, total phenolic and flavonoid content of ethanolic, acetonic, ethyl acetate and hexane extracts were well discussed. The factors that could have contributed to expected results were outline in this chapter. Gas chromatography Mass spectroscopy (GC-MS), Liquid Chromatography-Photodiode-array-Mass spectroscopy (LC-PDA-MS) and Fourier transform Infrared (FTIR) were instrumental techniques that were used to identified compounds that are found in the heartwood of *A. tortilis*. Findings on anti-termite activity of heartwood from *A. tortilis* was evaluated and this activity was related to the compounds that were identified in *A. tortilis* heartwood

4.1 Extraction yield

The relationship between polarity of different solvents (ethanol, acetone, ethyl acetate and hexane) and amount of yield was evaluated when the fine powder of *Acacia tortilis* heartwood was extracted separately with the above-mentioned solvents. The study revealed that extraction yield increases with increase in polarity of solvents. Ethanolic extract showed highest extraction yield (10.75%), followed by acetone (7.40%), ethyl acetate (7.30%) and hexane (0.15%). The percentage yield of extracts (Figure 11) was obtained by calculation using the formula below;

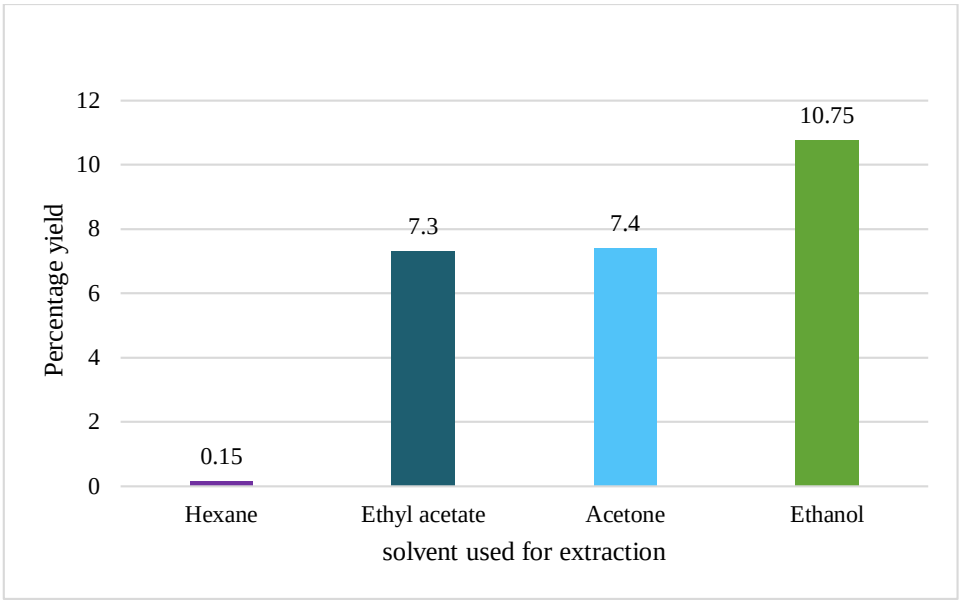


Figure 11: Percentage extraction yield

In the present study effect of solvents polarity on the extraction yield was determined and the more polar solvent, ethanol gave the highest yield but non-polar solvent, hexane gave the lowest yield as illustrated in Figure 11. Different solvents have different capability and ability of extracting certain quantity of extracts as well as influencing components and families of phytochemicals. Additionally, differences in the dielectric constants of solvents which contributes to difference in polarities of solvents resulted in different extraction yields (Bamidele, 2017). This could be the reason why ethanol with high dielectric constants hence more polar extracted high quantity of yield than other solvents used for extraction in the present study. According to Bamidele (2017) solvents such as water, methanol and ethanol are reliable and effective solvents for extracting the polar phytochemical constituents from plants sample.

The present study result is consistent with other authors (Rai *et al*., 2020; Vieitez *et al*., 2018) where they obtained that polar solvents extracted appreciable amount of yield than the comparable non polar solvents. This is because polar solvents have the ability to increase the cell permeability and get into the inner cells of the sample hence

extracting more phytochemical compounds (Patle *et al*., 2020). However, this may also mean that non polar compounds are in low concentration in *A. tortilis* heartwood while polar compounds are major compounds in the heartwood since ethyl acetate and acetone with intermediate polarities also gave good extraction yield. For example, Pinitol was identified as major compound this compound is polar in nature and it was in highest percentage 84 % in ethanolic extract (table 4).

4.2 Phytochemical screening

Secondary metabolites present and those not present in acetonic, ethyl acetate, ethanolic and hexane extracts of *Acacia tortilis* are summarized in the table 1. The results revealed presence of different bioactive compounds in the heartwood of the plant.

Table 1: Results for phytochemical screening of *A. tortilis* crude extracts

Serial NO.	Constituents	Test performed	Hexane extract	Ethyl acetate extract	Acetonic Extract	Ethanolic extract
1.	Flavonoids	Alkaline reagent test	-	+	+	+
2.	Phenols	Ferric Chloride test	-	+	+	+
3.	Alkaloids	Wagner's reagent test	+	-	-	-
4.	Tannins	Ferric Chloride test	_	+	+	+
5.	Cardiac glycosides	Keller Killiani test	-	+	+	+
6.	Saponins	Froth test	+	+	+	+
7.	Terpenoids	Salkowski's test	+	+	+	+

Key: (+) shows compound is present while (-) means it is absent.

The results showed that *Acacia tortilis* heartwood contains different classes of secondary metabolites like phenols, flavonoids, saponins, tannins, alkaloids, cardiac glycosides and terpenoids as shown in (Table 1). There is agreement between the results

obtained in the current study and the study conducted by Ali *et al*. (2019) on the leaf and the bark extract of *A. tortilis* which revealed that both leaf and barks extract showed presence of phenols, flavonoids, glycosides, tannins, steroids and saponins whereas the alkaloids and anthraquinones were absent in these crude extracts. Absence of alkaloids and anthraquinones could suggest that these secondary metabolites were not extracted by the solvents. Presence of compounds in one solvent and absent in another is affected by solvent polarity i.e polar solvents extract polar compounds and vice versa (Patle et al., 2020).

The results concurred with to study by Kubmarawa (2012) where phytochemicals compositions were investigated on water and n-hexane extracts of the leaves, root and stem bark of *A. tortilis*. N-hexane revealed absence of flavonoids saponins, tannins while volatile oil and alkaloids were present. A review study conducted by Subhan (2016) reported that Acacia species are very rich sources of biochemical compounds such as phenols, alkaloids, saponins, terpenoids, sterols, polysaccharides, non-protein amino acids and fatty acids. Ullah *et al*. (2020) reported that there are more than 100 flavonoids for example-: flavans, flavanones, flavonols, flavones and their glycosides have been reported to be found in different Acacia species. The present study revealed that different solvents have ability to extract different phytochemicals from the plant sample which contribute to variation of yield extracted by solvents in Figure 11.

4.3 Total phenolic content and Total flavonoid content

4.3.1 Total phenolic content

Total Phenolic content (TPC) was obtained by calculation using the equation of ($y = 0.0149x - 0.0705$, $R^2 = 0.9957$). Figure 12 below shows the standard calibration curve. The results showed that total phenolic content in *Acacia tortilis* heartwood was of the order $10.7 \pm 0.10 < 157.6 \pm 0.06 < 169.3 \pm 0.15 < 350.1 \pm 0.10$ mg of gallic acid

equivalent per gram (mg GAE/g) for hexane, ethyl acetate, acetonic and ethanolic extract (Figure 14). From the results ethanolic extract exhibited high phenolic content while the hexane extract contained lowest TPC. The present study revealed that the TPC content increases with increase in solvent polarity.

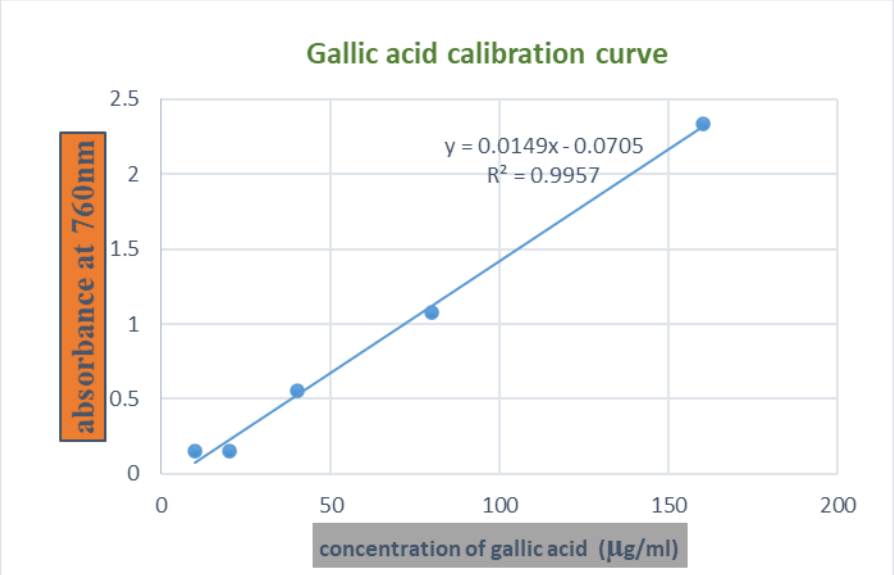


Figure 12: Standard calibration curve for determination of TPC

The variation in the TPC content is highly dependent on the solvent polarity and extraction ability of the solvents as it is indicated in various studies (Abdul Khalil *et al*., 2009; Bamidele, 2017; Kamtekar *et al*., 2014). Madjid *et al*. (2015) suggest that solvents like methanol, ethanol, acetone and ethyl acetate are commonly used for the extraction of phenols from plant samples. From the current findings ethanolic extract had highest TPC (350.1 ± 0.10 mg GAE/g) of dry extract almost twice of that exhibited in ethyl acetate and acetone and very low in hexane extract (10.7 ± 0.10 mg GAE/g) indicates that ethanolic extract contains more of the phenolic compounds. This is consistent with the results of Subhan (2016) where the polar methanolic extract of *A. pcynatha* leaf showed the highest TPC content of 60.4 mg GAE/g of dry extract while non polar hexane and dichloromethane extracts exhibited low TPC content of 0.1 and

1.0 mg GAE/ g of dry extract respectively. However, the findings were inconsistent with the results of *A. ataxacantha* obtained by Madjid *et al*. (2015) where the ethyl acetate showed maximum content of 74.18 mg GAE/g followed by methanolic with 36.51 mg GAE/g, 80 % ethanolic extract revealed 33.48 mg GAE/g and dichloromethane 28.14 mg GAE/g and N- Hexane 18.06 mg GAE/g showed low TPC content. This inconsistency is due to difference in the protocol for extraction where the present study used separate Soxhlet extraction method whereas that study used successive maceration method.

When Ali *et al*. (2019) conducted a study on variation of TPC based on leaf and bark of *A. tortilis*, the bark extract had high content 287.03 GAE/g compared to the leaf extract which contained 125.90 mg GAE/g. In another study by Yildiz *et al*. (2018), the bark extract of *A. dealbata* contained high TPC content 321.499 mg GAE/g, followed by heartwood with 31.893 mg GAE/g of the this plant which was 00 times lower than what the current study obtained in the heartwood of *A.tortilis* while the sapwood was with lowest TPC content 2.076 mg GAE/g.

4.3.2 Total flavonoid content

Total flavonoid content (TFC) in hexane, ethyl acetate, acetonic and ethanolic extracts were 0.7 ± 0.06, 14.7 ± 0.15, 19.2 ± 0.15 and 21.1 ± 0.17 mg of quercetin equivalents (QE)/g of dry extract respectively (Figure 14). These values were obtained by calculation using the obtained equation ($y=0.0136x- 0.00021$, $R^2= 0.9998$ in figure 13 below. The TFC in ethanolic extract was slightly higher among the four tested extracts and lowest in hexane extract whereas ethyl acetate contained approximately 5 mg QE/g of dry extract lower than acetonic extract.

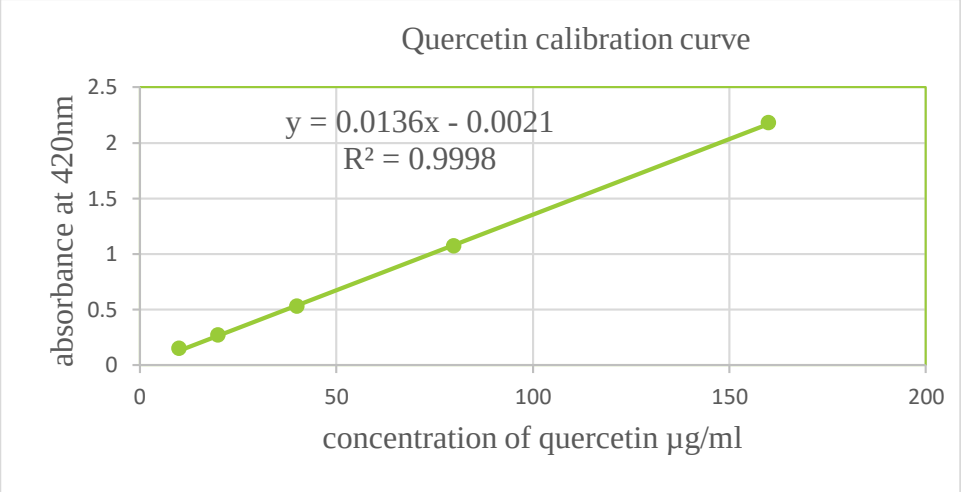


Figure 13: Standard curve for determination of TFC

The findings of the current study revealed that the TFC of the extracts varied with respect to the solvent of extraction figure 14. The samples extracted with polar solvent ethanol showed highest TFC of 21.1 ± 0.17 QE /g of dry extract. This is similar to a study where TFC in more polar methanol fraction of *A. catechu* was 3.2 mg QE/g and in non-polar chloroform fraction of *A. nilotica* exhibited the lowest flavonoid content 1.02 mg QE/g (Sulaiman *et al*., 2014). In another study by Ali *et al*. (2019), the leaves of *A. tortilis* with 77.0 mg QE/g was higher than the current study by almost 64 mg QE/g, however the barks of *A. tortilis* exhibited 3.30 mg QE/g which was lower than TFC in the heartwood of the present study. The variation of quantity of TFC in the parts of the similar plant can be attributed due to type of solvent used for extraction as well as method of extraction and difference in the composition of extractives in these two parts. When the amount of TFC observed in the current study is compared to what was obtained by Suleiman *et al*. (2020), the heartwood of *A. tortilis* contains more amount of TFC than that from unifloral honey from both *A. tortilis* and *A. ziziphus* which revealed 5.5 mg QE/100 g and 7.29 mg QE/100 g respectively.

Most of the flavonoids occur in plants as their glycoside derivatives and according to (Dirar *et al*., 2019) aqueous ethanolic extracts and acetonic extracts have mostly shown high total flavonoid content. Results provided evidence that the selection of solvent for extraction should be guided by the types of phytochemical constituents expected to be obtained from the plant samples. From the present study ethanolic, acetonic and ethyl acetate extracts contained appreciable amount of flavonoids content. However, ethanol was excellent solvent for extraction of flavonoids.

The results of extraction yield were in line with total phenolic and total flavonoid content results where ethanolic extract with highest yield exhibited highest content of flavonoids and phenols (Figure 14).

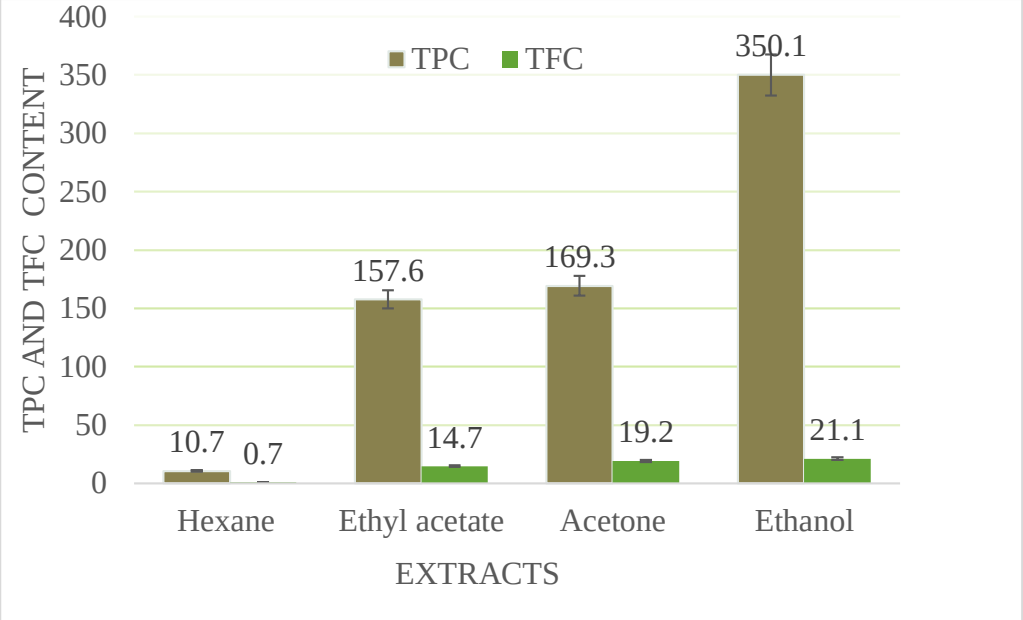


Figure 14: Total flavonoid and total phenolic content

4.3.3 Correlation between total phenolic content (TPC) and total flavonoid content (TFC)

Estimated quantity of flavonoid within total phenolic content was evaluated by calculating flavonoid to phenolic (F/P) ratio (Table 2 below). The highest F/P ratio was

observed in ethanolic extract of *A. tortilis* 60 % which indicated that flavonoid compounds are in high percentage compared to other phenolic compounds. Correlation between TPC and TFC in *A. tortilis* heartwood extracts was performed using Pearson correlation and a linear regression correlation with a coefficient R= 0.881 was obtained. The finding revealed that there was a strong positive correlation between TPC and TFC at 95% confidence interval with ($P < 0.05$). The correlation depicts that the extract with high TPC contain high flavonoid content while the p value showed that there is a significant difference between TPC and TFC of the four extracts

Table 2: Percentage correlation between total phenolic content and total flavonoid

Extract	F/P	% F/P
Hexane	0.07	7%
Ethyl acetate	0.09	9%
Acetone	0.11	11%
Ethanol	0.61	61%

Key: F/P: Flavonoid to phenolic ratio

4.4 Ultraviolet -Visible spectroscopy (UV-Vis) analysis

The UV-visible analysis was performed to establish the presence or absence of compounds with π-bonds, lone pair of electrons, chromophores and aromatic rings in hexane, ethyl acetate, acetonic and ethanolic extracts. Peaks at maximum wavelength (λ_{max}) in the UV-Vis spectra were 338, 338, 339 and 341 nm with the absorption intensity of 0.377, 0.925, 0.489 and 0.376 respectively in the same order of above-mentioned solvents. Peaks were also exhibited at λ_{max} 483, 489, 457 and 490 nm for ethyl acetate, acetonic and ethanolic extract. The absorbances of these peaks were 0.288, 0.146, 0.145 and 0.116 respectively. There was a peak at 450 nm for both ethanolic and ethyl acetate extracts but with different absorbance 0.168 and 0.383

respectively (see table 3 below). The UV-Vis spectra of *A. tortilis* extracts is shown in figure 15 below.

Table 3: Spectra data of hexane, ethyl acetate, acetonic and ethanolic extracts in the UV-Vis

Extract	**Maximum wavelength (λ_{max})**	**Absorbance**
Hexane	338 nm	0.377
Ethyl acetate	338 nm	0.925
	450 nm	0.383
	483 nm	0.228
Acetonic	339 nm	0.489
	457nm	0.145
	490 nm	0.116
Ethanolic	341 nm	0.376
	450 nm	0.168
	489nm	0.146

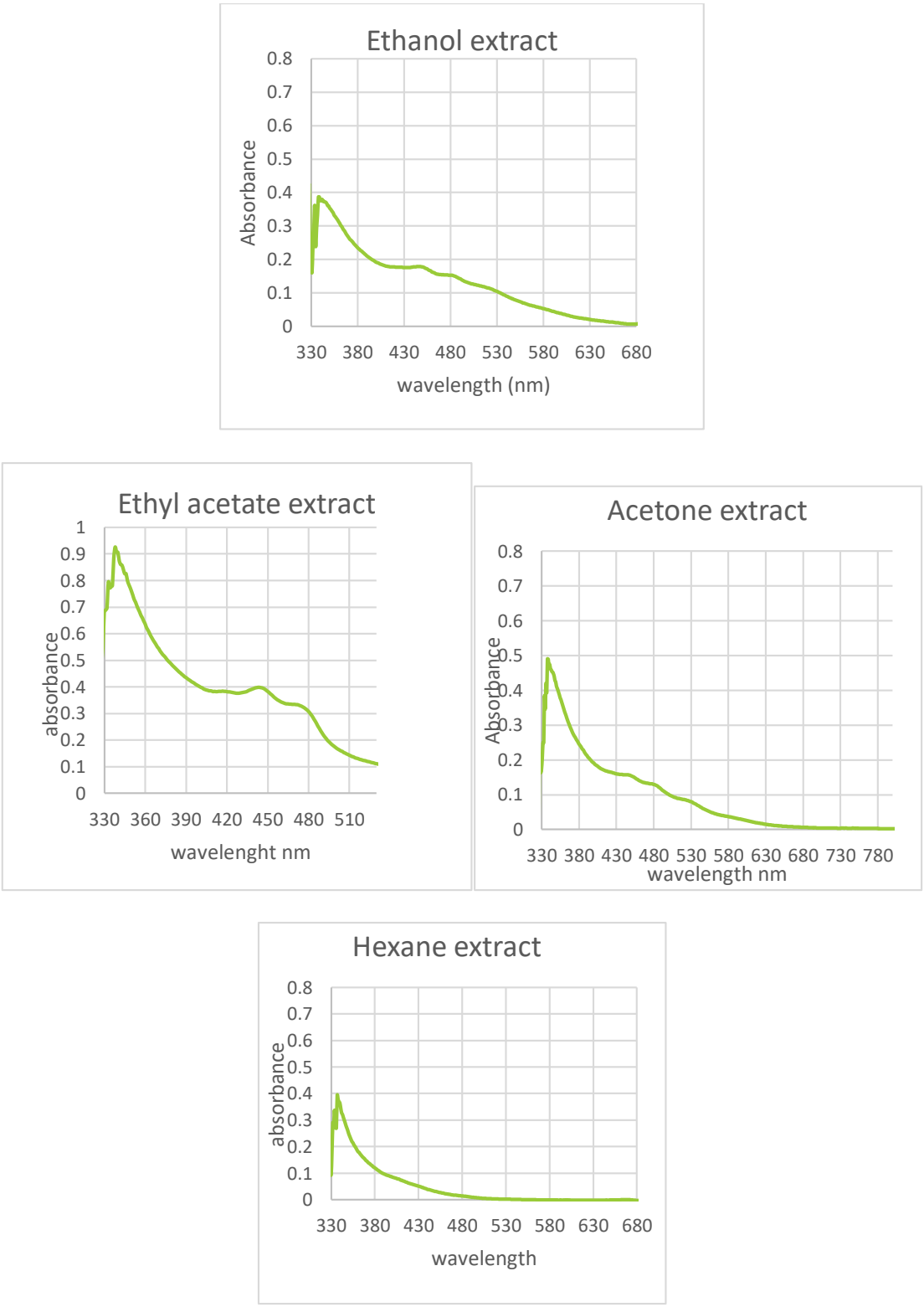


Figure 15: UV-VIS spectral analysis of ethanolic, ethyl acetate, acetonic and hexane extract of *A. tortilis* heartwood extract

The UV-Vis spectra indicates presence of double or triple bond and heteroatoms, according Jain *et al.* (2016), occurrence of one or more peaks in the wavelength between 200 and 400 nm is linked to presence or existence of double or triple bond and heteroatoms S, N, O. This is evidential because the compounds obtained by GC-MS and LC-PDA-MS revealed compounds like D-pinitol, catechin, afzelechin and fatty acids, sterols and terpenoids with oxygen heteroatom and presence of double bonds. Peaks at λ_{max} 338, 339 and 341 nm can be attributed to π -π^* transition of C=C double bond while peaks in the range of 450 to 490 nm is assigned to transitions of lone pair of electrons i.e n-π^* in ethyl acetate, acetonic and ethanolic extract extracts. These peaks in range between 450 and 490 nm were absent in hexane extract. This may suggest that there were no

n-π^* transitions vis-à-vis heteroatoms O_2. Solvent polarity affects extractions of compounds which contribute to extraction of different compounds which have different absorption of UV-VIS which leads to different in maximum wavelength.

4.5 Fourier transform infrared spectroscopy analysis

Figure 16 below shows FTIR spectra for hexane, acetonic, ethyl acetate and ethanolic extracts obtained from the FTIR scan. From the results three extracts; ethanolic, ethyl acetate and acetonic extracts revealed almost similar spectra showing the functional groups of compounds present in the three extracts are similar however this similarity was not observed in hexane extract. Hexane extract showed different FTIR spectra profile indicating dissimilarity in the compounds extracted by hexane due to its non-polarity. This dissimilarity between hexane and the other three extracts is consistent to the phytochemical screening and GC-MS results, where hexane extract showed different compounds obtained in acetone, ethyl acetate and ethanolic extracts (Table 4).

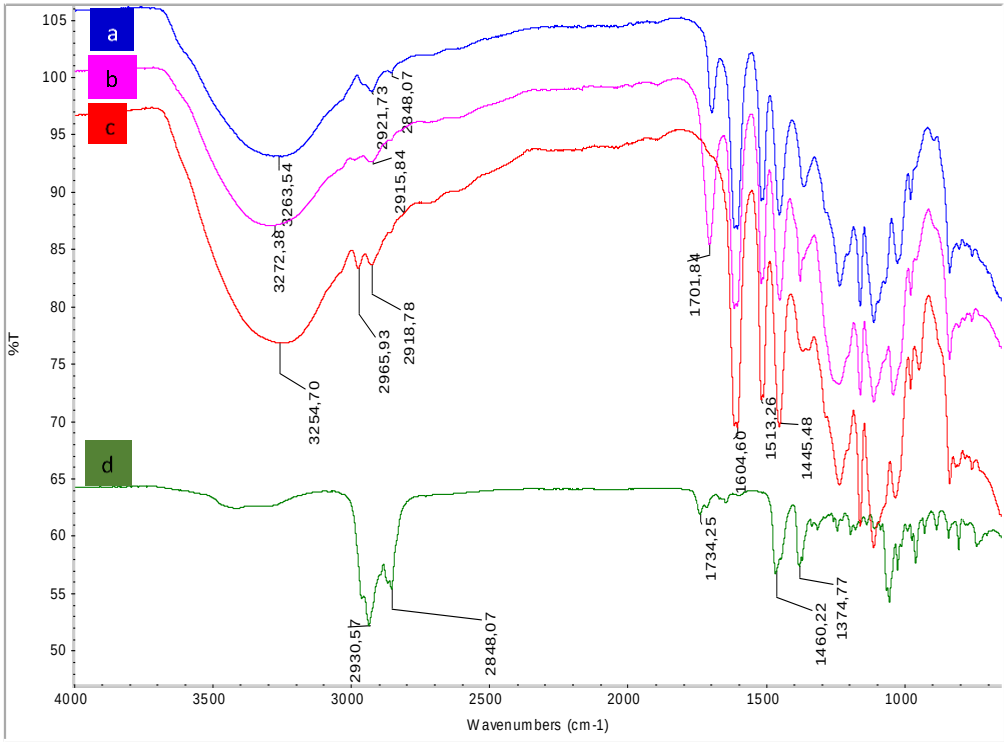


Figure 16: FTIR spectra for four extracts

KEY: a: acetone; **b**: ethyl acetate; **c**: ethanol; d: hexane extracts

FTIR spectra of acetonic, ethanolic and ethyl acetate extracts showed occurrence of peaks at 3254, 3263 and 3272 cm^{-1} respectively indicating stretching vibration of the O-H group. According to Patle *et al*. (2020), the O-H stretching frequencies range always occurs between 3700 and 2500 cm^{-1}. The peaks between 2965 and 2845 cm^{-1} for the four extracts indicated the presence of CH_2 asymmetric and symmetric stretching. Peak at 1701 cm^{-1} was assigned to stretching of the carbonyl (C=O) group for acetonic, ethyl acetate and ethanolic extract and three prominent peaks at 1604, 1513 and 1445 cm^{-1} were due to vibration of C=C of aromatic skeleton which are characteristic peaks of the flavanol structure (Sirmah *et al*., 2018). The results of the present study is almost similar to FTIR spectra of *A. tortilis* obtained by Charis *et al*. (2020), particularly the absorption bands in acetonic, ethyl acetate and ethanolic extracts. For hexane extract peaks at 1460 cm^{-1} and 1374cm^{-1} in hexanic extract

indicated presence of C-H bend. The ethanol, ethyl acetate and acetone are suggested to be suitable solvents for extraction because these shoes solvents exhibited functional groups like OH which are reported to be responsible for termiticidal activity of plant extracts.

4.6 Gas Chromatography- Mass Spectroscopy (GC-MS) analysis

Gas chromatography spectra of *Acacia tortilis* heartwood extracts are displayed in Figure 17 while mass spectra is shown in (Figure 18). Compounds that were extracted by hexane, ethyl acetate, acetone and ethanol solvents are showed in (Table 4) with their retention time, molecular weight and percentage peak area. The data indicated that the heartwood of *A. tortilis* is composed of flavonoids, fatty acids, isonitol and triterpenoids.

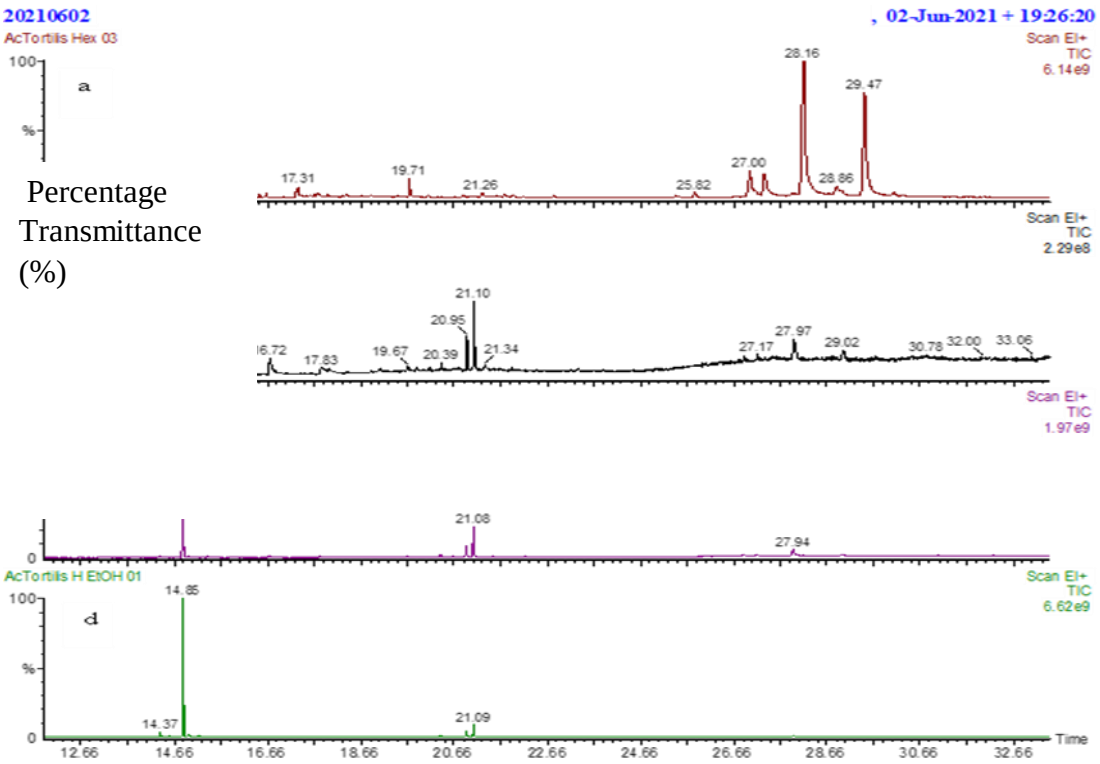


Figure 17: GC chromatogram for a; hexane, b; ethyl acetate, c; acetone and d; ethanol extract

Table 4: Retention time (RT), compound name and percentage peak area of hexane, acetonic, ethanolic and ethyl acetate extracts

Serial No	RT	Compound name	% Peak area				Molecular weight
			Hexane extract	Ethyl acetate extract	Acetonic extract	Ethanolic extract	
1.	3.11	Benzoic acid	-	0.9%	-	-	122.12
2.	8.37	Glycerol	-	6%	-	-	92.09
3.	14.39	9-12,15 octadecatrienoic acid	-	1%	-	-	278.43
4.	14.87	Pinitol	-	28%	59%	84%	194.18
5.	15.31	Phthalic acid	1.4%	-	-	-	166.14
6.	16.07	Hexadecanoic acid	2 %	-	-	-	256.40
7.	16.72	Hexadecanoic acid	-	3%	-	-	256.40
8.	17.31	Oleic acid	2.8	-	-	-	282.47
9.	19.71	3',8,8'-Trimethoxy-3-piperidin-1-yl-2,2'-binaphthyl-1,1',4,4'-tetrone	2.3%	-	-	-	487.50
10.	20.95	Epicatechin	-	4%	5%	5%	290.26
11.	21.01	Catechin	-	8%	13%	11%	290.26
12.	27.00	campesterol	8.9%	-	-	-	400.68
13.	27.94	β-sitosterol	-	5%	-	-	414.71
14.	27.97	Linoleic acid	-	4%	-	-	280.44
15.	28.16	γ -sitosterol	42.2%	-	-	-	414.71
16.	28.86	Botulin	3.7%	-	-	-	442.72
17.	29.47	Lupeol	33.3%	-	-	-	426.72
18.	35.51	Rhodoxanthin	-	7%	-	-	562.82

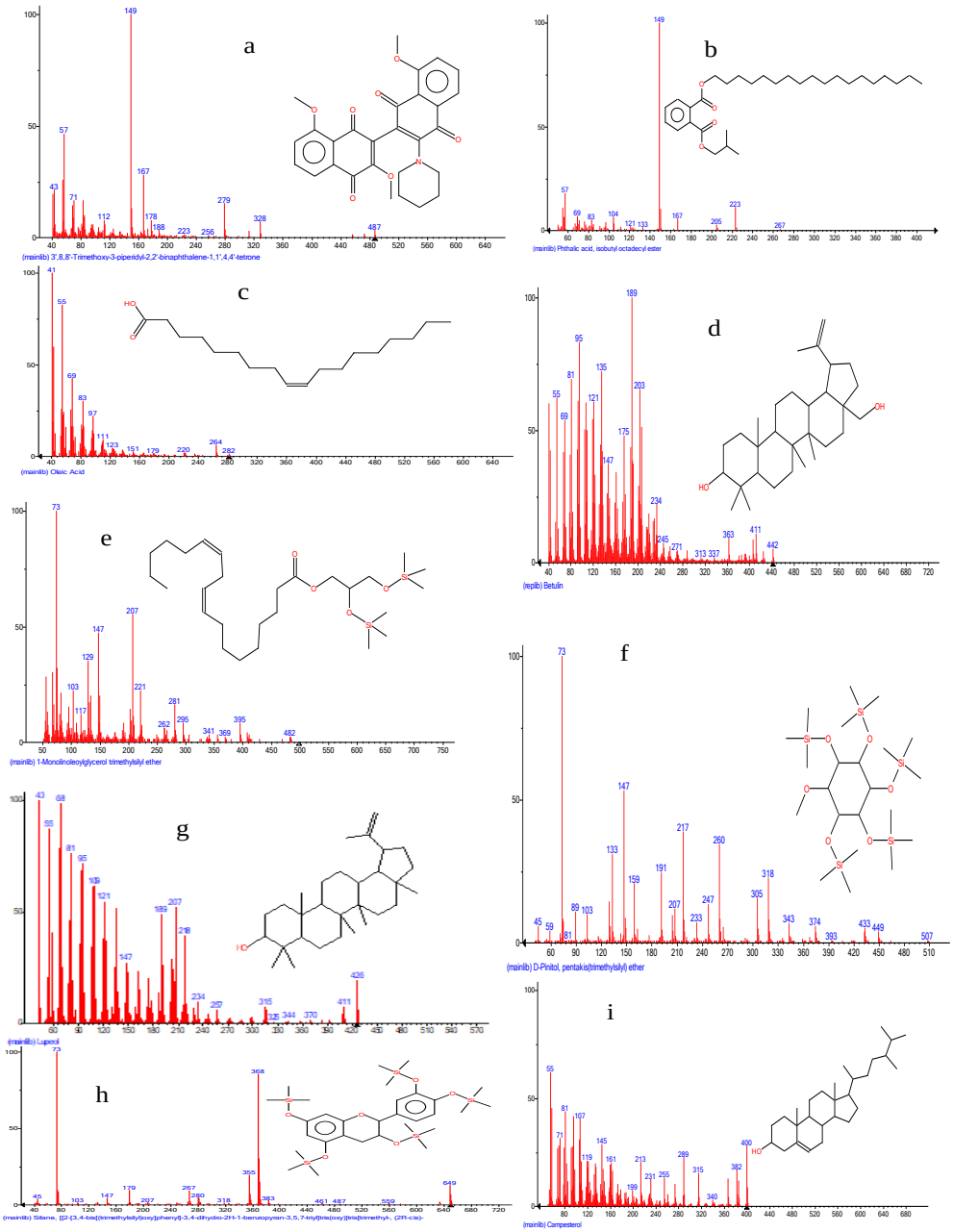
Key: A sign (-) means the compound is not present in the extract

The data in the current study indicated that the heartwood of *A. tortilis* is composed of flavonoids, fatty acids, isonitol and triterpenoids. Heartwood of *A. tortilis* revealed presence of isonitol compound called pinitol which was a predominant compound in ethanol, acetone and ethyl acetate with 84% in ethanolic extract, 59% acetonic extract while ethyl acetate had the least amount of 28%. However, pinitol was not present in the hexane extracts. Pinitol could be absent in hexane extract because of structure of this compound i.e cyclic compound with 5 hydroxyl group which makes it to be more polar hence soluble in polar solvent (ethyl acetate, acetone and ethanol). However, this compound was highly soluble in ethanol which could suggest that the compound is more polar hence soluble in ethanol than in ethyl acetate.

Apart from pinitol, fatty acids like linoleic acid and 9-12,15 octadecatrienoic acid were identified to be present only in ethyl acetate extract, while oleic acid and phthalic acid revealed to be present only in hexane extract. Hexadecanoic acid was eluted in both ethyl acetate extract at 16.72 mins and hexane extract at 16.07 mins. This compound was eluted at different retention times likely because ethyl acetate extract was derivatized while hexane extract was not derivatized before running in the GC-MS. On the other hand, fatty acids were insoluble in ethanol and acetone solvent.

Triterpenoids such as campesterol, lupeol and botulin were found to be present in hexane extract with lupeol in high percentage 33.3%. In addition to triterpenoids a 3',8,8'-Trimethoxy-3-piperidin-1-yl-2,2'-binaphthyl-1,1',4,4'-tetrone was exhibited in hexane extract. β-sitosterol (5%) and γ-sitosterol (42.2 %) were observed in ethyl acetate and hexane extracts respectively. Two types of catechin were observed at retention time of 20.95 and 21.01 minutes in ethyl acetate, acetonic and ethanolic extracts. The two compounds were suggested to be catechin at 20.95 mins and epicatechin at 21.01 mins this because of close retention time which could suggest that

the two compounds are isomers. The low composition was observed in ethyl acetate extract i.e epicatechin (4%) and catechin (8%) (Table 6). In addition, ethyl acetate extracts showed presence of benzoic acid and rhodoxanthin.



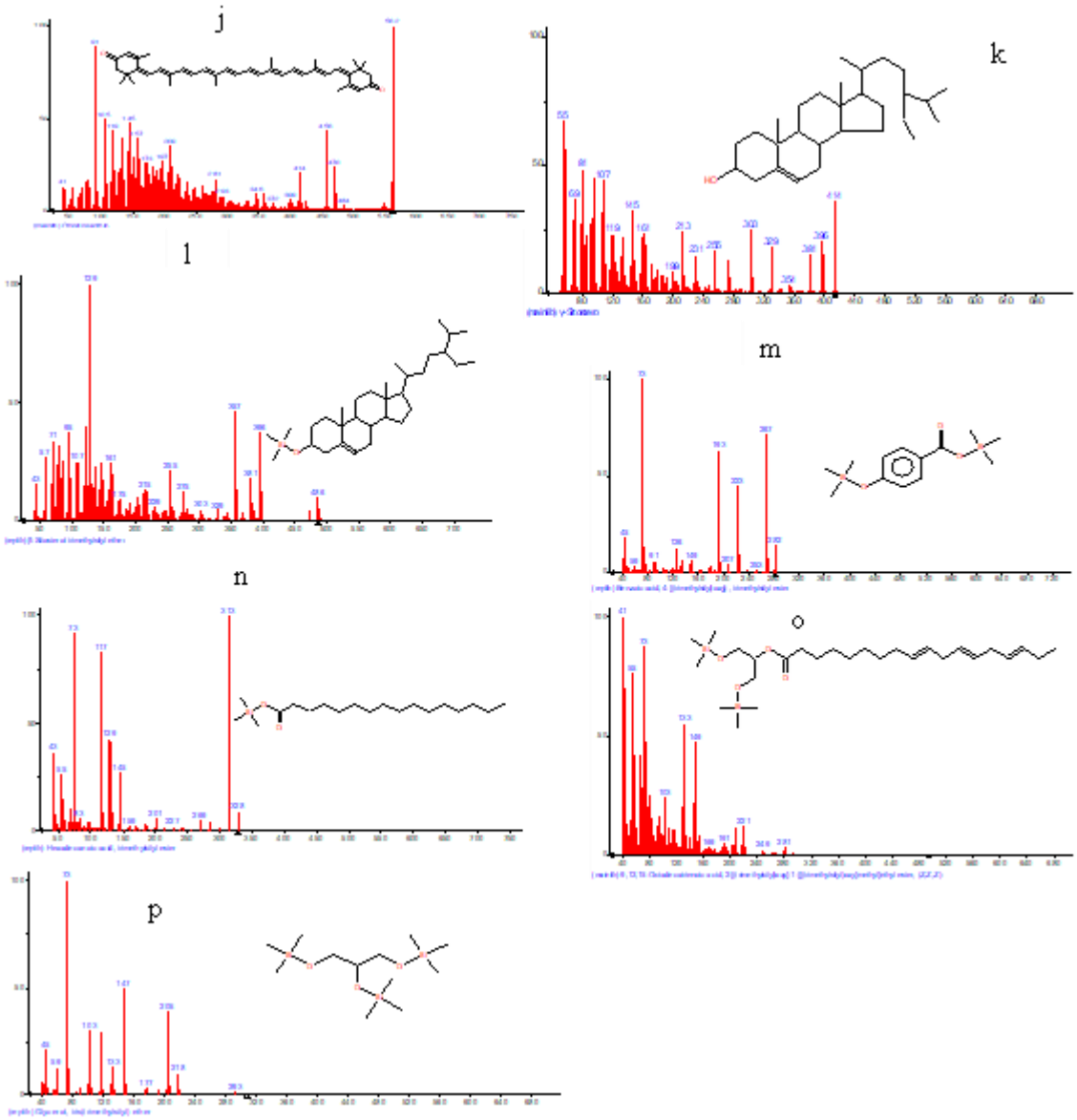


Figure 18: MS spectra and chemical structure of compounds in ethanol, ethyl acetate, hexane and acetone extract of *A. tortilis* heartwood extractives. Compounds with Trimethylsilyl (TMS) derivatizes were for ethyl acetate, ethanolic and acetonic extract after derivatization

Key: a-3',8,8'-Trimethoxy-3-piperidin-1-yl-2,2'-binaphthyl-1,1',4,4'-tetrone, **b**-Phthalic acid, **c**-Oleic acid, **d**-Botulin, **e**-Linoleic acid with 2TMS, **f**-D-pinitol with 5TMS, **g**-Lupeol, **h**-Catechin with 5TMS, **i**-Campesterol, **j**-Rhodoxanthin, **k**-γ- Sitosterol, **l**- β-Sitosterol with 1TMS, **m**-Benzoic acid with 2TMS, **n**-Hexadecanoic acid with 1TMS, **o**-9-12,15 octadecatrienoic with 2TMS acid and **p**-Glycerol with 3 TMS.

D-pinitol is known as 3-O-methyl-D-chiro- inositol which belongs to the family of inositol, they are generally cyclitol (cyclic polyol) and they occur naturally in plants. It has been reported to possess many therapeutic properties like anti-diabetic, anti-inflammatory activity, antioxidant, hepatoprotective, immuno-modulator, anti-cancer and anti-osteoporosis (Srivastava *et al*., 2020). It has been well stated that D-pinitol accumulates in large quantities in Acacia species due to water stress environment in order to help in osmoregulation (Liu *et al*., 2008). Perhaps this is the reason why it is found in high quantities in the heartwood of *A. tortilis* with 84% in ethanolic extract. Seigler (2003) also reported that (+)-Pinitol has been found in the bark of *A. longissima*, *A. mearnsii*, *A. obtusifolia*, and *A. orites* and *A. sieberiana var. woodii*. D-pinitol was also isolated in *Acacia senegal* root heartwood (Jain, 2012). In the present study ethanolic extracts showed highest amount of D-pinitol with 84% followed by acetonic 59% and lastly ethyl acetate extract with 28% (Table 4).

In the current study hexadecanoic acid, oleic acid, 9-12, 15 octadecatrienoic acid and linoleic acids are fatty acids that were observed to be present in *A. tortilis* heartwood. In a study by Moghanloo *et al*. (2015), they reported that fatty acids such as linoleic acid (70.0%), palmitic acid (20.6%) and vaccinic acid (2.1%) were present in *A.tortilis* seed. On the other hand, approximately 100 to 110 fatty acids have been isolated from different species of genus Acacia (Madjid O. *et al*., 2020).

Two catechins that were eluted in different but close retention times is an indication of presence of a catechin stereoisomer called epicatechin. Catechin has been reported to be present in the bark of *A. farnesiana, A. tortilis* and *A. longifolia* (Gabr *et al*., 2018).

In hexane extract, triterpenoids were dominant with γ- sitosterol observed in largest amount of 42.2 % while lupeol was 8.3 % which was actually lower than γ-sitosterol

(Table 5). Campesterol on the other hand was at 8.9 % and botulin was 3.7 %. β-sitosterol has been reported to be found in *A. tortilis* leaves (Muhaisen, 2021). On the other hand, other species of Acacia i.e *A. nilotica* and *A. hybrid* has been reported to have triterpenoid compounds (Misran, 2011).

4.7 Liquid Chromatography-Photodiode Array-Mass spectroscopy (LC-PDA-MS) Analysis

Results from the LC-PDA-MS revealed that ethyl acetate, acetonic and ethanolic extract is composed of catechin, afzelechin-epicatechin, afzelechin, afzelechin dimer, ellagitannin, and gallotannin. These compounds were detected based on the retention time in the liquid chromatography and mass to charge ratio (*m/z*) in the mass spectroscopy. Identification of compounds was based on reference to literature. LC chromatogram profile is shown in figure 19 and retention time, percentage peak area and *m/z* of identified compounds are shown in Table 6 below.

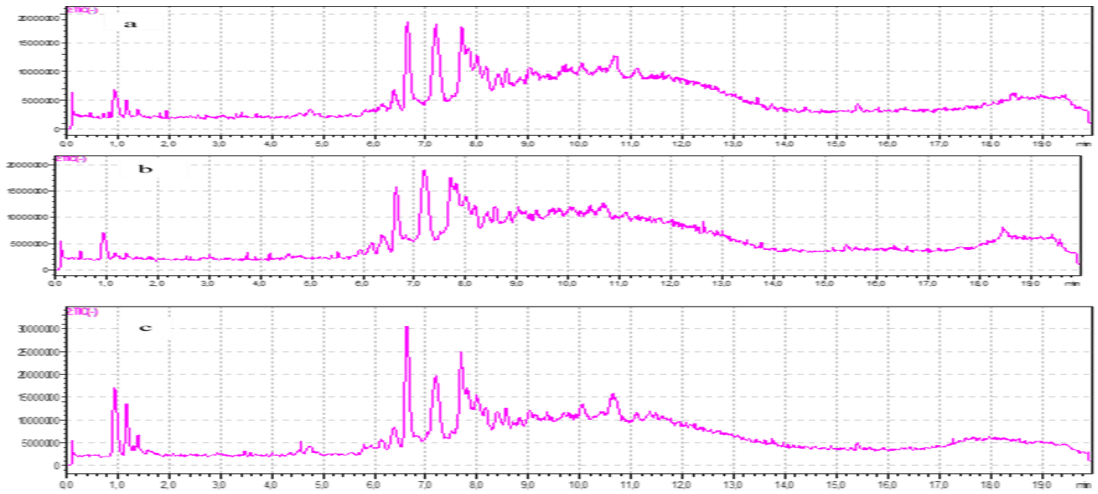


Figure 19: LC chromatogram of a: acetone, b: ethyl acetate and c: ethanol extract

Table 5: Charge/mass ratio, wavelength, identified compounds and the quantity (% peak area) from the extracts of *A. tortilis* heartwood using the LC-PDA-MS

Retention time	**Charge/ mass ratio (*m/z*) in the negative mode**	**Max wavelength (nm)**	**Tentative identified compounds**	**% Peak area of extracts**		
				Acetonic	**Ethanolic**	**Ethyl acetate**
4.5	289	278	Catechin	0.2	0.4	0.1
6.5	561	280	(Epi)afzelechin-epicatechin	1.1	1.3	0.8
6.6	273	280	Afzelechin	2.7	3.8	2.9
7.2	545	279	(Epi)afzelechin dimer	4.8	4.9	6.3
9.0	817	279	Ellagitannin	1.3	1.4	2.5
10.6	801	279	Gallotannin	5.3	5.6	1.2

Catechin was eluted at retention time 4.5 minutes with a *m/z* of 289 in negative mode while in the PDA detector there was an absorbance at maximum wavelength of 278 nm (Figure 20). This is evidential that the compound eluted is catechin. As well the *m/z* is in agreement with other researchers (Chang & Wu, 2011). At retention time 6.6 minutes a compound with *m/z* of 273 (Figure 19) in the negative mode was observed. The compound was tentatively identified as afzelechin. This is in reference to the work by de Souza *et al*., (2008).

Two proanthocyanidin dimers were detected in the LCP-PDA- MS. One at *m/z* 545 and observed at 279 nm in the PDA detector was suggested to be Afzelechin dimer and the other one was Afzelechin–Catechin at *m/z* 561 and an absorbance at maximum wavelength of 280 nm. The suggestion of names of compounds eluted was in accordance with Souza *et al*., 2008. Two hydrolysable tannins were also detected to be present in the heartwood of *A. tortilis* with *m/z* of 817 and 801 at retention time 9.0 and 10.6 minutes respectively. A compound with *m/z* of 801 was tentatively identified as gallotannin. This is in agreement with what was observed by Wyrepkowski *et al*. (2014). They identified a compound with *m/z* of 801 and UV peak at 273 nm as gallotannin. A compound with *m/z* of 817 was assigned to ellagitannin and this was also in agreement to a study done by Lee *et al*. (2005).

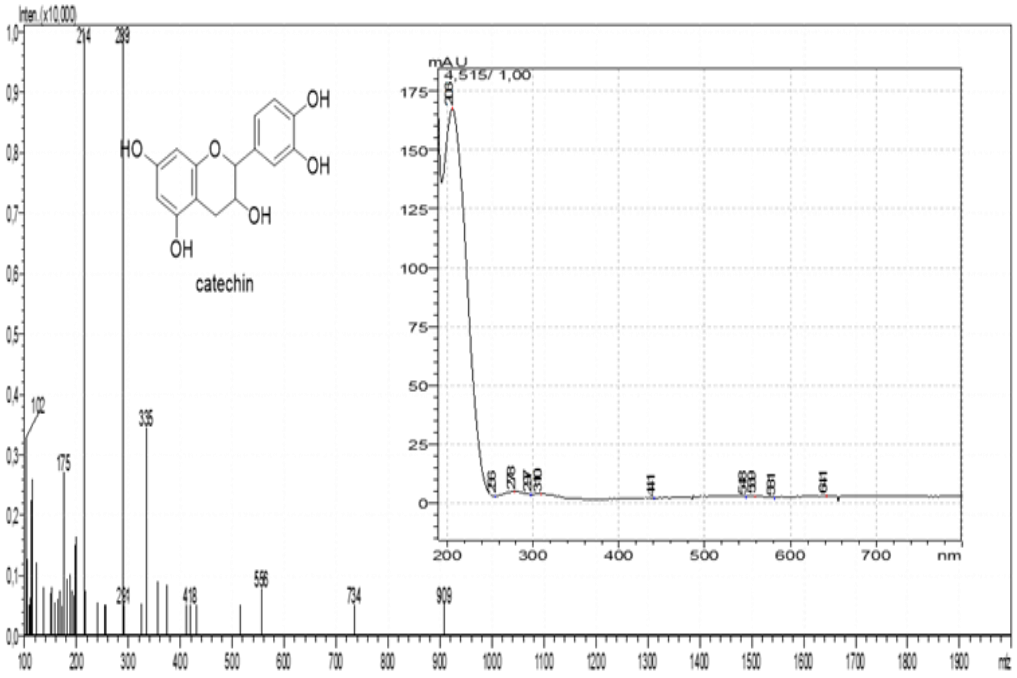


Figure 20: MS spectra, PDA spectra and chemical structure of catechin

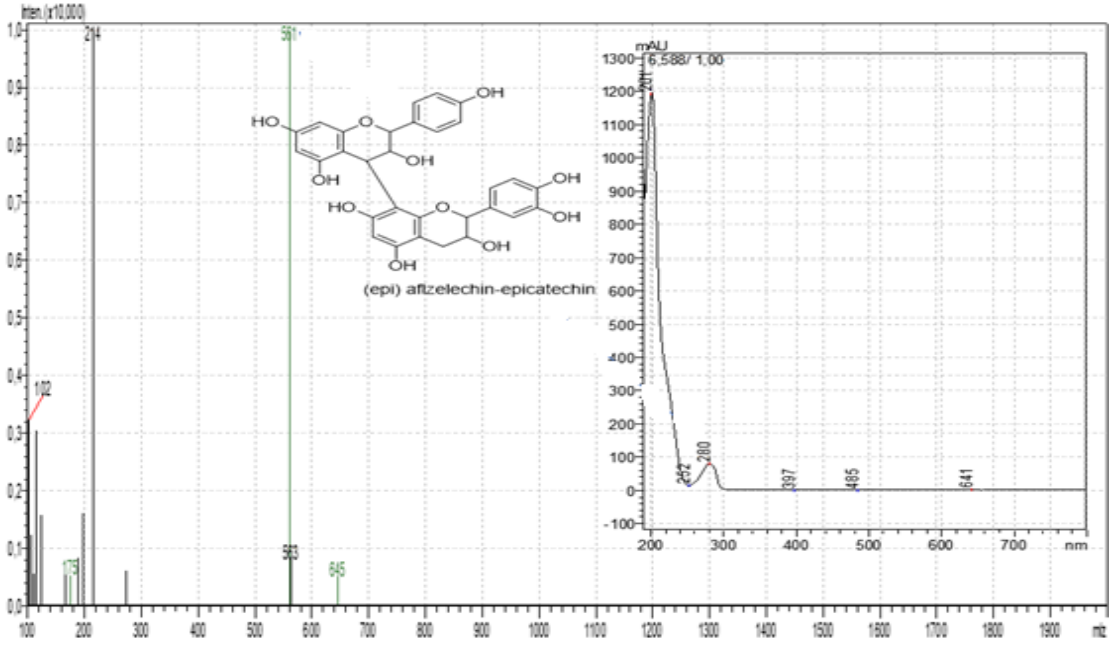


Figure 21: MS spectra, PDA spectra and chemical structure of (epi)afzelechin-epicatechin

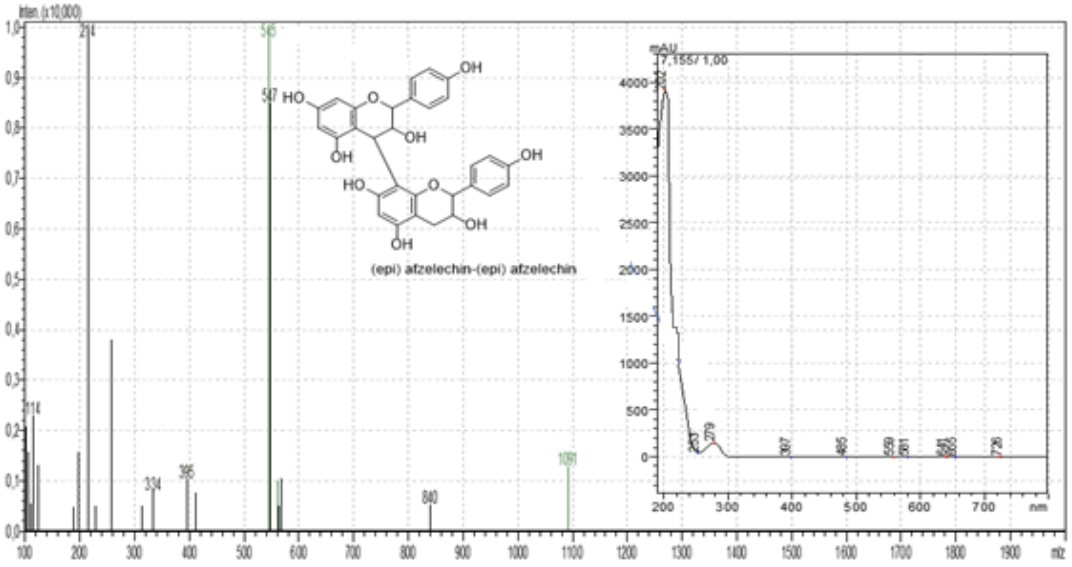


Figure 22: MS spectra, PDA spectra and chemical structure of (epi) afzelechin- (epi) afzelechin

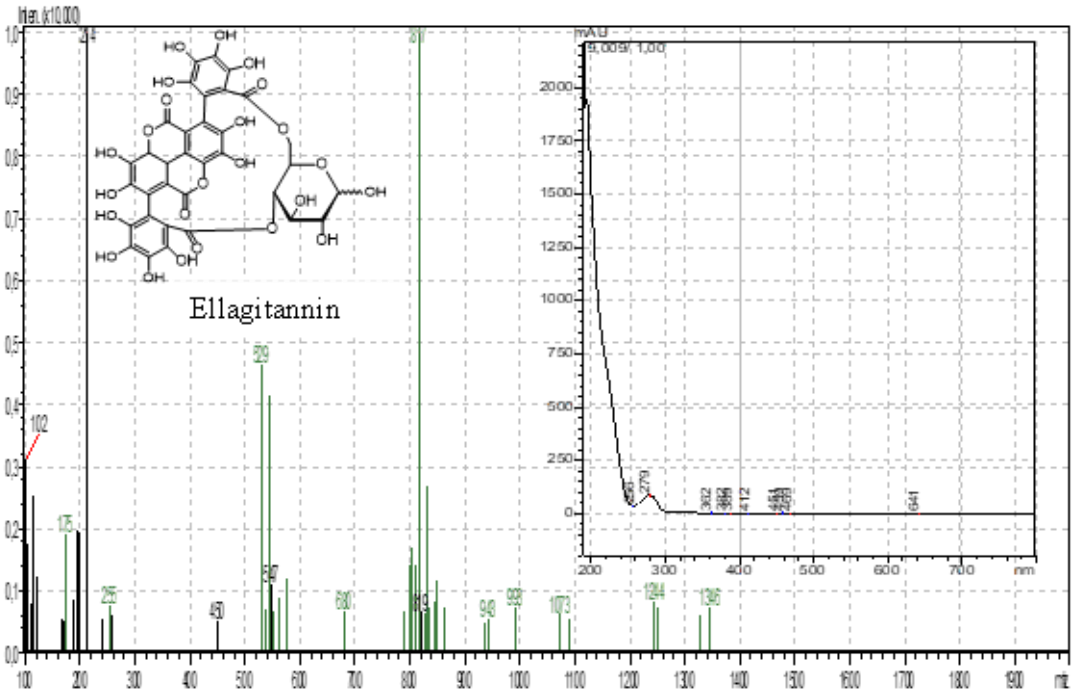


Figure 23: MS spectra and PDA spectra of ellagitannin

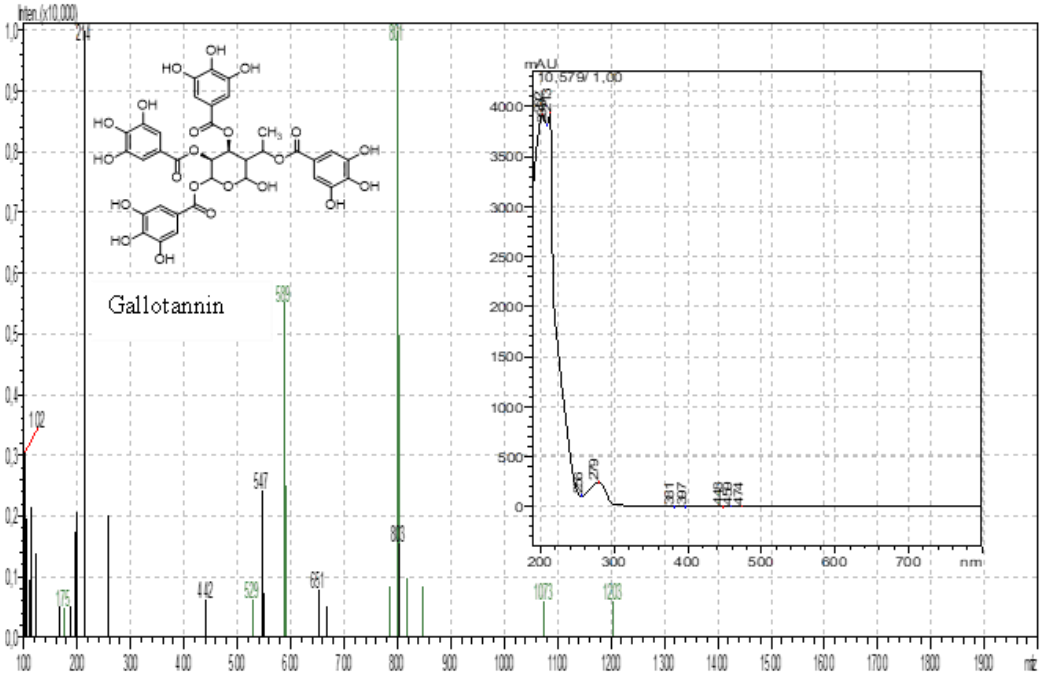


Figure 24: MS spectra and PDA spectra of Gallotannin

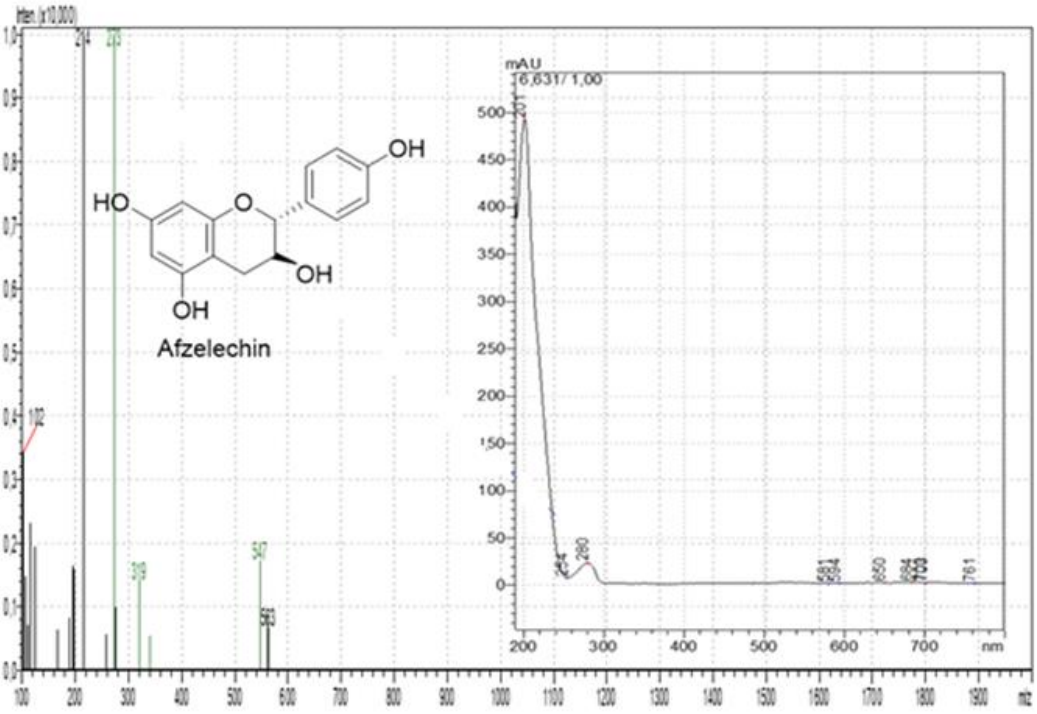


Figure 25: Mass spectra, PDA spectra and chemical structure of Afzelechin

4.8 Anti Termite Activity of *Acacia tortilis*

Results of anti-termite activity tests of *Acacia tortilis* heartwood and *Pinus sylvestris,* which was used as a positive control, were evaluated by calculation of percentage

weight loss after field exposure to termite (*Macrotermes natalensis*) attack. Figure 26 below shows the extent of *Macrotermes natalensis* attack of wood blocks after 2 months of field exposure while Figure 27 below shows the calculated percentage weight losses from the second to fifth month.

Figure 26: Extent of termite attack on blocks after field exposure for 2 months

Key: **U3 and U24:** Unextracted blocks of *A. tortilis*, **H3 and H19**: hexane extracted blocks of *A. tortilis*, **A21 and A5:** acetonic extracted blocks of *A. tortilis*, **E5 and E15:** ethyl acetate extracted blocks of *A. tortilis*, **T10 and T24:** ethanolic extracted blocks of *A. tortilis* and **P16 and P2:** *P. sylvestris* blocks **(unextracted).**

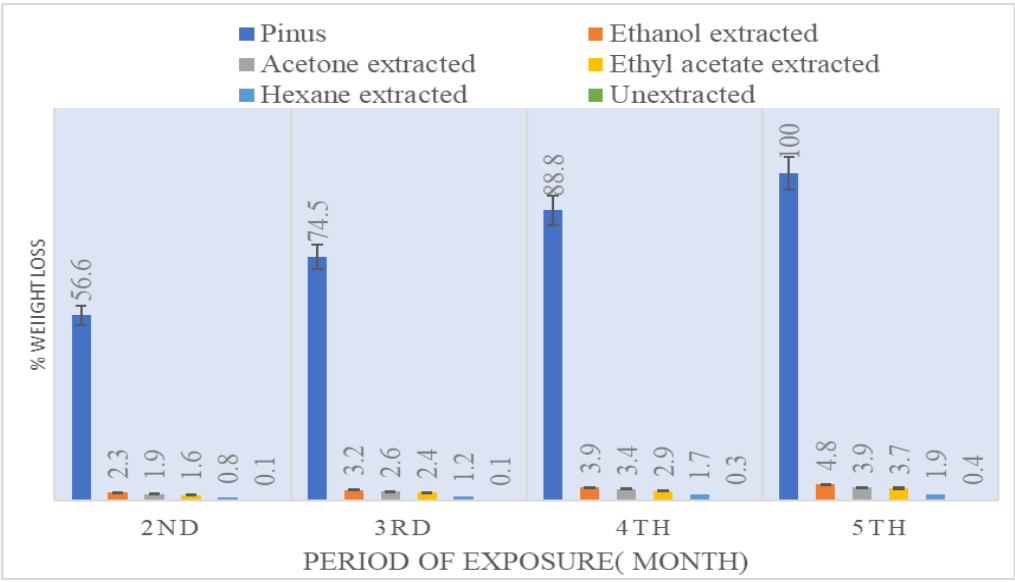


Figure 27: Weight loss of blocks after field exposure for five months

Key: Hexane, ethyl acetate, acetone and ethanol extracted were *A. tortilis* that were Soxhlet extracted before termite field exposure and Pinus Sylvestris was not extracted.

It is seen that *P. sylvestris* was highly susceptible to *Macrotermes natalensis* attack (Figure 26). However, the *A. tortilis* was resistant when exposed to similar termite species that was exposed to *P. sylvestris*. After calculating the weight losses (Figure 27) after 5 months of exposure, it was evident that *M. natalensis* generally did not attack the unextracted *A. tortilis* heartwood blocks but strongly attacked *P. sylvestris* (100%) while extracted *A. tortilis* heartwood blocks became susceptible to *M. natalensis* depending on the solvent of extraction. The *A. tortilis* blocks that were extracted with hexane were least attacked (1.9%), followed by ethyl acetate (3.7%), acetonic (3.9 %) and ethanolic extracted showed more attack (4.8 %).

The results obtained are in line with the amount of extract yield (Figure 11) where the hexane with low yield (0.15%) showed lowest attack and ethanol that gave high yield (10.75%) was preferably attacked by *M. natalensis.* This suggests that when most of the compounds responsible for anti-termite activity are removed from the wood, it becomes susceptible to termite attack as in the case of the ethanolic extracted blocks. From the GC-MS and LC-PDA-MS analyses, the extractives from the ethanolic extract were polar in nature. This suggests that the termiticidal activity of *A. tortilis* heartwood can be attributed to polar compounds like catechin, epicatechin afzelechin and hydrosable tannin. This is further confirmed where extracts which showed high total phenolic content (TPC) of 350.1 ± 0.10, 169.3 ± 0.15, and 157.6 ± 0.06 mg GAE/g for ethanolic, acetonic and ethyl acetate respectively of dry extract became most susceptible to termites this indicated that these solvents could have extracted phenols that are present in the heartwood of *A. tortilis* that plays a role in the protection of wood against termite attack. Wood blocks extracted by hexane solvent which had the least TPC of 10.7 ± 0.10 mg GAE/g of dry extract were least attacked by termites.

The huge weight loss of *P. sylvestris* (100%) after five months of exposure to termite attack in the field shown in (Figure 27) than both extracted and unextracted indicated that *A. tortilis* is resilient against *Macrotermes natalensis* attack which may be related to extractive components in *A. tortilis* heartwood. This is supported by a study by Mburu *et al*. (2007),in this study they reported that *Prunus africana* is highly resistant to termite attack and it is due to presence of extractive components in the heartwood. According to Nascimento *et al*., (2013) suggested that when extractives are removed from wood, it decreases its resilience to termite attack. This could be the reason why extracted wood blocks of *A. tortilis* showed weight loss as seen in Figure 27. Further confirmation of contribution of extractives to anti-termite activity was shown by Hassan *et al*. (2018) when they evaluated heartwood extractives of *Tectona grandis* Linn against *Heterotermes indicola.* In their study, they reported that termites generally did not attack un-extracted wood of *T. grandis*, instead they attacked more of the extracted wood 15.6%.

Removal of extractives from *A. tortilis* heartwood subsequently led to weight losses of wood blocks. On the other hand, the weight losses were dependable on the solvent used for extraction. Wood blocks extracted with hexane revealed low percentage weight loss of 1.9 % after five months of exposure. This is attributed to the fact that hexane did not extract all of the compounds present in the heartwood which are responsible for anti-termite activity of *A. tortilis*. While, wood extracted with ethanol, ethyl acetate and acetone showed moderate weight loss (Figure 27). This demonstrated that these solvents are able to extract some of extractives present in the heartwood of *A. tortilis*, which could be possessing anti-termite activity. This results were in agreement with previous studies where anti-termite activity of heartwood of *Calophyllum inophyllum* L was determined and attack of wood blocks was solvent dependable (Kadir *et al*.,

2015). Samples extracted with methanol resulted in a higher susceptibility of *C. inophyllum to C. curvignathus* (45.49%) followed by ethanol (21.96%) and samples extracted with petroleum ether were least susceptible (6.64%).

Natural repellency and toxic properties of wood resilient to termite attack is related to presence of terpenoids, quinones, flavonoids and fatty acids (Ganapaty *et al*., 2004). Therefore, weight loss of extracted wood blocks of *A. tortilis* (Figure 27 above) can be attributed to removal of compounds like β- sitosterol, γ sitosterol, lupeol, afzelechin, hexadecanoic acid, catechin, epicatechin, D-pinitol, ellagitannin and gallotannin (Table 5 and 6). Compound like catechin has been reported to exhibit anti-termite, insecticidal and antioxidant activities (Tascioglu *et al*., 2012). Whereas in a study by Adfa *et al*. (2017), it was found out that isolated (+)-catechin from *Toona sinensis* had the highest anti-termite activity. Therefore, the removal of catechin and epicatechin could be the reason why the wood blocks that were extracted with acetone, ethyl acetate and ethanol solvents were attacked more by termites than those extracted with hexane because there was absence of these compounds in hexane extracts.

The termiticidal activity of catechin and afzelechin could be due to presence of hydroxyl groups at C-5 and C-7 in the A-ring as well as 3',4'-dihydroxylated B-ring (Nascimento, et al., 2013). According to (Ismayati *et al.*, 2018), hydroxyl group in C-5 and C-7 of A ring of compound is important for anti-termite activity. This is because hydroxyl groups inhibit digestive enzymes like β-Glucosidase by binding to them, this eventually affects the digestion of nutrients by termite (Ares, 2017).

Catechin is known to be monomeric unit of condensed tannin. These tannins play important role in plants since they have repellency activity against insects. 'Astringency' nature of tannins makes the plant tissue inedible and they either

precipitate or immobilize enzymes hence preventing attack of host by the parasite (Ohmura & Ohara, 2000). In addition, tannins inhibit growth of insects and inhibit β-glucosidase and many other cellulose-degrading enzymes. Apart from these properties of tannins mentioned above, it has been reported that phenolic compounds act as inhibitor of enzymes in the termites' gut leading to dead of termites when they digest these compounds (Zulfiqar *et al.*, 2020). The phenolic compounds binds to the active site of enzymes leading to inhibition of the detoxification function of the enzyme (Hassan *et al*., 2018). Hassan *et al*. (2017) reported that phenolic compounds like flavonoids present in naturally durable wood are not just exhibiting anti-termite activity but as well they are rich source of antioxidants with radical scavenging activity which may lead to termite mortality. The authors added that phenolic compounds present in the heartwood also have effect on termites because they kill protozoan fauna that helps in their digestion.

Previously, it has been reported that methylated cyclitols such as pinitol are usually broken down only by a few number of microorganisms, suggesting that they might contribute to resistance of wood tissues to fungal attack (Silva *et al*., 2007). The predominance of pinitol in the ethanolic, acetonic and ethyl acetate extracts (table 4) is a clear indication that the susceptibility of the wood blocks to termite attack could be due to removal of pinitol. According to the research by Veillon (2011), toxicity of Myo-inositol compound was conducted and it was found that this compound increase the mortality of Formosan subterranean termite as well as decreases the feeding behavior of termites.

Decanoic acid is a carboxylic acid that has a characteristic smell. The smell is assumed to stimulate the nerve of an insect so as to either attract or repel insects (Sudrajat *et al*., 2018). Therefore, the susceptibility to termite attack of wood extracted with ethyl

acetate can be related to removal of hexadecanoic acid (Table 4). Whereas those wood blocks extracted with hexane could be attributed to removal of lupeol, β- sitosterol and γ-sitosterol. These three compounds have been reported to be toxic, and possess antifeeding and repellent properties against termites and other insects (Stone *et al*., 2020).

CHAPTER FIVE

CONCLUSION AND RECOMMENDATIONS

5.1 Conclusions

1) From the findings, the study concludes that *Acacia tortilis* is composed of useful secondary metabolites such as flavonoid, phenol, tannins, alkaloids, saponins and terpenoids. These compounds were extractable depending on the solvent of extraction. Difference in the solubility of these phytochemical compounds contributes to difference in extraction yield and total phenolic and flavonoid content.
2) It is concluded that *A. tortilis* contains extractive components like catechin, epicatechin, hexadecanoic acid, sterol, pinitol, ellagitannin and gallotannin that could be responsible for resilience of *A. tortilis* to termite attack.
3) The test of anti-termite activity in the field showed that wood blocks that were extracted with different solvents showed weight loss that was dependable on the extractive content in the wood blocks. Therefore, the current study concludes that extractives play important role in protection of wood against termites.

5.2 Recommendations

1) Isolation of pure compounds from crude extracts and bioassay of isolated compounds to be conducted in order to understand the anti-termite activity of identified compounds if it is synergistic or it is by a single compound that were identified to be present in the extract.
2) The field tests can be conducted for a longer period of time to evaluate if environmental factors like temperature, rainfall and relative humidity affects termite ability to attack *A. tortilis* heartwood.

3) Efficacy of anti-termite activity of *A. tortilis* extracts against termites can be investigated considering impregnation of non-durable wood species with *A. tortilis* extracts and commercially known termicides.

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