THÈSE

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Et
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Etude de la variabilité et modification chimique du Mesquitol, extractible polyphénolique de *Prosopis juliflora:* Application à la préservation du bois

Variability study and chemical modification of Mesquitol, a polyphenolic extractive of *Prosopis juliflora:* Application to wood preservation

Spécialité : Sciences du Bois et des Fibres

Par

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Applied research generates improvements, not breakthrough. Great scientific advances spring from pure research… Jacques Cousteau

DEDICATION

This thesis is dedicated to my parents, Mr. and Mrs. Chepkwony for their unwavering support, love and encouragement throughout this work. Mum, may you get well soon.

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LIST OF ABBREVIATIONS

ANR : Anthocyanidin reductase

ANS : Anthocyanidin synthase

ASE : Accelerated Solvent Extraction

CCK : Cummins Cogeneration Kenya

CHI : Chalcone isomerase

CHS : Chalcone synthase

CLHP-SM : Chromatographie en phase liquide haute performance - spectrométrie de masse en tandem

^{13}C NMR : Carbon Nuclear Magnetic Resonance

Cp : *Coniophora puteana*

Cv : *Coriolus versicolor*

DHF : Dihydroflavonol

DNA : Deoxyribonucleic acid

DPPH : 2, 2-diphenyl-1-picrylhydrazyl

FAEP : Fuelwood Afforestation Extension Project

F3H : Flavanone-3-hydroxylase

F3'H : Flavonoid-3'-hydroxylase

FLS : Flavonol synthase

FNR : Flavanone 4-reductase

FT-IR : Fourier Transform Infrared

GC-MS : Gas chromatography – mass spectrometry

GISD : Global Invasive Species Database

^{1}H NMR : Proton Nuclear Magnetic Resonance

LAR : Leucoanthocyanidin reductase

LC-ESI-MS/MS: Liquid Chromatography Electrospray Ionization Tandem Mass Spectrometry

LERMaB : Laboratoire d'Etude et de Recherche sur le Matériau Bois

MAE : Microwave-Assisted Extraction

NIST : National Institute for Standards and Technology

QM : Quinone Methide

RDA : Retro-Diels-Alder

Rf : Retention factor

R.H. : Relative humidity

SN_2 : Bimolecular substitution

t_a : Ambient temperature

TBAI : Tetra-n-butylammonium iodide

TFA : Trifluroacetic acid

TIC : Total Ion Current

TMS : Tetramethylsilane

UFGT : UDP-glucose flavonoid 3-O-glucosyltransferase

UV : UltraViolet

WL : Weight Loss

WPG : Weight Percentage Gain

WPGAL : Weight Percentage Gain after Leaching

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CHAPITRE 1

INTRODUCTION GÉNÉRALE

1.0 INTRODUCTION GÉNÉRALE

Prosopis juliflora est un arbuste, ou parfois un arbre, réputé pour son extrême adaptation aux terres arides. Il a donc été introduit au Kenya pour lutter contre la désertification (Pasiecznik, 2001). Traditionnellement, *P. juliflora* est utilisé comme remède contre diverses maladies telles que la dysenterie, la rougeole, le rhume, le goût, les maux de gorge, la diarrhée, les excroissances, la grippe, les affections dermatologiques, l'indigestion, les problèmes oculaires comme la cataracte, l'inflammation et la cicatrisation des plaies (Khandelwal *et al.* 2015).

Il a récemment suscité un vif intérêt pour les scientifiques en raison de l'un de ses constituants : reconnu pour ses propriétés antioxydantes (Lakshmibai, 2016; Sirmah, 2011; Azam *et al*., 2011). En effet, une étude antérieure réalisée au LERMab (Laboratoire d'Etudes et de Recherche sur le Matériau Bois) a montré que le bois de cœur de *P. Juliflora* contient de grandes quantités de Mesquitol, un dérivé naturel relativement rare de type flavan-3-ol, identifié sous le nom de 2- (3,4-dihydroxyphényl) chromane-3,7,8- triol, également connu sous le nom de (-) - mesquitol (Sirmah *et al*., 2009).

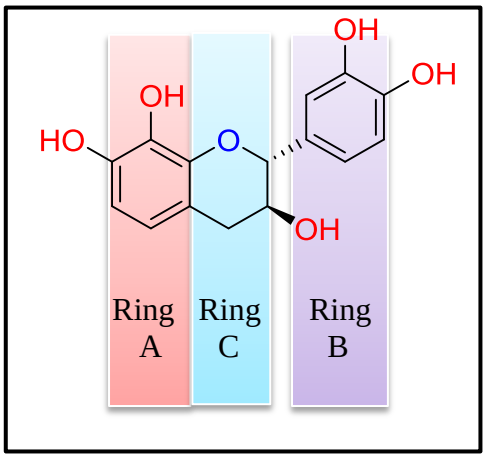


Fig **1:** Structure du (-)-mesquitol

Au fil des années, l'espèce *P. juliflora* est devenue une menace majeure pour l'agriculture, étant une espèce très invasive qui se répand dans les champs cultivés, les pâturages, les zones humides et les rives des lacs (Muturi, 2012; Shackleton *et al*., 2014). Selon Muturi (2012), les forêts riveraines et les zones humides saisonnières sont les zones les plus envahies par *Prosopis* au Kenya.

Cette invasion du *P. juliflora* est également favorisée par sa propriété allélopathique qui influence la croissance des plantes qui poussent dans son voisinage par la libération d'allélochimiques, à savoir des produits chimiques toxiques produits par une plante dans le but d'exercer un effet physiologique néfaste sur les individus d'une autre espèce (Santosh et Gowsiya, 2013).

Une enquête réalisée auprès des communautés locales autour du lac Baringo au Kenya a montré que 85 à 90% des personnes sondées étaient favorables à l'éradication complète de l'espèce envahissante *Prosopis* (Mwangi and Swallow, 2008). Un rapport connexe décrit les difficultés d'une communauté connue sous le nom d'Ilchamus au Kenya à vendre leur viande en raison du mauvais goût résultant de la consommation de *P. juliflora* par le bétail (BBC news report, 2006; UNEP 2004).

Les efforts visant à éradiquer complètement la plante ont jusqu'à présent échoué, principalement en raison de la pénétration profonde de ses racines pivotantes qui pourraient atteindre les nappes phréatiques à plus de 50 mètres de profondeur (Raven *et al.*, 2005). Selon des études réalisées en Érythrée par Bokrezion (2008), abattre les arbres de *Prosopis* s'est également révélé inutile. Il a signalé qu'une majorité des Érythréens avaient pris les choses en main et avaient tenté d'éradiquer les plants de *Prosopis*, mais qu'après de nombreuses tentatives, très coûteuses, le *Prosopis* avait repoussé dans les semaines qui avaient suivi (Bokrezion, 2008).

La prolifération incontrôlée de *P. juliflora* sur les terres arables ne répond pas à l'objectif initial, i.e. la lutte contre la désertification, et constitue à présent une nuisance. Cette étude vise donc à explorer des voies d'utilisation de cette essence à travers la valorisation du mesquitol qui est l'un des extractifs du *P. juliflora.* Cette molécule présente des propriétés antioxydantes, ce qui permet d'envisager plusieurs applications potentielles dans l'industrie alimentaire, les cosmétiques ou comme matière première pour des matériaux innovants.

Par conséquent, cette étude tente de répondre à une problématique actuelle du Kenya en utilisant une plante nuisible en tant que matière première d'intérêt pour la génération de produits à valeur ajoutée. L'abondance du mesquitol dans le bois de coeur, facile à extraire juste par extraction au solvent, justifie également le choix de cette plante spécifique.

Les objectifs spécifiques de l'étude étaient:-

1) Déterminer le meilleur solvant et la meilleure méthode d'extraction du mesquitol.
2) Etudier la variabilité intraspécifique géographique et naturelle des substances extractibles de *P. juliflora.*
3) Mettre au point des méthodes de synthèse de produits dérivés du mesquitol
4) Caractériser les propriétés des dérivés synthétisés et évaluer le potentiel de certains des dérivés à utiliser comme agent antifongique.

L'étude de *P. juliflora* dans le but de valoriser le mesquitol a été réalisée en deux parties complémentaires. La première partie traite de la variabilité intraspécifique naturelle des substances extractibles sur plusieurs échantillons de *P. juliflora* venant d'arbres de différentes régions du Kenya. L'objectif est d'étudier la variabilité de la distribution des substances extractibles du bois au sein de plusieurs arbres de *P. juliflora* des trois différentes régions géographiques du Kenya afin de mieux comprendre la disponibilité de cette ressource en vue de réfléchir à sa mobilisation pour une valorisation industrielle ultérieure.

Des échantillons de plantes ont été collectés dans trois zones géographiques différentes; le comté de Baringo (latitude 0 ° 28 '0 "N, longitude 35 ° 58' 0" E), le comté de Garissa (latitude 0 ° 27 '09 "S, longitude 39 ° 38' 45" E) et le comté de Turkana (latitude 03 ° 09 'N, longitude 35 ° 21' E) au Kenya. Les échantillons provenaient de petits arbres âgés de moins de 4 ans et de grands arbres âgés de plus de 8 ans.

Le test de variabilité a été réalisé en comparant les pourcentages de rendement en substances extractibles du bois utilisant différents solvants (dichlorométhane, acétone, toluène: éthanol (2:1) et eau), puis en identifiant, quantifiant et comparant certains composés présents dans les différents extraits provenant de différentes régions géographiques du Kenya par une chromatographie en phase liquide haute performance en phase inverse couplée à une ionisation par électrospray - spectrométrie de masse en tandem - CLHP-SM. Cette partie impliquait également le développment d'un protocole d'extraction du mesquitol

La deuxième partie de l'étude portait sur la modification chimique du mesquitol. Quelques dérivés d'intérêt ont été ciblés pourraient accroître la lipophilie du mesquitol. L'objectif principal de ces modifications était de pouvoir utiliser ces dérivés pour agir en synergie grâce à leurs propriétés anti-oxydnates avec des agents antifongiques permettant ainsi de diminuer la quantité de ces derniers dans les formulations utilisées pour la préservation du bois.

Le fait de les hydrophober devrait permettre une utilisation du matériau bois en extérieur, car le composé serait ainsi moins lessivable. Des mélanges de tebuconazole (un biocide du commerce utilisé pour le traitement du bois) et différentes formulations de mesquitol et de son dérivé ont également été testés contre le champignon *Coriolus versicolor*. Le mesquitol et son dérivé ont été ajoutés en tant qu'antio-xydant pour agir en synergie avec le composé anti-fongique (biocide).

1.1 GENERAL INTRODUCTION

Prosopis juliflora is a shrub, or sometimes a tree, known to be highly adapted to dry lands and was therefore introduced in Kenya to curb desertification (Pasiecznik, 2001). *P. juliflora* is traditionally used as a remedy to various diseases like dysentery, measles, cold, gout, sore throat, diarrhea, excrescences, flu, dermatological ailments, indigestion, eye problems like cataracts, inflammation and wound healing (Khandelwal *et al.*, 2015).

It has recently generated a lot of interest to scientists due to the presence of the mesquitol compound that has been found to have antioxidant properties (Lakshmibai, 2016; Sirmah, 2011; Azam *et al.*, 2011). Previous study at LERMab (Laboratoire d'Etudes et de Recherches sur le Matériau Bois) has showed that *P. Juliflora* heartwood contains high amounts of mesquitol, which is a natural relatively rare flavan-3-ol compound identified as 2-(3,4-dihydroxyphenyl)chromane-3,7,8-triol otherwise known as (-)- mesquitol (Sirmah *et al.*, 2009).

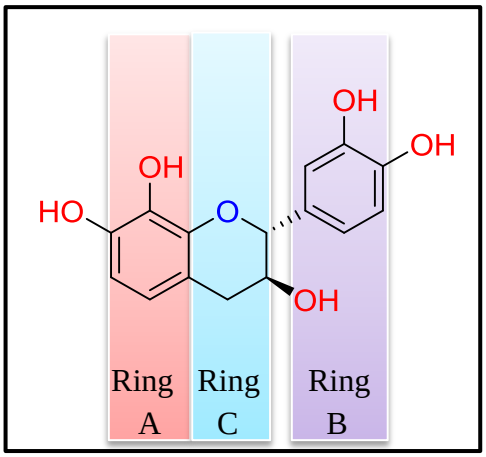


Fig **2:** Structure of (-)-mesquitol

Over the years, the *P. juliflora* species has become a major threat to many livelihoods mainly due to its uncontrolled ability to spread into crop fields, grazing areas, wetlands and lake shore areas (Muturi, 2012; Shackleton *et al.*, 2014). According to Muturi (2012), the riverine forests and the seasonal wetlands are the areas that are mostly invaded by *P. juliflora* in Kenya. This invasion of *P. juliflora* is known to be aided by its allelopathic property which influences the growth of plants growing in its vicinity by the release of allelochemicals (toxic chemicals produced by a plant to exert a detrimental physiological effect on individuals of another species) (Santosh and Gowsiya, 2013).

A survey done of local communities around Lake Baringo in Kenya showed that 85-90 % of respondents to a questionnaire favored complete eradication of the invasive *P. juliflora* species (Mwangi and Swallow, 2008). A related report was also recorded where a community known as Ilchamus in Kenya complained that it was hard to sell the meat of the livestock due to the meat having a bad taste as a result of the livestock feeding the *P. juliflora* (BBC news report 2006; UNEP 2004).

Efforts to eradicate the plant completely have failed mostly because of its deep penetrating taproots which have been reported to be able to reach water tables at a depth of more than 50 meters (Raven *et al*., 2005). Cutting down the *Prosopis* trees has also proved to be futile according to studies done in Eritrea by Bokrezion (2008). He reported that a majority of the people living in Eritrea had taken matters in their own hands and tried to eradicate the *P. juliflora* plants but after a long and strenuous attempt, which was very costly, the *P. juliflora* re-grew in a week's time even after they had been cut down (Bokrezion, 2008).

The uncontrolled spread of *P. juliflora* into arable land makes it fail its intended purpose of controlling desertification hence becoming more of a nuisance. This study therefore, aimed at harnessing the plant for raw material in order to consider potential valorization. Mesquitol exhibits antioxidant properties hence it leads to several potential applications in food industry, cosmetics or as starting material for innovative materials. The study therefore, proves as a timely research having availed valuable data that will address the plant's transformation from a weed to a raw material of importance. Abundance of mesquitol in the heart wood, that is easy to extract just by solvent extraction, further justifies the choice of this specific plant for the purpose of valorization of materials of plant origin.

The specific objectives of the study were to:-

1) Determine the best solvent and method of extraction for mesquitol.
2) Establish the geographical and natural intraspecific variability of extractives from *P. juliflora*.
3) Synthesize different mesquitol derivatives by hemi-synthesis using mesquitol as the starting material.
4) Characterize the properties of the synthesized derivatives and assess the potential of some of the derivatives as antifungal agents for wood preservation.

The exploration of *P. juliflora* with the aim of valorizing the mesquitol compound was done in two complementary parts. The first part dealt with the natural intraspecific variability of extractives from *P. juliflora* trees. The aim of this was to study the variability of wood extractives distribution within *P. juliflora* trees from three different geographical regions in Kenya in order to better understand the availability of this resource in view of considering its mobilization for a later industrial valorization. Plant samples were collected from three different geographical areas; Baringo County (latitude 0° 28' 0" N, longitude 35° 58' 0" E), Garissa County (latitude 0° 27' 09" S, longitude 39° 38' 45" E) and Turkana County (latitude 03° 09' N, longitude 35° 21' E) in Kenya. The samples were both from the small trees aged less than 4 years and the big trees aged more than 8 years old. The variability test was done by comparing the percentage yields of extractives using different solvents (dichloromethane, acetone, toluene: ethanol (2:1) and water) followed by identifying, quantifying and comparing some compounds found in the different extracts from the different geographic regions by use of the LC-ESI-MS/MS. This part also involved developing an extraction method for mesquitol.

The second part of the study dealt with the chemical modification of mesquitol. A few derivatives of interest were targeted which could increase the lipophilicity of the mesquitol compound. The main aim of this was to be able to use these derivatives as potential antifungal agents for wood preservation especially in outdoor use which could aid in decreasing leachability. Mixtures of tebuconazole (a commercial biocide used for wood treatment) and different formulations of mesquitol and its derivative were also tested against the white rot fungus (*Coriolus versicolor*). Mesquitol and its derivative were added as an antioxidant to act synergistically with the anti-fungal (biocide) compound.

CHAPTER 2

LITERATURE REVIEW

2.0 LITERATURE REVIEW

2.1 Introduction

The study of flavonoid chemistry has generated a lot of interests to scientists over the years due to their versatile health benefits like anti-inflammatory, antioxidative activity and heart disease prevention (Kumar and Pandey 2013). Due to their structural nature, most flavonoids allow for chemical modifications leading to several researches being carried out in an attempt to search for new compounds which have useful physiological properties.

This study focused on doing a substantive study on a plant species known as *Prosopis juliflora.* The study highlights the several phenolic compounds present in this plant while focusing on one of its extractives known as mesquitol (a flavan-3-ol). Minimal study has been done on this particular flavan-3-ol and therefore this chapter discusses the state of the art that has been carried out so far.

2.2 Wood composition

Wood is a sustainable resource that is mainly composed of four major parts which are the bark, the sapwood, the heartwood and the pith respectively from the outside to the inside. The general anatomy of wood is shown in figure 3 below (Merriam-Webster, 2006). The different parts can usually be distinguished by color but some species especially the tropical woods do not show a color difference between the sapwood and the heartwood making them indistinguishable (Tchinda, 2015).

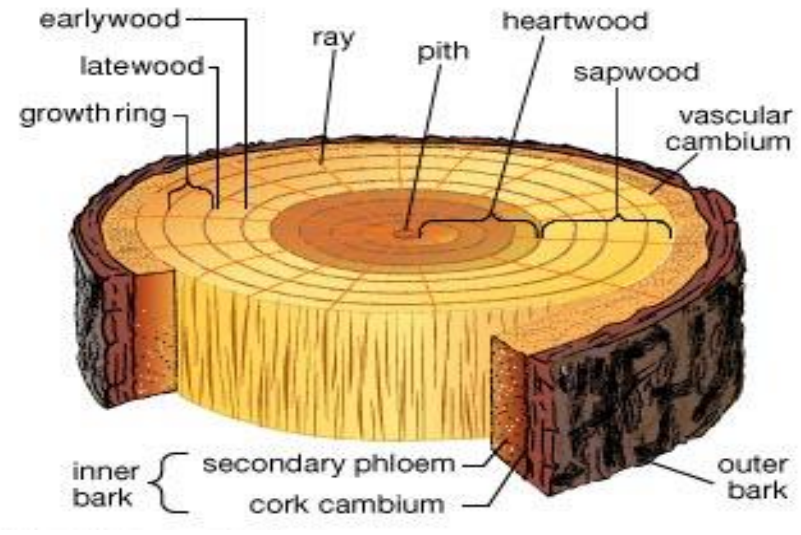


Fig **3**: A cross-sectional cut showing wood anatomy (Merriam-Webster, 2006)

The chemical composition of wood varies from one species to another, the different tree parts, the geographic location, climate and the soil conditions (Mounguengui, 2008). Generally, wood composition is around 6 % hydrogen, 50 % carbon, 44 % oxygen and little quantities of inorganics (Rowell, *et al.*, 2005).

The chemical compounds present in wood comprise of cellulose, hemicellulose and lignin. Cellulose is present in the highest percentage of about 40 – 45 percent of the weight of a piece of wood (Conners, 2015), with softwoods having a higher percentage compared to the hardwoods (Rowell, *et al.*, 2005). It is the compound that gives strength to wood since it is particularly strong in tension. Hemicellulose is a branched carbohydrate polymer that is also present in wood. It is usually approximately 18 – 35 % of wood and is less strong than cellulose and has more ability to absorb water since it is hygroscopic (Conners, 2015). Lignin on the other hand, is an amorphous polymer made up of phenolic molecules which is present in wood at a percentage of approximately 20 – 35 % of wood. Lignin and hemicellulose are both known to stiffen individual wood fibers by acting as adhesives that hold the fibers together (Conners, 2015; Alonso *et al.*, 2012). Lignin has been found to contain approximately 40 % of the possible energy of the biomass due to its high carbon content (Alonso *et al.*, 2012).

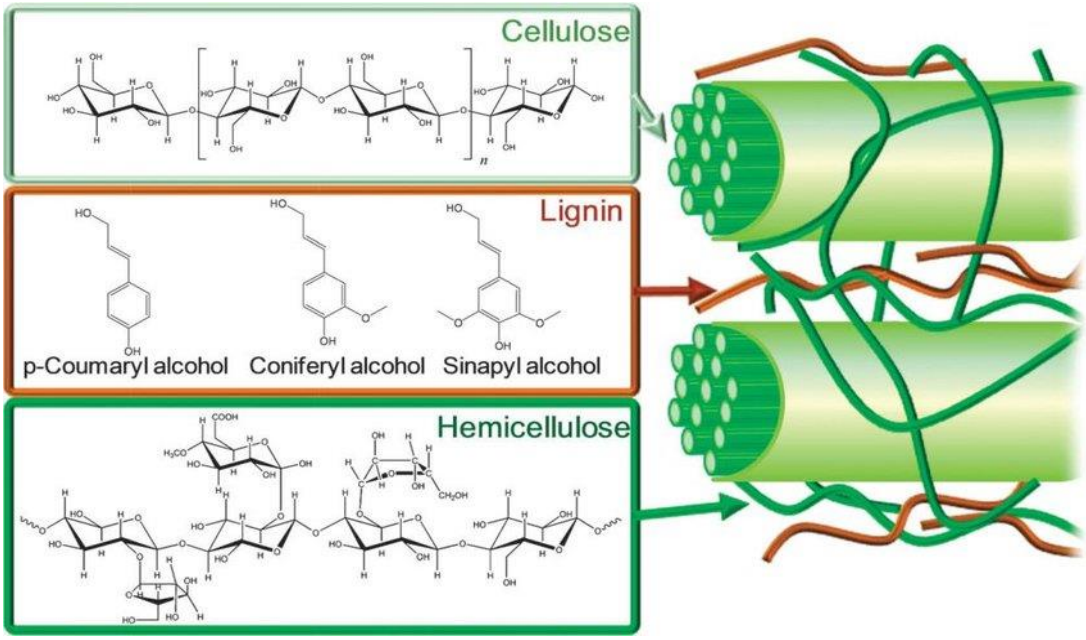


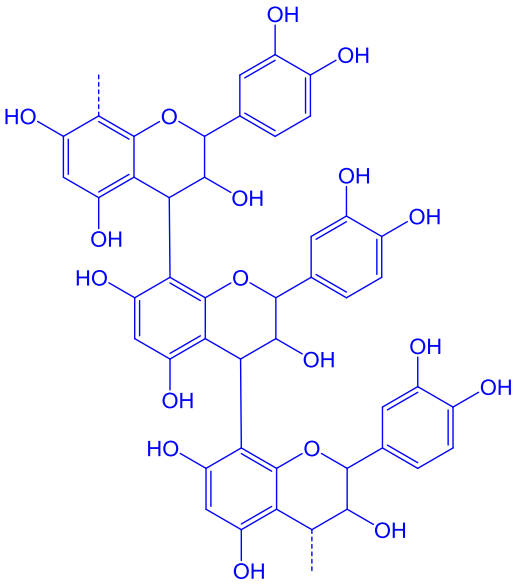
Fig **4:** Structure of lignocellulosic biomass with cellulose, hemicellulose, and lignin (Alonso *et al.*, 2012)

Other compounds present in wood which can be extracted using solvents are known as extractives. They are normally non-structural wood molecules that represent a small fraction in wood (Valette, *et al.*, 2017; Nascimento *et al.*, 2013). They can be present in small or large amounts of about 3 – 8 % in hardwoods of temperate regions and up to 18 – 22 % in hardwoods of tropical regions (Conners, 2015; Pecha and Perez, 2015; Willför *et al.*, 2003). About 3-15 % extractives have been found in *Prosopis juliflora* (Prabha *et al.*, 2014).

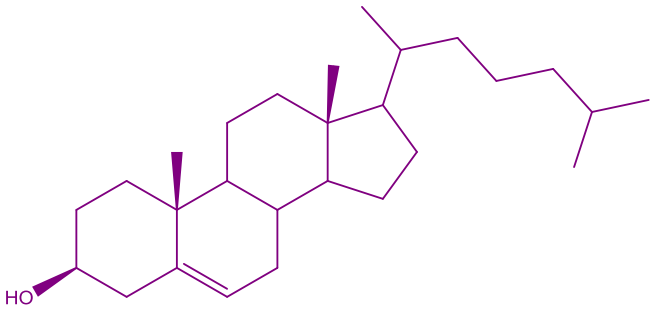
The extractives are sometimes classified according to their polarity and / or solubility in the solvent that has been used to extract them, for example, an extractive could either be water-soluble, ethanol-soluble or ether-soluble (Rowell, *et al*., 2005). Some of the roles played by extractives in plants are well known however, in other cases, the reason for their presence is not clear. Some extractives like resins are used to waterproof boats and as binders. Other extractives have found application in pharmacology, cosmetics and even as wood preservatives (Rowell, *et al*., 2005) and are also known to be responsible for odor, color and other physical and mechanical properties in trees (Conners, 2015).

Some extractives have been shown to inhibit the growth of fungi hence making them decay resistant (Tchinda *et al*., 2018). A strong correlation has also been established between the presence of extractives and the durability of wood (Kirker *et al.*, 2013; Pometti *et al.*, 2010). Kirker found out this when he evaluated the fungal attack of both white-rot and brown-rot fungi on eight different wood blocks from naturally durable species while comparing them with the ones that had been chemically extracted. These wood blocks were from *Callitrosis n., Juniperus v., Thuja p., Juniperus o., Robinia p., Prosopis g., Paulownia t.* and *Catalpa spp.* The results showed higher weight loss in the blocks which had no extractives hence indicating that extractives are primarily responsible for durability (Kirker *et al.,* 2013). On the other hand, the same experiment was done by Sirmah 2009 using wood blocks from *Prosopis juliflora* heartwood. He did not observe the different mass losses between the blocks whose extractives had been extracted with those which had the extractives, hence, concluding that the durability of *P. juliflora* was not solely due to the presence of extractives (Sirmah, 2009).

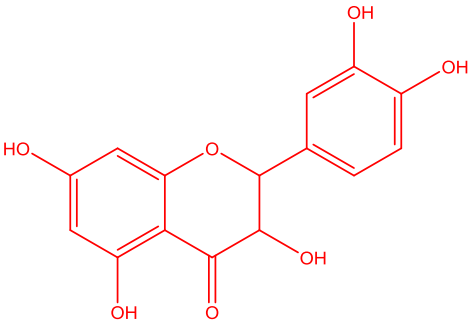
Some examples of extractives found in wood include terpenes and terpenoids, tannins and flavonoids, phenolics, lignans, quinones, essential oils, pectins, mucilages, glycosides and saponins, fatty acids, sterols, proteins, gums, resins, simple sugars, starches, fats and waxes (Pattiya, 2018). Specific structures of some of these extractives are shown in figure 5.



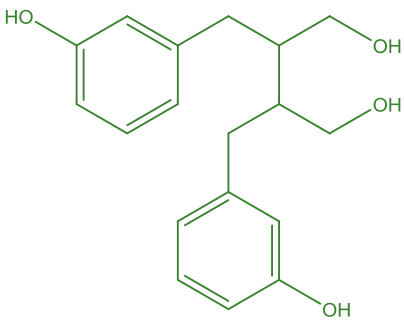
Condensed tannin
(Procyanidin)



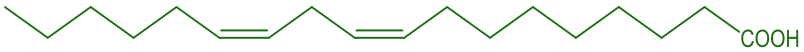
Sterol
(Cholesterol)



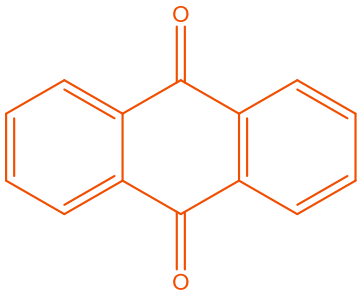
Flavonoid
(Taxifolin)



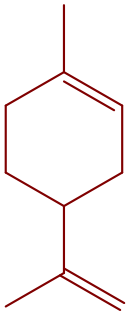
Lignan
(Enterodiol)



Fatty acid
(Linoleic acid)



Quinone
(Anthraquinone)



Terpene
(Limonene)

Fig **5:** Specific examples of some wood extractives

In wood, the phenolic extractives, which protect the lignocellulose from fungal and microbial attack, are found mostly in the heartwood and bark while the waxes are found in the parenchyma cells (Pecha and Perez, 2015). The heartwood contains many high molecular weight polyphenols and other aromatic compounds. This is the reason why the heartwoods of many species have a dark color and are resistant to decay (Bajpai, 2018).

2.3 *Prosopis juliflora* studies

Prosopis juliflora, an exogenous plant that is normally known as 'mathenge' in Kenya is believed to have been first introduced in Kenya in 1973. The reason for this was to rehabilitate the quarries near the coastal regions (Jama and Zeila, 2005; Oduor and Githiomi, 2013; Mwangi and Swallow, 2008). It was later introduced to Baringo County in the early 1980's by the Fuel wood Afforestation Extension Project (FAEP) with the aim of reducing soil erosion and combating desertification (Lenachuru, 2003; Dubow, 2011). Other common names of the *P. juliflora* are: Mesquite, Mexican thorn, cashaw (English); algarrobo, mezquite, trupillo, espino real, cuji yaque (Spanish); bayahonde (French Caribbean), vilayati babul (Hindi, Punjabi) (Pasiecznik *et al*., 2004).

The *P. juliflora* is found either as a shrub, or sometimes a tree (figure 6 and 7) and is known to be native to Central America, the Caribbean and northern South America. It belongs to the family of *Leguminosae* and about 44 species of the genus *Prosopis* have been identified so far (Pasiecznik *et al*., 2004). It is known to be highly adapted to dry lands because of its deep taproots which tolerate dry as well as waterlogged soils. These taproots have been reported to grow downwards in search of water tables to a depth of up to 50 meters so as to aid it access sub-surface waters. It also has well-developed lateral roots which enables it to compete with the grasses (Pasiecznik *et al*., 2001).

Fig **6**: *Prosopis juliflora* tree

Fig **7**: *Prosopis juliflora* shrub

2.3.1 Morphological description of *Prosopis juliflora*

Prosopis species are known to be of varying sizes, predominantly xerophilous and aculeate (Pragnesh and Jaya, 2013). The trees are usually evergreen leguminous (this is typical of the trees in arid and semi-arid regions) and have been reported to grow up to 10-15 m high (Shackleton *et al*., 2014). The shrubs however, grow around 3-5 m high (Pasiecznik *et al*., 2001).

The *P. juliflora* are known to have long, sharp and strong poisonous thorns measuring about 1 to 5 cm. The leaves are pinnately compound with around 13-25 pairs of leaflets arranged on one or two pairs of pendulous rachis (Shackleton *et al*., 2014). Its fruits are long, cylindrical green pods which turns yellow upon ripening. These pods of about 10-20 cm in length are sweet to taste and contains around 10-20 hard oval or elliptic seeds (2.5-7 mm long) that are hard to extract (Pasiecznik *et al.,* 2001). Figure 8 shows the fruit pods of *P. juliflora* that have been harvested and the ground flour obtained from the pods which some locals mix it with wheat flour and use it as food.

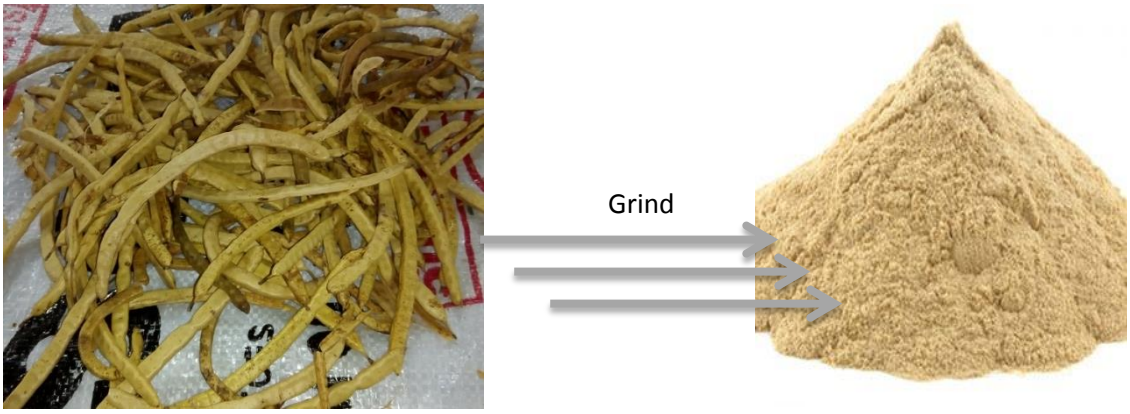


Fig **8:** Ripened fruit pods and ground flour of *Prosopis juliflora*

2.3.2 Habitat and geographic distribution of *P. juliflora* in Kenya

Being a drought tolerant species, *Prosopis juliflora* can thrive in most soils. This includes sandy, rocky, poor and saline soils within an altitude range of 300-1900 m above sea level (Pasiecznik *et al.,* 2001). Since its introduction to Kenya, the *P. juliflora* has slowly found its way to spread to a wide area over a period of time. Some of the areas that have been infested so far are shown in green in figure 9.

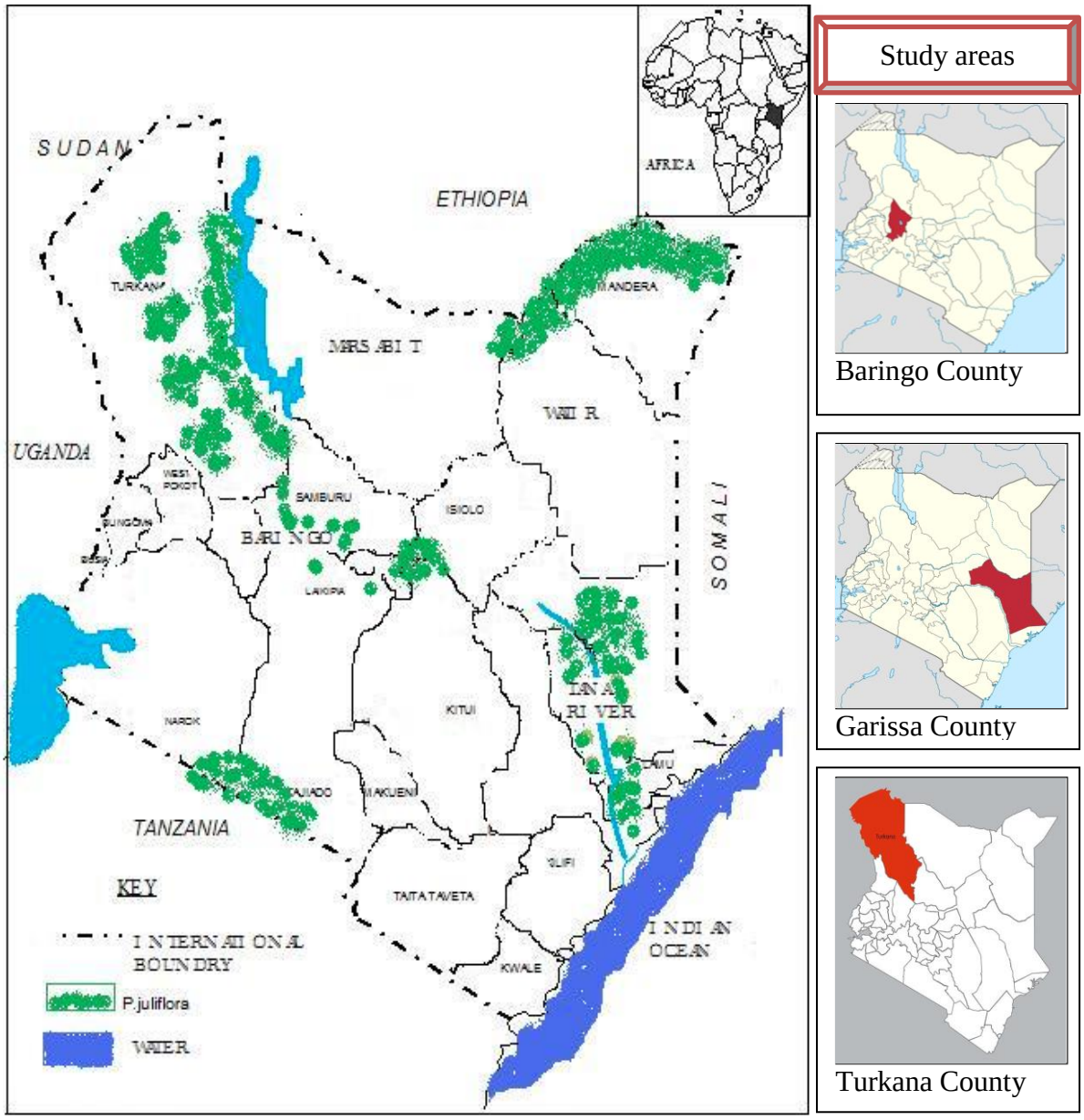


Fig **9**: Kenyan map showing areas infested by *Prosopis juliflora* (Sirmah, 2009)

The study area of this research was in three different Counties in Kenya. These were Baringo County (latitude 0° 28' 0" N, longitude 35° 58' 0" E), Garissa County (latitude 0° 27' 09" S, longitude 39° 38' 45" E) and Turkana County (latitude 03° 09' N, longitude 35° 21' E).

Baringo is a county which is situated 270 Km north-west of Nairobi capital city at an altitude of 1067 m above sea level. It has a population of 555,561 people according to the 2009 national census, who mostly practice agriculture and livestock keeping. The county has two distinct weather patterns of cold and hot seasons. The cold months are usually June and July with temperatures of about 25 °C. The hot months are January and February with

temperatures between 30 °C to 35 °C. Annually, this county receives rainfall between 1000 mm and 1500 mm in the highland regions and 600 mm in the lowlands regions (K.I.G, 2015).

Garissa County on the other hand is a semi-arid area situated 330 Km north-east of Nairobi capital city at an altitude of 147 m above sea level. As per the national census (2009), the county registered a population of 923,060 people. Rainfall in this county is usually short torrential downpour making it hard to practice agriculture hence, the residents mostly practice livestock keeping. The county receives an average rainfall of 275 mm per year with the southern parts of the county receiving more rainfall than the northern parts. Garissa County generally experiences high temperatures throughout the year ranging from 20 °C to 39 °C with the hottest months being September and January to March (GCID, 2018).

Turkana County, the second largest county in Kenya by land area, is situated 700 Km north-west of Nairobi capital city at an altitude of about 1138 m above sea level. It is known to be one of the driest counties of Kenya with a population of 855,399 people according to the 2009 national census. The residents of this county are mostly nomadic pastoralists who mainly keep camels, goats, donkeys and cattle. This county receives an unpredictable rainfall pattern of about 150 – 400 mm annually although at times the county receives no rain in a whole year. The temperatures experienced have been estimated to be between 25 °C and 35 °C with very high temperatures being experienced during the day and moderate temperatures during the night (K.I.G, 2015).

2.3.3 Negative aspects of *Prosopis juliflora*

Chilume (2016) posted a feature entitled 'When a blessing becomes a curse – A case study of invasive *Prosopis juliflora*' in the University of Auckland blog. She talks about the drastic changes over the years that have been caused by the wide spread of *P. juliflora* after its introduction in Botswana. She highlights the fact that there is a public outcry from the locals living in the invaded areas who prefer complete eradication of the plant (Chilume, 2016). This cry is shared by many in several other countries like Eritrea, India and Kenya (Bokrezion, 2008; Patnaik *et al*., 2017; Mwangi and Swallow, 2008).

The growing dislike towards *P. juliflora* has emerged due to its invasive property that has seen it rapidly spreading into areas where it was not initially intended to grow like forests, rangelands and croplands as seen in figure 10. Because of it being an aggressive invader, it

has been declared among the most invasive species in the Global Invasive Species Database (GISD, 2019).

Fig **10**: Pictures of *Prosopis juliflora* showing different invasion into undesirable areas like roads and sea shores

The introduction of *P. juliflora* to areas which are far from their original habitats have ended up causing some great havoc by affecting both the natural vegetation and also causing a decrease in land value towards livestock grazing (Golubov *et al.,* 2001). It has ended up destroying nearby roads, invading into the sea shores and even invading into the homes of the locals. Following a court case in 2008 petitioned by some residents from Baringo County in Kenya (where *P. juliflora* is known to have spread greatly) the *P. juliflora* was finally declared as a noxious weed in Kenya under the Suppression of Noxious Weeds Act (CAP 325) laws of Kenya.

There are several factors favoring its rapid distribution in the environment; *P. juliflora* has the capability of easily adapting to a range of climatic conditions, it has an effective dispersal mechanism, has a large seed bank in the soil environment, they grow very fast and show allelopathic effect (Abdulahi, *et al.,* 2017). The mechanism of the allelopathic effect is still unknown but it is thought to be one of the reasons that influence the diversity, productivity and reproduction of plants (Santos *et al*., 2017).

One of the reason for introducing the *Prosopis* tree in Kenya was to reduce soil erosion but however, it has been found to actually encourage erosion because the under-story of herbaceous plants are eliminated on account of the *Prosopis* (Aboud *et al*., 2005). This might be due to the fact that the *P. juliflora* contains allelochemicals in various quantities. This was proved by a study by Santosh and Gowsiya (2013) who carried out a study of the allelochemicals in soils that were found under the canopy of *P. juliflora* and those that were found outside the canopy. They observed that the soil that was found under the canopy contained a lot more allelochemicals which inhibited the growth of other plant species like grasses around the *P. juliflora* (Santosh and Gowsiya, 2013).

The *P. juliflora* has also been known to cause neurotoxic damage to animals after they have consumed the plant. This was according to a study done by Silva *et al* (2013), who showed that the ingestion of *P. juliflora* invoked intoxication in animals which was depicted by neuromuscular alterations caused by mechanisms which have not yet been well understood. He attributed this to the presence of piperidine alkaloids (juliprosopine and juliprosine) in *P. juliflora* which could be the primary cause of the neurotoxic damage (Silva *et al*., 2013).

Another negative aspect of *P. juliflora* is brought about by its long, strong and prickly thorns. Some local residents living near areas invaded by the *P. juliflora* have protested about the plant's strong and poisonous thorns. Some claim that the thorns destroy the teeth of the

livestock feeding on them especially the goats. The reason why this occurs has not been proved exactly. The cause might probably be that the goats are pricked by the thorns during feeding and the thorns become stuck in the gums causing teeth decay. However, Masakha and Wegulo (2015) claims that the goats lose their teeth probably due to the high sugar content that is found in the pods of *P. juliflora*. This high sugar content enhances the activity of the rumen bacterial cellulase which causes tooth decay in goats and leads to high mortality cases (Masakha and Wegulo, 2015). When humans are pricked by the thorns, it causes serious inflammations which have been found to take almost a week to subside and if left untreated, the infections are known to cause gangrene that leads to amputation of limbs (Patnaik *et al*., 2017). In addition, the thorns are known to cause car tyre punctures and even destruction of fishing nets (Pasiecznik *et al*., 2001).

Other negative aspects of *P. juliflora* include: invasion into parts of wildlife reserves and national parks threatening biodiversity, decrease levels of water table due to great intake by *P. juliflora* on account of the presence of its deep tap roots (Selvan *et al.*, 2018). Dense stands of *P. juliflora* clog irrigation channels, cut off roads and impede smaller trails completely affecting access to water sources, pasture, croplands and fishing areas (Pasiecznik *et al*., 2001). It is also used as a hideout by cattle rustlers and wild animals (Jama and Zeila, 2005).

2.3.4 Positive aspects of *Prosopis juliflora*

Despite the negative aspects of *P. juliflora* mentioned before, there exist positive aspects of *P. julfilora* which are mostly due to the reasons why it was introduced to many arid areas. According to Dubow (2011), sixty one percent (61%) of the respondents to a questionnaire in Garissa County reported that they use *P. juliflora* in one way or the other, with most of them using it as furniture and building materials. He reported that many residents also use it for fuel wood and for making charcoal; although some key informants of the questionnaire reported that most of the people who made the charcoal preferred making them using other indigenous trees since they were of better quality and fetched a higher price (Dubow, 2011).

Studies have shown moreover that the *P. juliflora* has high calorific value and hence its wood is good as fuel wood (Oduor and Githiomi, 2013). The study found the calorific value of *Prosopis* fuel wood as 4.952 Kcal with the calorific value for its charcoal as 7.854 Kcal. The average calorific values generally for wood range from 3.5 to 5.0 Kcal whereas that of charcoal range from 5.0 to 9.0 Kcal (Oduor and Githiomi, 2013).

They calculated the average calorific value as follows:

Calorific value (Cal/g) = [(water equivalent + water quantity of inner cylinder) x (rise in temperature – correlation value)] / Quantity of sample

Traditionally, *Prosopis juliflora* is used as a remedy to various diseases like dysentery, cold, diarrhea, excrescences, flu, gout, sore throat, dysentery, hoarseness, measles, dermatological ailments, cataracts, indigestion, skin lesions and inflammation. The water extract decoction of the leaves and seeds are normally used as disinfectant, wound healing and to treat scurry. The folk give children with weight deficiency or retardation in motor development syrup made from the *P. juliflora* ground pods. This syrup is believed to increase lactation (Khandelwal *et al.*, 2015).

Decoction from the *P. juliflora* has soothing, antiseptic and astringent properties. The plant is also known for its antimicrobial activity according to a recent study which observed high antibacterial activity in aqueous fractions as compared to solvent fractions (Khandelwal *et al.*, 2015; Thakur *et al*., 2014). *Prosopis* is also known to present anti-termite and anti-fungal properties (Sirmah *et al*., 2008).

Patulitrin, a flavonoid that has been isolated from the flowers and fruits of *P. juliflora*, have shown to have significant activity against the lung carcinoma. Alkaloids extracted from the *P. julifora* leaves were also found to be preferentially cytotoxic to human T-cell leukemia (Molt-4) cells in a dose and time dependent manner with the absence of genotoxicity (Khandelwal *et al.*, 2015; Sathiya and Muthuchelian, 2011).

The alkaloids from *P. juliflora* have also been found to show antimalarial, analgesic, antispasmodic and antiarrhythmic activities (Khandelwal *et al.*, 2015). The alkaloid juliflorine, has been found as a cure of the Alzheimer disease. It inhibits acetylcholine esterase at cholinergic brain synapses (Henciya *et al*., 2017).

The pods of *P. juliflora* have been found not only to be used as animal feeds but also some natives living in *Prosopis* invaded areas grind the pods to make flour that they use for consumption (Choge *et al.*, 2007). This flour has been found to be gluten free with about 7 – 8 % protein (Felker *et al*., 2003; Felker *et al.*, 2013). This makes it a better alternative for people who would like to avoid gluten and white flours that have recently been shown to have some negative health hazards. This flour has also been found to have high solute fiber and is a great alternative to regular sweeteners in baked goods and other foods (Felker *et al*., 2013;

Bigne *et al.*, 2016). Choge *et al.* (2007) however, reported that the flour from the *P. juliflora* contained some Ochratoxin A and aflatoxins that could be harmful to human health. However, the study did not determine whether the toxins could have been obtained by long periods of storage or due to wrong conditions of storage or if they were present also in freshly harvested pods (Choge *et al.,* 2007). The *P. juliflora* seeds have also been reported to be a potential source of seed gum (Khandelwal *et al*., 2015).

Another study by Marquez *et al*. (2016) showed that the pod extract from *P. juliflora* could disrupt female but not male fertility (Marquez *et al*. 2016). This study evaluated fertility in both male and female rats treated with mesquite pod extracts. They compared its effects with those of daidzein and estradiol and were able to show that the mesquite pod extracts decreases fertility in female but not male rats. The study is yet to be done on humans (Marquez *et al*. 2016).

In an effort to manage the invasive species through utilization, Haile *et al.* (2018) made some studies on the use of the *P. juliflora* pods mash for the production of biofuel energy. He found out that the pod mash of *P. juliflora* can be used in the production of bio-ethanol. His preliminary analysis also of the solid waste after hydrolysis suggested that there was a possibility of using it as solid fuel hence alleviating major disposal problems (Haile *et al*., 2018). A company also known as the Cummins Cogeneration Kenya (CCK) recently put up a factory in Baringo County in Kenya with the main aim of using *P. juliflora* as a source of biomass for electricity production. Its operations however, have yet to start.

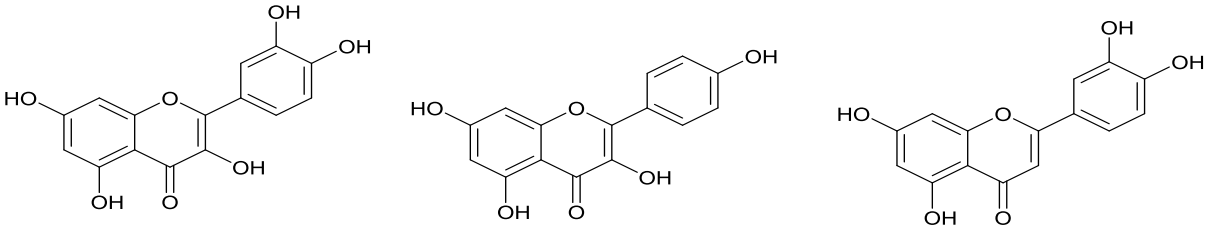
The other positive aspect of *P. juliflora* is that it is in nature known as an important species, not only as it provides shelter to many species of animals in very dry areas but also because it is known to be a nitrogen fixer hence, *Prosopis* has been noted to improve the fertility and physical characteristics of soils in which they grow (Norma, 2007; Pasiecznik *et al*., 2004; Golubov *et al.,* 2001).

The most recent positive aspect of *Prosopis* was in a study which used it for the green synthesis of gold nanoparticles. The study used the naturally occurring leaf extracts from *P. juliflora* as a reducing agent while using water as a benign solvent. This fabrication of gold nanoparticles was carried out by reducing aurum chloride with the aqueous extract of *P. juliflora* leaves. The biologically synthesized *Prosopis juliflora* - gold nanoparticles are believed to have potential in antibacterial application and are promising and effective antimicrobial agent against the *E. coli* and *B. subtilis* (Rao *et al*., 2017).

2.3.5 Compounds identified in *Prosopis juliflora*

Prosopis juliflora has been found to contain 40-45 % cellulose, 25-30 % of hemicellulose and 11-28 % lignin (Prabha *et al.,* 2014). In addition to this, a lot of compounds have been identified and isolated from the *P. juliflora* plant. Singh did quantitative analysis of the total metabolites present in different parts of the plant. The study showed that the leaves and the pods of *P. juliflora* were the richest source of plant metabolites, followed by the flowers then the roots and finally the stem. The phytochemical analysis of the extracts used in the study showed the presence of phenolics, alkaloids, flavonoids, terpenes and steroids (Singh, 2012).

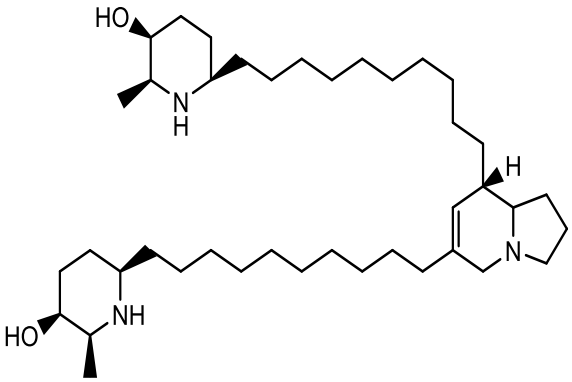
P. juliflora is widely known for the presence of flavonoids which are responsible for various activities like antioxidant activity. Khandelwal *et al.* (2016), isolated and identified three types of flavonoids from a methanol extract; Quercetin, Kaempferol and Luteolin in *P. juliflora*. The quantity of quercetin was 2.6 mg / g dry weight, kaempferol (1.7 mg / g dry weight) and luteolin (1.3 mg / g dry weight) (Khandelwal *et al.,* 2016; Prabha *et al.,* 2014).



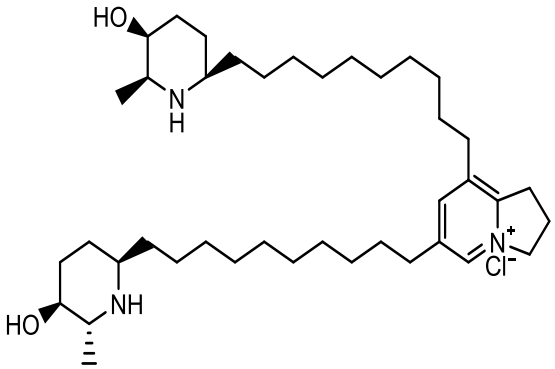
Quercetin Kaempferol Luteolin

Fig **11:** Structures of some flavonoids found in *P. juliflora*

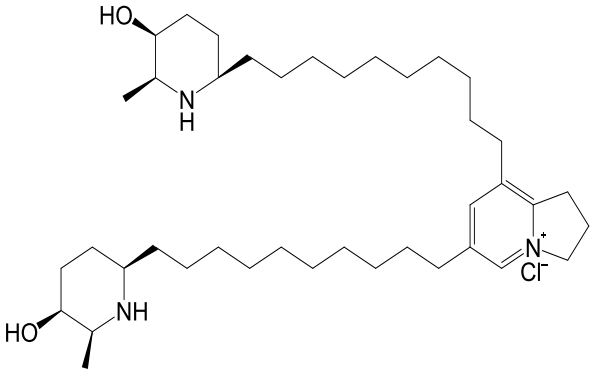
Several alkaloids have also been isolated from leaf extracts and they show antibacterial activities (Dos Santos *et al.,* 2013; Prabha *et al.,* 2014; Silva *et al*., 2013; Henciya *et al*., 2017). According to Dos Santos, the main constituents of alkaloids in the *P. juliflora* were juliprosopine, juliflorine, prosoflorine and juliprosine. He however, did not quantify these alkaloids (Dos Santos *et al.,* 2013). The structures of these constituents of alkaloids are shown in figure 12 below.



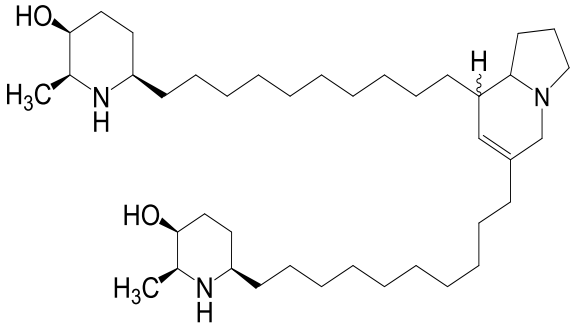
Juliprosopine



Prosoflorine



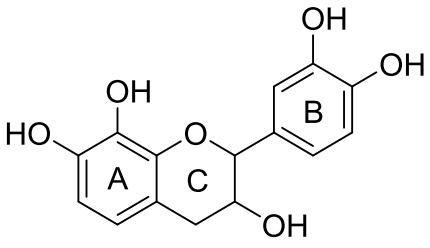
Juliprosine



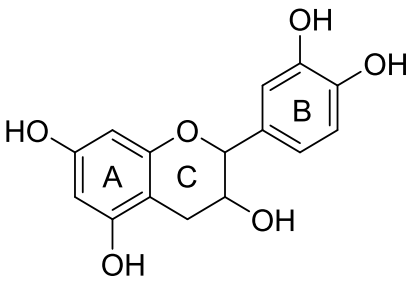
Juliflorine

Fig **12:** Structures of some alkaloids found in *P. juliflora*

A study done by Sirmah (2009) showed the presence of three flavan-3-ols; mesquitol, catechin and 4'- *O*-methylgallocatechin in *P. juliflora*, see figure 13.



Mesquitol



Catechin

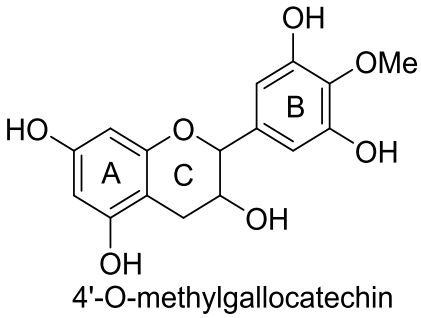


Fig **13:** Structures of flavonoids found in the stem of *P. juliflora*

2.4 Flavonoid studies

Since mesquitol is a type of flavonoid (a flavan- 3-ol), this section introduces briefly what flavonoids are, some of its uses, its biosynthesis and some of its chemical reactivities focusing mostly on the flavanols.

Flavonoids are essential constituents of the cells of all higher plants and are a class of natural products which has generated a lot of scientific interests (Havsteen, 2002). They are low-molecular-weight phenolic compounds that are mostly found in fruits, vegetables and some beverages like tea (Panche *et al*., 2016). About 10,000 flavonoids have been identified so far but it is believed that this number will continue to increase as time goes by. The flavonoids are known to be the third largest group of natural products after terpenoids and alkaloids. The alkaloids identified so far are about 12,000 while the terpenoids are about 30,000 (Martens *et al*., 2010; Dixon and Pasinetti, 2010).

Flavonoids normally contain 15 carbon atoms in their basic skeleton in the form of C6-C3-C6 carbon. They occur naturally as glycosides (conjugated form attached to sugar) or as aglycones (without attached sugar) (Santos *et al*., 2017; Veitch *et al*., 2008; Martens *et al*., 2010). They can be classified into several groups whose biological and chemical properties can be different in each group. These are the flavanols, flavonols, anthocyanidins, flavones, flavanones and chalcones. Some flavonoids are classified as aurones, which is a group whose structure differs slightly from the other flavonoids and is not found in all species (Winkel-Shirley, 2001; Brodowska, 2017), figure 14 below.

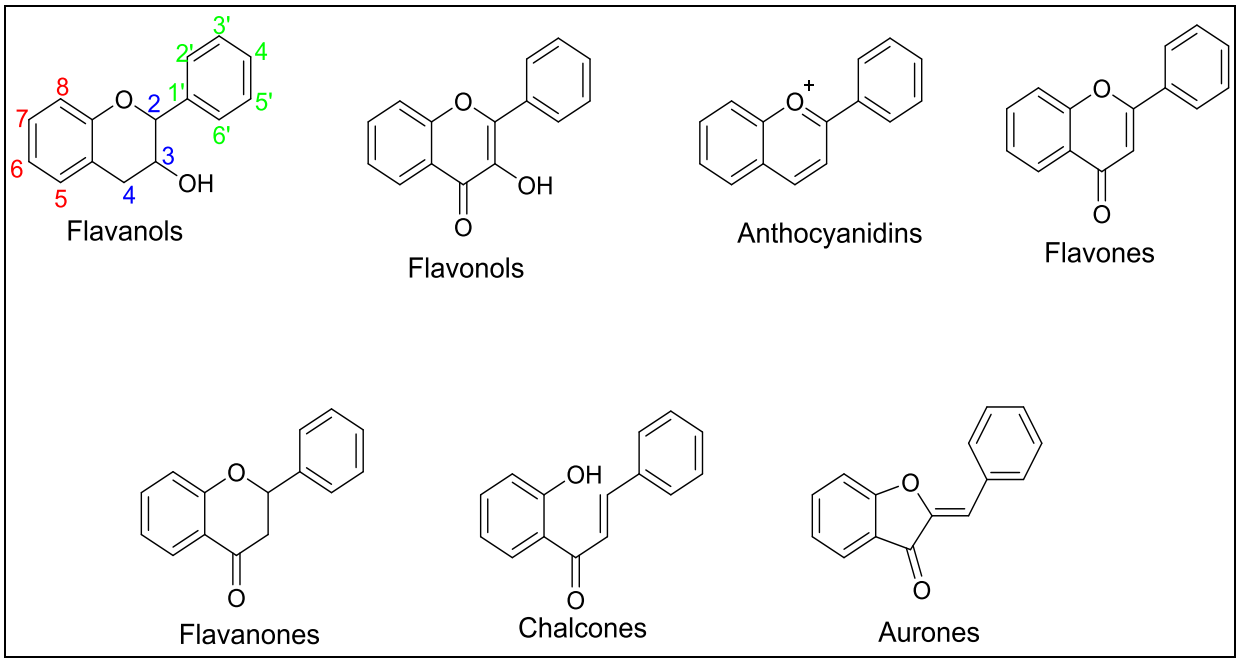


Fig **14:** General structures of the some classes of flavonoids

The flavan-3-ols, for example catechin and epicatechin, represents the largest class of naturally occurring monomeric flavonoids. They have raised a lot of interest generally as they are the constituent units of proanthocyanidins (Marais *et al*, 2006; Ferreira *et al.*, 2005). Proanthocyanidins (condensed tannins) are a group of polyphenols that are oligomers and polymers of monomeric flavonoids linked through a particular single bond (B linkages) and double (A linkages) bonds. They are present in various plants as a defense against biotic and abiotic stressors (Rauf *et al*., 2019; Beecher, 2004). Oligomeric proanthocyanidins are primarily known for their antioxidant activity. They are also known to demonstrate antibacterial, anti-inflammatory, antiviral, anti-allergic, anticarcinogenic and vasodilatory actions. They also inhibit lipid peroxidation, capillary permeability and platelet aggregation (Fine, 2000). Figure 15 below shows the different linkages within proanthocyanidins.

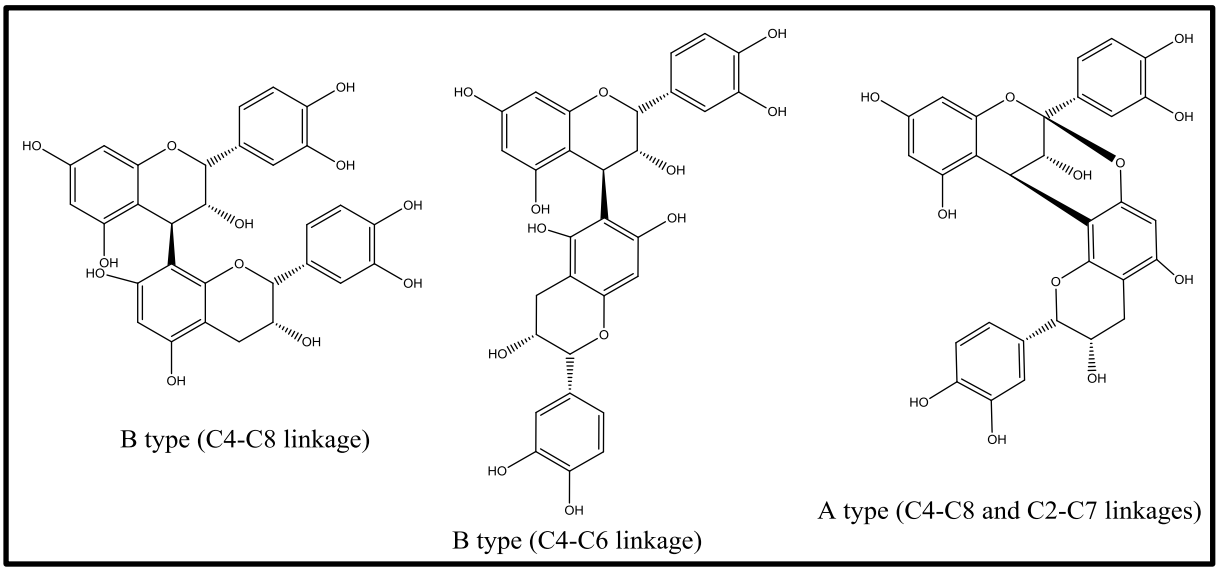


Fig **15:** Linkages within proanthocyanidins

2.4.1 Role of flavonoids

Flavonoids are known to play a variety of biological roles in plants, animals and bacteria. These biological functions are linked to their potential cytotoxicity against cancer cells and their capacity to interact with enzymes by binding to proteins via non-covalent or covalent bonds sequestering proteins into soluble or insoluble complexes. This binding of flavonoids to proteins significantly influences the antioxidant and antibacterial properties since the binding engages the same functional groups that are involved in redox cycling (Williams *et al.*, 2004; Arts *et al.*, 2002; Brudzynski and Alvarez, 2015).

The sparked interest of flavonoids to both scientists and non-scientists is hugely because they account for the coloration found in plants (Tanaka *et al.*, 2008). Other classes of pigments that allows for coloration in plants are the carotenoids, betalains and chlorophyll (Tanaka *et al.*, 2008; Feild *et al.*, 2001). Due to this pigmentation in plants, flavonoids have become the main focus in the attempt of modifying the color of flowers by genetic engineering. According to Tanaka, it has become feasible by modifying the pathway through genetic engineering (Tanaka *et al.*, 1998).

The different range of colors provided by flavonoids in plants aids in attracting insects for pollination and dispersal of seeds (Winkel-Shirley, 2001; Santos *et al.*, 2017; Havsteen, 2002). These flavonoids act as key-modulators of plant communication with insects and microbes, either as attractants or repellants (Dixon and Pasinetti, 2010; Mandal *et al.*, 2010). They have a role in the protection of plants against microbial invasions as they accumulate

phytoalexins in response to microbial attack. Phytoalexins are lipophilic low-molecular-mass antimicrobial stress metabolites that are accumulated by plants at infection sites to concentrations which inhibits the development of fungi and bacteria (Harborne and Williams, 2000; Winkel-Shirley, 2001; Ahuja *et al.*, 2012). The structure of a phytoalexin is shown in figure 16 below.

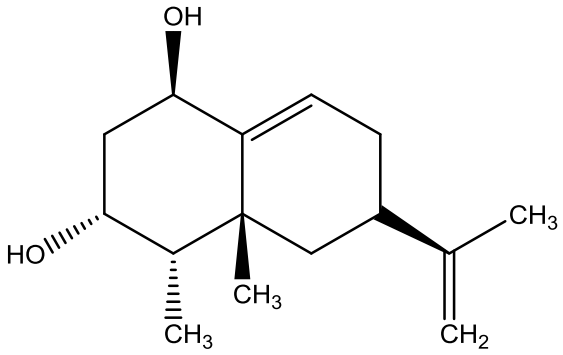


Fig **16:** Phytoalexin structure

It is believed that plants exposed to drought stress develop different strategies to tolerate the lack of water during their development. Varela made a study and suggested that the production and accumulation of flavonoids in plants could be one of the strategies used by plants to avoid the oxidative damage caused by drought (Varela *et al.*, 2016). This might explain the high quantity of flavonoids in *Prosopis juliflora* trees which are known to be drought-resistant.

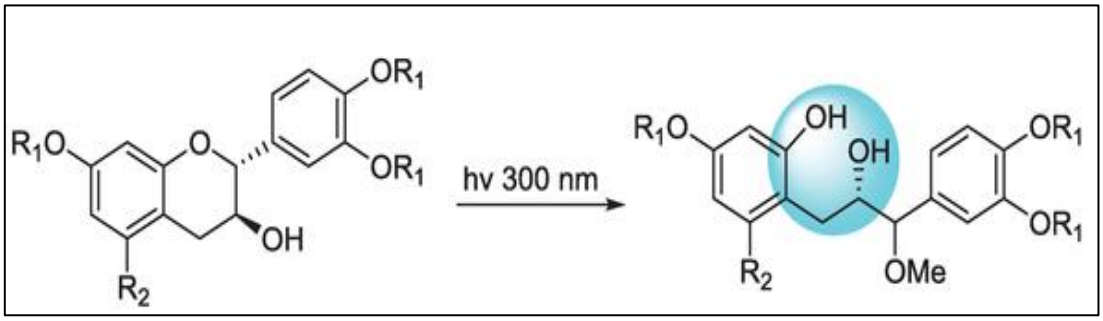
The other major reason for the increased interest of flavonoids to scientists is because of the association of flavonoids with the health benefits of food such as fruits, vegetables, chocolate and wine. This is because they are natural antioxidants since they have the ability to sequester free radicals (Gupta *et al*., 2016). The ingestion of these flavonoids by humans helps reduce heart disease, prevent cancer and slow down the aging processes in cells responsible for degenerative diseases (Gupta *et al*., 2016). This has been shown in some studies where consumption of tea (*Camellia sinensis*) has led to protection against cancer and cardiovascular diseases. These health effects have been attributed to the tea catechins which have shown to have inhibitory activities against tumorigenesis (Meng *et al*., 2001; Bigelow and Cardelli, 2006; Ju *et al*., 2007; Braicu *et al*., 2013). This has therefore led to an increased interest in using flavonoids as drugs for therapy and also as dietary supplements for prevention of diseases (Harborne and Williams, 2000).

Flavonoids are advocated for use against fungal pathogens of man because of their capability to inhibit spore germination of plant pathogens. Other biological and pharmacological activities exhibited by flavonoids include cytotoxic, antiviral, antibacterial, anti-inflammatory, antiallergic, antipyretic, antithrombotic, cardioprotective, analgesic, hepatoprotective, neuroprotective, antimalarial, anti-mutagenic, antitrypanosomal and antiamebial properties (Ferreyra e*t al.*, 2012; Rajbhar *et al*., 2014; Winkel-Shirley, 2001; Panche *et al*., 2016).

2.4.2 Stability of flavonoids

One of the biggest problems in flavonoids is that they are very susceptible to humidity, light and air induced oxidation. Plants absolutely depend on light for their development and light is known to be one of the most important environmental factors that affect the biosynthesis of flavonoids with high solar radiation tending to increase flavonoid content in fruits (Zoratti *et al*., 2014). Howbeit the stability of flavonoids during storage can be affected by either oxygen or by light (Chaaban *et al*., 2017).

UV light from sunlight is an essential requirement by plants mainly for photosynthesis but more exposure of the harmful UV rays is known to cause damage to DNA, protein and the cell membranes of plants (Cetinkaya *et al*., 2017). While absorbing the UV light, flavonoids may undergo a transformation as illustrated in scheme 1.



Scheme **1**: Photochemistry of flavan-3-ol (Cetinkaya *et al.*, 2017)

The degradation of flavonoids is also known to occur depending on the molecular structure of the flavonoid. Most flavonoids having a hydroxyl group in position 3 and a double bond between C2-C3 are known to have decreased flavonoid stability (Chaaban *et al*., 2017).

Biesaga 2011 reported that the flavonoid stability is affected by the extraction mode with significant decomposition being found in samples obtained through ultrasonic-assisted extraction, maceration and microwave-assisted extraction. Out of the four methods of

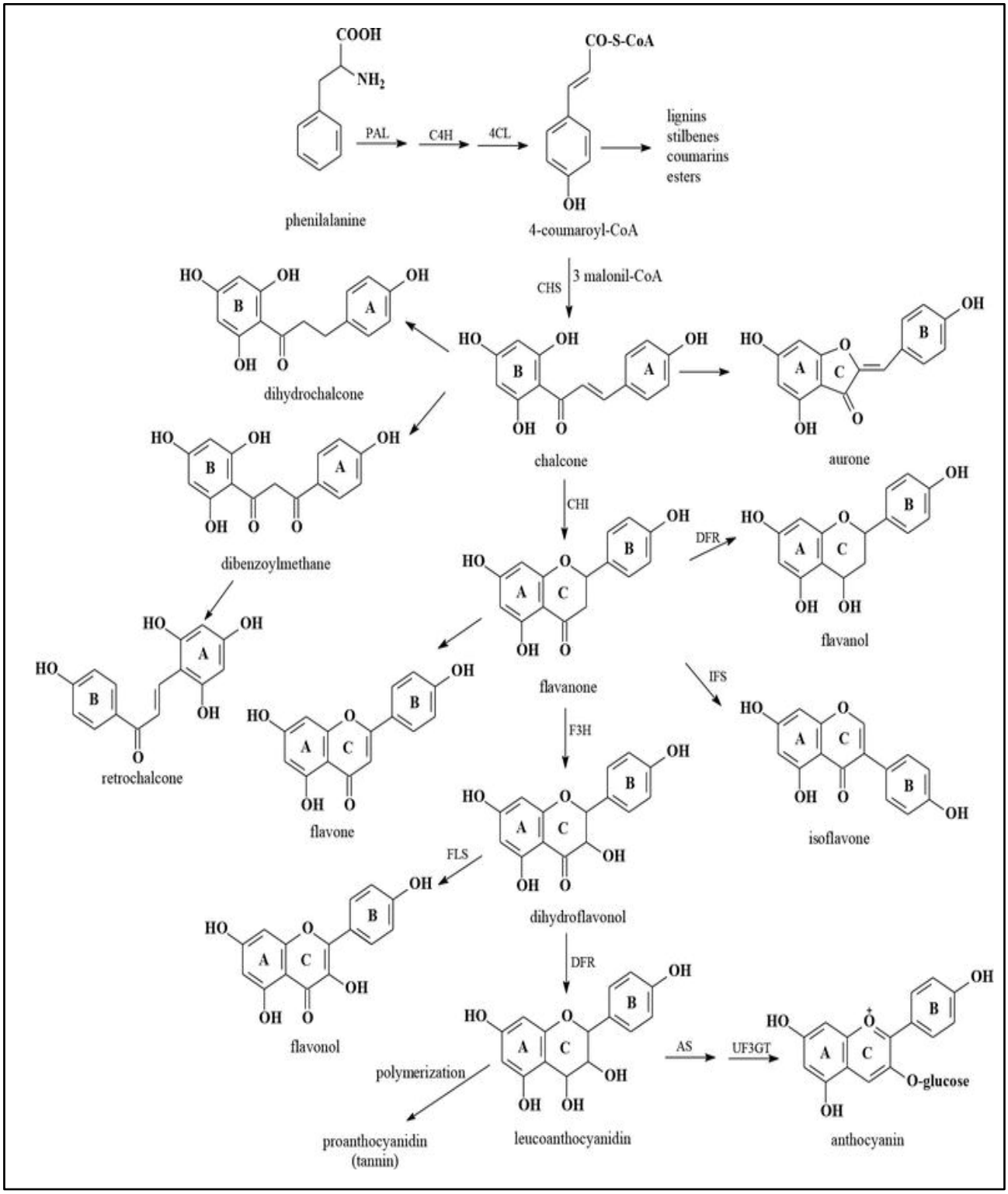
extraction that she used, the traditional soxhlet extraction method observed flavonoids with the best stabilities (Biesaga, 2011).

The acylated flavonoid glycosides are usually unstable at increased temperatures and are therefore frequently thermally degraded during the process of drying of the plant tissues (Marais *et al*, 2006).

2.4.3 Biosynthesis of flavonoids

The biosynthesis of flavonoids in plants is believed to evolve early during land plant evolution (Santos *et al.*, 2017). They are generally synthesized through the phenylpropanoid pathway, starting from phenylalanine and transforming it into 4-coumaroyl-CoA, which finally enters the flavonoid biosynthesis pathway (Havsteen, 2002; Winkel-Shirley, 2001). The phenylalanine comes from shikimic acid pathway (Raffa *et al*., 2017).

A simple schematic of the phenylpropanoid pathway is shown in scheme 2.



Scheme **2**: Simple schematic of the phenylpropanoid biosynthetic pathway. CHS, chalcone synthase; CHI, chalcone isomerase; ANS, anthocyanidin synthase; FLS, flavonol synthase; F3H, flavanone-3-hydroxylase; F3'H, flavonoid-3'-hydroxylase; ANR, anthocyanidin reductase; DHF, dihydroflavonol; UFGT, UDP-glucose flavonoid 3-O-glucosyltransferase; FNR, flavanone 4-reductase; LAR, leucoanthocyanidin reductase (Santos *et al.*, 2017)

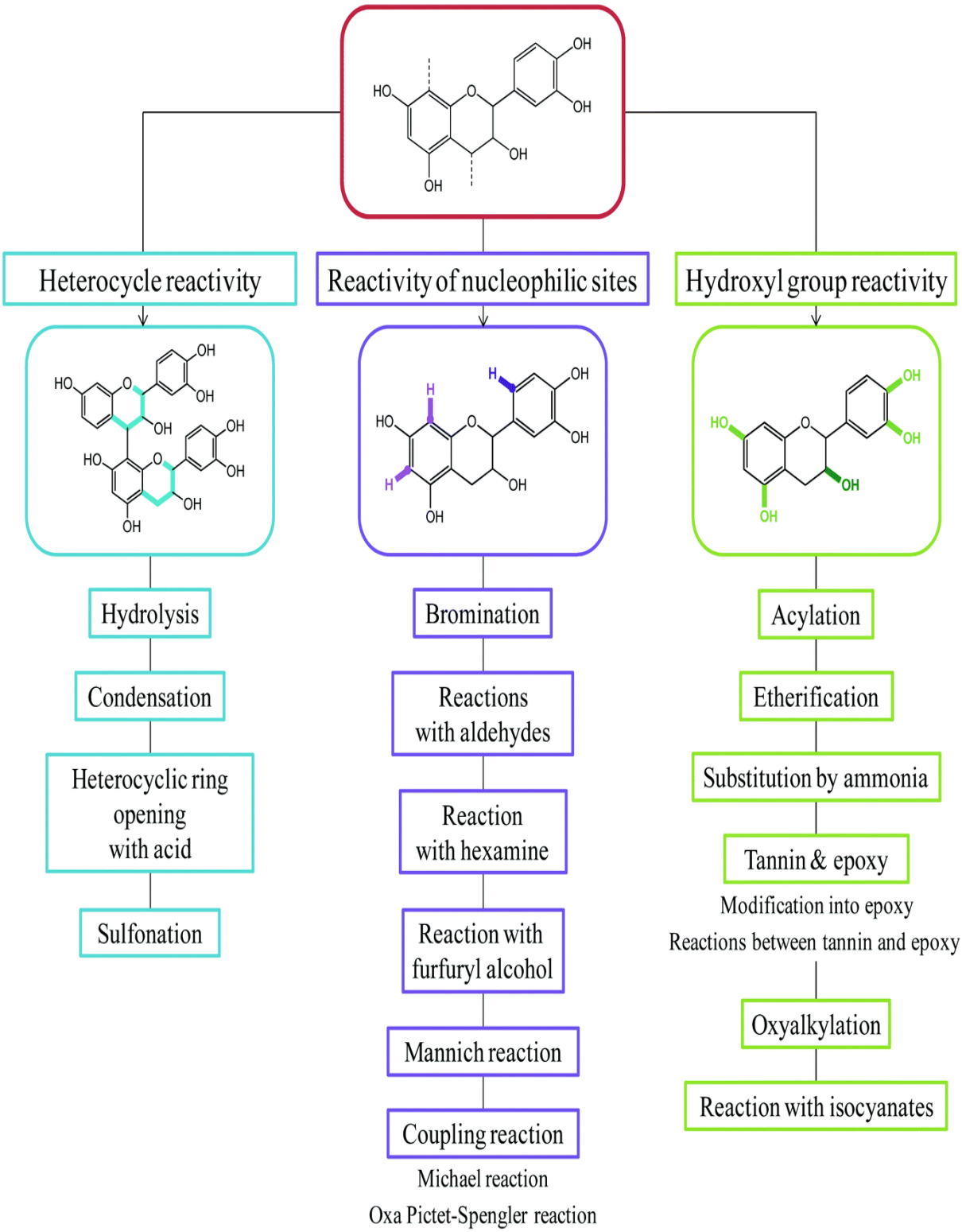
Most enzymes of flavonoid biosynthesis are highly stereoselective and / or stereospecific (Marais *et al*, 2006). Some of the key-enzymes that catalyzes some of the reactions in the pathway are; phenylalanine amonialyase, cinnamate 4-hydroxylase, 4-coumaroyl-coenzyme A ligase and chalcone synthase. The first enzyme in the phenylpropanoid pathway (phenylalanine amonialyase) is being developed as a detoxification treatment for phenylketonuria, a congenital disease of phenylalanine metabolism (Ferrer *et al*., 2008). It catalyzes the conversion of the phenylalanine to *trans*-cinnamic acid (Sharma *et al*., 2019). The cinnamate 4-hydroxylase catalyse the hydroxylation of *trans*-cinnamic acid to *p*-coumaric acid (Chen *et al*., 2017). The chalcone synthase enzyme produces chalcone scaffolds from which all flavonoids derive (Ferreyra *et al*., 2012).

2.4.4 Chemical reactivity studies of flavonoids

During the last few decades, chemical modifications have been made on flavonoids in a view of developing new derivatives that are believed to have better bioactivity, better formulation properties and less sensitive than the corresponding flavonoids. For example, the antioxidant activity of flavonoids usually depends on their structure. Therefore, any chemical modification of these flavonoids like alkylation or glycosylation will lead to either an increase or a decrease in their antioxidant activity (Chaaban *et al*., 2017; Cruz *et al*., 2015).

Chemical modifications of flavonoids have been made possible with the presence of phenolic and hydroxyl groups in the flavonoids structures. This study notes in detail a few of such derivatives that have been synthesized so far with their reaction schemes. Flavonoids usually vary from one another by either the saturation of the ring C, the number of hydroxyl groups present or the position of the aromatic ring B, which can either be bonded to ring C ether at position C-2 or C-3. Their modifications therefore, can be either methoxylation or hydroxylation or *C*-glycosylation on a carbon atom or by *O*-glycosylation of the hydroxyl groups (Marais *et al*, 2006). Furthermore, alkyl groups can be covalently bonded to the flavonoid moieties (Marais *et al*, 2006).

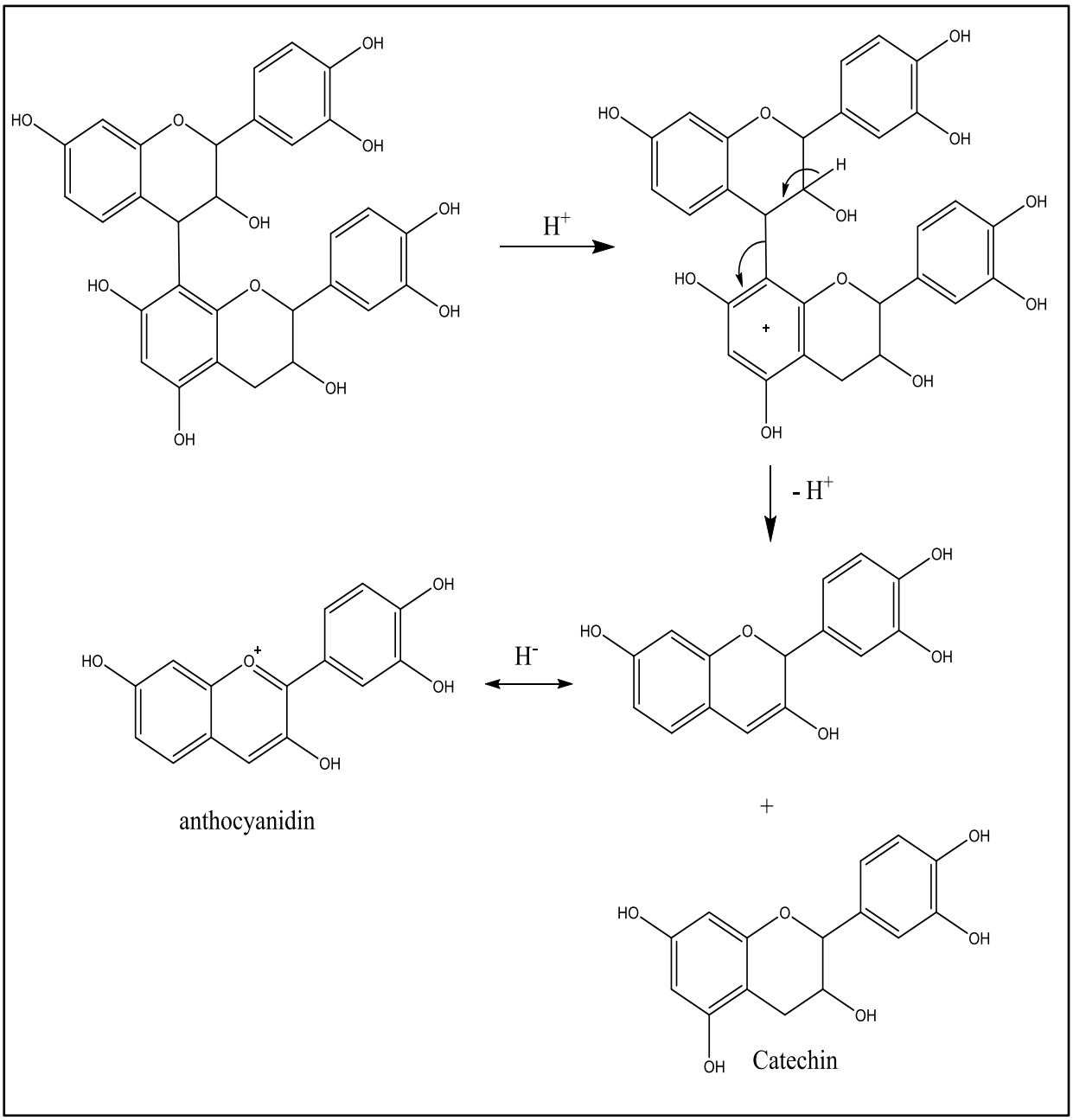
Generally, the chemical modifications of flavonoids can be categorized into three major groups; opening of the heterocycle ring leading to alteration of the chemical structure, reactivity of the nucleophilic sites at the aromatic rings and finally reactions can take place directly with the OH group (Arbenz and Avérous, 2015; Kalia and Avérous, 2016). The reactions are summarized in scheme 3 where catechin compound has been used as an example to show the possible chemical modifications of flavonoids.



Scheme **3**: Possible chemical modifications of flavonoids (Arbenz and Avérous, 2015)

2.4.4.1 Heterocycle reactivity

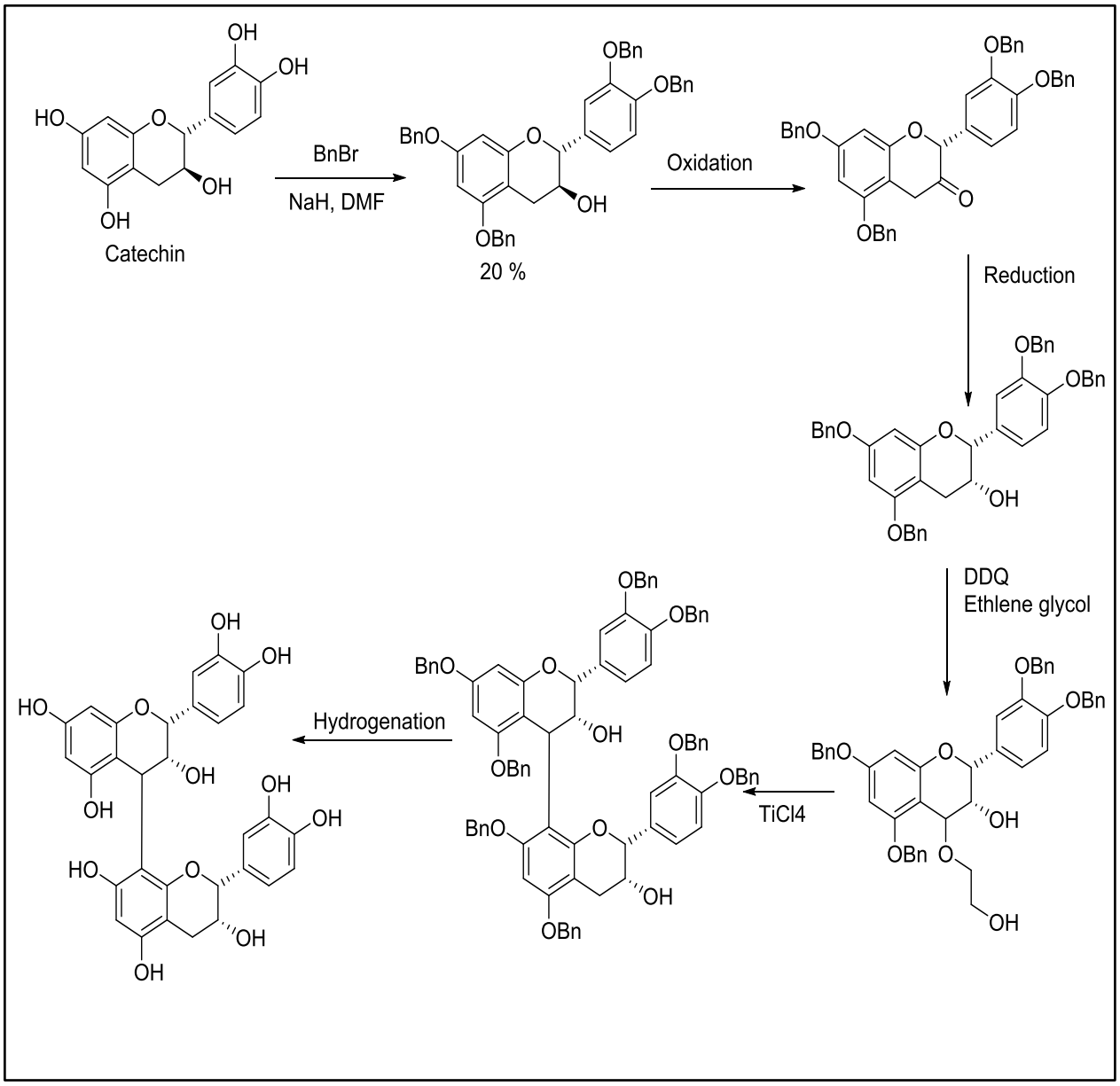
Monomers of some flavonoids can be formed from their corresponding dimers through degradation under acidic media. An example of this is shown in scheme 4 (Arbenz and Avérous, 2015).



Scheme **4:** Degradation of catechin dimer under acidic medium (Arbenz and Avérous, 2015)

On the same note, studies have shown that it is also possible to synthesize the dimers of some flavonoids from their monomers (Higashino, *et al.*, 2018; He, *et al.*, 2008; Es-Safi *et al.*,

2007; Ferreira and Slade, 2002). Scheme 5 shows an example of the coupling of two catechin monomers.



Scheme **5:** Synthesis of catechin dimer from its monomer

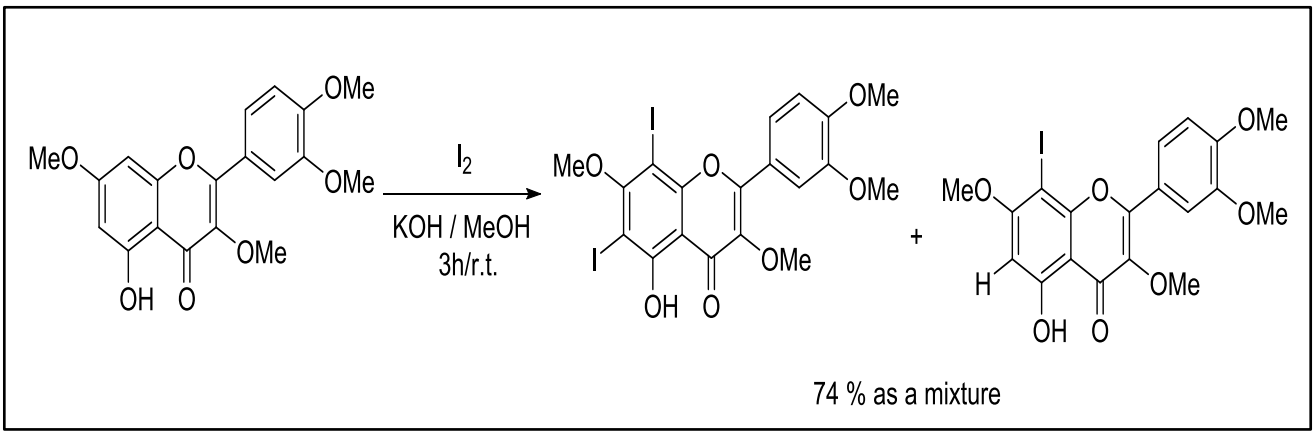
Different lewis acids can be used in the coupling between the electrophile and the nucleophile for example; tin tetrachloride ($SnCl_4$), titanium tetrachloride ($TiCl_4$), or trimethylsilyl triflate (Higashino *et al*., 2018). The proanthocyanidins are known to possess strong antioxidant activity and they naturally occur as a mixture of various analogues from plants making its purification very difficult (Higashino *et al*., 2018).

2.4.4.2 Reactivity of the nucleophilic sites

The nucleophilic sites are present in flavonoids due to the presence of phenol groups that allows the possibility of electrophilic aromatic substitutions reactions to occur (Arbenz and Avérous, 2015). Phenol groups are ortho and para directors and they therefore activate the benzene ring in flavonoids. A lot of reactions have been made on flavonoids by reacting the nucleophilic sites especially the C6 and C8 positions of the A-ring.

The nucleophilicity of flavonoids stems from its resonance between the benzene ring and the free electron pair on the phenolic oxygen. This increases the electron delocalization and confers the position of substituent adjacent to the hydroxyl group a partial negative charge and thus a nucleophilic character (Cheynier *et al*., 2010; Andersen and Markham 2006).

An example of this kind of reaction is shown in scheme 6 below.

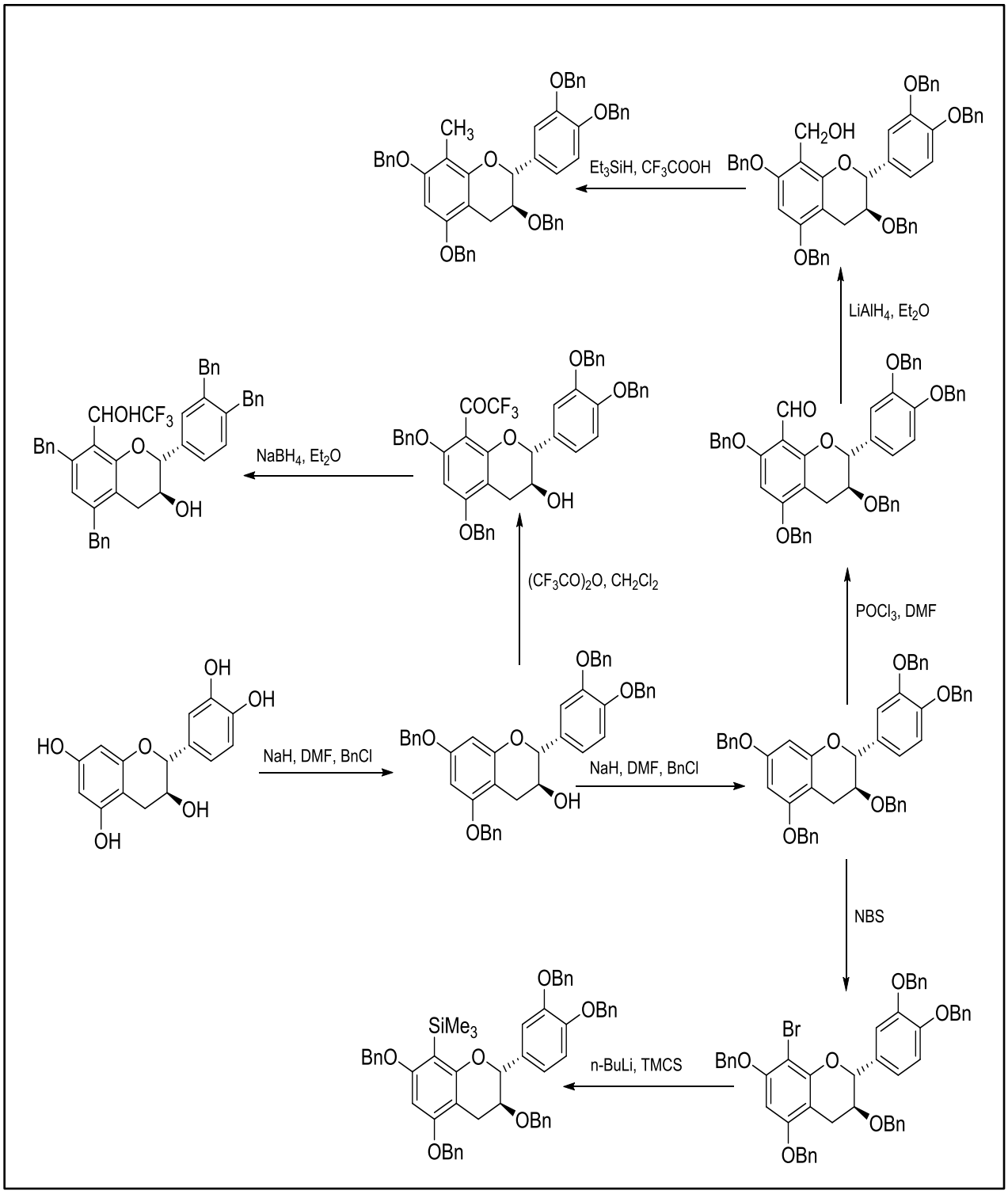


Scheme **6:** Iodination of quercetin (Carvalho, *et al*., 2009)

Carvalho (2009) was able to synthesize the first iodination reaction of quercetin. The iodine was added to both position C-6 and C-8 of the flavonoid as per scheme 6. He used iodine because it is relatively cheap and readily available (Carvalho, *et al*., 2009).

The synthesis of these new derivatives of natural flavonoids can be used as intermediates in synthetic procedure by allowing introduction of new groups with the aim of increasing the biological activity of flavonoids (Carvalho, *et al*., 2009).

Scheme 7 below shows some possible synthetic pathways for catechin with addition of several groups at position C8.

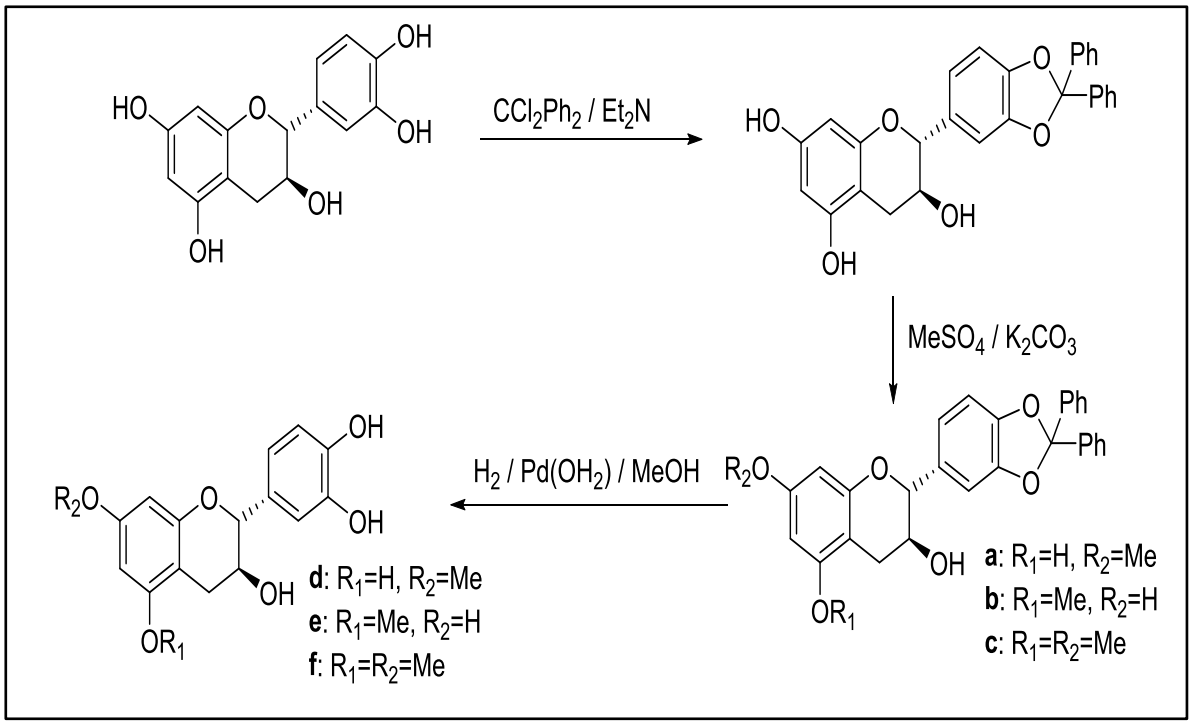


Scheme **7:** Synthesis pathways of catechin monomer (Es-Safi *et al*., 2007)

2.4.4.3 Reactivity at the hydroxyl groups

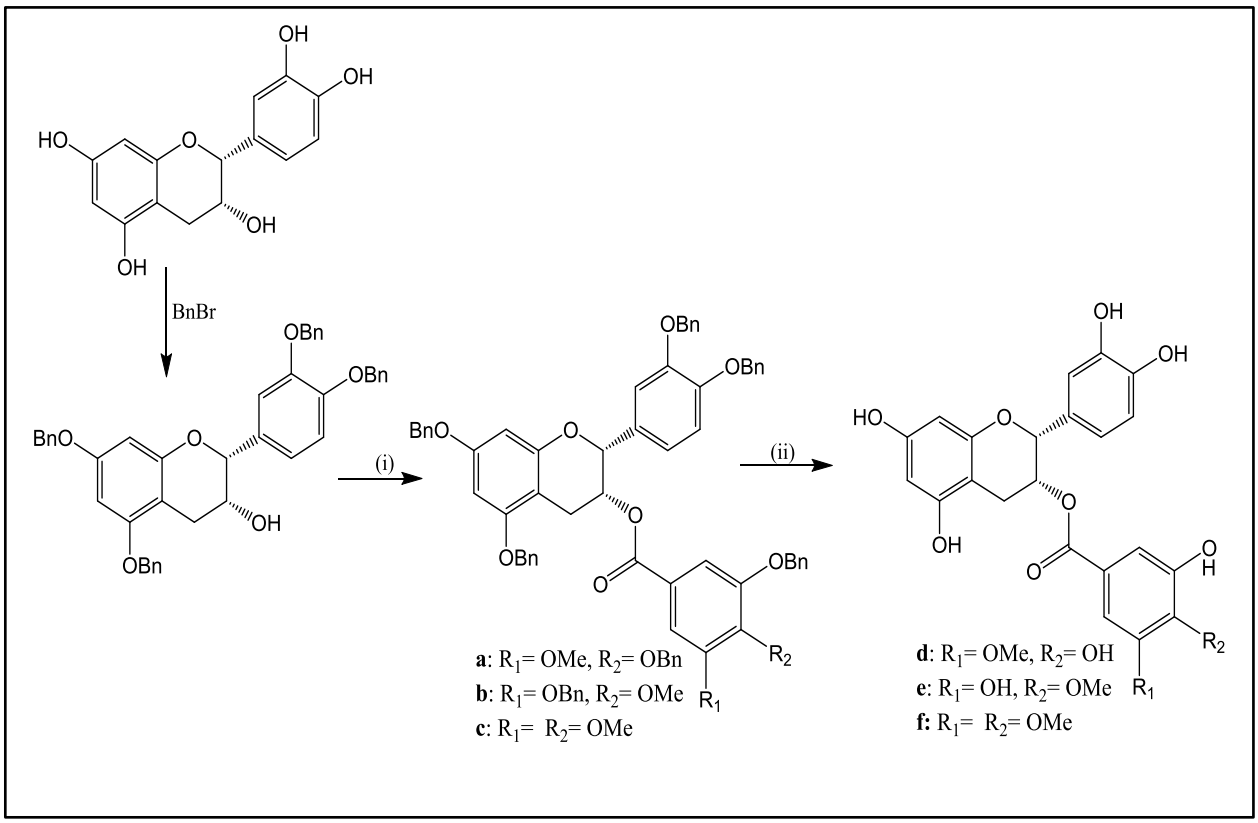
The phenolic hydroxyl groups are the most reactive groups in flavonoids and its reactivity stems from the acidity of the phenolic hydroxyl groups (Andersen and Markham 2006; Panche, *et al*., 2016).

Scheme 8 shows an example of a selective methylation of the phenolic hydroxyl groups of catechin. The reaction started by the protection of the hydroxyl groups at the B-ring of the flavonoid, followed by methylation and finally deprotection by performing a hydrogenation reaction over palladium catalyst.



Scheme **8**: Selective methylation of the phenolic hydroxyl groups of catechin (Ferreira and Slade 2002)

The aliphatic hydroxyl group can also be modified by first protecting the phenolic hydroxyl groups. This leads to several intermediate steps that will finally end by the deprotection reaction. Wan *et al*. (2006), was able to synthesize different methylated catechins at the 3-OH aliphatic position. These derivatives allowed them to evaluate the role of biomethylation in affecting the biological effects of tea consumption. They established that the addition of a methyl group led to decreased proteasome-inhibitory activity *in vitro*. This inhibitory activity decreased more as the number of the methyl groups increased (Wan *et al*., 2006). The reaction scheme is shown in scheme 9.

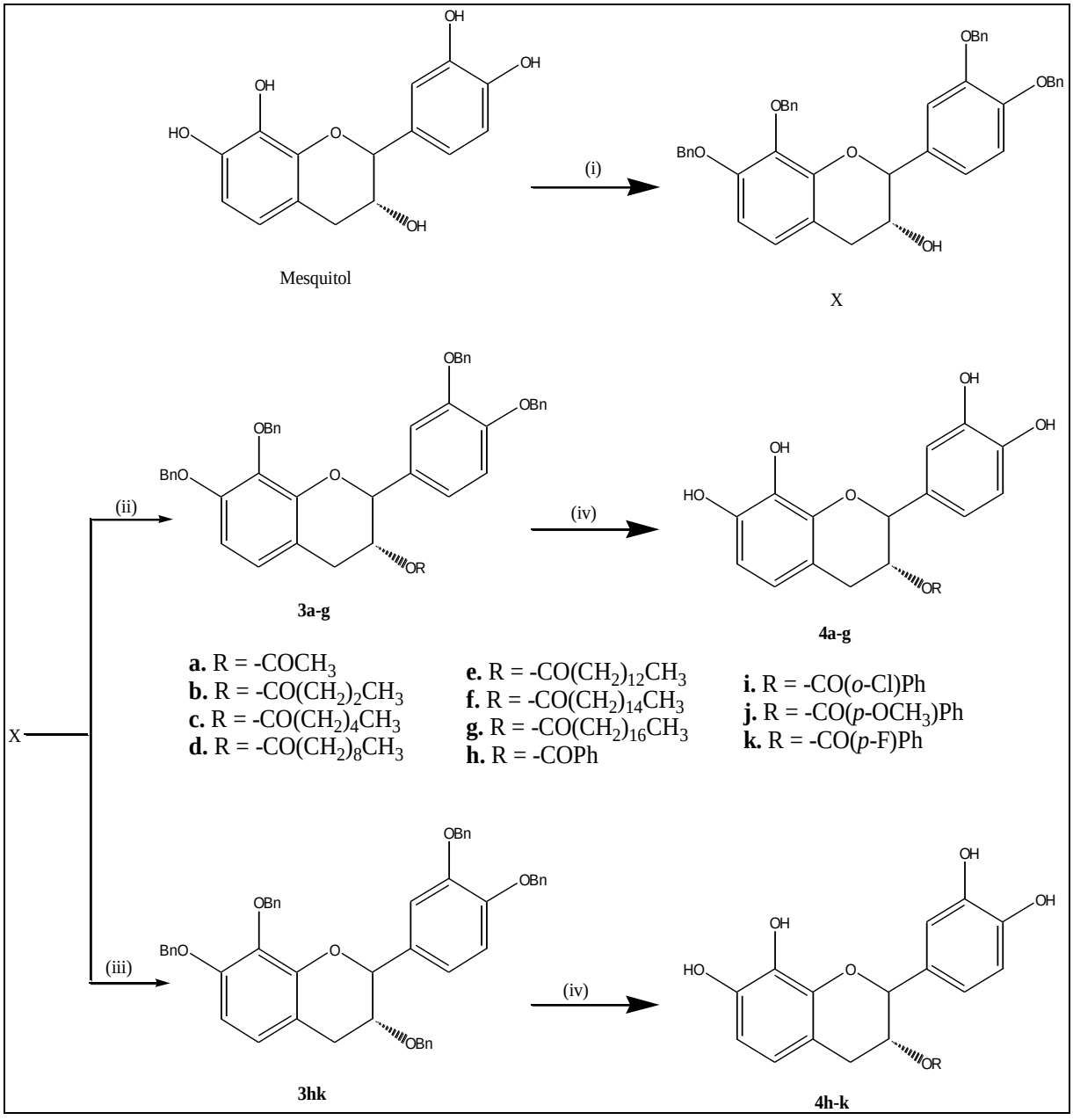


Scheme **9:** Different methylation pathways of catechin. Conditions and reagents: (i) Acid chloride, DMAP and CH_2Cl_2, room temperature (ii) H_2 / Pd $(OH)_2$ and solvent mixture of MeOH:THF (Wan *et al*., 2006).

Having found that (-)-mesquitol elicited better free radical scavenging activity and showed glucosidase inhibitory activity better than (-)-epicatechin but did not elicit xanthine oxidase inhibitory activity due to the presence of free hydroxyl group at C-3 position, Rao 2003 chemically prepared 3-O-aliphatic acyl esters of mesquitol. The aim of this was to obtain derivatives that had xanthine oxidase inhibitory property (Rao *et al*., 2003).

He observed that the acylation of the 3-OH group significantly enhanced glucosidase inhibition and displayed xanthine oxidase inhibitory potential favoring the antioxidant activity. Xanthine oxidase is considered to be the biological source of free radicals (Rao *et al*., 2003).

Scheme 10 shows the reaction scheme for the production of the 3-O-aliphatic acyl esters of mesquitol. The reactions started with protection of the phenolic group, followed by esterification reactions and finally deprotection.



Scheme **10:** Reaction scheme of various mesquitol derivatives. Reagents and conditions: (i) $PhCH_2Br$, K_2CO_3, acetone, reflux; (ii) corresponding aliphatic acid, DCC, DMAP, CH_2Cl_2; (iii) corresponding aromatic acid chloride, Et_3N, CH_2Cl_2; (iv) H_2, 10% Pd-C, MeOH (Rao, *et al.*, 2003).

2.5 Wood preservation

Wood is normally used for many applications. It has been used as a renewable source of energy and as engineering materials for many years and nowadays it is still an essential building material because of its qualities like processability, its light weight, good mechanical properties, sustainability and technological applicability (Jones and Brischke, 2017).

With proper use conditions, wood can be maintained for a long period of time but under unfavorable conditions, it can be damaged and destroyed readily (Thomasson *et al*., 2015). The free hydroxyl groups in wood from the phenolic molecules can absorb water increasing the moisture content (to about 20 %) making it susceptible to fungi and bacterial attack. This attack leads to the degradation of the cell walls hence wood degradation (Ramage *et al.*, 2017).

Fig **17**: Wood degraded by fungi (Wikimedia commons, 2019)

Wood degrading fungi have the capability of breaking down the complex polymers which make up the wood structure. Fungi require adequate moisture, ambient temperature and oxygen for it to thrive on wood. Sapwood generally has more nutrients like carbohydrates than heartwood, hence are more susceptible to fungi attack (Ujang *et al*., 2007).

Fungi which cause wood decay are divided into three categories; the basidiomycetes which cause the brown and white rot decay, the ascomycetes and anamorphic fungi which cause soft rot decay and finally the sap stain and mould fungi which does not cause decay but causes

severe discoloration of wood. The ascomycetes' spores are produced within microscopic cells called asci while the basidiomycetes' spores develop on projections that grow out from the basidia microscopic cells (Ujang *et al*., 2007).

Wood can also be degraded by wood boring beetles, termites, carpenter ants, insects and marine-borers.

For improvement of some of the wood properties like resistance to biological degradation, UV resistance, dimensional and thermal stability and mechanical properties and to give a longer service life, various treatments should be applied to it before use (Jones and Brischke, 2017). Thus wood preservatives are applied according to how and where the wood products will be used and the expected conditions of exposure to wood destroying agents (Thomasson *et al.,* 2015).

Wood treatment involves the modification of cell wall (either by thermal or chemical modification), impregnation or coating (Ramage *et al.,* 2017; Jones and Brischke, 2017). In thermal modification, the chemical and anatomical properties of wood are both changed during heat treatment. Although the wood becomes more brittle and shows lower bending and tensile strength, polycondensation reactions of lignin results in higher strength in the longitudinal direction and an elevation in compressive strength and stiffness (Ramage *et al.,* 2017).

For the chemical modification: applied chemical reagents react with the hydroxyl and phenyl groups of the cell wall polymers. This leads to the blockage of the hydroxyl groups hence reduction of hygroscopicity. Chemical modification results in permanent change to the molecular structure and leads to good durability and dimensional stability without loss of strength as compared to wood treated by thermal modification (Ramage *et al.,* 2017).

Wood impregnation generally entail the filling of the cavities and lumina in the cell walls with bulking chemicals that hardly react with the wood polymers but elevates the wood density and clog up pathways for water. After impregnation, the permeability of wood decreases leading to better resistance to water and fungi. The impregnating preservatives could be able to kill fungi, bacteria and insects directly, or are fire retardant causing thermal stability (Ramage *et al.,* 2017).

Wood can also be preserved by coating. This is applying or painting a layer on the surface of wood, supplying a physical barrier against weathering and degradation (Bulian and Graystone, 2009).

The synthetic organic and inorganic preservatives can however, be very harmful to human health and the environment. Several studies have considered the use of wood extractives as possible alternatives for wood preservation as natural preservatives (Nascimento, *et al*., 2013; Tascioglu *et al*., 2013). Many extractives from wood have therefore, found application as wood preservatives. The study by Tascioglu (2013) suggested that some commercial plant extracts can be used as environmentally friendly alternative wood preservative against common wood decay fungi. The study used extracts from *mimosa, quebracho* and *Pinus brutia* to test for wood preservative properties. He used them to treat both sapwoods of pine and beech wood blocks. The study observed low mass losses especially for the *quebracho* and *mimosa* extracts treated wood blocks at 9 % and 12 % concentration levels against both the brown rot and white rot fungi (Tascioglu *et al*., 2013).

CHAPTER 3

MATERIALS AND METHODS

3.0 MATERIALS AND METHODS

3.1 Reagents and apparatus

3.1.1 Reagents

Formic acid, methanol, ethyl acetate and acetonitrile used were HPLC grade and were purchased from Merck Company, Germany. Dichloromethane, acetone, ethyl acetate, toluene, ethanol, hexane, anhydrous acetonitrile, methanol and tetrahydrofuran solvents were analytical grade and were purchased from Merck Company, Germany. All the reagents used (capric acid, lauric acid, myristic acid, DMAP, DCCI, CS_2CO_3, TBAI, $Pd(OH)_2/C$, benzyl bromide, TFA, phenyl isocyanate, tebuconazole, HCl and succinic anhydride were commercially obtained and were not purified whatsoever. $MgSO_4$ and Na_2SO_4 salts were purchased from Merck Company, Germany. The water used for extraction was deionized in the laboratory using the Purelab option-Q Elga water deionizer. Analytical TLC was performed on Merck silica gel 60 F 254 plates and vanillin and UV (detection was done at 254 nm) were used as visualizers. FT-IR spectra were recorded with a Perkin Elmer FT-IR spectrophotometer and water extracts were dried using a Christ Beta 2-8 LD plus lyophilizer.

Statistical analysis

Tukey's test analysis was used to compare the data of the amounts of mesquitol of both the small and big trees in the three geographic regions and in the different plant parts. The correlation of the mesquitol quantities between the small trees and the big trees was also computed using ANOVA. The analyses were conducted using R statistical software.

3.1.2 NMR analyses

The NMR spectra (^{1}H NM Rand ^{13}C NMR) were done using a Bruker 400 MHz and were recorded using $CDCl_3$, CD_3OD, D_2O, acetone or DMSO deuterated solvents. The chemical shifts (δ) were expressed in parts per million and the coupling constants (*J*) in hertz. The shifts were reported relative to the solvent peak. Multiplicities were recorded as singlets (s), doublets (d), triplet of doublets (td), quartets (q), doublets of doublets (dd) and multiplets (m).

3.1.3 GC-MS analyses

GC-MS analyses were performed using a Clarus 500 GC gas chromatograph (Perkin Elmer Inc., USA) which was coupled to a Clarus 500 quadrupole MS mass spectrometer (Perkin Elmer Inc., USA). Gas chromatography was done on a 95 % dimethyl polysiloxane and 5 % diphenyl fused-silica capillary column (Elite-5ms, 60 m x 0.25 mm, 0.25 mm film thickness, Perkin Elmer Inc, USA). The GC was equipped with an electronically controlled split / split less injection port. The injection (injection volume of 1 µL) was performed at 250°C in the split mode (split flow of 20 mL/min). Helium was used as carrier gas, with a constant flow of 1.2 mL/min.

The temperature program of the oven was 200°C for a constant period of 4 min, 200°C to 330°C at a rate of 5°C/min and then finally constant for 330°C. Ionization was achieved under the electron impact mode (ionization energy of 70 eV). The source and transfer line temperatures were 250°C and 330°C, respectively. Detection was done in scan mode of m/z 35 to m/z 700 a.m.u. The detector was switched off in the initial 10 min (solvent delay). A volume of 100 ml of trimethylchlorosilane (BSTFA / 1% TMCS) was added to 1 to 2 mg of each sample to form its tetramethylsilyl derivatives. These were then heated at 60 °C for 12 hours. Finally, 1 ml of ethyl acetate was added to each sample before aspiration into the GC-MS. The compounds present in the extracts were identified using the NIST library.

3.1.4 LC-ESI-MS/MS analyses

Liquid chromatography-mass spectrometry analyses of samples were carried out using a Shimadzu (Noisiel, France) LC-20A ultra-HPLC (UHPLC) system equipped with an auto sampler and interfaced to a PDA UV detector SPD-20A, followed by an LC-MS 8030 triple-quadruple mass spectrometer. The separation was carried out at a flow rate of 0.4 mL/min on a Luna C18 analytical column (inner diameter, 150 mm by 3 mm; Phenomenex, Le Pecq, France) using a 10 minutes gradient as follows: starting from 2 % of acetonitrile which contained 0.1 % formic acid in water also containing 0.1 % formic acid solution, acetonitrile proportion was increased linearly to 20 % in 3 min then to 80 % in 6.25 min. Initial conditions were then reached in 0.25 min. The injection volume was 1 µL. UV-visible spectra were recorded between 190 and 800 nm.

Negative and positive electrospray mass spectrometric analyses were performed at a unit resolution between 100 and 2,000 m/z at a scan speed of 15,000 U/s. The desolvation line and

heat-block temperatures were 250°C and 400°C, respectively. Nitrogen was used as a nebulizing (3 liters/min) and drying (15 liters/min) gas. The ion spray voltage was +/- 4,500V. Approximately 10 mg of each of the acetonic extracts was dissolved in 1000 µl of methanol before aspiration into the LC-MS. Identification was achieved by comparison of experimental retention times and UV-MS spectra to bibliographic data and standard compounds.

3.1.5 Sample preparation

The plant samples were collected from their natural habitats in Baringo County (latitude 0° 28' 0" N, longitude 35° 58' 0" E), Garissa County (latitude 0° 27' 09" S, longitude 39° 38' 45" E) and Turkana County (latitude 03° 09' N, longitude 35° 21' E) in Kenya. The samples were separated into two categories of ages; small trees, aged less than four years and the big trees, aged more than eight years. Each sample was thereafter separated into five different plant parts: the bark, sapwood, knot wood, heartwood and the pith. They were air dried, then dried at 103°C until weight stabilization and were then ground differently into fine powders using a Fritsch pulverisette 9 laboratory vibrating cup mill at 1200 rpm for 1 minute and passed through a 115-mesh sieve.

3.2 Optimum extraction method for mesquitol

The first objective of this study was to determine the best extraction method for mesquitol with the aim of proposing the best alternative for a subsequent industrial application. Comparisons were done using different solvents and extraction methods. There is a growing demand in industries for more environmentally friendly solvents which are less expensive and non-toxic. Use of large amounts of organic solvents for extraction is hazardous and also leads to increase cost of solvent waste disposal (Ho *et al*., 2008).

The extraction solvents used for this study were; water, ethanol and a mixture of both ethanol and water in a ratio of 1:3 respectively. The best industrial solvent which is an alternative to the usual solvents is normally water because of its low cost and its non-toxic character (Ho *et al*., 2008). This is the reason water was used in the study.

3.2.1 Solvent extraction methods

Solvent extraction method is mainly used in the extraction of phenolic compounds from their plant sources because of their ease of use, efficiency, and wide applicability (Rajbhar *et al.*, 2014).

In this study, different extraction methods were studied using one sample from the heartwood of the big trees from Baringo County. The extraction methods carried out were; soxhlet extraction, maceration extraction, microwave-assisted extraction and accelerated solvent extraction using the Dionex ASE extractor.

The percentage yields of the extractives were calculated using the formula:

$$\% \text{ yield} = \frac{mf}{mi} \times 100\ \%$$

Where mf was the weight of the extractives after extraction

mi was the weight of the sample powder before extraction.

3.2.1.1 Soxhlet extraction method

In this study, triplicate extractions were performed on a one litre soxhlet apparatus on 35 g of sample powder using four solvents (ethanol, water, ethanol: water (1:3) and acetone). The solvents used each measured 600 ml.

Figure 18 shows the general Soxhlet extraction set-up used in the study.

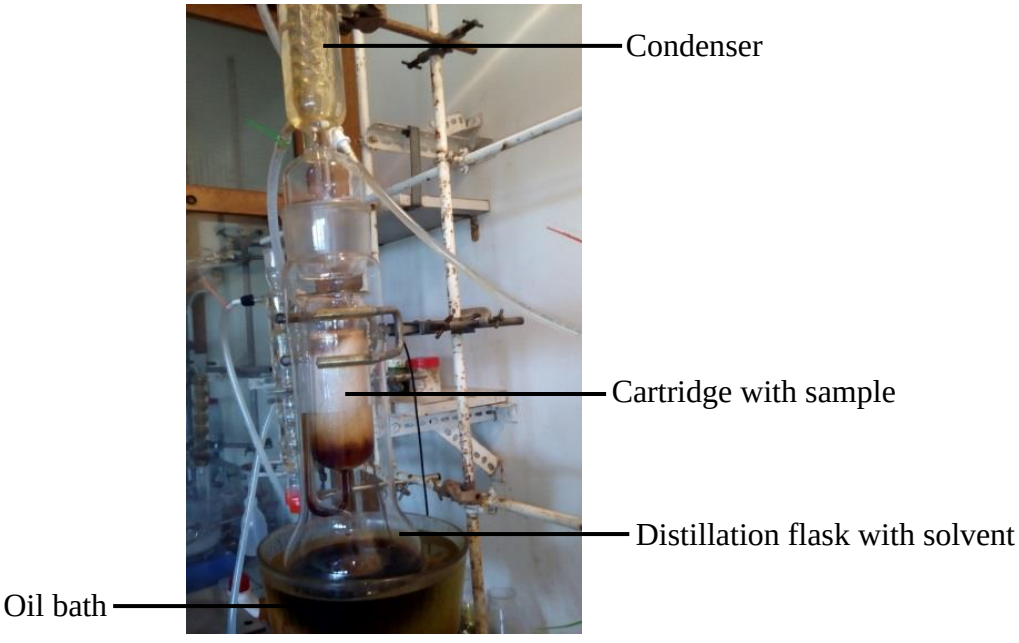


Fig **18:** Soxhlet apparatus set-up

Each extraction was done for six hours and one cycle was completed after every five minutes. Timing started after the first cycle of extraction was obtained. The solvent extracts were then concentrated at low pressure using the Buchi rotavapor to give a dark-brown extract and the water extracts were dried using a lyophilizer.

3.2.1.2 Maceration method

Triplicate extractions were performed using an Ika labortechnik RW 20 overhead stirrer apparatus that was used for the mechanical stirring of the extracts. Fifty grams of the solid sample of *P. juliflora* was weighed and dissolved in 500 ml of solvent. The solvents used were ethanol, water, ethanol: water (1:3) and acetone. Figure 19 shows the maceration extraction set-up using the Ika labortechnik RW 20 apparatus.

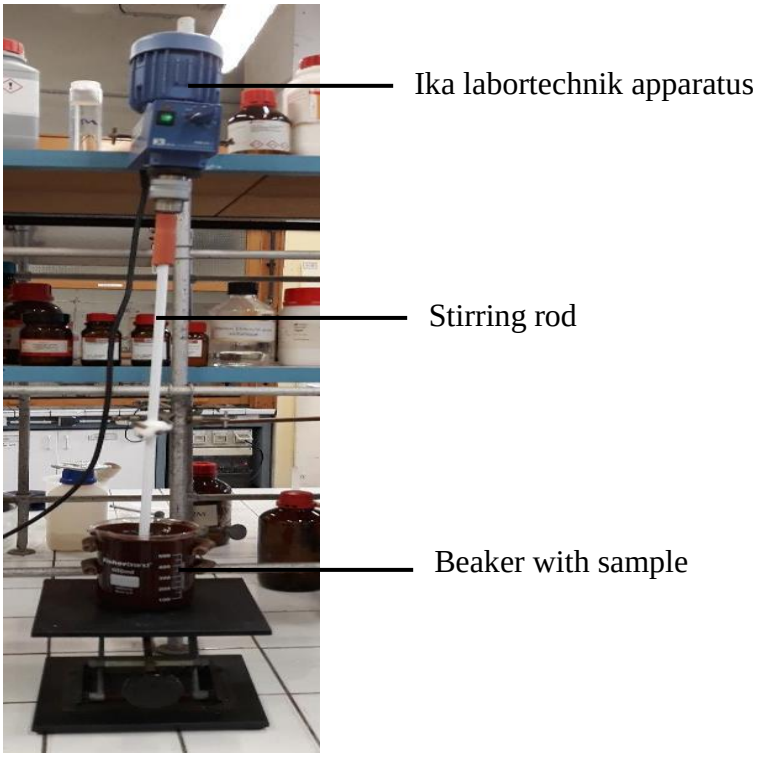


Fig **19:** Maceration extraction using a mechanical overhead stirrer

Each extraction was done for twenty four hours and this was followed by filtration using a filter paper. The filtrates were then concentrated at low pressure using the Buchi rotavapor to give a dark-brown extract while the water extracts were dried using a lyophilizer.

3.2.1.3 Microwave-assisted extraction method (MAE)

In this study, triplicate extractions were performed on 2 g of sample powder. The sample to solvent ratio was 1:10 (w/v). The solvents used were ethanol, water, ethanol: water (1:3) and acetone). Figure 20 shows the microwave reactor monowave 450 that was used for the microwave-assisted extraction.

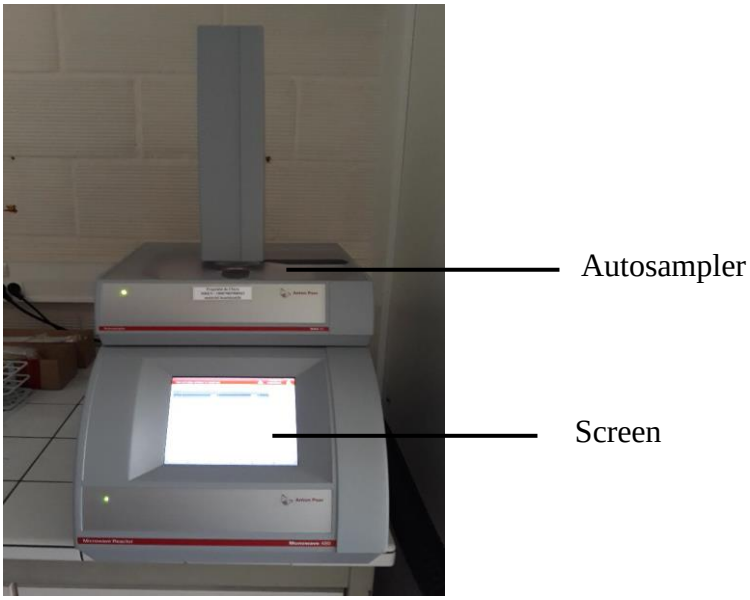


Fig **20:** A microwave reactor monowave 450

Two parameters of the MAE method were investigated; microwave power and irradiation time. The different microwave powers that were used were 200 W, 400 W and 800 W. Two different irradiation times of 10 mins and 20 mins were also investigated. This was to obtain an optimum MAE extraction method. An agitation speed of 1000 rpm was used in each extraction.

After every extraction, the vials were allowed to cool before filtration. The filtrates were afterwards concentrated at low pressure using the Buchi rotavapor to give a dark-brown extract. The water samples were dried using a lyophilizer.

3.2.1.4 Accelerated solvent extractor (ASE)

Triplicate extractions were performed on a Dionex Accelerated solvent extractor on 10 g of sample powder using different solvents which were; ethanol, water, ethanol: water (1:3) and acetone. Figure 21 shows the Dionex ASE that was used for the extraction.

Fig **21:** A Dionex Accelerated Solvent Extractor

The sample is placed in an extraction cell which is made of stainless steel and placed on the cell tray. The extractor adds the solvent after which the cell is pressurized and heated to the desired temperature (50 – 100 °C). This is followed by the extraction of the sample statically for a certain period of time after which the extract is removed from the extraction cell and the cell is flushed with fresh solvent. This can be repeated several times depending on the desired number of cycles. After the extraction is complete, compressed nitrogen is used to drain all the solvent from the cell into the vials in the vial tray.

In this study, an ASE Dionex extraction method was developed and optimized. The extractions were done on a 34 ml cell size at 100 °C under 3 static cycles of 5 minutes each and after each extraction the organic solvents were evaporated under vacuum using a rotary evaporator. The water samples were dried using a lyophilizer.

3.3 Geographical and natural intraspecific variability of *P. juliflora* extractives

A substantive study was done on the geographical and intra-specific variability of wood extractives from *Prosopis juliflora* from three different regions in Kenya; Baringo County, Garissa County and Turkana County. The aim of this was to show the variability of extractives present in *P. julifora* trees from different geographic habitats, the age of the tree and the different parts of the tree's stem with the main emphasis on the mesquitol compound. This was done by comparing the percentage yields of extractives using different solvents followed by identifying, quantifying and comparing some compounds found in the different extracts. The structures of the compounds were tentatively identified by a reversed-phase liquid chromatography coupled to electrospray ionization – tandem mass spectrometry.

3.3.1 Extraction method for variability studies

Among the extraction methods, the ASE Dionex extraction method was found to be fast and efficient method for extraction compared to the other three extraction methods yielding high yields and was therefore chosen as the extraction method used for the extraction of the different samples for the geographical and intra variability study.

An ASE Dionex extraction method was therefore developed where serial extractions of the different samples were done using various organic solvents in order of their increasing polarity (dichloromethane, acetone, followed by toluene / ethanol (2/1, *v/v*) and finally water). These samples included the samples from both the big and small trees collected from the three Counties that had been each separated into five different parts (bark, sapwood, knot wood, heartwood and pith). The extractions were performed on a 34 mL cell size on 8 g of sample powder at 100°C under three static cycles of 5 minutes each. After each extraction the organic solvents were evaporated under vacuum using a rotary evaporator. The water samples were dried using a lyophilizer.

3.3.2 Analyses of *P. juliflora* extractives

GC-MS analyses were performed on all the extracts that had been obtained from the dionex ASE extraction. Two mg of each extract was used for the GC-MS analyses and some of the compounds present identified using the NIST library.

Since most of the compounds could not be identified with the GC-MS, a LC-ESI-MS/MS was thereafter used. The GC-MS analyses had shown that the acetonic extracts contained high quantities of phenolic compounds which are abundant in *P. juliflora*. The study therefore used the acetonic extracts from all the samples to perform the LC-ESI-MS/MS analyses. The LC-ESI-MS/MS has higher sensitivity, selectivity and robustness than the GC-MS (Scherbaum *et al.*, 2008).

The identification of compounds present in the different stem parts of the *P. juliflora* and the quantification of the three major flavan-3-ols was therefore done using the LC-ESI-MS/MS in the negative ion mode. This is because it has been demonstrated that the negative ion mode has higher sensitivity and selectivity compared to the positive ion mode detection which generates a higher background signal (Gu *et al.*, 2003, Sun *et al.*, 2002). Another reason for doing the quantification in the negative ion mode was also because of the free phenol groups present in most of the compounds identified since acids deprotonate easily in the negative ion

mode while they form adducts with cations in the sample or mobile phase in the positive mode (Swatsitang *et al.*, 2000).

3.4 Extraction and isolation of mesquitol for chemical modification

3.4.1 Extraction and isolation of mesquitol

Mesquitol was extracted in bulk through soxhlet extraction. Eighty (80) g of the ground sample was extracted using a one litre soxhlet apparatus. Extraction was first done using dichloromethane solvent to remove the fatty acids and waxes and then followed by acetone solvent. The dichloromethane extraction was done for two hours and the acetone extraction was done for six hours.

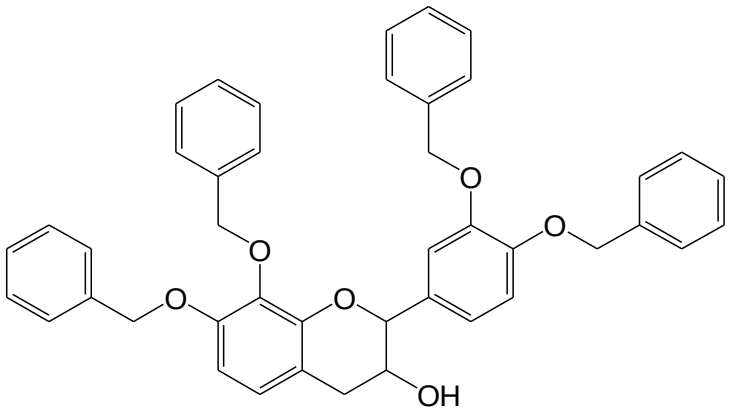
This was followed by concentrating the extract to dryness using a rotavapor to obtain a dark-brown sticky paste. This was fast purified by washing with diethyl ether using a silica-bed fritted glass funnel of Pyrex 3 under low pressure. The filtrate was concentrated on a Buchni rotavapor to give a yellow solid sample. The NMR confirmed the yellow sample obtained as pure mesquitol.

3.4.2 Chemical modification of mesquitol

Several targeted chemical reactions of mesquitol were done. The reactions were done at the 3-OH aliphatic position. The study looked at reactions of mesquitol with carboxylic acids, anhydrides and amines. The characterization of the mesquitol derivatives and their intermediates were done by proton and carbon NMR, elemental analyses, FT-Infrared spectroscopy and determination of melting points for solids.

This section summarizes the experimental procedure for each reaction with the characterization obtained for each compound. All the compounds obtained were purified using a classical column filled with silica gel.

Compound **[21]**



TBAI (2.76 mmol, 0.8 eq) was mixed with 1 g of mesquitol in a two-neck round bottomed flask. The mixture was dissolved in 20 ml acetone under nitrogen environment. CS_2CO_3 base (13.78 mmol, 4 eq) was added while agitating and this was followed by the addition of benzyl bromide (27.56 mmol, 8 eq) drop wise. The reaction was carried out for 24 hours at ambient temperature after which the solvent was evaporated under vacuum using a Buchni rotavapor. The solid residue obtained was dissolved in 100 ml of ethyl acetate and then washed two consecutive times using 50 ml of water. The remaining extra base that was dissolved in the aqueous phase was separated from the organic phase. The organic phase was recuperated and dried by $MgSO_4$. Filtration was then done and the residue rinsed with ethyl acetate. The filtrate was finally concentrated to dryness using a rotavapor and the solid product formed purified by a classical column on silica gel. Visualization was done by vanillin which revealed the desired compound **[21]** at an Rf of 0.25 with an eluent system of (7:3) hexane: ethyl acetate. The characterization of compound **[21]** is given below.

MW: 650.76; Color: White; Mpt: 150 °C; Percentage yield: 66 %

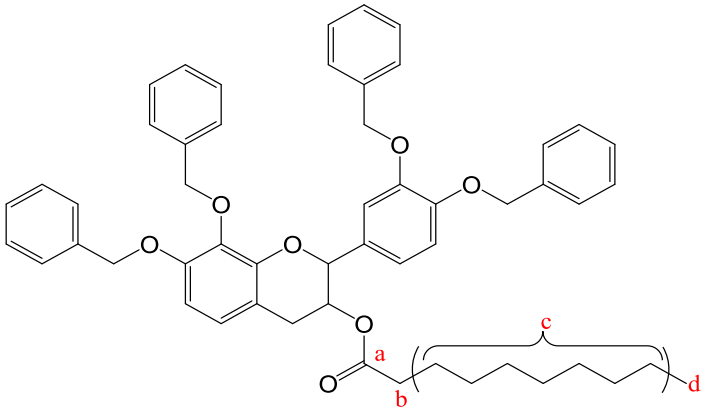
^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 7.5-7.1 (20 H, m, H^{Ph}), 7.1-6.9 (3 H, m, $H^{2', 5', 6'}$), 6.74 (1 H, m(para), *J* 8.5 Hz, H^5), 6.58 (1 H, m(para), *J* 8.6 Hz, H^6), 5.3-5.0 (8 H, m, $H^{\underline{CH}Bn}$), 4.71 (1 H, d, *J* 7.8 Hz, H^2), 3.97 (1 H, td, H^3), 2.99 (1 H, dd, *J* 5.1 and 14.4 Hz, H^4), 2.81 (1 H, dd, *J* 8.2 and 14.4 Hz, H^4).

^{13}C NMR (400 MHz, $CDCl_3$) δ (ppm): δ (ppm): 81.57 (C-2), 68.17 (C-3), 32.29 (C-4), 124.01 (C-5), 107.86 (C-6), 149.01 (C-7), 137.86 (C-8), 149.22 (C-9), 114.97 (C-10), 131.17 (C-1'), 113.80 (C-2'), 151.37 (C-3'), 148.15 (C-4'), 114.51 (C-5'), 120.32 (C-6'), 75.11-71.29 (C-$\underline{C}H_2$Bn), 128.58-127.21 ($\underline{C}$H-Ph), 137.36-136.90 ($\underline{C}$-Ph).

Elemental analysis: Calcd. (%) for $C_{43}H_{38}O_6$: C 79.36, H 5.89, Found: C 79.10, H 5.90.

IR (υ_{max}/cm^{-1}): 3550 – 3300 (OH), 3100 – 3000 (CH aliphatic), 1610 (C=O).

Compound **[22]**



Benyzlated mesquitol (0.5 g) was dissolved in 40 ml of anhydrous acetonitrile in a two-neck round-bottom flask. Under nitrogen environment, DMAP (1 mmol, 1.3 eq) was rapidly added followed by addition of DCCI (1 mmol, 1.3 eq) and finally capric acid (1 mmol, 1.3 eq). The reaction was then purged with nitrogen for about 1 minute after which it was carried out at reflux (80 °C) for 12 hours. After this period elapsed, the reaction mixture was cooled to room temperature and the precipitate formed filtered off under vacuum. The precipitate was rinsed abundantly with acetonitrile. The filtrate obtained was then concentrated to dryness using a rotavapor and the solid product formed finally purified by a classical column on silica gel. Visualization was done by vanillin which revealed the desired compound **[22]** at an Rf of 0.27 with an eluent system of (9:1) hexane: ethyl acetate. Characterization of compound **[22]** is given below.

MW: 805.01; Color: White; Mpt: 88 °C; Percentage yield: 79 %

^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 7.4-7.1 (20 H, m, H^{Ph}), 6.95 (1 H, s, $H^{2'}$), 6.9-6.75 (2 H, m, $H^{5', 6'}$), 6.6 (1 H, m, H^5), 6.5 (1 H, m, H^6), 5.2 (1 H, td, H^3), 5.1-4.95 (9 H, m, H^2, $H^{\underline{CH}Bn}$), 2.8 (1 H, dd, H^4), 2.65 (1 H, dd, H^4), 1.35 (2 H, m, H^{CH2}), 1.25-1.1 (14 H, m, H^{CH2}), 0.8 (3H, t, H^{CH3}).

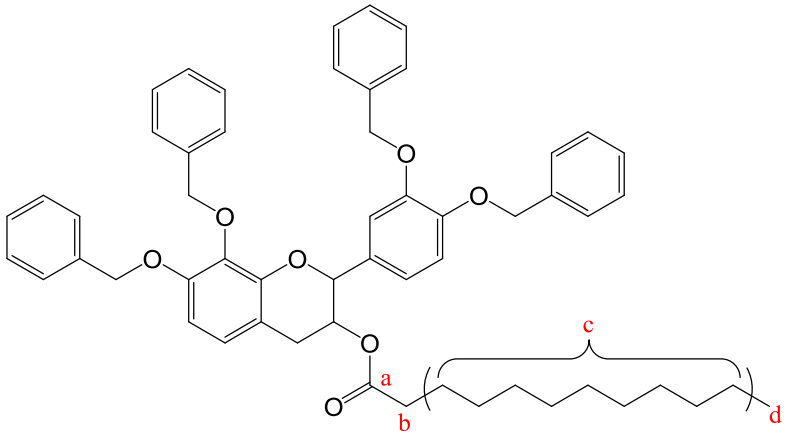
^{13}C NMR (400 MHz, $CDCl_3$) δ (ppm): δ (ppm): 78.15 (C-2), 69.04 (C-3), 31.86 (C-4), 123.75 (C-5), 107.46 (C-6), 147.72 (C-7), 137.34 (C-8), 148.83 (C-9), 114.84 (C-10), 131.49 (C-1'), 113.19 (C-2'), 151.47 (C-3'), 137.86 (C-4'), 113.48 (C-5'), 119.59 (C-6'), 75.07-71.27 (C-

$\underline{C}H_2Bn$), 128.57-127.25 ($\underline{C}H$-Ph), 137.23-136.71 ($\underline{C}$-Ph), 173.09 (C-a), 34.34 (C-b), 30.94-22.67 (C-c), 14.10 (C-d).

Elemental analysis: Calcd. (%) for $C_{53}H_{56}O_7$: C 79.08, H 7.01, Found: C 78.75, H 7.16.

IR (υ_{max}/cm^{-1}): 2950 – 2850 (CH aliphatic), 1729 (C=O), 1609 (C=C)

Compound **[23]**



The same reaction as for compound **[22]** was carried out but lauric acid (1 mmol, 1.3 eq) was used instead of capric acid. After column purification, visualization was also done by vanillin which revealed the desired compound **[23]** at an Rf of 0.28 with an eluent system of (9:1) hexane: ethyl acetate. Characterization of compound **[23]** is given below.

MW: 833.06; Color: White; Mpt: 89 °C; Percentage yield: 87 %

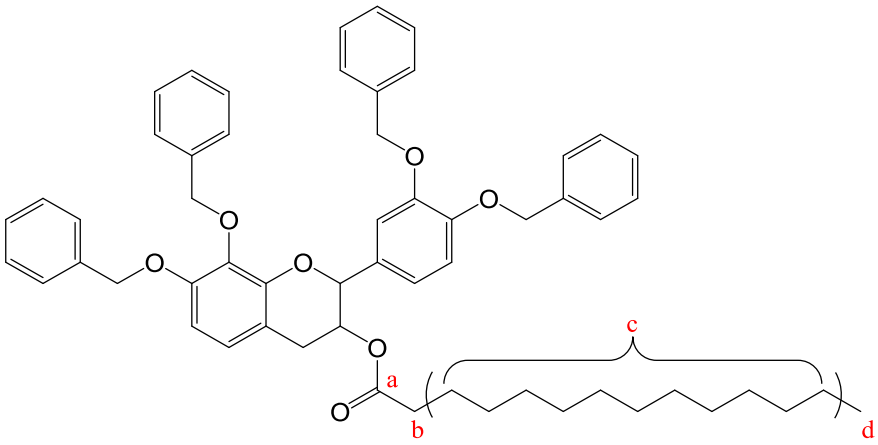
^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 7.4-7.1 (20 H, m, H^{Ph}), 6.95 (1 H, s, $H^{2'}$), 6.9-6.75 (2 H, m, $H^{5', 6'}$), 6.6 (1 H, m, H^5), 6.45 (1 H, m, H^6), 5.2 (1 H, td, H^3), 5.1-4.95 (9 H, m, H^2, $H^{\underline{CH}Bn}$), 2.8 (1 H, dd, H^4), 2.65 (1 H, dd, H^4), 1.4 (2 H, m, H^{CH2}), 1.25-1.1 (18 H, m, H^{CH2}), 0.8 (3H, t, H^{CH3}).

^{13}C NMR (400 MHz, $CDCl_3$) δ (ppm): δ (ppm): 78.16 (C-2), 69.05 (C-3), 31.91 (C-4), 123.76 (C-5), 107.46 (C-6), 147.72 (C-7), 137.34 (C-8), 148.84 (C-9), 114.84 (C-10), 131.49 (C-1'), 113.20 (C-2'), 151.47 (C-3'), 137.86 (C-4'), 113.48 (C-5'), 119.60 (C-6'), 75.07-71.27 (C-$\underline{C}H_2Bn$), 128.57-127.25 ($\underline{C}H$-Ph), 137.22-136.70 ($\underline{C}$-Ph), 173.10 (C-a), 34.35 (C-b), 32.81-22.69 (C-c), 14.12 (C-d).

Elemental analysis: Calcd. (%) for $C_{55}H_{60}O_7$: C 79.30, H 7.01, Found: C 78.72, H 7.68.

IR (υ_{max}/cm^{-1}): 2950 – 2850 (CH aliphatic), 1727 (C=O), 1657 (C=C)

Compound **[24]**



The same reaction as for compound **[22]** was carried out but myristic acid (1 mmol, 1.3 eq) was used instead of capric acid. After column purification, visualization was also done by vanillin which revealed the desired compound **[24]** at an Rf of 0.33 with an eluent system of (9:1) hexane: ethyl acetate. Characterization of compound **[24]** is given in below.

MW: 861.11; Color: White; Mpt: 91 °C; Percentage yield: 76 %

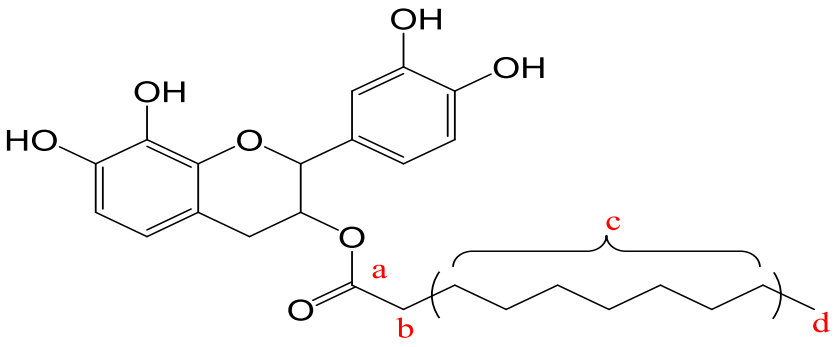
^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 7.4-7.1 (20 H, m, H^{Ph}), 6.9 (1 H, s, $H^{2'}$), 6.85-6.75 (2 H, m, $H^{5', 6'}$), 6.6 (1 H, m, H^{5}), 6.5 (1 H, m, H^{6}), 5.2 (1 H, td, H^{3}), 5.1-4.95 (9 H, m, H^{2}, $H^{\underline{CH}Bn}$), 2.8 (1 H, dd, H^{4}), 2.65 (1 H, dd, H^{4}), 1.35 (2 H, m, H^{CH2}), 1.25-1.1 (22 H, m, H^{CH2}), 0.8 (3H, t, H^{CH3}).

^{13}C NMR (400 MHz, $CDCl_3$) δ (ppm): δ (ppm): 78.18 (C-2), 69.06 (C-3), 31.95 (C-4), 123.77 (C-5), 107.48 (C-6), 147.74 (C-7), 137.36 (C-8), 148.86 (C-9), 114.85 (C-10), 131.50 (C-1'), 113.22 (C-2'), 151.49 (C-3'), 137.88 (C-4'), 113.50 (C-5'), 119.62 (C-6'), 75.09-71.28 (C-$\underline{C}H_2$Bn), 128.59-127.27 ($\underline{C}$H-Ph), 137.25-136.74 ($\underline{C}$-Ph), 173.10 (C-a), 34.36 (C-b), 32.82-22.72 (C-c), 14.15 (C-d).

Elemental analysis: Calcd. (%) for $C_{57}H_{64}O_7$: C 79.10, H 7.49, Found: C 79.18, H 7.62.

IR (υ_{max}/cm^{-1}): 2950 – 2850 (CH aliphatic), 1729 (C=O), 1612 (C=C)

Compound **[25]**



Under nitrogen environment, 0.5 g of compound **[22]** was dissolved in a 100 ml solution of a mixture of MeOH: THF (1:1). Palladium (II) hydroxide on carbon (0.37 mmol, 0.6 eq) was added as a catalyst. This was followed by addition of hydrogen gas (1 atm). Purging of the nitrogen gas from the reaction vessel was done for about 1 minute. The reaction was carried out for 4 hours after which the Pd catalyst was filtered off on a celite-bed. Rinsing was done by excess ethanol. The filtrate was finally concentrated to dryness using a rotavapor and the solid product formed purified by a classical column on silica gel. Visualization was done by vanillin which revealed the desired compound **[25]** at an Rf of 0.46 with an eluent system of (5:5) hexane: ethyl acetate. Characterization of compound **[25]** is given below.

MW: 444.52; Color: White; Mpt: 95 °C; Percentage yield: 61 %

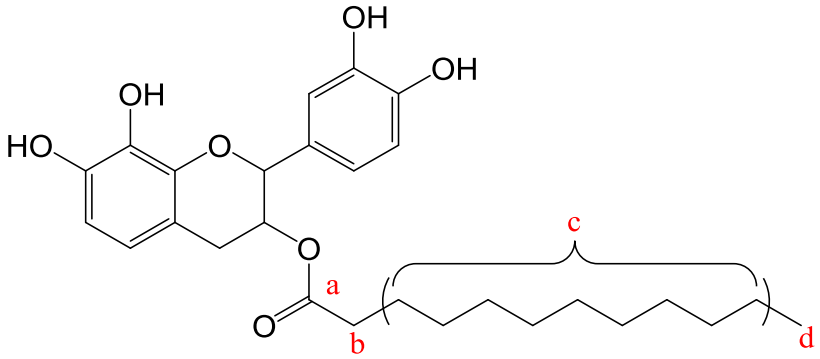
^{1}H NMR (400 MHz, DMSO) δ (ppm): 8.9 (1 H, s, $H^{0H\text{-}Ph}$), 8.85 (1 H, s, $H^{0H\text{-}Ph}$), 8.7 (1 H, s, $H^{0H\text{-}Ph}$), 8.2 (1 H, s, $H^{0H\text{-}Ph}$), 6.8-6.65 (3 H, m, $H^{2', 5', 6'}$), 6.3 (2 H, s, $H^{5, 6}$), 5.2 (1 H, td, *J* 6.8 and 5.2 Hz, H^{3}), 5 (1 H, d, *J* 6.6 Hz, H^{2}), 2.85-2.75 (1 H, dd, *J* 5.0 and 16.3 Hz, H^{4}), 2.75-2.6 (1 H, dd, *J* 7.2 and 16.1 Hz,H^{4}), 2.2 (2 H, t, H^{CH2}), 1.4 (2 H, m, H^{CH2}), 1.3-1.1 (12 H, m, H^{CH2}), 0.85 (3 H, t, H^{CH3}).

^{13}C NMR (400 MHz, DMSO) δ (ppm): δ (ppm): 78.77 (C-2), 69.17 (C-3), 31.85 (C-4), 119.96 (C-5), 108.70 (C-6), 144.09 (C-7), 131.28 (C-8), 141.08 (C-9), 115.30 (C-10), 130.11 (C-1'), 111.37 (C-2'), 143.59 (C-3'), 142.73 (C-4'), 113.41 (C-5'), 119.69 (C-6'), 173.51 (C-a), 34.36 (C-b), 29.37-22.69 (C-c), 14.11 (C-d).

Elemental analysis: Calcd. (%) for $C_{25}H_{32}O_{7}$: C 67.55, H 7.26, Found: C 66.46, H 7.43.

IR (υ_{max}/cm^{-1}): 3400 – 3100 (OH), 2950 – 2850 (CH aliphatic), 1739 (C=O), 1630 (C=C).

Compound **[26]**



The same reaction as for compound **[25]** was carried out while using compound **[23]** as the starting material. Palladium (II) hydroxide on carbon (0.36 mmol, 0.6 eq) was added as a catalyst. Visualization was done by vanillin which revealed the desired compound **[26]** at an Rf of 0.23 with an eluent system of (7:3) hexane: ethyl acetate. Characterization of compound **[26]** is given below.

MW: 472.57; Color: White; Mpt: 97 °C; Percentage yield: 78 %

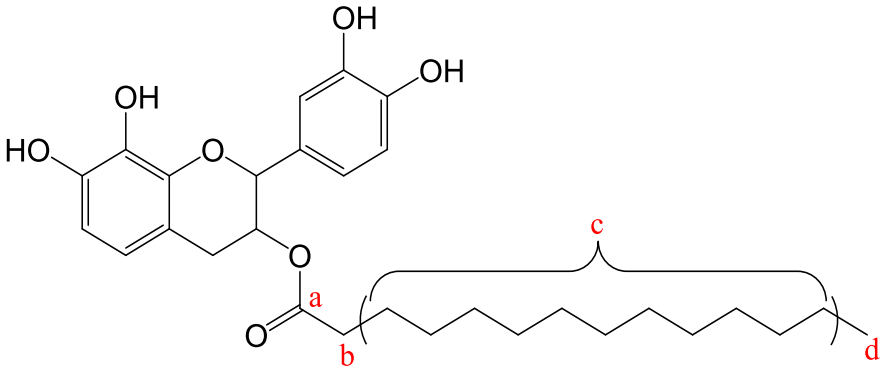
^{1}H NMR (400 MHz, DMSO) δ (ppm): 8.9 (1 H, s, $H^{0H\text{-}Ph}$), 8.85 (1 H, s, $H^{0H\text{-}Ph}$), 8.7 (1 H, s, $H^{0H\text{-}Ph}$), 8.2 (1 H, s, $H^{0H\text{-}Ph}$), 6.8-6.65 (3 H, m, $H^{2', 5', 6'}$), 6.3 (2 H, s, $H^{5, 6}$), 5.2 (1 H, td, *J* 6.8 and 5.3 Hz H^{3}), 5 (1 H, d, *J* 6.6 Hz H^{2}), 2.85-2.75 (1 H, dd, *J* 5.0 and 16.2 Hz H^{4}), 2.75-2.6 (1 H, dd, *J* 5.0 and 16.1 Hz H^{4}), 2.2 (2 H, t, H^{CH2}), 1.4 (2 H, m, H^{CH2}), 1.3-1.1 (16 H, m, H^{CH2}), 0.85 (3 H, t, H^{CH3}).

^{13}C NMR (400 MHz, DMSO) δ (ppm): δ (ppm): 77.76 (C-2), 68.12 (C-3), 31.68 (C-4), 118.92 (C-5), 107.63 (C-6), 143.19 (C-7), 130.23 (C-8), 139.95 (C-9), 114.25 (C-10), 129.09 (C-1'), 110.22 (C-2'), 142.62 (C-3'), 141.67 (C-4'), 112.36 (C-5'), 118.58 (C-6'), 172.36 (C-a), 33.33 (C-b), 30.89-21.69 (C-c), 13.10 (C-d).

Elemental analysis: Calcd. (%) for $C_{27}H_{36}O_{7}$: C 68.62, H 7.68, Found: C 68.71, H 7.80.

IR (υ_{max}/cm^{-1}): 3400 – 3100 (OH), 2950 – 2850 (CH aliphatic), 1734 (C=O), 1626 (C=C).

Compound **[27]**



The same reaction as for compound **[25]** was carried out while using compound **[24]** as the starting material. Palladium (II) hydroxide on carbon (0.35 mmol, 0.6 eq) was added as a catalyst. Visualization was done by vanillin which revealed the desired compound **[27]** at an Rf of 0.31 with an eluent system of (7:3) hexane: ethyl acetate. Characterization of compound **[27]** is given in below.

MW: 500.62; Color: White; Mpt: 100 °C; Percentage yield: 82 %

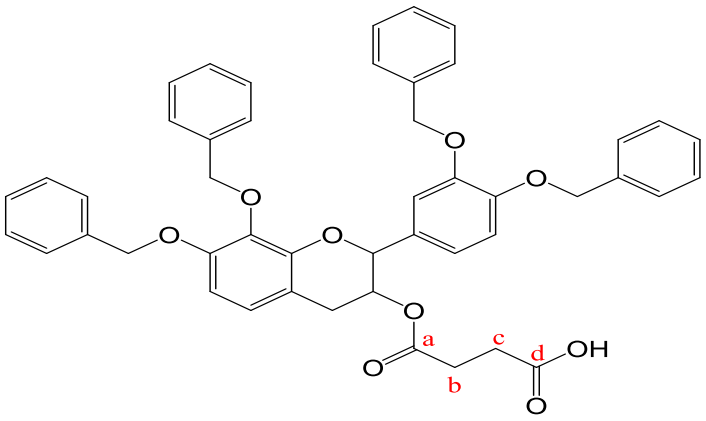
^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 6.8-6.7 (3 H, m, $H^{2', 5', 6'}$), 6.4-6.3 (2 H, m, $H^{5, 6}$), 5.2 (1 H, td, H^{3}), 5 (1 H, d, H^{2}), 2.9-2.8 (1 H, dd, H^{4}), 2.75-2.6 (1 H, dd, H^{4}), 2.1 (2 H, t, H^{CH2}), 1.35 (2 H, m, H^{CH2}), 1.3-1.1 (20 H, m, H^{CH2}), 0.85 (3 H, t, H^{CH3}).

^{13}C NMR (400 MHz, $CDCl_3$) δ (ppm): δ (ppm): 78.74 (C-2), 69.26 (C-3), 31.93 (C-4), 119.96 (C-5), 108.77 (C-6), 144.16 (C-7), 131.32 (C-8), 141.16 (C-9), 115.30 (C-10), 129.99 (C-1'), 111.42 (C-2'), 143.64 (C-3'), 142.73 (C-4'), 113.39 (C-5'), 119.66 (C-6'), 173.71 (C-a), 34.37 (C-b), 32.67-22.69 (C-c), 14.12 (C-d).

Elemental analysis: Calcd. (%) for $C_{29}H_{40}O_7$: C 69.58, H 8.05, Found: C 69.49, H 8.28.

IR (υ_{max}/cm^{-1}): 3400 – 3100 (OH), 2950 – 2850 (CH aliphatic), 1737 (C=O), 1615 (C=C).

Compound **[28]**



Under nitrogen environment using a two-necked round bottom flask, 1 g of the benzylated mesquitol (1.54 mmol) was dissolved in 20 ml of dichloromethane at ambient temperature. Succinic anhydride (3.07 mmol, 2 eq) was added followed by addition of DMAP (3.07 mmol, 2 eq). The reaction was carried out for 48 hours at ambient temperature while being followed by TLC. After this period, the reaction was stopped and 1 M HCl was added to the reaction mixture until a pH of 1 was obtained. The organic phase was then extracted using 100 ml of ethyl acetate and was dried using Na_2SO_4. The sulphate was then filtered while rinsing with ethyl acetate. The filtrate was finally concentrated to dryness using a rotavapor and the solid product formed purified by a classical column on silica gel. Visualization was done by vanillin which revealed the desired compound **[28]** at an Rf of 0.55 with an eluent system of (5:5) hexane: ethyl acetate. Characterization of compound **[28]** is given below.

MW: 750.83; Color: brownish white; Mpt: 123 °C; Percentage yield: 73 %

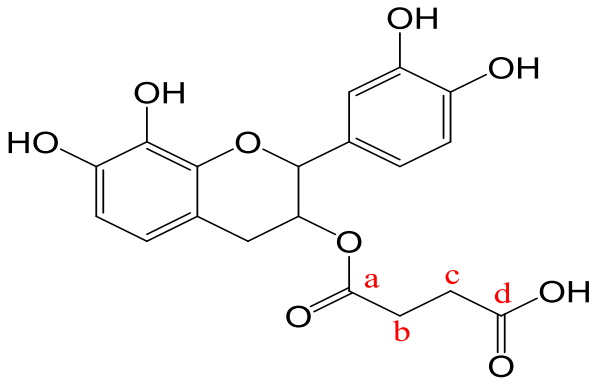
^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 7.4-7.1 (20 H, m, H^{Ph}), 6.9-6.75 (3 H, m, $H^{2, 5', 6'}$), 6.55 (1 H, m, H^5), 6.45 (1 H, m, H^6), 5.2 (1 H, td, H^3), 5.1-4.9 (9 H, m, H^2, $H^{\underline{CH}Bn}$), 2.8-2.75 (1 H, dd, H^4), 2.7-2.6 (1 H, dd, H^4), 2.4 (4 H, m, H^{CH2}).

^{13}C NMR (400 MHz, $CDCl_3$) δ (ppm): δ (ppm): 78.20 (C-2), 69.92 (C-3), 30.10 (C-4), 124.10 (C-5), 118.05 (C-6), 147.84 (C-7), 137.40 (C-8), 149.04 (C-9), 114.90 (C-10), 131.80 (C-1'), 113.36 (C-2'), 151.60 (C-3'), 138.01 (C-4'), 113.57 (C-5'), 118.20 (C-6'), 75.01-71.26 (C-$\underline{CH_2}$ Bn), 128.90-127.40 (C-Ph), 137.40-136.82 (C- Ph), 172.10 (C-a), 29.20 (C-b), 28.01 (C-c), 177.80 (C-d).

Elemental analysis: Calcd. (%) for $C_{47}H_{42}O_9$: C 75.18, H 5.64, Found: C 75.10, H 5.65.

IR (υ_{max}/cm^{-1}): 2950 – 2850 (CH aliphatic), 1729 (C=O ester), 1714 (C=O acid), 1607 (C=C).

Compound **[29]**



Under nitrogen environment, 0.5 g of compound **[28]** was dissolved in a 100 ml solution of a mixture of ethanol. Palladium (II) hydroxide on carbon (0.47 mmol, 0.7 eq) was added as a catalyst. This was followed by addition of hydrogen gas (1 atm). Purging of the nitrogen gas from the reaction vessel was done for about 1 minute. The reaction was carried out for 12 hours after which the Pd catalyst was filtered off on a celite-bed. Rinsing was done by excess ethanol. The filtrate was finally concentrated to dryness using a rotavapor. Since the product formed was very polar, a quick purification was done by filtration on a silica gel bed on a fritted glass funnel under vacuum. The solvent used was a mixture of dichloromethane: methanol (6:4). Visualization was done by vanillin which revealed the desired compound **[29]** at an Rf of 0.14 with an eluent system of (10:1) dichloromethane: methanol. Characterization of compound **[29]** is given below.

MW: 390.34; Color: brownish white; Mpt: 154 °C; Percentage yield: 74 %

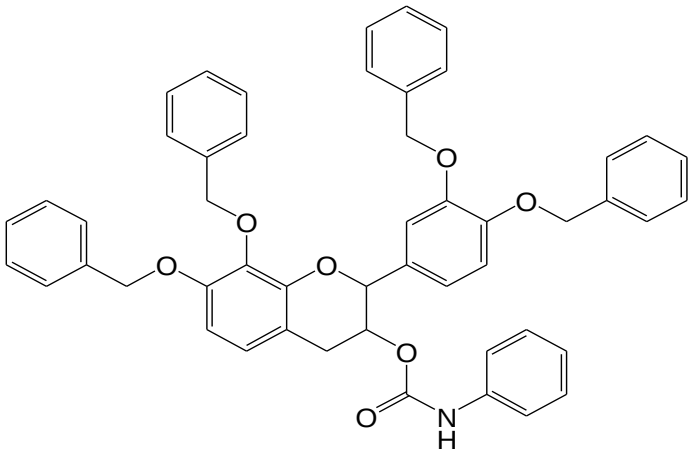
^{1}H NMR (400 MHz, CD_3OH) δ (ppm): 6.75-6.5 (3 H, m, $H^{2', 5', 6'}$), 6.25 (2 H, s, $H^{5, 6}$), 5.2 (1 H, td, H^{3}), 5 (1 H, d, H^{2}), 2.8-2.7 (1 H, dd, H^{4}), 2.7-2.55 (1 H, dd, H^{4}), 2.4 (4 H, m, H^{CH2}).

^{13}C NMR (400 MHz, CD_3OH) δ (ppm): δ (ppm): 77.39 (C-2), 69.45 (C-3), 30.69 (C-4), 119.16 (C-5), 109.05 (C-6), 145.63 (C-7), 133.54 (C-8), 142.83 (C-9), 115.35 (C-10), 129.71 (C-1'), 110.57 (C-2'), 145.08 (C-3'), 144.94 (C-4'), 113.99 (C-5'), 118.92 (C-6'), 172.57 (C-a), 30.18 (C-b), 27.59 (C-c), 176.10 (C-d).

Elemental analysis: Calcd. (%) for $C_{19}H_{18}O_9$: C 58.46, H 4.65, Found: C 51.80, H 4.80.

IR (υ_{max}/cm^{-1}): 3700 – 3000 (OH), 2950 – 2850 (CH aliphatic), 1729 (C=O ester), 1713 (C=O acid), 1609 (C=C).

Compound **[30]**



Into a two-neck round bottomed flask, phenyl isocyanate (1.54 mmol, 2 eq) was weighed and triflouroacetic acid (0.38 mmol, 0.5 eq) added under nitrogen environment. Agitation was done at room temperature for 10 minute after which benzylated mesquitol (0.5g, 0.77 mmol) was dissolved in 10 ml of dichloromethane and added drop-wise to the reaction mixture. The reaction was then carried out for 24 hours and finally concentrated to dryness by a rotavapor and the solid product formed purified by a classical column on silica gel. Visualization was done by vanillin which revealed the desired compound **[30]** at an Rf of 0.27 with an eluent system of (9:1) hexane: ethyl acetate. Characterization of compound **[30]** is given below.

MW: 769.88; Color: White; Percentage yield: 51 %

^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 7.0-6.96 (25 H, m, H^{Ph}), 6.88-6.79 (3 H, m, $H^{2, 5', 6'}$), 6.6 (1 H, m, H^{5}), 6.5 (1 H, m, H^{6}), 5.3 (1 H, td, H^{2}), 5.29 (1 H, q, H^{3}), 5.06-4.98 (8H, m, $H^{\underline{CH}Bn}$), 2.73 (1 H, dd, H^{4}), 2.70 (1 H, dd, H^{4}).

^{13}C NMR (400 MHz, $CDCl_3$) δ (ppm): δ (ppm): 77.74 (C-2), 70.51 (C-3), 31.15 (C-4), 128.04 (C-5), 107.42 (C-6), 147.56 (C-7), 137.75 (C-8), 148.55 (C-9), 119.35 (C-10), 131.62 (C-1'), 113.11 (C-2'), 148.59 (C-3'), 138.29 (C-4'), 113.58 (C-5'), 128.0 (C-6'), 74.69 – 70.64 (C-$\underline{C}H_2$Bn), 12916-128.18 ($\underline{C}$H-Ph), 137.65-136.38 (C-Ph).

IR (υ_{max}/cm^{-1}): 3350 – 3300 (NH stretch), 2926 – 2850 (CH aliphatic), 1692 (C=O amide), 1597 (C=C aromatic).

3.5 Durability Tests

Biological tests were carried out under laboratory conditions to evaluate the ability of mesquitol and some of the targeted mesquitol derivatives to protect beech wood and pine wood blocks exposed to the white rot fungus (*Coriolus versicolor*) and brown rot fungus (*Coniophora puteana*) respectively against decay.

In this study, different formulations were impregnated into wood blocks and the fungicidal efficiencies of these treatments were evaluated.

3.5.1 Formulations

The study of the wood decay test was done according to the European standard EN 113 procedure (EN 113, 1996). Treatment was done on beech wood (*Fagus sylvatica L.*) and pine sapwood (*Pinus sylvestrifs L.*).

For the treatment on beech wood, six formulations were prepared. Solutions of 0.1 % and 0.01 % tebuconazole, solutions of 5 % of both mesquitol and compound **[25]**, solution of a mixture of 0.01 % tebuconazole and 1.5 % mesquitol and finally a solution of a mixture of 0.01 % tebuconazole and 1.5 % compound **[25]**. These formulations were all prepared directly in ethanol solvent.

For the treatment on pine wood, solutions of 5 % and 10 % of mesquitol, compound **[25]** and compound **[26]** and were also prepared directly in ethanol solvent.

The chemical structures of compounds **[25]** and **[26]** are shown in figure 22.

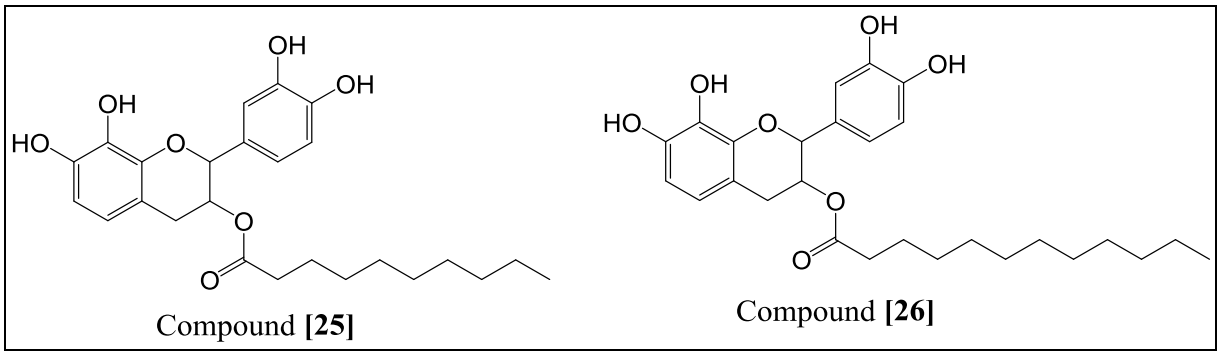


Fig **22:** Chemical structures of compounds used for wood decay test

3.5.2 Wood impregnation

Six treatments each (of different formulations) were investigated for the beech wood and pine wood. All the blocks of wood used, each measuring 2.5 cm x 1.5 cm x 0.5 cm, were first oven dried at 103 °C for 24 hours before they were weighed (m_0). The dried blocks were then placed in a beaker inside a desiccator and subjected to a 5 mbar vacuum for 20 minutes.

The blocks were then impregnated by suction with the different formulations each at a time for 2 hours under vacuum. Glass beads had been placed in the beaker on top of the wood blocks to ensure that the blocks remained totally submerged in the solutions during the impregnation period. For each formulation, impregnation was done on 12 wood blocks. After the impregnation, the wood blocks were removed, dried with absorbent paper and finally oven dried at 103 °C for 48 hours after which their weight taken (m_1). The percentage of weight percentage gain after impregnation (WPG) was thereafter calculated as per the formula below:

$$WPG\ (\%) = \frac{m_1 - m_0}{m_0} \times 100\ \%$$

Where; m_0 = Initial oven-dried weight before impregnation

m_1 = Oven-dried weight after impregnation

3.5.3 Leaching process

The leaching process was carried out according to the ENV 1250-2 procedure (ENV 1250-2, 1994). For each formulation that had been used for impregnation, six wood blocks were leached by immersing the blocks in 60 ml distilled water and subjecting them to six leaching periods while mechanically shaking them. The first period was for 1 hour, followed by 2 hours and then 4 hours. The water was changed after every period of time.

The blocks were then removed and left without water for 16 hours after which the leaching process was resumed for another 8 hours, followed by 16 hours and finally for 48 hours with water being changed after every period. At the end of the leaching process, all the wood blocks were removed, dried with absorbent paper and finally oven dried at 103 °C for 48 hours after which their weight taken (m_2).

The percentage of weight percentage gain after leaching (WPGAL) was thereafter calculated as per the formula below:

$$WPGAL\ (\%) = \frac{m_2 - m_0}{m_0} \times 100\ \%$$

Where; m_0 = Initial oven-dried weight before impregnation

m_2 = Oven-dried weight after leaching

3.5.4 Wood decay test

Mesquitol and one of its derivatives, compound **[25]** (a C_{10} chain length conjugation) were used as formulations against the *Coriolus versicolor* fungal strain. The formulations also involved mixtures containing tebuconazole, a widely known and used fungicide. This was done to evaluate the synergistic effect or additive effects of the fungicide.

The test against the *Coniophora puteana* fungal strain, involved formulations of mesquitol and two derivatives obtained from conjugation of two fatty chain lengths of C_{10} and C_{12} (compound **[25]** and compound **[26]**).

Two treated and one untreated wood blocks were placed in a petri dish with sterile culture medium (prepared from 2.5 % potato dextrose agar and 4% malt extract in distilled water) that had been previously exposed to the fungal strains for duration of 2 weeks. Three replicates of each formulation were done both for the treated and the leached wood blocks. Three replicates of the control were also made by placing three untreated wood blocks on the mycelia. The petri dishes were sealed and incubated at 22 °C under controlled humidity conditions of 70 % R.H. for a period of 12 weeks.

After the incubation period, the wood blocks were removed from the mycelia, dried at 103°C for 48 hours and finally their weight measured (m_3). The fungicidal efficiencies of the wood treatment using the different formulations were determined by the weight loss obtained after the fungi attack as per the formula below:

$$WL\ (\%) = \frac{m_0 - m_3}{m_0} \times 100\ \%$$

Where; WL = Weight loss caused by fungi attack

m_0 = Initial oven-dried weight before impregnation

m_2 = Oven-dried weight after fungi attack

CHAPTER 4

RESULTS AND DISCUSSION

4.0 RESULTS AND DISCUSSION

4.1 Optimum extraction methods for mesquitol

4.1.1 Soxhlet extraction method

Soxhlet extraction method is one of the oldest conventional extraction methods that have been well established especially for the extraction of solid samples. Its advantages are that it can allow for many extractions by use of multiple Soxhlet assemblies and the cost of the apparatus is quite low. On the contrary, it is time-consuming and uses large volumes of solvents leading to increase cost of solvent waste disposal. It is also labour intensive.

The percentage yields obtained from the soxhlet extraction method were calculated and tabulated in table 1.

Table **1**: Percentage yields (%) of extractives by Soxhlet extraction method

Solvents	**1st extraction % yield**	**2nd extraction % yield**	**3rd extraction % yield**	**Mean % yield ± SD**
Water	12.68	11.71	12.34	12.24 ± 0.49
Ethanol	22.80	21.43	20.00	21.41 ± 1.40
Water:ethanol (3:1)	17.57	18.00	17.71	17.76 ± 0.22
Acetone	10.40	10.29	9.14	9.94 ± 0.70

SD – standard deviation

4.1.2 Maceration extraction method

Maceration extraction method is also one of the traditional methods of extraction just like the soxhlet extraction. It is known to be the easiest and the simplest method but just like the soxhlet extraction, it requires use of large volumes of solvents which leads to problems like high increase in cost of solvent waste disposal (Azwanida, 2015). It also has the disadvantage of long extraction time and low extraction efficiency (Zhang *et al.*, 2018).

The percentage yields obtained from the maceration extraction method were calculated and tabulated in table 2.

Table **2**: Percentage yields (%) of extractives by maceration extraction method

Solvents	**1st extraction % yield**	**2nd extraction % yield**	**3rd extraction % yield**	**Mean % yield ± SD**
Water	3.50	3.60	4.00	3.70 ± 0.26
Ethanol	10.92	10.26	10.60	10.59 ± 0.33
Water:ethanol (3:1)	8.14	8.20	8.80	8.38 ± 0.36
Acetone	4.14	4.80	4.40	4.45 ± 0.33

SD – standard deviation

4.1.3 Microwave-assisted extraction method

Microwave-assisted extraction (MAE) method is considered as one of the fastest extraction methods that not only has reduced extraction time and high reproducibility but also uses very low amounts of solvents. The major disadvantage of this extraction method is that after completion of the extraction method, the solvent has to be removed physically from the sample matrix prior to analysis. This leads to extra steps which consumes a lot of time (Dean and Xiong, 2000; Rajbhar, *et al*., 2014).

The MAE uses microwave radiation which interacts with the dipoles of polarizable and polar materials causing heating at the surfaces of these materials. The microwave electromagnetic waves induce a dipole rotation of the molecules causing a disruption of the hydrogen bonding which enhances solvent penetration into the matrix. For non-polar solvents, there is poor heating since energy is transferred only by dielectric absorption which regards the capability of a solvent to dissolve ionized solutes. Hence this method favors polar molecules and solvents that have high dielectric constants. A higher dielectric constant correlates with a higher ability of the solvent to dissolve salts (Azwanida, 2015).

The percentage yields obtained from the microwave-assisted extraction method were calculated and tabulated in table 3.

Table **3**: Percentage yields (%) of extractives by microwave-assisted extraction method (MAE)

		Water		Ethanol		Water / Ethanol (3:1)		Acetone	
Power	**Time / Test**	**10 mins**	**20 mins**	**10 mins**	**20 mins**	**10 mins**	**20 mins**	**10 mins**	**20 mins**
200 W	**1**	7.50	7.00	13.00	13.50	16.00	16.50	3.50	4.50
	2	7.50	7.00	12.00	14.50	14.50	16.00	3.50	4.00
	3	7.00	6.50	12.00	14.00	15.00	16.00	3.00	4.00
	Mean ± SD	7.33 ± 0.29	6.83 ± 0.29	12.33 ± 0.58	14.00 ± 0.50	15.17 ± 0.76	16.17 ± 0.29	3.33 ± 0.29	4.17 ± 0.29
400 W	**1**	6.50	7.00	13.00	13.00	14.50	17.00	2.00	3.50
	2	6.00	6.00	14.00	13.50	15.00	17.00	3.00	4.00
	3	6.00	6.00	13.50	14.50	15.00	16.50	3.50	4.00
	Mean ± SD	6.17 ± 0.29	6.33 ± 0.58	13.50 ± 0.50	13.67 ± 0.76	14.83 ± 0.29	16.83 ± 0.29	2.83 ± 0.76	3.83 ± 0.29
800 W	**1**	6.00	6.50	13.50	13.50	15.00	17.00	2.50	4.00
	2	6.50	7.00	12.50	13.00	16.00	16.00	3.00	4.50
	3	6.50	6.50	12.50	12.00	16.00	17.00	4.00	3.50
	Mean ± SD	6.33 ± 0.29	6.67 ± 0.29	12.83 ± 0.58	12.83 ± 0.76	15.67 ± 0.58	16.67 ± 0.58	3.17 ± 0.76	4.00 ± 0.50

4.1.3.1 Comparison of different parameters in the Microwave-assisted extraction

Comparison of the different irradiation times, solvents and the microwave power of the microwave-assisted extraction was graphically done and represented in figure 23.

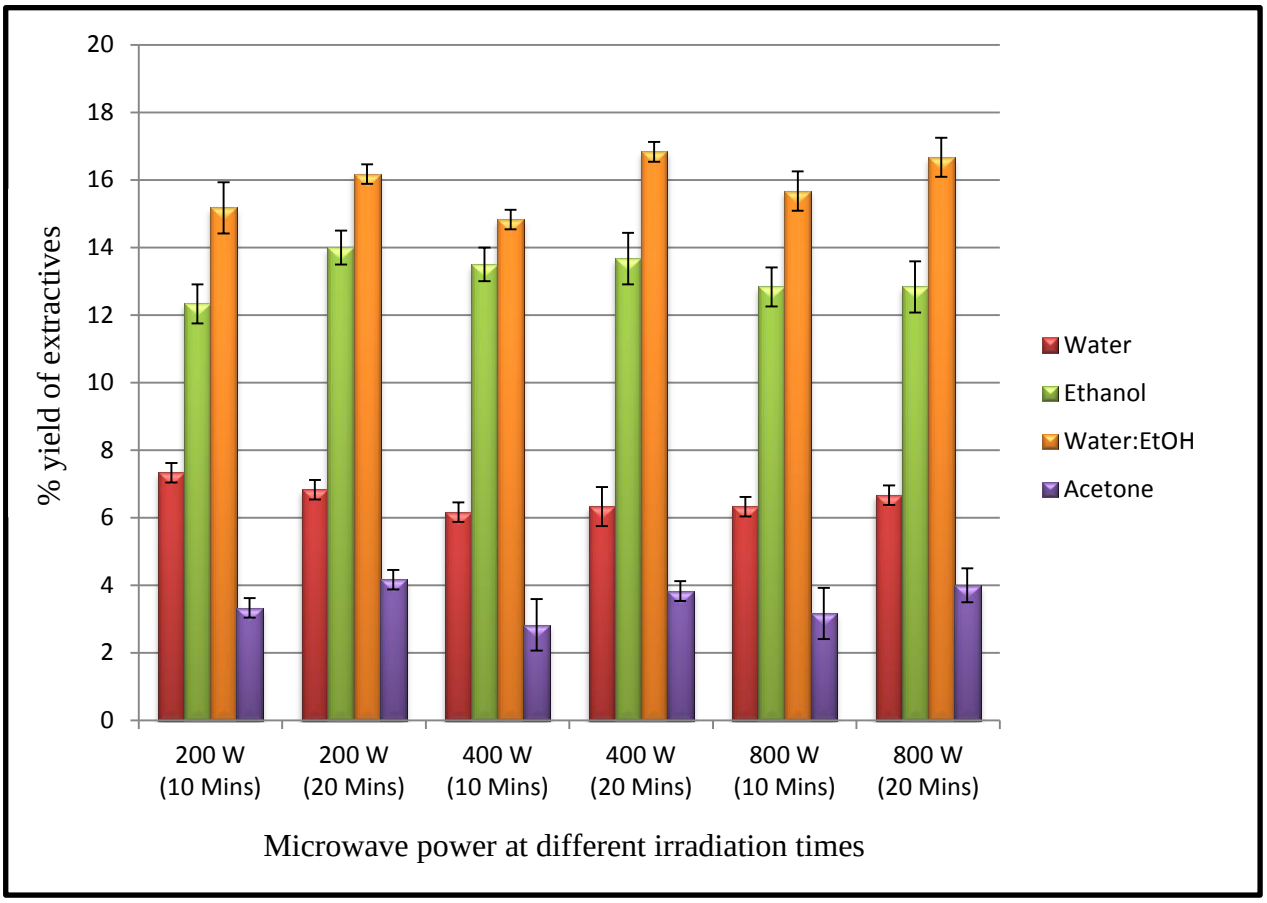


Fig **23:** Comparison of the different irradiation times, solvents and the microwave power in the MAE

From figure 23, there is a clear distinction of the influence of the selectivity of the solvent type on the extraction yield of the extractives. Acetone solvent has the least extraction yields. The reason for this is that the MAE is known to be solvent selective and favors polar solvents (Azwanida, 2015). Acetone being less polar than ethanol and water would have less interaction with the microwave irradiations. This leads to poor dielectric absorption hence low partition of the analytes from the sample matrix to the solvent hence lower yields of extractives.

The solvents that gave high yields were ethanol and the mixture of water and ethanol. The organic solvent (ethanol) allowed the extraction of maximum amounts of polar organic molecules in the samples.

The different irradiation times does not show any big difference in the percentage of the extraction yields, with just a slight yield increase of the extraction yields at 20 minutes compared to 10 minutes.

The variation of the microwave power also shows no significant difference in the extraction yields hence for the comparisons of this extraction method with the rest of the extraction methods the extraction yields at 200W (to ensure that no extractives were degraded) and at 10 minutes (shortest time) were used.

4.1.4 Extraction by Accelerated solvent extraction (ASE)

ASE is known to be one of the most efficient forms of liquid solvent extractions for solid and semi-solid samples. It uses organic solvents at high temperatures above their boiling points and at high pressures hence increase extraction efficiency. The elevated temperature increases the extraction kinetics and the high pressure keeps the solvents above their boiling points by maintaining them in a liquid state leading to rapid extractions. The closed system allows very high pressures to be reached (100 – 140 atm). The ASE is also advantageous for using minimal amount of solvents and short extraction time periods.

The percentage yields obtained from the accelerated solvent extraction method were calculated and tabulated in table 4.

Table **4**: Percentage yields (%) of extractives by accelerated solvent extraction method (ASE)

Solvents	**1st extraction % yield**	**2nd extraction % yield**	**3rd extraction % yield**	**Mean % yield ± SD**
Water	15.0	13.0	14.0	14.00 ± 1.00
Ethanol	16.3	16	16.5	16.27 ± 0.25
Water:ethanol (3:1)	19.6	19	18.7	19.10 ± 0.46
Acetone	9.1	10	9.5	9.53 ± 0.45

For the determination of the best extraction method for mesquitol from *P. juliflora*, two comparisons were done. One was on the percentage yields of the extractives in each extraction method while considering the solvent used for extraction. The second comparison was done to determine the purity of mesquitol in the different extracts. This was done by performing the ^{1}H NMR analyses and comparing the spectra of the various extracts.

4.1.5 Comparison of percentage yields of different extraction methods

Comparison of the percentage yields of the different extraction methods while considering the solvent used for extraction was done and represented graphically in figure 24.

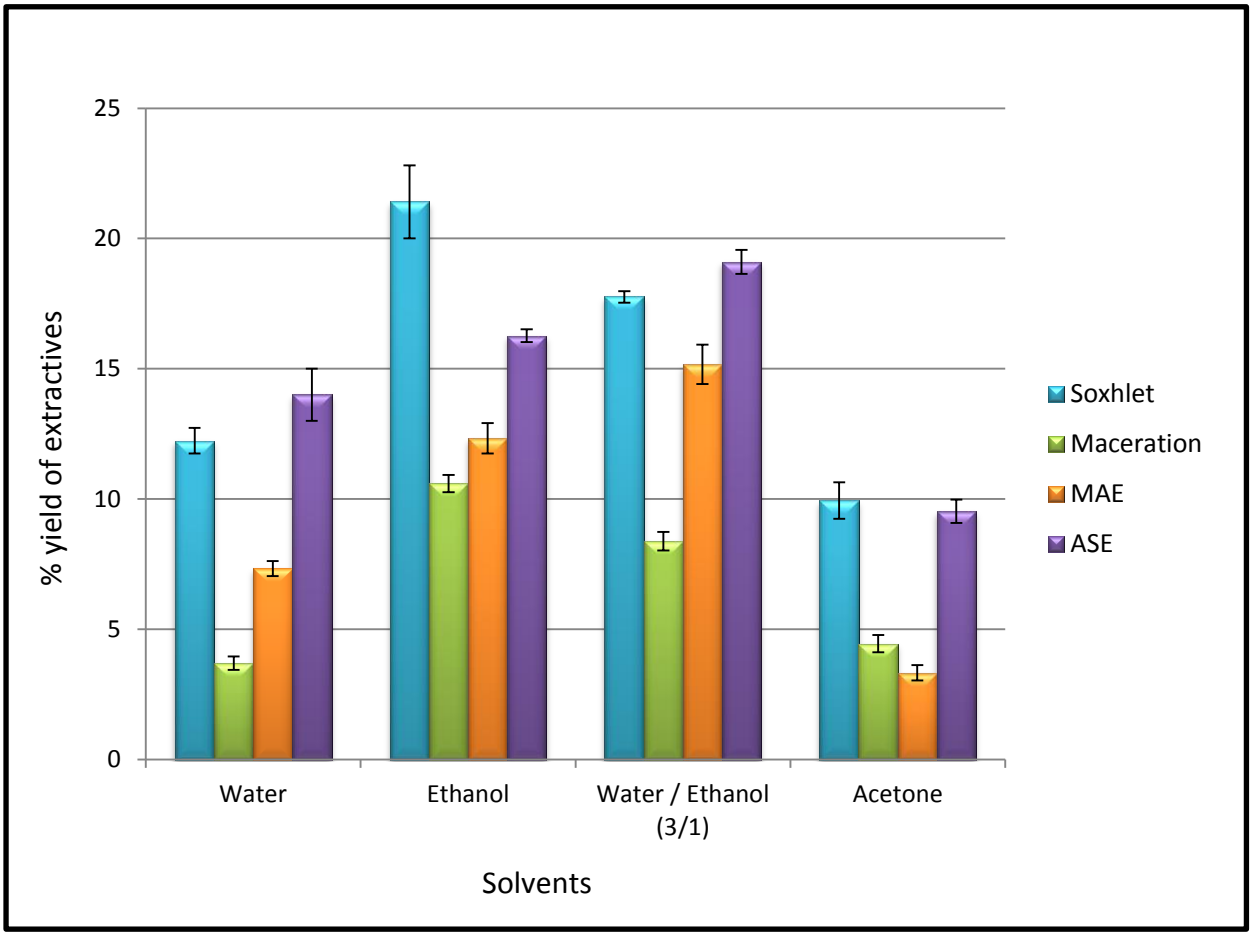


Fig **24:** Comparison of the percentage yields of extractives from *P. juliflora* using different extraction methods

The results shown in figure 24 were the best results obtained from each extraction method and the extraction parameters like the extraction time, temperature, pressure and power of irradiation were all optimized to get those results.

Extraction methods that use solvents in their procedures are usually known to be influenced critically by the solvents types. This was observed for the four different methods of extraction used as there was a huge difference among the four solvents used. Ethanol and the mixture of water and ethanol (3:1) gave better yields of extractives in all the extraction methods compared to water and acetone.

The best solvent that gave the highest yield of extractives in the two conventional methods (soxhlet and maceration) was ethanol (21.41 % and 10.59 % respectively). For the MAE and ASE, the mixture of water and ethanol gave the highest yield of extractives (12.83 % and 16.27 % respectively).

Acetone gave low yields of extractives in all the extraction methods with the least being found in the acetonic extract from the microwave-assisted extraction (3.33 %) and the highest from soxhlet extraction (9.94 %). This was expected since extractions by the MAE favor polar media which was not the case for acetone (Azwanida, 2015).

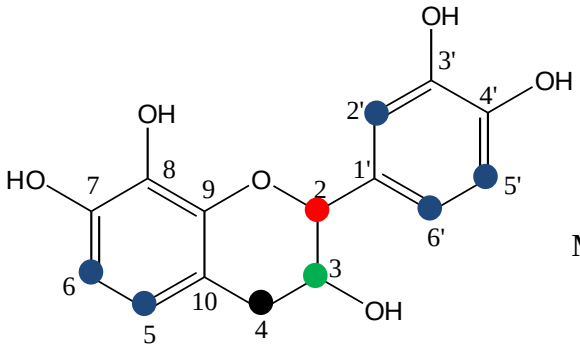
In comparison of the percentage yields, soxhlet extraction method and the ASE Dionex extraction gave higher yields compared to the other two. They are therefore, the preferred methods for extraction. Comparing the two, the ASE Dionex extraction is the most preferred method of extraction since soxhlet extraction poses a lot of disadvantages like use of huge amounts of solvents for extraction plus very long extraction times and several rounds sometimes needed.

Generally, maceration extraction method gave the lowest percentage yields in all the extraction methods tested.

4.1.6 Comparison of ^{1}H NMR spectra of the extracts

All the extraction methods used resulted in crude extracts containing a mixture of metabolites. The ^{1}H NMR analyses were done to determine the purity of the extracts as to which of them contained pure mesquitol.

Pure mesquitol was obtained from column purification of an acetonic extract from *Prosopis juliflora*. Its ^{1}H NMR was first done and was then compared to the ^{1}H NMR of the rest of the extracts.



Mesquitol

The ^{1}H NMR characterization of pure mesquitol is given below;

^{1}H NMR (400 MHz, DMSO) δ (ppm): 8.95 (I H, s, H^{OH}), 8.9 (I H, s, H^{OH}), 8.7 (I H, s, H^{OH}), 8.2 (I H, s, H^{OH}), 6.8-6.6 (3 H, m, $H^{2', 5', 6'}$), 6.3 (2 H, s, $H^{5, 6}$), 4.95 (1 H, s, H^{OH}), 4.7 (1 H, d, *J* 7.6 Hz, H^{2}), 3.9 (1 H, td, *J* 5.2 and 6.8 Hz, H^{3}), 2.8 (1H, dd, *J* 5.2 and 16.9 Hz, H^{4}), 2.6 (1 H, dd, *J* 8.3 and 16.9 Hz, H^{4}).

^{1}H NMR spectra of the extracts were compared with the mesquitol spectrum. Different comparisons were done according to the different solvents used for extraction (water, ethanol, water: ethanol and finally acetone).

Mesquitol is a typical flavonoid that shows ^{1}H NMR characteristic peaks of a flavanol structure with a characteristic doublet (d) from the proton at position 2 (4.7 ppm), a triplet of doublets (td) from the proton at position 3 (3.9 ppm) and two doublet of doublets (dd) caused by the two protons at position 4 (2.8 and 2.6 ppm). These peaks were used for the comparisons of the different extracts of the different extraction methods.

The peaks corresponding to these four peaks in the different extracts have been circled as shown in figure 25, 26, 27 and 28. The peaks circled in black belong to the protons found at the benzene ring on carbon 5, 6, 2', 5'and 6'. The peaks circled in red belong to the proton found at the carbon 2. The peaks circled in green belong to the proton found at the carbon 3 while the peaks circled in blue belong to the two protons found at the carbon 4.

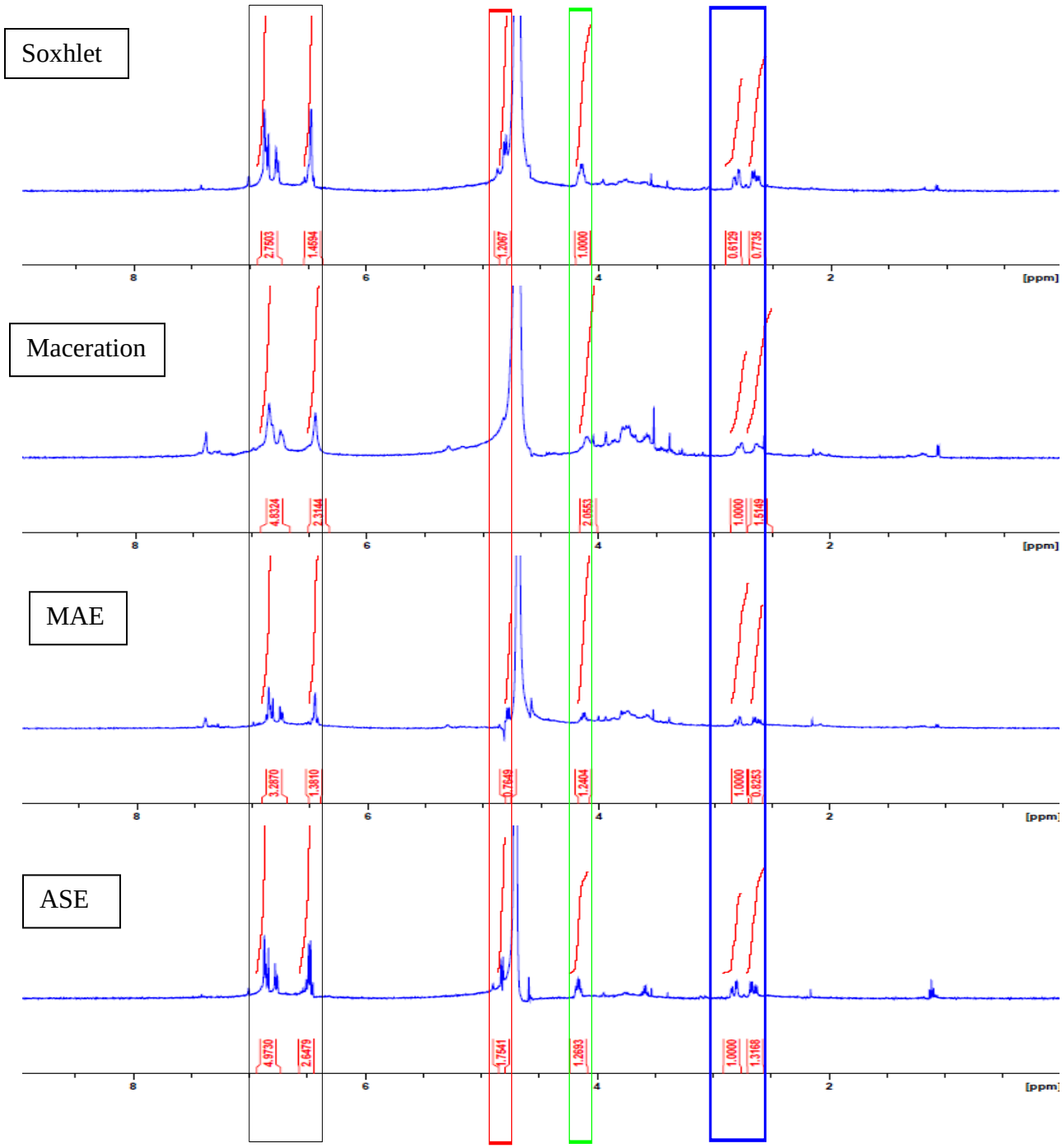


Fig **25:** ^{1}H NMR spectra of the aqueous extracts of different extraction methods using D_2O

Clear and more resolved peaks were seen with ASE and soxhlet extracts showing high purity of mesquitol by use of these methods.

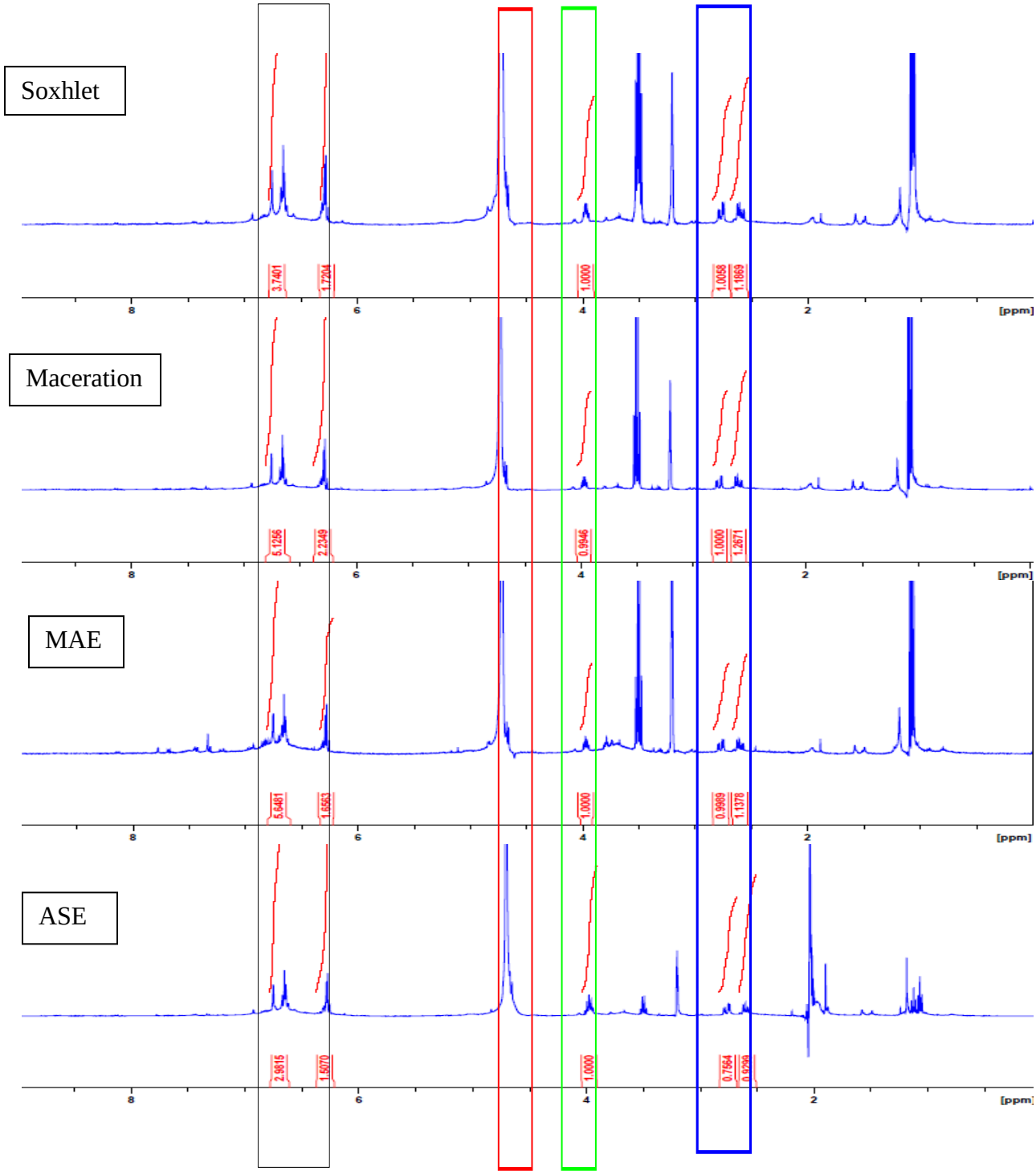


Fig **26:** ^{1}H NMR spectra of the ethanolic extracts of different extraction methods using CD_3OD solvent

All the extraction methods showed the characteristic mesquitol peaks with the doublet for proton at position 2 being masked by the solvent peak.

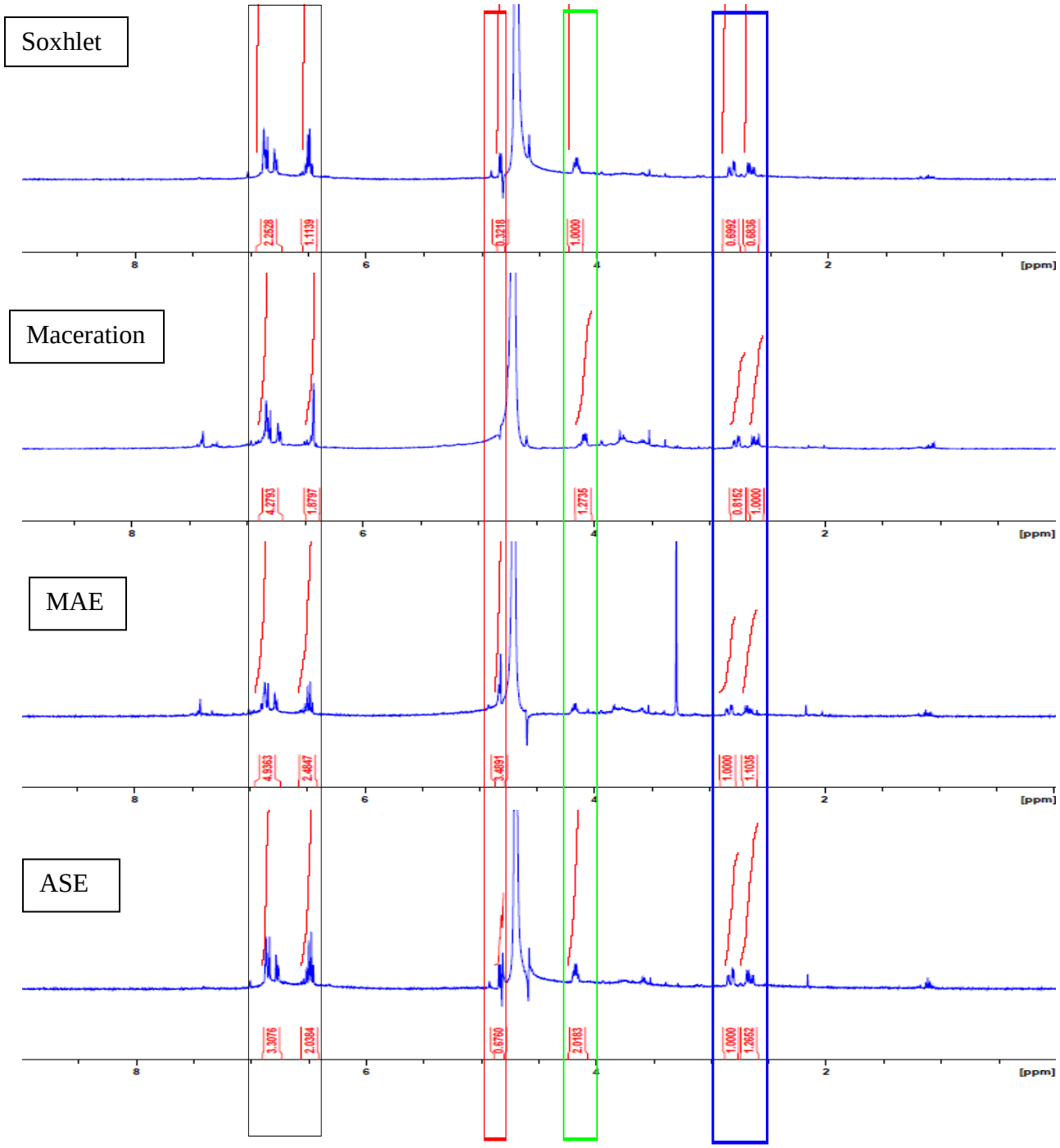


Fig **27:** ^{1}H NMR spectra of the water: ethanol (3:1) extracts of different extraction methods using D_2O

All the extraction methods showed high purity of mesquitol present.

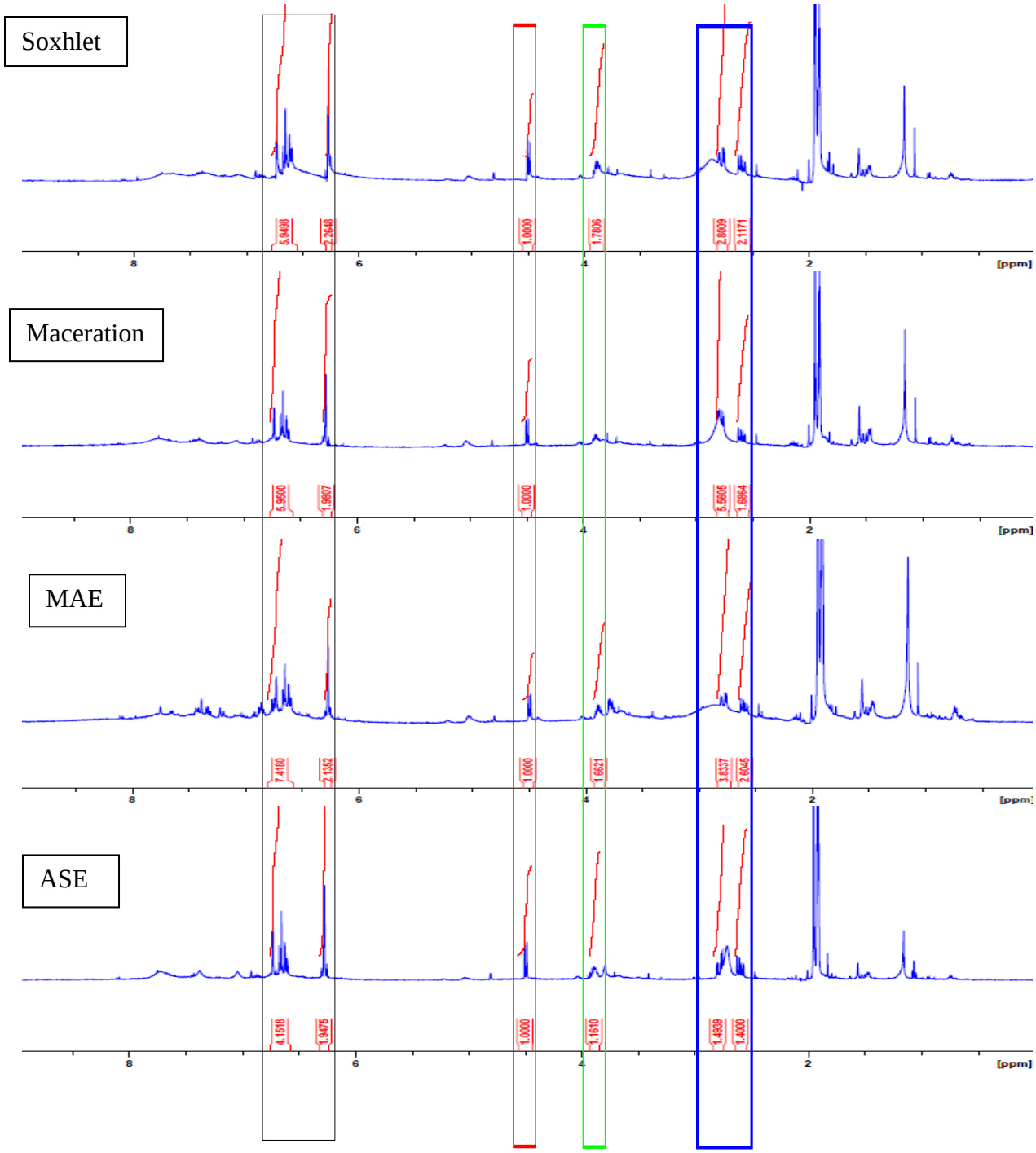


Fig **28:** ^{1}H NMR spectra of the acetonic extracts of different extraction methods using deuterated acetone

The ^{1}H NMR spectra from the extracts obtained from ASE and soxhlet extraction methods were more resolved and therefore had better purity compared to the extracts from maceration and MAE methods.

The two extraction methods (ASE and soxhlet) had earlier been found to give higher percentage yields of extractives which show that the higher the percentage yields of extractives, the better the purity. The ethanolic extracts however showed resolved peaks for all the extraction methods. This was expected for the ethanol solvent since it being a polar organic solvent will extract almost all the organic molecules present including all the mesquitol.

4.2 Geographical and natural intraspecific variability of *P. juliflora* extractives

Various parameters were studied in an effort to evaluate the geographical and natural intraspecific variability of the *P. juliflora* extractives. This included the comparison of the percentage yields of the extractives depending on the geographic region, the age and part of the tree and the type of solvent used for the extraction. Four solvents had been used for a serial extraction in increasing polarity. These were dichloromethane, acetone, toluene: ethanol (2:1) and finally water.

Identification of the different compounds found in the different parts of the *P. juliflora* was done which was followed by the quantification and comparison of some major compounds found in the *P. juliflora* samples from different geographical regions.

4.2.1 Variability of percentage yields of extractives

The percentage yields of the extractives obtained from the ASE extraction method were calculated and tabulated in table 5. These values were reported as the mean value of three replicates of extraction. The percentage yields were tabulated according to the different solvents used (subsequent extractions had been done), the age of the trees and the different parts of the tree.

Table 5: Percentage yields (%) of extractives of *P. juliflora* samples

		Baringo County		Turkana County		Garissa County	
Plant part	**Solvent / Solvent mixture**	**Small trees**	**Big trees**	**Small trees**	**Big trees**	**Small trees**	**Big trees**
Bark	Dichloromethane	1.86±0.18	1.72±0.92	1.63±0.00	1.42±0.51	1.27±0.09	1.21±0.26
	Acetone	6.58±0.73	6.59±0.64	4.46±2.13	6.50±0.57	5.31±1.39	5.11±0.77
	Toluene: Ethanol	1.92±0.19	0.99±0.23	1.63±0.66	1.13±0.25	1.28±0.54	1.44±0.27
	Water	8.28±048	6.99±1.00	3.13±0.78	3.13±0.90	13.21±1.90	16.42±2.22
Sap wood	Dichloromethane	0.63±0.23	0.52±0.11	0.56±0.06	0.46±0.07	0.57±0.18	0.70±0.07
	Acetone	1.30±0.18	1.42±0.02	2.07±6.64	1.38±0.22	1.97±0.18	1.61±0.13
	Toluene: Ethanol	0.65±0.06	0.40±0.09	1.05±0.53	0.38±0.00	0.66±0.15	0.74±0.11
	Water	4.78±0.56	4.21±0.43	7.44±0.38	3.13±0.25	5.05±1.09	4.92±1.49
Knot wood	Dichloromethane	1.09±0.24	0.99±0.04	1.07±0.12	0.71±0.19	1.70±1.00	1.00±0.00
	Acetone	9.91±1.96	12.62±0.58	8.17±1.71	4.79±0.19	17.73±0.72	8.67±1.21
	Toluene: Ethanol	0.75±0.05	0.68±0.10	1.05±0.30	0.54±0.14	0.94±0.31	0.58±0.07
	Water	7.08±0.10	7.44±0.80	7.13±1.07	4.63±1.09	8.52±1.65	7.67±1.38
Heart wood	Dichloromethane	2.60±1.51	2.25±1.02	1.08±0.26	0.75±0.13	1.48±0.78	1.38±0.13
	Acetone	8.73±0.54	16.42±1.41	6.58±0.38	9.46±0.69	14.80±0.39	8.92±0.36
	Toluene: Ethanol	2.13±0.62	1.88±1.56	0.88±0.38	0.58±0.07	0.85±0.09	0.63±0.25
	Water	5.10±0.62	7.47±1.42	7.67±1.28	4.46±051	6.18±0.65	5.83±0.38
Pith	Dichloromethane	1.77±0.03	1.09±0.28	1.00±0.25	0.79±0.07	1.48±0.46	1.13±0.13
	Acetone	12.17±0.83	17.92±2.33	6.54±0.92	5.96±0.83	16.57±0.74	8.13±1.32
	Toluene: Ethanol	1.87±0.78	0.87±0.03	0.88±0.33	0.54±0.07	1.05±0.30	0.58±0.47
	Water	5.48±1.12	7.34±1.39	8.21±2.06	4.33±2.06	7.81±0.58	6.83±0.47

Comparisons of the percentage yields were graphically done and represented in figures 29, 30, 31 and 32. Comparisons were done according to the four different solvents used (dichloromethane, acetone, toluene / ethanol and water).

Extractives were present in high amounts in the acetonic and the water extracts. For the bark, the water extracts gave the highest yields compared to all other solvents apart from the samples from Turkana County, where acetone gave a higher yield. There was a considerable difference in the water extracts from the bark of *P. juliflora* from Garissa County, which gave very high yields of extractives compared to the rest of the Counties. This shows that *P. juliflora* from this County contains a lot of polar compounds. This was later confirmed by the GC-MS which showed high amounts of sugars that resulted from fragmentation of the glycosylated flavonoids.

The sapwood generally had low amounts of extractives with the water solvent showing a higher percentage compared to the rest of the solvents. Water is known to extract polar compounds like sugars and gums constituted of polysaccharides. This was also confirmed by the GC-MS analyses, where presence of sugars dominated in the sapwood.

The higher yields of extractives from the knot wood, heartwood and pith were obtained with acetone in all the trees from the different counties. These high yield values may be explained by the presence of high amounts of flavonoids being extracted by the acetone solvent as seen in the GC-MS.

Compared to the rest of the solvents used for extraction, toluene: ethanol solvent gave lower percentage yields of extractives in all the regions (0.4 - 2.1 %) indicating that most of the organic soluble extractives had already been extracted before by dichloromethane and acetone. Figure 29 below shows the graphical representation of the percentage yields of the different sites for dichloromethane extracts.

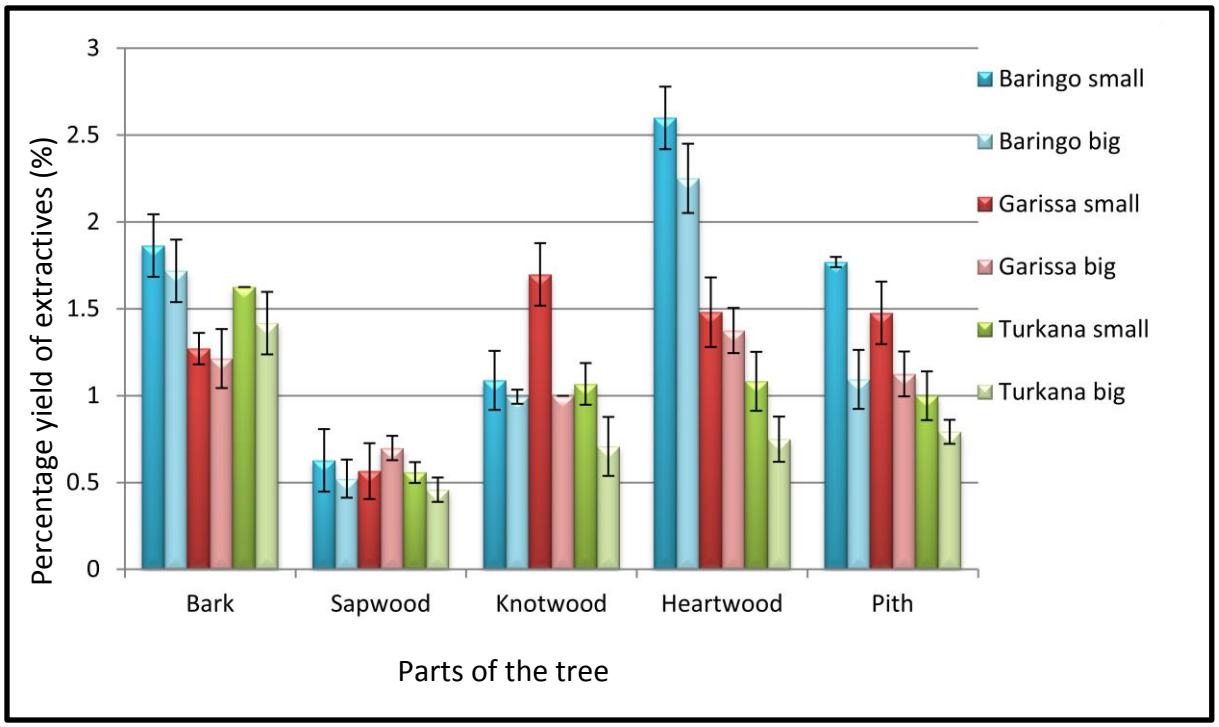


Fig **29**: Percentage yields of different geographical sites for dichloromethane extracts

Generally, the small trees have higher percentage yields than the big trees apart from the sapwood yields from Garissa County. The highest yields from Baringo County were obtained from the heartwood, while Turkana County showed highest yields of extractives in the bark. The sapwood gave the lowest yields in all the geographic regions.

Figure 30 shows the graphical representation of the percentage yields of the different sites for the acetone extracts.

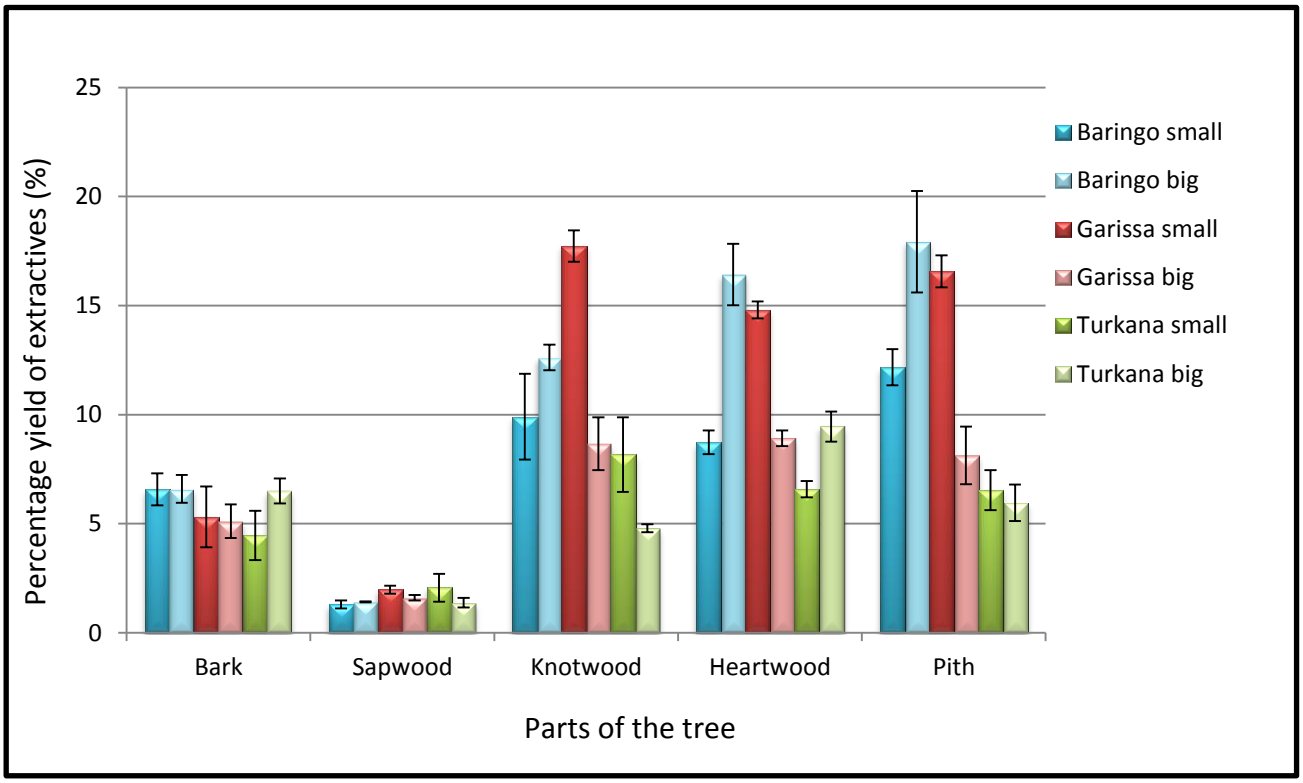


Fig **30:** Percentage yields of different geographical sites for acetone extracts

Acetone solvent was found to be the best solvent for the extraction of extractives from *P. juliflora* since it gave high yields of extractives in all the trees from the different areas as compared to the dichloromethane and toluene: ethanol solvents. These high yield values may be explained by the presence of high amounts of flavonoids being extracted by the acetone solvent as seen in the GC-MS.

The knot woods, heartwoods and the pith gave high yields as compared to the bark and the sapwood with Turkana County generally showing lower yields.

Figure 31 shows the graphical representation of the percentage yields of the different sites for toluene: ethanol extracts.

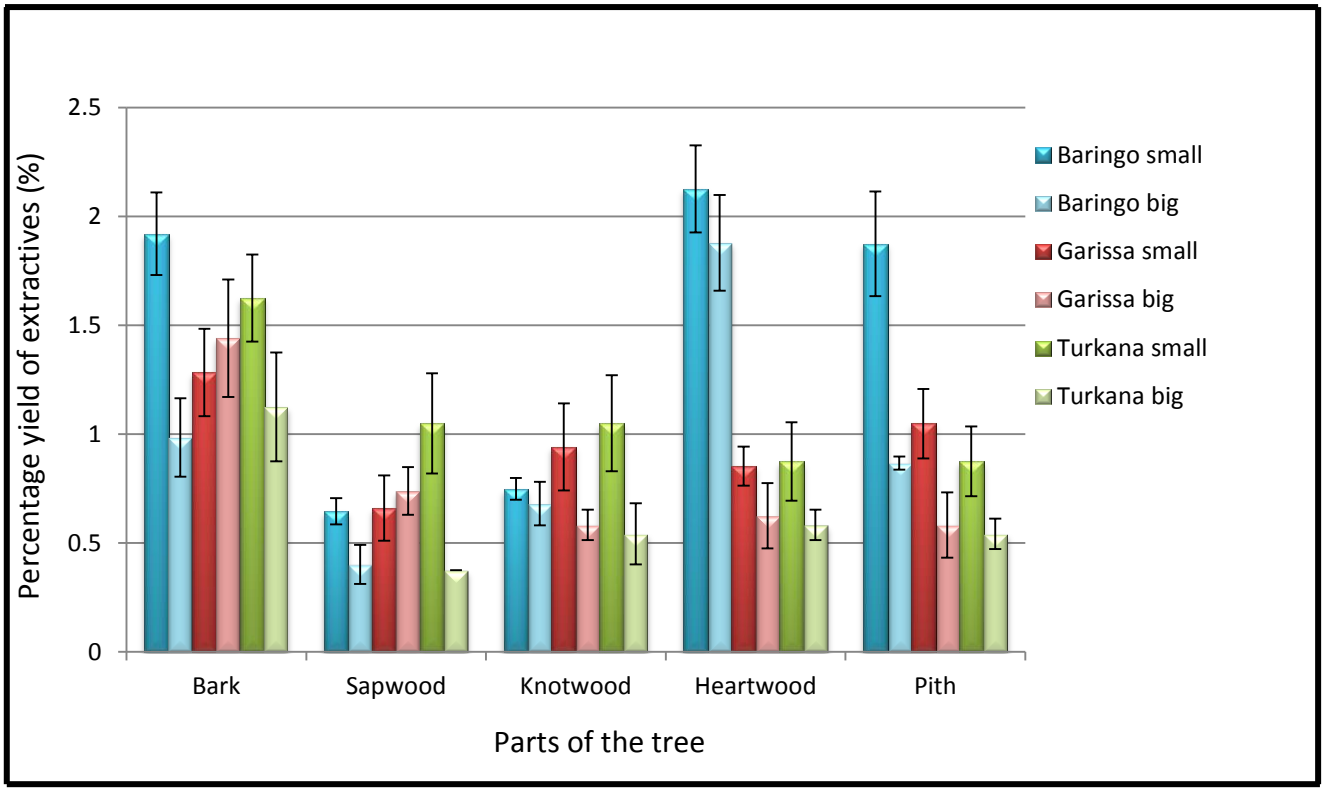


Fig **31:** Percentage yields of different geographical sites for toluene/ethanol (2:1 v/v) extracts

Compared to the rest of the solvents used for extraction, toluene: ethanol solvent gave the least percentage yields of extractives in all the regions (0.4 - 2.1 %) making it the least desirable solvent for extraction of extractives from *P. juliflora.*

Figure 32 shows the graphical representation of the percentage yields of the different sites for water extracts.

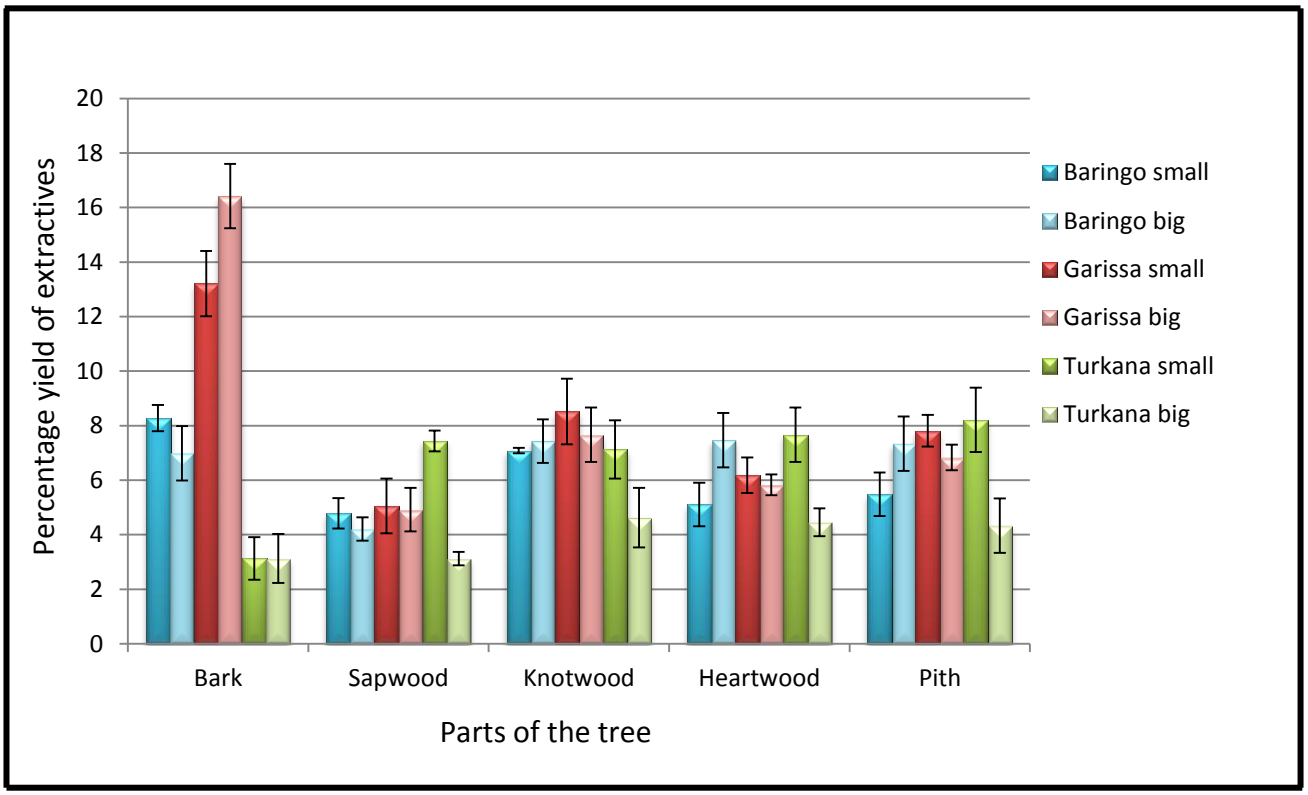


Fig **32:** Percentage yields of different geographical sites for water extracts

The water extracts showed high percentage yields compared to the dichloromethane and toluene: ethanol solvents. Water, being a polar solvent, is believed to extract all the remaining polar molecules that were still present in *P. juliflora* samples.

The bark samples from Garissa County probably had a lot of polar compounds since they gave the highest percentage yields compared to the other regions (13 and 16 %).

Compared to the other solvents, for only the sapwood samples, water extracts gave the highest percentage yields of 4 -7 %.

4.2.2 Identification of compounds present in *P. juliflora* by GC-MS

According to the NIST library, the dichloromethane extracts showed the presence of mostly fatty acids, sterols, terpenes, alcohols and sugars. The acetone extracts, toluene / ethanol extracts and water extracts were mostly composed of sugars and flavonoids as previously recorded by Sirmah *et al.*, (2011).

Mesquitol was found to be present in all parts of the trees' stem with the highest yields in the heartwood, pith and knot wood. All the sapwoods were found to contain very little flavonoids and were composed mainly of sugars. The water extracts of the pith were mostly composed of

mesquitol only. The different classes of extractives obtained from the three counties depending on the solvent used, age and part of the tree have been shown in annex 1.

The sample extracts observed for the GC-MS analyses generally showed the presence of three flavan-3-ols present in all the three counties. These were catechin, mesquitol and 4'-*O*-methylgallocatechin. Of these, catechin was observed first with a retention time of 20.61 minutes, followed by mesquitol with a retention time of 20.82 minutes and finally 4'-*O*-methylgallocatechin was observed with a retention time of 21.60 minutes. These retention times were observed while using a fused-silica capillary column.

A sample of one of the GC chromatograms of the TMS derivatives showing these three flavan-3-ols is shown in figure 33.

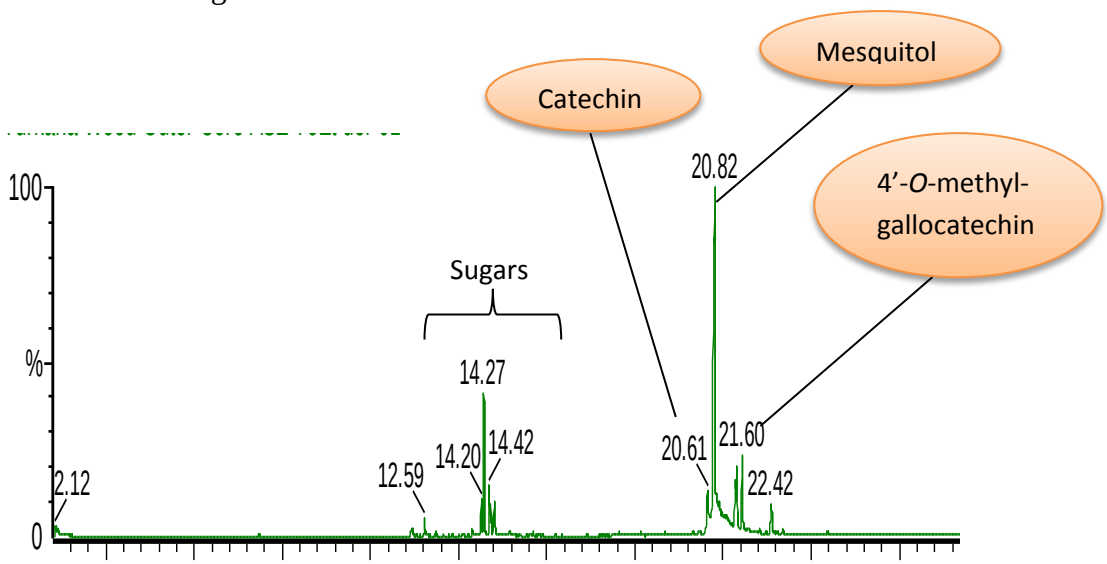


Fig **33:** GC chromatogram of the acetonic extract of Turkana big tree heartwood

The mass spectra of the three major flavan-3-ols showed almost similar MS fragmentation patterns as shown in figure 34, 35 and 36.

The mass spectrum of the penta-TMS derivative of mesquitol was m/z (%): 650 (M^+), 383.

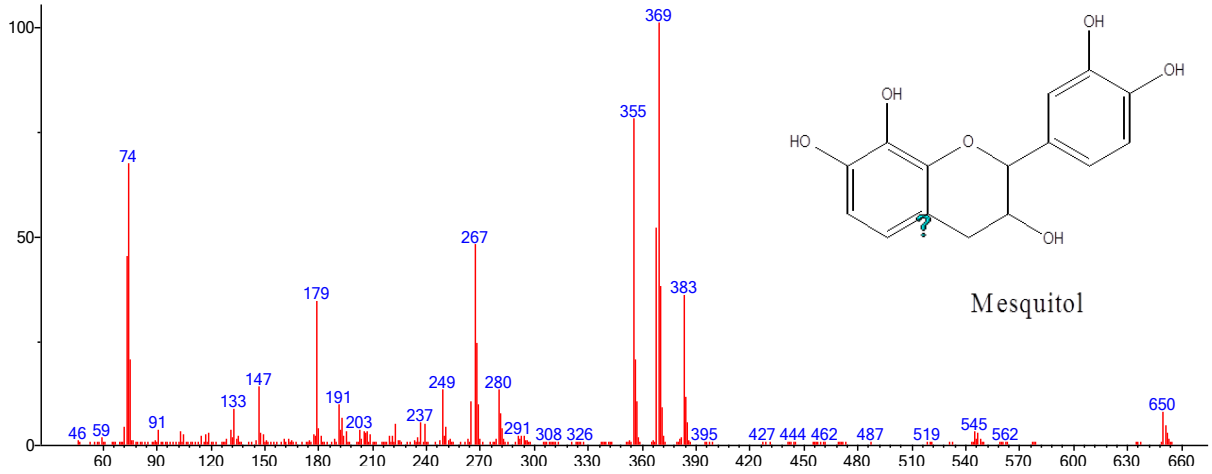


Fig **34:** MS chromatogram of the penta-TMS derivative of mesquitol

The mass spectrum for catechin was similar to that of mesquitol since it has similar fragmentation pattern. This is because it only differs from mesquitol by the position of the hydroxyl OH at the A-ring. The MS spectrum of the penta-TMS derivative of catechin is shown in figure 35.

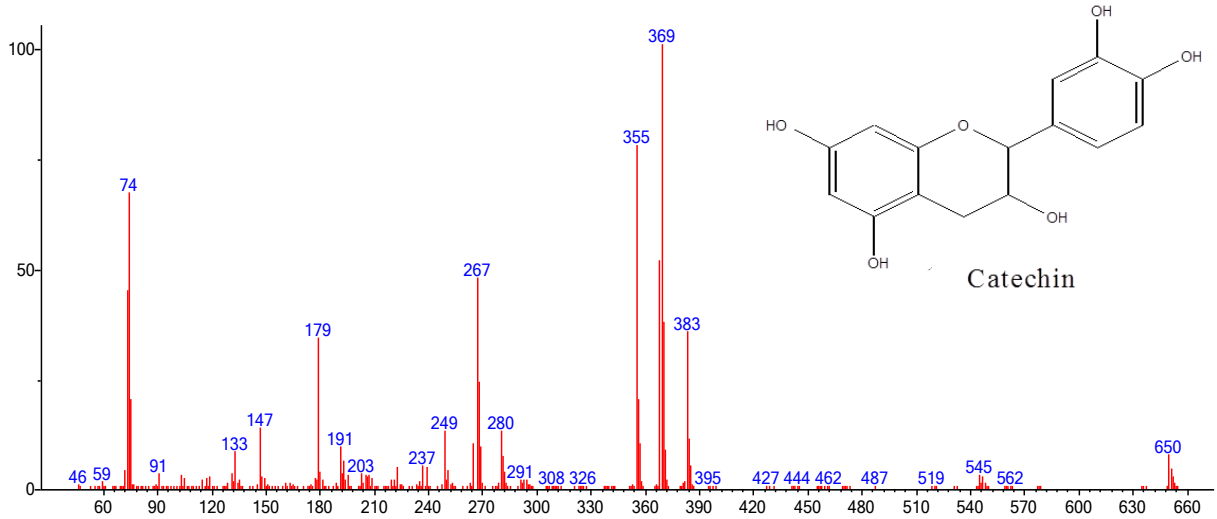


Fig **35:** MS chromatogram of the penta-TMS derivative of catechin

The MS spectrum of the penta-TMS derivative of 4'-*O*-methylgallocatechin was m/z (%): 680 (M^+), 398, 368, 297, 267, 73 (100). This corresponds to the data reported by Sirmah (2009) on the presence of 4'-*O*-methylgallocatechin in *P. juliflora.* Its MS spectrum is shown in figure 36.

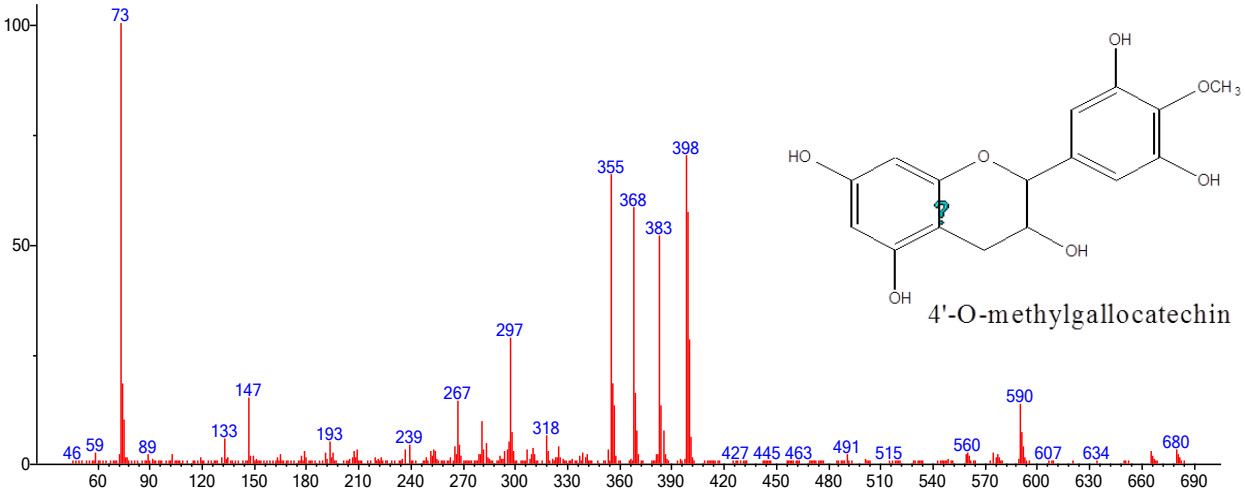


Fig **36:** MS chromatogram of the penta-TMS derivative of 4'-*O*-methylgallocatechin

A further study on the estimation of the percentages of the three flavan-3-ols was done using the GC-MS. This was done so as to later compare with the percentages present while using the LC-ESI-MS/MS which allowed for the comparison of the aglycone flavonoids with the glycosylated flavonoids since the GC-MS fragments of glycosylated compounds uses the electron impact mode unlike the electron ion spray of the LC-ESI-MS/MS.

The estimation of the percentages of the three main flavanols relative to TIC (total ion current) was done for the acetonic extracts and the threshold relative area for all the samples was set at 1.0. These percentages were recorded in table 6.

Table **6**: Percentages of the three main flavan-3-ols in acetonic extracts from different plant parts of *P. juliflora* of the three counties

		Baringo County		Turkana County		Garissa County	
Plant part	**Flavan-3-ol**	**Small trees**	**Big trees**	**Small trees**	**Big trees**	**Small trees**	**Big trees**
Bark	Mesquitol	6	23	0	1	19	0
	Catechin	0	0	0	0	0	0
	4'-*O*-methylgallocatechin	45	32	71	14	30	29
Sapwood	Mesquitol	6	0	0	1	8	16
	Catechin	1	0	0	0	0	0
	4'-*O*-methylgallocatechin	0	4	1	0	0	0
Knot wood	Mesquitol	48	57	22	63	63	81
	Catechin	8	15	0	5	2	2
	4'-*O*-methylgallocatechin	11	0	19	0	15	12
Heartwood	Mesquitol	74	74	43	68	42	70
	Catechin	7	14	0	6	3	9
	4'-*O*-methylgallocatechin	17	8	40	6	11	7
Pith	Mesquitol	70	78	45	68	62	74
	Catechin	10	22	3	4	5	7
	4'-*O*-methylgallocatechin	15	0	30	8	25	11

The 4'-*O*-methylgallocatechin was found in higher percentages in the bark compared to the other plant parts. It was also found more in the small trees compared to the big trees. No catechin was observed in the bark and the sapwood with just 1 % from the small trees from Baringo County. Very high percentages of mesquitol were observed in the knot wood, heartwood and the pith of all the different geographic regions. The small trees from Turkana County had small percentages of mesquitol in these plant parts with the least being 22 % from the knot wood. The highest percentage of mesquitol was observed from the knot wood from the big trees from Garissa County (81 %) followed by the pith from the big trees from Baringo County (78 %).

4.2.3 Identification and quantification of compounds by LC-ESI-MS/MS

4.2.3.1 Identification of compounds

Identification of the compounds present in the different stems of the *P. juliflora* was achieved by comparison of experimental retention times and UV-MS spectra to bibliographic data and by the use of some standard compounds.

From the calculation of the percentage yields of the extractives, the acetonic extracts were found to have the highest yields of extractives as compared to the other solvents. These acetonic extracts were therefore subjected to LC-ESI-MS/MS analyses in order to confirm the exact kind of compounds present in the *P. juliflora.* Majority of these compounds that were tentatively identified were phenolic compounds and their glycosylated derivatives. According to Sherwood and Bonello (2013), the sugar unit of phenolic glycoside serves to improve solubility for storage in cell organs.

The LC chromatograms from the different regions were studied and analyzed as shown in figures 37, 39, 41, 42, 44 and 45. The selected chromatograms are examples of some of the LC chromatograms obtained and show the peaks corresponding to the different compounds that were tentatively identified. Some peaks were found in higher percentages in one geographic region compared to the others while some peaks were found only in some regions and were absent in some. All the LC chromatograms obtained from the different acetonic extracts depending on the different geographic area, age of the tree and the different plant parts are shown in annex 2.

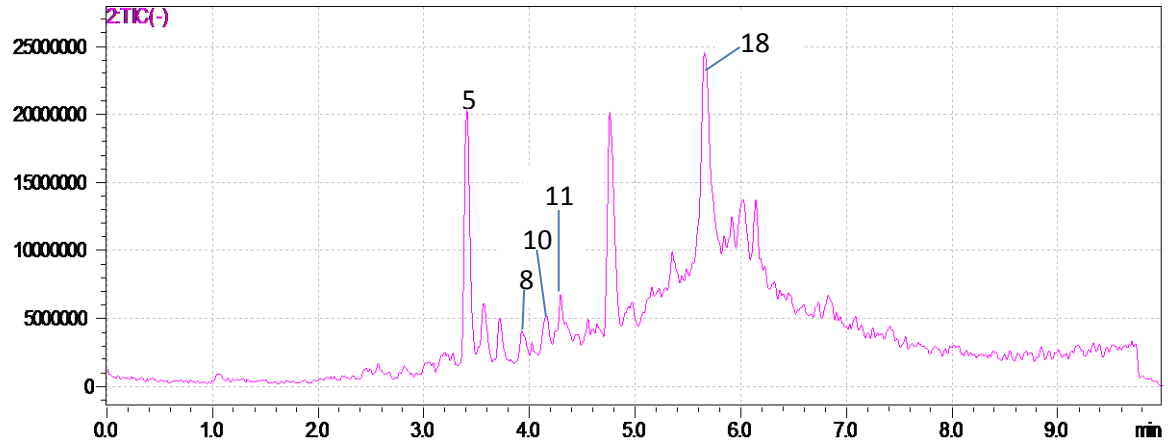


Fig **37**: LC chromatograms of the acetonic extract from the bark of the big trees from Turkana County

All the bark extracts were impure and had a mixture of many compounds. Figure 37 above shows some characteristic peaks observed in the barks from all the three different regions. The structures of the five compounds tentatively identified in the bark of *P. juliflora* are as shown in figure 38 below.

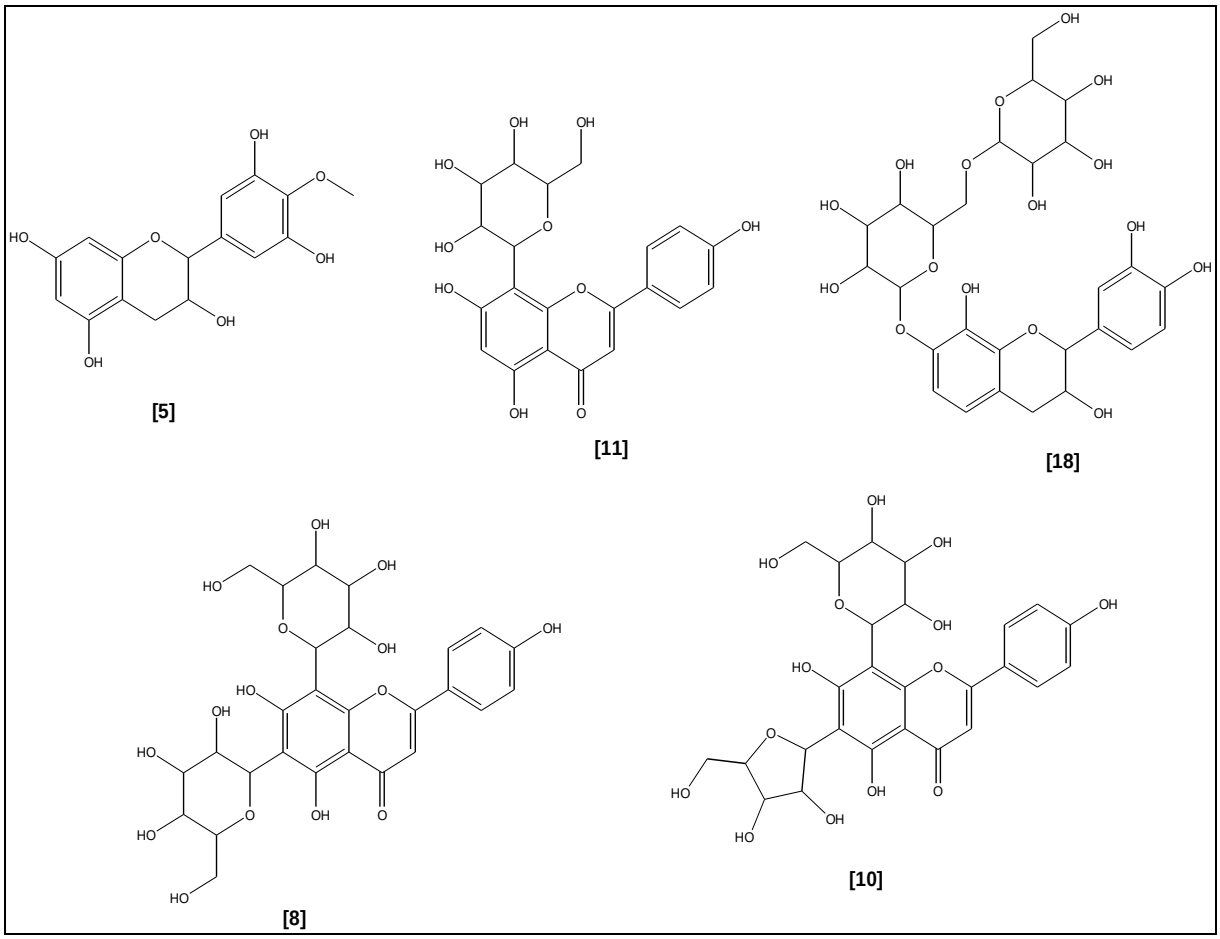
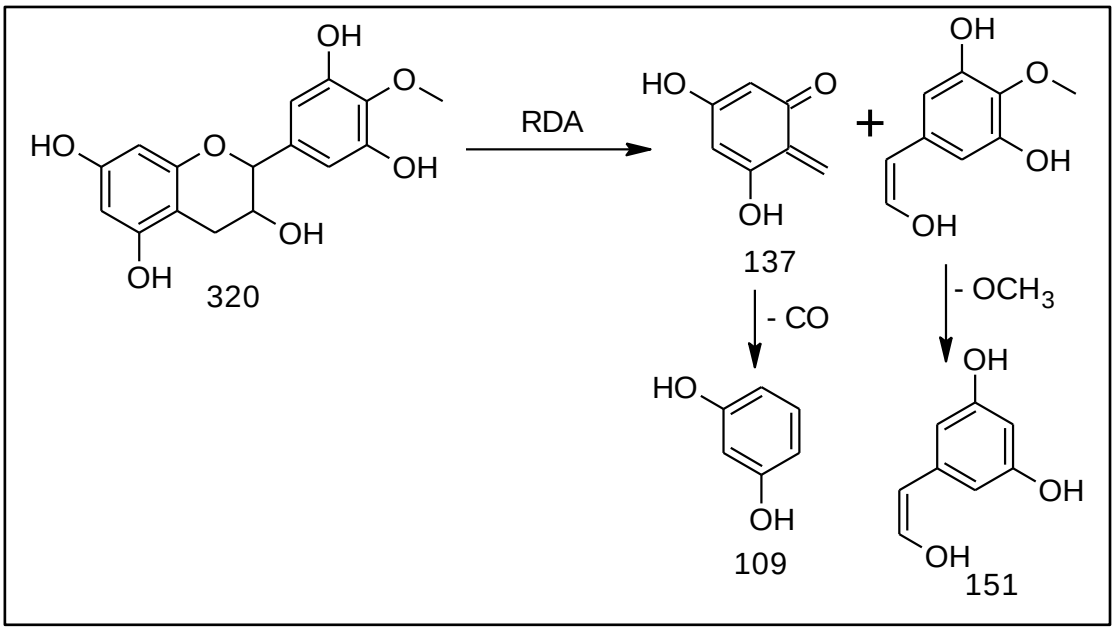


Fig **38:** Structures of compounds tentatively identified in the bark of *P. juliflora*

The most abundant peaks in the barks were peak **5** (4'-*O*-methylgallocatechin) with ranges between 18 – 28 %; peak **18** (mesquitol diglucoside) with ranges of 21 – 29 % and peak **11** (apigenin glucoside) with ranges of 7 – 20 %.

Bark samples from Baringo County contained only glycosylated form of mesquitol **[18]**. Turkana County had very little free mesquitol in the bark (3 %) while only small trees from Garissa County had free mesquitol (19 %), thus in relatively high amounts.

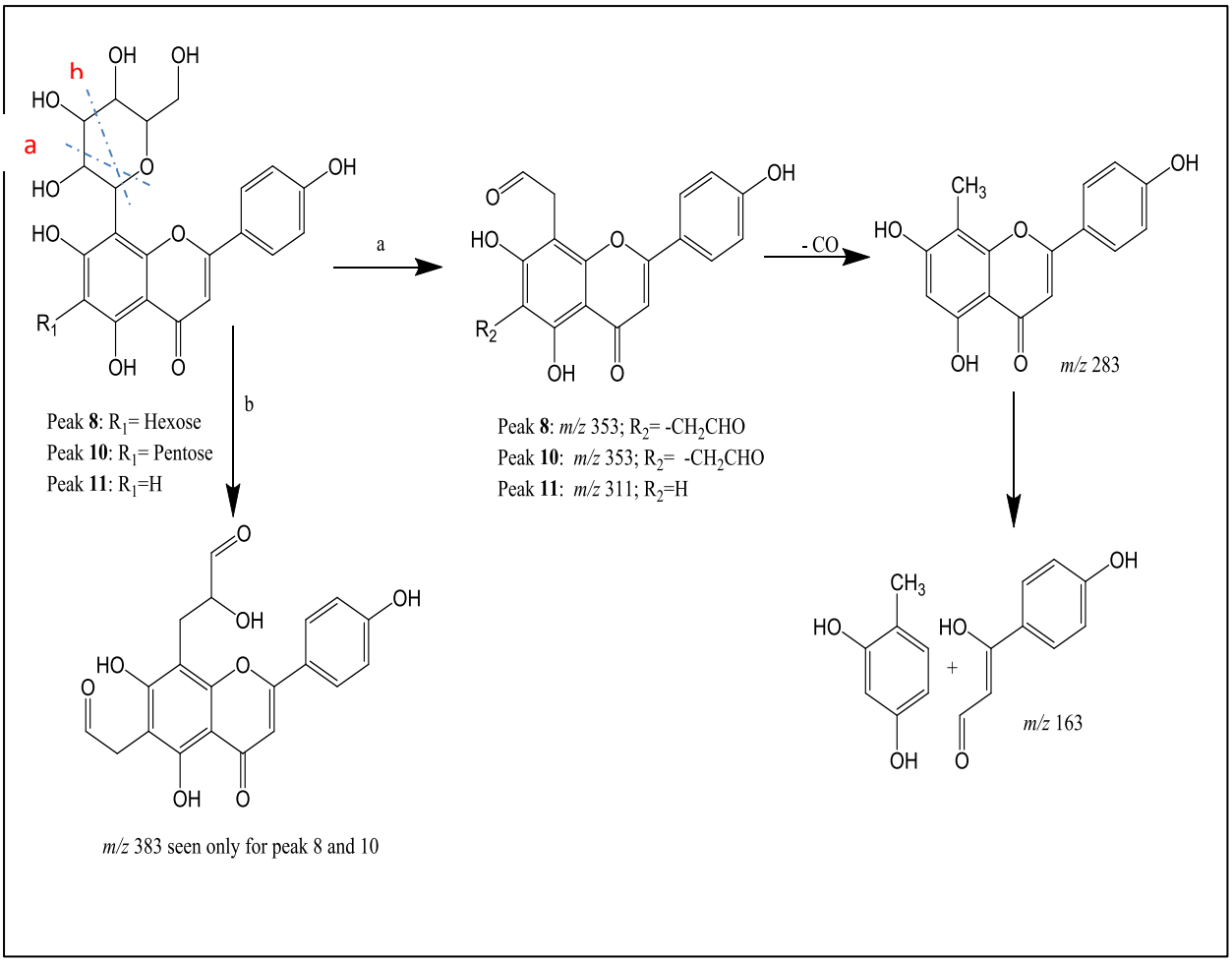
Peak **5**, identified as 4'-*O*-methylgallocatechin had a retention time 3.40 min; UV 278 nm; MS, 319 [M-H]; MS/MS [M-H]$^-$, 166, 151, 137, 109. Presence of this compound was first cofirmed in *P. juliflora* by the use of the GC-MS. This compoud was identified as 4'-*O*-methylgallocatechin with fragmentation pattern shown in scheme 11.



Scheme **11:** MS fragmentation pattern of 4'-*O*-methylgallocatechin

Its MS fragmentation pattern started from a retro-Diels Alder reaction which is typical of flavan-3-ols (Pati *et al*., 2006) followed by loss of CO and methoxy groups thus resulting in ionized molecules of m/z 137, 109 and 151. It was more abundant in the small trees from Turkana County.

Peak **8, 10** and **11**, were identified as glycosylated apigenin with peak **8** containing a di-hexose moiety, peak **10** had a pentose and an hexose moiety while peak **11** had one hexose moiety. The proposed fragmentation patterns of these compounds are shown in scheme 12.



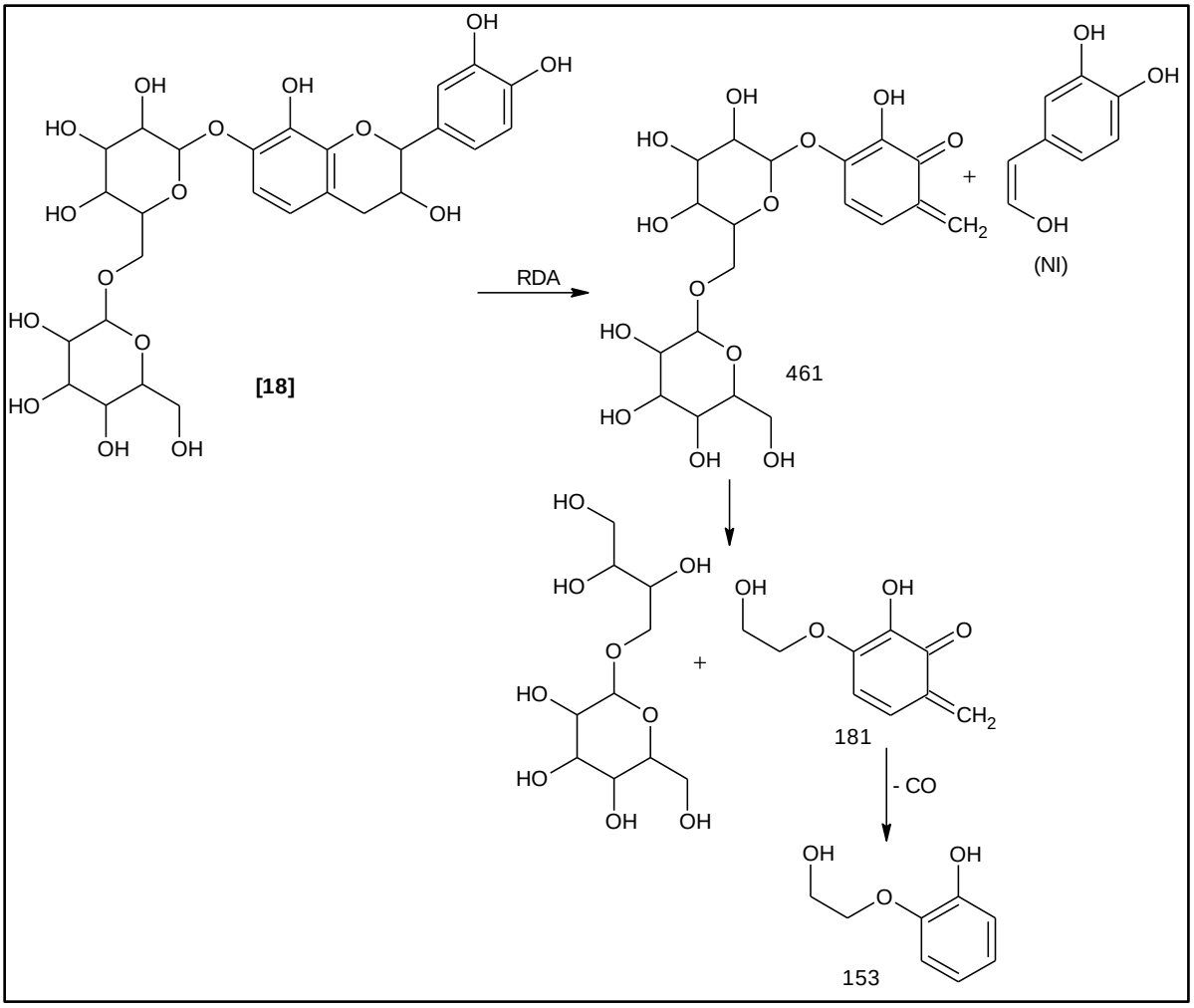
Scheme **12:** Fragmentation patterns of the various glycosylated apigenin

For the three peaks, the MS fragmentation pattern started firstly with the fragmentation of the sugar moieties. Peak **8** and **10** fragmented resulting in two product ions of *m/z* 353 and *m/z* 383. The *m/z* 335 observed in these two peaks are attributed to the loss of H_2O moiety of 18 Da from the *m/z* ion 353. For peak **11**, only the *m/z* 353 is observed which further fragments into products ions of *m/z* 283 and 163 as shown in scheme 12.

The sugar moeities (hexose and pentose) of these compounds did not fragment from the main aglycone forming two sugar moieties of -162 Da and -132 Da respectively as it normally does for most glycosylated compounds. This is explained by Kumar, 2017, who describes this as a characteristic fragmentation pattern of di *C*-glycosyl flavones. The reason for this is that *C*-glycosidic bonds are more stable than *O*-glycosidic bonds and therefore fragmentation pathway of *O*-glycosylated flavonoids begins with the cleavage of glycosidic bonds and removal of the sugar moieties. In *C*-glycosylated flavonoid, complete removal of the sugar

moiety is not observed rather than the elimination of fragments or several fragments of the sugar ring (Kumar, 2017; Bonta, 2017). This fragmentation pathway also agrees to a study done by Ferreres *et al.,* 2003. The glycosyl groups of C-glycosides (just like in this study) are usually connected to the C6 and or C8 (Feng *et al*., 2016).

Peak **18** was attributed to mesquitol diglucoside which was observed to occur in high quantities in the bark of *P. juliflora*. It was found to have a retention time of 5.67 min; UV maxima of 274 and 278 nm; MS 613 [M-H]; MS/MS $[M-H]^-$, 461, 181, 153. Its fragmentation pattern is shown in scheme 13 and it is also a *C*-glycosylated flavonoid.



Scheme **13:** Fragmentation pattern of the mesquitol diglucoside

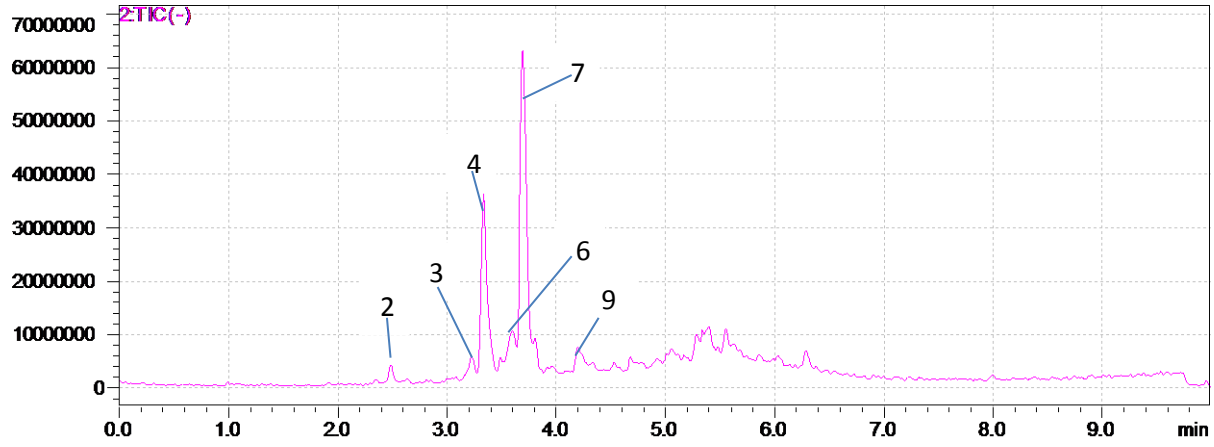


Fig **39:** LC chromatogram of the acetonic extract from the heartwood of the small trees of Turkana County

Figure 39 shows characteristic chromatograms observed for the heartwoods from the different geographic regions. The knot woods and the piths also showed almost similar chromatograms. The structures of the identified compounds are as shown in figure 40 below.

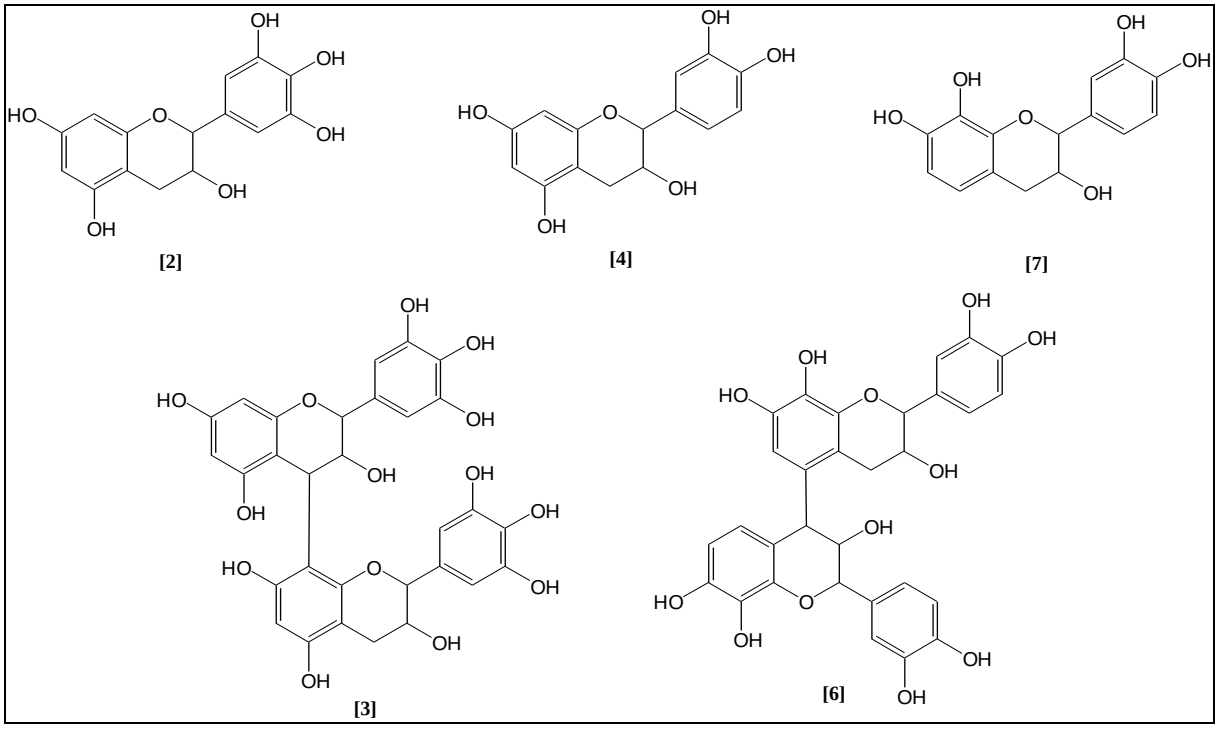
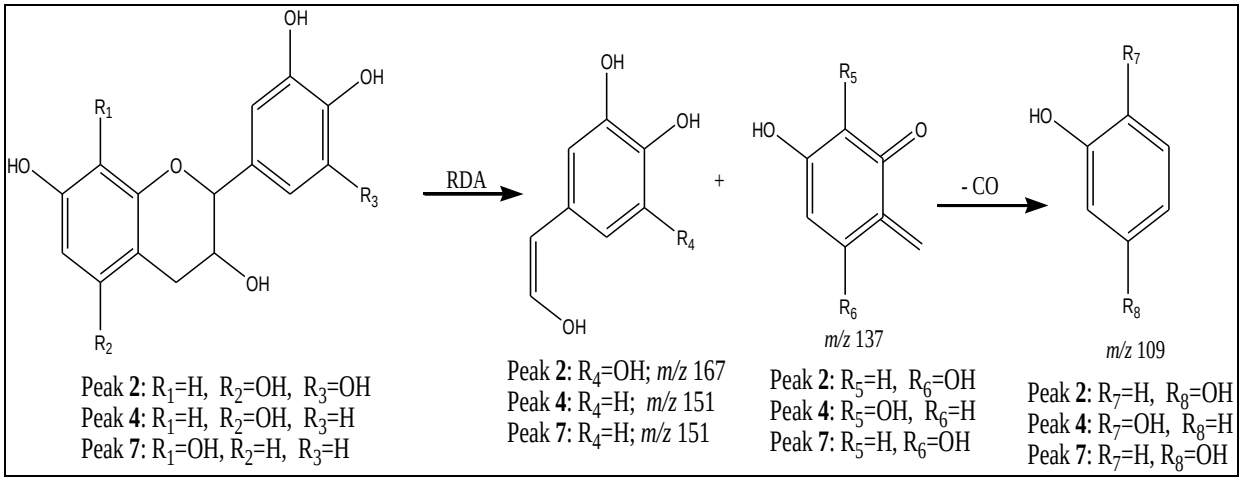


Fig **40:** Structures of compounds tentatively identified in the heartwood of *P. juliflora*

Peak **9**, was observed with a retention time of 4.24 min; MS 287 [M-H]; MS/MS [M-H]$^-$, 242, 185, 153, 125, 123. Although this compound is unknown it was found only in the big trees from Turkana County.

Peak **2**, which was identified as gallocatechin, was observed in small quantities and was found only in the small trees from all regions (1-4 %). It is interesting to note that this compound was not found in the bark and sapwood but was only present in the knot wood, heartwood and pith.

The chromatograms of the small trees were dominated by catechin (peak **4**) and mesquitol (peak **7**). In all the sites, the amount of peak **4**, which is catechin, was higher in small trees but decreased greatly in amounts in the big trees. The fragmentation patterns of peak **2**, **4** and **7** are shown in scheme 14 below.

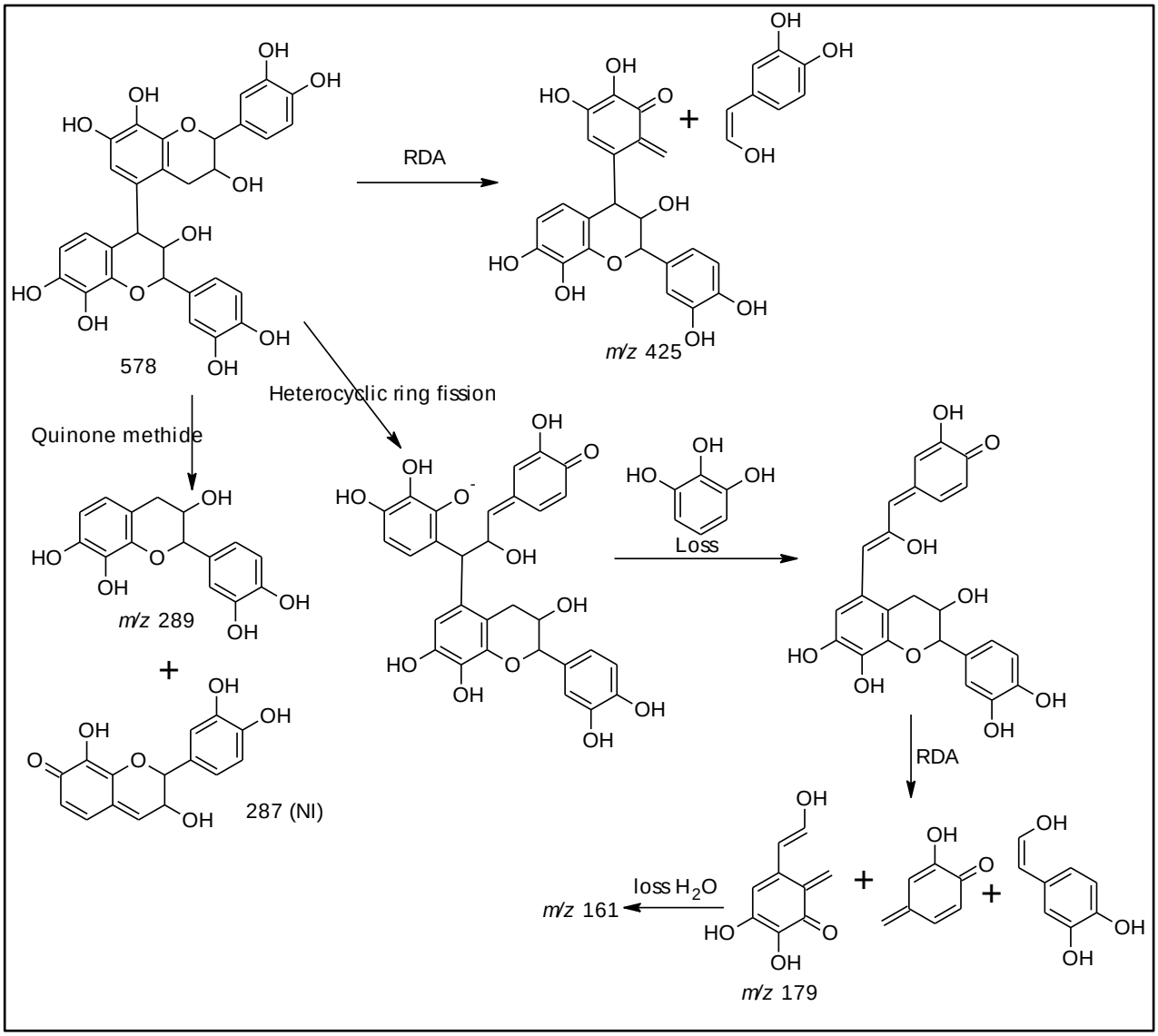


Scheme **14:** Fragmentation patterns of gallocatechin, catechin and mesquitol

The fragmentation started from a RDA resulting in product ions of *m/z* 167 for peak **2** and *m/z* 151 for peak **4** and **7**. For peak **2** (gallocatechin), product ions of *m/z* 139 and *m/z* 111 were also observed. This was due to the loss of a CO moiety for the first one and two CO moieties for the later daughter ion. A subsequent product ion of *m/z* 137 was also observed for peak **4** and **7**. This ion fragmented further by a loss of 28 Da (CO) to give fragments of *m/z* 109 which was also observed.

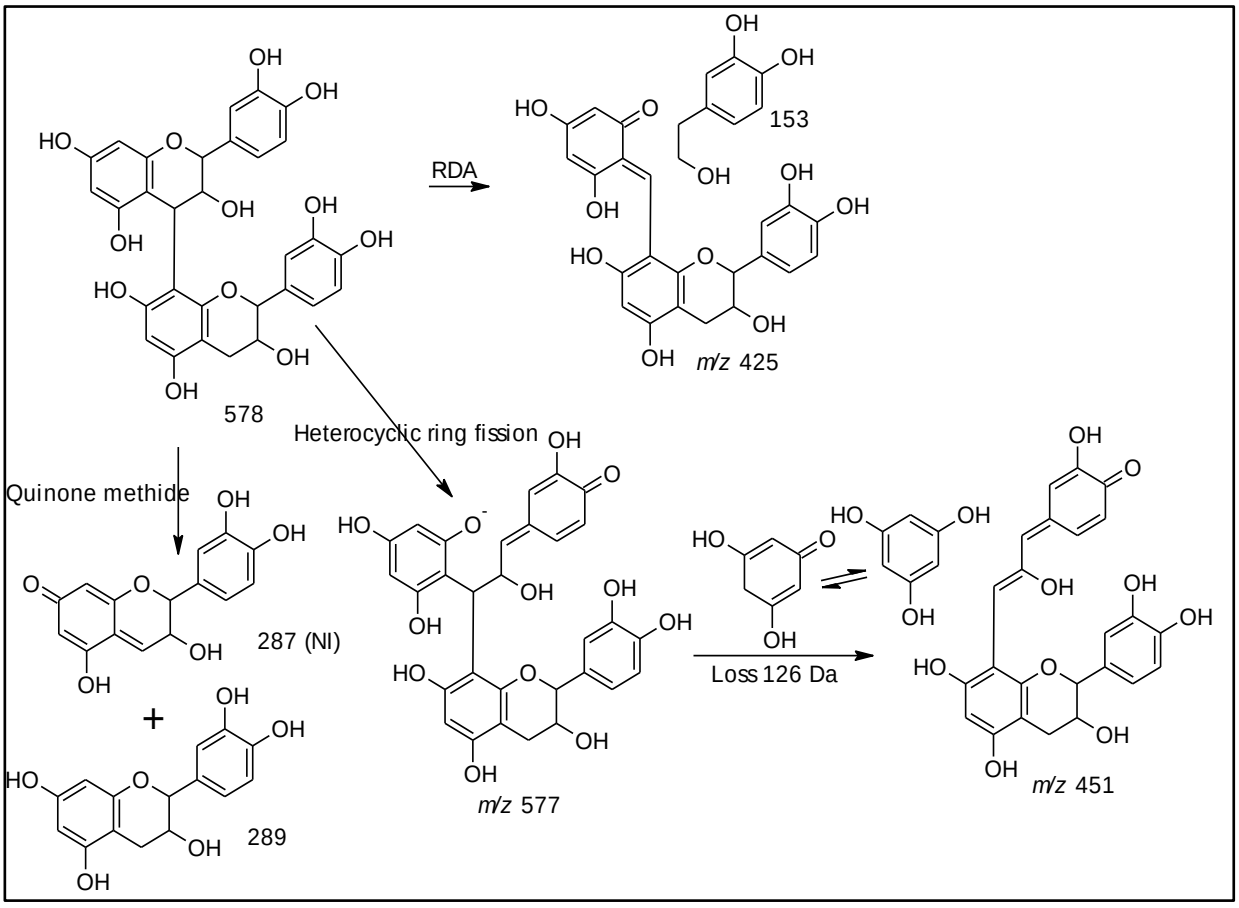
For the first time, a new compound believed to be a B-type proanthocyanidin of mesquitol **[6]** was identified. The identification was based on the MS/MS diagnostic ions that resulted from retro-Diels-Alder reaction, heterocyclic ring fission and quinone methide cleavage where the cleavage occurs at the bond connecting the two flavanol molecules forming a molecule with a quinone in its structure as shown in scheme 15.

This compound was present in the knot wood, heartwood and pith of all regions. The amounts present were between 2 - 7 %. The 7 % was observed in the samples of both the big and small trees from Turkana County.



Scheme **15:** Fragmentation pattern of the novel B-type proanthocyanidin of mesquitol

Peak **3**, which was also a catechin proanthocyanidin, was identified. It had the same fragmentation pattern with a slight difference as shown in scheme 16. Whereas as for the mesquitol dimer, the molecule with an *m/z* 451 was observed to further fragment to *m/z* 179 and *m/z* 161 and this fragmentation was not observed for the catechin dimer. This compound had a retention time of 3.23 min; UV maxima 277 nm; MS, 577 [M-H]; MS/MS $[M-H]^-$, 451, 425, 288, 126.



Scheme **16:** Fragmentation pattern of the catechin dimer

This fragmentation pattern on the retro-Diels Alder reaction is similar to the one that has been proposed by Demarque *et al.* (2015).

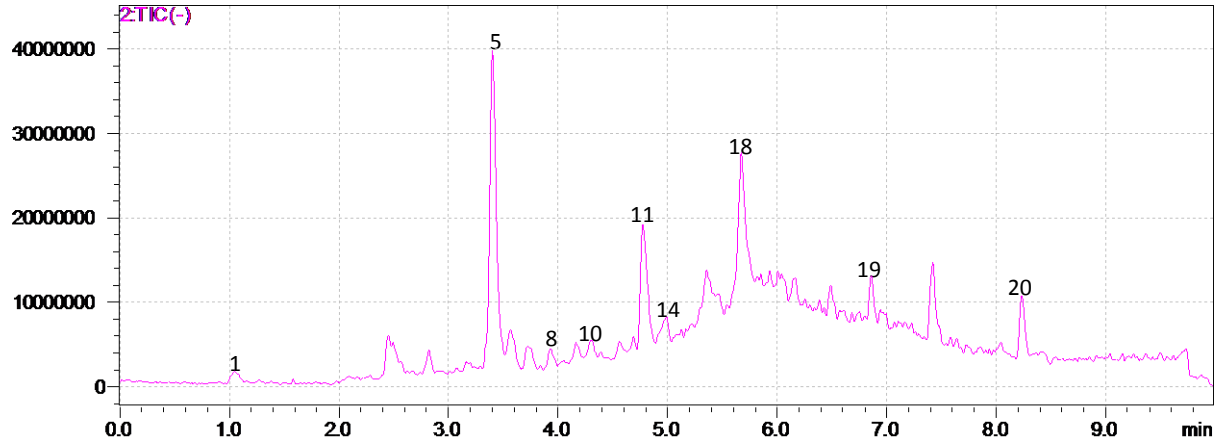
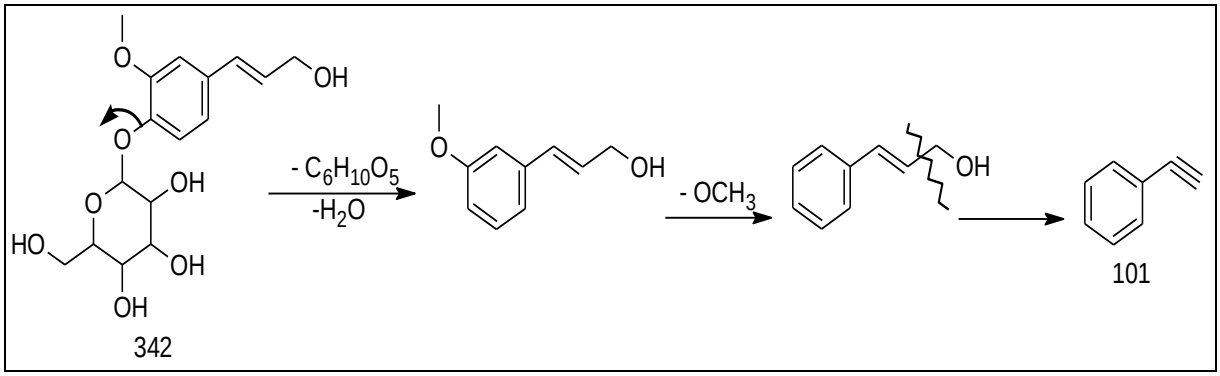


Fig **41:** LC chromatogram of the acetonic extract from the bark of the small trees of Turkana County

Figure 41 shows some extra peaks found in *P. juliflora.* Peak **1** was observed mostly in the sapwoods and barks from all regions, with it dominating more in the sapwoods than the barks. It was tentatively identified as abietin (coniferin). It was abundant in the sapwood of Garissa and Baringo counties (13 – 25 %). Turkana County showed little amounts of coniferin in their sapwood (5 %).

This compound was observed at a retention time of 1.06 min; UV 284 nm and MS, 341 [M-H]; MS/MS [M-H]$^-$, 101 and had a fragmentation pattern as shown in scheme 17 below.



Scheme **17:** Fragmentation pattern of coniferin

There is a chemical compound in the extract with a retention time of 8.25 mins that is still unknown (peak **20**). This compound was found present only in the bark and sapwood samples from the small trees of Turkana County and was absent in Baringo and Garissa Counties. More studies should be done in its identification, the role it plays in the tree and why it is found only in Turkana County. Peak **19** was also unknown.

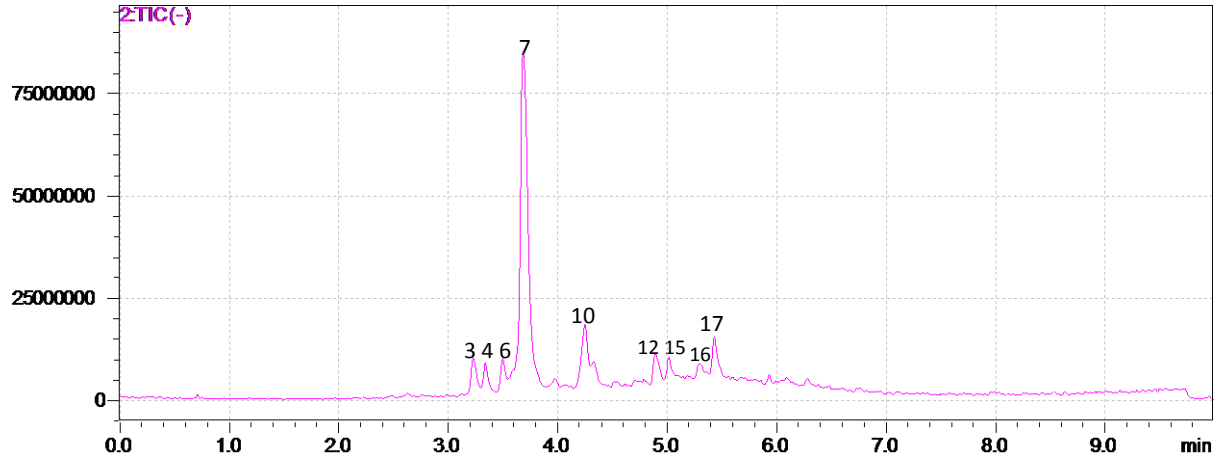


Fig **42:** LC chromatogram of the acetonic extract from the heartwood of the big trees of Turkana County

Figure 42 above shows some extra peaks observed from *P. juliflora.* Peak **15** and **17** were identified as taxifolin and kaempferol respectively and their structures are shown in figure 43 below. Peak **12** and **16** were both unknown and were found only in the knot wood, heartwood and the pith from all regions

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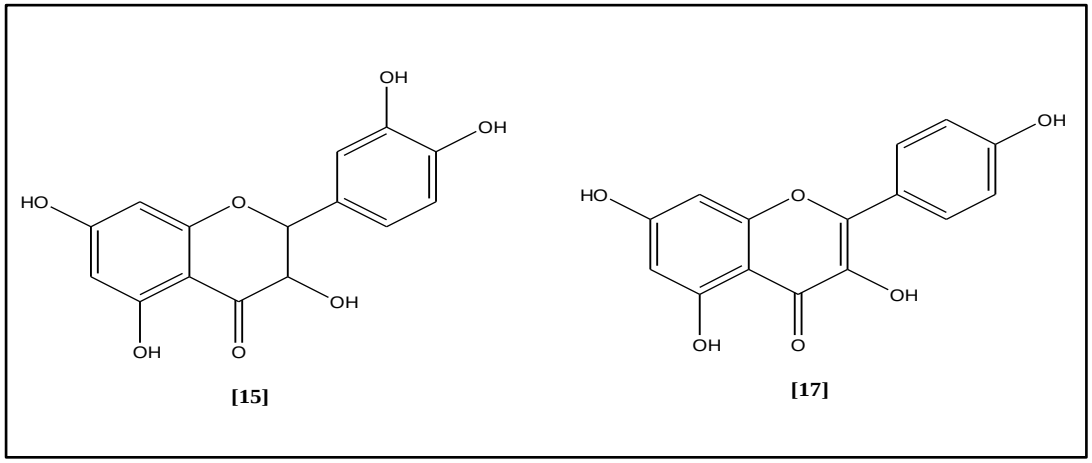
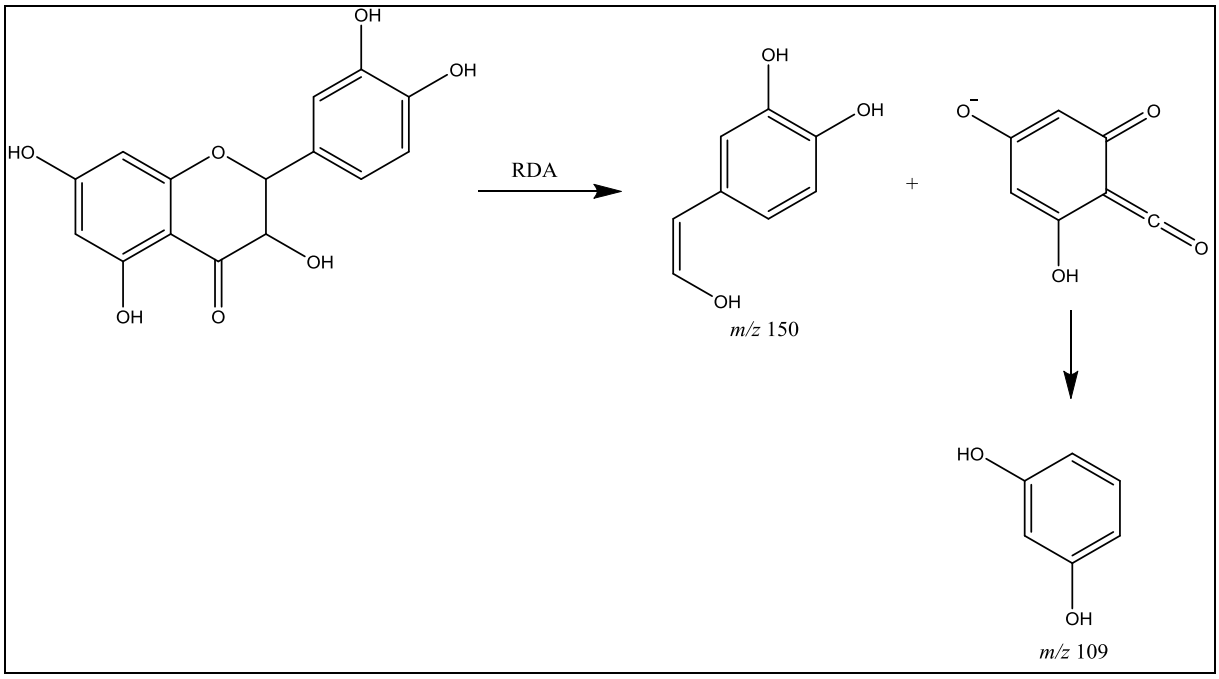


Fig **43:** Structures of the flavonoids (taxifolin **[15]**, kaempferol **[17]**) identified in *P. juliflora*

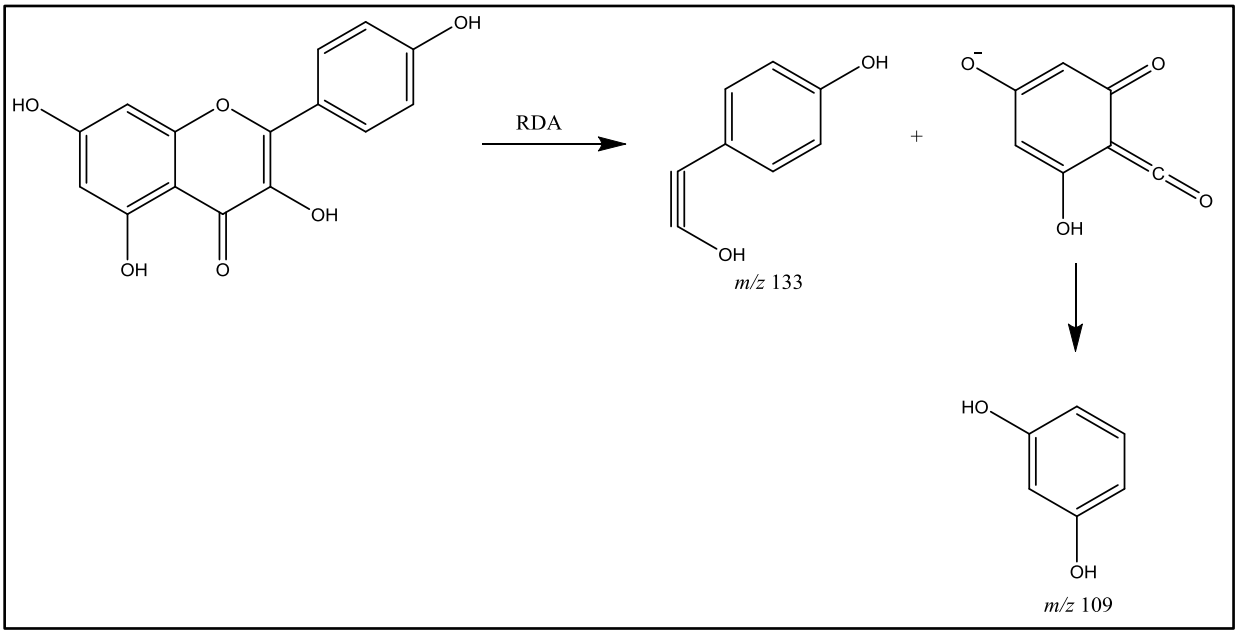
Peak **15** had a retention time 5.02 min; UV 285 nm; MS, 303 [M-H]; MS/MS [M-H]$^-$, 257, 150, 109 with a fragmentation pattern shown in Scheme 18.



Scheme **18:** Fragmentation pattern of taxifolin

The fragment with an *m/z* 257 observed was obtained by the loss of a CO and H_2O moieties from the M-H ion of *m/z* 303.

Peak **16** had a retention time of 5.43min; UV maxima of 264 and 312 nm; MS, 285 [M-H]; MS/MS $[M-H]^-$, 256, 239, 227, 133, 117, 109. The fragmentation pattern is shown in Scheme 19.



Scheme **19:** Fragmentation pattern of kaempferol

The *m/z* 256 was attributed to loss of –COH moiety, fragment *m/z* 239 was attributed to loss of two OH moities while *m/z* 227 was attributed to loss of a CO and COH moities.

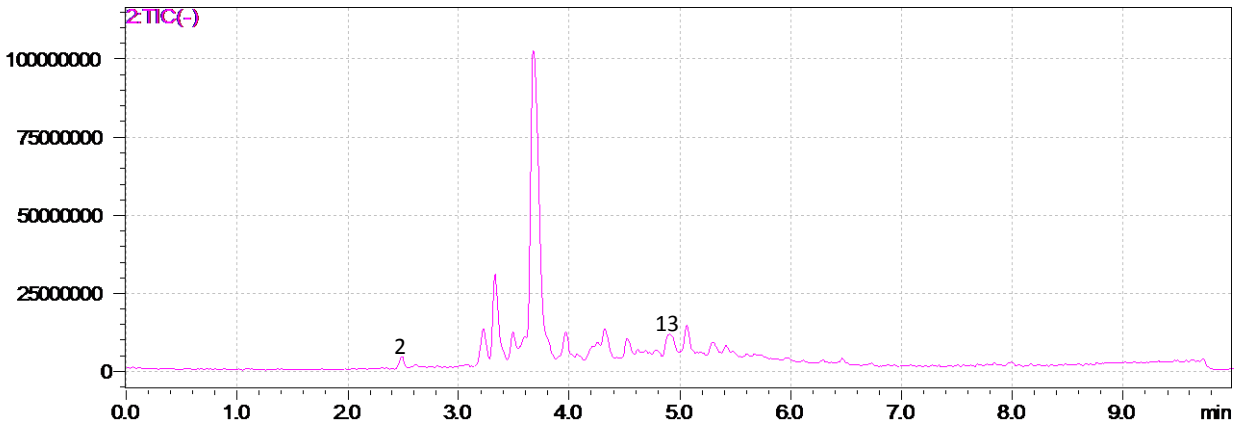
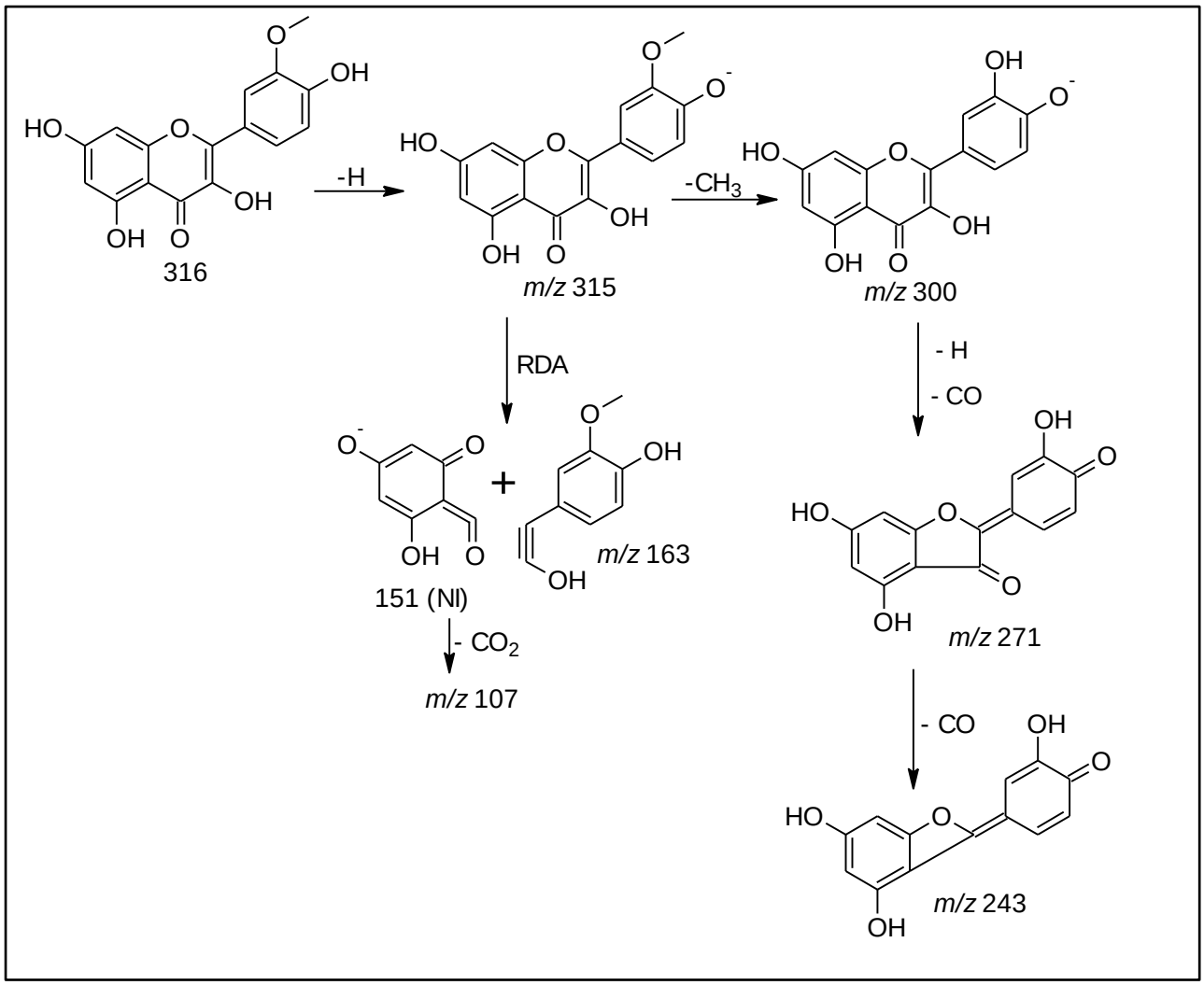


Fig **44:** LC chromatogram of the acetonic extract from the heartwood of the small trees of Baringo County

Figure 44 above shows a chromatogram consisting of a rare peak **13**. This peak was very small and seemed insignificant but it was identified since it was found only in the knot wood and heartwood samples from Baringo County at around 4 - 7 %. It was not observed at Garissa County and Turkana County. This peak was identified as isorhamnetin with a $[M-H]^-$ peak of 315 Da. Isorhamnetin is usually known as the methylated metabolite of quercetin.

Its fragmentation pattern agrees to what was obtained by Chen *et al.*, 2015 and is shown in Scheme 20.



Scheme **20:** Fragmentation pattern of isorhamnetin

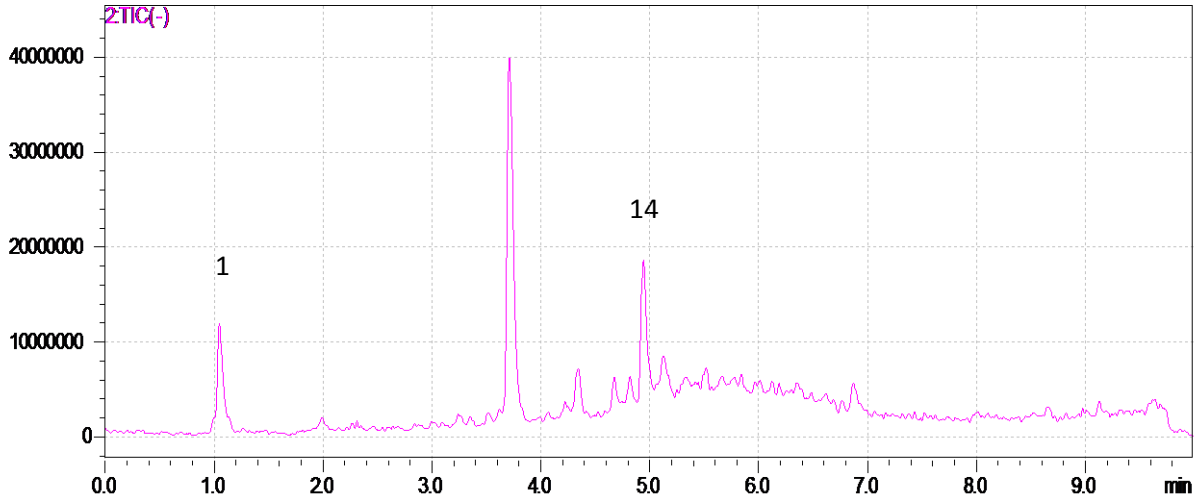
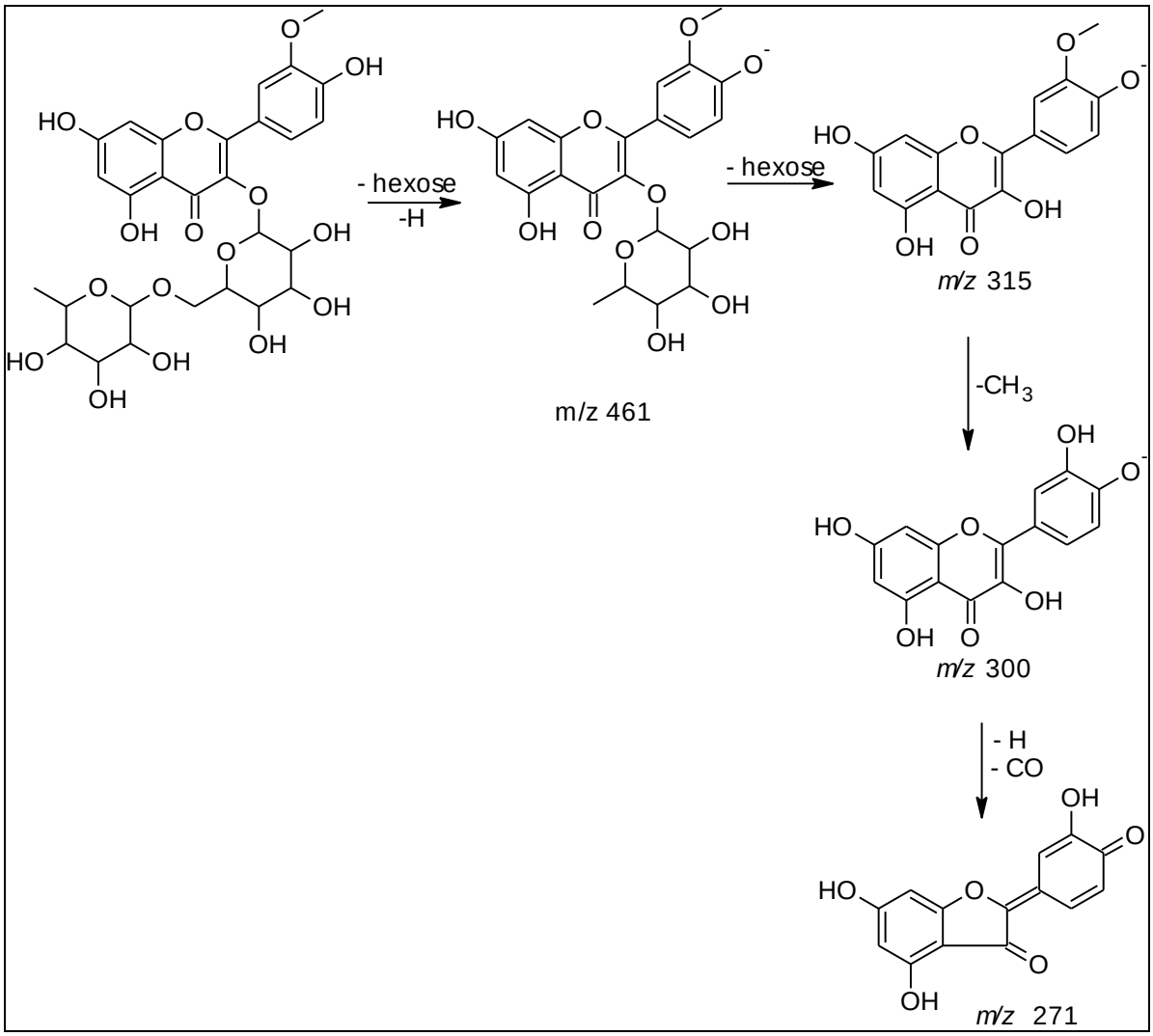


Fig **45:** LC chromatogram of the acetonic extract from the sapwood of the big trees of Garissa County

Although Garissa and Turkana Counties did not contain isorhamnetin, a glycosylated isorhamnetin was found only in Garissa County in high amounts of 14 % (Peak **14**) as shown in figure 45 above. The presence of this compound in *P. juliflora* agrees to what had been reported by Prabha *et al*., 2014 on the compounds present in *P. juliflora.*

It had a retention time of 4.94 min; UV 329 nm and a [M-H] of 623; MS/MS $[M-H]^-$, 461, 161, 135, 133. This compoud was identified as isorhamnetin-3-rutinoside with fragmentation pattern as shown in scheme 21.



Scheme **21:** Fragmentation pattern of isorhamnetin-3-rutinoside

A summary of the 20 peaks that were tentatively identified have been tabulated in table 7 below according to their corresponding peak numbers.

Table **7**: Compounds present in the *Prosopis juliflora* by the use of LC-ESI-MS/MS

Peak number	t_R (min)	λ_{max} (nm)	MW	$[M-H]^-$	MS/MS Peaks MS^2 ions	Tentative Identification
1	1.06	284	342	341	101	Abietin (coniferin)
2	2.40	280	306	305	167; 139; 111; 109	Gallocatechin
3	3.23	277	578	577	577; 451; 425; 288; 126	Catechin dimer
4	3.32	278	290	289	151; 137; 109	Catechin
5	3.40	278	320	319	166 ; 151; 137; 109	4'-*O*-Methylgallocatechin
6	3.49	277	578	577	425 ; 301 ; 289 ; 179 ; 161	Mesquitol dimer
7	3.70	278	290	289	151; 137; 109	Mesquitol
8	3.93	271, 331	594	593	383; 353; 335	6,8-di *C*-glucosyl apigenin (vicenin 2)
9	4.24		288	287	242; 185; 153; 125; 123	Unknown
10	4.30	271, 335	564	563	383; 353; 335	6-C-glucosyl-8-C-pentosyl apigenin or 6-C-pentosyl-8-C-glucosyl apigenin
11	4.77	269, 336	432	431	311; 283; 269; 163; 119	Apigenin glucoside
12	4.90		302	301	245; 227; 199; 149; 132; 121; 109	Unknown
13	4.92	281	316	315	315; 300; 271; 243; 163 ; 151; 107	Isorhamnetin
14	4.94	329	624	623	461 ; 315 ; 300 ; 271 ; 255	Isorhamnetin-3-rutinoside
15	5.02	285	304	303	257; 150; 109	Taxifolin
16	5.31	281	330	329	144	Unknown
17	5.43	264, 312	286	285	256; 239; 227; 133; 117; 109	Kaempferol
18	5.67	274, 278	614	613	461; 181; 153	Mesquitol diglucoside
19	6.86	--	306	305	305; 159; 147; 145; 132; 129; 119	Unknown
20	8.25	--	432	431	431; 413; 269; 255; 243; 169	Unknown

4.2.3.2 Quantification of the three major flavan-3-ols

Having confirmed the presence of the compounds in the different samples, quantification of the three major flavan-3-ols observed has been done. These quantifications were done in the negative ion mode of the LC-ESI-MS/MS. The three flavan-3-ols were mesquitol, catechin and 4'-*O*-methylgallocatechin. Catechin was observed first of the three flavan-3-ols with a retention time of 3.3 min followed by 4'-*O*-methylgallocatechin which was observed at a retention time of 3.4 min. Mesquitol was lastly observed at a retention time of 3.7 min.

All the three favan-3-ols showed strong UV absorption (λ_{max}) at 278 nm. This is in agreement with the fact that the aromatic phenol groups of organic compounds absorbs strongly near 280 nm (Kalsi, 2005).

Mesquitol quantification

A systematic study of the percentages of mesquitol present in *P. juliflora* showed that it is the most abundant compound in most parts of the stem with high amounts being found in the knot wood, heartwood and pith. These results corroborated with those reported by Sirmah *et al.*, 2008 and Odero *et al.*, 2017. Mesquitol's percentage in the acetonic extract ranged from 31 – 72 % in the knot wood, heartwood and pith. Small amounts of mesquitol were also detected in the bark and in the sapwood. Percentages of free mesquitol in acetonic extracts from the various parts and regions were tabulated in table 8.

Table **8**: Percentage of mesquitol in acetone extracts identified by LC-ESI-MS/MS in the different parts of *P. juliflora* of the three counties

Plant part	Baringo County		Turkana County		Garissa County	
	Small trees	**Big trees**	**Small trees**	**Big trees**	**Small trees**	**Big trees**
Bark	0	0	0	3	19	0
Sapwood	33	22	18	19	44	43
Knot wood	62	58	31	61	55	57
Heartwood	61	69	47	63	67	72
Pith	63	57	51	59	58	65

Percentages of mesquitol were however lower than those measured by GC-MS suggesting that mesquitol may exist under different forms especially as its glycoside form.

From the knot wood, heartwood and the pith, the small trees from Turkana County showed lower percentages of mesquitol in the acetonic extract compared to the other counties.

Statistical data

From the Anova test done using the results obtained from table 8, it shows that the amounts of mesquitol present in the acetonic extracts of small trees are significantly different ($p = 0.002$) in the three regions and are also significantly different ($p = 0.000$) in the plant parts.

The amounts of mesquitol present in the acetonic extracts of big trees are significantly different ($p = 0.001$) in the three regions and are also significantly different ($p = 0.000$) in the plant parts.

There is a very strong positive correlation ($r = 0.91$) between amounts of mesquitol present in the acetonic extracts between small trees and big trees from the three regions.

Table 8 shows that mesquitol is present in the barks of only the big trees from Turkana County and the small trees of Garissa County in small quantities of 3 and 19 % respectively. On the surface, it seems that mesquitol is not present in the barks of *P. juliflora* however, after a systematic study of the LC chromatograms of the barks; it was revealed for the first time that the mesquitol compound is present in the barks of *P. juliflora* in its glycosylated form with the molecular structure shown as compound **[18]** in figure 38. The percentages of the glycosylated mesquitol in the acetonic extracts from the barks of the *P. juliflora* were therefore estimated in the negative mode and tabulated in table 9.

Table **9**: Percentage of glycosylated mesquitol in acetone extracts identified by LC-ESI-MS/MS in the bark of *P. juliflora* from the three counties

Plant part	Baringo County		Turkana County		Garissa County	
	Small trees	**Big trees**	**Small trees**	**Big trees**	**Small trees**	**Big trees**
Bark	30	22	24	29	21	28

Having estimated the percentages of mesquitol present in the acetonic extracts, the study went ahead and calculated from this data the amounts of the mesquitol present in *P. juliflora*. These were represented in percentages in table 10.

Table **10**: Estimation of percentages of mesquitol in *P. juliflora* (%)

Plant part	Baringo Small trees	Baringo Big trees	Turkana Small trees	Turkana Big trees	Garissa Small trees	Garissa Big trees
Bark	2.0	1.5	1.1	2.1	2.1	1.4
Sapwood	0.4	0.3	0.4	0.3	0.9	0.7
Knot wood	6.1	7.3	2.5	2.9	9.8	4.9
Heartwood	5.3	11.3	3.1	6.0	9.9	6.4
Pith	7.7	10.2	3.3	3.5	9.6	5.3

The big trees had slightly higher amounts of mesquitol in the knot wood, heartwood and the pith on both Turkana and Baringo Counties compared to the small trees. This was contrary to the samples from Garissa County where the opposite effect was observed.

An interesting thing was the small amounts of mesquitol present in the samples from Turkana County. The exact factors that could lead to these differences among the counties are not yet completely understood, but a possible explanation could be the higher altitude of Turkana location compared to Baringo and Garissa Counties which may account for the decrease in mesquitol.

Catechin quantification

Catechin was also identified in the *P. juliflora* and quantified. It was found in little amounts in some parts of the knot wood, heartwood and the pith. In the sapwood, it was present only in the small trees from Baringo and Garissa Counties. An interesting result was the absence of catechin in the barks.

The percentages of catechin in acetonic extracts from the various plant parts and Counties were presented in table 11.

Table **11**: Percentage of catechin in acetone extracts identified by LC-ESI-MS/MS from the various plant parts in the three counties

Plant part	Baringo County		Turkana County		Garissa County	
	Small trees	Big trees	Small trees	Big trees	Small trees	Big trees
Bark	0	0	0	0	0	0
Sapwood	8	0	14	0	0	0
Knot wood	11	0	16	5	14	5
Heartwood	13	5	26	3	9	6
Pith	10	0	24	3	15	5

The big trees from all the quantities had really very small amounts of 0 – 6 % compared to the small trees which had a range of 9 – 26 % in the knot wood, heartwood and the pith.

The samples from big trees from Baringo County had no catechin apart from their heartwood which only had 5 % present. The highest percentages of catechin were found in the small trees from Turkana County where the heartwood had 26 % of catechin.

A keen study of the LC chromatograms of the knot wood, heartwood and pith from the different regions revealed some interesting results in the difference between amounts of mesquitol and catechin present in *P. juliflora*. Comparisons of the big and small trees were done. Figure 46 below shows the comparisons between the heartwood of the small trees and the big trees from the three counties.

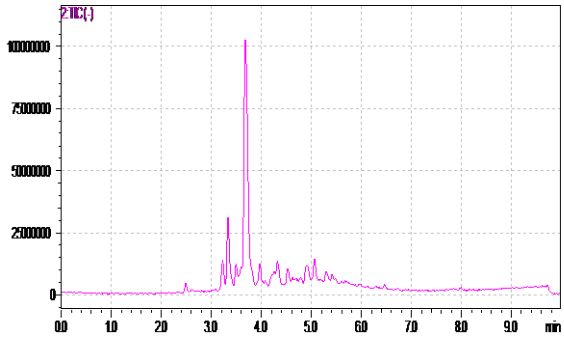
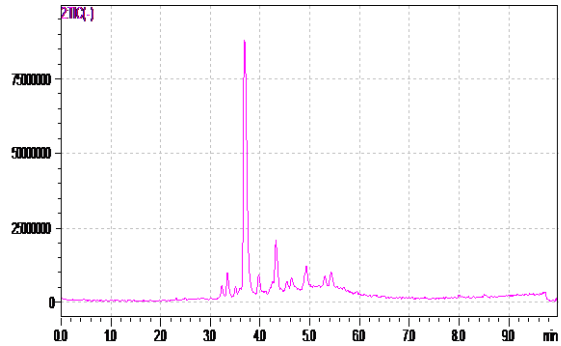
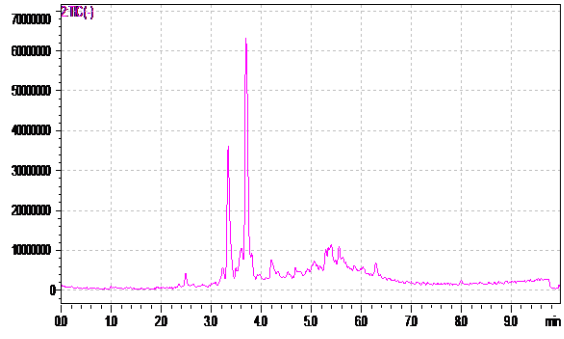
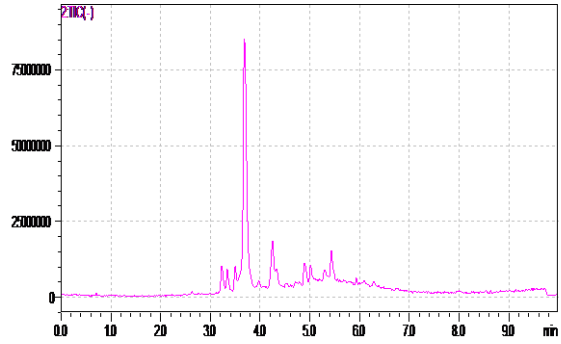
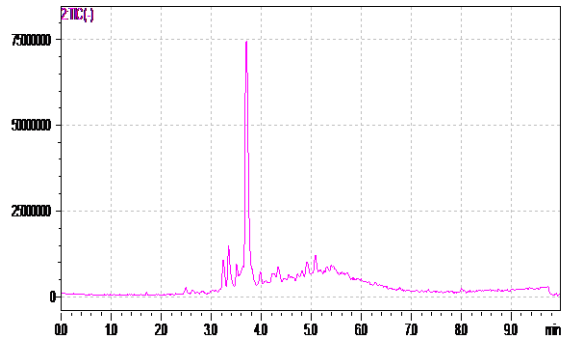
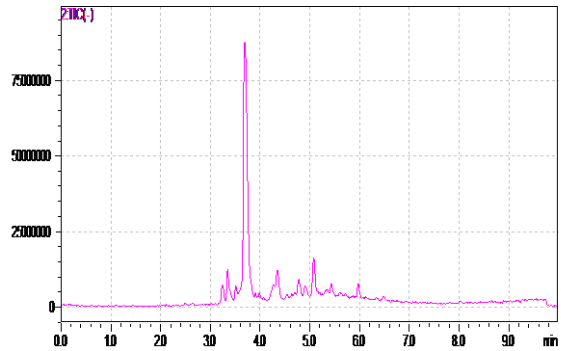
County	(a) Small trees	(b) Big trees
Baringo		
Turkana		
Garissa		

Fig **46:** LC chromatograms of the acetonic extracts showing comparisons between the heartwood of the small trees and the big trees from the three counties

The heartwoods like knot woods and piths of the big trees were generally dominated by only one peak corresponding to mesquitol (retention time of 3.7 min). On the other hand, the extractives of small trees were dominated by two peaks for catechin and mesquitol. The ratios of these two compounds are shown in table 12.

It was interesting to note that in all the counties, the amounts of catechin, was higher in small trees but its amounts decreased significantly in big trees. Cornelia *et al*. (2013), argues that catechin can play two different roles in plants according to its stereochemistry.

The (+)-catechin has antioxidant properties whereas the (–)-catechin induces oxidation and cellular death in root cells of neighboring plants (Cornelia *et al*., 2013). The absolute configuration of asymmetric carbons present in catechin isomer present in the *P. juliflora* is yet to be established. The study also hypothesizes that the catechin may be in higher amounts in the small trees and tends to disappear in the big trees probably because the catechin molecule might be a precursor molecule in the biosynthesis of mesquitol. This hypothesis however is yet to be proved.

Ratios of mesquitol and catechin in the knot wood, heartwood and pith for the different counties are as shown in table 12.

Table **12**: Ratios of mesquitol: catechin present in the heartwood of the three different counties

County	**Knot wood**		**Heartwood**		**Pith**	
	Small trees	**Big trees**	**Small trees**	**Big trees**	**Small trees**	**Big trees**
Baringo	6:1	No catechin	5:1	16:1	6:1	No catechin
Garissa	4:1	11:1	8:1	13:1	4:1	15:1
Turkana	2:1	12:1	2:1	19:1	2:1	22:1

Results showed that mesquitol dominated in the big trees. Catechin was present in huge amounts in the small trees from Turkana County and its ratio was half that of mesquitol (2:1) in all the different parts of the stem. Catechin amounts decreased drastically in the big trees.

4'-*O*-methylgallocatechin quantification

4'-*O*-methylgallocatechin was the most abundant free flavonoid that was found in the barks of *P. juliflora* with ranges between 18 – 28 %. The percentages of 4'-*O*-methylgallocatechin in acetonic extracts from different parts of *P. juliflora* and counties found in the bark and sapwood were tabulated in table 13.

Table **13**: Percentage of 4'-*O*-methylgallocatechin in *P. juliflora* as per LC-ESI-MS/MS

Plant part	Baringo County		Turkana County		Garissa County	
	Small trees	Big trees	Small trees	Big trees	Small trees	Big trees
Bark	18	23	20	28	26	19
Sapwood	9	13	23	0	0	0

In all cases, 4'-*O*-methylgallocatechin was more abundant in barks of the different trees independently from the counties. Similarly to catechin, 4'-*O*-methylgallocatechin was completely absent from the sapwood of Garissa trees. Catechin was consequently probably a precursor of 4'-*O*-methylgallocatechin.

4.3 Chemical modification of mesquitol

Chemical modification of mesquitol compound was done with the aim of synthesizing different mesquitol derivatives by hemi-synthesis using mesquitol as the starting material.

Generally, polyphenols are soluble in water while sparingly soluble in lipophilic media. This makes them less effective in their application in any lipophilic systems like fats and oils, cosmetic formulations and lipid-based foods (Cruz *et al*., 2015). Although they are known to be good antioxidants, this factor limits their applications hence the need to modify their structure to increase their lipophilicity in order to expand their industrial applications.

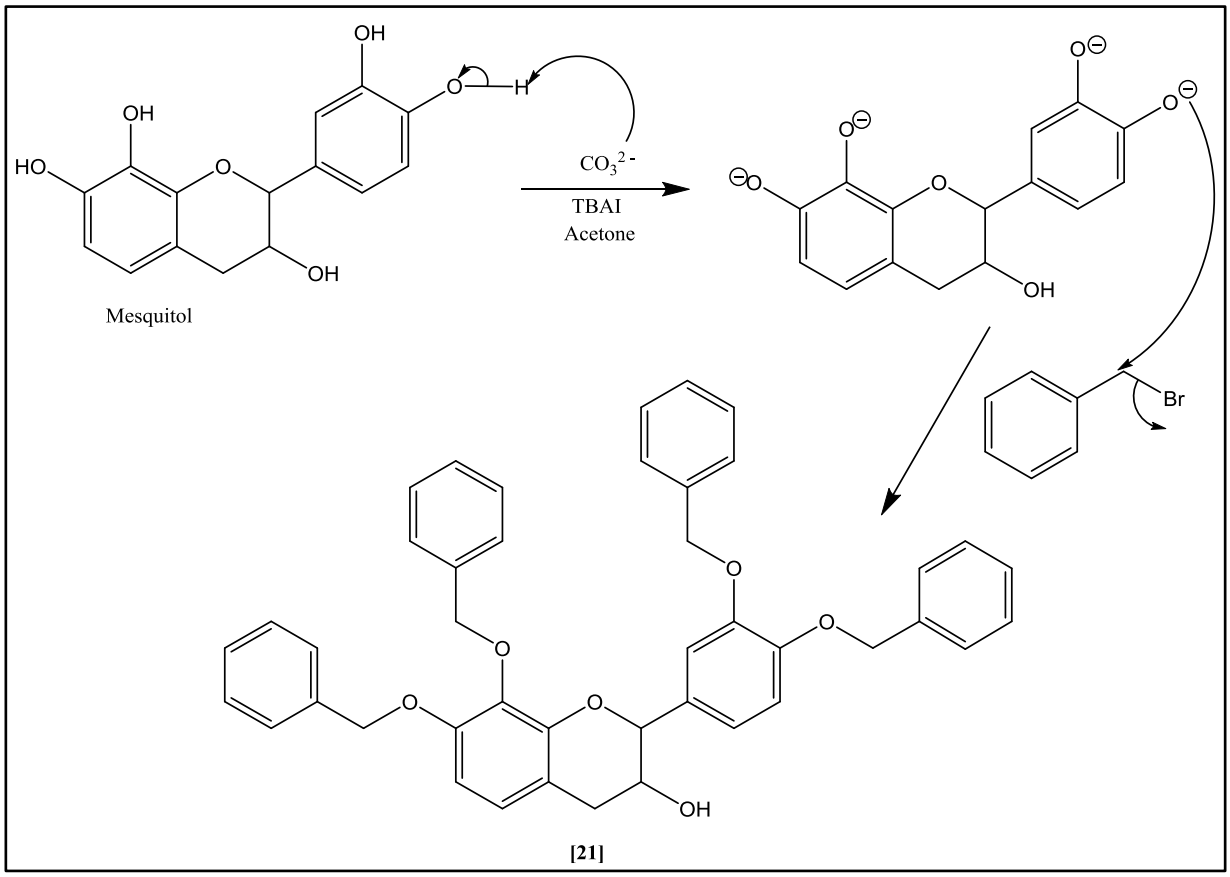
4.3.1 Chemical modification of mesquitol by use of carboxylic acids

Mesquitol was used as a starting material for chemical modifications through Steglich esterification reactions allowing the conjugation to fatty acids in order to gain hydrophobicity. The conjugation of the fatty acids was done at the aliphatic hydroxyl (3-OH) group. Three different acids were used for the aliphatic conjugation (capric acid, lauric acid and myristic acid). The process involved three steps; protection of the phenolic hydroxyl groups through benzylation, followed by esterification process and finally deprotection.

Step 1: Benzylation (Protection of the phenolic hydroxyl groups)

This was done by reacting mesquitol with benzyl bromide. The phenolic hydrogens are more acidic than the alcoholic hydrogen since the phenolic hydrogens are stabilized by resonance effect. The acidity is increased by the intramolecular hydrogen bonding in the catechol structure of the phenolic hydroxyl groups which enhances their nucleophilicity under basic conditions and therefore increases the capacity of it becoming ionized in the presence of a

base yielding its phenoxide ion. The reaction mechanism of this reaction is shown in scheme 22.



Scheme **22:** Reaction mechanism of the benzylation reaction

The phenol hydroxyl groups were deprotonated by the base as shown in the mechanism. Ionic reactants are often soluble in aqueous phase but insoluble in organic phase in the absence of a phase transfer catalyst, hence, TBAI was used as the catalyst to aid at solubilizing the Cs_2CO_3 salt into the organic phase. The benzyl bromide reacted with the phenoxide anions of the mesquitol in a SN_2 reaction forming a benzylated compound labeled as compound **[21]**.

One of the disadvantages for this kind of reactions (which requires initial protection and deprotection steps afterwards) is the production of low yields of products caused by the multiple steps followed. The study therefore, aimed at performing this reaction step with variant reaction conditions to determine the best conditions that resulted in high yields.

Table 14 shows the different reaction conditions tested for the first reaction step of benzylation of mesquitol.

Table **14**: Percentage yields obtained in the benzylation reaction by different reaction conditions

Mesquitol (g)	**$C_6H_5CH_2Br$** (eq)	**CS_2CO_3** (eq)	**K_2CO_3** (eq)	**TBAI** (eq)	**Temperature** (°C)	**% yield** (%)
1	8	4	-	0.8	t_a	66
1	8	4	-	-	t_a	51
1	8	-	4	4	t_a	58
1	4.5	-	7.5	-	reflux	62

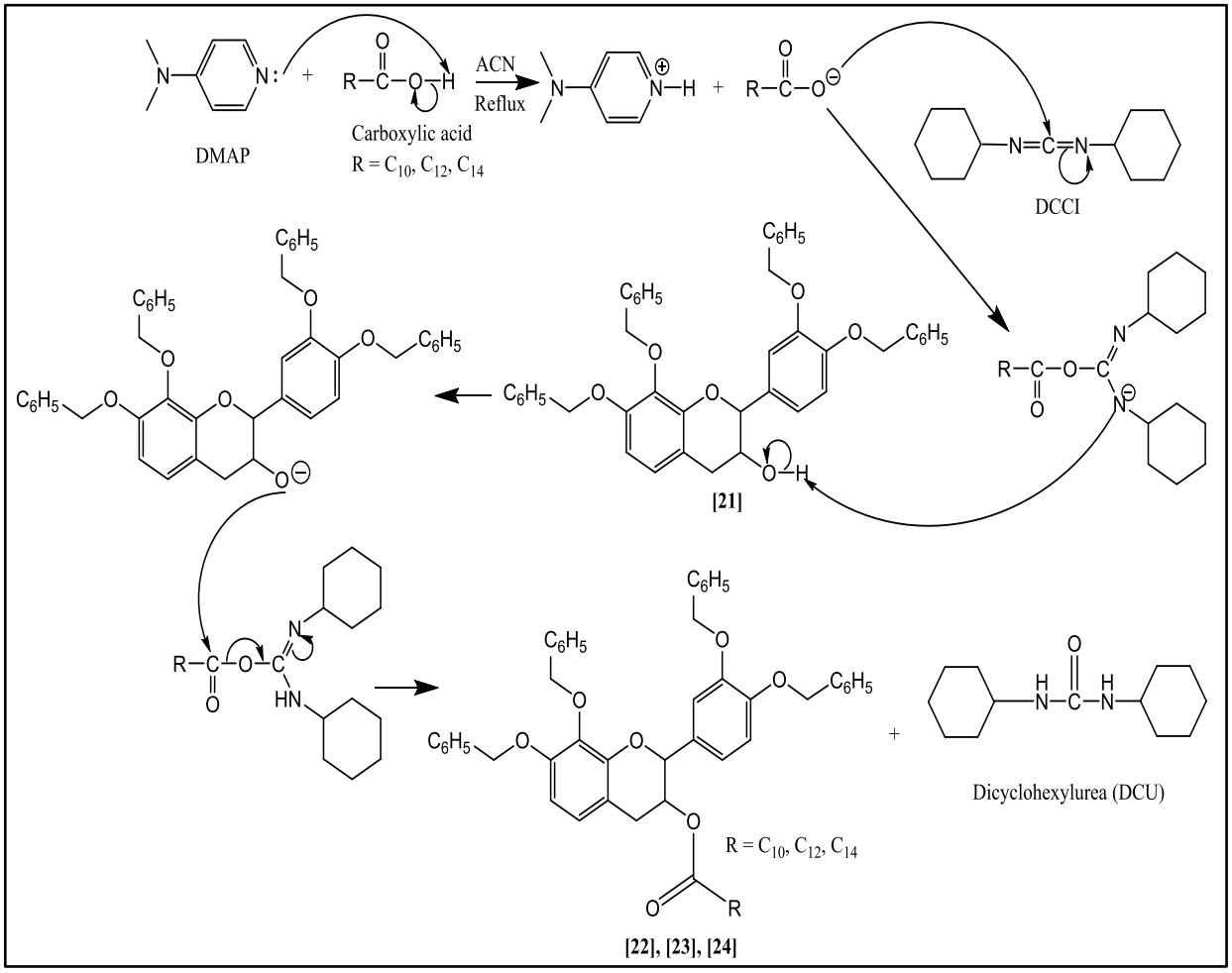
Key

1. $C_6H_5CH_2Br$ – Benzyl bromide
2. CS_2CO_3 – Cesium carbonate
3. K_2CO_3 – Potassium carbonate
4. TBAI – Tetra-n-butylammonium iodide
5. t_a – Ambient temperature

All the reactions were done in 24 hours. The preferred reaction condition for the study was the one that gave the highest yield of 66 %. Cesium is the most electropositive of readily available metals and forms ionic compounds only so that the carbonate ion can exert its full basicity hence, Cs_2CO_3 was preferred as a base instead of the Na and K carbonates and the yield obtained by using Cs_2CO_3 was slightly higher than that obtained when K_2CO_3 was used.

Step 2: Esterification process

Steglich esterification reaction is a form of esterification reaction that uses dicyclohexylcarbodiimide (DCCI) as a coupling reagent. In this reaction, the benzylated mesquitol was reacted with carboxylic acid in the presence of DCCI and DMAP. Three different carboxylic acids were used. These were; capric acid, lauric acid and myristic acid. The reaction mechanism is given in scheme 23.



Scheme **23:** Steglich esterification of benzylated mesquitol

DMAP is a nucleophilic catalyst which is a very effective acyl transfer agent. It is more basic than pyridine due to the resonance stabilization of the NMe_2 group. The reaction led to the formation of acylated benzylated mesquitol compounds. The compounds labeled **[22]**, **[23]** and **[24]** formed had different fatty acid chains of 10, 12 and 14 respectively added to the benzylated mesquitol.

The driving force of this reaction is the formation of a very stable urea by-product.

In this reaction step, the percentage yields of a reaction differed according to the length of the fatty acid used as shown in table 15 below.

Table **15**: Percentage yields obtained in the esterification reactions while using carboxylic acids of different chain lengths

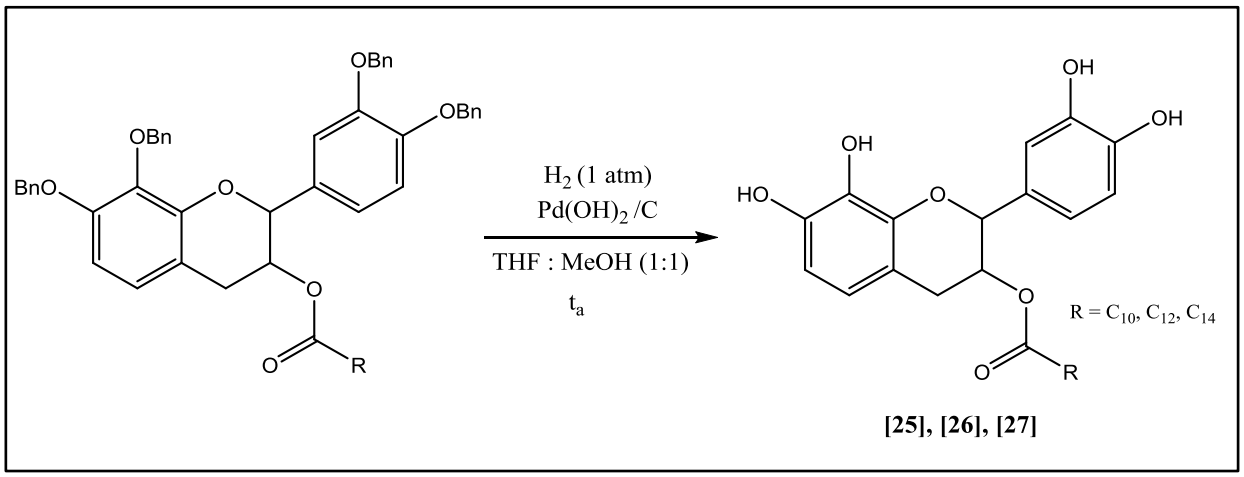
Carboxylic acid	Carbon chain length	Percentage yield (%)
Capric acid	10	79
Lauric acid	12	87
Myristic acid	14	76

All of the percentage yields are close to 80 % which are very good isolated yield values.

Step 3: Deprotection (catalytic hydrogenation reaction)

The esterification reaction was finally followed by a deprotection step that involved the removal of the benzyl groups.

Deprotection of the phenolic hydroxyl groups was done once the fatty chain had been attached to the mesquitol compound at the aliphatic 3-position. This was done using palladium hydroxide on carbon catalyst and using hydrogen gas at 1 atm. The reaction set-up is shown in scheme 24 below.



Scheme **24:** Catalytic hydrogenation reaction

The final products were labeled **[25]**, **[26]** and **[27]** which had different fatty acid chains of carbon atoms 10, 12 and 14 respectively added to mesquitol.

The hydrogenation reaction also had the percentage yields differing according to the length of the fatty acid used. As the number of carbon atoms increased, the percentage yield on the other hand also increased as shown in table 16 below.

Table **16**: Percentage yields obtained in the hydrogenation reactions while using carboxylic acids of different chain lengths

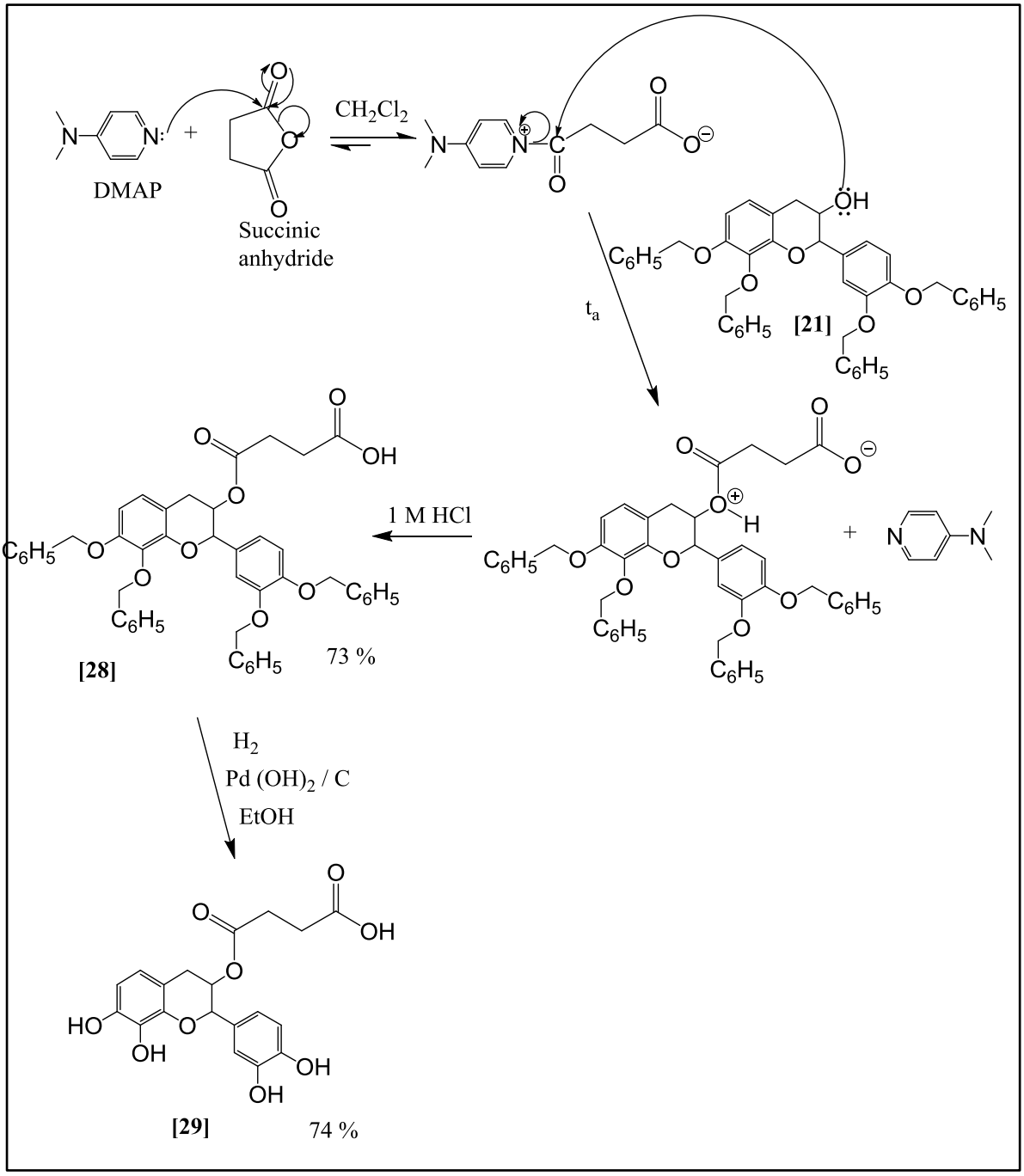
Carboxylic acid	Carbon chain length	Percentage yield (%)
Capric acid	10	61
Lauric acid	12	78
Myristic acid	14	82

4.3.1.1 Chemical modification of mesquitol with anhydrides

Succinic anhydride was used in the study of chemical reactions of mesquitol with anhydrides. Normally acid anhydrides react with alcohols to form esters and carboxylic acids in the presence of a base catalyst.

Succinic anhydride was chosen because it gives an additional free carboxylic acid function on the structure. It is mostly used as a linker between two moieties. The 4-carbon linker possess a suitable length for minimizing steric hindrance and is advantageous in that it has the potential of allowing further derivatization at the carboxylic functional group. For example, the addition of an amide functional group leading to formation of peptides that have widespread uses as therapeutic molecules, detergents, lubricants and new polymeric materials.

Reaction of succinic anhydride with benzylated mesquitol was performed. The reaction also involved the protection and deprotection steps. Benzyl bromide was used as the protecting group and this was followed by the reaction of the benzylated mesquitol with succinic anhydride as per the reaction scheme 25.



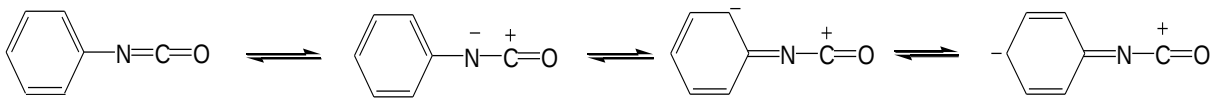
Scheme **25:** Reaction of benzylated mesquitol with succinic anhydride

DMAP was used as a catalyst for the reaction. The reaction first started by the reaction of DMAP with succinic anhydride increasing the electrophilicity of one of the carbonyl groups which allows for attack by the benzylated mesquitol which acts as the nucleophile. This result in the formation of a zwitterion which is acidified by hydrochloric acid to form a product labeled **[28]**. The final step involved the deprotection step using hydrogen gas at atmospheric pressure with palladium catalyst giving rise to the final product labeled **[29].**

4.3.1.2 Chemical modification of mesquitol with isocyanates

Isocyanates are electrophiles which are reactive towards a variety of nucleophiles like alcohols. They react with alcohols forming a urethane link and can therefore aid in the formation of polyurethranes since the urethane group is the major repeating unit in polyurethanes. Polyurethrane chemistry is of interest to most scientists since it leads to formation of a variety of polymers used in various ways like appliances, medical devices, textiles and fibers, electronics and many more. The polyurethanes are produced by polymerization of isocyanate with a polyester or polyether polyol (Akindoyo *et al*., 2016).

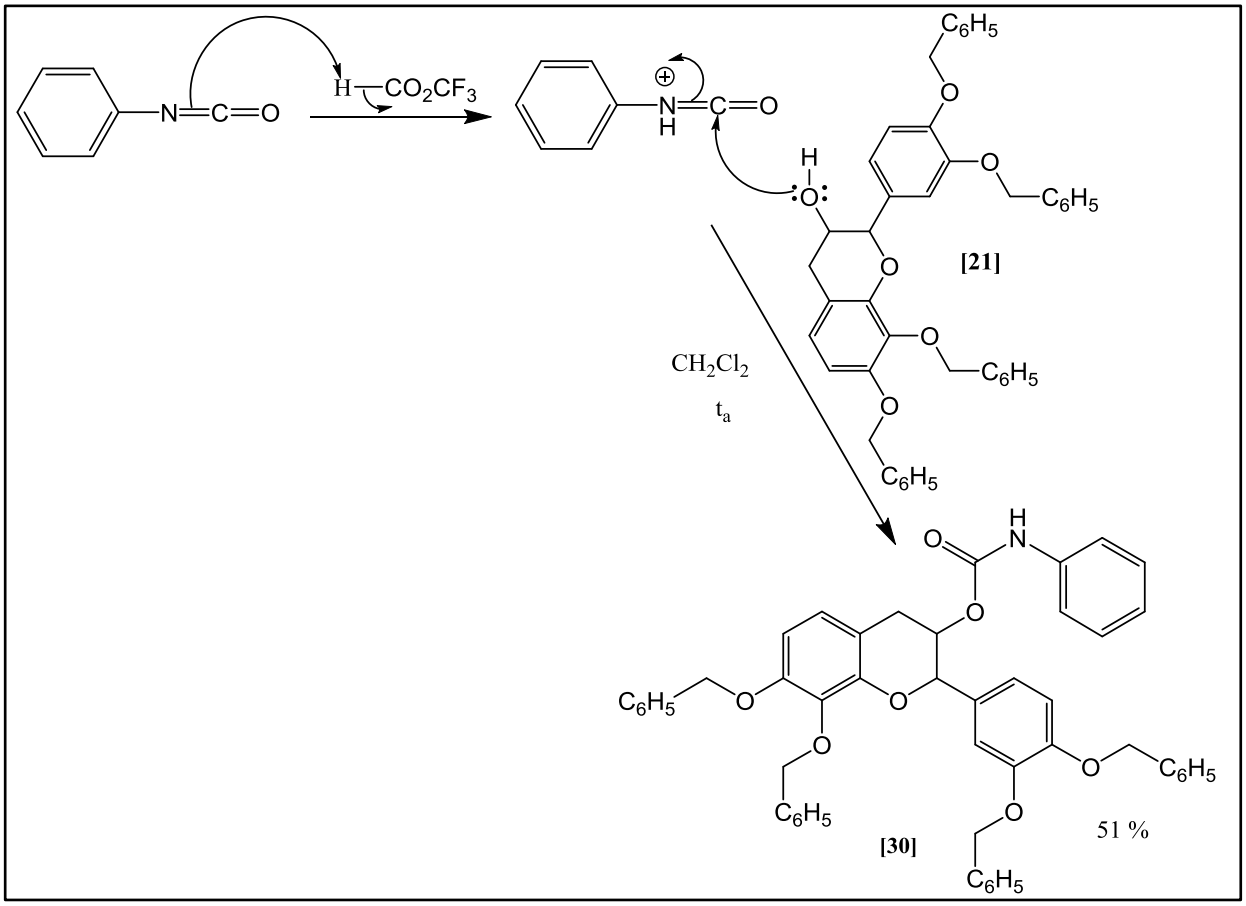
This study used phenyl isocyanate to study the reaction of 3-OH group of mesquitol with isocyanates. The aromatic isocyanate was chosen since it is more reactive than aliphatic or cycloaliphatic isocyanates. This is because aromatic substituent gets the negative charge delocalized into the benzene electron ring hence increasing the positive charge of the carbon atom as shown in scheme 26. The reactivity of isocyanates is also increased by substituents that improve the positive load on the NCO group carbon atom.



Scheme **26:** Electron delocalization of the phenyl isocyanate

Apart from the electronic effect, steric hindrance also affects the reactivity of isocyanates. Bulky groups near the reaction site reduce the reactivity of the isocyanate.

The reaction mechanism for this reaction is shown in scheme 27.



Scheme **27:** Reaction of benzylated mesquitol with phenyl isocyanate

The reaction of benzylated mesquitol with phenyl isocyanate was first attempted without use of trifluoroacetic acid (TFA) but after 24 hours of reaction, no product was formed. TFA was therefore used as an acid catalyst since isocyanate reactions are susceptible to catalysis. The catalyst polarizes the isocyanate making the C=N bond more susceptible to nucleophilic addition of the hydroxyl group. The percentage yield of the product obtained was 51 %.

The main aim of adding an isocyanate to mesquitol in this study was to obtain a carbamate compound that could be used as a wood preservative with increased activity from the mesquitol compound. Carbamates have been widely used as wood preservatives since they can interact with wood through hydrogen bonding. A study by Zhenhua and Dong reported increased dimensional stability and mechanical properties of wood after they impregnated poplar wood with polyurethane that had been made from polymeric methylene bis phenylisocyanate and polyethylene glycol (Zhenhua and Dong, 2007). The study therefore synthesized this carbamate compound.

Isocyanates are however known to be toxic and can present respiratory hazards as particulates, vapors or aerosols. The most tragic industrial chemical accident was seen in 1984

in India, where there was release of methyl isocyanate that killed almost 4000 people. The effects were both acute and long term (Obach and Kalgutkar, 2010). The study therefore tried synthesizing urethane links through reaction of the 3-OH group of mesquitol with dimethyl carbonate followed by reaction with butylamine so as to avoid the use of the toxic and environmentally unfriendly isocyanates. However, efforts to perform this reaction were not successful and more studies should be done on this.

4.4 Durability tests

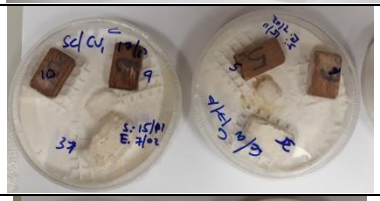
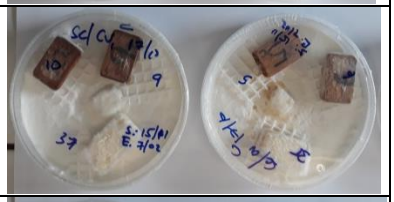
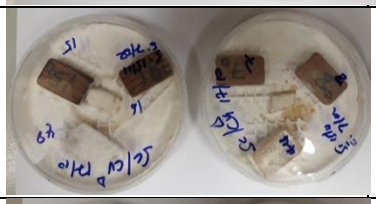
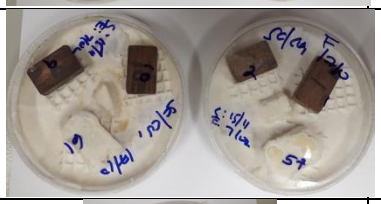
Wood is susceptible to degradation by several microorganisms. Although all the mechanisms that cause the biochemical degradation are not yet known, the peroxidases in white rots and hydroxyl radicals in brown rots are known to play a big role in the degradation of wood. The degradation involves oxidative processes that are initiated by free radicals which lead to wood depolymerization. These free radicals can be stabilized by addition of various addictives like antioxidants. The phenolic antioxidants, in this case mesquitol and its derivatives, act as hydrogen donors. Hydrogen donors block chain oxidation reactions by functioning as a "trap" of radicals. The phenolic antioxidants deactivate the peroxide radicals involved in the degradation by reacting with peroxy radicals to form hydroperoxides hence avoiding abstraction of an hydrogen from the polymer backbone and consequently its degradation.

The ability of mesquitol and some of the targeted mesquitol derivatives to protect against fungi were investigated.

Formulations of mesquitol and Compound **[25]** were used to protect beech wood exposed against the white rot fungus (*Coriolus versicolor*). The synergistic effect of the two compounds with tebuconazole was also studied. Formulations of mesquitol, compound **[25]** and compound **[26]** were used to protect pine wood blocks exposed against the brown rot fungus (*Coniophora puteana*).

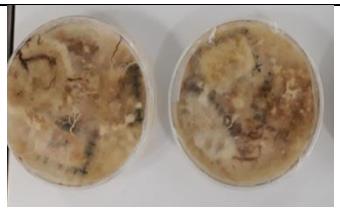
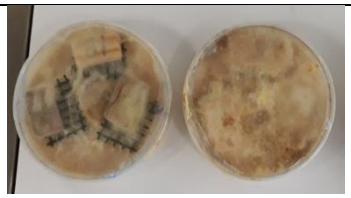
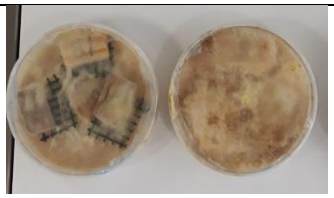
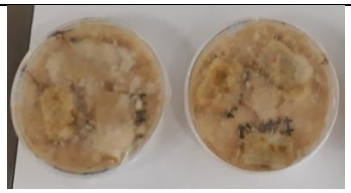
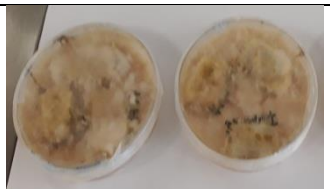
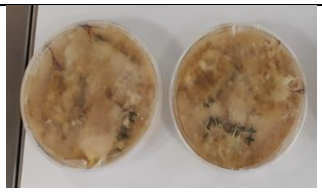
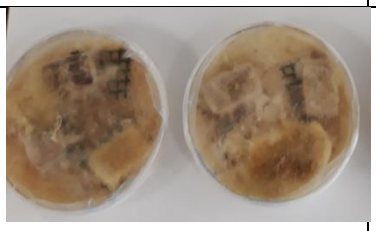
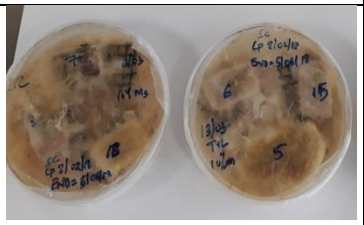
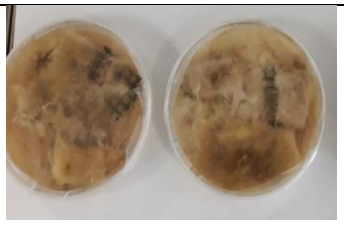
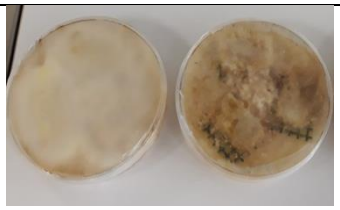
The advantage of using mesquitol and its derivatives is that unlike the widely used fungicides, they exhibit some antioxidant properties which when used together with the common fungicides; they are likely to increase their biological properties through synergistic or additive effects.

Figure 47 and 48 shows the various mycelia with the wood blocks after every 4 weeks of exposure to fungi attack. Figure 47 shows the attack by the *Coriolus versicolor* while figure 48 shows the attack by the *Coniophora puteana.*

Sample	Period of time					
	4 weeks exposure		8 weeks exposure		12 weeks exposure	
	Treated	Treated+leached	Treated	Treated+leached	Treated	Treated+leached
A						
B						
C						
D						
E						
F						
G						

(A) 0.1% Tebuconazole; **(B)** 0.01% Tebuconazole; **(C)** 0.01% Tebuconazole + 1.5% Mesquitol-C10; **(D)** 0.01% Tebuconazole + 1.5% Mesquitol; **(E)** 5% Mesquitol-C10; **(F)** 5% Mesquitol; **(G)** Control

Fig **47:** Fungicidal decay test on beech wood exposed to *Coriolus versicolor* fungal strain.

Sample	Period of time					
	1st Month		2nd Month		3rd Month	
	Treated	Treated+leached	Treated	Treated+leached	Treated	Treated+leached
H						
I						
J						
K						
L						
M						
N						

(H) 5% Mesquitol; **(I)** 5% Mesquitol-C10; **(J)** 5% Mesquitol-C12; **(K)** 10% Mesquitol; **(L)** 10% Mesquitol-C10; **(M)** 10% Mesquitol-C12; **(N)** Control

Fig **48:** Fungicidal decay test on pine wood exposed to *Coniophora puteana* fungal strain.

The synergistic effect of mesquitol and compound **[25]** with tebuconazole against the *Coriolus versicolor* fungal strain was investigated and the effect of the treatment was evaluated according to the weight loss obtained due to fungal attack. Mesquitol and compound **[25]** were also tested alone to evaluate their effect on the fungal development. These results were graphically represented in figure 49.

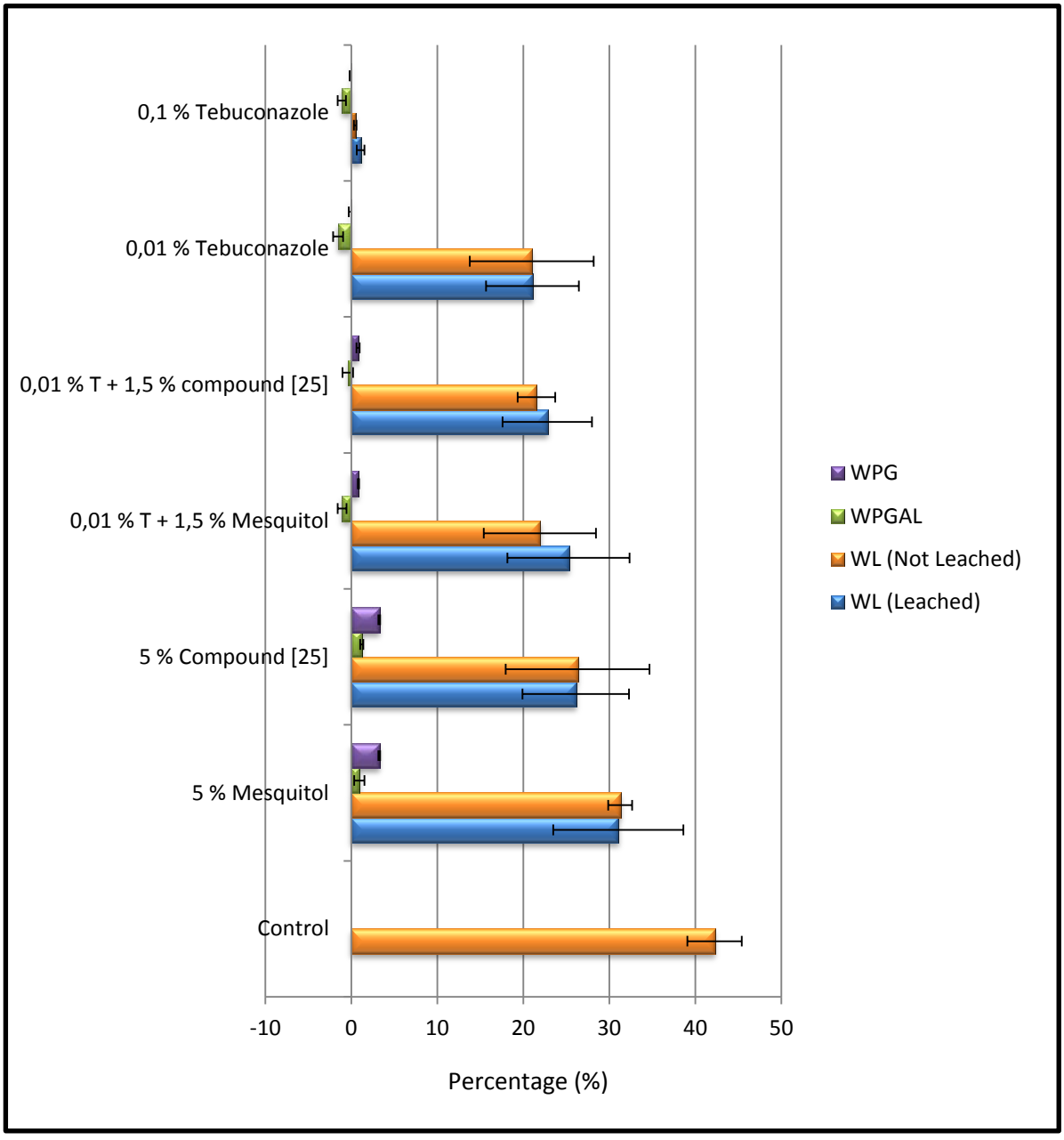


Fig **49:** Wood treatment on *Coriolus versicolor* fungal strain showing the WPG, WPGAL, and WL values

No significant weight loss was observed for wood blocks treated with 0.1 % tebuconazole however, as the concentration of the tebuconazole was decreased to 0.01 %, a weight loss of 21 % was observed for both the leached and unleached wood blocks. Since this low

concentration of 0.01 % was unable to prevent effectively wood degradation caused by the white rot fungus, it was therefore used so as to observe any possible synergies with mesquitol and its derivative.

The weight loss obtained from mesquitol and its derivative when tested alone, were unable to effectively prevent wood degradation against the white rot fungus. The use of their mixtures with tebuconazole allowed reduced weight losses.

The synergistic effect of tebuconazole combined with mesquitol and compound **[25]** was observed as shown in figure 49. According to Tallarida 2001, a mixed treatment is considered to be in synergy when the isoeffective concentration of the combination is significantly lower than those of compounds acting alone (Tallarida, 2001). This was observed in figure 49 where the weight losses obtained from the mixture of tebuconazole with mesquitol and also with compound **[25]** were lower than when they were tested alone.

It is also evident that when the aliphatic chain length is added to mesquitol, the weight loss of the wood blocks reduces slightly in amount compared to when mesquitol is used alone.

A further study was done using different formulations of mesquitol, compound **[25]** and compound **[26]**. This was to investigate the effect of these treatments on a brown-rot fungus (*Coniophora puteana*), the effect of increase in the concentration of the treatments and the effect of increasing the aliphatic chain length on the mesquitol derivatives. These results were graphically represented in figure 50.

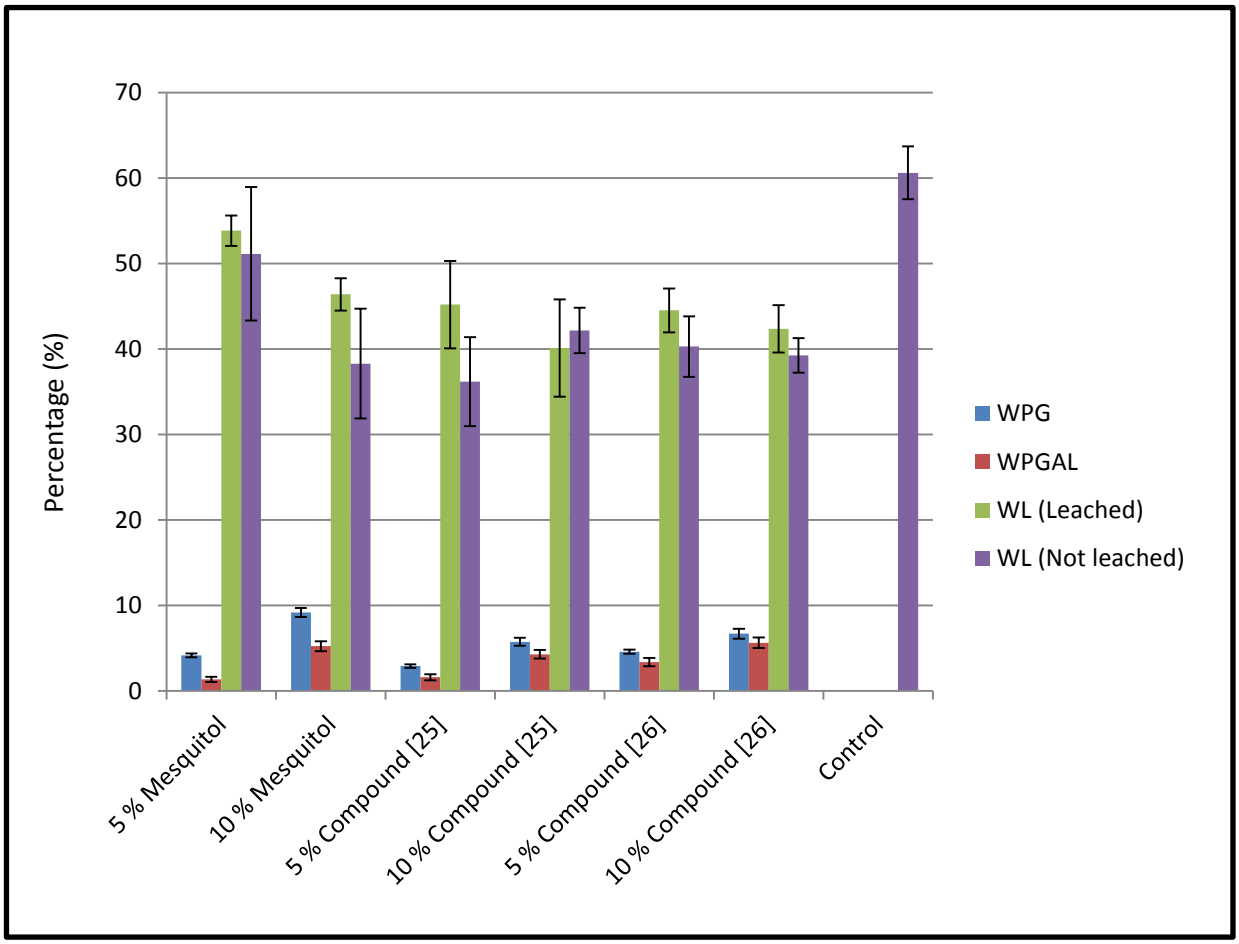


Fig **50:** Wood treatment on *Coniophora puteana* fungal strain showing the WPG, WPGAL, and WL values

The weight loss obtained from mesquitol and its derivatives tested alone, were unable to effectively prevent wood degradation against the brown rot fungus. These weight losses depended on the concentration of the treatment used and were generally lower at higher concentrations.

It is evident also that there is decreased leaching as the alkyl chain increases. The values of the weight percent gain after leaching for treatment with mesquitol compound are lower than for the corresponding grafted compounds. Mesquitol is easily leached unlike when conjugated to long chain fatty acids. The mass loss obtained after the fungi attack by mesquitol showed significant decrease when its concentration was increased, this shows potential of it being used as a wood preservative.

Due to their lipophilic part, from the fatty chain length of the carboxylic acids, the mesquitol derivatives increased wood durability by decreasing leachability compared to mesquitol for outdoor wood uses. They also diminish intake of water by the wood blocks leading to low water content hence creating unfavourable conditions for the fungi attack and development.

CHAPTER 5

CONCLUSION AND RECOMMENDATIONS

5.0 CONCLUSION

The accelerated solvent extraction (ASE) method for mesquitol extraction was found to be the most efficient method compared to the other three methods used (maceration, soxhlet and microwave-assisted extraction methods). This was due to the; high yields obtained from this extraction method, the use of less solvents and the short time periods required for extractions. Extraction methods that use solvents in their procedures generally are known to be influenced critically by the solvents types. A mixture of ethanol: water gave the highest yield of extractives (19.10 %) by the use of the ASE method. The percentage of ethanol used in the mixture was quite low (25 %) hence, this solvent mixture is highly recommended for use industrially for obtaining high yields of extractives from *P. juliflora*. The other solvents used for the ASE method had yields of 14 % for water, ethanol had 16.27 % and acetone had 9.53 %.

LC-ESI-MS/MS analytical method was employed to analyze the compounds present in *P. juliflora*. As a result 20 peaks were detected out of which 15 were tentatively identified either as flavonoid aglycones or some of their glycosylated forms. Among these compounds identified, a new B-type proanthocyanidin of mesquitol has been reported here for the very first time. Quantification of some of the identified compounds was done in the negative ion mode of the LC-ESI-MS/MS. A systematic study on the mass spectra and the observed fragmentations of the flavonoids showed that mesquitol compound is the most abundant compound in *P. juliflora* with high amounts being found in the heartwood and pith of the acetonic extract (47 – 72 %).

Mesquitol abundance was also found to vary greatly depending on the age of the tree and on the geographical areas. The highest amounts of mesquitol were found in the heartwood from the big trees in Baringo County where mesquitol could be extracted with unusual high yield of 11 % which makes it the most likely site for harnessing of mesquitol. The least amounts of mesquitol not taking into account the bark and the sapwood, was in the knot wood from small trees of Turkana County (31 %). However, this County (Turkana) had the highest amounts of catechin (26 %) and 4'-*O*-methlgallocatechin (28 %) as compared to the other counties. Catechin was generally found more in the small trees compared to the big trees with a range of 0 – 6 % in the big trees and 9 – 26 % in the big trees (not taking into account the bark and the sapwood).

Despite the fact that samples from Turkana County showed low amounts of mesquitol and generally had low percentage yields, it was realized that the species from this County had the highest diversity of extractives with some of these extractives lacking or found only in very small amounts in the other Counties for example the unknown compound represented by peak **[20]** which was present only in this County. It is also interesting to note that the new B-proanthocyanidin of mesquitol was identified for the first time in samples from Turkana County and was found with the highest quantity yield of 7 %. Since Turkana County is found in the highest altitude compared to Garissa and Baringo Counties, the study therefore presumes that the diversity of extractives in *P. juliflora* trees varies from one geographic region to another and that it increases as the altitude increases and at the same time mesquitol amounts decreases as the altitude increases. However, this is yet to be fully established by taking into account several other factors like climate, rainfall, surrounding vegetation or type of soil in these areas.

The chemical modification of mesquitol allowed the introduction of various functional groups through formation of compound **[25]**, compound **[26]** and compound **[27]** from esterification with carboxylic acids of different chain lengths, compound **[29]** from reaction with succinic anhydride and compound **[30]** from reaction with phenyl isocyanate.

Biological tests indicate that mesquitol, compound **[25]** and compound **[26]** possess antifungal properties and can therefore be advocated for use as wood preservative used in combination with low concentrations of the widely used fungicide since 1.5 % mesquitol and compound **[25]** showed synergistic effect when used together with 0.01 % tebuconazole. It is also evident that when an aliphatic chain length is conjugated with mesquitol, the mass loss obtained after the fungal attack decreases significantly especially comparing the leached wood blocks. This shows that addition of the chain length increased the lipophilicity property of mesquitol hence reducing the wood preservative agent from being leached. The strong hydrophobic character of the mesquitol derivatives reduces humidity uptake by the wood blocks leading to low water content which create unfavorable conditions for the development of fungi.

The wood decay antifungal tests done in this study discovered that mesquitol extractive might not be solely involved in the durability of the *Prosopis* species. This is because there was a higher weight loss that was obtained when mesquitol was used as a wood treatment agent without the presence of tebuconazole.

The efficiency of the compounds used as treatment in this study however showed lower weight loss when used against *Coriolus versicolor (Cv)* fungal strain compared to the *Coniophora puteana (Cp)* fungal strain.

5.1 RECOMMENDATIONS

Having observed the variability of extractives in *P. juliflora* according to their geographical location, the study could not establish the exact reasons that caused this variability. More studies should therefore be done on how altitude, rainfall and climatic factors affect the distribution and species richness pattern of *P. juliflora* trees.

Studies should also be done to address the contribution of catechin to the invasiveness of *P. juliflora* and also to determine if catechin is a precursor in mesquitol biosynthesis since its amounts is high in the small trees but decreases significantly in the big trees.

Considering the fact that the mesquitol derivatives obtained from conjugation of a fatty chain length at the aliphatic OH group of mesquitol showed some antifungal activity, more wood decay test studies should be carried out using derivatives of mesquitol obtained from conjugation of the fatty chain lengths at the aromatic OH groups of mesquitol. This will entail detailed studies about mesquitol reactivity for chemo-selective functionalization of some phenol groups (taking care to preserve the antioxidant property). These new derivatives might show better activity as wood preservatives since more than one fatty chain lengths can be added to the aromatic OH groups of mesquitol hence significantly reduce leaching. Further studies on derivatization of mesquitol by introducing different functional groups could also be carried out.

The study recommends the hemi-synthesis of the mesquitol dimer that had been observed to be present, although in small amounts, in the *P. juliflora*. This is for further investigation of the properties of the new dimer.

The study finally recommends more biological activities to be carried out on the mesquitol derivatives. These derivatives should be targeted for various applications in the agro-food, detergent and cosmetics (liposoluble antioxidants, gelling molecules with antioxidant properties).

REFERENCES

REFERENCES

Abdulahi, M. M., Ute, J. A. and Regasa, T. (2017). *Prosopis juliflora:* Distribution, impacts and available control methods in Ethiopia. Tropical and Subtropical Agroecosystems, 20: 75-89.

Aboud, A.A., P.K. Kisoyan, and D.L. Coppock. 2005. Agro-pastoralists' wrath for the Prosopis tree: The case of the IL Chamus of Baringo District, Kenya. Research Brief 05-02-PARIMA. Global Livestock Collaborative Research Support Program. University of California at Davis.

Ahuja, I., Kissen, R., and Bones, A. M. (2012). Phytoalexins in defense against pathogens. *Trends in plant science.* Vol 17 (2). Pp 73-90. DOI: 10.1016/j.tplants.2011.11.002.

Akindoyo, J. O., Beg, M. D. H, Ghazali, S., Islam, M. R., Jeyaratnama, N. and Yuvaraj, A. R. (2016). Polyurethane types, synthesis and applications. RSC Advances. Vol 6. DOI: 10.1039/c6ra14525f.

Alonso, D. M., Wettstein, S. G. and Dumesic, J. A. (2012). Bimetallic catalysts for upgrading of biomass to fuels and chemicals. *Chemical Society Reviews.* 41(24). Pp 7965–8216.

Arbenz, A. and Avérous, L. (2015). Chemical modification of tannins to elaborate aromatic biobased macromolecular architectures. *Green Chem*. 17, 2626. DOI: 10.1039/c5gc00282f.

Arts, M.J.T.J., Haenen, G.R.M.M., Wilms, L.C., Beetstra, S,A,J,N,, Heijnen,C.G.M., Voss, H.P. and Bast, A. (2002). Interactions between Flavonoids and Proteins: Effect on the Total Antioxidant Capacity. *J. Agric. Food Chem.* DOI: 10.1021/jf010855a.

Andersen, Ø. M and Markham, K. R, (eds). (2006). Flavonoids: chemistry, biochemistry and applications. *CRC press*: Taylor and Francis Group. Pp 2, 104, 286.

Azam, M. M., Tewari, J. C., Singh, Y. and Roy, M. M. (2011). *Prosopis juliflora, A rich Source of Antioxidant Product.* Central Arid Zone Research Institute.

Azwanida NN (2015) A Review on the Extraction Methods Use in Medicinal Plants, Principle, Strength and Limitation. *Med Aromat Plants* 4: 196. doi:10.4172/2167-0412.1000196.

Bajpai, P. (2018). Biermann's handbook of pulp and paper. (3 ed). Pp 19-74. Doi:10.1016/B978-0-12-814240-0.00002-1.

BBC News report. (2006). Killer weed hits Kenyan herders. Available at: news.bbc.co.uk (Accessed: 20 May 2019).

Beecher, G. R. (2004). Proanthocyanidins: Biological Activities Associated with Human Health. *Physiology and Biochemistry*. 42 (1). Pp 2-20. DOI: 10.1080/13880200490893474.

Biesaga, M. (2011). Influence of extraction methods on stability of flavonoids. *Journal of Chromatography A*. 1218, 2505–2512. DOI:10.1016/j.chroma.2011.02.059.

Bigelow, R. L. H. and Cardelli, J. A. (2006). The green tea catechins, (-)-Epigallocatechin-3-gallate (EGCG) and (-)-Epicatechin-3-gallate (ECG), inhibit HGF/Met signaling in immortalized and tumorigenic breast epithelial cells. *Oncogene,* 25, 1922–1930.

Bigne, F., Puppo, M. C. and Ferrero, C. (2016). Fibre enrichment of wheat flour with mesquite (*Prosopis* spp.): Effect on bread making performance and staling. Food Science and Technology. Vol 65. Pp 1008-1016. https://DOI: org/10.1016/j.lwt.2015.09.028.

Bokrezion, H. (2008). The ecological and socio-economic role of *Prosopis juliflora* in Eritrea. *Academic Dissertation, Johannes Gutenberg-Universität Mainz, Germany, (PhD report).*

Bonta, R. K. (2017). Application of HPLC and ESI-MS techniques in the analysis of phenolic acids and flavonoids from green leafy vegetables (GLVs). *Journal of Pharmaceutical Analysis*. Pp. 349-364.

Braicu, C., Ladomery, M. R, Chedea, V. S., Irimie, A. and Berindan-Neagoe, I. (2013). Review: The relationship between the structure and biological actions of green tea catechins. *Food Chemistry*, 141 (2013) 3282–3289.

Brodowska, K. M. (2017). Natural flavonoids: classification, potential role, and application of flavonoid analogues. *European Journal of Biological Research*. 7 (2), 108-123. DOI: 10.5281/zenodo.545778.

Brudzynski, K., Maldonado-Alvarez, L. (2015). Polyphenol-protein complexes and their consequences for the redox activity, structure and function of honey. A Current view and new hypothesis – a Review. *Pol. J. Food Nutr. Sci.* Vol. 65(2). Pp 71–80. DOI: 10.1515/pjfns-2015-0030.

Bulian, F. and Graystone, J. A. (2009). Wood coatings: Theory and practice. Elsevier. Pp 2-10.

Carvalho, M. G., Silva, V. S., Silva, T. N. M. S., Camara, C. A. and Braz-Filho, R. (2009). New iodine derivatives of flavonol and isoflavone. *Anais da Academia Brasileira de Ciências* 81(1): 21-28**.**

Cetinkaya, H., Kulak, M., Karaman, M., Karaman, H. S. and Kocer, F. (2017). Flavonoid accumulation behavior in response to the abiotic stress: Can a uniform mechanism be illustrated for all plants? *IntechOpen.* DOI: 10.5772/68093.

Chaaban, H., Ioannou, I., Paris, C., Charbonnel, C., Ghoul, M. (2017). The photostability of flavanones, flavonols and flavones and evolution of their antioxidant activity. *Journal of Photochemistry and Photobiology A: Chemistry.* 336; 131–139.

Chen, Y., Yu, H., Wu, H., Pan, Y., Wang, K., Jin, Y. and Zhang, C. (2015). Characterization and Quantification by LC-MS/MS of the Chemical Components of the Heating Products of the Flavonoids Extract in Pollen Typhae for Transformation Rule Exploration. *Molecules* 2015, *20*, 18352-18366; DOI: 10.3390/molecules201018352.

Cheynier, V., Schneider, R., Salmon, J. M., & Fulcrand, H. (2010). Chemistry of wine. *Elsevier ltd.*

Chilume, T. (2016, September 30). When a blessing becomes a curse – A case study of invasive *Prosopis juliflora*. [Blog post]. Retrieved from https://aucklandecology.com

Choge, S.K., Pasiecznik, N.M., Harvey, M., Wright, J., Awan, S.Z. and Harris, P.J.C. (2007). *Prosopis* pods as human food, with special reference to Kenya. Water SA Vol. 33 (3). DOI: 10.4314/wsa.v33i3.49162.

Conners, T. (2015). Introduction to Wood Structure and Characteristics. Agriculture and Natural Resources Publications. Pp 104.

Cornelia, B., Ladomery, M.R., Chedea, V., Irimie, A. (2013). The relationship between structure and biological actions of green tea catechins. *J. Food Chemistry*, vol 141, pg 3282 – 3289. Elsevier.

Cruz, L., Fernandes, V. C., Araújo, P., Mateus, N. and Freitas, V. (2015). Synthesis, characterisation and antioxidant features of procyanidin B4 and malvidin-3-glucoside stearic acid derivatives. *Food Chemistry* 174, 480–486.

Dean, J. R., & Xiong, G. (2000). Extraction of organic pollutants from environmental matrices: selection of extraction technique. *TrAC Trends in Analytical Chemistry*, *19*(9), 553-564.

Demarque, D. P., Crotti, A. E. M., Vessecchi, R., Lopesa, J. L. C. and Lopes, N. P. (2015). Fragmentation reactions using electrospray ionization mass spectrometry: an important tool for the structural elucidation and characterization of synthetic and natural products. *Natural Product Reports*, 33, 432. DOI: 10.1039/c5np00073d.

Dixon, R. A. and Pasinetti, G. M. (2010). Flavonoids and isoflavonoids: from plant biology to agriculture and neuroscience. *Plant Physiology.* 154, 453-457.

Dos Santos, E. T., Pereira, M. L. A., da Silva, C. F. P., Souza-Neta, L. C., Geris, R., Martins, D., and Figueiredo, M. P. (2013). Antibacterial activity of the alkaloid-enriched extract from *Prosopis juliflora* pods and its influence on in vitro ruminal digestion. *International journal of molecular sciences*, *14* (4), 8496-8516.

Dubow, A.Z. (2011). Mapping and managing the spread of *Prosopis Juliflora* in Garissa County, Kenya. Masters desertification. Kenyatta University.

EN 113 (1996) Wood preservatives—determination of toxic values of wood preservatives against wood destroying basidiomycetes cultured on agar medium.

ENV 1250-2 (1994) Wood preservatives—methods for measuring losses of active ingredients and other preservative ingredients from treated timber—part 2: laboratory method for obtaining samples to measure losses by leaching into water or synthetic sea water.

Es-Safi, N. E., Ghidouche, S. and Ducrot, P. H. (2007). Flavonoids: Hemisynthesis, Reactivity, Characterization and Free Radical Scavenging Activity. *Molecules 12* (9), 2228-2258. DOI: 10.3390/12092228.

Feild, T. S., Lee, D. W., and Holbrook, N. M. (2001). Why leaves turn red in autumn. The role of anthocyanins in senescing leaves of Red-osier dogwood. *Plant Physiol.* 127, 566–574.

Felker, P., Grados, N., Cruz, G. and Prokopiuk, D. (2003). Economic assessment of production of flour from *Prosopis alba* and *P. pallida* pods for human food applications. *Journal of Arid Environments.* Volume 53:4, 517-528. DOI:10.1006/jare.2002.1064.

Felker, P., Takeoka, G. and Dao, L. (2013). Pod mesocarp flour of North and South American species of leguminous tree *Prosopis* (Mesquite): Composition and Food Applications, Food Reviews International, 29:1, 49-66, DOI: 10.1080/87559129.2012.692139.

Feng, W., Hao, Z. and Li, M. (2016). Isolation and Structure Identification of Flavonoids. *IntechOpen* DOI: org/10.5772/67810

Ferreira, D., Marais, J. P. J. and Slade, D. (2005). Heterogeneity of the interflavanyl bond in proanthocyanidins from natural sources lacking C-4 (C-ring) deoxy flavonoid nucleophiles. *Phytochemistry*. 66 2216–2237. DOI:10.1016/j.phytochem.2005.01.014.

Ferreira, D. and Slade, D. (2002). Oligomeric proanthocyanidins: naturally occurring O-heterocycles. *Natural Products Report.*19**,** 517-541. DOI:10.1039/B008741F

Ferrer, J., Austin, M., Stewart, C. J., and Noel, J. (2008). Structure and function of enzymes involved in the biosynthesis of phenylpropanoids. *Plant Physiol. Biochem.* 46, 356–370.

Ferreres, F., Silva, B. M., Andrade, P. B., Seabra, R. M. and Ferreira, M. A. (2003). Approach to the Study of *C*-Glycosyl Flavones by Ion Trap HPLC-PAD-ESI/MS/MS: Application to Seeds of Quince (*Cydonia oblonga*) *Phytochem. Anal*. 14, 352–359. DOI: 10.1002.pca.727.

Ferreyra, M. L. F., Rius, S. P. and Casati, P. (2012). Flavonoids: biosynthesis, biological functions, and biotechnological applications. *Frontiers in Plant Science.* 3:1-15. DOI.org/10.3389/fpls.2012.00222.

Fine, A.M. (2000). Oligomeric Proanthocyanidin Complexes: History, Structure, and Phytopharmaceutical Applications. *Altern Med Rev.* 5(2):144-151.

GISD (2019). Global Invasive Species Database online data sheet. *Prosopis juliflora.* http://issg.org/database/species/List.aspttp. Accessed March 2019.

GCID (2018). Garissa County Integrated Development Plan 2018 – 2022. http://www.globalcrrf.org/crrf_document/garissa-county-integrated-development-plan-2018-2022. Accessed April 2019.

Golubov, J., Mandujano M, Eguiarte L. (2001). The paradox of mesquites (*Prosopis spp*.): invading species or biodiversity enhancers? *Boletı´N De La Sociedad Bota´ Nica De Me´ Xico*, 2001, 69, 23.

Gu, L., Kelm, M.A., Hammerstone, J.F., Zhang, Z., Beecher, G., Holden, J., Haytowitz, D. and Prior, R.L. (2003). Liquid chromatographic/electrospray ionization mass spectrometric studies of proanthocyanidins in foods. *J. Mass spectrom.* 38: 1272 – 1280. DOI: 10.1002/jms.541.

Gupta, J., Gupta, A., Gupta, A. K. (2016). Flavonoids: its working mechanism and various protective roles. *International Journal of Chemical Studies*. (4), 190-198.

Haile, M., Hishe, H. and Gebremedhin, D. (2018). *Prosopis juliflora* Pods Mash for Biofuel Energy Production: Implication for managing invasive species through utilization. Int. Journal of Renewable Energy Development 7 (3) : 205-212.

Hanson, W. (2003). *Natural products, the secondary metabolites*. Royal Society of Chemistry.

Harborne, J.B. and Williams, C. A. (2000.) Advances in flavonoid research since 1992. Phytochemistry 55 (2000). 481-504.

Havsteen, B. H. (2002). The biochemistry and medical significance of the flavonoids. *Pharmacology and Therapeutics*. 96, 67-202.

He, F., Pan, Q. H., Shi, Y. and Duan, C. Q. (2008). Chemical synthesis of proanthocyanidins *in vitro* and their reactions in aging wines. *Molecules.* 13, 3007-3032. DOI: 10.3390/molecules13123007.

Henciya, S., Seturaman, P., James, A. R., Tsai, Y., Nikam, R., Wu, Y. C., Dahms, H. U. and Chang, F. R. (2017). Biopharmaceutical potentials of *Prosopis spp*.(*Mimosaceae, Leguminosa*). *Journal of food and drug analysis.* 25. Pp 187-196.

Higashino, Y., Okamoto, T. , Mori, K., Kawasaki, T. , Hamada, M., Nakajima, N. and Saito, A. (2018). Regioselective Synthesis of Procyanidin B6, A 4-6-Condensed (+)-Catechin Dimer, by Intramolecular Condensation. *Molecules* *23*(1), 205. DOI: 10.3390/molecules23010205.

Jama B and Zeila A. (2005). Agroforestry in the drylands of eastern Africa: a call to action. ICRAF Working Paper – no. 1. Nairobi: World Agroforestry Centre.

Jones, D. and Brischke, C. (eds). (2017). Performance of Bio-based building materials. Woodhead publishing. Pp 32-33.

Ju, J., Lu, G., Lambert, J. G. and Yang, C. S. (2007). Review: Inhibition of carcinogenesis by tea constituents. *Seminars in Cancer Biology*, 17 (2007) 395–402.

Kalia, S. and Avérous, L. (eds) (2016). Biodegradable and biobased polymers for environmental and biomedical applications. Wiley Scrivener publishing. 108 – 111.

Kalsi, P.S. (2005). Spectroscopy of Organic compounds (6^{th} ed). New age International publishers. Pp 45 – 51.

Khandelwal, P., Sharma, R. A., and Agarwal, M. (2015). Pharmacology and Therapeutic Application of *Prosopis juliflora*: A Review. *Journal of Plant Sciences*. Vol. 3 (4). Pp. 234-240. DOI: 10.11648/j.jps.20150304.20.

Khandelwal, P., Sharma, R. A., & Agarwal, M. (2016). Isolation and identification of flavonoids from *Prosopis juliflora*. *Mintage Journal of Pharmaceutical and Medical Sciences*, 1-3.

K.I.G. Kenya Information Guide. (2015). http://www.kenya-information-guide.com

Kirker, G.T., Blodgett, A.B., Arango, R.A., Lebow, P.K., and Clausen, C.A. (2013). The role of extractives in naturally durable wood species. *International Biodeterioration and Biodegradation*. 82: 53-58.

Kumar, B. R. (2017). Application of HPLC and ESI-MS techniques in the analysis of phenolic acids and flavonoids from green leafy vegetables (GLVs). *Journal of pharmaceutical analysis*, *7*(6), 349-364.

Kumar, S. and Pandey, A. K. (2013). Chemistry and Biological activities of Flavonoids: An Overview. *Scientific World Journal*. DOI: 10.1155/2013/162750.

Lakshmibai, R., Amirtham, D., and Radhika, S. (2016). Preliminary phytochemical analysis and antioxidant activities of *Prosopis juliflora* and *Mimosa pudica* leaves. *International Conference on Industrial Scientific Research Engineering.* (pp. 040-044).

Lenachuru, C. (2003). "Impacts of Prosopis species in Baringo District." In: S.K. Choge and B.N. Chikamai (eds) Proceedings of Workshop on Integrated Management of Prosopis Species in Kenya. Jointly published by the Global Environmental Facility (GEF), the Kenya Forestry Research Institute (KEFRI), and the Kenya Forest Department (FD), Nairobi.

Li, H. and Deinzer, M.L. (2008). The mass spectral analysis of isolated hops A-type proanthocyanidins by electrospray ionization tandem mass spectrometry. *J. Mass Spectrom,* 43: 1353–1363. DOI: 10.1002/jms.1411.

Liu, F., Chen, J.R., Tang, Y.H., Chang, H.T., Yuan, Y.M. and Guo, Q. (2018). Isolation and characterization of cinnamate 4-hydroxylase gene from cultivated ramie (*Boehmeria nivea*). *Agriculture and Environmental Biotechnology.* Pp 324-331. DOI: 10.1080/13102818.2017.1418675.

Mandal, S. M., Chakraborty, D. and Dey, S. (2010). Phenolic acids act as signaling molecules in plant- microbe symbioses. *Plant Signaling and Behavior.* 5 (4), 359–368. DOI: 10.4161/psb.5.4.10871

Marais, J. P., Deavours, B. E. T. T. I. N. A., Dixon, R. A., and Ferreira, D. (2006). The stereochemistry of flavonoids. In *The science of flavonoids* (pp. 1-46). Springer, New York, NY.

Martens, S., Preuß, A. and Matern, U. (2010). Multifunctional flavonoid dioxygenases: flavonols and anthocyanin biosynthesis in *Arabidopsis thaliana L*. *Phytochemistry*. 71:1040-1049.

Marquez, S. R., Hernandez, E., Solano, F., Romero, C., Lopez, G., Juarez-Rojas, L., and Delgadillo, J. E. A. (2016). Mesquite (*Prosopis juliflora*) pod extract decreases fertility in female but not male rats. *African Journal of Pharmacy and Pharmacology*, *10* (46), 984-994.

Masakha, E.J. and Wegulo, F.N. (2015). Socioeconomic Impacts of Prosopis Juliflora on the People of Salabani Location, Marigat District, Baringo County in Kenya. *Journal of Natural Sciences Research.* Vol.5 (19).

Meng, X., Lee, M.J., Li, C., Sheng, S., Zhu, N., Sang, S., Ho, C.T., Yang, C.S. (2001). Formation and identification of 4'-O-methyl-(-)-epigallocatechin in humans. *Drug Metabolism Disposition*, 29 (6): 789-93.

Merriam-Webster.com. 2019. https://www.merriam-webster.com (10 May 2019).

Mounguengui, W. S. (2008). *Caractérisation HPLC de marqueurs permettant de prévoir l'évolution de certaines propriétés macroscopiques du bois lors de différents processus de dégradation*. Academic dissertation, Université de Lorraine, (PhD report).

Muturi, G. M. (2012). *Ecological impacts of Prosopis invasion in Riverine Forests of Kenya*. *Academic Dissertation, Wageningen University, (PhD report).*

Mwangi, E., and Swallow, B. (2008). *Prosopis juliflora* invasion and rural livelihoods in the Lake Baringo area of Kenya. *Conservation and Society*, *6*(2), 130.

Nascimento, M. S., Santana, A. L. B. D., Maranhão, C. A., Oliveira, L. S. and Bieber, L. (2013). Phenolic extractives and natural resistance of wood, biodegradation - Life of Science, Rolando Chamy and Francisca Rosenkranz, *IntechOpen*, DOI: 10.5772/56358.

Norma, A., Maríada, G. C., José, A. R., Nestor, N. J., Jesús, H. C., and Laura, S. G. (2007). Antioxidant activity of polyphenolic extract of monofloral honeybee-collected pollen from mesquite (*Prosopis juliflora*, Leguminosae). *Journal of Food Composition and Analysis.* 20:119-124.

Obach, R.S. and Kalgutkar, A.S. (2010). General Principles. *Comprehensive Toxicology*. Vol 1. Pp 309-347.

Odero, M.P., Munyendo, W. L. and Kiprop, A. K. (2017). Quantitative Analysis of the Flavonoid Mesquitol in the Medicinal Plant *Prosopis juliflora* with Seasonal Variations in Marigat, Baringo County-Kenya. *Science Journal of Analytical Chemistry*, 5(6): 107-112. DOI: 10.11648/j.sjac.20170506.16.

Oduor, N. M. and Githiomi, J. K. (2013). Fuel-wood energy properties of *Prosopis juliflora* and *Prosopis pallida* grown in Baringo District, Kenya. African Journal of Agricultural Research. Vol. 8(21), pp. 2476-2481, DOI: 10.5897/AJAR08.221

Panche, A. N., Diwan, A. D. and Chandra, S. R. (2016). Flavonoids: an overview. *Journal of Nutritional Science*. 5 (47). 1 – 15. DOI: 10.1017/jns.2016.41

Pasiecznik, N. M., Felker, P., Harris, P. J., Harsh, L., Cruz, G., Tewari, J. C., & Maldonado, L. J. (2001). *The 'Prosopis juliflora' - 'Prosopis pallida' Complex: A Monograph* (Vol. 172). Coventry: HDRA.

Pasiecznik, N. M, Harris, P. J. and Smith, S. J. (2004). Identifying Tropical *Prosopis* Species: A Field Guide. HDRA, Coventry, UK. ISBN 0 905343 34 4.

Patnaik, P., Abbasi, T. and Abbasi, S. A. (2017). *Prosopis* (*Prosopis juliflora*): blessing and bane. Tropical Ecology 58(3): 455–483.

Pattiya, A. (2018). Direct thermochemical liquefaction for energy applications. Pp 3-28. Doi.org/10.1016/B978-0-08-101029-7.00001-1.

Pecha, B. and Perez, M. G. (2015). Bioenergy. Biomass to biofuels. Pp 413-442. Doi: 10.1016/13978-0-12-407909-0.00026-2.

Pometti, C.L., Palanti, S., Pizzo, B., Charpentier, J.-P., Boizot, N.,Resio, C. and Saidman, B.O. (2010). Durability of five native argentine wood species of the genera *Prosopis* and *Acacia* decayed by rot fungi and its relationship with extractive content. Biodegradation. 21, 753-760. DOI: org/10.1007/s10532-010-9340-5.

Pragnesh, N. D. and Jaya, B. (2013). *Prosopis julifera*: A review. *International Journal of Chemical Studies*. 1 (3). Pp 181 – 196.

Prabha, D. S., Dahms, H. U., Malliga, P. (2014). Pharmacological potentials of phenolic compounds from *Prosopis spp*.-a review. *Journal of Coastal Life Medicine*. 2(11): 918-924

Raffa, D., Maggio, B., Raimondi, M. V., Plescia, F. and Daidone, G. (2017). Recent discoveries of anticancer flavonoids. *European Journal of Medicinal Chemistry*. Vol 142, 213-228.

Rajbhar, K., Dawda, H. and Mukundan, U. (2014). Polyphenols: Methods of extraction. *Scientific reviews and chemical communications*. 5(1), 1-6.

Ramage, M. H., Burridge, H., Busse-Wicher, M., Fereday, G., Reynolds, T., Shah, D. U., ... & Allwood, J. (2017). The wood from the trees: The use of timber in construction. *Renewable and Sustainable Energy Reviews*, *68*, 333-359.

Rao, K.S.V.K., Reddy, P. R. S., Sekhar, E. C. (2017). Fabrication of gold nanoparticles from *Prosopis juliflora* leaves extract by green method for potential antibacterial application. *Indian journal of Advances in Chemical Science*. 5(2), 102-107.

Rao, R. J., Tiwari, A. K., Kumar, U. S., Reddy, S. V., Ali, A. Z. and Rao, J. M. (2003). Novel 3-O-Acyl Mesquitol Analogues as Free-Radical Scavengers and Enzyme Inhibitors: Synthesis,Biological Evaluation and Structure–Activity Relationship. *Bioorganic and Medicinal Chemistry Letters*. 13. Pp 2777–2780.

Rauf, A., Imran, M., Abu-Izneid, T., Haq, L., Patel, S., Pan, X., Naz, S., Silva, A. S., Saeed, F. and Suleria, H. A. R. (2019). Proanthocyanidins: A comprehensive review. *Biomedicine & Pharmacotherapy*. Vol 116, DOI: 10.1016/j.biopha.2019.108999.

Raven, P. H., Evert, R.F., Eichhorn, S.E. (2005). "Chapter 24". Biology of plants (7th ed.). New York, USA. Freeman. Pp. 528-546.

Rowell, R. M., Pettersen, R., Han, J. S., Rowell, J. S, and Tshabalala, M. A. (2005). Handbook of wood chemistry and wood composites. CRC Press LLC. Pp 35 – 49.

Sathiya, M. and Muthuchelian, K. (2011). Anti-tumor potential of total alkaloid extract of *Prosopis juliflora DC*. leaves against Molt-4 cells *in vitro*. *African Journal of Biotechnology*. Vol. 10 (44), pp. 8881-8888. DOI: 10.5897/AJB10.875.

Santos, E. L., Maia, B. H. L. N. S., Ferriani, A. P. and Teixeira, S. D. (2017). Flavonoids: Classification, Biosynthesis and Chemical ecology. *IntechOpen*. DOI:10.5772/67861

Santosh K.M. and Gowsiya S. (2013). Testing the allelopathic effect of aqueous extract of leaves of *P.juliflora* on the growth of BGA. *Indian Journal of Fundamental and Applied Sciences*. Vol 3, pp 252 – 255.

Scherbaum E., Anastassiades M., Schüle. E., Bauer, N., Ellendt, K. and M. Wieland. (2008). GC-MS or LC-MS(/MS) - Which Technique is More Essential? *CRL for Pesticide Residues using Single Residue Methods Germany.*

Selvan, T.R., Parthiban, K.T. and Palanikumaran, B. (2018). *Prosopis juliflora* –A myth and reality to the current development scenario in Tamil Nadu. *Int. J. Pure App. Biosci*.6 (1): 1088-1092. DOI 10.18782/2320-7051.6151.

Shackleton, R. T., Le Maitre, D. C., Pasiecznik, N. M., and Richardson, D. M. (2014). *Prosopis*: a global assessment of the biogeography, benefits, impacts and management of one of the world's worst woody invasive plant taxa. *AoB Plants*, Volume 6, 27. DOI: org.bases-doc.univ-lorraine.fr/10.1093/aobpla/plu027.

Sharma, A., Shahzad, B., Rehman, A., Bhardwaj, R., Landi, M. and Zheng, B. (2019). Response of phenyl propanoid pathway and the role of polyphenols in plants under abiotic stress. *Molecules*. Vol *24* (13). DOI: 10.3390/molecules24132452.

Sherwood , P., Bonello, P. (2013) . Austrian pine phenolics are likely contributors to systemic induced resistance against *Diplodia pinea*. *Tree Physiol.* 33 (8). 845-854. doi.org/10.1093/treephys/tpt063.

Silva, V. D. A., Pitanga, B. P., Nascimento, R. P., Souza, C. S., Coelho, P. L. C., Menezes-Filho, N., and Costa, S. L. (2013). Juliprosopine and Juliprosine from *Prosopis juliflora* leaves induce mitochondrial damage and cytoplasmic vacuolation on cocultured glial cells and neurons. *Chemical research in toxicology*, *26* (12), 1810-1820.

Singh, S. (2012). Phytochemical analysis of different parts of *Prosopis juliflora*. *Int J Curr Pharm Res*, *4*(3), 59-61.

Sirmah, P.K. (2009). *Valorisation du Prosopis juliflora comme alternative à la diminution des ressources forestières au Kenya.* Academic dissertation, Université de Lorraine, (PhD report).

Sirmah, P., Mburu, F., Iaych, K., Dumarçay, S., and Gérardin, P. (2011). Potential antioxidant compounds from different parts of *Prosopis juliflora*. *Journal of tropical forest science*, 187-195.

Sirmah, P., Muisu, F., Mburu, F., Dumarçay, S., and Gérardin, P. (2008). Evaluation of *Prosopis juliflora* properties as an alternative to wood shortage in Kenya. *Bois et forêts des tropiques*, *298* (4), 25-35.

Sun, W. and Miller, J. M. (2002). Tandem mass spectrometry of the B-type procyanidins in wine and B-type dehydrodicatechins in an autoxidation mixture of (+)-catechin and (−)-epicatechin. *J. Mass Spectrom.* 38: 438–446.

Swatsitang, P., Tucker, G., Robards, K., Jardine, D. (2000). Isolation and identification of phenolic compounds in *Citris sinensis. Analytical chemistry. Acta.* 417, 231.

Tallarida, R. J. (2001). Drug synergism: its detection and applications. *Journal of Pharmacology and Experimental Therapeutics*, *298*(3), 865-872.

Tanaka, Y., Sasaki, N., Ohmiya, A. (2008). Biosynthesis of plant pigments: anthocyanins, betalains and carotenoids. *The Plant Journal*. 54:733-749. DOI: 10.1111/j.1365-313X.2008.03447.x

Tanaka, Y., Tsuda, S. and Kusumi, T. (1998). Metabolic Engineering to Modify Flower Color. *Plant Cell Physiol.* 39(11): 1119-1126.

Tascioglu, C., Yalcin, M., Sen, S. and Akcay, C. (2013). Antifungal properties of some plant extracts used as wood preservatives. *International Biodeterioration and Biodegradation.* 85. Pp 23 – 28.

Tchinda, J. B. S. (2015). Caractérisation et valorisation des substances extractibles de cinq essences camerounaises majeures de l'industrie du bois. Academic dissertation, Université de Lorraine, (PhD report).

Tchinda, J. B. S., Ndikontar, M. K., Belinga, A. D. F., Mounguengui, S., Njankouo, J. M., Durmaçay, S. and Gerardin, P. (2018). Inhibition of fungi with wood extractives and natural durability of five Cameroonian wood species. *Industrial Crops and Products.* 123. Pp 183 – 191.

Thakur, R., Singh, R., Saxena, P., & Mani, A. (2014). Evaluation of antibacterial activity of Prosopis juliflora (SW.) DC. leaves. *African Journal of Traditional, Complementary and Alternative Medicines*, *11*(3), 182-188.

Thomasson, G, Capizzi, J., Dost, F., Morrell, J. and Miller, D. (2015). Wood Preservation and Wood Products Treatment. Training Manual Oregon State University.

UNEP, United Nations Environment Programme (2004).

Valette, N., Perrot, T., Sormani, R., Gelhaye, E. and Morel-Rouhier, M. (2017). Antifungal activities of wood extractives. *Fungal biology reviews.* 31. Pp 113 – 123.

Varela, M. C., Arslan, I., Reginato, M. A., Cenzano, A. M., Luna, M. V. (2016). Phenolic compounds as indicators of drought resistance in shrubs from Patagonian shrublands (Argentina). *Plant Physiology and Biochemistry.* 104; 81- 91.

Veitch, N. C., and Grayer, R. J. (2008). Flavonoids and their glycosides, including anthocyanins. *Natural product reports, 25* (3), 555-611.

Wan, S. B., Dou, Q. P. and Chan, T. H. (2006). Regiospecific and enantioselective synthesis of methylated metabolites of tea catechins. *Tetrahedron*. 62. Pp 5897–5904.

Wikimedia commons . (2019). https://commons.wikimedia.org/wiki/File.

Willför, S. M., Ahotupa, M. O., Hemming, J. E., Reunanen, M. H. T., Eklund, P. C., Sjöholm, R. E., Eckerman, C. S. E., Pohjamo, S. P. and Holmbom, B. R. (2003). Antioxidant activity of knotwood extractives and phenolic compounds of selected tree species. *J. Agric. Food Chem.*, 51 (26), 7600–7606. DOI: 10.1021/jf030445h.

Williams, R. J., Spencer, J. P. and Rice-Evans, C. (2004). Flavonoids: antioxidants or signalling molecules? *Free Radical Biology and Medecine*. 36 (7), 838–849. DOI: 10.116/j.freeradbiomed.2004.01.001.

Winkel-Shirley, B. (2001). Flavonoid biosynthesis. A colorful model for genetics, biochemistry, cell biology, and biotechnology, *Plant Physiol* **126**: 485-493.

Xiao, W., Han, L. and Shi, B. (2008). Microwave-assisted extraction of flavonoids from *Radix Astragali*. *Separation and Purification Technology*. 62, 614–618. DOI:10.1016/j.seppur.2008.03.025.

Zhang, Q. W., Lin, L. G. and Ye, W. C. (2018). Techniques for extraction and isolation of natural products: a comprehensive review. *Chin Med.* 13: 20. DOI: 10.1186/s13020-018-0177-x.

Zhenhua, G. and Dong, L. (2007). Chemical modification of poplar wood with foaming polyurethane resins. *J Appl Polym Sci*. 104:2980–2985.

Zoratti, L., Karppinen, K., Escobar, A. L. and Jaakola, L. (2014). Light-controlled flavonoid biosynthesis in fruits. *Frontiers in Plant Science.* 5 (534). DOI: 10.3389/fpls.2014.00534.

ANNEXES

ANNEX 1: GC-MS tables showing the different classes of extractives in *P. juliflora*

GC-MS BARINGO ANALYSES
DCM extract from small trees

Retention time	Relative area	% Yield	Fragments	Identified product	Nist Match
16.44	215417568	11.72	117, 328	Fatty acid (Palmitic acid, TMS)	877
17.54	212033552	11.53	117, 234, 352	Fatty acid (Linoleic acid, TMS)	905
21.63	289353696	15.74	117, 440	Fatty acid (Tetracosanoic acid)	803
23.97	445710784	24.25	117, 468	Fatty acid (Hexacosanoic acid)	854
26.51	135047216	7.35	129, 343, 472	Sterol (Campesterol, TMS)	870
26.80	373948128	20.34	129, 355, 484	Sterol (Stigmasterol, TMS)	848
27.54	166815952	9.07	129, 357, 486	Sterol (β-Sitosterol TMS)	817
Total Area	1838326896	100			

Bark Acetone extract from small trees

Retention time	Relative area	% Yield	Fragments	Identified product	Nist Match
14.15	188857824	2.52	204, 217	Sugar	729
14.40	180436496	2.41	204, 217	Sugar	768
14.48	624211328	8.34	204, 217	Sugar	831
14.63	731631552	9.78	204, 217	Sugar	899
14.76	248874464	3.33	204, 217	Sugar	706
15.02	373037440	4.99	305, 361	Sugar	622
15.14	141600096	1.89	217, 305	Sugar	732
15.45	114221688	1.53	217, 361	Sugar	811
18.51	549701760	7.35	191, 179	Lignan	535
21.03	487128160	6.51	368, 650	Mesquitol	768
21.90	3545511168	47.40	368, 398, 680	Methylgallocatechin	698
22.28	295094272	3.94	456, 648	Gallocatechin	537
Total Relative Area	7480306248	100			

Bark Toluene/Ethanol extract from small trees

Retention time	Relative area	% Yield	Fragments	Identified product	Nist Match
13.58	261970272	3.82	204, 217	Sugar	905
14.40	294306176	4.29	204, 217	Sugar	786
14.49	1822945408	26.59	204, 217	Sugar	856
14.65	2162405120	31.54	204, 217	Sugar	857
15.46	565808192	8.25	217, 307	Sugar	763
21.02	180512832	2.63	368, 650	Mesquitol	788
21.86	1291294208	18.84	368, 398, 680	Methylgallocatechin	754
21.91	195921584	2.86	368, 398, 680	Methylgallocatechin (isomers)	724
22.27	80579288	1.18	456, 648	Gallocatechin	547
Total Relative Area	6855743080	100			

Bark Water extract from small trees

Retention time	Relative area	% Yield	Fragments	Identified product	Nist Match
14.35	1271812	83.76	204, 217	sugar	802
15.38	246551	16.24	204, 217	sugar	637
Total Relative Area	1518362	100			

GC-MS BARINGO ANALYSES
Bark DCM extract from big trees

Retention time	Relative area	% Yield	Fragments	Identified product	Nist Match
16.43	119124160	9.84	117, 328	Fatty acid (Palmitic acid, TMS)	869
20.97	120231728	9.93	368, 650	Catechin	778
23.88	223023488	18.42	117, 468	Fatty acid (Hexacosanoic acid)	826
25.24	234776496	19.39	468	Alcohol (Octacosane)	664
26.71	108988672	9.00	129, 355, 484	Sterol (Stigmasterol, TMS)	783
28.45	404889280	33.43	411, 442	Triterpene (Betulin)	731
Total Relative Area	1211033824	100			

Bark Acetone extract from big trees

Retention time	Relative area	% Yield	Fragments	Identified product	Nist Match
14.45	318797536	9.74	204, 217	Sugar	842
14.59	503042944	15.37	204, 217	Sugar	887
14.73	162112672	4.95	204, 217	Sugar	712
18.48	104332648	3.19	179	Lignan	622
20.98	742669440	22.69	368, 650	Catechin	720
21.79	1039296576	31.76	368, 398, 680	Methylgallocatechin	618
21.86	402161600	12.29	368, 398, 680	Methylgallocatechin (isomers)	730
Total Relative Area	3272413416	100			

Bark Toluene/Ethanol extract from big trees

Retention time	Relative area	% Yield	Fragments	Identified product	Nist Match
14.11	257835808	4.93	204, 217	Sugar	726
14.36	289010624	5.53	204, 217	Sugar	792
14.45	1047056128	20.03	204, 217	Sugar	850
14.60	1568024192	29.99	204, 217	Sugar	858
14.73	298160000	5.70	204, 217	Sugar	711
15.42	294065888	5.62	217, 307	Sugar	802
18.48	244992736	4.69	179	Lignan	615
20.98	404751424	7.74	368, 650	Catechin	773
21.78	607235712	11.61	368, 398, 680	Methylgallocatechin	701
21.85	217329664	4.16	368, 398, 680	Methylgallocatechin (isomers)	732
Total Relative Area	5228462176	100			

Bark Water extract from big trees

Retention time	Relative area	% Yield	Fragments	Identified product	Nist Match
14.43	54686836	16.77	204, 217	sugar	826
14.58	35118176	10.77	204, 217	sugar	880
14.71	37248924	11.42	204, 217	sugar	688
15.29	14238185	4.37	204, 217	sugar	780
20.96	29178154	8.95	368, 650	Catechin	759
21.75	131416280	40.29	368, 398, 680	Methylgallocatechin	
21.82	24281610	7.44	368, 398, 680	Methylgallocatechin (isomers)	796
Total Relative Area	326168165	100			

GC-MS BARINGO ANALYSES
Sapwood DCM extract from small trees

Retention time	Relative area	% Yield	Fragments	Identified product	Nist Match
10.46	449931392	16.98	288	2-Isopropyl-3-ketobutyrate	739
14.71	306834752	11.58	204, 217	Sugar	686
16.42	429986720	16.23	117, 328	Fatty acid (Palmitic acid)	875
17.52	297159968	11.21	117, 234, 352	Fatty acid (Linoleic acid)	871
17.56	295047200	11.13	117, 354	Fatty acid (Oleic acid)	873
26.72	432295040	16.31	129, 355, 484	Sterol (Stigmasterol)	814
28.45	438767648	16.56	411, 442	Triterpene (Betulin)	718
Total Relative Area	2650022720	100			

Sapwood Acetone extract from small trees

Retention time	Relative area	% Yield	Fragments	Identified product	Nist Match
13.57	62812036	0.65	204, 217	Sugar	870
14.03	89314000	0.92	204, 217	Sugar	881
14.19	129336608	1.33	204, 217	Sugar	670
14.26	122709768	1.26	204, 217	Sugar	688
14.41	357458688	3.68	204, 217	Sugar	894
14.51	3485501440	35.85	204, 217	Sugar	786
14.67	4272438784	43.95	204, 217	Sugar	822
14.76	71164008	0.73	204, 217	Sugar	739
15.00	78183328	0.80	204, 217	Sugar	662
16.00	70590736	0.73	204, 217	Sugar	819
19.86	295674528	3.04	204, 217	sugar	868
20.88	64141072	0.66	368, 650	Catechin	736
21.04	621922304	6.40	368, 650	Mesquitol	751
Total Relative Area	9721247300	100			

Sapwood Toluene/Ethanol extract from small trees

Retention time	Relative area	% Yield	Fragments	Identified product	Nist Match
13.60	126078456	0.69	204, 217	Sugar	660
13.70	283619968	1.55	204, 217	Sugar	651
13.89	766516544	4.18	204, 217	Sugar	633
14.06	242694816	1.32	204, 217	Sugar	653
14.14	688437888	3.76	204, 217	Sugar	705
14.21	2309489408	12.60	204, 217	Sugar	685
14.36	1949391104	10.64	204, 217	Sugar	717
14.43	384429248	2.10	204, 217	Sugar	706
14.50	3452746752	18.84	204, 217	Sugar	849
14.66	5115373568	27.91	204, 217	Sugar	847
14.73	291620736	1.59	204, 217	Sugar	761
14.78	1901916800	10.38	204, 217	Sugar	683
14.88	447204224	2.44	204, 217	Sugar	686
15.64	194656784	1.06	204, 217	Sugar	891
15.98	172492544	0.94	204, 217	Sugar	852
Total Relative Area	18326668840	100			

Sapwood Water extract from small trees

Retention time	Relative area	% Yield	Fragments	Identified product	Nist Match
14.41	137569168	2.93	204, 217	Sugar	894
14.50	1473352320	31.34	204, 217	Sugar	843
14.65	1579136512	33.59	204, 217	Sugar	873
15.66	102416680	2.18	204, 217	Sugar	885
19.87	1293116160	27.50	204, 217	Sugar	815
19.91	116207328	2.47	204, 217	Sugar	782
Total Relative Area	4701798168	100			

GC-MS BARINGO ANALYSES
Sapwood DCM extract from big trees

Retention time	Relative area	% Yield	Fragments	Identified product	Nist Match
14.46	453963552	8.39	204, 217	Sugar	787
14.61	565791232	10.46	204, 217	Sugar	903
16.45	1113254016	20.58	117, 328	Fatty acid (Palmitic acid)	836
17.56	1235788928	22.85	117, 234, 352	Fatty acid (Linoleic acid acid)	881
17.59	623121920	11.52	117, 354	Fatty acid (Oleic acid)	834
18.15	380190848	7.03	204, 217	Sugar	794
26.80	401050528	7.41	129, 355, 484	Sterol (Stigmasterol)	840
28.56	636030272	11.76	411, 442	Triterpene (Betulin)	730
Total Relative Area	5409191296	100			

Sapwood Acetone extract from big trees

Retention time	Relative area	% Yield	Fragments	Identified product	Nist Match
14.15	1104811392	3.34	204, 217	Sugar	716
14.26	540568768	1.64	204, 217	Sugar	743
14.44	3836653568	11.61	204, 217	Sugar	679
14.51	6714191872	20.32	204, 217	Sugar	613
14.68	6735219712	20.39	204, 217	Sugar	730
14.78	3854435584	11.67	204, 217	Sugar	699
15.01	700352384	2.12	204, 217	Sugar	780
15.15	1906258688	5.77	204, 217	Sugar	854
15.20	1620547456	4.91	204, 217	Sugar	713
15.83	1592753664	4.82	204, 217	Sugar	767
19.55	984467264	2.98	204, 217	Sugar	775
19.85	1621084800	4.91	204, 217	sugar	833
19.91	459472608	1.39	204, 217	sugar	785
21.04	1366887936	4.14	368, 650	Mesquitol	695
Total Relative Area	33037705696	100			

Sapwood Toluene/Ethanol extract from big trees

Retention time	Relative area	% Yield	Fragments	Identified product	Nist Match
14.15	719686208	4.74	204, 217	Sugar	710
14.24	475041248	3.13	204, 217	Sugar	731
14.42	2248023040	14.79	204, 217	Sugar	760
14.49	3602187776	23.70	204, 217	Sugar	648
14.64	1847532800	12.16	204, 217	Sugar	868
14.77	2079293568	13.68	204, 217	Sugar	690
14.80	1011767808	6.66	204, 217	Sugar	715
15.13	534239392	3.52	204, 217	Sugar	864
15.18	639611008	4.21	204, 217	Sugar	886
15.25	486250784	3.20	204, 217	Sugar	824
15.82	555475520	3.65	204, 217	Sugar	825
19.55	999575168	6.58	204, 217	Sugar	767
Total Relative Area	15198684320	100			

Sapwood Water extract from big trees

Retention time	Relative area	% Yield	Fragments	Identified product	Nist Match
14.20	14796969	6.42	204, 217	Sugar	894
14.30	30351990	13.18	204, 217	Sugar	844
14.42	34110476	14.81	204, 217	Sugar	897
15.01	19243410	8.35	204, 217	Sugar	890
15.68	27228148	11.82	204, 217	Sugar	871
18.98	9115870	3.96	204, 217	Sugar	816
19.35	31108138	13.51	204, 217	Sugar	769
19.63	54524540	23.67	204, 217	Sugar	895
19.71	9844043	4.27	204, 217	Sugar	747
Total Relative Area	230323584	100			

GC-MS BARINGO ANALYSES
Knotwood DCM extract from small trees

Retention time	Relative area	% Yield	Fragments	Identified product	Nist Match
14.46	240166256	4.72	204, 217	Sugar	690
16.45	669967808	13.16	117, 328	Fatty acid (Palmitic acid)	881
17.56	854394624	16.78	117, 234, 352	Fatty acid (Linoleic acid)	884
17.59	255425280	5.02	117, 354	Fatty acid (Oleic acid)	881
20.83	343261248	6.74	368, 650	Catechin	597
21.04	1104816256	21.69	368, 650	Mesquitol	721
22.75	254600528	5.00	439	Alcohol (Hexacosane)	837
25.31	241891856	4.75	468	Alcohol (Octacosane)	824
26.80	407508416	8.00	129, 355, 484	Sterol (Stigmasterol)	841
28.56	720718016	14.15	411, 442	Triterpene (Betulin)	742
Total Relative Area	5092750288	100			

Knotwood Acetone extract from small trees

Retention time	Relative area	% Yield	Fragments	Identified product	Nist Match
14.14	264721632	1.58	204, 217	Sugar	733
14.40	534399136	3.19	204, 217	Sugar	757
14.48	1619926144	9.66	204, 217	Sugar	847
14.64	1534178432	9.15	204, 217	Sugar	881
14.76	1210547456	7.22	204, 217	Sugar	704
20.88	1283555072	7.65	368, 650	Catechin	713
21.15	8149453312	48.59	368, 650	Mesquitol	715
21.94	1954288512	11.65	368, 398, 680	Methylgallocatechin	792
22.28	221290032	1.32	456, 648	Gallocatechin	566
Total Relative Area	16772359728	100			

Knotwood Toluene/Ethanol extract from small trees

Retention time	Relative area	% Yield	Fragments	Identified product	Nist Match
11.46	380151040	1.44	156, 273	L-Proline, 5-oxo-1-(trimethy	920
14.15	532100160	2.01	204, 217	Sugar	740
14.41	1197364992	4.53	204, 217	Sugar	775
14.49	5791772160	21.89	204, 217	Sugar	694
14.67	5910007296	22.34	204, 217	Sugar	773
14.77	1187228928	4.49	204, 217	Sugar	713
15.23	315614400	1.19	204, 217	Sugar	848
15.99	412802240	1.56	204, 217	Sugar	826
20.88	1149282560	4.34	368, 650	Catechin	764
21.15	7721249280	29.18	368, 650	Mesquitol	686
21.94	1863126272	7.04	368, 398, 680	Methylgallocatechin	605
Total Relative Area	26460699328	100			

Knotwood Water extract from small trees

Retention time	Relative area	% Yield	Fragments	Identified product	Nist Match
18.93	318718	12.63	204, 217	Sugar	626
20.70	2205067	87.37	368, 650	Mesquitol	726
Total Relative Area	2523785	100			

GC-MS BARINGO ANALYSES
Knotwood DCM extract from big trees

Retention time	Relative area	% Yield	Fragments	Identified product	Nist Match
16.42	270559968	8.57	117, 328	Fatty acid (Palmitic acid)	888
17.53	337649312	10.70	117, 234, 352	Fatty acid (Linoleic acid)	761
20.82	260132176	8.24	368, 650	Catechin	782
21.00	1205341056	38.20	368, 650	Mesquitol	767
21.60	140104832	4.44	117, 440	Fatty acid (Tetracosanoic acid)	616
23.89	146267344	4.64	117, 468	Fatty acid (Hexacosanoic acid)	763
26.71	244403248	7.75	129, 355, 484	Sterol (Stigmasterol)	832
27.45	151815984	4.81	129, 357, 486	Sterol (β-Sitosterol)	763
28.45	399245664	12.65	411, 442	Triterpene (Betulin)	731
Total Relative Area	3155519584	100			

Knotwood Acetone extract from big trees

Retention time	Relative area	% Yield	Fragments	Identified product	Nist Match
20.84	1089359104	20.60	368, 650	Catechin	736
21.06	4199218944	79.40	368, 650	Mesquitol	667
Total Relative Area	5288578048	100			

Knotwood Toluene/Ethanol extract from big trees

	Relative area	% Yield	Fragments	Identified product	Nist Match
Retention time	813464384	9.74	204, 217	Sugar	823
14.45	1261195008	15.10	204, 217	Sugar	898
14.60	636715520	7.63	204, 217	Sugar	705
14.73	1185952128	14.20	368, 650	Catechin	730
20.84	4452927488	53.33	368, 650	Mesquitol	755
21.06	8350254528	100			
Total Relative Area					
	5				
Compounds detected					

Knotwood Water extract from big trees

	Relative area	% Yield	Fragments	Identified product	Nist Match
Retention time	7157621	100.00	368, 650	Mesquitol	727
20.97	7157621				
Total Relative Area					
	1				
Compounds detected					

GC-MS BARINGO ANALYSES
Small trees
Heartwood DCM extract

Retention time	Relative area	% Yield	Fragments	Identified product	Nist Match
15.51	100767024	3.92	55, 207, 125, 136	Farnesyl acetone	846
16.44	108632768	4.22	117, 328	Fatty acid (Palmitic acid)	871
17.55	136219872	5.29	117, 234, 352	Fatty acid (Linoleic acid)	891
20.84	230348912	8.95	368, 650	Catechin	634
21.06	1295944704	50.37	368, 650	Mesquitol	685
22.75	137163840	5.33	439	Alcohol (Hexacosane)	819
25.33	120083024	4.67	468	Alcohol (Octacosane)	618
26.54	115591240	4.49	129, 343, 472	Sterol (Campesterol)	784
26.82	244615584	9.51	129, 355, 484	Sterol (Stigmasterol)	819
27.57	83559432	3.25	129, 357, 486	Sterol (β-Sitosterol)	721
Total Relative Area	2572926400	100			

Heartwood Acetone extract

Retention time	Relative area	% Yield	Fragments	Identified product	Nist Match
20.89	174560320	5.01	368, 650	Catechin	775
21.09	2696689408	77.40	368, 650	Mesquitol	729
21.92	612877760	17.59	368, 398, 680	Methylated gallocatechin	677
Total Relative Area	3484127488	100			

Heartwood Toluene/Ethanol extract

Retention time	Relative area	% Yield	Fragments	Identified product	Nist Match
14.50	1023747264	8.96	204, 217	Sugar	855
14.65	1014226176	8.87	204, 217	Sugar	897
20.89	708330560	6.20	368, 650	Catechin	748
21.16	6731028480	58.88	368, 650	Mesquitol	616
21.70	95048696	0.83		Unknown compound	
21.96	1675109504	14.65	368, 398, 680	Methylgallocatechin	723
22.28	184203456	1.61	456, 648	Gallocatechin	
Total Relative Area	11431694136	100			

Heartwood Water extract

Retention time	Relative area	% Yield	Fragments	Identified product	Nist Match
21.04	48746352	100.00	368, 650	Mesquitol	757
Total Relative Area	48746352				

GC-MS BARINGO ANALYSES
Big trees
Heartwood DCM extract

Retention time	Relative area	% Yield	Fragments	Identified product	Nist Match
20.89	613351360	16.61	368, 650	Catechin	778
21.11	3078227712	83.39	368, 650	Mesquitol	698
Total Relative Area	3691579072	100			

Heartwood Acetone extract

Retention time	Relative area	% Yield	Fragments	Identified product	Nist Match
14.50	40906760	0.95	204, 217	Sugar	828
14.65	118753144	2.76	204, 217	Sugar	896
20.89	625698304	14.54	368, 650	Catechin	732
21.11	3188530688	74.09	368, 650	Mesquitol	717
21.93	329739264	7.66	368, 398, 680	Methylated gallocatechin	811
Total Relative Area	4303628160	100			

Heartwood Toluene/Ethanol extract

Retention time	Relative area	% Yield	Fragments	Identified product	Nist Match
14.49	875116544	7.30	204, 217	Sugar	863
14.65	1488272256	12.42	204, 217	Sugar	887
20.90	1497094400	12.49	368, 650	Catechin	732
21.16	6992776192	58.36	368, 650	Mesquitol	702
21.94	1056821440	8.82	368, 398, 680	Methylated gallocatechin	754
22.29	72371808	0.60	456, 648	Gallocatechin	570
Total Relative Area	11982452640	100			

Heartwood extract

Retention time	Relative area	% Yield	Fragments	Identified product	Nist Match
21.031	12100701	100.00	368, 650	Mesquitol	688
Total Relative Area	12100701				

GC-MS BARINGO ANALYSES
Small trees
Pith DCM extract

Retention time	Relative area	% Yield	Fragments	Identified product	Nist Match
16.44	164826880	6.47	117, 328	Fatty acid (Palmitic acid)	880
17.54	278657312	10.93	117, 234, 352	Fatty acid (Linoleic acid)	893
20.78	244521904	9.59	368, 650	Catechin	686
21.00	1564292224	61.36	368, 650	Mesquitol	732
26.72	149910608	5.88	129, 355, 484	Sterol (Stigmasterol)	812
28.45	147025504	5.77	411, 442	Triterpene (Betulin)	684
Total Relative Area	2549234432	100			

Pith Acetone extract

Retention time	Relative area	% Yield	Fragments	Identified product	Nist Match
20.83	1198349312	10.52	368, 650	Catechin	745
21.11	8016253440	70.39	368, 650	Mesquitol	688
21.88	1765340928	15.50	368, 398, 680	Methylgallocatechin	742
22.21	164792320	1.45	456, 648	Gallocatechin	564
22.72	244363632	2.15	267, 368, 664	Taxifolin	580
Total Relative Area	11389099632	100			

Pith Toluene/Ethanol extract

Retention time	Relative area	% Yield	Fragments	Identified product	Nist Match
14.45	585407680	6.68	204, 217	Sugar	846
14.60	722418560	8.25	204, 217	Sugar	891
20.83	699623360	7.99	368, 650	Catechin	762
21.08	5591749120	63.82	368, 650	Mesquitol	648
21.87	1162302208	13.27	368, 398, 680	Methylgallocatechin	759
Total Relative Area	8761500928	100			

Pith Water extract

Retention time	Relative area	% Yield	Fragments	Identified product	Nist Match
20.966	12096426	100	368, 650	Mesquitol	704
Total Relative Area	12096426				

GC-MS BARINGO ANALYSES
Big trees
Pith DCM extract

Retention time	Relative area	% Yield	Fragments	Identified product	Nist Match
20.83	400888224	19.93	368, 650	Catechin	788
21.00	1610315008	80.07	368, 650	Mesquitol	681
Total Relative Area	2011203232	100			

Pith Core Acetone extract

Retention time	Relative area	% Yield	Fragments	Identified product	Nist Match
20.84	1073067776	21.59	368, 650	Catechin	750
21.04	3897044480	78.41	368, 650	Mesquitol	687
Total Relative Area	4970112256	100			

Pith Toluene/Ethanol extract

Retention time	Relative area	% Yield	Fragments	Identified product	Nist Match
14.36	526271520	5.17	204, 217	Sugar	732
14.44	925549440	9.10	204, 217	Sugar	858
14.59	870895488	8.56	204, 217	Sugar	898
14.72	1597692672	15.70	204, 217	Sugar	734
20.84	1316432384	12.94	368, 650	Catechin	710
21.06	4938122240	48.53	368, 650	Mesquitol	663
Total Relative Area	10174963744	100			

Pith Water extract

Retention time	Relative area	% Yield	Fragments	Identified product	Nist Match
20.98	159985168	100	368, 650	Mesquitol	687
Total Relative Area	159985168				

GC-MS GARISSA ANALYSES
Small trees
Bark DCM extract

Retention time	Relative area	% Yield	Fragments	Identified product	Nist Match
14.54	28076482	3.59	204, 217	Sugar	872
16.40	26494834	3.39	117, 328	Fatty acid (Palmitic acid)	668
21.74	125227200	16.03	368, 398, 680	Methylgallocatechin	599
26.39	42336136	5.42	129, 343, 472	Sterol (Campesterol)	697
26.66	128187080	16.41	129, 355, 484	Sterol (Stigmasterol)	805
27.39	49272588	6.31	129, 357, 486	Sterol (β-Sitosterol)	682
27.84	68874048	8.82		Linoleic acid	680
28.38	312736096	40.03		Betulin	734
Total Relative Area	781204464	100			

Bark Acetone extract

Retention time	Relative area	% Yield	Fragments	Identified product	Nist Match
14.34	105359648	2.03	204, 217	Sugar	791
14.43	505786624	9.75	204, 217	Sugar	842
14.58	978195904	18.86	204, 217	Sugar	896
14.71	230377808	4.44	204, 217	Sugar	701
14.97	375643904	7.24	305, 361	Sugar	643
18.47	234022112	4.51	191, 179	Lignan	609
19.49	50831768	0.98	217, 361	Sugar	779
19.79	177824816	3.43	217, 361	Sugar	848
19.85	74014464	1.43	217, 361	Sugar	782
20.80	38202892	0.74	368, 650	Catechin	738
20.96	810560512	15.63	368, 650	Mesquitol	755
21.77	1239466880	23.90	368, 398, 680	Methylgallocatechin	549
21.83	286380320	5.52	368, 398, 680	Methylgallocatechin isomer	762
22.18	79621288	1.54	456, 648	Gallocatechin	557
Total Relative Area	5186288940	100			

Bark Toluene/Ethanol extract

Retention time	Relative area	% Yield	Fragments	Identified product	Nist Match
14.10	13616484	1.02	204, 217	Sugar	694
14.35	53491896	3.99	204, 217	Sugar	766
14.43	177582656	13.25	204, 217	Sugar	819
14.58	343893920	25.67	204, 217	Sugar	889
14.71	189783552	14.16	204, 217	Sugar	687
14.98	25288236	1.89	305, 361	Sugar	654
18.48	27636432	2.06	191, 179	Lignan	611
19.50	22624804	1.69	217, 361	Sugar	766
19.80	51451108	3.84	217, 361	Sugar	823
19.86	23493240	1.75	217, 361	Sugar	730
20.96	148262960	11.07	368, 650	Mesquitol	779
21.76	221501504	16.53	368, 398, 680	Methylgallocatechin	605
21.83	41220396	3.08	368, 398, 680	Methylgallocatechin isomer	790
Total Relative Area	1339847188	100			

Bark Water extract

Retention time	Relative area	% Yield	Fragments	Identified product	Nist Match
14.43	1348471168	16.78	204, 217	sugar	836
14.59	1728552320	21.50	204, 217	sugar	881
14.70	640526592	7.97	204, 217	sugar	701
14.99	1967184256	24.47	204, 217	sugar	621
19.79	955829120	11.89	204, 217	sugar	848
20.94	465787968	5.79	368, 650	Mesquitol	782
21.76	932052160	11.59	368, 398, 680	Methylgallocatechin	616
Total Relative Area	8038403584	100			

GC-MS GARISSA ANALYSES
Big trees
Bark DCM extract

Retention time	Relative area	% Yield	Fragments	Identified product	Nist Match
14.57	164760128	10.88	204, 217	Sugar	892
16.40	122144872	8.07		Palmitic acid	859
17.50	108452088	7.16		Linoleic acid	871
20.94	104689352	6.92	368, 650	Mesquitol	780
21.74	421811456	27.86	368, 398, 680	Methylgallocatechin	628
26.67	110404368	7.29		Stigmasterol	813
28.41	481678720	31.82		Betulin	726
Total Relative Area	1513940984	100			

Bark Acetone extract

Retention time	Relative area	% Yield	Fragments	Identified product	Nist Match
14.35	113575512	2.84	204, 217	Sugar	788
14.43	529957120	13.24	204, 217	Sugar	850
14.58	812116416	20.29	204, 217	Sugar	903
14.71	283510144	7.08	204, 217	Sugar	692
14.98	575732032	14.39	305, 361	Sugar	629
18.48	211224832	5.28	191, 179	Lignan	619
19.50	52659496	1.32	217, 361	Sugar	765
19.80	163329456	4.08	217, 361	Sugar	845
19.86	62876900	1.57	217, 361	Sugar	784
20.96	136646912	3.41	368, 650	Mesquitol	782
21.77	939931200	23.49	368, 398, 680	Methylgallocatechin	554
21.84	57914016	1.45	368, 398, 680	Methylgallocatechin isomer	795
22.20	62576784	1.56	456, 648	Gallocatechin	560
Total Relative Area	4002050820	100			

Bark Toluene/Ethanol extract

Retention time	Relative area	% Yield	Fragments	Identified product	Nist Match
14.43	1074609792	19.75	204, 217	Sugar	835
14.58	1523595392	28.00	204, 217	Sugar	883
14.73	430555968	7.91	204, 217	Sugar	609
14.97	421118976	7.74	305, 361	Sugar	636
19.80	607663296	11.17	217, 361	Sugar	831
20.95	308987168	5.68	368, 650	Mesquitol	777
21.77	1074650752	19.75	368, 398, 680	Methylgallocatechin	588
Total Relative Area	5441181344	100			

Bark Water extract

Retention time	Relative area	% Yield	Fragments	Identified product	Nist Match
14.43	781380224	14.87	204, 217	Sugar	835
14.58	848799808	16.16	204, 217	Sugar	896
14.70	392701472	7.47	204, 217	Sugar	698
14.99	1872959616	35.65	204, 217	Sugar	619
19.49	243587056	4.64	204, 217	Sugar	805
19.79	555647936	10.58	204, 217	Sugar	878
21.75	558662720	10.63	368, 398, 680	Methylgallocatechin	601
Total Relative Area	5253738832	100			

GC-MS GARISSA ANALYSES from small trees
Sapwood DCM extract

Retention time	Relative area	% Yield	Fragments	Identified product	Nist Match
14.35	266006176	8.07	204, 217	Sugar	895
14.43	546056832	16.57	204, 217	Sugar	801
14.46	181335312	5.50	204, 217	Sugar	868
14.59	1124541056	34.13	204, 217	Sugar	896
16.42	396792128	12.04	117, 328	Fatty acid (Palmitic acid)	877
17.51	480374784	14.58	117, 234, 352	Fatty acid (Linoleic acid)	897
17.55	299857376	9.10	117, 354	Fatty acid (Oleic acid)	876
Total Relative Area	3294963664	100			

Sapwood Acetone extract

Retention time	Relative area	% Yield	Fragments	Identified product	Nist Match
12.47	262013568	2.01	204, 217	Sugar	765
14.21	292410432	2.24	204, 217	Sugar	704
14.36	955468288	7.32	204, 217	Sugar	824
14.44	2248781824	17.22	204, 217	Sugar	752
14.61	3220396288	24.67	204, 217	Sugar	857
14.73	1486942464	11.39	204, 217	Sugar	700
15.09	316592928	2.43	204, 217	Sugar	864
15.14	802209088	6.14	204, 217	Sugar	854
15.78	764464960	5.86	204, 217	Sugar	833
19.50	259877216	1.99	217, 361	Sugar	796
19.80	1096207616	8.40	217, 361	sugar	852
19.86	315793888	2.42	217, 361	sugar	798
20.97	1034184640	7.92	368, 650	Mesquitol	730
Total Relative Area	13055343200	100			

Sapwood Toluene/Ethanol extract

Retention time	Relative area	% Yield	Fragments	Identified product	Nist Match
14.38	2016278400	8.33	204, 217	Sugar	762
14.45	3834582528	15.85	204, 217	Sugar	693
14.63	5406531072	22.35	204, 217	Sugar	753
14.72	913280512	3.77	204, 217	Sugar	714
15.11	858033344	3.55	204, 217	Sugar	884
15.15	1630106240	6.74	204, 217	Sugar	713
15.23	567905280	2.35	204, 217	Sugar	892
15.61	428330560	1.77	204, 217	Sugar	850
15.80	1745243392	7.21	204, 217	Sugar	770
15.96	292587712	1.21	204, 217	Sugar	831
16.60	572799040	2.37	204, 217	Sugar	896
19.50	340086144	1.41	204, 217	Sugar	811
19.83	3961894656	16.37	217, 361	Sugar	677
19.87	1081271168	4.47	217, 361	Sugar	781
20.97	546382016	2.26	368, 650	Mesquitol	701
Total Relative Area	24195312064	100			

Sapwood Water extract

Retention time	Relative area	% Yield	Fragments	Identified product	Nist Match
14.58	77962360	37.38	204, 217	Sugar	889
14.73	51920896	24.90	204, 217	Sugar	856
15.61	19478258	9.34	204, 217	Sugar	854
16.60	59178568	28.38	204, 217	Sugar	886
Total Relative Area	208540082	100			

GC-MS GARISSA ANALYSES from big trees
Sapwood DCM extract

Retention time	Relative area	% Yield	Fragments	Identified product	Nist Match
14.43	431909664	8.64	204, 217	Sugar	831
14.59	783294592	15.66	204, 217	Sugar	905
16.42	903445504	18.06	117, 328	Fatty acid (Palmitic acid)	885
17.52	1145536640	22.90	117, 234, 352	Fatty acid (Linoleic acid)	881
17.55	638030528	12.76	117, 354	Fatty acid (Oleic acid)	892
17.71	296844160	5.94	117, 356	Fatty Acid (Octadecanoic acid)	882
20.96	239409104	4.79	368, 650	Mesquitol	783
26.70	233912128	4.68	129, 355, 484	Sterol (Stigmasterol)	814
27.44	156336272	3.13	129, 357, 486	Sterol (β-Sitosterol)	721
28.43	172683440	3.45	411, 442	Triterpene (Betulin)	716
Total Relative Area	5001402032	100			

Sapwood Acetone extract

Retention time	Relative area	% Yield	Fragments	Identified product	Nist Match
10.96	151244832	1.85	147, 233	Malic acid	866
14.35	463042656	5.65	204, 217	Sugar	848
14.44	1252300672	15.28	204, 217	Sugar	788
14.60	2092600576	25.53	204, 217	Sugar	894
14.72	1225664128	14.95	204, 217	Sugar	674
15.14	196077792	2.39	204, 217	Sugar	893
15.78	165928144	2.02	204, 217	Sugar	912
19.50	269385376	3.29	204, 217	Sugar	800
19.80	822182336	10.03	204, 217	Sugar	842
19.86	288373440	3.52	204, 217	Sugar	786
20.81	139722880	1.70	368, 650	Catechin	761
20.97	1130822528	13.79	368, 650	Mesquitol	750
Total Relative Area	8197345360	100			

Sapwood Toluene/Ethanol extract

Retention time	Relative area	% Yield	Fragments	Identified product	Nist Match
13.57	209839248	6.25	204, 217	Sugar	666
13.63	139857904	4.17	204, 217	Sugar	689
13.67	356882592	10.63	204, 217	Sugar	668
13.84	403366432	12.02	204, 217	Sugar	650
13.90	54966288	1.64	204, 217	Sugar	681
13.96	83816112	2.50	204, 217	Sugar	661
14.01	194039552	5.78	204, 217	Sugar	634
14.15	962494848	28.67	204, 217	Sugar	657
14.19	59646300	1.78	204, 217	Sugar	690
14.21	56090120	1.67	204, 217	Sugar	710
14.31	97052984	2.89	204, 217	Sugar	688
14.40	50996436	1.52	204, 217	Sugar	698
14.58	437143296	13.02	204, 217	Sugar	881
14.73	250682816	7.47	204, 217	Sugar	702
Total Relative Area	3356874928	100			

Sapwood Water extract

Retention time	Relative area	% Yield	Fragments	Identified product	Nist Match
14.43	485049888	8.81	204, 217	Sugar	795
14.58	736716864	13.38	204, 217	Sugar	904
14.70	489388576	8.89	204, 217	Sugar	709
19.11	654088128	11.88	204, 217	Sugar	818
19.50	1063624256	19.32	204, 217	Sugar	774
19.79	1440511104	26.16	204, 217	Sugar	816
19.85	636198912	11.56	204, 217	Sugar	783
Total Relative Area	5505577728	100			

GC-MS GARISSA ANALYSES
Small trees
Knotwood DCM extract

Retention time	Relative area	% Yield	Fragments	Identified product	Nist Match
17.45	16607470	4.26		Linoleic acid	826
20.55	25040728	6.42	368, 650	Catechin	603
20.75	220754688	56.59	368, 650	Mesquitol	804
25.93	20167554	5.17		terpene	817
26.20	21339910	5.47		terpene	821
26.26	14659185	3.76		terpene	683
26.39	23567282	6.04		Stigmasterol	827
27.97	47934452	12.29		terpene	879
Total Relative Area	390071269	100			

Knotwood Acetone extract

Retention time	Relative area	% Yield	Fragments	Identified product	Nist Match
14.34	8109753	3.00	204, 217	Sugar	734
14.48	10055120	3.72	204, 217	Sugar	861
20.75	203402576	75.22	368, 650	Mesquitol	782
21.58	48850144	18.06	368, 398, 680	Methylgallocatechin	807
Total Relative Area	270417593	100			

Knotwood Toluene/Ethanol extract

Retention time	Relative area	% Yield	Fragments	Identified product	Nist Match
13.96	14799678	2.76	204, 217	Sugar	691
14.27	97937864	18.27	204, 217	Sugar	844
14.42	180416384	33.66	204, 217	Sugar	914
14.61	20888144	3.90	204, 217	Sugar	719
20.76	176337248	32.90	368, 650	Mesquitol	792
21.60	45621844	8.51	368, 398, 680	Methylgallocatechin	816
Total Relative Area	536001162	100			

Knotwood Water extract

Retention time	Relative area	% Yield	Fragments	Identified product	Nist Match
19.03	498210	39.91		unknown compound	
19.05	168740	13.52		unknown compound	
19.07	160324	12.84		unknown compound	
19.25	168352	13.49		unknown compound	
19.46	74872	6.00		unknown compound	
19.48	177907	14.25		unknown compound	
Total Relative Area	1248406	100			

GC-MS GARISSA ANALYSES
Big trees
Knotwood DCM extract

Retention time	Relative area	% Yield	Fragments	Identified product	Nist Match
16.33	20327418	8.99		Palmitic acid	878
17.43	33937760	15.00		Linoleic acid	858
17.63	23332500	10.31		Octadecanoic acid	856
20.57	17506670	7.74	368, 650	Catechin	582
20.75	44462496	19.65	368, 650	Mesquitol	793
26.12	13992188	6.18		Campesterol	754
26.38	42952708	18.99		Stigmasterol	820
28.03	29721652	13.14		α-Amyrin	684
Total Relative Area	226233392	100			

Knotwood Acetone extract

Retention time	Relative area	% Yield	Fragments	Identified product	Nist Match
20.77	1750309120	96.46	368, 650	Mesquitol	770
21.58	64311584	3.54	368, 398, 680	Methylgallocatechin	768
Total Relative Area	1814620704	100			

Knotwood Toluene/Ethanol extract

Retention time	Relative area	% Yield	Fragments	Identified product	Nist Match
13.36	51012816	3.45		Xylitol (5TMS)	914
14.43	51317896	3.47	204, 217	Sugar	889
20.59	98000288	6.63	368, 650	Catechin	737
20.79	1132639616	76.60	368, 650	Mesquitol	709
21.58	145748400	9.86	368, 398, 680	Methylgallocatechin	684
Total Relative Area	1478719016	100			

Knotwood Water extract

Retention time	Relative area	% Yield	Fragments	Identified product	Nist Match
20.69	6011563	100	368, 650	Mesquitol	787
Total Relative Area	6011563	100			

GC-MS GARISSA ANALYSES
Small trees
Heartwood DCM extract

Retention time	Relative area	% Yield	Fragments	Identified product	Nist Match
20.57	23882092	9.42	368, 650	Catechin	574
20.76	183832224	72.55	368, 650	Mesquitol	791
26.14	10876327	4.29		Campesterol	759
26.40	25162182	9.93		Stigmasterol	812
27.11	9651363	3.81		β-Sitosterol	646
Total Relative Area	253404188	100			

Heartwood Acetone extract

Retention time	Relative area	% Yield	Fragments	Identified product	Nist Match
14.59	69633456	3.86	204, 217	Sugar	609
20.78	848226624	47.00	368, 650	Mesquitol	753
21.42	669784576	37.11		unknown	
21.59	217185952	12.03	368, 398, 680	Methylgallocatechin	828
Total Relative Area	1804830608	100			

Heartwood Toluene/Ethanol extract

Retention time	Relative area	% Yield	Fragments	Identified product	Nist Match
14.26	145549264	11.18	204, 217	Sugar	881
14.41	285609024	21.94	204, 217	Sugar	930
20.77	715529664	54.96	368, 650	Mesquitol	799
21.58	155228320	11.92	368, 398, 680	Methylgallocatechin	824
Total Relative Area	1301916272	100			

Heartwood Water extract

Retention time	Relative area	% Yield	Fragments	Identified product	Nist Match
20.69	5676107	83.61	368, 650	Mesquitol	783
21.52	1113067	16.39	368, 398, 680	Methylgallocatechin	645
Total Relative Area	6789173	100			

GC-MS GARISSA ANALYSES
Big trees
Heartwood DCM extract

Retention time	Relative area	% Yield	Fragments	Identified product	Nist Match
16.44	2640504	2.93		Palmitic acid	629
20.58	5609598	6.22	368, 650	Catechin	611
20.75	48066156	53.33	368, 650	Mesquitol	796
22.40	8690745	9.64		alcohol	666
24.94	9294237	10.31		alcohol	695
26.14	5006996	5.56		Campesterol	654
26.39	10823183	12.01		Stigmasterol	743
Total Relative Area	90131419	100			

Heartwood Acetone extract

Retention time	Relative area	% Yield	Fragments	Identified product	Nist Match
20.58	229766816	11.65	368, 650	Catechin	626
20.83	1554964992	78.81	368, 650	Mesquitol	751
21.59	188305168	9.54	368, 398, 680	Methylgallocatechin	834
Total Relative Area	1973036976	100			

Heartwood Toluene/Ethanol extract

Retention time	Relative area	% Yield	Fragments	Identified product	Nist Match
14.26	90325448	4.25	204, 217	Sugar	869
14.41	138522080	6.52	204, 217	Sugar	927
20.60	219959888	10.35	368, 650	Catechin	716
20.84	1485352064	69.92	368, 650	Mesquitol	732
21.59	146150032	6.88	368, 398, 680	Methylgallocatechin	823
22.40	44162392	2.08		flavonoid	716
Total Relative Area	2124471904	100			

Heartwood Water extract

Retention time	Relative area	% Yield	Fragments	Identified product	Nist Match
20.54	1066652	4.04	368, 650	Catechin	626
20.67	23673922	89.76	368, 650	Mesquitol	806
21.51	1632900	6.19	368, 398, 680	Methylgallocatechin	734
Total Relative Area	26373473	100			

GC-MS GARISSA ANALYSES
Small trees
Pith DCM extract

Retention time	Relative area	% Yield	Fragments	Identified product	Nist Match
14.62	3897524	2.10	204, 217	Sugar	649
20.57	9238814	4.98	368, 650	Catechin	590
20.76	108741472	58.56	368, 650	Mesquitol	806
24.96	7600976	4.09		alcohol	622
26.14	14029341	7.55		Campesterol	772
26.39	29321968	15.79		Stigmasterol	793
27.10	12870312	6.93		β-Sitosterol	701
Total Relative Area	185700407	100			

Pith Acetone extract

Retention time	Relative area	% Yield	Fragments	Identified product	Nist Match
14.43	112780896	4.68	204, 217	Sugar	907
20.60	137029776	5.69	368, 650	Catechin	727
20.82	1547029888	64.19	368, 650	Mesquitol	766
21.62	613403584	25.45	368, 398, 680	Methylgallocatechin	819
Total Relative Area	2410244144	100			

Pith Toluene/Ethanol extract

Retention time	Relative area	% Yield	Fragments	Identified product	Nist Match
14.25	368784352	13.22	204, 217	Sugar	870
14.42	704776704	25.26	204, 217	Sugar	915
20.59	81723648	2.93	368, 650	Catechin	617
20.79	1172901760	42.03	368, 650	Mesquitol	752
21.60	462247168	16.57	368, 398, 680	Methylgallocatechin	832
Total Relative Area	2790433632	100			

Pith Water extract

Retention time	Relative area	% Yield	Fragments	Identified product	Nist Match
20.69	4011396	81.83	368, 650	Mesquitol	768
21.52	890543	18.17	368, 398, 680	Methylgallocatechin	717
Total Relative Area	4901939	100			

GC-MS GARISSA ANALYSES
Big trees
Pith DCM extract

Retention time	Relative area	% Yield	Fragments	Identified product	Nist Match
20.57	16847972	7.28	368, 650	Catechin	592
20.75	87260464	37.69	368, 650	Mesquitol	782
22.38	7708051	3.33		alcohol	728
24.92	8658940	3.74		alcohol	649
26.12	21940536	9.48		Campesterol	823
26.38	52778748	22.80		Stigmasterol	820
27.09	14226494	6.15		β-Sitosterol	704
28.02	22072112	9.53		Betulin	673
Total Relative Area	231493317	100			

Pith Acetone extract

Retention time	Relative area	% Yield	Fragments	Identified product	Nist Match
20.57	21961698	3.98	368, 650	Catechin	650
20.76	492215904	89.10	368, 650	Mesquitol	755
21.58	38242416	6.92	368, 398, 680	Methylgallocatechin	770
Total Relative Area	552420018	100			

Pith Toluene/Ethanol extract

Retention time	Relative area	% Yield	Fragments	Identified product	Nist Match
12.21	65884904	3.41	204, 217	Sugar	866
12.57	62835576	3.26	204, 217	Sugar	920
14.26	66203376	3.43	204, 217	Sugar	876
14.41	96542912	5.00	204, 217	Sugar	917
20.59	133676808	6.93	368, 650	Catechin	753
20.79	1331481728	69.00	368, 650	Mesquitol	766
21.58	172961360	8.96	368, 398, 680	Methylgallocatechin	829
Total Relative Area	1929586664	100			

Pith Water extract

Retention time	Relative area	% Yield	Fragments	Identified product	Nist Match
20.70	7345591	100.00	368, 650	Mesquitol	780
Total Relative Area	7345591	100			

GC-MS TURKANA ANALYSES from small trees

Bark DCM extract

Retention time	Relative area	% Yield	Fragments	Identified product	Nist Match
16.41	6817704	2.36	117, 328	Fatty acid (Palmitic acid)	783
17.52	6356239	2.20	117, 234, 352	Fatty acid (Linoleic acid)	713
22.39	7463202	2.59	439	Alcohol (Hexacosane)	709
24.93	42377436	14.69	468	Alcohol (Octacosane)	606
26.13	19187756	6.65	129, 343, 472	Sterol (Campesterol)	762
26.39	58222736	20.19	129, 355, 484	Sterol (Stigmasterol)	857
27.10	25605676	8.88	129, 357, 486	Sterol (β-Sitosterol)	697
27.49	18219166	6.32		Linoleic acid	661
28.05	85765832	29.74		Betulin	703
30.55	18373918	6.37		Linoleic acid	622
Total Relative Area	288389665	100			

Bark Acetone extract

Retention time	Relative area	% Yield	Fragments	Identified product	Nist Match
14.20	11999539	1.53	204, 217	Sugar	857
14.27	76044296	9.68	204, 217	Sugar	922
14.43	116171976	14.79	204, 217	Sugar	692
14.57	50435220	6.42	204, 217	Sugar	692
20.76	20256228	2.58	368, 650	Mesquitol	782
21.56	510558208	65.00	368, 398, 680	Methylgallocatechin	607
Total Relative Area	785465467	100			

Bark Toluene/Ethanol extract

Retention time	Relative area	% Yield	Fragments	Identified product	Nist Match
14.27	238943168	25.38	204, 217	Sugar	857
14.42	303227872	32.21	204, 217	Sugar	882
20.75	14984060	1.59	368, 650	Mesquitol	794
21.54	384325824	40.82	368, 398, 680	Methylgallocatechin	801
Total Relative Area	941480924	100			
Compounds detected	6				

Bark Water extract

Retention time	Relative area	% Yield	Fragments	Identified product	Nist Match
14.30	29295436	40.60	204, 217	Sugar	866
14.45	42866516	59.40	204, 217	Sugar	888
Total Relative Area	72161952	100			

GC-MS TURKANA ANALYSES from bg trees

Bark DCM extract

Retention time	Relative area	% Yield	Fragments	Identified product	Nist Match
19.33	25180060	7.27	218	β-Amyrin	810
20.32	30608862	8.84	218	α-Amyrin	808
24.94	22528922	6.51	468	Alcohol (Octacosane)	802
26.13	31188594	9.01	129, 343, 472	Sterol (Campesterol)	735
26.40	42447656	12.26	129, 355, 484	Sterol (Stigmasterol)	827
27.10	43767268	12.64	129, 357, 486	Sterol (β-Sitosterol)	720
27.51	24737136	7.14		Linoleic acid	674
28.04	125832488	36.34		Betulin	697
Total Relative Area	346290986	100			

Bark Acetone extract

Retention time	Relative area	% Yield	Fragments	Identified product	Nist Match
14.27	75662304	24.01	204, 217	Sugar	868
14.42	57492248	18.25	204, 217	Sugar	905
14.48	6345181	2.01	204, 217	Sugar	734
20.76	3357829	1.07	368, 650	Mesquitol	697
21.53	64572324	20.49	368, 398, 680	Methylgallocatechin	470
28.05	107665176	34.17		Linoleic acid	671
Total Relative Area	315095062	100			

Bark Toluene/Ethanol extract

Retention time	Relative area	% Yield	Fragments	Identified product	Nist Match
13.36	9383263	1.92		Xylitol	879
13.82	17324488	3.54		D-Glucitol	892
14.27	256291280	52.39	204, 217	Sugar	869
14.43	181077856	37.02	204, 217	Sugar	920
21.54	25115718	5.13	368, 398, 680	Methylgallocatechin	440
Total Relative Area	489192605	100			
Compounds detected	6				

Bark Water extract

Retention time	Relative area	% Yield	Fragments	Identified product	Nist Match
14.82	159254768	83.49		unknown compound	
21.53	31481568	16.51	368, 398, 680	Methylgallocatechin	593
Total Relative Area	190736336				

GC-MS TURKANA ANALYSES from small trees
Sapwood DCM extract

Retention time	Relative area	% Yield	Fragments	Identified product	Nist Match
14.27	173057248	13.18	204, 217	Sugar	872
14.42	173773456	13.23	204, 217	Sugar	918
15.50	136790000	10.42		Unknown compound	
16.28	219788160	16.73		Palmitic acid	902
17.39	177839184	13.54		Linoleic acid	917
17.42	169393200	12.90		trans-9-octadecenoate	898
26.40	146150864	11.13		Stigmasterol	884
28.05	116595744	8.88		Betulin	700
Total Relative Area	1313387856	100			

Sapwood Acetone extract

Retention time	Relative area	% Yield	Fragments	Identified product	Nist Match
13.95	405568608	8.75	204, 217	Sugar	709
14.02	761396544	16.42	204, 217	Sugar	652
14.16	319447104	6.89	204, 217	Sugar	673
14.20	263745456	5.69	204, 217	Sugar	714
14.28	729280640	15.73	204, 217	Sugar	823
14.33	91391064	1.97	204, 217	Sugar	666
14.46	1646059648	35.50	204, 217	Sugar	914
14.59	378186592	8.16	204, 217	Sugar	706
14.69	41853560	0.90	204, 217	Sugar	672
Total Relative Area	4636929216	100			

Bush Sapwood Toluene/Ethanol extract

Retention time	Relative area	% Yield	Fragments	Identified product	Nist Match
14.36	2763506176	40.59	204, 217	Sugar	844
14.54	3381570816	49.67	204, 217	Sugar	878
14.60	139762576	2.05	204, 217	Sugar	732
15.46	80558464	1.18	204, 217	Sugar	884
15.64	85101152	1.25	204, 217	Sugar	904
15.80	135410032	1.99	204, 217	Sugar	858
19.63	131273880	1.93	204, 217	Sugar (disaccharide)	906
20.75	90506080	1.33	368, 650	Mesquitol	765
Total Relative Area	6807689176	100			

Sapwood Water extract

Retention time	Relative area	% Yield	Fragments	Identified product	Nist Match
14.29	1197734	24.99		Unknown compound	
14.31	860968	17.96	204, 217	Sugar	587
14.55	1092979	22.80	204, 217	Sugar	736
14.60	892027	18.61	204, 217	Sugar	700
14.70	340777	7.11	204, 217	Sugar	644
14.71	409082	8.53	204, 217	Sugar	641
Total Relative Area	4793567	100			

GC-MS TURKANA ANALYSES from big trees
Sapwood DCM extract

Retention time	Relative area	% Yield	Fragments	Identified product	Nist Match
11.75	15354787	5.32		Vanillin	876
14.29	12010981	4.16	204, 217	Sugar	715
16.57	45277740	15.68		Unknown compound	
26.15	25471482	8.82		Campesterol	809
26.41	53501504	18.52		Stigmasterol	852
27.11	32885782	11.39		β-Sitosterol TMS	772
27.55	15293002	5.29		Linoleic acid	649
28.06	89025360	30.82		Betulin	708
Total Relative Area	288820638	100			

Sapwood Acetone extract

Retention time	Relative area	% Yield	Fragments	Identified product	Nist Match
13.36	114310248	7.90		D-(+)-Arabitol	920
13.82	29213622	2.02		D-Glucitol	909
14.00	15945112	1.10	204, 217	Sugar	674
14.20	55282712	3.82	204, 217	Sugar	712
14.29	661618240	45.70	204, 217	Sugar	879
14.43	498585792	34.44	204, 217	Sugar	921
14.58	31504560	2.18	204, 217	Sugar	724
15.25	26240540	1.81		Mannitol, (6TMS)-	902
20.77	15046206	1.04	368, 650	Mesquitol	754
Total Relative Area	1447747032	100			

Sapwood Toluene/Ethanol extract

Retention time	Relative area	% Yield	Fragments	Identified product	Nist Match
13.36	254586640	5.80		L-(-)-Arabitol	921
13.82	129637288	2.96		D-Glucitol	904
13.99	177125568	4.04	204, 217	Sugar	663
14.16	60476916	1.38	204, 217	Sugar	683
14.22	211367152	4.82	204, 217	Sugar	729
14.32	1789457024	40.79	204, 217	Sugar	886
14.46	1257799296	28.67	204, 217	Sugar	904
14.59	56546736	1.29	204, 217	Sugar	692
15.25	218414816	4.98		Mannitol	920
15.47	103274832	2.35	204, 217	Sugar	859
15.81	128095312	2.92	204, 217	Sugar	876
Total Relative Area	4386781580	100			

Sapwood Water extract

Retention time	Relative area	% Yield	Fragments	Identified product	Nist Match
13.52	719073	5.69	204, 217	Sugar	764
14.36	4846304	38.35	204, 217	Sugar	731
14.60	1122365	8.88	204, 217	Sugar	665
15.43	1361689	10.78	204, 217	Sugar	661
15.45	646052	5.11	204, 217	Sugar	666
19.32	1739016	13.76	204, 217	Sugar	631
19.59	2201160	17.42	204, 217	Sugar	611
Total Relative Area	12635658	100			

GC-MS TURKANA ANALYSES
Small trees
Knotwood DCM extract

Retention time	Relative area	% Yield	Fragments	Identified product	Nist Match
16.30	100626696	20.84		Palmitic acid	820
17.44	180888752	37.46		(E)-9-Octadecenoic acid	892
17.62	18241290	3.78		Octadecanoic acid	847
20.77	19330914	4.00	368, 650	Mesquitol	762
24.94	39463312	8.17		Octacosane	635
26.13	17284410	3.58		Campesterol	839
26.40	60833744	12.60		Stigmasterol	853
27.10	17428414	3.61		β-Sitosterol	767
28.04	28768316	5.96		Betulin	684
Total Relative Area	482865848	100			

Knotwood Acetone extract

Retention time	Relative area	% Yield	Fragments	Identified product	Nist Match
13.94	134172472	6.23	204, 217	Sugar	694
14.00	44641360	2.07	204, 217	Sugar	656
14.16	37949544	1.76	204, 217	Sugar	686
14.19	60144252	2.79	204, 217	Sugar	731
14.26	237853168	11.04	204, 217	Sugar	774
14.43	498727168	23.14	204, 217	Sugar	911
14.56	201060800	9.33	204, 217	Sugar	697
20.76	438010496	20.32	368, 650	Mesquitol	773
21.39	32921896	1.53		flavonoid	627
21.60	469666016	21.79	368, 398, 680	Methylgallocatechin	645
Total Relative Area	2155147172	100			

Knotwood Toluene/Ethanol extract

Retention time	Relative area	% Yield	Fragments	Identified product	Nist Match
13.94	107562776	3.77	204, 217	Sugar	696
13.99	220939088	7.75	204, 217	Sugar	648
14.15	94254552	3.31	204, 217	Sugar	698
14.19	93580672	3.28	204, 217	Sugar	733
14.28	749032128	26.27	204, 217	Sugar	873
14.44	1233928832	43.28	204, 217	Sugar	921
14.56	351939744	12.34	204, 217	Sugar	692
Total Relative Area	2851237792	100			

Bush Knotwood Outer core Water extract

Retention time	Relative area	% Yield	Fragments	Identified product	Nist Match
20.72	686156	51.40	368, 650	Mesquitol	640
21.53	648904	48.60	368, 398, 680	Methylgallocatechin	666
Total Relative Area	1335060	100			

GC-MS TURKANA ANALYSES
Big trees
Knotwood DCM extract

Retention time	Relative area	% Yield	Fragments	Identified product	Nist Match
20.78	11205530	20.70	368, 650	Mesquitol	758
26.16	6473660	11.96		Campesterol	714
26.42	16385507	30.28		Stigmasterol	788
27.13	4517336	8.35		β-Sitosterol	651
28.06	15538386	28.71		Linoleic acid	676
Total Relative Area	54120419	100			

Knotwood Acetone extract

Retention time	Relative area	% Yield	Fragments	Identified product	Nist Match
14.26	84469376	5.09	204, 217	Sugar	855
20.61	97097960	5.85	368, 650	Catechin	713
20.83	1199601408	72.30	368, 650	Mesquitol	747
21.61	224699200	13.54	368, 398, 680	Methylgallocatechin	683
22.41	53396176	3.22		flavonoid	
Total Relative Area	1659264120	100			

Knotwood Toluene/Ethanol extract

Retention time	Relative area	% Yield	Fragments	Identified product	Nist Match
13.35	79604304	4.04		Xylitol (5TMS)	915
14.28	488565408	24.82	204, 217	Sugar	814
14.42	291909344	14.83	204, 217	Sugar	899
20.61	54216992	2.75	368, 650	Catechin	758
20.81	878286848	44.61	368, 650	Mesquitol	763
21.60	120585976	6.13	368, 398, 680	Methylgallocatechin	844
22.42	55437824	2.82		flavonoid	
Total Relative Area	1968606696	100			

Knotwood Outer Water extract

Retention time	Relative area	% Yield	Fragments	Identified product	Nist Match
14.84	3371603	17.02	204, 217	Sugar	547
20.69	14757506	74.49	368, 650	Mesquitol	789
21.52	1683424	8.50	368, 398, 680	Methylgallocatechin	774
Total Relative Area	19812533	100			

GC-MS TURKANA ANALYSES
Small trees
Heartwood DCM extract

Retention time	Relative area	% Yield	Fragments	Identified product	Nist Match
11.44	100185080	37.83		Unknown compound	
16.33	16601558	6.27		Palmitic acid	868
17.49	17998606	6.80		Linoleic acid	807
20.77	15239419	5.75	368, 650	Mesquitol	806
26.14	23281786	8.79		Campesterol	806
26.40	51849320	19.58		Stigmasterol	847
27.10	22865036	8.63		β-Sitosterol	727
28.04	16808772	6.35		Linoleic acid	672
Total Relative Area	264829577	100			

Heartwood Acetone extract

Retention time	Relative area	% Yield	Fragments	Identified product	Nist Match
14.26	95411296	3.78	204, 217	Sugar	837
14.41	128455736	5.10	204, 217	Sugar	917
14.56	139177520	5.52	204, 217	Sugar	681
20.60	66457292	2.64	368, 650	Catechin	708
20.81	769356096	30.52	368, 650	Mesquitol	719
21.38	161456016	6.40		flavonoid	
21.66	1160804864	46.04	368, 398, 680	Methylgallocatechin	811
Total Relative Area	2521118820	100			

Heartwood Toluene/Ethanol extract

Retention time	Relative area	% Yield	Fragments	Identified product	Nist Match
12.58	185703088	5.75		Arabose	936
14.28	670787200	20.75	204, 217	Sugar	878
14.44	885952000	27.41	204, 217	Sugar	914
20.80	667823872	20.66	368, 650	Mesquitol	775
21.64	821696640	25.42	368, 398, 680	Methylgallocatechin	809
Total Relative Area	3231962800	100			

Heartwood Water extract

Retention time	Relative area	% Yield	Fragments	Identified product	Nist Match
20.67	1074632	70.44	368, 650	Mesquitol	681
21.49	450880	29.56	368, 398, 680	Methylgallocatechin	675
Total Relative Area	1525512	100			

GC-MS TURKANA ANALYSES
Big trees
Heartwood DCM extract

Retention time	Relative area	% Yield	Fragments	Identified product	Nist Match
20.77	41413772	41.61	368, 650	Mesquitol	790
21.45	14354319	14.42		Unknown compound	
24.95	7614277	7.65		Linoleic acid	526
26.15	9917328	9.96		Campesterol	726
26.41	18763034	18.85		Stigmasterol	792
27.11	7467193	7.50		β-Sitosterol	672
Total Relative Area	99529923	100			

Heartwood Acetone extract

Retention time	Relative area	% Yield	Fragments	Identified product	Nist Match
20.62	148051376	5.18	368, 650	Catechin	717
20.85	2067090688	72.39	368, 650	Mesquitol	745
21.42	409519520	14.34		Unknown compound	
21.60	154658000	5.42	368, 398, 680	Methylgallocatechin	811
22.42	76214040	2.67		flavonoid	668
Total Relative Area	2855533624	100			

Heartwood Toluene/Ethanol extract

Retention time	Relative area	% Yield	Fragments	Identified product	Nist Match
14.27	155828000	8.20	204, 217	Sugar	863
14.56	54956276	2.89	204, 217	Sugar	698
20.61	100611104	5.30	368, 650	Catechin	722
20.82	1423060992	74.90	368, 650	Mesquitol	712
21.60	107523352	5.66	368, 398, 680	Methylgallocatechin	828
22.42	58048496	3.06		flavonoid	723
Total Relative Area	1900028220	100			

Heartwood Water extract

Retention time	Relative area	% Yield	Fragments	Identified product	Nist Match
20.77	38578912	81.98	368, 650	Mesquitol	793
21.61	8477971	18.02	368, 398, 680	Methylgallocatechin	791
Total Relative Area	47056883	100			

GC-MS TURKANA ANALYSES
Small trees
Pith DCM extract

Retention time	Relative area	% Yield	Fragments	Identified product	Nist Match
11.47	91649296	27.64		Unknown compound	
16.32	39996924	12.06		Palmitic acid	893
17.64	19634494	5.92		Linoleic acid	716
26.13	22659394	6.83		Campesterol	775
26.38	62420936	18.82		Stigmasterol	808
27.09	50969632	15.37		β-Sitosterol	718
28.03	44271880	13.35		Linoleic acid	670
Total Relative Area	331602556	100			

Pith Acetone extract

Retention time	Relative area	% Yield	Fragments	Identified product	Nist Match
13.93	50354684	2.04	204, 217	Sugar	705
14.26	87740920	3.56	204, 217	Sugar	830
14.41	58299164	2.37	204, 217	Sugar	908
14.56	117951880	4.79	204, 217	Sugar	702
20.61	91446528	3.71	368, 650	Catechin	760
20.86	934186816	37.93	368, 650	Mesquitol	739
21.67	1122849408	45.59	368, 398, 680	Methylgallocatechin	804
Total Relative Area	2462829400	100			

Pith Toluene/Ethanol extract

Retention time	Relative area	% Yield	Fragments	Identified product	Nist Match
12.18	65147820	1.92	204, 217	Sugar	899
12.58	192570848	5.68	204, 217	Sugar	914
14.19	78059168	2.30	204, 217	Sugar	732
14.27	651109056	19.19	204, 217	Sugar	860
14.44	845987200	24.94	204, 217	Sugar	916
14.56	197126640	5.81	204, 217	Sugar	701
20.80	756917440	22.31	368, 650	Mesquitol	761
21.62	605308544	17.84	368, 398, 680	Methylgallocatechin	826
Total Relative Area	3392226716	100			

Pith Water extract

Retention time	Relative area	% Yield	Fragments	Identified product	Nist Match
20.76	57864	100	368, 650	Mesquitol	
Total Relative Area	57864	100			

GC-MS TURKANA ANALYSES
Big trees
Pith DCM extract

Retention time	Relative area	% Yield	Fragments	Identified product	Nist Match
20.77	12153943	29.46	368, 650	Mesquitol	746
24.95	7754484.5	18.80		Linoleic acid	525
26.15	7474927.5	18.12		Campesterol	690
26.41	13865593	33.61		Stigmasterol	760
Total Relative Area	41248948	100			

Pith Acetone extract

Retention time	Relative area	% Yield	Fragments	Identified product	Nist Match
20.61	63721152	4.45	368, 650	Catechin	749
20.83	1152857472	80.48	368, 650	Mesquitol	745
21.61	123067400	8.59	368, 398, 680	Methylgallocatechin	840
22.43	92880136	6.48		flavonoid	727
Total Relative Area	1432526160	100			

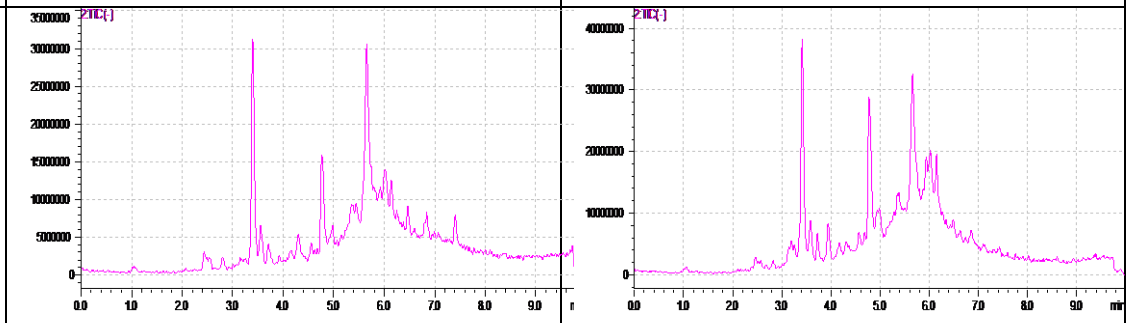
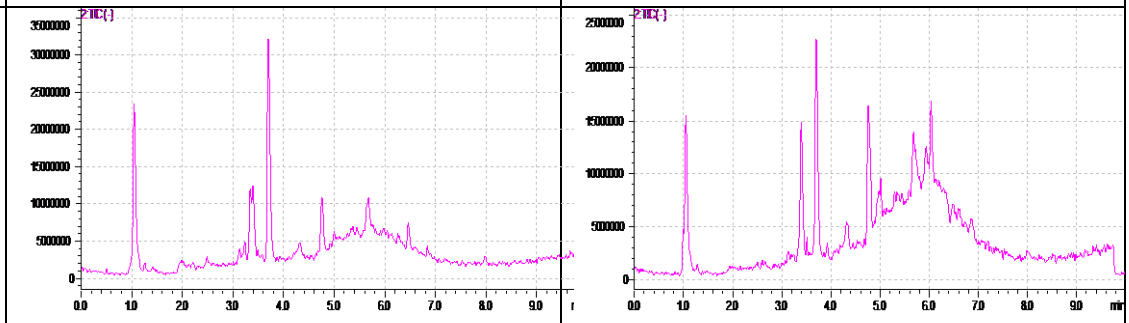
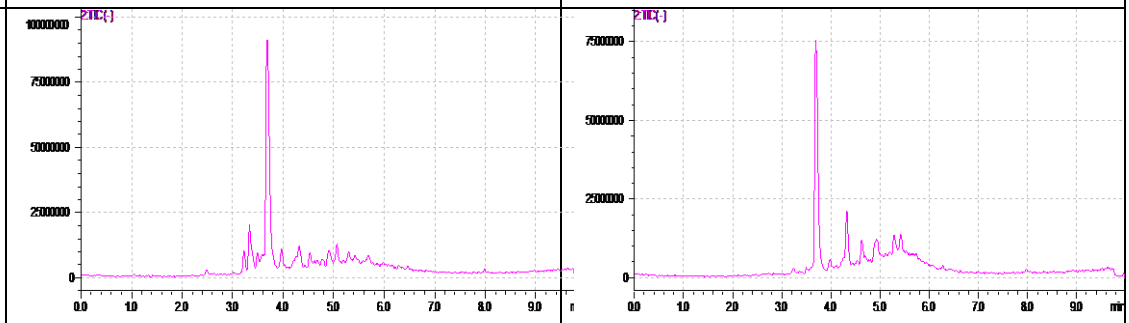
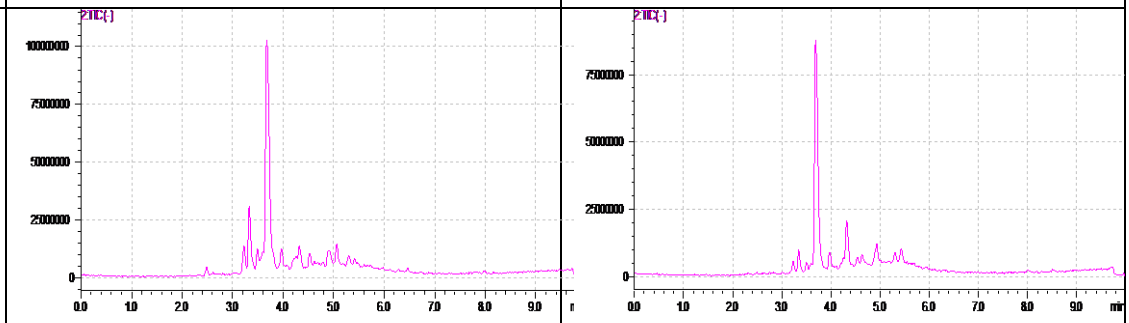
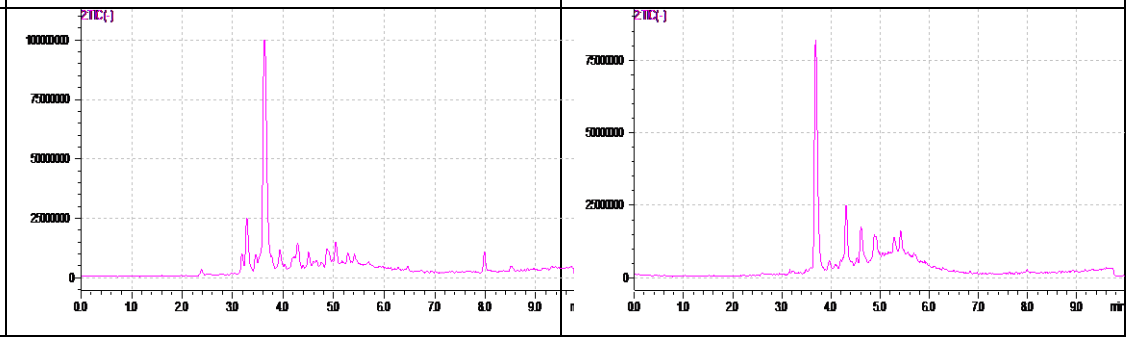
Pith Toluene/Ethanol extract

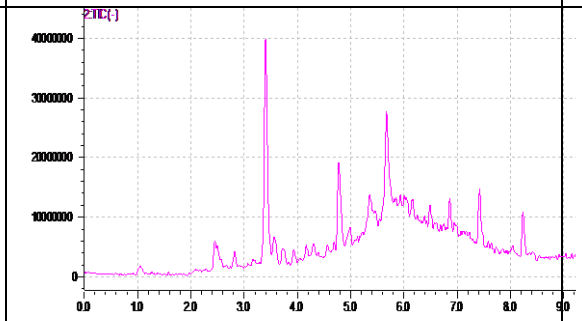
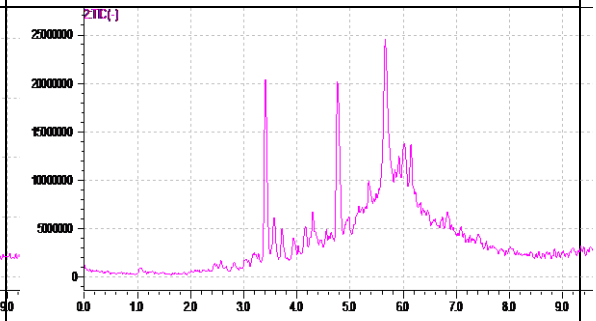
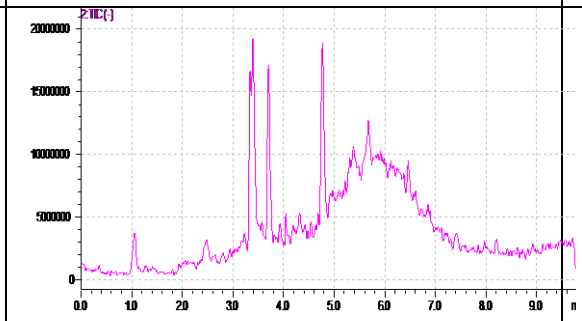
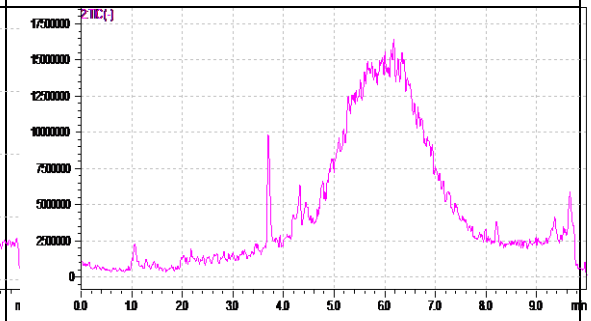
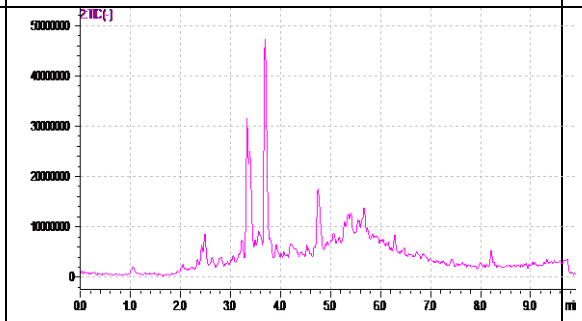
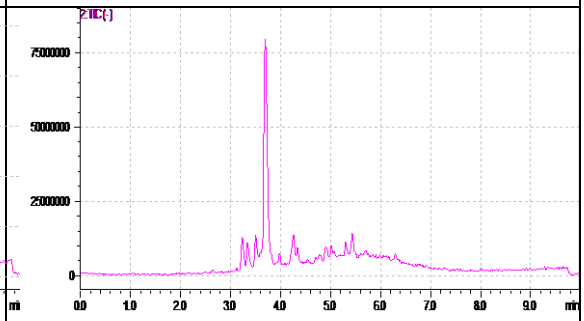
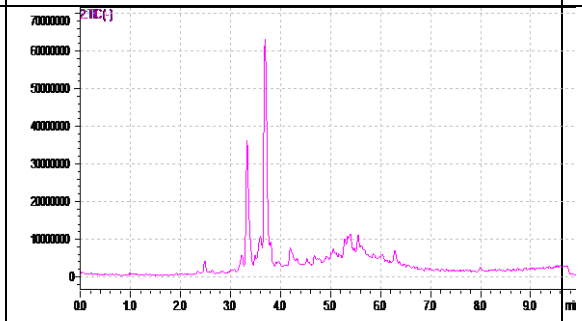
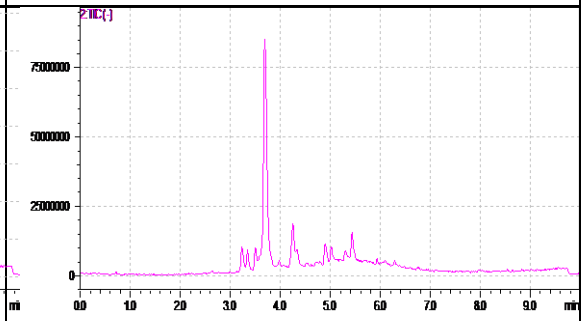
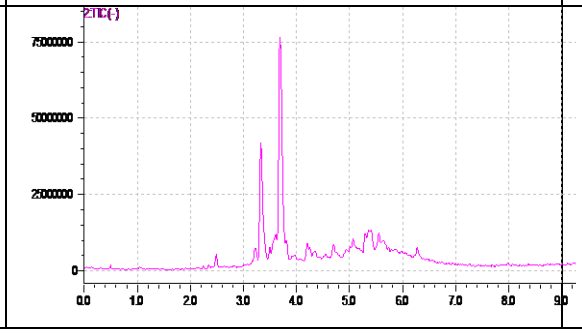
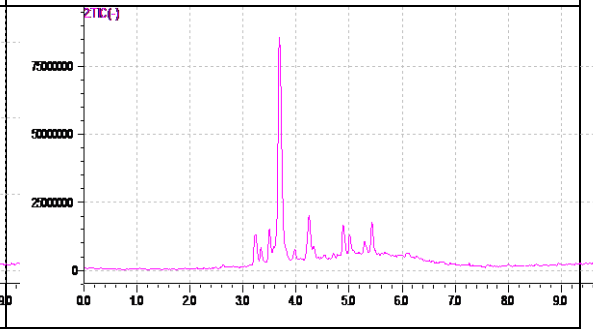
Retention time	Relative area	% Yield	Fragments	Identified product	Nist Match
12.59	57730444	2.55	204, 217	Sugar	919
14.28	192377744	8.48	204, 217	Sugar	849
14.43	67761784	2.99	204, 217	Sugar	925
20.62	123855112	5.46	368, 650	Catechin	740
20.84	1596402304	70.40	368, 650	Mesquitol	742
21.60	112188016	4.95	368, 398, 680	Methylgallocatechin	710
22.43	117383264	5.18		flavonoid	730
Total Relative Area	2267698668	100			

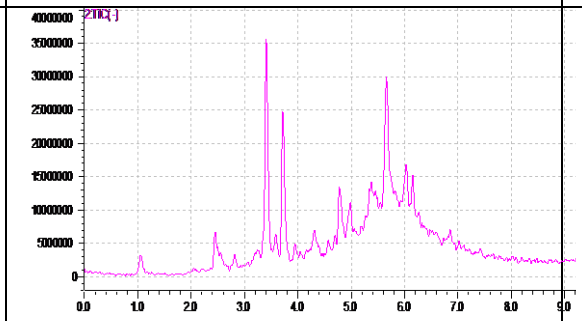
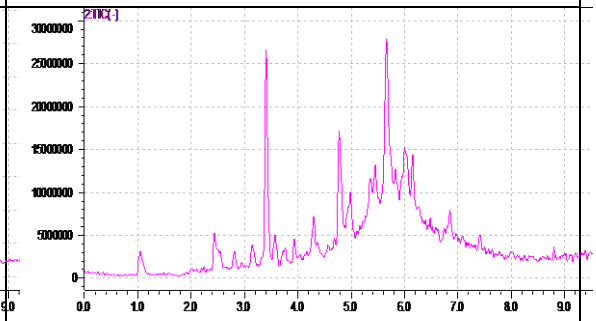
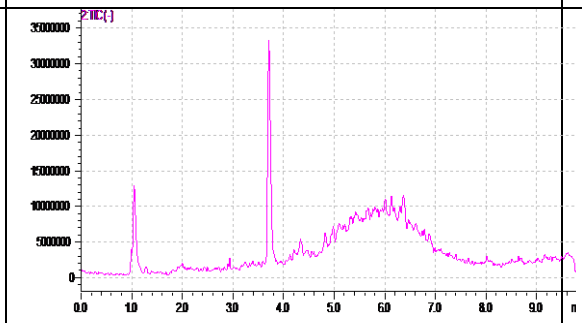
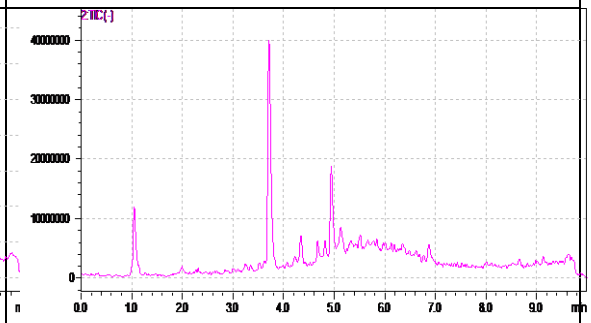
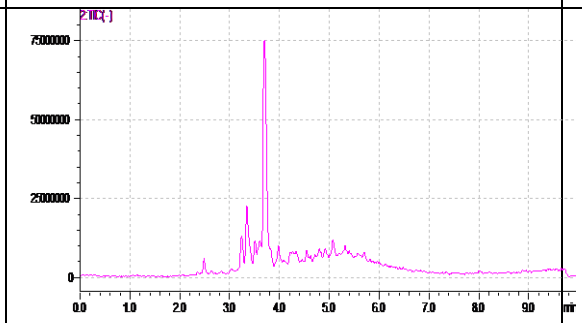
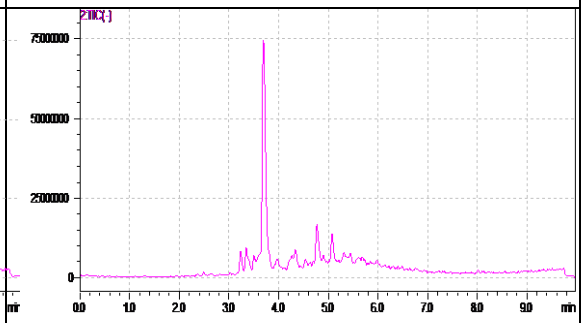
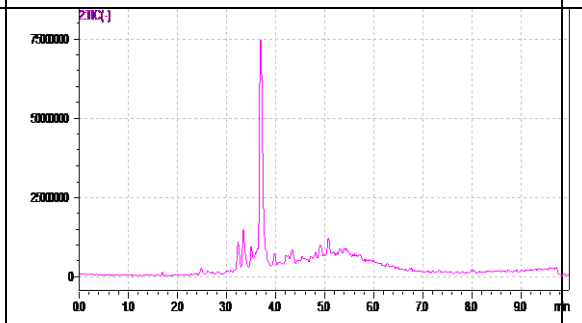
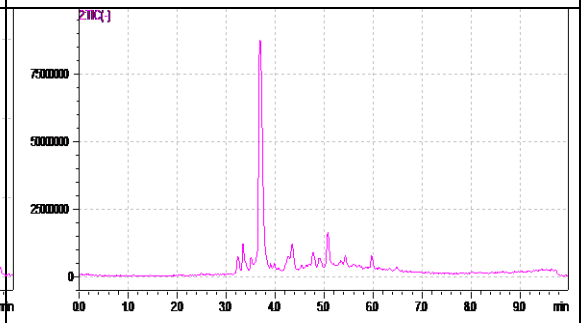
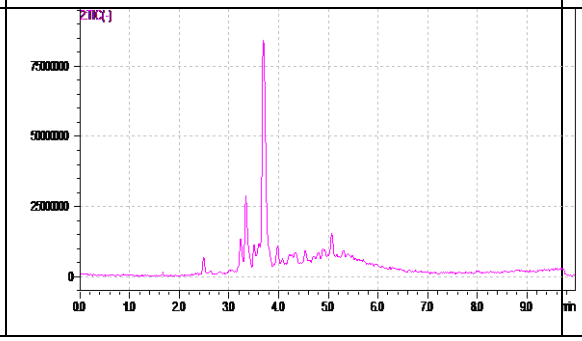
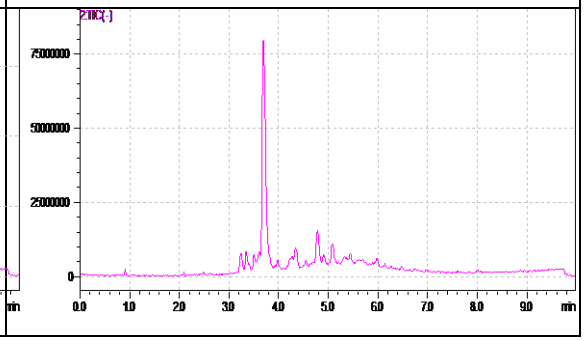
Pith Water extract

Retention time	Relative area	% Yield	Fragments	Identified product	Nist Match
20.77	46795840	89.04	368, 650	Mesquitol	782
21.62	5760964	10.96	368, 398, 680	Methylgallocatechin	788
Total Relative Area	52556803.5	100			

ANNEX 2: LC chromatograms obtained from the different acetonic extracts.

	BARINGO COUNTY	
	Small trees	Big trees
Bark		
Sap wood		
Knot wood		
Heart wood		
Pith		

	TURKANA COUNTY	
	Small trees	Big trees
Bark		
Sap wood		
Knot wood		
Heart wood		
Pith		

	GARISSA COUNTY	
	Small trees	Big trees
Bark		
Sap wood		
Knot wood		
Heart wood		
Pith		

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RÉSUMÉ

Prosopis juliflora a été classé parmi les pires espèces envahissantes au monde et par conséquent, cette espèce se répand rapidement dans des zones destinées à l'agriculture ou le pâturage. Cette étude visait donc à trouver des solutions face cette menace à travers la valorisation du composé le plus abondant de *P. juliflora* : le mesquitol. En effet, des travaux antérieurs effectués au laboratoire (LERMaB, France) ont permis de mettre en évidence que le bois de *Prosopis juliflora* contient des quantités importantes de mesquitol (2-(3,4-dihydroxyphenyl)chromane-3,7,8-triol), un flavonoïde relativement rare présentant des propriétés antioxydantes. La stratégie mise en place vise donc à utiliser ce composé en tant que matière première pour obtenir, par modifications chimiques, des dérivés antifongiques pour des applications dans la préservation du bois.

En l'absence de données précises sur les proportions de mesquitol dans *P. juliflora* ou sur la variabilité en fonction de l'emplacement géographique de l'arbre ou l'âge de l'arbre, la première partie de l'étude a été consacrée à l'établissement de ces données. Pour cela des échantillons de *P. juliflora* de trois différentes régions géographiques du Kenya (Baringo County, County Garissa et Turkana County) ont été collectés. Les arbres âgés de moins de quatre ans et ceux âgés de plus de huit ans ont été étudiés. Chaque échantillon a été séparé en cinq parties : l'écorce, l'aubier, les nœuds, le bois de cœur et la moelle. Des tests de variabilité ont été réalisé en comparant les rendements d'extractions avec différents solvants (dichlorométhane, acétone, toluène : éthanol (2:1) et eau). Les extraits ont été caractérisés par GC-MS et la HPLC-MS. La quantification de certains des composés identifiés a été effectuée en mode d'ionisation négative en HPLC-MS. Les résultats ont été comparés en fonction des différentes régions géographiques, de l'âge de l'arbre et des différentes parties de la tige, l'accent étant mis principalement sur le mesquitol. L'étude des spectres de masse a permis de mettre en lumière la richesse de *P. juliflora* en composés phénoliques. Parmi ces composés, un dimère de mesquitol a été identifié pour la première fois. Les résultats ont également montré que le mesquitol est bien le composé le plus abondant dans *P. juliflora*. Les rendements d'extractions les plus élevés ont été obtenus dans les extraits acétoniques du bois de cœur et de la moelle (47 - 72%). Des variations de son abondance ont également été observées en fonction de l'âge de l'arbre et de la zone géographique : les taux les plus élevés ont été observés dans le bois de coeur des grands arbres de Baringo.

La seconde partie de l'étude porte sur l'utilisation du mesquitol comme matière première pour la synthèse, par modifications chimiques, de différents dérivés dans le but d'améliorer la lipophilie de ce composé, ce qui pourrait contribuer à réduire sa lixiviabilité. Les réactions du mesquitol avec divers acides carboxyliques ont permis d'obtenir des dérivés greffés avec des chaînes grasses en position aliphatique. Les propriétés antifongiques de certains de ces dérivés ont été testées pour évaluer leur potentiel pour des applications dans la protection du bois. D'après les résultats de cette étude, il est évident que les dérivés du mesquitol obtenus par réaction avec des acides carboxyliques peuvent être utilisés comme agents de préservation du bois lorsqu'ils sont utilisés avec de faibles quantités de tébuconazole.

ABSTRACT

Earlier work from the laboratory (LERMaB, France) helped to highlight that the heartwood of *Prosopis juliflora* contains significant amounts of mesquitol, a relatively rare flavonoid with antioxidant properties, otherwise identified as 2-(3,4-dihydroxyphenyl)chromane-3,7,8-triol. *P. juliflora* has been rated among the worst world invasive species and therefore this study aimed at finding solutions to this menace that is fast spreading into areas that are otherwise intended for useful purposes like farming or grazing. The study's strategy was to use mesquitol, the most abundant compound in *P. juliflora*, as a raw material to obtain some targeted derivatives through chemical modification envisaging their potential valorization in use as antifungal agents for wood preservation.

With no data existing on the exact amounts of mesquitol present in *P. juliflora* or its variability depending on the geographic location of the tree or the tree's age, the study commenced by first availing this data. This was done by a substantive study on *P. juliflora* stem samples that had been collected from three different geographic regions in Kenya (Baringo County, Garissa County and Turkana County). Trees aged less than four years and those aged more than eight years were studied. Each sample had been further divided into five different stem parts that included the bark, sapwood, knot wood, heartwood and the pith. The variability test was done by comparing the percentage yields of extractives using different solvents (dichloromethane, acetone, toluene: ethanol (2:1) and water). This was followed by the identification of the compounds present in the extracts using both GC-MS and LC-ESI-MS/MS. Quantification of some of the identified compounds was done in the negative ion mode of the LC-ESI-MS/MS. Comparisons of some of these compounds were done according to the different geographic regions, the age of the tree and the different parts of the stem with the main emphasis on mesquitol.

A systematic study on the mass spectra and the observed fragmentations illustrated a rich array of phenolic compounds present in *P. juliflora.* Among these compounds is a mesquitol dimer, which has been identified for the very first time here. The results also showed that mesquitol compound is the most abundant compound in *P. juliflora* with high amounts being found in the heartwood and pith of the acetonic extract (47 – 72 %). Its abundance was also found to vary depending on the age of the tree and on the geographical area with the highest amounts of mesquitol being found in the heartwood from the big trees from Baringo County.

The second part of the study focused on the chemical modification of mesquitol targeting specific derivatives which could enhance the lipophilicity of mesquitol hence help in decreasing leachability. Various reactions of mesquitol with various carboxylic acids were performed allowing grafting of long fatty chains (lipophilic part) onto mesquitol through the aliphatic 3-OH position. Some of these derivatives were used to evaluate their ability to protect wood against fungal attack from both a white-rot and a brown-rot fungus. From the results of this study, it is evident that mesquitol derivatives obtained from reaction with carboxylic acids can be used as wood preservatives when used together with low amounts of tebuconazole.